Kinetic Heterogeneity of Low Density Lipoproteins in Primary Hypertriglyceridemia

Gloria Lena Vega and Scott M. Grundy

The kinetics of two subfractions of low density lipoproteins (LDL) were examined in nine patients with primary hypertriglyceridemia. LDL was subjected to equilibrium ultracentrifugation, and three patterns of LDL subfractions were noted. The LDL of five patients with moderate hypertriglyceridemia (plasma triglycerides (TG) ranging from 333 to 580 mg/dl) appeared to contain two distinct subfractions. One was less dense and had a high TG content; the other was more dense and had a reduced content of all lipids, particularly cholesterol. Each subfraction was labeled separately and was reinjected into the patient. Of the two subfractions, the more dense LDL usually had a higher fractional catabolic rate (FCR), although the turnover rates of both subfractions for these hypertriglyceridemic patients were higher than normal. Two other patients with mild hypertriglyceridemia had only a single LDL after gradient equilibrium ultracentrifugation. This fraction was divided into less dense and more dense subfractions, and their FCR was determined. In both patients, turnover rates of the two subfractions were similar and both were in the normal range. Finally, two more patients with mildly elevated TG had a very dense LDL, besides having a single, less dense band. For both patients, the FCR for the less dense and very dense subfractions were similar, although the denser LDL had a greater fraction in the extravascular compartment. Thus, patients with primary moderate hypertriglyceridemia often have distinct subfractions that have different turnover rates. For patients with mild hypertriglyceridemia, the LDL is more homogenous, and its subfractions are kinetically similar. (Arteriosclerosis 6:395–406, July/August 1986)

The presence of subspecies of low density lipoproteins (LDL) was first described several years ago, and since then different species of LDL have been demonstrated by immunological, electrophoretic, and ultracentrifugal methods. Heterogeneity of LDL has been reported for patients with hypertriglyceridemia, for normolipidemic patients with increased LDL apoprotein B (apo B), for patients with diabetes mellitus, and even for normal subjects. Fisher introduced the term "polydisperse" LDL to signify the presence of more than one species of LDL in patients with hypertriglyceridemia or diabetes mellitus; this heterogenous LDL contrasted to the monodisperse LDL of normolipidemic subjects.

Because of differences in the physical and chemical properties of subclasses of LDL, we can ask whether all species of LDL have similar kinetic properties. Fisher et al. pioneered investigations on the kinetics of polydisperse LDL. They showed that a group of patients with hypertriglyceridemia had at least two species of LDL, and these subspecies differed not only in physicochemical properties, but also in kinetic behavior. Therefore, we have examined further the metabolism of LDL in primary hypertriglyceridemia. Patients with both moderate and mild hypertriglyceridemia were studied. Their LDL were subjected to equilibrium ultracentrifugation. Patients with moderate hypertriglyceridemia demonstrated two apparently distinct subfractions of LDL; these differed both in chemical composition and in turnover rates. In contrast, patients with mild hypertriglyceridemia had a more homogenous LDL, although in some, a small, very dense subfraction was noted in addition to the major subfraction. The kinetic heterogeneity of LDL appeared minimal in patients with mild hypertriglyceridemia.

Methods

Patients

Nine men participated in studies conducted on the metabolic ward of the Veterans Administration Medical Center and in the General Clinical Research Center of the Parkland Memorial Hospital, Dallas, Texas. All patients had
hypertriglyceridemia at the time of screening for study. All had a history of coronary heart disease (CHD) defined as documented myocardial infarction or previous coronary artery surgery. Neither myocardial infarction nor coronary surgery had occurred in the 6 months preceding the study, nor had the patients received hypolipidemic drugs during this same period. None had diabetes mellitus or diseases of the gastrointestinal tract, liver, or endocrine system. The research protocol was approved by the appropriate institutional review boards, and all patients gave informed consent to participate in the study.

**Experimental Design**

This study was designed to examine the metabolism of subfractions of autologous LDL in patients with primary hypertriglyceridemia. To obtain subfractions, each patient's LDL was subjected to equilibrium ultracentrifugation. In these patients, three different patterns of LDL subfractions were noted. Five patients apparently had two distinct bands of LDL; two others had only a single band of LDL; and two patients had a single major band and a small quantity of a very dense band of LDL. The patients were grouped according to their pattern of subfractions, and different studies were carried out on each pattern as described below.

**Study 1. LDL Kinetics in Patients with Two Distinct LDL Subfractions**

Group 1 consisted of Patients 1 to 5 (Table 1). These patients maintained a definite hypertriglyceridemia throughout their study. The LDL of each patient was separated into subfractions that appeared as two distinct bands upon equilibrium centrifugation; details of the isolation procedure are described below. The less dense of the two subfractions, designated LDL-1, had a density of 1.032 g/ml or less. The denser subfraction, LDL-2, had a mean density ranging from 1.041 to 1.045 g/ml. After isolation of the two subfractions, one aliquot of each was taken for chemical analysis and another was radioiodinated with either $^{131}$I or $^{125}$I. The two labeled subfractions were reinjected simultaneously into the patient, and the disappearance from plasma of each radioisotope was measured for 20 days.

**Study 2. LDL Kinetics in Patients with a Single LDL Subtraction**

Group 2 consisted of two patients (6 and 7). They sustained relatively mild hypertriglyceridemia throughout their study. Each had a single band of LDL after equilibrium ultracentrifugation. This single fraction was divided into two subfractions which were designated LDL-1 and LDL-2. These subfractions had densities less than and greater than 1.036 g/ml, respectively. Both subfractions were analyzed for composition and were differentially radioiodinated; they were reinjected into the patient, and the disappearance of radioactivity from plasma was followed.

**Study 3. LDL Kinetics in Patients with a Very Dense LDL Subfraction**

Patients 8 and 9 (Group 3) also had mild hypertriglyceridemia throughout their study. Both had a small, but apparently discrete, subfraction of LDL of relatively high density (d > 1.040 g/dl); this small band, called LDL-3, was present in addition to a single larger and less dense subfraction, designated LDL-1/2. The latter resembled the single large band in the patients in Study 2. LDL-1/2 and LDL-3 were isolated separately, analyzed, differentially labeled, and reinjected; their decay curves were compared.

**Measurements of Lipoprotein Concentrations and Composition**

**Plasma Total and Lipoprotein Lipids**

Plasma total cholesterol (TC), triglycerides (TG), and lipoprotein cholesterol (C) were estimated every 3 days throughout the turnover studies. Total cholesterol and triglycerides were measured enzymatically as described recently. LDL cholesterol levels were determined as follows. Very low density lipoproteins (VLDL) and intermediate density lipoproteins (IDL) (d < 1.019 g/ml) were isolated by ultracentrifugation. Cholesterol was measured in the infranatant fraction. High density lipoprotein (HDL) cholesterol was measured after precipitation of apo B-containing lipoproteins of whole plasma with phosphotungstic acid and manganese. It has been reported that HDL cholesterol levels determined with this method average 2 to 5 mg/dl lower than those obtained with heparin-manganese precipitation. LDL cholesterol was estimated as the difference between the cholesterol in the d < 1.019 infranatant and in HDL.

**Isolation of LDL Subfractions for Turnover Studies**

Subfractions of LDL were obtained by equilibrium ultracentrifugation using a modification of the method of Lee et al. To carry out equilibrium centrifugation of LDL, the conditions for formation of an appropriate gradient were first established. To obtain a broad gradient over the LDL density range (1.019 to 1.063 g/ml), a mock solution of

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yrs)</th>
<th>Body weight (kg)</th>
<th>Body mass index kg/m²</th>
<th>Screening plasma lipids</th>
<th>Cholesterol (mg/dl)</th>
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</thead>
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<tr>
<td></td>
<td></td>
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<td>TC, TG, HDL, IDL</td>
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<tr>
<td>Group 1</td>
<td></td>
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<tr>
<td>1</td>
<td>52</td>
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<td>49</td>
<td>75</td>
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<td>239</td>
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<td>Group 2</td>
<td></td>
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</tr>
<tr>
<td>6</td>
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<tr>
<td>Group 3</td>
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<td>8</td>
<td>54</td>
<td>86</td>
<td>27.1</td>
<td>380</td>
<td>258</td>
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<tr>
<td>9</td>
<td>45</td>
<td>67</td>
<td>22.7</td>
<td>246</td>
<td>270</td>
</tr>
</tbody>
</table>
NaBr-NaCl of density 1.045 g/ml was prepared. This solution was subjected to ultracentrifugation in a fixed angle 60 Ti rotor (37.5 ml capacity) at 58,000 rpm for 40 hours at a temperature of 12°C. Runs were made in quadruplicate. After ultracentrifugation, 2 ml were aspirated sequentially starting from the top of the tube. The refractive index of each fraction was measured with a Bausch and Lomb refractometer (Model Abbe-3L) at 25°C. These refractive indices were converted to density by the following equation:

\[ p_{25} = 0.1767\Delta p_5 - 0.1767 \]  

where \( r = 0.99 \), \( p_{25} \) is the density of 25°C, and \( \Delta p_5 \) is the refractive index difference between NaBr solution and that of water. Equation 1 was derived from the refractive index and densities for NaBr listed in the International Critical Tables. The refractive index of water averaged 1.3330. NaCl was present in relatively small quantities, and it was assumed to make a negligible contribution in the density gradient of NaBr formed during ultracentrifugation. The densities for each successive 2 ml aliquot for the four tubes were averaged, and a linear regression analysis of volume vs. density was carried out. A regression line was constructed and was used to determine the density of the LDL subfractions in the study. The equation for this regression line was:

\[ p_{25} = 0.001 v + 1.0289 \]  

where \( r = 0.99 \), \( p \) is the density, and \( v \) is the volume removed from the top of the tube. In the fixed-angle tubes used to isolate the LDL subfractions, the density at the midpoint of the tubes was 1.045 g/ml.

The subfractions of LDL were isolated from 200 ml of plasma. VLDL + IDL (d < 1.019 g/ml) was removed initially in a 60 Ti rotor. The infranatant was adjusted to a density of 1.045 g/ml. This was done by combining two parts of infranatant (d < 1.019 g/ml) with one part of NaBr-NaCl solution (d = 1.097 g/ml). This mixture also was subjected to ultracentrifugation in a fixed angle 60 Ti rotor (37.5 ml capacity) at 58,000 rpm for 40 hours at a temperature of 12°C.

In Patients 1 to 5, two subfractions of LDL (called LDL-1 and LDL-2) were discernible after equilibrium ultracentrifugation. They consisted of two yellow bands separated by a clear region. They were divided through the clear region. Their densities were estimated by their position within the gradient, according to Equation 2. Each subfraction was collected by aspiration with a sterile syringe and blunt needle; each was then re centrifuged for 20 hours at a density of 1.070 g/ml at a temperature of 12°C in an SW 41 rotor. Thereafter, the lipoproteins were dialyzed extensively against 0.15 M NaCl and 0.3 mM disodium EDTA (pH 7.4). One aliquot of the subfraction was set aside for analysis of chemical composition; another containing 3 to 5 mg of apo B was radioiodinated and used in the turnover studies.

In two patients (6 and 7), onlv a single band of LDL was discernible after equilibrium ultracentrifugation. This band was divided into two subfractions with the division being made at density of 1.036 g/ml, according to Equation 2. The less dense subfraction was designated LDL-1, and the more dense subfraction was designated LDL-2. These subfractions were prepared for reinjection as described above.

Two patients (7 and 8) had one major band of LDL, designated LDL-1/2; this band was in the typical density range. Another small subfraction, having a density greater than 1.04 g/ml, was present; it was called LDL-3. LDL-1/2 and LDL-3 were isolated separately, washed, and concentrated by ultracentrifugation at a density of 1.07 g/ml, and then were prepared for injection as described above.

**Chemical Analysis of LDL Subfractions**

On subfractions of LDL, measurements were made of total cholesterol, triglycerides, phospholipid, and protein. Apo B as a component of total protein was determined as described recently. The fatty acid content of LDL cholesterol ester was approximated as LDL cholesterol × 0.48; this assumes that cholesterol in LDL is 70% esterified. The apoprotein pattern of each fraction was examined after electrophoresis of LDL in a polyacrylamide gel gradient containing sodium dodecyl sulfate (SDS-PAGE). Also each subfraction of LDL was tested for immunoactivity against apo B and human serum albumin. All subfractions reacted positively to apo B but not to albumin. Apolipoproteins other than apo B could not be detected by SDS-PAGE in the washed subfractions of LDL used as tracers in any studies. Dr. Petar Alapouvic kindly examined the LDL-3 fraction of Study 3 for immunoreactivity against an Lp(a) antibody. In Patient 8, the band of LDL-3 was immunoreactive, but in Patient 9, the LDL-3 band was not immunoreactive against Lp(a).

**Kinetic Studies of LDL**

Subfractions of LDL were radioiodinated with either 125I or 131I, as described previously. In these studies, LDL-1 was always labeled with 131I, and LDL-2, with 125I. Approximately 50 μCi of each tracer was used. Blood samples were collected at 10, 20, and 30 minutes and at 1, 2, 3, 4, 8, 12, and 24 hours; then every 12 hours up to the 4th day of the turnover; thereafter samples were collected every day for the next 16 days.

The die-away curves for individual subfractions of LDL were subjected to multicompartmental analysis by using a 2-pool model with one intravascular and one extravascular pool. The fractional catabolic rate (FCR) for each fraction (or subfraction) was estimated by using the CONSAM program (Convorsational V-ision of the SAAM Modeling Program) developed by Berman et al. Analyses were carried out in a Vax 11/780 computer. FCIs for LDL subfractions were compared using the Fisher F-test; this statistical test was used to evaluate whether there was a unique solution for each data set constituting the isotope decay curves, and if so, whether the two curves were significantly different at a p value of less than 0.05. The extravascular/intravascular distributions of LDL were estimated from the steady-state equations of the Matthews analysis.
### Study 1. Metabolism of Two Apparently Discrete Subfractions of LDL

The five patients of Study 1 had a mean plasma triglyceride of 424 ± 28 (±SEM) mg/dl (Table 2); the average plasma total cholesterol (182 ± 35 mg/dl) and LDL cholesterol (98 ± 32 mg/dl) were within the normal range and HDL cholesterol levels averaged 21 ± 4 mg/dl. Each patient had two subfractions of LDL. The mean density of LDL-1 was 1.031 ± 0.001 g/ml, and that of LDL-2 was 1.042 ± 0.001 g/ml. On the average, LDL-2 had a significantly higher percentage of apo B than LDL-1, but LDL-1 had a higher percentage of triglycerides. The percentages of cholesterol in LDL-1 and LDL-2 were similar; the same was noted for phospholipids. The ratio of apo B-to-cholesterol for LDL-2 was significantly higher than for LDL-1. None of the patients had abnormally high concentrations of total LDL-apo B, i.e., levels over 120 mg/dl.

The die-away curves for the tracers of LDL-1 and LDL-2 are presented in Figures 1 and 2. For all patients, both curves were biexponential. They decayed at relatively rapid rates, as reflected by their high FCRs. The FCRs for the different subfractions in these patients ranged from 0.545 to 1.8 pools/day. In contrast, in a previous study from our laboratory, 15 normotriglyceridemic men of similar age had FCRs of 0.04 to 0.28 pools/day.

### Table 2. Lipid Concentrations and Chemical Composition of LDL Subfractions in Patients 1–5 (Study 1)

<table>
<thead>
<tr>
<th>Property</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma and lipoprotein lipids (mg/dl ± SD) (n = 6)</td>
<td>222 ± 18</td>
<td>175 ± 19</td>
<td>130 ± 14</td>
<td>207 ± 33</td>
<td>178 ± 22</td>
<td>182 ± 35</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>580 ± 230</td>
<td>333 ± 51</td>
<td>450 ± 93</td>
<td>359 ± 58</td>
<td>397 ± 149</td>
<td>424 ± 98</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>109 ± 6</td>
<td>57 ± 13</td>
<td>77 ± 2</td>
<td>139 ± 24</td>
<td>107 ± 22</td>
<td>96 ± 32</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>19 ± 1</td>
<td>22 ± 2</td>
<td>18 ± 1</td>
<td>19 ± 3</td>
<td>27 ± 4</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>LDL properties</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density (g/ml)</td>
<td>1.031</td>
<td>1.041</td>
<td>1.031</td>
<td>1.045</td>
<td>1.030</td>
<td>1.031 ± 0.001</td>
</tr>
<tr>
<td>Apo B</td>
<td>19.7</td>
<td>28.4</td>
<td>22.0</td>
<td>20.0</td>
<td>27.2</td>
<td>23.8 ± 1.8</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>22.8</td>
<td>28.9</td>
<td>27.3</td>
<td>23.1</td>
<td>26.3</td>
<td>33.3 ± 6.7</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>27.5</td>
<td>9.5</td>
<td>32.3</td>
<td>21.6</td>
<td>10.2</td>
<td>5.7 ± 4.6</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>18.9</td>
<td>20.0</td>
<td>32.4</td>
<td>24.1</td>
<td>23.9</td>
<td>21.7 ± 2.2</td>
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<tr>
<td>Cholesterol FA</td>
<td>11.0</td>
<td>13.9</td>
<td>13.2</td>
<td>11.4</td>
<td>12.7</td>
<td>15.9 ± 2.5</td>
</tr>
<tr>
<td>Apo B/chol ratio</td>
<td>0.86</td>
<td>0.98</td>
<td>0.81</td>
<td>0.96</td>
<td>0.87</td>
<td>1.04 ± 0.71</td>
</tr>
<tr>
<td>LDL-apo B conc (mg/dl)</td>
<td>24</td>
<td>66</td>
<td>26</td>
<td>12</td>
<td>21</td>
<td>51</td>
</tr>
</tbody>
</table>

*LDL-2 greater than LDL-1 by paired t test (p < 0.05).
†LDL-1 greater than LDL-2 by paired t test (p < 0.05).

**Results**

**Figure 1.** Isotope decay curves for LDL subfractions for Patients 1 to 3 of Study 1. The fractional catabolic rates (FCRs) of LDL-2 were greater than LDL-1 in these three patients. Likewise, the extravascular (EV) fraction was higher for LDL-2 than LDL-1.
METABOLISM OF LDL SUBFRACTIONS  

Vega and Grundy

Figure 2. Isotope decay curves for LDL subfractions for Patients 4 and 5 of Study 1. In Patient 4, the fractional catabolic rates (FCRs) for LDL-1 and LDL-2 were similar, but the extravascular (EV) fraction was much greater for LDL-2. In Patient 5, the FCR for LDL-2 was higher than for LDL-1, but the EV fraction was greater for LDL-1.

had an average FCR for LDL of $0.30 \pm 0.04$ (SEM) pools/day (range = 0.25 to 0.40 pools/day).

Patients 1 to 4 had FCRs that were significantly higher for LDL-2 than for LDL-1 as demonstrated by Fisher's F-test.30 In Patient 5, the FCRs for the two subfractions were not significantly different. The distributions of intravascular mass of apo B (mass fraction) between LDL-1 and LDL-2 were variable. Also, in four patients (1,2,3,5) the extravascular fraction apparently was greater for LDL-2 than for LDL-1.

Study 2. Metabolism of Subfractions from a Single Band of LDL

Data for Patients 6 and 7 are shown in Table 3 and Figure 3. Their TG levels throughout the study averaged 236 \pm 55 and 196 \pm 22 mg/dl, respectively. These patients had only a single band of LDL upon equilibrium centrifugation. This band was divided at density 1.036 g/ml into subfractions with mean densities for LDL-1 and LDL-2 of 1.032 g/ml and 1.039 g/ml, respectively. The apo B/cholesterol ratios generally were higher for LDL-2. In both patients, the FCRs for LDL-2 were only marginally higher than those of LDL-1. In neither patient was the FCR abnormally high. For both patients, the extravascular fraction of LDL-2 was greater than for LDL-1.

Study 3. Metabolism of Very Dense LDL

Two patients (8 and 9) had a small, but apparently distinct, subfraction of LDL of unusually high density (Table 4). Their TG levels averaged 310 \pm 77 and 208 \pm 19 mg/dl, respectively. These patients had only a single band of LDL upon equilibrium centrifugation. This band was divided at density 1.036 g/ml into subfractions with mean densities for LDL-1 and LDL-2 of 1.032 g/ml and 1.039 g/ml, respectively. The apo B/cholesterol ratios generally were higher for LDL-2. In both patients, the FCRs for LDL-2 were only marginally higher than those of LDL-1. In neither patient was the FCR abnormally high. For both patients, the extravascular fraction of LDL-2 was greater than for LDL-1.

Table 3. LDL Metabolism In Two Patients with a Single LDL Fraction (Study 2)

<table>
<thead>
<tr>
<th>Property</th>
<th>Patient 6</th>
<th>Patient 7</th>
</tr>
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<tbody>
<tr>
<td>Total cholesterol</td>
<td>253 ± 17</td>
<td>171 ± 27</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>236 ± 55</td>
<td>196 ± 22</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>181 ± 9</td>
<td>109 ± 21</td>
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<tr>
<td>HDL cholesterol</td>
<td>37 ± 4</td>
<td>28 ± 3</td>
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LDL properties

<table>
<thead>
<tr>
<th>Subfraction</th>
<th>LDL-1</th>
<th>LDL-2</th>
<th>LDL-1</th>
<th>LDL-2</th>
</tr>
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<tbody>
<tr>
<td>Density (g/ml)</td>
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<td>1.039</td>
<td>1.032</td>
<td>1.039</td>
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<tr>
<td>Composition (%)</td>
<td></td>
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<tr>
<td>Apo B</td>
<td>20.5</td>
<td>25.2</td>
<td>20.3</td>
<td>25.1</td>
</tr>
<tr>
<td>Total chol</td>
<td>31.8</td>
<td>29.9</td>
<td>31.4</td>
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<td>Triglyceride</td>
<td>11.7</td>
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<td>Phospholipid</td>
<td>21.0</td>
<td>19.7</td>
<td>23.4</td>
<td>24.1</td>
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<tr>
<td>Chol ester FA</td>
<td>15.3</td>
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<tr>
<td>Apo B/chol ratio</td>
<td>0.65</td>
<td>0.84</td>
<td>0.65</td>
<td>0.85</td>
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</table>

LDL-apo B conc (mg/dl) | 64.0 | 48.0 | 51.3 | 29.7 |

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mg/dl, respectively, during the turnover study. The mean densities of LDL-3 were 1.059 and 1.050 g/ml for Patients 8 and 9, respectively, and in both cases, apo B/cholesterol ratios for LDL-3 were very high (1.26 and 1.07, respectively). In both patients the percentage of TG in LDL-3 was higher than in LDL-1 (12).

The turnover rates of their very dense subfractions (LDL-3) were compared to those of less dense LDL (LDL-1/2) (Figure 4). The FCRs for LDL-3 were similar to those for LDL-1/2; but the extravascular fraction of LDL-3 was essentially double that of LDL-1/2; this was reflected by the high tail on the curves of LDL-3.

**Discussion**

This investigation in patients with primary hypertriglyceridemia demonstrated that subfractions of LDL, isolated
Metabolism of LDL Subfractions

Vega and Grundy

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Figure 4. Isotope decay curves for LDL-1/2 and LDL-3 in Study 3 (Patients 8 and 9). The fractional catabolic rates (FCRs) for LDL-3 were slightly lower than for LDL-1/2, but the extravascular (EV) fractions were much higher for LDL-3. Most of the LDL mass was in LDL-1/2.

by differences in density, do not exhibit identical kinetic behavior. Their differential turnover rates almost certainly reflect differences in their chemical compositions. This demonstration that subfractions of LDL are kinetically different may have implications for the overall kinetic analysis of LDL metabolism. The greatest differences in kinetic behavior of subfractions of LDL were noted for patients with moderate hypertriglyceridemia who had apparently discrete subfractions of LDL. To add to the complexity of LDL metabolism in these patients, they had high turnover rates for both species of LDL, which were superimposed upon differences in turnover rates for the subfractions. On the other hand, patients with mild hypertriglyceridemia seemingly had a less complex picture of LDL metabolism. Therefore, in the discussion to follow, the results for patients with different patterns of subfractions of LDL will be considered separately.

Metabolism of Polydisperse LDL

Fisher et al. used the term "polydisperse" to signify the marked heterogeneity in particle size of LDL from hypertriglyceridemic patients, as revealed by analytical ultracentrifugation. Most normolipidemic patients, in contrast, have a more homogenous or "monodisperse" LDL. Polydisperse LDL have been noted in several studies to have abnormalities in chemical composition. Upon analytical ultracentrifugation, polydisperse LDL appear as a spectrum of particles without distinct subfractions. However, Krauss and Burke have shown that LDL subjected to equilibrium density ultracentrifugation exhibit more distinct subfractions not revealed by analytic ultracentrifugation. They suggest the term "paucidisperse" to signify an LDL fraction containing sets of discrete particles in LDL. Thus, as demonstrated by these workers and the current study, polydisperse LDL can be separated by equilibrium ultracentrifugation into subfractions that are more distinct than can be demonstrated by analytic ultracentrifugation.

In the present study, LDL from five patients with moderate hypertriglyceridemia were separated into two subfractions of different density. Both subfractions had relatively high ratios of apo B to cholesterol. The less dense subfraction was enriched in triglycerides, and the more dense subfraction was poor in lipids and had an unusually high ratio of apo B to cholesterol. Previously, Teng et al. had reported that denser LDL in patients with hyperapobetalipoproteinemia has a high content of apo B. Many patients with hyperapobetalipoproteinemia probably have familial combined hyperlipidemia — a condition in which very dense LDL have also been reported. Since our hypertriglyceridemic patients had CHD, they too probably had familial combined hyperlipidemia.

In Study 1, both subfractions of LDL had relatively high turnover rates; however, the FCRs calculated for LDL-2 generally were higher than those for LDL-1. Therefore, two questions should be differentiated. First, why do many patients with hypertriglyceridemia have unusually high turnover rates of all subfractions of LDL? And second, why are the FCRs for LDL-2 usually greater than for LDL-1? Both questions can be considered.

High Turnover Rates of Polydisperse LDL

Several mechanisms could contribute to the high turnover rates of all low density particles noted in patients with...
moderate hypertriglyceridemia. For example, the number of LDL receptors could be increased. When hypertriglyceridemia is due to an increased secretion of VLDL into plasma, these VLDL may carry an excess of hepatic cholesterol; this could reduce hepatic concentrations of cholesterol and trigger an increase in synthesis of LDL receptors. Also, patients with hypertriglyceridemia frequently have elevated excretion rates for cholesterol and bile acids which likewise could deplete the liver of cholesterol and thereby stimulate the synthesis of LDL receptors.

Alternatively, polydisperse LDL might have an unusually high affinity for LDL receptors. There are several ways in which the affinity of LDL for LDL receptors might be increased. First, the apo B of polydisperse LDL could be unusually exposed on surface of the lipoprotein particles so that it has a high affinity for LDL receptors. The possibility of enhanced exposure of apo B is suggested by the increased LDL-apo B/cholesterol ratios in these patients (Table 2). However, this mechanism is not supported by investigations in tissue culture. Klienman et al. reported that LDL from hypertriglyceridemic patients do not have enhanced binding to LDL receptors in cultured fibroblasts. Thus, a paucity of cholesterol in LDL may not of itself promote the binding of LDL to LDL receptors. If not, other causes of increased affinity might be considered. For example, polydisperse LDL in its native state might contain apo E that would promote binding to LDL receptors. We could not detect apo E on the washed LDL used for reinjection, but preparative ultracentrifugation may have sheared it off. If apo E is lost during ultracentrifugation, this also could account for poor binding of isolated LDL in tissue culture. After reinjection of labeled LDL into patients, apo E may have recombined with LDL causing it to be cleared rapidly.

We also might consider whether polydisperse LDL is removed at increased rates other than by LDL receptors. For instance, nonspecific endocytosis of LDL might be increased; or LDL could be cleared by chylomicron-remnant receptors or macrophage receptors. An alternate pathway for removal of LDL was explored for patients with severe hypertriglyceridemia by Shepherd et al. These workers used cyclohexanedione-modified LDL to prevent uptake of LDL by LDL receptors. Normally, less than 20% of the plasma pool of modified LDL is removed per day, and presumably this reflects the activity of non LDL-receptor pathways; in contrast, a much higher percentage of modified LDL was removed each day in hypertriglyceridemic patients. Thus, the abnormal LDL of hypertriglyceridemic patients could be cleared at a high rate by alternate pathways.

Supporting evidence that compositional abnormalities of LDL contribute to high turnover rates in many hypertriglyceridemic patients comes from studies during treatment with gemfibrozil. We have shown recently that gemfibrozil therapy tends to "normalize" the composition of LDL of hypertriglyceridemic patients by reducing LDL-apo B/cholesterol ratios; simultaneously, there is a reduction in FCR for LDL. Similar changes in LDL kinetics, associated with fibric acid therapy, have been reported by others. The reduction in FCR for LDL during fibric acid therapy is consistent with the concept that normalizing the composition of LDL causes LDL to be cleared through its normal pathways.

**Differential Turnover Rates for LDL Subfractions**

Next we can consider the reasons for the apparently greater fractional clearance for LDL-2 compared to LDL-1 for most patients in Study 1. One possible cause for the slower clearance of LDL-1 could be that it must be converted, at least in part, to LDL-2 before it can leave the circulation; a delay in conversion would cause LDL-1 to be cleared more slowly than LDL-2. Clearly, the lower FCR for LDL-1 implies that it had a lower affinity for removal sites than LDL-2.

Differences in the FCRs for LDL-1 and LDL-2 could have implications for analysis of LDL kinetics in hypertriglyceridemic patients who have at least two species of LDL. This potential problem was recognized previously by several investigators, and they have proposed several models to account for LDL kinetics in these patients. An accurate estimate of production rate of total LDL-apo B depends upon choice of the correct model. At least six different models for LDL can be considered (Figure 5). From the current data, the production of total LDL-apo B can be estimated for the first three models, as shown in Table 5.
and the approaches employed for these calculations are described in the Appendix.

**Model A.** Most previous investigators of LDL kinetics have used whole LDL labeled with a single isotope, and the data have been analyzed by the 2-compartment model of Matthews,27 i.e., by Model A of Figure 5. This model assumes a single homogeneous pool of LDL in plasma and a single extravascular exchange pool. Estimates of production rates for total LDL-apo B for Patients 1 to 5, using Model A, are given in Table 5. Since LDL from these patients was not homogeneous, an isotopic decay curve for whole LDL does not reveal subfractions of LDL that differ in kinetic behavior; and therefore use of the 2-pool model may not give a precise estimate of the production rate, or transport rate, of total LDL-apo B.

**Model B.** An alternate way to analyze the current data for Patients 1 to 5 is by Model B. This model assumes that turnovers of LDL-1 (L-1) and LDL-2 (L-2) are completely independent; i.e., each subfraction has a separate input and exit, and the two compartments are not connected. If this model pertains, the production rate of total LDL-apo B would equal the sum of the turnover rates of the two subfractions; this sum is given in Table 5. The summed transport rates for LDL-apo B closely approximate the transport rate of total LDL-apo B obtained by Model A. Still, Model B could overestimate the total transport of LDL-apo B if one subfraction is partially or completely transformed into the other, and models incorporating interconversions must be considered.

**Model C.** One such model was proposed by Fisher et al.14 These workers labeled apo B endogenously with radioactive leucine. They isolated two subfractions of LDL from hypertriglyceridemic patients by sequential ultracentrifugation and defined each as an intravascular compartment. The less dense subfraction appeared to be a precursor of the more dense one; the latter usually comprised the larger percentage of the total intravascular pool. For most patients, almost none of the less dense subfraction appeared to be removed directly from the circulation, but instead it was converted to the more dense fraction. The proposed model thus corresponds essentially to Model C (Figure 5). According to their model,14 L-1 had no exchange compartment. Also, since most L-1 was converted to L-2, total production of LDL-apo B should be equivalent to the transport through L-2. This value, assuming no direct exit from L-1, is shown for Patients 1 to 5 as Model C-I in Table 5. In several instances, the transport rates through LDL-2 were substantially lower than the total production rate of LDL-apo B estimated by models A and B. Therefore, if Model C-I, having no exit from L-1, is correct, analysis of data by Model A or B could grossly overestimate the production rate of total LDL-apo B in these patients.

Another possibility is that a portion of L-1 is removed directly from the circulation, without conversion to L-2. This modification of Model C is designated Model C-II. As indicated in the Appendix, Model C-II is more consistent with the observed data than is Model C-I. In a word, for most patients the pool size of L-1 was too large to fit Model C-I, and thus a portion of L-1 must have been removed directly. Transport rates for total LDL-apo B can be approximated for Model C-II (Table 5), but the calculations must be made on unproven assumptions because of a lack of direct data on conversion of L-1 into L-2. In general, Model C-II gives higher values for total transport than those of Model C-I, but lower than those obtained by Models A and B.

**Model D.** Another model for metabolism of LDL-apo B, proposed by Goebel and Berman45 is Model D (Figure 5). These workers injected exogenously labeled LDL, and the presence of subfractions of LDL was detected by the pattern of excretion of radioactivity in urine compared to the plasma decay curves. Patients who had a constant urine/plasma (U/P) ratio were presumed to have a homogeneous LDL, but if the U/P ratio was inconstant, heterogeneity in LDL kinetics was assumed to be present. For patients with apparently heterogeneous LDL, two pools of LDL were assigned to the plasma compartment. The turnover rate of one pool generally was estimated to be approximately twice that of the other. The "fast" pool apparently had no exchange compartment while the "slow" pool did. The subfractions isolated in the current study clearly did not correspond to the two compartments of the model of Goebel and Berman.45 We found no evidence for a major subfraction without an exchange pool, and our data raise the question of whether such a pool in LDL is possible; all of our kinetic curves were biexponential, and thus seemingly had an exchange compartment. Also, there was not a great difference in FCRs of our two subfractions, as was implied by the Goebel-Berman model.45 If LDL subfractions with markedly different turnover rates do exist, therefore, they apparently are not density dependent. For this reason, Model D does not fit the current data.

**Model E.** Still another possible configuration is Model E. If one subfraction of LDL were to be removed from the circulation very rapidly, it should be present in low concentrations, and if input into this fraction were high, calculation of turnover of LDL by any other model might seriously underestimate the production of LDL-apo B. This possibil-
ity has been suggested by the reports of Thompson et al.\textsuperscript{46} and Witzum et al.\textsuperscript{47} However, in our studies, we could detect no subtraction of LDL that had a very high turnover rate compared to another; in other words, we found no evidence for a "very-fast" pool of LDL that would seriously underestimate production rates of total LDL-apo B when they are calculated by other models in Figure 5. Consequently, Model E is not consistent with our data. In our view, before a "very-fast" pool of LDL can be accepted, it must be demonstrated to exist by direct isolation and appropriate turnover studies.

\textbf{Model F.} A final model to explain LDL kinetics is Model F. This may be the preferred model to explain current and previous data of hypertriglyceridemic patients with polydisperse LDL. Two pools of LDL are shown, and a conversion of L-1 to L-2 is allowed as suggested by previous studies.\textsuperscript{14, 48} Also a reverse conversion of L-2 to L-1 cannot be ruled out. Direct removal from L-1 appears to be required by the current data. Because of biexponential curves of L-1 in our patients, an exchange pool was added to this compartment; the need for an exchange pool for L-1 was especially notable in Patient 5 in whom conversion of LDL-1 into LDL-2 seemed minimal (Figure 2). Finally, the possibility of direct input of L-2 has not been ruled out. If there is a conversion of L-1 to L-2, there would be a lower production rate for total LDL-apo B than estimated by Models A and B. This was illustrated by Model C-II (Table 5). However, any overestimation of transport by Models A or B, because of the partial conversion of L-1 to L-2 and the delay therefrom would be mitigated if there is direct synthesis of L-2 or reverse transport from L-2 to L-1. Certainly if Model F pertains, transport rates would be underestimated by using Model C-I. Furthermore if model F pertains, true values for transport of total LDL-apo B should lie between values obtained by Model B and Model C-II, but in the present study, data are not available to make an accurate estimate of transport using Model F. In our view, future studies should test Model F as being the most likely to accurately reflect the metabolism of LDL-apo B when at least two species of LDL can be demonstrated. To employ this model and obtain accurate transport rates of LDL-apo B, rates of interconversion between the subfractions must be measured throughout the turnover study.

\textbf{Metabolism of Monodisperse (Single Band) LDL}

Fisher et al.\textsuperscript{12} have reported that patients with normal plasma lipids generally have monodisperse LDL by analytical ultracentrifugation. Two of our patients (6 and 7), who had relatively mild hypertriglyceridemia during their study, appeared to have only a single band of LDL by equilibrium ultracentrifugation. Thus, hypertriglyceridemia, particularly when it is mild, is not invariably associated with polydisperse LDL. In Patients 6 and 7, the single band of LDL was divided into two subfractions — one of greater and one of lesser density. The chemical compositions of these two subfractions were not identical in that apo B/cholesterol ratios were somewhat higher for LDL-2. In neither patient were the FCRs for either LDL fraction abnormally high, as in Patients 1 to 5. Furthermore, the turnover rates for LDL-2 were only marginally higher than those of LDL-1. Because of this similarity in turnover rates of the two subfractions, the 2-pool model for LDL-apo B (Model A, Figure 5) should provide a fairly accurate estimate of the turnover of total LDL-apo B in patients with monodisperse LDL. This conclusion also was reached by Fisher et al.\textsuperscript{12} and Beltz et al.\textsuperscript{46} for LDL metabolism in normotriglyceridemic patients who have essentially normal turnover rates for LDL.

\textbf{LDL Kinetics in the Presence of a Very Dense Band of LDL}

In two patients with mild hypertriglyceridemia (8 and 9), there was a small subtraction of LDL that had an unusually high density. Although this band did not have a density greater than 1.063 g/ml, as reported for Lp(a),\textsuperscript{49} it nevertheless was antigenically positive for Lp(a) and in our studies, we could detect no subfraction of LDL that had a very high turnover rate compared to another; in other words, we found no evidence for a "very-fast" pool of LDL that would seriously underestimate production rates of total LDL-apo B when they are calculated by other models in Figure 5. Consequently, Model E is not consistent with our data. In our view, before a "very-fast" pool of LDL can be accepted, it must be demonstrated to exist by direct isolation and appropriate turnover studies.

\textbf{Conclusions}

This study provides further evidence that LDL is not kinetically homogeneous. The greatest heterogeneity of LDL appears to occur in patients with distinct hypertriglyceridemia, and more research is needed to obtain an adequate model for metabolism of LDL in this category of patient. As shown in Table 5, a 2-pool model (Model A) appears adequate for evaluating turnover of LDL. Certainly the presence of Lp(a) or related complexes in LDL may introduce a minor error in the analysis of LDL kinetics, but overall, the metabolism of monodisperse LDL is less complex than that of polydisperse LDL. Finally, the mechanisms responsible for these different types of LDL present a challenge for future investigation.

\textbf{Appendix}

The turnover of the whole LDL fraction, according to Model A, was calculated from the curve that would have been obtained had the whole LDL fraction been labeled with a single isotope. This curve could easily be constructed from the curves of the two subfractions as follows: The percent injected dose for the whole LDL fraction remaining
In plasma at a given time was equal to the mass fraction of LDL-1 times the percent injected dose of labeled LDL-1 remaining in plasma plus the mass fraction of LDL-2 times the percent injected dose of labeled LDL-2 remaining in plasma. The resulting die-away curve was then analyzed by the 2-pool model of Matthews27 (Model A). The transport of total LDL-apo B was determined by multiplying the estimated FCR by the plasma pool size of LDL-apo B. The latter was estimated as the product of the concentration of LDL-apo B and the plasma volume as determined by the dilution of radioactivity at the 10-minute sample.17

For Model B, the apparent transport rates for each subfraction were calculated as the product of pool sizes of each subfraction times the FCR of the subfraction. Each FCR was estimated by the 2-pool model.27 The pool sizes of the subfractions were estimated as described above. The total transport of LDL-apo B was taken to the numerical sum of the transport rates of the two subfractions. This calculation assumed that each subfraction contained no further kinetic heterogeneity and that neither was converted into the other.

For Model C, it was first assumed that L-1 has no direct exit, as postulated for most patients by Fisher et al.14 This configuration is Model C-I. It assumes that all of L-1 is converted to L-2 before exit. Therefore, for Model C-I, the total transport of LDL-apo B is equivalent to transport of L-2. In other words, the total transport of LDL-apo B would equal the turnover rate of L-2.

If Model C-I is correct, the transport rate for L-1 also should equal the transport rate of L-2. Furthermore, the difference in FCRs observed for L-1 and L-2 should closely approximate the FCR for the conversion of L-1 into L-2. Therefore, the pool size of L-1 should equal the transport rate through L-1 (or L-2) divided by the FCR for the conversion of L-1 into L-2. For most patients, the observed pool sizes for L-1 were much greater than required by this estimation; this means that much of L-1 must have been removed from the circulation (without conversion to L-2). Therefore, Model C-I must be modified to allow for direct exit from L-1. This modified model is designated Model C-II. It allows all L-2 to be derived from L-1, but the excess pool of L-1, not required for conversion to L-2, is postulated to exit directly at the FCR observed for L-1. Thus, total transport for LDL-apo B was calculated as the transport for L-2 plus the catabolic rate for L-1 not converted to L-2. It should be noted that this calculation represents an approximation based on assumptions about conversions of L-1 into L-2 which was not directly examined.

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References


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