Fenofibrate and LDL Metabolic Heterogeneity in Hypercholesterolemia

M.J. Caslake, C.J. Packard, A. Gaw, E. Murray, B.A. Griffin, B.D. Vallance, and J. Shepherd

Metabolic heterogeneity in low density lipoprotein (LDL) may be detected by examination of the daily urinary excretion rate of radioactivity after injection of trace-labeled lipoprotein. Two distinct pools are observed within LDL. The first (pool A) is cleared rapidly from the plasma, whereas the second (pool B) is catabolized more slowly. In the present study we examined LDL metabolism in seven hypercholesterolemic subjects (six women and one man) before and during fenofibrate therapy. Comparison with normocholesterolemic individuals showed that the pretreatment high LDL levels in the hypercholesterolemic subjects resulted from an accumulation of apoprotein–LDL (apo-LDL) mass in pool B (2,077±174 mg versus 787±70 mg in normal subjects, p<0.002). Pool A apo-LDL was present at normal levels (~1,000 mg), although its fractional catabolic rate was reduced (0.39±0.06 versus 0.61±0.03 pool/day in normal subjects, p<0.01). Fenofibrate therapy (100 mg t.i.d. for 8 weeks) produced substantial reductions in plasma cholesterol (29%; p<0.001), triglycerides (36%; p<0.001), and LDL cholesterol (30%; p<0.001). The latter was associated with a 30% decrease in circulating apo-LDL mass (2,312±200 mg versus 3,279±264 mg before treatment, p<0.005). This resulted from a combination of two effects. First, although overall LDL apoprotein B production did not change, there was a shift from pool B to pool A. Pool A input was 400±74 mg/day pretreatment versus 706±62 mg/day on fenofibrate; pool B input was 422±35 mg/day pretreatment versus 258±41 mg/day on the drug. At the same time, catabolism of pool A rose from 0.39±0.06 to 0.66±0.08 pool/day (p<0.05). We hypothesize that the shift from pool B to pool A resulted from a drug-induced decrease in the particle size of very low density lipoprotein made by the liver, which in turn favored the formation of more rapidly catabolized LDL. Overall, the rate of apo-LDL degradation by the receptor route (as detected using a combination of native and 1,2-cyclohexanedione-modified LDL tracers) rose 43% on the drug, whereas the amount cleared by the receptor-independent pathway did not change. Fenofibrate, therefore, appears not only to promote LDL catabolism via the receptor-mediated pathway but also, by lowering plasma triglyceride levels, inhibits the formation of slowly metabolized, potentially atherogenic LDL particles. (Arteriosclerosis and Thrombosis 1993;13:702–711)

KEYWORDS • apolipoprotein B • kinetics • drugs • multicompartmental modeling • fractional catabolic rate

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Low density lipoprotein (LDL), the major cholesterol carrier in human plasma, plays a critical role in the initiation and progression of atherosclerotic lesions. Until recently, studies of the structure, function, and metabolism of this lipoprotein assumed it to be a homogeneous entity. However, it has now been demonstrated that LDL exists in the plasma of normal and hyperlipidemic individuals as a heterogeneous population of particles that are usually divided into three subfractions on the basis of size and density.2–4 Preliminary investigations have indicated that these fractions have distinct properties. LDL-I, the largest and most buoyant, is present in high concentrations in young women,4 whereas LDL-II is the major species in most subjects.3–5 LDL-III appears to be specifically elevated when plasma triglyceride levels are raised6,7 and has been associated with increased risk of myocardial infarction.4,6,7

Concomitant with these studies on LDL structure, there has been a reevaluation of the in vivo kinetic behavior of the apoprotein component of this lipoprotein (apo-LDL). Classically, analysis of LDL turnover has used the Matthews model,8 which assumes that tracer LDL is homogeneous and is cleared from the circulation by a single catabolic mechanism. Recent detailed investigations,9–12 however, have tested the validity of this hypothesis and found it to be unwarranted in most subjects. Radioactivity released from the breakdown of trace-labeled LDL appears quantitatively in the urine. Examination of the daily rate of urinary radioactivity excretion relative to plasma radioactivity levels (urine:plasma ratio) has shown that instead of being constant, as predicted by Matthews,8 this parameter decreases over the period of the turnover. Therefore, new models have been formulated to describe LDL metabolism9–12 in which there are two plasma components, A and B. The first, pool A, has a rapid clearance rate and high affinity for LDL receptors; the second, pool B, has a slower catabolic rate and a reduced level of receptor-mediated catabolism.12 In one study, clearance of apo-LDL by the slower route was entirely restricted to the receptor-independent route.11
It is important to determine how these metabolically defined entities respond to the conditions that give rise to increased risk of coronary heart disease and whether they are affected by dietary and pharmacological stimuli.

The fibrates are a family of hypolipidemic drugs that lower plasma triglycerides by promoting the lipolysis of triglyceride-rich particles through activation of lipoprotein lipase and by the inhibition of very low density lipoprotein (VLDL) synthesis and secretion as a result of decreased free fatty acid flux to the liver. In addition, it has been suggested that they are able to suppress hepatic cholesterol synthesis and stimulate LDL receptor activity. The effect that fibrates have on LDL varies and depends on the initial plasma triglyceride level. In severely hypertriglyceridemic subjects, LDL particles are small and dense, and the low level of LDL in this condition is due to hypercatabolism of this species, probably by receptor-independent pathways. We found previously that fibrate treatment suppressed this high rate of clearance, and we attributed this effect to a reduction in the amount of LDL degraded by the receptor-independent pathway, whereas LDL catabolism into the receptor route was increased. The overall impact of these changes is to increase the initially low levels of plasma LDL cholesterol, alter LDL composition, and increase the average LDL particle size. Conversely, in hypercholesterolemic, normotriglyceridemic subjects, initially elevated LDL cholesterol levels are decreased by fibrate therapy. In a previous investigation of the mechanism of action of bezafibrate, we ascribed this effect to increased clearance of LDL through the receptor pathway. At the time, we surmised that the drug operated to suppress hepatic cholesterol synthesis and so activate clearance of LDL by receptors. However, in the light of present knowledge of the heterogeneity of LDL, it is pertinent to ask whether it was the receptor or the ligand itself that changed during fibrate therapy. It is arguable that the drug, by changing LDL composition and size, increased the propensity of the particles for degradation by the receptor mechanism. In the present study we examined this possibility by using fenofibrate as a hypolipidemic agent in a group of hypercholesterolemic, normotriglyceridemic subjects. The results are consistent with the view that the nature of the lipoprotein ligand was altered during therapy.

Methods

Subjects

Eight subjects (one man and seven women), aged 48–66 years, were recruited from attendees at a lipid clinic. Despite a 3-month standard cholesterol-reducing diet, the subjects showed persistent type IIa hyperlipidemia (cholesterol >7.0 mmol/L; triglycerides <2.3 mmol/L), which on the basis of appropriate tests of hepatic, renal, endocrine, and hematological function could not be attributed to an underlying disease. None of the subjects was receiving drug therapy known to affect lipoprotein metabolism, including hormone replacement therapy. The seven women were all postmenopausal. Subjects diagnosed as suffering from familial hypercholesterolemia on the basis of family history or tendon xanthomata were excluded.

Dietary control was maintained by frequent counseling throughout the study, which was conducted on an outpatient basis. In previous studies this arrangement maintained the steady-state conditions required for kinetic analysis. Body weight and plasma lipoprotein concentrations, measured serially throughout each study period, remained constant.

Thyroidal uptake of radiodiode released from lipoprotein catabolism was prevented by the administration of potassium iodate (90 mg twice daily) for 3 days before and 1 month after injection of the radiolabeled tracers.

The study was approved by the Ethics Committee of Glasgow Royal Infirmary, and written informed consent was obtained from each volunteer.

Study Outline

The study was carried out in two phases. In the first, subjects underwent four serial measurements of plasma lipids and lipoproteins and determination of native and 1,2-cyclohexanedione–treated, radiolabeled LDL turnover. These measurements were repeated in the second phase after 8 weeks of therapy with fenofibrate at 300 mg/day (given as 100 mg t.i.d.). One of the women completed the baseline phase of the study but after 2 days of fenofibrate therapy complained of upper gastrointestinal upset and nonspecific flu-like symptoms. Therapy was discontinued and these symptoms resolved. When fenofibrate was restarted at a reduced dose the complaints recurred. The medication was withdrawn and the patient was discontinued from further investigation.

Turnover Protocol

The LDL turnover protocol is detailed elsewhere. Briefly, LDL was isolated from fasting plasma by rate zonal ultracentrifugation, which allowed the selection of the major species of LDL for each individual. Peak fractions from the zonal rotor effluent were concentrated fivefold by pressure filtration and dialysis against 0.15 M NaCl, 0.01% (wt/vol) Na₂EDTA, pH 8.1. The selection of only two or three fractions corresponding to the top of the LDL peak allowed us to minimize potential contamination with lipoprotein(a) (Lp(a)). This purified LDL was divided into two aliquots that were labeled separately with 125I and 131I by a modification of the iodine monochloride method.

Tracer LDL was separated from unbound radioactivity by filtration through a Sephadex G25 column using 0.15 M NaCl, 0.01% (wt/vol) Na₂EDTA, pH 8.1, as the eluate. More than 95% of the label was found in the apoprotein fraction of the lipoprotein. The arginine residues on the apoprotein component of 131I-labeled tracer were then modified with 1,2-cyclohexanedicarboxylic acid. This procedure generates a lipoprotein that is unable to interact with LDL receptors on cells and so when it is injected in vivo it acts as a tracer for the measurement of receptor-independent catabolism. Its use has been discussed in detail previously. Alternative methods based on glycosylation of tracer LDL give lower values for receptor-independent clearance, and hence higher receptor-mediated catabolic rates. The choice of blocking agent does not significantly alter interpretation of the results.

After sterilization (0.22-μm filters, Millipore, Molsheim, France), 25 μCi of each tracer was injected...
Analyses

Plasma lipid and lipoprotein concentrations were measured on four occasions during each study phase according to the Lipid Research Clinics protocol. ApoB (apoB) was measured by immunonephelometry (Orion Diagnostics, Espoo, Finland) and Lp(a) by enzyme-linked immunosorbent assay (Enzyquick Lp[a], Immuno, Dunton Green, UK). LDL was isolated by sequential ultracentrifugation at a density of 1.019–1.063 g/mL, and its protein content was determined by a modification of the Lowry method. The masses of cholesterol, triglycerides, free cholesterol, and phospholipids in LDL were determined by commercially available enzymatic colorimetric assays (BCL, Lewes, UK).

The completeness of the urine collections was checked by the measurement of the 24-hour urinary excretion of creatinine. ApoE phenotypes were determined by Western blotting using an adaptation of the method of Havekes et al. Five subjects were homozygous for E_3; subject A.M. was E_3/E_4 and A.O`C. was E_2/E_3.

Kinetic Analysis

Plasma and urine radioactivities were determined on 2-mL aliquots at the end of the turnover period using a twin-channel gamma spectrometer (Packard, Downers Grove, III.). These data were analyzed on the basis of the model shown in Figure 1 to generate kinetic rate constants for apo-LDL catabolism using the SAAM 30 computer program. The model divides plasma LDL into two compartments (A and B) that are permitted to differ in their elimination rates. Pool B undergoes exchange with an extracellular compartment. The model is free to distribute both ^125I and ^131I initial radioactivity (and apo-LDL mass) between pools A and B to achieve the best fit to both plasma and urine data. This approach, similar to that described by Goebel et al.9 Foster et al.10 and ourselves,12 assumes that particles within the LDL spectrum are labeled uniformly in the radioiodination procedure. Since they exist within a narrow size range and their compositions are similar,2,4 this is a reasonable premise. Both pools A and B are believed to be degraded by the receptor and nonreceptor routes, but the probability of receptor clearance in pool A is much higher than pool B.

The need to subject LDL kinetic data to this complex process arises from the observation that the urine:plasma radioactivity ratio (a daily index of LDL catabolic potential) is not constant during the turnover. This parameter is initially high but falls to a lower constant value, usually in 8–10 days. This phenomenon occurred in the baseline investigations and was even more marked during fenofibrate therapy (Figure 2 and "Appendix").

Apo-LDL mass was calculated as the product of the apoprotein concentration in LDL (d, 1.019–1.063 g/mL) and the plasma volume. This was obtained by isotope dilution of the LDL tracer 10 minutes after injection; for subjects B.R., H.M., A.M., and A.O`C. the value was in good agreement with that calculated as % of body weight. For M.W. the agreement was poor because the subject was substantially obese (body mass index, 32.7), but the isotope dilution value agreed with her plasma volume calculated as % of her ideal body weight.

Technical problems produced low values for the plasma volume in subjects C.A. and W.C., and their estimates were derived from body weight.

Statistical Analysis

Statistical analysis on data obtained in the two phases of the fenofibrate study was performed by Student's t test for paired data, whereas the comparisons between the kinetic parameters obtained in normal subjects studied under this protocol and subjects on fenofibrate therapy were ascertained by Student's two-sample t test. Values are reported as mean±SEM.

Results

The lipid and lipoprotein profiles of the subjects during the control and fenofibrate phases of the study are shown in Table 1. All were hypercholesterolemic due to an elevation in LDL and had normal levels of plasma triglycerides and VLDL cholesterol. Drug therapy significantly lowered plasma cholesterol and triglyceride levels by 29% and 35%, respectively (p<0.001). The fall in the cholesterol content of VLDL paralleled that of plasma triglycerides (36%; p<0.01), and there was no change in the VLDL cholesterol to plasma triglyceride ratio (0.39 before and during therapy). Fenofibrate induced a decrease in LDL cholesterol (from 6.0±0.39 mmol/L to 3.86±0.25 mmol/L; p<0.001), whereas plasma apoB as measured by immunonephelometry fell by 33% (p<0.001; Table 1). In these normotriglycerideremic (mainly female) subjects the initial high density lipoprotein (HDL) cholesterol level

![Figure 1. Model of apoprotein–low density lipoprotein (apo-LDL) metabolism shows apo-LDL in the plasma divided into two components, A and B. Pool A (compartment 5) is more rapidly metabolized. Pool B (compartment 1) exchanges with the extravascular (E.V.) space (compartment 2). The model is similar to that used by Goebel et al,9 Foster et al,10 and ourselves.12 U(5) and U(1) represent input rates for pools A and B, respectively. L(3,5) and L(3,1) are elimination constants for the fast and slowly metabolized pools, respectively. Compartment 3 is the body iodide pool, which is cleared into the urine (compartment 4) at 2.5 pools/day.](http://atvb.ahajournals.org/)

Downloaded from http://atvb.ahajournals.org/ by guest on November 8, 2017
FIGURE 2. Line graphs show plasma and urine radioactivity decay curves after injection of $^{125}$I-native low density lipoprotein (LDL) in B.R. before and during fenofibrate therapy. Subject received a bolus dose of $^{125}$I-LDL tracer, and daily urine and plasma samples were taken for 24 days. Urine was collected for each 24-hour period, and the urinary radioactivity excretion rate was determined. The urine/plasma ratio was calculated as the urinary loss divided by the plasma radioactivity at the beginning of the collection period. Panel A: Plasma radioactivity decay curves before fenofibrate (○) and on fenofibrate (●) and urine radioactivity excretion curves before fenofibrate (○) and on fenofibrate (●). Panel B: Urine:plasma ratio before fenofibrate (○) and on fenofibrate (●).

was within normal limits and was unchanged by the drug. Fenofibrate therapy for 8 weeks also produced no significant changes in Lp(a) levels (86 ± 22, range 11–141 mg/100 mL before therapy; 91 ± 18, range 12–140 mg/100 mL on fenofibrate). Analysis of the composition of the LDL fraction showed no effect of fibrate therapy. The percentage of protein in LDL was 25.0 versus 25.9 (before and during therapy, respectively); the percentage of free cholesterol was 11.9 versus 12.0; the percentage of esterified cholesterol was 34.7 versus 33.0; the percentage of triglycerides was 6.5 versus 6.9; and the percentage of phospholipids was 21.9 versus 22.1, indicating that the reduction in LDL cholesterol was due to a diminished number of particles in the circulation.

The kinetic parameters of apo-LDL metabolism derived by multicompartmental analysis of the plasma and urine data (using the model in Figure 1) for both tracers are presented in Table 2 in a format that permits comparison with earlier results.16 Fenofibrate therapy was associated with a 65% increase in the fractional catabolic rate of apo-LDL. This change was apparently responsible for the 30% decrement in the concentration of apo-LDL in plasma, because fenofibrate did not significantly affect the total apo-LDL synthesis. When LDL catabolism was divided into receptor-dependent versus receptor-independent pathways, it was observed that the fractional catabolic rate for the former was increased by 105% (0.263 ± 0.028 pool/day on fenofibrate versus 0.128 ± 0.011 pool/day before drug; p < 0.002) and the latter by 27% (0.160 ± 0.018 pool/day on fenofibrate versus 0.126 ± 0.019 pool/day before drug; p < 0.001). Furthermore, the amount of apo-LDL degraded by the receptor route rose by 45% (p < 0.05; Table 2), whereas that removed by the receptor-independent pathway was unchanged.

Examination of the plasma decay curves in conjunction with the daily urinary radioactivity excretion rates (shown for a typical subject in Figure 2A and for others in the "Appendix") revealed that fenofibrate perturbed LDL metabolism in a complex fashion. During the control turnover, the amount of radioactivity excreted in the urine each day was virtually a fixed proportion of the radioactivity present in the plasma. The calculated urine:plasma ratio (Figure 2B), a daily index of catabolic potential, fell only slightly from a peak value of 0.34 at 2–4 days after injection to 0.26 by days 10–12. On fenofibrate, however, the relation between the urine and plasma decay curves was altered (Figure 2A). During the first 5 days of the turnover, the plasma curve showed more rapid decay than during the control phase. This change was due to a catabolic event rather than enhanced extravascular exchange because there was a concomitant increase in urinary radioactivity excretion. The urine:plasma ratio in this patient (Figure 2B) on fenofibrate was initially high (0.60), but it showed a substantial fall during the turnover period to approximately the same final value as in the control phase.

The kinetic behavior of pools A and B in the hypercholesterolemic subjects at baseline and on drug therapy are shown in Table 2 and Figure 3. In the control situation, pool A (the more rapidly cleared species) accounted for 1,200 mg, or approximately one third, of the circulating mass of apo-LDL. Its mean transport rate was 400 mg/day and it had a fractional catabolic rate of 0.39 pool/day. The mean mass of apo-LDL in pool B was approximately 2,000 mg, and in most subjects this was the major apo-LDL component. Its fractional catabolic rate was half that of pool A (0.2 pool/day), whereas its rate of production was similar (422 mg/day). Fenofibrate treatment (Table 3) significantly increased the transport of pool A by 77% (p < 0.005) and at the same time significantly decreased the transport of pool B by 39% (p < 0.02). The overall result showed no difference in the transport of total
apo-LDL (Table 2). The fractional clearance rate of pool B was not altered, whereas that of pool A rose by 69% on fenofibrate. The net effect of these changes was that while the computed mass of apo-LDL in pool A remained constant, that of pool B was reduced by approximately 50%.

Mean values from 10 normolipidemic individuals with plasma triglyceride values in the range seen in the present group during treatment are shown in Figure 3. Compared with these, the hypercholesterolemic subjects closely resembled that of the normal subjects, there is still much to be learned about the pathophysiology of this lipoprotein and its role in the atherosclerotic process. Recognition that the lipoprotein class is structurally and metabolically heterogeneous has been a significant step forward. Foster et al. described a number of two-compartment models of apo-LDL metabolism that were all capable of accommodating urine and plasma radioactivity data. One of these, the arrangement shown in Figure 1 (Model B in Foster et al.), was adopted for the present work as well as a previous study because it was compatible with the LDL subsystem in a much larger model that we developed.

### Table 1. Effect of Fenofibrate on Plasma Lipids and Lipoproteins in Hypercholesterolemia

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>Weight (kg)</th>
<th>Cholesterol (mmol/L)</th>
<th>Triglycerides (mmol/L)</th>
<th>VLDL-C (mmol/L)</th>
<th>LDL-C (mmol/L)</th>
<th>HDL-C (mmol/L)</th>
<th>ApoB (mg/100 mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.A.</td>
<td>63</td>
<td>M</td>
<td>77.5</td>
<td>8.00</td>
<td>6.04</td>
<td>1.74</td>
<td>0.91</td>
<td>0.75</td>
<td>0.45</td>
</tr>
<tr>
<td>B.R.</td>
<td>64</td>
<td>F</td>
<td>60.0</td>
<td>8.20</td>
<td>5.30</td>
<td>1.30</td>
<td>0.89</td>
<td>0.35</td>
<td>0.29</td>
</tr>
<tr>
<td>W.C.</td>
<td>66</td>
<td>F</td>
<td>68.0</td>
<td>7.89</td>
<td>5.54</td>
<td>2.10</td>
<td>1.19</td>
<td>0.79</td>
<td>0.45</td>
</tr>
<tr>
<td>H.M.</td>
<td>65</td>
<td>F</td>
<td>59.5</td>
<td>6.33</td>
<td>5.33</td>
<td>1.63</td>
<td>1.10</td>
<td>0.67</td>
<td>0.31</td>
</tr>
<tr>
<td>M.W.</td>
<td>53</td>
<td>F</td>
<td>78.5</td>
<td>8.50</td>
<td>5.60</td>
<td>1.59</td>
<td>0.96</td>
<td>0.61</td>
<td>0.34</td>
</tr>
<tr>
<td>A.M.</td>
<td>63</td>
<td>F</td>
<td>64.5</td>
<td>9.50</td>
<td>7.26</td>
<td>1.20</td>
<td>0.88</td>
<td>0.30</td>
<td>0.34</td>
</tr>
<tr>
<td>A.O'C.</td>
<td>48</td>
<td>F</td>
<td>58.2</td>
<td>7.67</td>
<td>5.26</td>
<td>1.88</td>
<td>1.51</td>
<td>1.04</td>
<td>0.66</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td>8.01</td>
<td>7.71</td>
<td>1.63</td>
<td>1.06</td>
<td>0.64</td>
<td>0.41</td>
</tr>
<tr>
<td>±SEM</td>
<td>0.39</td>
<td>0.27</td>
<td>0.12</td>
<td>0.09</td>
<td>0.10</td>
<td>0.09</td>
<td>0.39</td>
<td>0.25</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*VLDL-C, very low density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; apoB, apoprotein B; C, control; F, fenofibrate; NS, not significant. Subjects' ages are given in years.*

### Table 2. Effects of Fenofibrate on LDL Metabolism in Hypercholesterolemia

<table>
<thead>
<tr>
<th>Subject</th>
<th>Apo-LDL mass (mg)</th>
<th>Fractional catabolic rate (pools/day)</th>
<th>Apo-LDL transport rate (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>F</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>F</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td></td>
<td>Receptor mediated</td>
</tr>
<tr>
<td>C.A.</td>
<td>4,092</td>
<td>2,790</td>
<td>0.239</td>
</tr>
<tr>
<td>B.R.</td>
<td>3,696</td>
<td>2,108</td>
<td>0.248</td>
</tr>
<tr>
<td>W.C.</td>
<td>3,993</td>
<td>2,439</td>
<td>0.242</td>
</tr>
<tr>
<td>H.M.</td>
<td>2,332</td>
<td>2,142</td>
<td>0.319</td>
</tr>
<tr>
<td>M.W.</td>
<td>2,662</td>
<td>1,995</td>
<td>0.239</td>
</tr>
<tr>
<td>A.M.</td>
<td>3,431</td>
<td>3,149</td>
<td>0.204</td>
</tr>
<tr>
<td>A.O'C.</td>
<td>2,747</td>
<td>1,562</td>
<td>0.295</td>
</tr>
<tr>
<td>Mean</td>
<td>3,279</td>
<td>2,312</td>
<td>0.255</td>
</tr>
<tr>
<td>±SEM</td>
<td>264</td>
<td>200</td>
<td>0.015</td>
</tr>
</tbody>
</table>

*Apo-LDL, apoprotein-low density lipoprotein; C, control; F, fenofibrate; NS, not significant. The apo-LDL mass is the apo-LDL concentration multiplied by plasma volume. The total fractional catabolic rate is the overall clearance (from pools A and B) of I25I-LDL. The receptor-independent fractional catabolic rate is the overall rate (from pools A and B) of the 1,2-cyclohexanedione-treated LDL tracer. The total LDL transport rate is the product of the total fractional catabolic rate and apo-LDL pool size. The receptor-mediated transport rate is the total LDL transport rate (I25I-LDL) minus the receptor-independent transport rate (131I-labeled cyclohexanedione-treated LDL).*
TABLE 3. Effects of Fenofibrate on LDL Metabolic Heterogeneity

<table>
<thead>
<tr>
<th>Subject</th>
<th>Computed mass (mg)</th>
<th>Fractional catabolic rate (pools/day)</th>
<th>Transport (mg/day)</th>
<th>Computed mass (mg)</th>
<th>Fractional catabolic rate (pools/day)</th>
<th>Transport (mg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C.A.</td>
<td>2,471</td>
<td>0.26</td>
<td>642</td>
<td>1,621</td>
<td>0.203</td>
<td>329</td>
</tr>
<tr>
<td>B.R.</td>
<td>2,064</td>
<td>0.29</td>
<td>595</td>
<td>1,632</td>
<td>0.200</td>
<td>321</td>
</tr>
<tr>
<td>W.C.</td>
<td>1,525</td>
<td>0.30</td>
<td>458</td>
<td>2,468</td>
<td>0.210</td>
<td>518</td>
</tr>
<tr>
<td>H.M.</td>
<td>583</td>
<td>0.67</td>
<td>391</td>
<td>1,749</td>
<td>0.200</td>
<td>353</td>
</tr>
<tr>
<td>M.W.</td>
<td>222</td>
<td>0.47</td>
<td>104</td>
<td>2,440</td>
<td>0.220</td>
<td>332</td>
</tr>
<tr>
<td>A.M.</td>
<td>695</td>
<td>0.28</td>
<td>197</td>
<td>2,736</td>
<td>0.180</td>
<td>502</td>
</tr>
<tr>
<td>A.O'C.</td>
<td>853</td>
<td>0.49</td>
<td>415</td>
<td>1,894</td>
<td>0.210</td>
<td>396</td>
</tr>
<tr>
<td>Mean</td>
<td>1,202</td>
<td>0.28</td>
<td>400</td>
<td>2,077</td>
<td>0.200</td>
<td>422</td>
</tr>
<tr>
<td>±SEM</td>
<td>315</td>
<td>0.06</td>
<td>74</td>
<td>174</td>
<td>0.005</td>
<td>35</td>
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<td>NS</td>
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<td>0.02</td>
</tr>
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apo-LDL, apoprotein-low density lipoprotein; C, control; F, fenofibrate; NS, not significant; pool A, computed mass (M5) and fractional catabolic rate L(3,5); pool B, computed mass (M1) and fractional catabolic rate L(3,1). The figures are derived by using the model in Figure 1.

Figure 3: Diagrams show summary kinetics of apoprotein-low density lipoprotein (apo-LDL) metabolism in hypercholesterolemic and normal subjects. Mean values for compartmental masses, synthetic inputs, and fractional catabolic rates are given for seven subjects before drug (control) and during fenofibrate therapy (fenofibrate) and for ten normal subjects (normal) (mean plasma triglycerides, 1.12 mmol/L; mean LDL cholesterol, 2.68 mmol/L) from a previous study.12 Note that the apo-LDL masses quoted for normal subjects are approximately 20% lower than those in Reference 12. The masses presented previously included a small component due to intermediate density lipoprotein (d, 1.006–1.019 g/mL) apoprotein. Those presented here are values directly determined by measurement of the protein content of the 1.019–1.063 g/mL density fraction. d, day; A, LDL pool A; B, LDL pool B. See text for details.
catabolic rate of about 0.2 pool/day, equivalent to the rate seen for pool B material. The model shown in Figure 1 has been used to explain the relation between plasma triglyceride concentration and LDL metabolism in normolipidemic subjects. In this earlier work it was observed that as plasma triglyceride levels rose, an increasing proportion of apo-LDL was located in the more slowly metabolized pool B. This component was present at about 400 mg of circulating mass in subjects with low plasma triglyceride levels (<1.0 mmol/L) but became predominant (1,500 mg of the circulating mass) at high normal plasma triglyceride values (1.5-2.0 mmol/L). Apo-LDL in pool A was relatively stable, accounting for about 1,000 mg in most subjects. Application of the model to the present study provided an intuitively acceptable mechanism for the action of fenofibrate. An alternative, such as Model C of Foster et al, in which pool A is converted to pool B by lipolysis, would predict that a drug like fenofibrate that enhances lipase action would increase pool B formation; clearly, this did not occur in our subjects.

The model has certain features that appear to have physiological significance. First, \( L(3,5) \) in Figure 1 is a key variable in controlling the rate of LDL catabolism. The variation in this rate constant, we believe, is linked to the activity of the receptor pathway. Second, overall LDL clearance is also influenced by the distribution of mass between pools A and B. This, in turn, is dependent on their relative synthesis rates and possibly, as described above, on the nature of the VLDL precursor made by the liver. Third, the fractional catabolic rate of pool B is constant in most subjects. This is a reflection of the observation that in normal subjects and in untreated and treated hypercholesterolemic subjects (Figure 2 and Table 3), parallel terminal exponentials appear after 8-10 days in both the plasma and urine radioactivity decay curves. These give a urine-plasma ratio of about 0.2 pool/day. Clearance of pool B material in our model is by both receptor-dependent and receptor-independent pathways, although others have restricted this to the receptor-independent pathway. We envisage that LDL derived from different sources has a variable apoprotein surface conformation and hence a differing probability of removal by the receptor or nonreceptor routes. A particle in pool A or pool B could be removed by either pathway, but the pool A material has a higher likelihood of interaction with receptors, whereas the opposite is true of pool B. This is consistent with the in vitro observations that LDL from virtually all subjects, regardless of lipoprotein pattern, has the abil-
ity to interact with receptors, but the efficiency of LDL interaction from hypertriglyceridemic subjects is less.\textsuperscript{36} The mass of pool B appears to regulate the overall plasma LDL level and hence atherogenic potential (Reference 12 and Table 3). Clearly, it is of interest to determine the nature of the LDL that contains this slowly metabolized apoB. Since its concentration is directly related to the plasma triglyceride level in the same way as small, dense LDL in studies from Austin et al\textsuperscript{4} and this laboratory,\textsuperscript{3} we initially speculated that the apo-LDL in pool B resided in the LDL-III species. However, upon quantification of the latter in a preliminary report,\textsuperscript{37} we concluded that the pool B mass exceeds that of the apoprotein in LDL-III. Our present hypothesis is that pool B is composed of the apoB in LDL-III and a substantial fraction of that in LDL-II. This will be tested in direct investigations of LDL subfraction metabolism.

In the model the overall fractional catabolic rate for apo-LDL can be perturbed by changing either $L(3,5)$ or the distribution between pools A and B. We examined the influence of these parameters on the daily urine:plasma ratio in a series of simulations (Figure 4). Changes in $L(3,5)$ affect the shape of the urine:plasma curve in a manner that is distinct from that which occurs when mass is shifted from pool A to pool B. Hence, these parameters can be derived unambiguously during the fitting process from the shape of the urine:plasma curve.

\textbf{Influence of Fenofibrate on LDL Metabolism}

The results of the present study using fenofibrate in moderately hypercholesterolemic subjects are virtually the same as those in a previous study\textsuperscript{16} that examined the effects of the drug bezafibrate on apo-LDL turnover. Both the fractional catabolic rate and the amount of apo-LDL degraded by the receptor route increased during therapy (Table 2). However, in this report we employed a more detailed kinetic analysis and concluded that the changes in LDL metabolism were due to a combination of two effects: a shift in transport favoring the formation of the rapidly metabolized species (pool A) and an increase in its rate of elimination. We observed in the previous study in normal subjects\textsuperscript{12} that $L(3,5)$ (the elimination rate from pool A) was much more variable than $L(3,1)$ (the elimination rate from pool B) and that when the turnover of native LDL and 1,2-cyclohexanedione-modified LDL were modeled together, the former pathway was more closely associated with receptor activity than the latter. We interpret the increase in $L(3,5)$ shown in Table 3 as an indication that receptors were stimulated by the drug, perhaps, as previously suggested, secondary to a fenofibrate-induced suppression of hepatic cholesterol synthesis.\textsuperscript{15,17} However, we speculate that the main reason for the 36% reduction in LDL cholesterol shown in Table 1 was that during therapy, the LDL particles formed were better ligands for receptors, as reflected in the relative increase in pool A material. Further, we speculate that this occurred because fenofibrate caused a reduction in triglyceride levels relative to apoB synthesis in the liver and hence promoted the secretion of smaller VLDL precursor particles. These, on the basis of previous studies,\textsuperscript{34,35} generate receptor-active LDL as the end product of intravascular lipolysis. Evidence to support this hypothesis comes from studies of type III\textsuperscript{38} and type IV\textsuperscript{39} hyperlipidemic subjects that show that fibrate treatment causes a reduction in the synthesis of large, triglyceride-rich VLDL (S, 60–400).

We favor the mechanism described above because it is concordant with the view of apoB metabolism derived from VLDL tracer studies.\textsuperscript{34,35,38,39} There are two alternative explanations for the effects of the drug on LDL metabolic heterogeneity. First, the reduction in plasma triglyceride circulating mass led to decreased neutral lipid exchange and a compositional change in LDL so that, during treatment, it became larger and more lipid rich and hence had an increased affinity for receptors.\textsuperscript{40} The second explanation is that increased lipoprotein lipase activity promoted the formation of receptor-active LDL regardless of the size and lipid content of the VLDL precursor.

Based on the previous study in normal subjects\textsuperscript{12} and the present report, we conclude that the kinetics of LDL are strongly influenced by plasma VLDL (triglyceride) levels. In hypercholesterolemia, fenofibrate lowers plasma triglyceride levels and corrects the underlying metabolic abnormality in LDL metabolism.

\textbf{Acknowledgments}

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Appendix

Line graphs show plasma and urine radioactivity decay curves after injection of $^{125}$I-native low density lipoprotein (LDL) in hypercholesterolemic subjects before and during fenofibrate therapy. Subjects received a bolus dose of $^{125}$I-LDL tracer, and daily urine and plasma samples were taken for 24 days. Urine was collected for each 24-hour period, and the urinary radioactivity excretion rate was determined. The results are shown for the remaining subjects C.A., W.C., H.M., A.M., M.W., and A.O'C. Results for B.R. are shown in Figure 2. Plasma radioactivity observed values: ■, before drug; ▲, on fenofibrate; urine radioactivity observed values: □, before drug; △, on fenofibrate; calculated values, ——.
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Fenofibrate and LDL metabolic heterogeneity in hypercholesterolemia.
M J Caslake, C J Packard, A Gaw, E Murray, B A Griffin, B D Vallance and J Shepherd

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