In Vivo Catabolism of Biologically Modified LDL

J. Fred Nagelkerke, Louis Havekes, Victor W.M. van Hinsbergh, and Theo J.C. van Berkel

Incubation of human low density lipoprotein (LDL) at 37°C in the presence of human umbilical vein endothelial cells (EC) caused a time-dependent shift in the charge and density of LDL. The physical changes of the human LDL occurred parallel with an increase in its clearance from the serum and uptake in the liver when injected into rats. The serum decay of the EC-modified LDL (44 hours incubation) was 20 times faster than for control LDL. EC-modified LDL, cleared from the blood, was quantitatively recovered in the liver. Isolation of the different liver cell types (parenchymal, Kupffer, and endothelial cells) after in vivo injection of 125I-EC-modified LDL showed that approximately 30 times more radioactivity was associated with the endothelial cells than with the parenchymal cells (per milligram of cell protein). In vitro experiments indicated that EC-modified LDL was processed by the rat liver endothelial cells via a high affinity, saturable pathway related to the pathway by which these cells processed acetyl-LDL. We concluded that, if EC-modified LDL is generated in vivo, the liver, and in particular the endothelial cell, forms the major protection system against the occurrence of atherogenic particles in the blood.


The mechanism by which low density lipoprotein (LDL) is cleared from the blood circulation has been a subject of intensive research for the last decade. The best known mechanism is the classical LDL-receptor pathway, initially described by Goldstein and Brown. However, in vivo studies indicate that the LDL clearance from the blood is not quantitatively mediated by the classical receptor. Therefore, other mechanisms have been proposed, generally called "nonreceptor" pathways. This name was based upon the observation that LDL, in which the recognition site for the classical LDL receptor is blocked, is nevertheless cleared from the circulation, although at a lower rate. One such additional clearance pathway, operating via a different receptor site, is the scavenger pathway. Its existence was proposed on the basis of the findings of Brown et al. that accumulation of cholesterol esters in macrophages, observed in patients with increased LDL levels, could only be provoked in vitro by incubation of macrophages with acetylated low density lipoprotein (acetyl-LDL), while native LDL was ineffective. The scavenger pathway is predominantly present in cell types belonging to the mononuclear phagocyte system. Furthermore, the receptor site is present in the foam cell, which is thought to be derived from cells belonging to this system.

Recently we demonstrated that human acetyl-LDL is almost completely cleared from the circulation by the liver within 3 minutes after injection into rats. Moreover, it was demonstrated that the rat liver endothelial cell is by far the most active liver cell type in the uptake of acetyl-LDL. In vitro studies indicated that a high affinity saturable receptor site for acetyl-LDL is present on the liver endothelial cell at a relatively high concentration.

In vivo acetylation of LDL is, however, doubtful and, therefore, the physiological importance of the scavenger pathway was unclear. Recently a more relevant biological modification of LDL, leading toward in vitro recognition by the scavenger receptor on macrophages, was reported by Henriksen et al. The modification was achieved by incubating the LDL with rabbit aortic or human umbilical vein endo-
thelial cells (EC-modified LDL). The purpose of this study was to investigate the behavior of EC-modified LDL in vivo and to correlate this with receptor studies in vitro. In addition, the in vivo and in vitro uptake and degradation rates of EC-modified LDL were compared with those of acetyl-LDL.

Methods

Isolation and Culture of Umbilical Vein Endothelial Cells

Human umbilical cords were kept after delivery in ice-cold cord buffer (140 mM NaCl, 4 mM KCl, 11 mM D-glucose, 10 mM Hepes, pH 7.3, 100 IU/ml penicillin, 0.10 mg/ml streptomycin). They were collected once a day from the hospital. Endothelial cells were isolated from veins as described by van Hinsbergh et al. Briefly, the vein was rinsed and incubated for 15 minutes at 37°C with 0.1% collagenase in M-199 medium at 37°C. The collected venous cells were centrifuged (5 minutes 200 g) and suspended in M-199 medium containing 20% human serum and 15 mM Hepes buffer. They were seeded rather densely (1–5 × 10⁶ cells/cm²) in six 2 cm² wells coated with a crude fibronectin solution. The coating was performed at 37°C for 30 minutes. The fibronectin solution was removed by aspiration immediately before the cells were seeded. The cells were cultured at 37°C in M-199 medium supplemented with 20% pooled human serum (not inactivated), 15 mM Hepes buffer, 100 IU/ml penicillin and 0.1 mg/ml streptomycin under 5% CO₂ in air. The medium (0.2 ml/cm²) was refreshed every 2 or 3 days. At confluency, the cells were used, or released with trypsin/EDTA and passaged with a 1:3 split ratio to obtain subcultures.

Low Density Lipoproteins

LDL was isolated from freshly prepared human serum by density gradient ultracentrifugation according to the method of Redgrave et al. followed by tube slicing. SDS-polyacrylamide gel electrophoresis showed only the presence of apo B. The LDL was immediately used for iodination by the ¹²⁵I-iodine monochloride method described by Bilheimer et al. After iodination, LDL was dialyzed against phosphate-buffered saline (PBS), without EDTA, for 4 hours (4 × 500 volumes). Thereafter, it was stabilized by the addition of 10 mg of bovine serum albumin (BSA) per ml and further dialyzed overnight against 500 volumes of PBS at 4°C. The specific activity ranged from 80 to 150 cpn/ng of LDL protein. The acid-soluble (noniodine) fraction was less than 0.1%. This ¹²⁵I-labelled LDL was used for endothelial cell modification within 2 days of storage at 4°C.

Endothelial Cell-Modified LDL

EC-modified LDL was prepared by incubating confluent endothelial cell cultures at 37°C with medium M-199 supplemented with 10 g/l BSA, instead of 20% human serum, and with 100 μg of LDL protein per ml medium. After the indicated time intervals, the medium was aspirated and stored at 4°C. The morphology of the cells was then examined by phase contrast light microscopy. At all time intervals, no morphological changes could be detected. Incubations of LDL in the same medium at 37°C but without cells were used as controls.

EC-modified LDL and control LDL preparations were, without prior isolation from the culture media, subjected to agarose electrophoresis according to the method of Demmacker. After electrophoresis, the agarose plate was dried by a stream of hot air and subjected to autoradiography. Simultaneously, 100 μl of the culture media were subjected to density gradient ultracentrifugation according to the method of Redgrave et al. After 14 hours of ultracentrifugation (4°C, mean RCF is 200,000 g) density fractions of 0.5 ml were collected using the method described by Groot et al. The fractions were measured for density using a DMA 602 M densitometer and counted for radioactivity. The acid soluble (noniodine) fractions of the EC-modified and control LDL samples were measured as described by Bierman et al.

Acetylation of LDL

LDL was acetylated with acetic anhydride as described by Basu et al.

Rat Liver Cells

Throughout this study 3-month-old male Wistar R1 rats (Centraal Proefdieren Bedzyf, Rotterdam, The Netherlands) were used. Rat liver endothelial, Kupffer, and parenchymal cells were prepared as previously noted. After injection of the indicated lipoprotein preparations, the cells were isolated with either pronase or collagenase by a low temperature procedure. Earlier studies showed that in vivo endocytosed material was not degraded during the applied isolation procedures, which led to a reliable determination of the contribution of each cell type to total liver uptake in vivo.

For in vitro studies cells were isolated with collagenase at 37°C as described earlier. We found that this procedure gives the best preservation of lipoprotein receptor activity.

Freshly isolated endothelial cells were incubated at 37°C in Hams F-10 medium containing 2% BSA and the indicated amounts of lipoproteins. Incubations were carried out in siliconized Sorvall tubes. At the indicated times, 1 ml samples were withdrawn, transferred to plastic Eppendorf tubes, and centrifuged for 2 minutes at 600 g. The cell pellets were suspended in 1 ml of medium, containing 50 mM Tris-HCl (pH 7.4), 0.15 M NaCl and 2 mg BSA; incubated for 5 minutes at 4°C, and centrifuged again. This washing procedure was repeated twice. The last washing was performed with similar medium without BSA for a reliable cell protein determination.
Degradation of the lipoproteins was measured according to the method of Bierman et al.\textsuperscript{14} To 0.5 ml of the first supernatant, 0.2 ml of 35\% trichloroacetic acid was added, followed by incubation for 15 minutes at 37°C; subsequently the mixture was centrifuged for 2 minutes at 15,000 \( g \). To 0.5 ml of the supernatants, 5 \( \mu l \) of 40\% potassium iodide and 25 \( \mu l \) of 30\% hydrogen peroxide were added. After 5 minutes at room temperature, 0.8 ml chloroform was added and the mixture was shaken for another 5 minutes. After centrifugation for 2 minutes at 15,000 \( g \), 0.4 ml of the aqueous phase (containing iodinated degradation products) and 0.5 ml of the chloroform phase (containing free iodine) were sampled. Radioactivity was counted in a LKB-Wallace ultra-gamma counter.

The viability of the cells before and after incubation was more than 95\% as judged by the Trypan blue exclusion test. The purity of the rat liver endothelial cell preparations was checked by a light microscope as described earlier\textsuperscript{6} and was always more than 95\% pure.

### Protein Determination

Protein determination was according to the method of Lowry et al.\textsuperscript{15} using BSA as a standard.

### Chemicals

Type I collagenase and BSA, Fraction V, were from Sigma Company, St. Louis, Missouri; B-grade pronase was obtained from Calbiochem-Behring Corporation, La Jolla, California; metrizamide was from Nyegaard and Company, A/S, Oslo, Norway; Ham’s F-10 was from Gibco-Europe, Hoofddorp, The Netherlands; and \( ^{125} \)I (carrier-free) in NaOH was from New England Nuclear, Dreieich, West Germany.

### Results

#### Characterization of the LDL Preparations

Human LDL incubated with human umbilical vein endothelial cells for increasing periods of time showed an increasing electrophoretic mobility on agarose gels (Figure 1). This effect was already observed after 9 hours of incubation. The mobility increased further with prolonged incubation times without reaching a plateau during the time studied. Note that LDL incubated for 44 hours at 37°C in the absence of cells also migrated slightly faster than LDL that was not incubated.

Density gradient ultracentrifugation demonstrated that after 44 hours of incubation, the buoyant density had shifted from the normal LDL range toward a mean density of \( d = 1.080 \) g/ml (Figure 2). The incubation of LDL in the absence of cells did not lead to a significant shift in buoyant density. The acid soluble (noniodine) radioactivity in all lipoprotein fractions was less than 1\% of the acid-precipitable radioactivity (Table 1). The modification was a result of a direct interaction between LDL and the umbilical vein endothelial cells. LDL that was incubated for 44 hours in medium in which endothelial cells were previously incubated was not significantly altered (data not shown).

### In Vivo Studies

Serum decay and liver uptake after intravenous injection into rats were determined with the same lipoprotein preparations that are characterized in Figures 1 and 2 and Table 1. The LDL preparations were injected into the recipient animals without prior isolation from the media. Figure 3 shows the serum decay of the \( ^{125} \)I-labeled EC-modified and control lipoproteins after injection into rats. (Routinely, a lipoprotein preparation containing 40–60 \( \mu g \) of apoprotein, specific activity 80–150 dpm/\( \mu g \) apoprotein was injected.) There was a strong effect of prolonged incubation of the LDL with the umbilical vein endothelial cells on the clearance from serum. Also the LDL that was incubated for 44 hours at 37°C in the absence of cells was cleared slightly faster from the circulation than LDL that was not incubated.

### Table 1. Water-Soluble (Noniodine) Radioactivity in the Lipoprotein Fractions

<table>
<thead>
<tr>
<th>Incubation time (hrs)</th>
<th>Presence of umbilical vein endothelial cells</th>
<th>Absence of umbilical vein endothelial cells</th>
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<tr>
<td>0</td>
<td>0.05</td>
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<tr>
<td>9</td>
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<td>44</td>
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Acid-precipitable and acid-soluble (noniodine) radioactivity was determined as in reference 14. Values are expressed as percentage of the acid-precipitable radioactivity.

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**Figure 1.** Agarose electrophoresis of \( ^{125} \)I-labeled EC-modified LDL and \( ^{125} \)I-labeled control LDL. After incubation in the presence or absence of cells at 37°C for the indicated time, 2 \( \mu l \) of medium was subjected to agarose electrophoresis according to the method of Demacker et al.\textsuperscript{12} After 3 hours of electrophoresis, the agarose plate was dried by hot air and subjected to autoradiography for 18 hours.
Figure 2. Density gradient ultracentrifugation of 125I-labeled EC-modified LDL and control LDL. After incubation in the presence or absence of cells at 37°C for the indicated time, 100 μl of medium was mixed with 4 ml KBr salt solution (density 1.21 g/ml) and subjected to density gradient ultracentrifugation according to the method of Redgrave et al.10 After 14 hours of ultracentrifugation (RCF 200,000 g, 4°C) the density fractions (0.5 ml) were collected using the apparatus described by Groot et al.13 connected to a fraction collector. Each fraction was measured for density and counted for radioactivity. In the figure only the fractions with densities between 1.00 and 1.12 g/ml are shown.

Figure 3. Serum decay of 125I-labeled lipoproteins after injection into rats. Blood samples were drawn as indicated. The samples were centrifuged for 2 minutes at 20,000 g and radioactivity was counted in the supernatants. The values are expressed as percentages of the injected dose. LDL was incubated with human umbilical vein endothelial cells for 9 (○), 23 (●) or 44 (▲) hours or in the absence of cells for 0 (△) or 44 (▲) hours. Three different batches of LDL were modified for different time periods. The results shown are from one batch. Experiments with the other two batches gave similar results.

clearance is, however, much slower than that of LDL incubated for 44 hours in the presence of cells.

To determine the kinetics of the liver association in vivo, the 125I-labeled lipoproteins (40–60 μg apoprotein) were injected into rats. At different time intervals after injection, a liver lobule was tied off and excised. After weighing the lobule and counting its radioactivity, the total liver uptake was extrapolated using the assumption that 3.75% of the total body weight is contributed by the liver.18 Figure 4 shows that the uptake of the EC-modified LDL by the liver increased with prolonged incubation of the LDL with the endothelial cells. Also the peak values of the lipoprotein content in the liver shifted to shorter time intervals after injection.

Determination of the cell types responsible for the liver uptake was performed by isolating the various cell types 10 minutes after the in vivo injection of the 125I-labeled lipoproteins (40 to 60 μg apoprotein) into rats (Figure 5). It appeared that the liver endothelial and parenchymal cells were mainly responsible for the increased liver association of EC-modified LDL. The relative contribution of these cell types to the uptake of EC-modified LDL appeared to be similar to that observed earlier for chemically modified LDL.6 Approximately 30 times more EC-modified LDL became associated with the endothelial cells than with the parenchymal cells (per mg cell protein). Taking into account the contribution of each cell type to the total liver protein,18 the total rat liver
endothelial cell population was the major site for the liver uptake of EC-modified LDL, similar to acetyl-LDL (Table 2). Figure 5 also shows that LDL incubated 44 hours in the absence of cells was taken up more than the nonincubated LDL. However, the presence of umbilical vein endothelial cells during incubation resulted in an uptake by endothelial and parenchymal cells that was about 10 times as high.

To determine the cellular metabolism of EC-modified LDL associated with the liver endothelial cells in vivo, we warmed the cells, isolated at 8°C, to 37°C. At different time intervals thereafter, we drew a sample and determined the cell-bound and excreted acid-soluble (noniodine) degradation products.

Figure 6 shows that the radioactivity initially bound to the cells appeared as acid-soluble (noniodine) radioactivity in the medium, indicating the degradation of the apoprotein.

To compare the intracellular processing rate of EC-modified LDL with acetyl-LDL, we repeated the experiment. This time acetyl-LDL was injected. One-half of the acetyl-LDL, initially cell-associated, was

| Table 2. Relative Contribution of the Different Liver Cell Types to the Total Uptake of EC-Modified LDL and Acetyl-LDL by Rat Liver |
|-----------------|-----------------|-----------------|
| Cell type       | EC-modified LDL incubation time (hrs) | Acetyl-LDL |
|                 | 9               | 22              | 44              |
| Parenchymal cells (%) | 33             | 34              | 37              | 38 |
| Endothelial cells (%)    | 49             | 49              | 51              | 53 |
| Kupffer cells (%)         | 18             | 17              | 12              | 9  |

The percentage was calculated by multiplying the values from Figure 4 with the amount of protein that each cell type contributes to total liver protein. The values are expressed as a percentage of the total injected dose.

Figure 4. Liver uptake of $^{125}$I-labeled lipoproteins after injection into rats. After injection, lobules were tied off and excised at the indicated times. Values are expressed as percentages of the injected doses. The lobules were not perfused; liver values here include the amount of lipoproteins present in the entrapped blood (approx. 9% of the serum value based upon $^3$H-albumin measurements). LDL was incubated with human umbilical vein endothelial cells for 9 (○), 22 (●) or 44 (▲) hours or in the absence of cells for 0 (▲) or 44 (▲) hours.

Figure 5. Cellular distribution of $^{125}$I-labeled LDL in liver after intravenous injection into rats. Ten minutes later perfusion was started by cannulation of the vena porta. The perfusion and cell isolation was performed at 8°C to prevent degradation of endocytosed LDL. Eight minutes after perfusion began a lobule was tied off and excised; then different cell types were isolated and separated. Values are expressed as percentages of the injected dose per mg liver or cell protein. From each lipoprotein fraction tested two independent total liver uptake values were obtained. The averages are shown at the top (left to right): 1.55 ± 0.15; 5.95 ± 1.15; 3.7 ± 0.1; 10.35 ± 0.35; 22.3 ± 4.4; 39.3 ± 2.25.
degraded within 10 minutes, although it took 30 to 40 minutes of incubation at 37°C before one-half of the cell-associated EC-modified LDL was degraded.

**Nature of the Recognition Site**

The time course of in vitro cell-association of the EC-modified LDL, (incubated for 44 hours) with isolated liver endothelial cells, indicates a linear increase with time up to 2 hours (Figure 7 A). In the insert the results of a similar experiment with acetyl-LDL as a substrate are shown. Here cell association was more rapid and reached a plateau after 30 minutes of incubation. The degradation rate of EC-modified LDL showed a lag-phase of 30 minutes before TCA-soluble (noniodine) degradation products were detected (Figure 7 B). In the insert the degradation rate of acetyl-LDL is shown, with degradation detectable between 10 and 30 minutes. Addition of the lysosomotropic agent chloroquine (50 \mu M) led to a virtually complete blockade of the degradation of EC-modified LDL, suggesting the involvement of the lysosomes (Figure 7 B).

**Figure 6.** Time course of intracellular processing of EC-modified LDL and acetyl-LDL by isolated liver endothelial cells after in vivo injection of lipoproteins. Rat liver endothelial cells were isolated from rats injected with 40–60 \mu g \textsuperscript{125}I-labeled lipoproteins 10 minutes before starting liver perfusion. The isolated pure endothelial cells were resuspended in buffer at 37°C. Samples were drawn at indicated times, the cells were centrifuged, and the amount of cell-associated and acid-soluble radioactivity in the supernatant was determined. Closed symbols represent cell-associated radioactivity; open symbols, acid-soluble (noniodine) radioactivity. Triangles indicate acetyl-LDL, circles indicate LDL coincubated with umbilical vein endothelial cells for 44 hours. Values are per mg/cell protein. The 100% value represents the amount of cell-bound radioactivity from the freshly prepared cells.

**Figure 7.** In vitro time course of cell association (A) and degradation (B) of EC-modified LDL (44 hours incubation) by isolated rat liver endothelial cells. Isolated cells were incubated at 37°C with 20 \mu g/ml EC-modified LDL. Values are the ng apoplipoprotein cell-associated (A) or degraded (acid-soluble radioactivity) (B) per mg cell protein. Cells were incubated in the absence (circles) or presence (squares) of 50 \mu M chloroquine. Inserts show the time course of in vitro association (A) and degradation (B) of acetyl-LDL by rat liver endothelial cells in vitro. Data is the same as in the main figures.
Figure 8. Cell association (A) and degradation (B) of $^{125}\text{I}$-modified LDL (44 hours incubation) by rat liver endothelial cells as a function of the apolipoprotein concentration. Values are expressed as ng apolipoprotein per mg cell protein. Incubation time was 2 hours at 37°C.

Figure 9. The effect of increasing concentrations of unlabeled acetyl-LDL on the cell association (●) and degradation (○) of $^{125}\text{I}$-labeled EC-modified LDL by rat liver endothelial cells. The concentration of $^{125}\text{I}$-EC-modified LDL was 10 µg/ml apolipoprotein. Cells were incubated for 2 hours at 37°C. Values are given as percentages of the control incubated in the absence of unlabeled acetyl-LDL. Insert shows a similar experiment for $^{125}\text{I}$-acetyl-LDL and unlabeled acetyl-LDL. Data is the same as in the main figure. ▲ = association, △ = degradation.

This study was undertaken to determine the in vivo behavior of biologically modified LDL. Recent studies by Henriksen et al. indicated that incubation of LDL with endothelial cells induces changes in structure that result in recognition by macrophages and subsequent degradation at three to five times the rate of unmodified LDL. This biologically modified LDL may be pathophysiologically significant. Earlier studies with macrophages indicated that the uptake and degradation of EC-modified LDL is exerted by a pathway that is shared to some extent by acetyl-LDL. The present study shows that in vivo EC-modified LDL was processed at similar cellular sites as acetyl-LDL. EC-modified LDL was rapidly cleared from the blood and recovered quantitatively in the liver (Figures 3 and 4) at 10 minutes after injection of the lipoproteins. At longer time intervals after injection, the uptake of the disappeared EC-modified LDL was no longer quantitative, probably because iodine-labeled degradation products of EC-modified LDL left the liver. This explanation is supported by the data on the in vitro degradation of in vivo recognized EC-modified LDL. Endothelial cells that were isolated 10 minutes after injection of EC-modified LDL, actively degraded the cell-associated radioactivity to acid-soluble products, which were subsequently released from the cells.

The contribution of the different cell types to total liver uptake was similar to that observed with acetyl-LDL. The rat liver endothelial cell was approximately 30 times more active per milligram of cell protein than the parenchymal cell. Prolonged coincubation of umbilical vein endothelial cells with LDL strengthened the acetyl-LDL character of the EC-modified LDL, i.e., a faster serum decay and increased liver uptake (up to 20-fold), although the clearance and liver uptake rate of the chemically generated acetyl-LDL was never reached. In the case of acetyl-LDL 80% of the injected dose is present in the liver, after 10 minutes, indicating a high capacity of the liver acetyl-LDL receptor. Because similar concentrations of EC-modified LDL and acetyl-LDL were injected, the lower uptake of EC-modified LDL as compared...
with acetyl-LDL could only be the result of a slower association rate.

These findings can be explained by the in vitro cell-association data. The dose response curves for EC-modified LDL and acetyl-LDL were similarly dependent on the substrate concentration; half-maximal cell-association and degradation occurred at about 10–20 μg apoprotein/ml for both modified forms of LDL. However, the maximal amount that became cell-associated was clearly different: 20,000 ng/mg cell protein for acetyl-LDL and 7500 ng/mg cell protein for EC-modified LDL at 2 hours after incubation. This results in a higher association rate for acetyl-LDL than for EC-modified LDL at comparable concentrations of EC-modified LDL and acetyl-LDL. This difference in maximal cell association rate is reflected in the longer time needed for EC-modified LDL to reach a steady-state level (determined by the cell-association rate versus the degradation rate). The competition experiments indicated that acetyl-LDL was a more efficient competitor for 125I-EC-modified LDL than for 125I-acetyl-LDL. With this model, one should expect that concentrations of EC-modified LDL higher than acetyl-LDL should be necessary to compete for 125I-acetyl-LDL cell-association, a finding presented earlier by Henriksen et al.7

In conclusion, it is evident that EC-modified LDL binds to the receptor on endothelial cells which also bind acetyl-LDL. Also in vivo association of EC-modified LDL with parenchymal cells can be explained by the presence of an acetyl-LDL binding site on these cells as demonstrated earlier.19

It should be noted that we have injected human lipoproteins into rats, which could be a potential problem due to the species difference. However, it was reported20 that upon acetylation, both rat and human LDL were processed in a similar fashion after injection into rats. During incubation of LDL with umbilical vein endothelial cells, two physical characteristics (charge and density) of the LDL particle change. Which, if either, of these changes induces the enhanced serum decay and liver uptake is not clear at the moment. Modification of the LDL by incubation with umbilical vein endothelial cells is a continuous process.8 The electrophoretic mobility and buoyant density gradually increases and there is a gradual increase in serum decay and liver uptake.

Besides the changes induced by incubation of LDL with umbilical vein endothelial cells, we also found that incubation of LDL for 44 hours at 37°C in the absence of cells slightly altered its behavior. The electrophoretic mobility and serum decay, as compared with native LDL were increased. The in vivo association was enhanced, but in contrast with EC-modified LDL, the live Kupffer cells were mostly responsible for the increased liver association of the 37°C incubated LDL.

Modified lipoproteins are considered potentially atherogenic because uptake of these particles can lead to accumulation of cholesterol esters in cells of the mononuclear phagocyte system.4 The uptake of these abnormal lipoproteins by macrophages in vivo might explain the formation of foam cells in the arterial wall.5 If EC-modified LDL is generated in vivo and enters the general circulation, it would very rapidly be removed by the liver. Therefore the liver, and in particular the liver endothelial cell, forms an important protection system against these pathophysiological lipoproteins. It is possible that a delicate balance between formation of these particles and the capacity of the liver to entrap them determines their pathological action. In this view, a small disturbance of the balance could give rise to prolonged circulation of these particles, thereby allowing the slow formation of atherosclerotic lesions.

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