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Satchell, L. and Leake, D. (2012) Oxidation of low density lipoprotein by iron at lysosomal pH: implications for atherosclerosis. Biochemistry, 51 (18). pp. 3767-3775. ISSN 0006-2960 doi: https://doi.org/10.1021/bi2017975 Available at https://centaur.reading.ac.uk/27950/

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To link to this article DOI: http://dx.doi.org/10.1021/bi2017975

Publisher: American Chemical Society

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Oxidation of Low-Density Lipoprotein by Iron at Lysosomal pH: Implications for Atherosclerosis

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ABSTRACT: Low-density lipoprotein (LDL) has recently been shown to be oxidized by iron within the lysosomes of macrophages, and this is a novel potential mechanism for LDL oxidation in atherosclerosis. Our aim was to characterize the chemical and physical changes induced in LDL by iron at lysosomal pH and to investigate the effects of iron chelators and α-tocopherol on this process. LDL was oxidized by iron at pH 4.5 and 37 °C and its oxidation monitored by spectrophotometry and high-performance liquid chromatography. LDL was oxidized effectively by FeSO₄ (5–50 μM) and became highly aggregated at pH 4.5, but not at pH 7.4. The level of cholesteryl esters decreased, and after a pronounced lag, the level of 7-ketocholesterol increased greatly. The total level of hydroperoxides (measured by the triiodide assay) increased up to 24 h and then decreased only slowly. The lipid composition after 12 h at pH 4.5 and 37 °C was similar to that of LDL oxidized by copper at pH 7.4 and 4 °C, i.e., rich in hydroperoxides but low in oxysterols. Previously oxidized LDL aggregated rapidly and spontaneously at pH 4.5, but not at pH 7.4. Ferrous iron was much more effective than ferric iron at oxidizing LDL when added after the oxidation was already underway. The iron chelators diethylenetriaminepentaaetic acid and, to a lesser extent, desferrioxamine inhibited LDL oxidation when added during its initial stages but were unable to prevent aggregation of LDL after it had been partially oxidized. Surprisingly, desferrioxamine increased the rate of LDL modification when added late in the oxidation process. α-Tocopherol enrichment of LDL initially increased the rate of oxidation of LDL but decreased it later. The presence of oxidized and highly aggregated lipid within lysosomes has the potential to perturb the function of these organelles and to promote atherosclerosis.

The accumulation and oxidation of low-density lipoprotein (LDL) in the arterial intima might play an important role in atherosclerosis.¹ There has been an enormous amount of work on the mechanisms by which LDL might be oxidized, but no consensus has emerged. Possible mechanisms involve iron, copper, caeruloplasmin, lipoxygenase, superoxide, peroxynitrite, and myeloperoxidase.²

There is evidence that LDL can be modified by nonoxidative mechanisms in the arterial wall so that it is endocytosed faster by macrophages. These include modification by sphingomyelinase,³ secretory phospholipase A₂,⁴ or cathepsin D.⁵ We have proposed that nonoxidatively modified LDL might be taken up by macrophages in atherosclerotic lesions and oxidized within lysosomes.⁶ Macrophages in culture were capable of taking up acetylated or aggregated LDL and oxidizing it in lysosomes.⁶ Lysosomes are a source of redox-active iron,⁷ and iron is present at elevated levels in atherosclerotic lesions.⁷ Lysosomal LDL oxidation may explain why oxidized LDL is present within lesions despite the apparently strong antioxidant protection within the arterial intima.⁸ Oxidized LDL formed within lysosomes might affect cell function or be released from cells into the interstitial fluid, both with potentially atherogenic consequences.

Oxidation of LDL by copper at pH 7.4 has been extensively characterized,⁹ whereas little is known about the chemical composition of LDL oxidized by iron under the acidic conditions present in lysosomes (pH ∼4.5). We report here the chemical changes in LDL oxidized by iron at lysosomal pH and the surprising effects of ferrous and ferric iron, iron chelators, and α-tocopherol.

MATERIALS AND METHODS

LDL Isolation. LDL (d = 1.019–1.063 g/mL) was isolated by sequential ultracentrifugation of pooled plasma from four healthy adult volunteers.¹⁰

Measurement of Conjugated Dienes. Conjugated diene formation was monitored at 234 nm.¹¹ LDL (50 μg of protein/mL) was oxidized by freshly dissolved FeSO₄ (5 μM) or FeCl₃ (5 μM) at 37 °C in washed Chelex-100-treated 150 mM NaCl/10 mM sodium acetate buffer (pH 4.5) or 150 mM NaCl/10 mM 3-(N-morpholino)propanesulfonic acid (MOPS) buffer (pH 7.4), in capped quartz cuvettes, in an automatic six-position (Lambda 2) or eight-position (Lambda Bio 40) spectrophotometer (Perkin-Elmer). The FeSO₄ or FeCl₃ stock solutions for the experiments at pH 4.5 were dissolved in buffer

Received: December 7, 2011
Revised: April 11, 2012
Published: April 11, 2012

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dx.doi.org/10.1021/bi2017975 | Biochemistry 2012, 51, 3767–3775
at pH 4.5, rather than in water, to prevent the rapid oxidation of ferrous ion that occurs at higher pH values.\textsuperscript{12} The change in attenuance at 234 nm was measured every minute at 37 °C, against reference cuvettes lacking LDL. The attenuance at time zero was subtracted from all values, except where stated.

Measurement of LDL Aggregation. LDL aggregation was monitored by continuously measuring light scattering at 680 nm and 37 °C in the spectrophotometers mentioned above.

LDL Oxidation for Compositional Analysis. LDL (250 μL at a concentration of 2 mg of protein/mL) was oxidized with freshly dissolved FeSO₄ (50 μM) at 37 °C in NaCl/sodium acetate buffer (pH 4.5). At various times over 48 h, the oxidation was stopped by addition of butylated hydroxytoluene (BHT, final concentration of 80 μM, from a 2 mM stock solution in ethanol) and EDTA (final concentration of 4 mM).

Preparation of Lipid Hydroperoxide-Rich and Oxysterol-Rich LDL. Lipid hydroperoxide-rich LDL and oxysterol-rich LDL were produced by oxidizing LDL with CuSO₄ (10 μM) at 4 and 37 °C, respectively, in 150 mM NaCl/10 mM MOPS buffer (pH 7.4), followed by dialysis in the presence of EDTA overnight to remove copper.\textsuperscript{14}

Total Hydroperoxide Content. Hydroperoxides (ROOH) were measured using a triiodide method.\textsuperscript{15}

Relative Electrophoretic Mobility. The relative electrophoretic mobility (REM) of oxidized LDL was measured using a Paragon Lipogel electrophoresis system (Beckman Instruments), according to the manufacturer’s instructions, with 3 μL of LDL (2 mg of protein/mL) applied to the origin and run at 100 V for 40 min. The distance that the samples migrated was divided by the distance migrated by native LDL.

Thiobarbituric Acid Reactive Substances (TBARS). TBARS were assessed as described previously.\textsuperscript{16}

HPLC Analysis. Lipids were extracted from oxidized LDL for HPLC analysis using methanol and hexane.\textsuperscript{17} The upper hexane layer was dried under a stream of nitrogen and redissolved in 200 μL of an acetonitrile/2-propanol/water mixture (44/54/2, by volume). Lipid species were separated by reverse phase HPLC in a Capital C18 column (250 mm × 4.6 mm, 5 μm particle size, 5 μm guard column) with a Perkin-Elmer 200 series HPLC system. Cholesterol and cholesteryl esters were detected at 210 nm using an acetonitrile/2-propanol/water mobile phase (44/54/2, by volume) and a flow rate of 1.5 mL/min. 7-Ketocholesterol and cholesteryl linolate hydroperoxide were detected at 234 nm using an acetonitrile/2-propanol/water mobile phase (50/48.8/1.2, by volume) and a flow rate of 1.2 mL/min. The identities of the peaks were...
confirmed by mass spectrometry (data not shown), and the lipids were quantified using commercially available standards.

**Enrichment of LDL with α-Tocopherol.** Blood was centrifuged at 1500 g for 30 min at 4 °C in the presence of 3 mM Na2EDTA. The plasma was incubated at 37 °C for 3 h with α-tocopherol (1 mM) dissolved in dimethyl sulfoxide (1% of the plasma volume) or with dimethyl sulfoxide alone (1% of the plasma volume).18 LDL was then isolated from the plasma.10 The α-tocopherol content of the LDL was measured by HPLC19 after extraction using methanol and hexane17 and drying the hexane using nitrogen. The extract was redissolved in ethanol and injected into the column described above. The mobile phase consisted of 99% methanol and 1% water (v/v), with a flow rate of 1 mL/min and detection at 298 nm.

**Statistical Analysis.** The mean and the standard error of the mean (SEM) of n independent experiments are given. Where appropriate, results were evaluated using a paired t test or one-way ANOVA, with a Dunnett’s post-hoc test.

### RESULTS

**Oxidation of LDL by Ferrous Iron at pH 4.5.** To compare the oxidation of LDL by iron at lysosomal pH with the oxidation at pH 7.4, LDL (50 μg of protein/mL) was oxidized in NaCl/sodium acetate buffer (pH 4.5) or NaCl/MOPS buffer (pH 7.4) at 37 °C in the presence of ferrous iron (FeSO₄, 5 μM). The oxidation was followed by measuring the formation of conjugated dienes, which absorb UV radiation at 234 nm. In agreement with previous results,6 LDL was oxidized effectively at pH 4.5 and much less so at pH 7.4 (Figure 1A). At pH 4.5, following a lag phase, conjugated diene formation proceeded rapidly and then more slowly. There was then a second rapid increase in attenuance (absorbance of UV plus UV scattering) with rapid minor fluctuations, consistent with UV scattering by aggregates, as the aggregates move in and out of the UV beam. This was followed by a decrease in attenuance, caused by the LDL aggregates, which were clearly visible by eye, sedimenting below the beam of UV radiation. Inverting the cuvette gently to
resuspend the aggregates increased the attenuation. Light scattering was also measured by continuously measuring the attenuation at 680 nm. Light scattering increased rapidly at ~200 min, corresponding with the second rapid phase at 234 nm, and decreased at ~500 min (Figure 1B). There was no light scattering in the absence of iron, showing that LDL aggregation and sedimentation were due to LDL oxidation (Figure 1B).

**LDL Aggregation at pH 4.5.** We tested if oxidized LDL would spontaneously aggregate at pH 4.5, regardless of whether iron was present. LDL that had previously been oxidized with CuSO₄ to give a species rich in lipid hydroperoxides (LOOH) or oxysterols followed by removal of the CuSO₄ was incubated at pH 4.5 or 7.4 and 37 °C. Both oxidized forms of LDL aggregated rapidly at pH 4.5, whereas native LDL did not (Figure 1C). Oxysterol-rich LDL aggregated even more rapidly than LOOH-rich LDL, indicating that the extent to which the LDL was oxidized affected its aggregation rate. In contrast, none of the lipoprotein species aggregated at pH 7.4 (Figure 1D).

**Oxidation of LDL by Ferrous or Ferric Iron.** It has been reported that phagolysosomes in macrophages contain ferrous iron and probably ferric iron. Iron will cycle between the two phases and that when LDL is oxidized sufficiently it is able to aggregate in the absence of iron, which is consistent with the results shown in Figure 1C.

Unlike DTPA, addition of desferrioxamine (100 μM) at the start of the incubation or during the rapid phase partially inhibited the oxidation but did not fully inhibit it (Figure 3B). Addition of desferrioxamine at the start of the slow phase of oxidation caused the rate of the reaction to decrease, but the LDL was able to aggregate and then sediment. Interestingly, adding desferrioxamine at the start of the aggregation phase actually caused a faster increase in attenuation (p < 0.05; n = 3 independent experiments). These data agree with the DTPA data in that the lag, slow, and rapid oxidation phases are dependent on iron. It appears that desferrioxamine is less able than DTPA to inhibit LDL oxidation and may actually promote LDL modification when LDL is sufficiently oxidized.

**Effect of α-Tocopherol Enrichment on the Oxidation of LDL by Iron.** α-Tocopherol can inhibit or sometimes promote LDL oxidation. We therefore investigated its effect on the oxidation of LDL by iron at pH 4.5. LDL was enriched with α-tocopherol via addition of α-tocopherol to plasma and isolation of the LDL. The α-tocopherol content was increased ~4.5 times from 6.5 ± 4.4 to 29.5 ± 9.1 nmol/mg of protein (p < 0.005; n = 6 independent experiments). As expected, α-tocopherol enrichment increased the length of the lag phase of oxidation of LDL by 5 μM CuSO₄ at pH 7.4 (Figure 4).
μ experiment with NaCl/sodium acetate buffer. LDL enriched with CuSO₄ (---). Oxidation was monitored by measuring the change in μ (pH 7.4) containing 5 and FeSO₄ were higher than those used for spectrophotometry ° the oxidation of the which had a lag period followed by a rapid phase of oxidation α-tocopherol-enriched LDL started immediately with no lag period (concentrations of LDL were measured by HPLC studies, because of sensitivity issues, and we therefore expected, cholesteryl arachidonate was oxidized somewhat reaction, which is initiated by the abstraction of a hydrogen atom by a free radical at a bisallylic methylene group. As hence, at the peak level of hydroperoxides, cholesteryl linoleate hydroperoxide accounted for most of the hydroperoxides. Other cholesteryl ester hydroperoxides and phospholipid, triacylglycerol, and protein hydroperoxides have not been measured here and may account for the remainder of the total hydroperoxides. Interestingly, in contrast to apparent CLOOH levels, total hydroperoxide levels did not decline much between 24 and 48 h.

The level of TBARS, which consist mainly of malondialdehyde, increased to 23.9 ± 2.9 nmol/ml of LDL protein at 24 h and subsequently decreased at 48 h (Figure 5G).

The electron negativity of the oxidized LDL was also assessed as a measure of the extent of LDL protein oxidation (Figure SH). The REM of the LDL, measured in agarose gels at pH 8.6, increased slightly as the duration of oxidation increased, reaching 1.3 ± 0.07 after 6 h. LDL oxidized for ≥12 h could not be loaded onto the gel effectively because the LDL was highly aggregated.

**DISCUSSION**

Lyosomal iron is a candidate for catalyzing the oxidation of LDL in vivo. Redox-active iron has been shown to be present in lysosomes, probably because of the degradation of ferritin, the turnover of iron-containing organelles or the phagocytosis of erythrocytes. Most of the labile iron in cells is present in lysosomes, and the concentration of chelatable iron in some endosomes/lysosomes is ~16 μM. The concentration of redox-active iron in lysosomes is therefore probably above 5 μM. We have used a concentration of FeSO₄ of 5 μM for the spectrophotometry studies and 50 μM for the HPLC studies (as the LDL concentration needed to be much higher for the HPLC studies, because of sensitivity issues, and we therefore needed to increase the FeSO₄ concentration, as well). These concentrations of FeSO₄ might be broadly consistent with the concentrations of redox-active iron in lysosomes (although the nature of the redox-active iron and its ligands in lysosomes are unclear).

In agreement with work conducted previously in this laboratory, LDL was oxidized effectively by 5 μM FeSO₄ at lysosomal pH (pH 4.5) but was not oxidized effectively at the pH of plasma or normal interstitial fluid (pH 7.4) (Figure 1A). In contrast, CuSO₄ oxidized LDL effectively at pH 7.4, but not so well at pH 4.5. Acidity increases the solubility of iron and should allow it to oxidize LDL more effectively. Extracellular fluids have a high antioxidant capacity, with the oxidation of LDL by transition metals being inhibited greatly by just a few percent of serum or interstitial fluid. Oxidation of LDL by iron within lysosomes might help to explain why LDL can be oxidized in atherosclerotic lesions in the presence of strong antioxidant protection in the interstitial fluid of the arterial intima.

The lipid composition of LDL oxidized by iron at lysosomal pH over time has been characterized (Figure 5). The levels of cholesteryl linoleate oxidation product, cholesteryl linoleate hydroperoxide (CLOOH), increased significantly for periods of oxidation of LDL...
≤24 h and then appeared to decrease again. The total level of hydroperoxides was measured using a triiodide assay and also increased up to 24 h. It then decreased only slowly over the next 24 h. During LDL oxidation, lipid hydroperoxides may decompose to form aldehydes, such as malondialdehyde and epoxy lipids. This decomposition probably accounted for the slow decrease in the total level of hydroperoxides after oxidation for 24 h. The levels of CLOOH may have declined faster after 24 h than the levels of total hydroperoxides because CLOOH might have become oxidized also on its cholesterol.
moiety, causing its HPLC retention time to change, although it may have retained the hydroperoxide group on its fatty acyl moiety and been detected in the triiodide assay.

When LDL is oxidized with copper at 37 °C and pH 7.4, lipid hydroperoxides are formed during the propagation phase of oxidation and then decompose fairly rapidly. In contrast, when LDL is oxidized by iron at 37 °C at acidic pH, the net hydroperoxide levels fall only slowly between 24 and 48 h (Figure S5), in agreement with our previous work. The lipid composition of LDL oxidized by iron at pH 4.5 and 37 °C for 12 h (Figure S) was similar to that of LDL oxidized by copper at pH 7.4 and 4 °C for 12 h, i.e., rich in hydroperoxides but low in oxysterols. The high levels of lipid hydroperoxides present in LDL oxidized by iron at lysosomal pH may confer atherogenic properties to the LDL, which differ from those of copper-oxidized LDL. Kritharides et al. showed that treatment of murine peritoneal macrophages with acetylated or aggregated LDL, subjected to mild oxidation, led to a lysosomal or prelysosomal accumulation of CLOOH and CLOH, which impaired the breakdown of cholesteryl esters. The accumulation of lipid hydroperoxides within the lysosome might therefore compromise lysosomal function. Furthermore, data presented here show that oxidized LDL aggregates spontaneously and rapidly at lysosomal pH (Figure 1C). This may weaken the ability of lysosomal enzymes to degrade it effectively. Atherosclerosis has previously been described as having features of a lysosomal storage disorder, whereby engorgement of lysosomes with lipids impairs their function, and our data lend further support to this concept.

A wide variety of aldehydes are formed from the decomposition of lipid hydroperoxides. A major aldehyde formed is malondialdehyde. The levels of TBARS (mainly malondialdehyde) increased after oxidation for ≤24 h by iron at pH 4.5 and then decreased (Figure S5). The decrease might be explained by the further reaction of malondialdehyde with amino groups of the lysyl residues of apolipoprotein B-100, which would abolish the positive charge of these residues.

The level of the cholesterol oxidation product 7-ketocolesterol remained very low for some time but then increased greatly (Figure SB). The level of 7-ketocholesterol was also increased in macrophages when they oxidized LDL in their lysosomes, and 7-ketocholesterol is present in much larger amounts in human atherosclerotic lesions than in normal arteries.

A slight increase in REM at pH 8.6 was detected after oxidation for ≤6 h (Figure SH). LDL oxidized for ≥12 h could not be loaded onto an agarose gel, because of its high level of aggregation, but it is possible that the net negative charge of LDL continued to increase. The isoelectric point of native LDL has been reported to be 5.1–5.8, so at pH 5.1–5.8, LDL will have no net charge. At pH 7.4 or 8.6 (the pH at which electrophoresis was conducted), LDL would have a net negative charge, but at the pH of lysosomes, ~4.5, it would have a net positive charge. The LDL particles would therefore repel each other; however, as oxidation proceeds and the net positive charge becomes smaller (Figure SH), the repulsion would weaken, and this may favor the aggregation of the LDL particles.

Oxidation of LDL by ferrous iron at lysosomal pH proceeds with lag, rapid oxidation, slow oxidation, aggregation, and sedimentation phases (Figure 1A), in agreement with our earlier findings. The pattern of oxidation with ferric iron was similar to that with ferrous iron; however, there was a decrease in the rates of the rapid and slow phases of oxidation and aggregation (Figure 2A). Kuzuya et al. found that ferrous iron oxidized LDL faster than did ferric iron in an unbuffered NaCl solution initially at pH 7.4. There is evidence that lysosomes contain cysteine and therefore have a reducing environment (this is controversial), and this would favor the ferrous oxidation state of iron. In fact, the phagolysosomes of macrophages are known to contain ferrous (and probably ferric) iron.

Iron may be able to initiate the oxidation of LDL by reacting with preexisting lipid hydroperoxides in the LDL particle.

\[
\begin{align*}
\text{Fe}^{2+} + \text{LOOH} &\rightarrow \text{Fe}^{3+} + \text{LO}^- + \text{OH}^- \quad \text{(fast)} \\
\text{Fe}^{3+} + \text{LOOH} &\rightarrow \text{Fe}^{2+} + \text{LOO}^- + \text{H}^+ \quad \text{(slow)}
\end{align*}
\]

Fe²⁺ is believed to react faster with lipid hydroperoxides (reaction 1) than does Fe³⁺ (reaction 2), as the rate constant for the analogous reaction of Fe²⁺ with H₂O₂ is 68 M⁻¹ s⁻¹ whereas that for Fe³⁺ with H₂O₂ is ~4 × 10⁻⁶ M⁻¹ s⁻¹. This may explain why the oxidation of LDL is slower with Fe³⁺. Interestingly, adding higher concentrations of Fe²⁺ shortened the lag phase and increased the rates of the rapid, slow, and aggregation phases (Figure 2B), but adding a higher concentration of Fe³⁺ had little effect (Figure 2C). This suggests that it is the concentration of Fe²⁺, rather than Fe³⁺, that determines the rate-limiting step in the oxidation of LDL. The results shown in panels D and E of Figure 2 are consistent with this suggestion. Adding extra Fe²⁺ at various times when the oxidation was already underway caused an immediate rapid burst of oxidation, whereas adding extra Fe³⁺ had little effect. These results suggest that the rapid phase of oxidation is mediated by the reaction of Fe²⁺ with LDL and the slow phase occurs when the levels of Fe³⁺ predominate.

DTPA is a metal ion chelator with a high affinity for iron and is able to bind both ferrous and ferric iron. Adding this chelator at the start of the oxidation or during the rapid oxidation phase caused great inhibition of LDL oxidation with iron at pH 4.5 (Figure 3A). When DTPA was added during the slower oxidation phase, the rate of change of attainment was decreased substantially, but the LDL was still able to aggregate, as indicated by the aggregation and sedimentation phases. These data suggest that the rapid and slower oxidation phases of LDL modification are iron-dependent and that LDL is able to aggregate only after it has been oxidized to a certain extent, corresponding to the slow oxidation phase. The aggregation phase can proceed in the absence of iron.

Adding another iron chelator, desferrioxamine, at the start of LDL oxidation or during the rapid or slow phases of oxidation caused a partial inhibition of LDL oxidation but was less effective than adding DTPA (Figure 3B). Desferrioxamine binds Fe²⁺ within seconds or less above pH 7.0 but requires many minutes at pH 4.5. Surprisingly, when desferrioxamine was added at the beginning of the aggregation phase of LDL modification, the rate of the modification was actually increased. Pro-oxidant effects of desferrioxamine have been reported. It might be argued that desferrioxamine was acting by pulling the equilibrium in eq 1 over to the right, as it binds Fe³⁺ with a higher affinity than Fe²⁺, and accelerating the breakdown of LOOH. Arguing against this is the fact that there is a rapid burst of LDL oxidation when Fe²⁺ is added to partially oxidized LDL in the absence of an added iron chelator (Figure 2D). The reason for the different behaviors of desferrioxamine and DTPA is unknown but may be related.
to their different stability constants for Fe$^{2+}$ and Fe$^{3+}$, their different standard reduction potentials, or the electron donating ability of desferrioxamine, which might reduce Fe$^{3+}$ to Fe$^{2+}$ and promote reaction 1.

Desferrioxamine injections inhibited atherosclerosis in cholesterol-fed rabbits. Desferrioxamine inhibits intralysosomal iron redox chemistry and inhibits the lysosomal oxidation of LDL in macrophages. Targeting iron chelators to lysosomes might be a potential strategy for slowing the lysosomal oxidation of LDL, if toxicity issues could be overcome.

$\alpha$-Tocopherol enrichment of LDL had complex effects on the oxidation of LDL by iron at pH 4.5, whereas its antioxidant effects on oxidation of LDL by copper at pH 7.4 were as expected (Figure 4). There was initially a pro-oxidant effect of $\alpha$-tocopherol enrichment on the oxidation of LDL by iron, followed by an antioxidant effect. The initial pro-oxidant effect might possibly be explained by tocopherol-mediated peroxidation, in which an $\alpha$-tocopheroxyl radical abstracts a hydrogen atom from a polyunsaturated fatty acyl group. There may later have been a net antioxidant effect of $\alpha$-tocopherol enrichment. Large clinical trials have shown that $\alpha$-tocopherol supplementation does not usually decrease the risk of cardiovascular disease.

The absence of an initial antioxidant effect of $\alpha$-tocopherol enrichment on oxidation of LDL by iron at lysosomal pH might possibly be relevant to this lack of protection. These data lend support to the finding that LDL can be oxidized within lysosomes. High levels of lipid hydroperoxides and 7-ketocholesterol can be formed in LDL by iron at lysosomal pH, and the LDL becomes highly aggregated and may therefore be difficult to degrade with lysosomal hydrolases. This suggests that lysosomal oxidation of LDL might lead to lysosomal lipid engorgement and lysosomal dysfunction in atherosclerosis.

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**Funding**

We are grateful to the British Heart Foundation for a Ph.D. studentship (grant number FS/05/040/18863) (L.S.).

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

We thank Ms. Helen A. Oakley and Ms. Jessica del Rio for skilfully isolating LDL and the volunteer blood donors from the University of Reading.

**ABBREVIATIONS**

CA, cholesteryl arachidonate; CL, cholesteryl linoleate; CLOOH, cholesteryl linoleate hydroperoxide; DTPA, diethylenetriaminepentaacetic acid; HPLC, high-performance liquid chromatography; LDL, low-density lipoprotein; MOPS, 3-(N-morpholino)propanesulfonic acid; REM, relative electrophoretic mobility; TBARS, thiobarbituric acid-reactive substances.

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