Receptor-independent Catabolism of Low Density Lipoprotein
IN VOLVEMENT OF THE RETICULOENDOTHELIAL SYSTEM*

Howard R. Slater, Christopher J. Packard, and James Shepherd
From the University Departments of Biochemistry and Medical Cardiology, Royal Infirmary, Glasgow G4 OSF, United Kingdom

In this study we examine the effects of intravenous ethyl oleate emulsions on the metabolism of native and cyclohexanedione-modified human low density lipoprotein in rabbits. Treatment produced a highly significant fall in receptor-independent catabolism as measured by the fractional clearance rate of cyclohexanedione-modified low density lipoprotein. Receptor-dependent catabolism (the difference between the fractional clearance rates of native and cyclohexanedione-modified low density lipoprotein) was variably affected with some animals showing a decrease in receptor activity. These data suggest that the reticuloendothelial system makes a substantial contribution to receptor-independent low density lipoprotein catabolism in the rabbit.

Tissue culture studies have shown (1) that a variety of animal cells possess a specific high affinity receptor for low density lipoprotein. Interaction of the lipoprotein with the receptor initiates a sequence of events which culminates in degradation of the particle with release of its cholesterol into the cell. This can be suppressed by chemical alteration of positively charged amino acid residues of arginine or lysine on the LDL protein which are functionally important for receptor recognition. In practice, 1,2-cyclohexanedione modification of 50% of the arginyl (2) or reductive methylation of 60% of the lysyl residues with formaldehyde/BH$_4$(3) abolishes receptor-lipoprotein interaction in vitro and delays clearance of the lipoprotein from the plasma of both animals (4, 5) and man (6) to an extent that is dependent on the quantitative significance of the receptor pathway in vivo. This approach has shown that, in all species examined, 30 to 50% of total LDL catabolism occurs via the high affinity receptor route. The removal mechanism(s) for the remaining lipoprotein are not clear but may include bulk fluid endocytosis, absorptive endocytosis (7), or other mechanisms not yet described.

Earlier studies from this laboratory (5) have suggested that, in the rabbit, reticuloendothelial tissues such as the spleen, liver, and lymph nodes are particularly active in the removal of cyclohexanedione-treated LDL from the circulation. We have therefore examined the involvement of the reticuloendothelial system in this process and have attempted to assess its contribution to LDL catabolism by observing the plasma clearance of LDL in animals whose RE activity has been suppressed by ethyl oleate (8, 9).

EXPERIMENTAL PROCEDURES
Preparation and Chemical Modification of LDL—Human LDL (1.030 < d < 1.050 kg/liter) was isolated from normal plasma by rate zonal ultracentrifugation (10), dialyzed against 0.1 M NaCl, 0.01% Na$_2$EDTA, pH 7.0, and labeled (11) with either $^{125}$I or $^{131}$I (7) at first (Radiochemical Centre, Amersham, United Kingdom). The labeled lipoprotein was then freed of unbound radiiodide by gel filtration through Sephadex PD-10 columns (Pharmacia (GB) Ltd., London, United Kingdom) using the above buffer for elution.

Modification of arginine residues on the radiolabeled LDL was effected by treatment with 1,2-cyclohexanedione (Fluorochem Ltd., Glossop, United Kingdom) as described previously (2, 6). Unbound cyclohexanedione was removed by gel filtration through a PD-10 column followed by dialysis against 0.15 M NaCl, 0.01% Na$_2$EDTA, pH 7.0.

Administration of Lipid Emulsions—A 10% emulsion of ethyl oleate or glycerol trioleate (Sigma Chemical Co., London, United Kingdom) was prepared by sonication in 0.15 M NaCl containing 0.7% Tween 20 (Sigma Chemical Co.) as stabilizer and injected into rabbits via a marginal ear vein at a dose of 1 ml of lipid/kg body weight.

In a preliminary study in which a trace amount of radiodinated ethyl oleate (12) was incorporated into the emulsion, 65% of the lipid was cleared from the plasma within 5 min and 96% by 90 min. At this time, less than 0.1% of the injected label co-precipitated with apolipoprotein B-containing lipoproteins on addition of heparin/MnCl$_2$ (13) to the plasma. The remaining radioactivity was widely distributed throughout the tissues of the body, with highest concentrations in the spleen, liver, lung, and lymph nodes.

Turnover Study Protocol—Adult male New Zealand White rabbits were purchased from Leslie Moore Ltd., Bradford, United Kingdom, and maintained on standard rabbit chow. Two days prior to and throughout each study, they were given 0.1 $\mu$g/liter of KI in their drinking water to prevent thyroidal uptake of radioiodide. Approximately 10 $\mu$Ci (200 $\mu$g of protein) each of LDL and CHD-LDL labeled with different isotopes of iodine were mixed, stabilized by membrane filtration (0.22 $\mu$m filters, Millipore Corp., Bedford, MA), and injected into a marginal ear vein. Blood samples were then collected from the opposite ear after 10 min and subsequently on eight occasions over the next 48 h. Plasma decay curves were constructed for both isotopes and used to calculate the fractional clearance rate of each tracer (14).

Before and after administration of the stabilized lipid emulsions, measurement was made of plasma lipoprotein levels and of various standard biochemical and hematological parameters.

RESULTS
In an earlier study (5), we have shown that the clearance of human LDL from the plasma of rabbits is indistinguishable from that of homologous rabbit lipoprotein and can be described in terms of a two-compartment model. Intercompartmental equilibration of an intravascularly injected radiolabeled LDL tracer is reached within 24 h and thereafter its elimination from the plasma is monoeponential.

Intravenous administration of an ethyl oleate emulsion during this terminal clearance phase (Fig. 1) slowed catabolism of the tracer. The half-lives of $^{125}$I-LDL before and after
oleate injection into two rabbits were 0.73, 0.53 and 1.6, 1.7 days, respectively. A further oleate injection 24 h after the first had no additional effect. Serial plasma cholesterol and triglyceride measurements were made over the course of the experiment. Following oleate injection, plasma cholesterol rose acutely, by 50% in one rabbit (Fig. 1) and 100% in the second, to a new steady state value. No consistent change was seen in triglyceride levels during this time. In order to confirm that the change in LDL metabolism resulted specifically from ethyl oleate treatment, rabbits were injected with an emulsion from which the lipid had either been omitted or had been replaced by glycerol trioleate. Neither of these treatments altered the rate of LDL clearance or the plasma cholesterol level (Fig. 2). Examination of a number of biochemical and hematological parameters before and 24 h after injection of ethyl oleate revealed no significant change in serum electrolytes, urea, creatinine, glucose, T₃, T₄, albumin, globulins, alkaline phosphatase, and γ-glutamyl transpeptidase. We therefore concluded that the observed change in lipoprotein metabolism did not apparently result from perturbation of hepatic thyroid or renal function. However, the treatment induced a leukocytosis (9) specifically affecting the lymphocyte and monocyte series. Blood hemoglobin and erythrocyte morphology were not changed.

The plasma clearance rates of native and CHD-modified LDL were measured in a group of 11 animals which had been given two injections of an ethyl oleate emulsion 24 and 48 h before the turnover study. Both tracers showed a reduced clearance in comparison to a control group of 14 animals (Fig. 3). The calculated fractional catabolic rate of native LDL (Table I) was 1.01 pools/day versus 1.5 pools/day in control animals, while CHD-LDL clearance fell by 35% in the oleate-treated animals (from 0.84 pool/day in the control situation to 0.55 pool/day after oleate treatment (t = 6.29, p < 0.001). LDL catabolism via the receptor pathway (calculated as the difference between the fractional clearance rates of native and CHD-LDL (5) showed a variable response when compared to the controls, inasmuch as 5 of the 11 animals gave a value for this parameter which was within one standard deviation of the control while in the remainder it was lower. The group as a whole showed a reduction which was significant at the 5% level.

Plasma and lipoprotein cholesterol levels were measured in these animals before and during oleate treatment (Table II). Total plasma cholesterol rose by 13%, this being attributable in its entirety to a 33% increase in circulating LDL cholesterol. High density lipoprotein cholesterol was not affected by the treatment, nor was there a consistent change in plasma triglyceride.

![Fig. 1. Acute effects of ethyl oleate on LDL catabolism in a rabbit. The plasma clearance of human 125I-LDL was followed in a rabbit for 48 h, by which time the decay was monoexponential. A 10% aqueous emulsion of ethyl oleate (1 ml of ethyl oleate/kg body weight) was then administered intravenously, followed by a second dose 24 h later. Serial measurements were made of plasma cholesterol and triglyceride over this period. Upper panel, plasma decay curve of 125I-LDL. Lower panel, plasma lipid measurements.](http://www.jbc.org/)

![Fig. 2. Effect of glycerol trioleate emulsion and Tween 20 on the plasma clearance of human LDL in rabbits. A rabbit received 10 ml of a 10% emulsion of glycerol trioleate 48 h after an injection of 125I-LDL as described in Fig. 1. The plasma clearance of the isotope was then followed for an additional 36 h. The above study was repeated in a second rabbit following injection of 10 ml of 0.7% Tween 20 alone.](http://www.jbc.org/)

![Fig. 3. Effects of intravenous ethyl oleate on the plasma clearance of native and cyclohexanedione-modified human LDL in rabbits. A 10% emulsion of ethyl oleate (1.0 ml of ethyl oleate/kg body weight) was administered intravenously 48 and 24 h prior to injection of 125I-LDL and 131I-cyclohexanedione-modified LDL (CHD-LDL). Plasma radioactivities were measured over the following 48 h and the derived fractional clearance rates compared with control values published previously (6). Open symbols, control animals; solid symbols, ethyl oleate-treated animals.](http://www.jbc.org/)
TABLE I
Effects of ethyl oleate on receptor-mediated and receptor-independent catabolism of human LDL in rabbits

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Fractional clearance rate of LDL</th>
<th>Receptor-independent</th>
<th>Receptor-mediated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.52</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.68</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.52</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.58</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.38</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.48</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.51</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.46</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.89</td>
<td>0.89</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.60</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>0.58</td>
<td>0.56</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± S.D. (n = 14) 0.55 ± 0.09 0.46 ± 0.27
Control value (n = 14) 0.84 ± 0.13 0.66 ± 0.19

* Plasma fractional clearance rate of 1,2-cyclohexanediol-treated LDL
* Plasma fractional clearance rate of native LDL minus that of cyclohexanediol-treated LDL.
* Unpaired t test versus controls, p < 0.001.
* Unpaired t test versus controls, p < 0.05.
* From Ref. 5.

TABLE II
Effects of ethyl oleate on plasma lipids and lipoproteins in rabbits

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total plasma cholesterol</th>
<th>LDL cholesterol</th>
<th>HDL cholesterol</th>
<th>Plasma tri-glyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (n = 11)</td>
<td>2.12 ± 0.95</td>
<td>1.03 ± 0.92</td>
<td>1.06 ± 0.24</td>
<td>0.69 ± 0.12</td>
</tr>
<tr>
<td>Ethyl oleate</td>
<td>2.40 ± 0.96</td>
<td>1.37 ± 0.89</td>
<td>0.95 ± 0.12</td>
<td>0.72 ± 0.23</td>
</tr>
</tbody>
</table>

Paired t test, control versus ethyl oleate treatment

p < 0.02  p < 0.01  NS  NS

* To convert to mg/dl, multiply by 88.5.
* To convert to mg/dl, multiply by 38.7.

DISCUSSION

There are at least two distinct routes of LDL catabolism in animals and man. The better understood of these is the receptor pathway described by Goldstein and Brown (1) which accounts for up to 50% of LDL catabolism. It appears to operate in response to the cells' cholesterol requirements. For example, expression of the pathway in cultured cells is inversely related to the availability of environmental LDL cholesterol (1); and modulation of the hepatic cholesterol pool in the intact animal by feeding bile acid sequestrants (5) or dietary cholesterol supplements (2) produces, respectively, an activation or suppression of the pathway in that organ. Furthermore, we have observed a strong negative correlation between the plasma LDL cholesterol concentration and its receptor-mediated fractional clearance rate in rabbits (r = 0.75, p < 0.01, n = 11) indicating that there exists a close regulatory association between receptor activity and LDL pool size.

By contrast, the receptor-independent degradation pathway(s) as measured by the fractional clearance rate of CHD-LDL in rabbits does not correlate with plasma LDL (r = 0.4, t = 1.6, n = 11), but appears to clear a fixed portion of the plasma LDL pool each day (i.e. 0.84 ± 0.13 pool/day, Table I). It is responsible in normal human subjects for approximately two-thirds of LDL catabolism (6) and is quantitatively even more significant in familial hypercholesterolemia patients (6, 15, 16) who degrade supranormal amounts of LDL (17, 18) despite their relative or absolute lack of specific LDL receptors (19).

Nevertheless, its location and role in lipoprotein metabolism is unclear. Earlier observations from this (5) and other laboratories (20) have shown that the liver, spleen, and lymph nodes are particularly active in assimilating from the plasma LDL which has been chemically modified to inhibit its receptor recognition, suggesting that the RE system might be functionally important in receptor-independent LDL catabolism. To investigate this possibility we made use of the suppressant effects of fatty acid alkyl monoesters (8, 9) on RE function in rabbits.

Ethyl oleate treatment specifically delayed the clearance of LDL and increased its plasma concentration without affecting HDL cholesterol or plasma triglyceride. This change was expressly dependent on ethyl oleate and could not be evoked by the substitution of glycerol trioleate (which is known not to inhibit RE function (8, 9)) or by injection of the stabilizing agent (Tween 20) alone.

We considered three potential mechanisms for the effect. First, the emulsion may have modified the composition of the LDL particle in such a way as to delay its catabolism. This is unlikely, since only trace amounts of radioactive lipid were found in the heparin/MnCl₂-precipitable plasma lipoproteins 90 min after injection of a labeled ethyl oleate emulsion. Moreover, the effect of the infusion persisted for more than 24 h, even though the plasma LDL pool would have turned over by this time (Table I). Second, infusion of a large bolus of lipid may have stimulated LDL synthesis, increasing its pool size and lowering its fractional clearance rate. However, as noted above, pool size and receptor-independent LDL catabolism are not apparently interdependent. Indeed, a number of human studies have underlined their independence (16, 21); and we have found that doubling the LDL pool size in rabbits by continuous infusion of the lipoprotein does not change acutely the removal rate of CHD-LDL. Consequently, we feel that a third mechanism, i.e. direct interference with the catabolic process, is the most likely explanation for the oleate-induced change in LDL metabolism.

So, how might ethyl oleate inhibit the catabolism of LDL? Stuart and co-workers (9) have shown that this lipid (but not glycerol trioleate) suppresses RE activity in animals, inhibiting their ability to clear colloidal carbon from the plasma. Our observations would link this suppression with a reduction in LDL catabolism by both receptor-dependent and receptor-independent pathways. The response of the receptor pathway was variable. In some treated animals, receptor activity continued at the same rate as normal while in others it was reduced (Table I). The latter may have resulted from either general receptor down-regulation in response to the increased plasma LDL level or from a direct toxic effect of ethyl oleate on RE cells (which have been reported to express receptor activity (22, 23)). In contrast, the ethyl oleate-induced reduction of receptor-independent LDL catabolism was evident in all animals and was highly significant, implicating the RE system in LDL clearance by this pathway.

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REFERENCES


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