Abnormal Composition of Hypertriglyceridemic Very Low Density Lipoprotein Determines Abnormal Cell Metabolism

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The metabolism of very low density lipoprotein (VLDL) from normolipemic (NTG) subjects, hypertriglyceridemic (HTG) subjects, and hypertriglyceridemic subjects treated with bezafibrate (BZ) was studied in cultured human skin fibroblasts. The binding, cell association, and proteolytic degradation of $^{125}$I-labeled lipoproteins and the capacity to regulate cellular sterol synthesis was determined with and without maximal stimulation of the lipoprotein by exogenous recombinant or plasmatic apolipoprotein (apo) E-3. The VLDL was separated into three density subfractions: I, II, and III. Multiple differences between HTG and NTG lipoproteins were found, which all reverted toward normal with therapy. Even in the presence of an optimal concentration of apo E-3, HTG-VLDL demonstrated 100% to 200% higher metabolic activities, indicating a better association or a better biological expression of apo E-3 at the surface of the lipoprotein. There was a strong and linear relationship between the cholesterol ester/protein ratios of the different VLDLs and their proteolytic degradations by the cells ($r=0.95$). Thus, the composition/structure alterations of VLDL appear to determine their apo E-3-dependent cellular catabolism. In addition, HTG-VLDLs not enriched with apo E-3 exhibited a capacity to down-regulate cellular sterol synthesis independently of their uptake and degradation by the cells. This abnormality appeared to reflect the ability of the VLDL to donate cholesterol to the cells and was not observed in receptor-negative cells. Thus, HTG-VLDL is much more capable than NTG-VLDL of introducing cholesterol to cells by at least two mechanisms: 1) accelerated uptake and degradation and 2) direct transfer of cholesterol to the cells. Both processes are potentially atherogenic and are reversible when triglyceride-lowering therapy is instituted.

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exogenous apo E-3 and that excessive transfer of cholesterol to the cells independent of uptake and degradation occurred with the HTG lipoprotein. Both abnormalities, which are potentially atherogenic, reverted to normal after BZ therapy was begun.

Methods

Patients

Seven male patients with primary endogenous HTG and 12 healthy NTG volunteers gave their consent to participate in the study. All the patients had HTG with normal or low LDL levels, and none of their family members had high LDL levels. Any secondary basis for hyperlipidemia was ruled out. The hyperlipidemic patients had been attending the Lipid Clinic at the Hadassah University Hospital, Jerusalem, for at least 6 months before the study. The patients' plasma lipid levels are shown in Table 1. Informed consent was obtained in all cases. All patients were normoglycemic, and their hepatic, renal, and thyroid functions were normal. None was receiving drug treatment for other conditions. Fastig plasma samples of 100 to 200 ml were obtained from an antecubital vein in the morning after a 14- to 16-hour fast. In each experimental HTG-VLDL or BZ-VLDL were examined together with a control normal VLDL isolated from an NTG subject.

Isolation and Characterization of Very Low and Low Density Lipoproteins

Blood was drawn into tubes containing ethylenediamine-tetraacetate (EDTA) (1 mg/ml), and plasma was separated promptly at 4°C. VLDL was separated at plasma density = 1.006 g/ml after 16 hours of centrifugation in a 60 Ti rotor at 45 000 rpm at 4°C in a Beckman L5-50 ultracentrifuge. The VLDL was spun once more at a density of 1.060 g/ml. VLDL density subfractions I, II, and III were prepared on an NaCl gradient in an SW-41 rotor following the procedure of Lindgren et al.17 LDL was separated at a density interval of 1.019 to 1.063 g/ml and was washed once at the high density solution. Lipoproteins were dialyzed against a solution of 0.15% NaCl, 20 mM Tris (pH 7.4), and 0.001% EDTA. All lipoprotein preparations were sterilized by passage through a 0.45 μm Millipore filter and were kept at 4°C. Lipoproteins were used within 10 days of preparation. Triglycerides were determined according to the U.S. Lipid Research Clinic's protocol with an Auto Analyzer AAII (Technicon, Tarrytown, NY).18 The cholesteryl ester and free cholesterol content in lipoproteins were determined enzymatically19 with a commercial kit (Boehringer Mannheim GmbH Diagnostics, Mannheim, FRG). Phospholipids were determined by the Bartlett procedure.20 VLDL and LDL protein were measured by the method of Lowry et al.21 with bovine serum albumin (BSA) as a standard. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of apolipoproteins on 10% gels was performed according to the methods described by Weber and Osborn.22 Sterols were separated by thin-layer chromatography on silica gel plates with the solvent system petroleum ether/diethyl ether/acetic acid, 80:20:1 (vol/vol/vol).23

Table 1. Characteristics of Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Plasma TG</th>
<th>Plasma C</th>
<th>HDL-C</th>
<th>Plasma TG</th>
<th>Plasma C</th>
<th>HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>H.Z.</td>
<td>46</td>
<td>348</td>
<td>275</td>
<td>36</td>
<td>173</td>
<td>244</td>
<td>45</td>
</tr>
<tr>
<td>S.I.</td>
<td>64</td>
<td>625</td>
<td>285</td>
<td>ND</td>
<td>269</td>
<td>234</td>
<td>25</td>
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<tr>
<td>S.I.</td>
<td>65</td>
<td>570</td>
<td>302</td>
<td>20</td>
<td>183</td>
<td>174</td>
<td>43</td>
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<tr>
<td>B.M.</td>
<td>60</td>
<td>396</td>
<td>252</td>
<td>33</td>
<td>94</td>
<td>244</td>
<td>51</td>
</tr>
<tr>
<td>C.I.</td>
<td>44</td>
<td>540</td>
<td>196</td>
<td>12</td>
<td>231</td>
<td>182</td>
<td>25</td>
</tr>
<tr>
<td>G.F.</td>
<td>52</td>
<td>475</td>
<td>207</td>
<td>34</td>
<td>200</td>
<td>194</td>
<td>39</td>
</tr>
<tr>
<td>R.M.</td>
<td>56</td>
<td>285</td>
<td>207</td>
<td>34</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Normals* (n=12)</td>
<td>—</td>
<td>127±18</td>
<td>135±8</td>
<td>41±3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Data are in mg/dl.

*Means±SEM.

TG=triglyceride, C=cholesterol, HDL=high density lipoprotein.

Apolipoprotein E

Recombinant apo E-3 was made in bacteria.24 The recombinant apo E-3 was identical to plasmatic apo E-3 in receptor assays and exhibited the same biological reactivity when tested in fibroblasts with VLDL and LDL. Plasmatic apo E-3 was prepared from the VLDL of a hyperlipidemic E-3/3 subject following the procedure described by Rail et al.25 The purity of apo E-3 thus obtained was determined by SDS-PAGE and was similar to that recently described.15

Iodination of Very Low and Low Density Lipoproteins

125I-labeled VLDL and LDL were prepared by the iodine monochloride method of MacFarlane26 as modified by...
The iodinated preparations were dialyzed extensively against 0.15 M NaCl and 0.001% EDTA (pH 7.4). The final specific activity varied between 300 and 600 cpm/ng protein and 50 and 500 cpm/ng protein for VLDL and LDL, respectively. In all preparations, >95% of the radioactivity was precipitated by trichloroacetic acid, and <5% was extractable by chloroform-methanol. All the iodinated VLDL and LDL preparations were sterilized by passage through a 0.45 μm Millipore filter and were used within 2 weeks of iodination. The preparations were kept sterile in tightly closed plastic tubes at 4°C. Labelled apo-proteins in the lipoproteins were determined after SDS-PAGE of 125I-labeled lipoproteins and counting of gel slices. The patterns of labeled apoproteins in preparations from HTG, BZ, or NTG subjects were similar. In VLDL-1, 125I apo B contributed 21.7% to 27.7% of the total radioactivity, and 125I apo C contributed 57.9% to 70.6%. In VLDL-II, the percentages were 27.5% to 34.7% and 51.5% to 56.9%, respectively. In VLDL-III, the percentages were 44.9% to 46.9% and 38.9% to 46.6%, respectively. Radioactivity in the gel slice that corresponded to apo E was less than 3.3% of the total gel radioactivity, a value not different from the background radioactivity on the gels.

In LDL, more than 87.5% of the total gel radioactivity was associated with apo B.

**Cultures of Human Skin Fibroblasts**

Human skin fibroblasts were prepared as previously described from skin biopsies obtained from the medial part of the forearm of normal adult male donors. The cells were cultured in plastic flasks (Falcon Labware, Becton Dickinson, Oxnard, CA) in modified Dulbecco-Vogt Medium containing 10% fetal calf serum (FCS) and were maintained in a humidified incubator (5% CO2) at 37°C. Fibroblasts from three to fifteen subcultures were used. After trypsinization, 3.5x10^6 cells were plated in 35 mm dishes (Falcon Labware) and were grown in 2 ml of medium containing 0.2% BSA. The amount of 125I lipoproteins bound to the cells was determined by the release of radioactivity after incubation of the washed cells at 4°C with medium containing 10 mg/ml of sodium heparin for 1 hour, according to the method of Goldstein et al. The heparin medium displaced more than 70% of bound 125I-VLDLs prepared from either patients or normal subjects as compared to a 40-fold excess of unlabeled LDL or apo E-3-enriched (4:10, wt/wt) VLDL. Labeled lipids in the cells were extracted with chloroform-methanol (1:1, vol/vol), and the cell residue was dissolved in 1 ml of 0.5 N NaOH. The cell-associated lipoprotein was determined as the radioactivity remaining in the delipidated cells. Dishes without cells and cultures incubated in the presence of a 40-fold excess of unlabeled lipoproteins (nonspecific values) were processed in parallel to the experimental samples. 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. The metabolic activities were determined in duplicate dishes. In some experiments, VLDL fractions were incubated with increasing amounts of apo E-3 (2 to 12 μg/ml); cellular binding, association, and degradation were determined as detailed above. In other experiments, the binding, association, and degradation of lipoproteins added at increasing concentrations (7.5 to 60 μg/ml) were measured.

**Incorporation of 14C-Acetate to Sterols**

The effect of added apo E-3 on the ability of lipoproteins to regulate sterol synthesis in up-regulated fibroblasts was determined after 6 hours of incubation with unlabeled VLDL fractions (30 μg of lipoprotein protein) or with LDL (15 μg of lipoprotein protein) in 1 ml of LPDS medium. Apo E-3 (recombinant or plasmatic) was added to the VLDL samples at a protein concentration of 6 μg/ml. After 6 hours, the medium was removed, and the cell layers were washed with PBS and 0.2% BSA and re-incubated for 2 hours at 37°C with medium containing 10 μCi of 2-14C-sodium acetate, 55 mCi/mmol. The 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-sterols. The values obtained from these cultures were taken as 100% values for the incorporation of 14C-acetate to sterols.

**Materials**

2-14C-acetate and 125I-Na were purchased from the Radiochemical Centre, Amersham, UK. Culture flasks and dishes were obtained from Falcon Labware. The medium and FCS were obtained from GIBCO Laboratories, Grand Island, NY. All other chemicals and reagents were of analytical grade.

**Statistical Analysis**

Evaluations of the significant differences between NTG, HTG, and BZ VLDLs were by paired t tests.
Table 2. Chemical Composition of Very Low Density Lipoprotein Subfractions from Normotriglyceridemic, Hypertriglyceridemic, and Bezafibrate-treated Subjects

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Subject (n)</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>NTG (9)</td>
<td>10.7±2.4</td>
<td>67.2±3.8</td>
<td>4.0±0.8</td>
<td>2.9±1.0</td>
<td>15.2±2.4</td>
</tr>
<tr>
<td></td>
<td>HTG (7)</td>
<td>7.2±1.2</td>
<td>63.9±2.0</td>
<td>9.5±2.8</td>
<td>4.3±0.8</td>
<td>15.1±2.4</td>
</tr>
<tr>
<td></td>
<td>BZ (6)</td>
<td>9.1±0.7</td>
<td>70.1±2.7</td>
<td>3.6±1.0</td>
<td>3.2±0.2</td>
<td>14.1±1.0</td>
</tr>
<tr>
<td>VLDL II</td>
<td>NTG (9)</td>
<td>13.3±1.0</td>
<td>57.0±3.2</td>
<td>6.2±1.1</td>
<td>3.3±0.7</td>
<td>20.2±2.5</td>
</tr>
<tr>
<td></td>
<td>HTG (7)</td>
<td>9.8±2.2</td>
<td>58.3±2.7</td>
<td>10.7±1.3</td>
<td>4.9±0.8</td>
<td>16.3±2.1</td>
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<tr>
<td></td>
<td>BZ (5)</td>
<td>11.0±0.8</td>
<td>64.1±3.6</td>
<td>5.5±1.3</td>
<td>3.2±0.4</td>
<td>16.2±1.4</td>
</tr>
<tr>
<td>VLDL III</td>
<td>NTG (9)</td>
<td>12.8±2.0</td>
<td>52.1±5.2</td>
<td>11.3±3.7</td>
<td>4.0±1.0</td>
<td>19.8±1.8</td>
</tr>
<tr>
<td></td>
<td>HTG (7)</td>
<td>11.3±2.0</td>
<td>51.2±3.5</td>
<td>13.2±2.5</td>
<td>6.0±1.0</td>
<td>18.3±3.0</td>
</tr>
<tr>
<td></td>
<td>BZ (5)</td>
<td>12.8±0.9</td>
<td>55.9±3.7</td>
<td>8.9±1.5</td>
<td>4.3±0.3</td>
<td>18.1±1.2</td>
</tr>
<tr>
<td>LDL</td>
<td>NTG (9)</td>
<td>20.8±2.0</td>
<td>8.3±2.6</td>
<td>37.6±4.6</td>
<td>7.8±0.8</td>
<td>25.5±2.7</td>
</tr>
</tbody>
</table>

Data are the means±SEM and are given as mg/100 mg lipoprotein. VLDL=very low density lipoprotein, TG=triglyceride, CE=cholesteryl ester, FC=free cholesterol, PL=phospholipid, NTG=normolipemic, HTG=hypertriglyceridemic, BZ=bezafibrate, LDL=low density lipoprotein.

Results

The chemical composition and SDS-PAGE of the NTG-, HTG-, and BZ-VLDL populations and of LDL are shown in Table 2 and Figure 1. Several differences are identified. As previously described, the total protein and triglyceride contents of the HTG-VLDLs were lower than the NTG-VLDLs, while both free and esterified cholesterol contents were higher. These differences were especially pronounced with VLDL-I but were also present in the other fractions. The VLDL populations from BZ-treated patients were generally similar to NTG-VLDL.

SDS-PAGE separation of apoproteins from VLDL demonstrated the presence of apo B, apo E, and apo Cs in all three NTG- and HTG-VLDL fractions. After centrifugation, NTG-VLDL I contained lower amounts of apo E as compared to NTG-VLDL III. HTG-VLDLs were rich with apo E and in contrast to NTG, HTG-VLDL I seemed to contain more apo E than did HTG-VLDL III. On the SDS-PAGE gradients, little or trace amounts of apo B-48 were identified with NTG-, HTG-, or BZ-VLDLs (data not shown).

Binding, cell association, and degradation of NTG-, HTG-, and BZ-VLDL I, II, and III and the effect of exogenous apo E-3 on these metabolic parameters are shown in Table 3. Without added apo E-3, the values for binding and cell association with all VLDL preparations were similar and very low. The degradation values, however, clearly demonstrated differences reflecting the source of the VLDL. In VLDLs obtained from HTG patients, proteolytic degradation was definitely identified and was three to five times higher than the VLDLs from the NTG individuals. These values, however, were about one-tenth those observed with normal LDL.

Exogenous plasmatic or recombinant apo E-3 induced a marked increase of the metabolic activity of all VLDL preparations. Binding values increased by three- to sixfold, cell association, by six- to tenfold, and degradation by 10- to 30-fold. Comparing the activities observed with the different preparations between subjects, four observations emerge: 1) In NTG-VLDL, the proteolytic degradation of VLDL III was considerably higher than VLDL I but similar in HTG. 2) In spite of the enhanced cellular metabolism of the apo E-3-enriched NTG-VLDL, the values were considerably lower than those of HTG-VLDL, especially VLDL I. 3) Even after the enrichment with apo E-3, the degradation values of HTG-VLDLs were similar or less than those of LDL. 4) After BZ therapy and normalization of plasma triglyceride levels, the metabolic activities of VLDL were similar to those observed with NTG-VLDLs. The ratios of proteolytic degradation to binding were calculated for each preparation and were found to be highest in the HTG-VLDL fractions. The difference was
Table 3. Effect of Exogenous Recombinant and Plasmatic Apolipoprotein E-3 on Cellular Metabolism of Very Low Density Lipoprotein Subtractions from Normotriglyceridemic, Hypertriglyceridemic, and Bezafibrate-treated Subjects

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Subject (n)</th>
<th>Binding</th>
<th>Cell association</th>
<th>Degradation</th>
<th>Degradation/binding</th>
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<tr>
<td>VLDL I</td>
<td>NTG (12)</td>
<td>18±5</td>
<td>51±5</td>
<td>25±3</td>
<td>129±20</td>
</tr>
<tr>
<td></td>
<td>HTG (7)</td>
<td>13±4</td>
<td>76±12</td>
<td>30±11</td>
<td>230±43</td>
</tr>
<tr>
<td></td>
<td>BZ (6)</td>
<td>14±4</td>
<td>47±12</td>
<td>19±3</td>
<td>99±27</td>
</tr>
<tr>
<td>VLDL II</td>
<td>NTG (12)</td>
<td>12±5</td>
<td>62±9</td>
<td>16±3</td>
<td>139±29</td>
</tr>
<tr>
<td></td>
<td>HTG (7)</td>
<td>11±3</td>
<td>69±5</td>
<td>25±10</td>
<td>226±20</td>
</tr>
<tr>
<td></td>
<td>BZ (6)</td>
<td>12±3</td>
<td>43±10</td>
<td>17±6</td>
<td>104±37</td>
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<tr>
<td>VLDL III</td>
<td>NTG (12)</td>
<td>18±5</td>
<td>86±12</td>
<td>17±5</td>
<td>149±19</td>
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<tr>
<td></td>
<td>HTG (7)</td>
<td>14±4</td>
<td>76±9</td>
<td>28±7</td>
<td>241±29</td>
</tr>
<tr>
<td></td>
<td>BZ (6)</td>
<td>29±10</td>
<td>95±39</td>
<td>20±5</td>
<td>136±30</td>
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<tr>
<td>LDL</td>
<td>NTG (10)</td>
<td>54±11</td>
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<td>113±15</td>
<td>1238±196</td>
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</table>

The values are the means±SEM and are given as ng lipoprotein protein/mg cell protein/6 hours.

The source of exogenous apo E-3 was recombinant E-3 in five experiments and plasmatic E-3 in two experiments. The data from the different experiments were indistinguishable, and therefore all values were pooled in the analysis. Very low density lipoprotein (VLDL) was used at a protein concentration of 15 μg/ml with or without the addition of exogenous apo E-3 (6 μg/ml). Low density lipoprotein (LDL) was used at a protein concentration of 10 μg/ml.

See the legend to Table 2 for an explanation of the abbreviations.

most striking with VLDL-I. BZ-VLDLs exhibited ratios similar to those observed with NTG-VLDLs.

The effect of increasing concentrations of exogenous plasmatic apo E-3 on the cellular proteolytic degradation of NTG- and HTG-VLDL fractions is shown in Figure 2. Apo E-3 stimulated both NTG- and HTG-VLDL III degradation to the same extent. In contrast, while NTG-VLDL I exhibited about one-half the activity of NTG-VLDL III, HTG-VLDL I exhibited a similar or even slightly higher activity than that of HTG-VLDL III. Of interest, HTG-VLDL was not degraded in receptor-negative cells with or without the addition of exogenous apo E-3 (data not shown). In another experiment, the concentration-dependent metabolism of NTG- and HTG-VLDLs was determined. Figure 3 presents the proteolytic degradation values from this experiment. At all concentrations tested, the proteolytic degradation of the lipoproteins decreased as follows: LDL>HTG-VLDL I>NTG-VLDL III>NTG-VLDL I.
Table 4. Effect of Exogenous Recombinant and Plasma Apolipoprotein E-3 on Regulation of Cholesterol Synthesis by Very Low Density Lipoprotein Subfractions from Normotriglyceridemic, Hypertriglyceridemic, and Bezafibrate-treated Subjects

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Subject (n)</th>
<th>-Apo E-3 (ng VLDL protein/mg cell protein/6 h)</th>
<th>+Apo E-3 (ng VLDL protein/mg cell protein/6 h)</th>
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</thead>
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<tr>
<td>VLDL I</td>
<td>NTG (8)</td>
<td>85.1 ± 8</td>
<td>54.2 ± 8</td>
</tr>
<tr>
<td></td>
<td>HTG (5)</td>
<td>56.2 ± 11</td>
<td>33.2 ± 7</td>
</tr>
<tr>
<td></td>
<td>BZ (4)</td>
<td>93.4 ± 7</td>
<td>69.5 ± 7</td>
</tr>
<tr>
<td>VLDL II</td>
<td>NTG (6)</td>
<td>91.6 ± 8</td>
<td>74.3 ± 12</td>
</tr>
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<td></td>
<td>HTG (5)</td>
<td>62.5 ± 13</td>
<td>35.0 ± 7</td>
</tr>
<tr>
<td></td>
<td>BZ (4)</td>
<td>90.3 ± 8</td>
<td>62.7 ± 4</td>
</tr>
<tr>
<td>VLDL III</td>
<td>NTG (8)</td>
<td>73.6 ± 7</td>
<td>45.2 ± 6</td>
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<td>HTG (5)</td>
<td>47.2 ± 15</td>
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<tr>
<td></td>
<td>BZ (4)</td>
<td>79.2 ± 3</td>
<td>39.0 ± 4</td>
</tr>
<tr>
<td>LDL</td>
<td>NTG (11)</td>
<td>29.3 ± 4</td>
<td>-</td>
</tr>
</tbody>
</table>

The values are the means±SEM and indicate the incorporation of 14C-acetate to sterols as percents of control. See the legend to Table 2 for an explanation of the abbreviations.

To ascertain that exogenous apo E-3 increases VLDL metabolism and not merely protein uptake, the effect on cellular sterol synthesis was determined (Table 4). Cholesterol synthesis was most effectively down-regulated by LDL with sterol synthesis of only 30% of that observed in cells incubated in the absence of lipoproteins. Without the addition of apo E-3, NTG-VLDL I and VLDL II had almost no effect on cholesterol synthesis, while some down-regulation was found with NTG-VLDL III. These findings agree with those from the proteolytic degradation studies, in which barely detectable metabolic activities were observed with NTG-VLDL I and VLDL II. The addition of exogenous apo E-3 increased considerably the capacity of NTG-VLDLs to down-regulate cholesterol synthesis. In contrast to NTG-VLDL, HTG subfractions were effective in regulating cholesterol synthesis even without the addition of exogenous apo E-3. In fact, HTG-VLDLs not enriched with apo E-3 down-regulated sterol synthesis almost as effectively as NTG-VLDL after the addition of apo E-3. No effects of HTG-VLDL on cellular sterol synthesis, however, were found in cells from a receptor-negative subject (data not shown). It is worthwhile pointing out that NTG-VLDL proteolytic degradation after the addition of apo E-3 was four- to sixfold higher than that of HTG-VLDL without the addition of apo E-3. As in the degradation studies, the addition of apo E-3 increased the capacity of HTG-VLDL to regulate cholesterol synthesis to levels markedly lower than NTG-VLDL. Subfractions obtained from BZ-treated subjects generally behaved similarly to NTG-VLDL.

To better understand the complex relationship between the degradation of the VLDLs and their capacity to regulate sterol synthesis, we examined the correlation between these two sets of data (Figure 4). It appears that the behavior of some preparations was distinctly different from the others. For NTG- and BZ-VLDL I and II and all the preparations after the addition of apo E-3, the linear relationships between degradation and regulation of sterol synthesis were evident. However, NTG- and BZ-VLDL

Figure 4. The relationships between proteolytic degradation of normotriglyceridemic (NTG), hypertriglyceridemic (HTG), and bezafibrate (BZ) very low density lipoprotein (VLDL) subfractions and their capacity to regulate sterol synthesis. This figure is based on data from 18 different VLDL fractions, and each value is a mean of six to nine individual preparations, NTG-, HTG-, and BZ-VLDL are represented by circles, squares, and triangles, respectively. Studies without exogenous apolipoprotein E-3 are shown as open symbols and studies with added exogenous apolipoprotein E-3 as closed symbols. The density subfractions are designated by Roman numerals. A linear regression analysis for data with the nine VLDL density subfractions studied with exogenous apolipoprotein E-3 is included, and the line of regression is shown (r²=0.78, p<0.01). The proteolytic degradations and regulations of sterol synthesis were determined after a 6-hour incubation of the VLDLs with up-regulated human skin fibroblasts as described in the Methods section.

III and all HTG-VLDL subfractions without additional exogenous E-3 appeared to possess a capacity to down-regulate sterol synthesis that was largely independent of the degradation values.

Discussion

It has been repeatedly shown that apo E molecules in VLDL serve as the main ligand for the interaction of the lipoprotein with cell receptors. 7-11,15,34,35 Both normal and HTG-VLDL populations contain apo B-100 and apo E. Yet, when prepared by ultracentrifugation, only the HTG lipoproteins exhibit a significant ability to react with B,E (LDL) receptors.11,12,13 This difference may reflect a higher content of apo E in HTG plasma36,37 or a smaller loss of apo E from HTG-VLDL during the centrifugation steps, or both. Neither mechanism, however, explains the two- to threefold difference of binding, association, and degradation of HTG-VLDL (especially fractions I and II) as compared to normal when both are maximally enriched with exogenous apo E-3. We offer two possible explanations of this phenomenon. The first is that HTG-VLDL has a larger capacity...
to bind apo E as compared to normal VLDL; the second is that HTG particles better express apo E. With either possibility, it must be assumed that structural and compositional differences between HTG and normal VLDL are responsible for a larger capacity to bind or for a higher biological expression of apo E on HTG-VLDL. Four compositional abnormalities were found in HTG-VLDL: low apoprotein and triglyceride contents and high free and esterified cholesterol concentrations. The cholesterol ester/protein ratio has been shown to serve as the best indicator for the degree of VLDL abnormality in the HTG state. In Figure 5, therefore, we plotted the relationship between proteolytic degradation and cholesterol ester/protein ratio in the nine VLDL preparations investigated (NTG-, HTG-, and BZ-VLDL-I, -II, and -III). A strong and highly significant relationship was found (Figure 5). This finding indicates that compositional and/or structural properties of VLDL determine or regulate the capacity of VLDL to interact (via apo E) with cellular B,E (LDL) receptors. The nature of such properties is obscure, but they must reflect the HTG state, because they disappear when plasma triglycerides are reduced during BZ therapy. These effects are especially pronounced for HTG- and BZ-VLDL-I when the compositional abnormalities and their reversal are particularly prominent, presumably because of the higher capacity of the lipoprotein to participate in cholesterol ester-triglyceride transfer reactions.

Our analysis of the binding, association, and degradation data uncovered another difference of the cellular metabolism of HTG-VLDLs: a higher degradation/binding ratio than that of normal or BZ-VLDLs. In studies on the metabolism of normal VLDL or LDL in fibroblasts, we had pointed out that this ratio is low in comparison to LDL, implying that only some of the particles bound to the fibroblast receptor are routed to lysosomes. The data contained here for apo E-3-enriched preparations demonstrate that while binding of HTG-VLDL to the cells is only moderately and inconsistently higher than normal or BZ-VLDL, both cell association and degradation are markedly and consistently higher, predominantly the degradation. The basis of this phenomenon is not clear but more efficient internalization and degradation of bound HTG particles, as compared to NTG- or BZ-VLDL are indicated. Because VLDL degradation is responsible for the shunt pathway, this phenomenon may partly explain the decreased LDL formation via the VLDL->LDL cascade in HTG states, found in some in vivo VLDL turnover studies.

In previous studies on the cellular metabolism of VLDL, either the binding/internalization/degradation assay or the effects on cellular sterol synthesis assay was used. In the present investigation, however, both assays were used and so can be compared. For normal and BZ-VLDLs and all preparations enriched with apo E-3, a linear relationship was found between the degradation and the ability to reduce cellular sterol synthesis. Since entry of cholesterol into the cells regulates HMG-CoA reductase activity, this finding reaffirms that receptor-dependent catabolism of the VLDLs followed by release of cholesterol is responsible for down-regulation of cellular sterol synthesis. With HTG-VLDLs, however, an unexpected deviation was found, and the particles demonstrated abilities to reduce sterol synthesis in great excess to the levels predicted from the entry of cholesterol into the cells by the degradation process. Thus, it can be inferred that HTG-VLDLs can serve as cholesterol donors independent of their lysosomal degradation. Excess free cholesterol in HTG-VLDL (Table 2) could be transferred to the cells as has been recently suggested for LDL. In VLDL, however, significant amounts of free cholesterol are dissolved in the triglyceride lipid core. Also, free cholesterol should enter receptor-negative cells, and that was not observed. Another possibility is the transfer of cholesterol ester molecules from lipoprotein to cells without the entry and lysosomal degradation of the particles. Such transfer was demonstrated for cholesterol ester-enriched chylomicrons in the perfused rat heart and for rat VLDL and chylomicrons in cultured cells. Studies are currently being conducted in our laboratory to determine whether a similar phenomenon occurs with human VLDL, whether functional receptors are necessary for this process, and whether HTG-VLDL behaves differently from NTG-VLDL. Regardless of the mechanism and nature of cholesterol molecules (free or esterified) that are transferred to the cells, the observations indicate that, in HTG states, such transfer may contribute significantly to the accumulation of cholesterol in cells and possibly to the development of atherosclerotic diseases.

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