Fenofibrate Reduces Low Density Lipoprotein Catabolism in Hypertriglyceridemic Subjects

James Shepherd, Muriel J. Castlake, A. Ross Lorimer, Barry D. Vallance, and Christopher J. Packard

This study examines the kinetic basis for the increment in plasma low density lipoprotein (LDL) levels that accompanies the fenofibrate treatment of severely hypertriglyceridemic (HTG) patients. Seven HTG men with a mean plasma triglyceride level of 1470 mg/dl were treated for 6 weeks. During treatment, their plasma triglyceride level fell by 77% and their cholesterol level by 41%. The fall in very low density lipoprotein (VLDL) cholesterol level was reciprocated by increments in the cholesterol level in both LDL and high density lipoproteins (HDL); the rise in HDL was confined to HDL3. LDL catabolism was examined before and during therapy using native and chemically modified tracers in an attempt to distinguish receptor-mediated from non-receptor-mediated clearance. In their basal state, the hypertriglyceridemic subjects overcatabolized both the native and the modified lipoprotein, implying that the non-receptor pathways were hyperactive. The mean fractional clearance rate of LDL via the receptor pathway was not significantly different from normal. Fenofibrate therapy corrected the patients' hypercatabolism, reducing the receptor-independent fractional clearance of apo LDL by 50% (from 0.48 to 0.24 pools/day; p < 0.05). The mean fractional catabolic activity of the receptor route did not change, but when the increment in the plasma apo LDL concentration was taken into account, it was clear that the drug treatment was associated with an increase in the net amount cleared by the receptor pathway and with a reduction of lipoprotein uptake into receptor-independent routes. (Arteriosclerosis 5:162-168, March/April 1985)

The metabolism of triglyceride-rich lipoproteins is a complex process mediated by several enzyme systems. It appears to occur in two stages. The first involves binding of the particles to lipoprotein lipase situated on the luminal endothelial surface of capillary beds. The enzyme hydrolyzes the lipoprotein triglyceride core, releasing redundant surface coat material which becomes incorporated into high density lipoproteins (HDL). The remnants produced by lipolysis have one of two fates. Some are catabolized directly from the plasma, while others are remodeled to form low density lipoproteins (LDL). Imbalance between synthesis and catabolism in this system may lead to hypertriglyceridemia in which very low density lipoproteins (VLDL) and possibly also chylomicrons accumulate in the circulation. In severe cases, there is an associated decrease in circulating LDL and HDL levels. Patients like these have a high risk of developing acute pancreatitis and therefore merit corrective intervention. The first line of treatment is dietary modification. Where this fails, the clinician may decide to initiate lipid-lowering drug therapy, and among the agents available to him are the recently introduced clofibrate analogues. They seem to produce their effects by activating the enzyme lipoprotein lipase, promoting clearance of the triglyceride-rich products and often altering the circulating pools of LDL and HDL. We still do not understand why LDL levels are low in severe hypertriglyceridemia nor why they rise...
when treatment is started. These two questions are addressed in the present investigation. Obviously, the concentration of any plasma protein is governed by its relative rates of synthesis and catabolism, and preliminary studies have suggested that grossly hypertriglyceridemic individuals overcatabolize LDL. It is known that this lipoprotein is cleared by at least two distinct mechanisms. One mechanism is mediated by high-affinity receptors on cell membranes and is thought to be responsible for the metered delivery of cholesterol to tissues that need the sterol for structural and metabolic purposes. When this process is defective (for example in individuals suffering from familial hypercholesterolemia) more LDL cholesterol is channelled into the alternative, poorly regulated receptor-independent routes. Since these subjects are predisposed to premature and accelerated vascular disease, receptor-independent mechanisms have been implicated in atherosclerosis.

The relative contribution made by each of these pathways to LDL catabolism as a whole can be assessed by using chemically modified LDL to trace the receptor-independent routes. The present report describes the derangement of LDL catabolism in grossly hypertriglyceridemic subjects and follows the response to fenofibrate therapy.

Methods

Subjects

Seven hypertriglyceridemic men were selected for study on the basis of plasma triglyceride levels greater than 400 mg/dl and LDL cholesterol levels less than 135 mg/dl. All had fasting chylomicronemia on agarose gel electrophoresis and raised circulating levels of VLDL as determined by the techniques described in previous publications. None abused alcohol or presented clinical or biochemical evidence of hepatic, endocrine, renal, or hematologic dysfunction. Specifically, they were neither hyperuricemic nor hyperglycemic (plasma glucose less than 120 mg/dl). This was dialyzed extensively against 0.15 M NaCl/0.01% Na₂ EDTA/0.01 M Tris HCl (pH 7.4). Aliquots of the material were labeled separately with 125I and 131I with the use of iodine monochloride. This resulted in incorporation of less than 5% of the label into material soluble in chloroform/methanol (1:1, vol/vol). The 31I-labeled tracer was then treated with 1,2-cyclohexanedione to block the arginyl residues on its protein moiety and provide a probe suitable for measuring receptor-independent LDL catabolism. The properties of this tracer are detailed in previous publications. Each subject received 25 μCi of his 125I-native LDL and 25 μCi of his 131I-cyclohexanedione-modified LDL by sequential intravenous injection. A 10-minute blood sample was then collected; thereafter, fasting plasma specimens were obtained at daily intervals over the next 14 days. Plasma decay curves of each tracer were analyzed by the procedure of Matthews by using a two-compartment model to obtain fractional clearance rates. The estimation error of these parameters was less than 5%. On the assumption that the clearance of the modified lipoprotein describes the activity of the receptor-independent catabolic pathways, receptor activity was estimated from the difference between the fractional clearance rates of the two tracers.

Plasma lipid and lipoprotein concentrations were measured serially on five occasions during each study phase according to the Lipid Research Clinics Protocol. Additionally, measurements were made of plasma HDL₃ and HDL₂ with an analytical ultracentrifugation technique. At the end of each study phase, the composition of LDL, isolated by rate zonal ultracentrifugation, was determined by measuring protein, phospholipid, triglyceride, and free cholesterol.

Turnover Protocol

It is well known that LDL, particularly in hypertriglyceridemic subjects, is polydisperse, and therefore it is inappropriate to use a fixed density interval to prepare a representative tracer for kinetic studies. Consequently, we used rate zonal ultracentrifugation to obtain a continuous spectral distribution of the lipoprotein and permit selection of the major species for radiolabeling. Fasting plasma from each volunteer was subjected to a preliminary separation in a Beckman Ti 60 rotor (18 hours, 40,000 rpm, 10°C) to remove the chylomicrons and VLDL. Rate zonal ultracentrifugation of the d>1.006 kg/liter infranatant was performed according to the method of Patsch et al. The peak fractions corresponding to the major LDL species were concentrated by pressure filtration (Amicon XM 100 filters, Amicon Corporation, Lexington, Massachusetts) to approximately 1.0 mg protein/ml. This was dialyzed extensively against 0.15 M NaCl/0.01% Na₂ EDTA/0.01 M Tris HCl (pH 7.4). Aliquots of the material were labeled separately with 125I and 131I with the use of iodine monochloride. This resulted in incorporation of less than 5% of the label into material soluble in chloroform/methanol (1:1, vol/vol). The 31I-labeled tracer was then treated with 1,2-cyclohexanedione to block the arginyl residues on its protein moiety and provide a probe suitable for measuring receptor-independent LDL catabolism. The properties of this tracer are detailed in previous publications. Each subject received 25 μCi of his 125I-native LDL and 25 μCi of his 131I-cyclohexanedione-modified LDL by sequential intravenous injection.
Table 1. Effect of Fenofibrate on the Plasma Lipids and Lipoproteins of Six Hypertriglyceridemic Men

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yr)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>Plasma triglyceride (mg/dl)</th>
<th>Plasma cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>Drug</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>40</td>
<td>178</td>
<td>82.6 ± 0.2</td>
<td>80.3 ± 0.3</td>
<td>1140 ± 370 170 ± 35</td>
</tr>
<tr>
<td>WA</td>
<td>36</td>
<td>175</td>
<td>74.8 ± 0.4</td>
<td>73.7 ± 0.2</td>
<td>400 ± 53 180 ± 60</td>
</tr>
<tr>
<td>GB</td>
<td>37</td>
<td>172</td>
<td>77.1 ± 0.3</td>
<td>78.3 ± 0.2</td>
<td>750 ± 195 210 ± 20</td>
</tr>
<tr>
<td>RB</td>
<td>38</td>
<td>176</td>
<td>76.2 ± 0.3</td>
<td>78.0 ± 0.4</td>
<td>3250 ± 975 295 ± 80</td>
</tr>
<tr>
<td>WD</td>
<td>48</td>
<td>168</td>
<td>60.3 ± 0.1</td>
<td>60.8 ± 0.3</td>
<td>1000 ± 20 210 ± 35</td>
</tr>
<tr>
<td>RR</td>
<td>46</td>
<td>175</td>
<td>77.1 ± 0.5</td>
<td>76.7 ± 0.2</td>
<td>1510 ± 415 275 ± 125</td>
</tr>
<tr>
<td>JS</td>
<td>67</td>
<td>171</td>
<td>66.7 ± 0.1</td>
<td>65.8 ± 0.2</td>
<td>2230 ± 130 1035 ± 205</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td>80.3 ± 0.3</td>
<td>78.3 ± 0.2</td>
<td>317 ± 12 186 ± 16</td>
</tr>
<tr>
<td>p (paired t test)</td>
<td></td>
<td></td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results

Table 1 shows the lipid and lipoprotein profiles of the subjects during the control and fenofibrate treat-
ment phases of the study. The drug affected all lipoprotein fractions in the plasma. Triglyceride levels fell 77% on average and there was a corresponding reduction in VLDL cholesterol ($p < 0.02$). This major decrease in the circulating mass of triglyceride-rich lipoproteins evoked reciprocal changes in the cholesterol-rich particles LDL and HDL. LDL cholesterol increased from a subnormal value of 74 mg/dl into the low normal range ($p < 0.02$). HDL cholesterol also rose by about 40% ($p < 0.01$) so that the LDL/HDL cholesterol ratio in the plasma was unchanged. Analytical ultracentrifugation revealed that the increment in the high density fraction was confined to HDL$_3$; the HDL$_2$, which was initially low in plasma concentration, remained unchanged.

Detailed analysis was made of the effects of treatment on LDL composition (Table 2) and on apo LDL kinetics (Table 3). Before treatment, the LDL particles in these grossly hypertriglyceridemic subjects were depleted in esterified cholesterol and relatively enriched in triglyceride. Drug therapy, in addition to raising the plasma LDL cholesterol concentration, also perturbed the composition of the particles. Their cholesteryl ester content rose 30% ($p < 0.05$), while triglyceride fell approximately 60% ($p < 0.05$) as one lipid presumably replaced the other in the hydrophobic core. In spherical particles like LDL, the ratio of core to coat components (cholesteryl ester + triglyceride)/(phospholipid + protein) is an indicator of size. Treatment with fenofibrate reduced the particles' coat phospholipid content by 22% ($p < 0.01$) and increased the above ratio from 0.62 to 0.79. Thus, therapy presumably enlarged the average LDL particle size.

Only part of the rise in plasma LDL cholesterol was due to the compositional changes seen in Table 2. Apo LDL kinetics were also affected, leading to an increment in the circulating mass of the lipoprotein (Table 3). Fenofibrate therapy increased the apo LDL concentration in the plasma by an average of 35% ($p < 0.05$), although there was variability within the group. Patient WA, whose initial apo LDL concentration was near normal, showed no change in this parameter, although there was a 300% increase in RB, the patient who showed the greatest response to therapy.

When the steady-state synthetic rate of apo LDL was calculated, it was normal in the basal phase and remained unaffected by fenofibrate treatment. Consequently, we expected that catabolism would

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**Table 1. (Continued)**

<table>
<thead>
<tr>
<th>VLDL cholesterol (mg/dl)</th>
<th>LDL cholesterol (mg/dl)</th>
<th>HDL cholesterol (mg/dl)</th>
<th>HDL$_2$ mass (mg/dl)</th>
<th>HDL$_3$ mass (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Drug</td>
<td>Control</td>
<td>Drug</td>
<td>Control</td>
</tr>
<tr>
<td>320 ± 12</td>
<td>31 ± 4</td>
<td>85 ± 16</td>
<td>100 ± 12</td>
<td>39 ± 6</td>
</tr>
<tr>
<td>54 ± 16</td>
<td>31 ± 8</td>
<td>124 ± 12</td>
<td>120 ± 8</td>
<td>46 ± 4</td>
</tr>
<tr>
<td>217 ± 39</td>
<td>46 ± 4</td>
<td>70 ± 19</td>
<td>132 ± 12</td>
<td>39 ± 1</td>
</tr>
<tr>
<td>542 ± 112</td>
<td>54 ± 4</td>
<td>35 ± 1</td>
<td>85 ± 8</td>
<td>22 ± 5</td>
</tr>
<tr>
<td>101 ± 66</td>
<td>39 ± 8</td>
<td>70 ± 12</td>
<td>100 ± 10</td>
<td>35 ± 3</td>
</tr>
<tr>
<td>290 ± 62</td>
<td>70 ± 31</td>
<td>120 ± 27</td>
<td>135 ± 27</td>
<td>46 ± 1</td>
</tr>
<tr>
<td>217 ± 8</td>
<td>108 ± 4</td>
<td>21 ± 1</td>
<td>50 ± 8</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>209 ± 150</td>
<td>54 ± 25</td>
<td>74 ± 35</td>
<td>104 ± 27</td>
<td>35 ± 12</td>
</tr>
</tbody>
</table>

---

**Table 3. (Continued)**

<table>
<thead>
<tr>
<th>Apo LDL absolute catabolic rate (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Native minus CHD LDL</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>3.7</td>
</tr>
<tr>
<td>3.1</td>
</tr>
<tr>
<td>5.0</td>
</tr>
<tr>
<td>1.3</td>
</tr>
<tr>
<td>3.2</td>
</tr>
<tr>
<td>7.3</td>
</tr>
<tr>
<td>3.8</td>
</tr>
<tr>
<td>3.9 ± 1.9</td>
</tr>
</tbody>
</table>

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<0.05 <0.05 -

0.6 ± 1.2 7.7 ± 1.7 13.3 ± 1.2
explain the changes in LDL. Before therapy, the mean apo LDL total fractional clearance rate in the subjects was about twice normal, although the values for this parameter varied over a fourfold range. In general, a low plasma apo LDL pool was associated with a high catabolic rate.

We used chemically modified (receptor-blocked) LDL in an attempt to uncover the mechanism responsible for the hypercatabolism. On the assumption that 1,2 cyclohexanedione-treated LDL traces the activity of pathways which do not involve the high affinity receptor, it was possible to calculate the contributions made by the receptor and nonreceptor routes in these subjects. In the basal state the average fractional clearance rate of the lipoprotein via its high affinity receptor was normal. Conversely, in only one subject was the clearance rate by nonreceptor mechanisms comparable to values obtained in normal individuals in our laboratory (Table 3). On average, it was increased 2.5-fold.

Fenofibrate therapy did not change apo LDL fractional catabolism via the receptor pathway but did reduce the activities of the alternate mechanisms to normal. So, in the group as a whole, the increase in plasma apo LDL concentrations that followed fenofibrate therapy arose from the suppression of the receptor-independent catabolism. Since, as noted earlier, the turnover rate of the apoprotein (in mg/kg/day) did not change, there apparently was a redistribution of the amounts channeled into each pathway during therapy. Table 3 shows that the absolute amount of LDL cleared by the receptor route increased significantly and that concomitantly there was a fall in that component handled by other, receptor-independent mechanisms.

**Discussion**

In severely hypertriglyceridemic subjects, plasma LDL characteristically is low in concentration and abnormal in composition, with a high triglyceride relative to cholesteryl ester (Table 2). The mechanisms responsible for these effects are caused by the combined actions of cholesterol ester transfer protein and plasma lipases. These authors envisage that in the presence of a large circulating mass of triglyceride-rich lipoproteins, the distribution of neutral lipid within the lipoprotein spectrum is distorted so that VLDL becomes relatively cholesteryl ester-enriched by changing a component of its hydrophobic triglyceride core into the receptor-independent catabolism of LDL and HDL. The triglyceride content of the latter is thereby enhanced, so that both become better substrates for lipase which shrinks them to particles of smaller than normal size.

Recent studies have indicated that low lipoprotein lipase activities may be crucial to the development of severe hypertriglyceridemia in nonobese subjects. This primarily results in high plasma concentrations of VLDL and chylomicrons, even after an overnight fast, but clearly the other lipoproteins in the bloodstream of these patients.

Therefore, in these patients the balance between the activities of both catabolic routes was deranged so that the uptake of lipoprotein into the nonreceptor pathway was disproportionately high. The factor(s) responsible for the increment in receptor-independent LDL catabolism are not known and, indeed, the mechanisms and tissues involved in the normal operation of the pathway are not established. Certainly, pinocytosis makes a contribution, but this is probably small. In a previous study, we suggested that the monocyte-macrophage system may play an important part. In support of this view, Ginsberg and his colleagues showed that clearance of LDL by the nonreceptor pathway is accelerated in patients with myeloproliferative disorders. Since grossly hypertriglyceridemic subjects commonly exhibit hepatosplenomegaly, it is tempting to speculate that an increase in LDL catabolism by the reticuloendothelial cells of these organs may be partly responsible for the low levels of the lipoprotein circulating in the bloodstream of these patients.

The above changes in LDL metabolism seen in hypertriglyceridemic patients are accompanied by significant alterations in the composition of the particles, which become smaller, denser, and enriched in triglyceride relative to cholesteryl ester (Table 2). The mechanisms responsible for these effects are not yet established, although it has been suggested by Deckelbaum and his colleagues that these effects are caused by the combined actions of cholesterol ester transfer protein and plasma lipases. These authors envisage that in the presence of a large circulating mass of triglyceride-rich lipoproteins, the distribution of neutral lipid within the lipoprotein spectrum is distorted so that VLDL becomes relatively cholesteryl ester-enriched by changing a component of its hydrophobic triglyceride core into the receptor-independent catabolism of LDL and HDL. The triglyceride content of the latter is thereby enhanced, so that both become better substrates for lipase which shrinks them to particles of smaller than normal size.
LDL (Table 3) reverted to normal. The cholesterol/triglyceride and cholesterol/protein ratios in the particle increased during fenofibrate therapy, as did the ratio of core/coat components, an index of the particle’s size. Additionally, both the fractional and absolute amounts of LDL that were cleared into the receptor-independent pathways fell to values approaching those found in normal subjects and so, in the face of maintained synthesis, the plasma LDL concentration rose. Concomitantly, there was a significant increment in the amount of LDL cleared into the receptor route. This usually implies an expanded cellular requirement for cholesterol and is consistent with the finding that fenofibrate inhibits the action of 3-hydroxy-3-methylglutaryl CoA reductase and therefore presumably suppresses endogenous sterol synthesis. The response of the receptor pathway to fenofibrate therapy is similar to that which we observed in a group of Type II hyperlipoproteinemic subjects prescribed an alternative clofibrate analogue, bezafibrate.

It should be noted that interpretation of our observations on these grossly hypertriglyceridemic subjects is based on our previous experiences with 1,2-cyclohexanediol-treated LDL in normal and hypercholesterolemic individuals. It is possible that the abnormal structure of LDL in the present group may produce aberrant behavior of the chemically modified probe. So, mechanisms other than hyperactivity of receptor-independent pathways may be responsible for the observed rapid catabolism of the cyclohexanediol-treated trace. These mechanisms include the incomplete receptor-blocking of the hypertriglyceridemic LDL and the generation of a probe that is recognizably different from normal to receptor-independent pathways.

Many of the chlorophenoxyisobutyric acid derivatives increase plasma HDL levels in humans, and Table 1 shows that fenofibrate produced the same effect. On average, HDL cholesterol rose by 42% (p < 0.01) in the seven hypertriglyceridemic subjects as a result of an increase in plasma HDL₂ and HDL₃, which was originally low in these patients, was not affected by the drug. Although the kinetic changes responsible for these effects were not measured, it is possible to make inferences from an earlier study designed to examine the influence of nicotinic acid on HDL metabolism in Type IV hyperlipoproteinemic subjects. In that situation, HDL₃ rose after a decrease in the fractional clearances of both A apolipoproteins. By extrapolation, it may be that fenofibrate decreases the catabolism of not only of LDL but also of HDL in our hypertriglyceridemic individuals. Certainly, evidence from the literature would indicate that the catabolism of LDL and HDL are linked in some obscure way so that the clearance rates of both fractions respond to metabolic perturbations in concert.

In a previous publication we proposed that the primary action of clofibrate and its analogues can be adequately explained on the basis of their effects on two key enzymes in lipoprotein metabolism — lipoprotein lipase and 3-hydroxy-3-methylglutaryl CoA reductase. The results of the present study remain consistent with that view and provide a working hypothesis to explain the actions of these drugs in the treatment of the various hyperlipoproteinemias.

Acknowledgments

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