# The effects of pH on the oxidation of low-density lipoprotein by copper and metmyoglobin are different

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**Abstract The amplification of low-density lipoprotein (LDL) peroxidation in vitro by copper and myoglobin are well-studied biochemical approaches for investigating the oxidative modification of LDL and its role in the pathogenesis of atherosclerosis. Since the acidity of the environment is increased in inflammatory sites, the aim of this study was to investigate the effects of acidic pH on the oxidisability of LDL mediated by the haem protein myoglobin in comparison with that of copper-mediated LDL oxidation. The results show that acidic pH enhances myoglobin-mediated LDL oxidation as measured by conjugated dienes, lipid hydroperoxides and electrophoretic mobility, whilst a retardation is observed with copper as pro-oxidant; the mechanism probably relates to the effects of pH on the decomposition and formation of lipid hydroperoxides and the relative influences of copper ions and of myoglobin under these conditions.**



*Key words:* Atherosclerosis; Acidic pH; Copper; Hydroperoxide; LDL oxidation; Myoglobin

## 1. Introduction

Cells of the type present in the arterial wall and found in atherosclerotic lesions, activated macrophages [1–3], endothelial cells [3,4], smooth muscle cells [5] and lymphocytes [6], have been reported to oxidise LDL in vitro. All these cells secrete superoxide radicals, hydrogen peroxide and hydrolytic enzymes [reviewed in [7]] but, superoxide and the hydrogen peroxide generated there from are not very reactive per se towards polyunsaturated fatty acids. Their reactivity may, in principle, be amplified in the presence of: (i) available delocalised haem proteins generating ferryl haem states [8–11], (ii) transition metal ions generating hydroxyl radical [12–16], or (iii) nitric oxide, forming peroxynitrite [17]. Superoxide radical is also more reactive with polyunsaturated fatty acids at lower pH values through its protonation [18,19].

Injury to cells and tissues may enhance the toxicity of the active oxygen species by releasing intracellular transition metal ions into the surrounding tissue from storage sites, decompartmentalised haem proteins or metalloproteins, for example, by interaction with accessible proteases or oxidants. Such delocalised iron and haem proteins have the capacity to decompose hydroperoxides to peroxyl and alkoxyl radicals, exacerbating the initial lesion. Thus, oxidation of LDL mediated by agents such as transition metal ions or haem proteins is dependent on the presence of preformed lipid hydroperoxides. In the presence of preformed lipid hydroperoxides, propagation of peroxidation can be effected in the vicinity of haem-containing and iron-containing species, generating alkoxyl and peroxyl radicals, which can amplify the damage by initiating further rounds of lipid peroxidation [20,21].

Since the acidity of the environment is increased in inflammatory and ischaemic sites [reviewed in [22]], the aim of this study was to investigate the effect of acidic pH on the oxidisability of LDL mediated by the haem protein metmyoglobin and to compare the mechanism with that of copper-mediated LDL oxidation. The results show that acidic pH enhances myoglobin-mediated LDL oxidation, whilst a retardation of the early and intermediate stages of LDL oxidation is observed with copper as pro-oxidant.

## 2. Materials and methods

### 2.1. LDL preparation

LDL was isolated by a modified version of the method of Chung et al. [23]. Venous blood obtained from humans was taken into acid citrate-dextrose solution with added EDTA 100 μM at 4°C. The plasma was separated by centrifugation. The LDL was isolated from the plasma by a single-step discontinuous density-gradient ultracentrifugation. The plasma density was adjusted to 1.3 g/ml by addition of NaBr according to Radding's equation. Ultracentrifuge tubes containing 20 ml of 0.9% NaCl solution (density = 1.006 g/ml) were underlain with aliquots of plasma (density = 1.3g/ml) and centrifuged at 149 000 × *g* for 1 h 45 min at 16°C in a Beckman L70 ultracentrifuge with a Type 70Ti rotor. The LDL fraction was removed and 20 ml aliquots placed in ultracentrifuge tubes containing 6 ml of NaBr solution with 100 μM EDTA (density = 1.154 g/ml). The tubes were made to volume with NaBr solution (density = 1.063 g/ml) and further centrifuged for 14 h at 149 000 × *g* at 16°C in Beckman L70 ultracentrifuge. The LDL fraction was recovered and dialysed for 6 h against 10 mM PBS with 10 μM EDTA (pH 7.4) at 4°C. The LDL solution was sterilised by passing through a 0.2 μm filter (Millipore).

### 2.2. Protein determination

The protein concentration of the LDL was determined by Markwell's modified Lowry procedure [24] using crystalline bovine albumin as standard.

### 2.3. Preparation of metmyoglobin

Metmyoglobin was purified prior to use [25] as follows: 10 ml of a freshly prepared solution of potassium ferricyanide (740 μM) in PBS was added to 10 ml of a 400 μM solution of metmyoglobin (from horse heart, Sigma), the solution mixed, left to stand at room temperature for 20 min then applied to a Sephadex G15 column and eluted with PBS. The first fraction was collected, mixed well and its absorbance read at 490, 560, 580 and at 700 nm to correct for background absorbance. The concentration of metmyoglobin in the column eluate was calculated from the extinction coefficients at each wavelength using the formula $[MetMb] = 146\ A_{490} - 108\ A_{560} + 2.1\ A_{580}$ [26]. Aliquots of metmyoglobin in PBS were stored at 4°C.

### 2.4. Oxidation conditions

LDL (125 μg protein/ml) was incubated with either 5 μM metmyoglobin [27] or 1.25 μM copper sulphate [28] in 150 mM NaCl 10 mM phosphate buffer solution (PBS), at 37°C, at pH 5, 6 and 7.4. Buffers were prepared under the different pH conditions by varying the relative proportions of the sodium dihydrogen phosphate and disodium hydrogen phosphate and the pH was adjusted at the incubation tem-

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perature (37°C). After the requisite incubation period, the LDL oxidation was stopped by adding butylated hydroxytoluene (BHT) (10 μM final concentration) prior to the determinations. Assays were performed on the same day. The stock solution of BHT was prepared in methanol.

*2.5. Total cholesterol determination*

Total cholesterol determination was carried out using an enzymic assay (Boehringer) [29]. The colorimetric kit consists of cholesteryl esterase, which hydrolyses cholesteryl esters and allows cholesterol oxidase to oxidise free cholesterol, generating a chromophore which absorbs at 500 nm.

*2.6. Conjugated diene formation*

The progress of LDL oxidation was recorded spectrophotometrically by continuous monitoring of conjugated diene formation [28]. Spectra were then recorded from 200-300 nm at 10 min intervals for 5 h, at 37°C, using an IBM PC2-linked Beckman DU65 spectrophotometer fitted with Quant 1 software. The spectra given represent increased conjugated diene formation, at the particular time point, above that of the control LDL sample. Absorbance values at 234 nm were measured as a function of time as a marker of the rate of conjugated diene formation.

*2.7. Measurement of lipid hydroperoxides*

In order to quantify lipid hydroperoxide formation, the xylenol orange (FOX) assay was performed [30]. Nine hundred microlitres of FOX reagent (100 μM xylenol orange, 250 μM ammonium ferrous sulphate, 90% methanol, 4 mM butylated hydroxytoluene and 25 mM $H_2SO_4$) were added to 100 μl of sample comprised of 90 μl of LDL (125 μg protein/ml) and 10 μl methanol, and the sample incubated at room temperature for 30 min. After centrifugation at 13 000 rpm for 5 min, the absorbance was read at 560 nm against a blank of methanol and FOX reagent. Ninety microlitres of LDL (125 μg protein/ml) were incubated with 10 μl of triphenylphosphine (TPP, a reductant of hydroperoxides), at room temperature for 30 min. Nine hundred microlitres of FOX reagent were added and the sample incubated at room temperature for a further 30 min. The sample was then centrifuged for 5 min and the absorbance was read at 560 nm. The content of hydroperoxides was determined by subtracting the absorbance of the solution reacted with TPP from that of the incubate in the absence of TPP and expressed as μM. Hydrogen peroxide was used to construct a standard curve using a range of 0-20 μM.

*2.8. Assay of lipid peroxidation products*

The modified thiobarbituric acid assay of [31] was used as a method for measuring lipid peroxidation. Briefly, 500 μl of 10% (w/v) trichloroacetic acid and 500 μl of thiobarbituric acid (TBA) (0.75 g dissolved in 100 ml of 0.1% HCl) were added to each 500 μl aliquot of sample and heated at 95°C for 20 min. The absorbance was measured at 532 nm and that at 580 nm was subtracted to eliminate any potential interfering contribution from the haem protein [32]. Hydrolysed 1,1,3,3-tetramethoxypropane (TMP) was used as standard. 90 μl of TMP were incubated with 3 ml of HCl (0.1 M) for 20 min at room temperature. Afterwards a standard curve in the range of 0-10 μM was constructed. The results were expressed as nmol of malondialdehyde (MDA) equivalents per mg of LDL protein.

*2.9. Relative electrophoretic mobility (REM)*

The Beckman Paragon® Lipoprotein System was used to monitor the LDL-REM. Five microlitres aliquots were run in barbital buffer (pH 8.6) at 100 V for 45 min and the lipoprotein fraction was stained using Sudan black B stain. The distance moved towards the anode by each sample in relation to that of the control was taken as REM.

*2.10. Statistical method*

Statistical significance was determined by using the unpaired Student's $t$ test and accepted at the $P < 0.05$ level. Because of the variability in the LDL derived from different individuals, due to such factors as diet, life-style, smoking, stress, etc., the experiments were performed in triplicate on one preparation and the data were expressed as mean ± SD.

## 3. Results

When the extent of LDL oxidation by myoglobin (5 μM) was assessed by conjugated diene formation, a progressive increase in the oxidation and an enhancement in the rate of the propagation phase was observed on reduction of the pH from 7.4 to 5 (Fig. 1). In contrast, applying copper (1.25 μM) as pro-oxidant, there was a progressive augmentation in the lag phase and decrease in the rate of the propagation phase as pH fell.

Fig. 2 shows the comparison between the time courses of hydroperoxide formation at pH 7.4 and at pH 5 for myoglobin- and copper-induced LDL oxidation. The oxidation rate was enhanced progressively in the case of myoglobin as pro-oxidant, attaining 28 and 65 μM levels at pH 7.4 and pH 5.0 respectively. The rate in the copper oxidation system, however, was more rapid at pH 7.4 and by 5 h 70% of the peroxides had decomposed. Thus, although the level appeared to be enhanced by copper-induced oxidation, this was due to a delayed peak in the levels of lipid hydroperoxide at acidic pH and to less breakdown of the lipid hydroperoxide by 5 h.

Comparison of the levels of decomposition products of lipid hydroperoxides produced on interaction of $Cu^{2+}$ with LDL to those with myoglobin are shown in Table 1. Although at pH 7.4 myoglobin generated less decomposition products of peroxides than $Cu^{2+}$, with the increase in hydrogen ion concentration to pH 5 the levels produced as a consequence of the myoglobin–LDL interaction were increased, implying an enhancement in the rate of formation and decomposition of peroxides under these conditions.

Incubation of LDL with 1.25 μM copper at pH 7.4 for 5 h caused an extensive modification of apolipoprotein–B100 compared to the response to myoglobin. As the pH decreased, the oxidative modification of the apolipoprotein–B100 was less extensive in the case of copper, whereas the myoglobin–LDL interaction was apparently more effective in modifying LDL apolipoprotein–B100 under acidic conditions (Table 1).

The oxidation of LDL led to the loss of total cholesterol with a more extensive oxidation at pH 7.4 by $Cu^{2+}$ than by myoglobin. However, the lower pH seemed to protect LDL

Table 1
Effect of acidic pH on TBARS and apolipoprotein–B-100 oxidative modification

| | TBARS | | | REM | | |
|---|---|---|---|---|---|---|
| | (nmol MDA/mg LDL protein) | | | | | |
| | (pH 5) | (pH 6) | (pH 7.4) | (pH 5) | (pH 6) | (pH 7.4) |
| LDL | 2.38 ± 0.40 | 2.38 ± 0.80 | 2.38 ± 0.60 | 1 | 1 | 1 |
| Mb-LDL | 50.97 ± 2.58 | 36.68 ± 0.99 | 14.48 ± 1.59 | 2.91 ± 0.17 | 3.47 ± 0.66 | 2.03 ± 0.41 |
| Cu-LDL | 48.40 ± 2.58$^{NS}$ | 47.41 ± 2.19** | 47.61 ± 2.99* | 2.13 ± 0.12** | 2.67 ± 0.19$^{NS}$ | 3.45 ± 0.19** |

LDL (125 μg protein/ml) was incubated in the presence of either 5 μM metmyoglobin or 1.25 μM copper sulphate at pH 5, 6 and 7.4 for 5 h at 37°C.

*$P < 0.0001$; **$P < 0.001$, Cu-LDL vs. Mb-LDL; NS, nonsignificant; data are mean ± SD ($n = 3$).

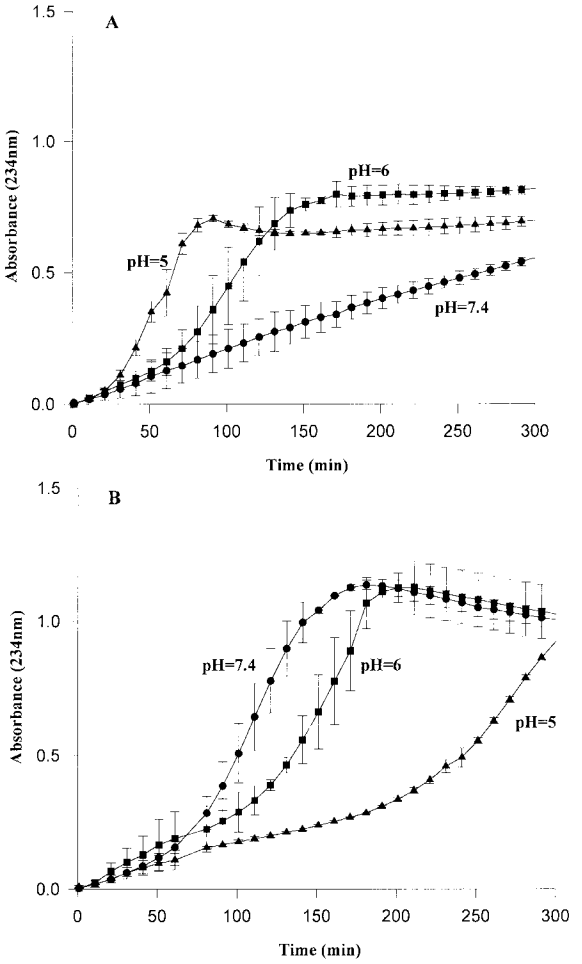


Fig. 1. The effect of pH on the LDL-conjugated diene formation. LDL (125 μg protein/ml) was incubated in the presence of either 5 μM metmyoglobin (A) or 1.25 μM copper (B) for 5 h at 37°C at pH 5 (▲), pH 6 (■) and pH 7.4 (•). The data represent the mean ± SD ($n = 3$).

total cholesterol when copper was used as a pro-oxidant, while the pro-oxidant activity of myoglobin on LDL cholesterol oxidation was exacerbated (Table 2).

## 4. Discussion

A number of redox active agents have been demonstrated to amplify LDL oxidation by catalysing the decomposition of pre-formed endogenous hydroperoxides, for example, copper ions [28], metmyoglobin [8–11] and haemoglobin [32] at pH 7.4. Recent studies have indicated that at acidic pH copper-mediated LDL oxidation is initially slower than at physiological pH [33]. It has been suggested that this may relate to the ability of copper to bind to the apolipoprotein–B-100 of LDL [34,35]. Nevertheless, although the initial oxidation by copper ions is slower at acidic pH, Morgan and Leake [33] noted a more rapid uptake by target macrophages at later time points of LDL modified under these conditions. In contrast, the rate of oxidation of LDL by iron ions alone, being slow under physiological conditions, is greater at acidic pH [33,36].

Caeruloplasmin (the plasma copper-containing protein) is also capable of catalysing LDL oxidation mediated by macrophages more rapidly after preincubation of the caeruloplasmin at acidic pH [37]. In addition, transferrin (the major iron-binding protein in plasma), co-incubated with LDL and cysteine at pH 5.5, but not pH 7.4, is capable of inducing the LDL oxidation, as detected by the formation of breakdown products of LDL peroxidation and altered surface charge on the apolipoprotein–B100 [38].

The results of this study show that copper ions at 1.25 μM are able to oxidise LDL (125 μg/ml) at pH 7.4 at a much high rate than the haem protein myoglobin (5 μM) under the same conditions, whereas acidic pH (5.0) enhances the myoglobin-mediated oxidation but retards that induced by copper. Studies of others have indicated that, at physiological pH, copper is more effective than iron in promoting LDL oxidation [15,39,40]. However, under acidic conditions iron and copper show opposing responses, iron becoming more effective in catalysing the oxidative modification of LDL, whereas copper becomes less effective (at least for the early stages of oxidation) [33,36].

The mechanism of action of myoglobin on LDL oxidation has been shown to be hydroperoxide-dependent [11], catalysing both the formation and decomposition of hydroperoxides. Interestingly, O'Brien [20] showed that haem proteins are

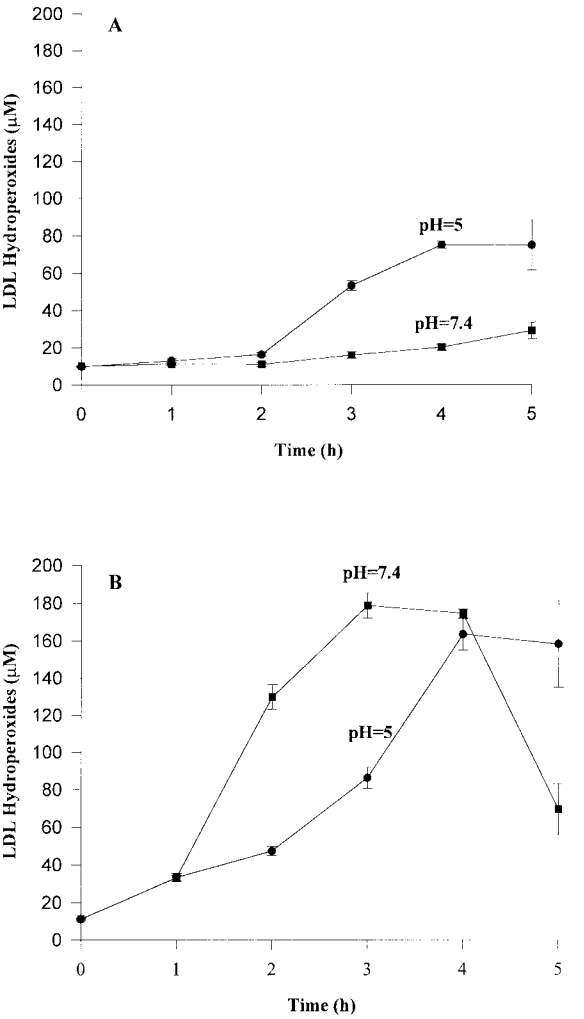


Fig. 2. The time course of LDL hydroperoxide formation at pH 5 (•) and 7.4 (■). LDL (125 μg protein/ml) was incubated in the presence of either 5 μM metmyoglobin (A) or 1.25 μM copper sulfates (B) at 37°C. The data represent the mean ± SD ($n = 3$).

Table 2
Effect of acidic pH on cholesterol oxidation

| | Loss of LDL total cholesterol (%) | |
|---|---|---|
| | (pH 5) | (pH 7.4) |
| Mb-LDL | 15.30 ± 3.18 | 8.45 ± 1.61 |
| Cu-LDL | 8.06 ± 2.84** | 12.24 ± 0.67* |

LDL (500 μg protein/ml) was incubated in the presence of either 20 μM metmyoglobin or 5 μM copper sulfate at pH 5 and 7.4 for 5 h at 37°C.
*$P < 0.01$; **$P < 0.05$, Cu-LDL vs. Mb-LDL; data are mean ± SD ($n = 3$) (native LDL total cholesterol = 1.79 ± 0.16 mg/mg LDL protein; $n = 6$).

more effective than inorganic metal ions, such as iron and copper at decomposing linoleic acid hydroperoxides under acidic conditions and proposed that the increased activity of the haem proteins at acidic pH may have been due to the increased exposure of the haem moiety. Oxidation induced by copper is influenced not only by the levels of pre-existing hydroperoxides but also on the potential interactions between copper and specific amino acid residues on the apolipoprotein–B100, which might be expected to show some pH dependency (especially histidine, for example) although this has not been well-defined. However, the oxidation by copper is clearly retarded at pH 5.0 compared with pH 7.4, as shown by the time-dependency of conjugated diene and hydroperoxide formation. The observation of different levels of lipid hydroperoxides at 5 h incubation but not of TBARS may be due to differences in the time courses for oxidation in which TBARS reach a maximum and then remain stable or only slowly decrease, whereas the lipid hydroperoxides reach a peak and gradually decline towards zero [33]. With metmyoglobin, however, the formation of conjugated dienes, lipid hydroperoxides. and TBARS are all increased at acidic pH.

Less is known about the oxidation of LDL cholesterol under acidic conditions. There is a tendency for the LDL total cholesterol to become more oxidised at acidic pH in the presence of myoglobin, compared to the situation with copper, where the loss of total cholesterol is less extensive at low pH consistent with the other markers of oxidation. However, this may be an underestimate of the extent of cholesterol oxidation since it has been suggested that the cholesterol oxidase assay also measures oxysterols generated during LDL oxidation [41]. Interestingly, Hazen et al. [42] reported that, at acidic pH, the LDL cholesterol seems to be a preferential target for myeloperoxidase, the phagocyte-derived haem protein recently identified in human atheroma.

The enhanced pro-oxidant ability of myoglobin at acidic pH might derive from the intrinsic property of haem proteins to catalyse peroxide formation and decomposition, since, for example, haemoglobin and cytochrome *c* [43] exhibit an enhanced pro-oxidant activity towards arachidonic acid under acidic conditions, and myoglobin towards microsomal membranes [44]. Caeruloplasmin under similar conditions does not display differential effects over the same range of pH values (Salah and Rice-Evans, unpublished observations). However, others have shown that pre-incubation of caeruloplasmin at acidic pH values prior to incubation with LDL and macrophages leads to enhanced uptake of the modified LDL by target macrophages [37], suggesting that the pretreatment modifies the caeruloplasmin in some way.

In vivo, acidosis within atherosclerotic lesions might have consequences for specific potential mechanisms of oxidation such as those mediated by nitric oxide [44] or the protonation of the superoxide anion [19,46] or the enhanced pro-oxidant action of haem proteins or iron in promoting peroxide decomposition. Other mechanisms might be retarded, for example, the early stages of copper-dependent LDL oxidation.

The findings here demonstrate the amplification of LDL oxidation by haem proteins in more acidic environments and the retardation of copper-mediated formation of LDL hydroperoxides. The implications for the catalysis of further oxidation of LDL containing minimal levels of pre-formed hydroperoxides (initially formed by lipoxygenase action, dietary intake of lipid peroxides or peroxynitrite-mediated oxidation) suggest that delocalised haem proteins, if present, may oxidise LDL more rapidly in regions with a slightly acidic pH, such as chronic inflammatory sites, or delocalised iron released from haem proteins in the presence of excess peroxide [10].

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## References

[1] S. Parthasarathy, D.J. Printz, D. Boyd, L. Joy, D. Steinberg, Arteriosclerosis 6 (1986) 505–510.
[2] D.S. Leake, S.M. Rankin, Biochem. J. 270 (1990) 741–748.
[3] J. Morgan, J.A. Smith, G.M. Wilkins, D.S. Leake, Atherosclerosis 102 (1993) 209–216.
[4] T. Henriksen, E.M. Mahoney, D. Steinberg, Proc. Natl. Acad. Sci. USA 78 (1981) 6499–6503.
[5] T. Henriksen, E.M. Mahoney, D. Steinberg, Arteriosclerosis 3 (1983) 149–159.
[6] D.J. Lamb, G.M. Wilkins, D.S. Leake, Atherosclerosis 92 (1992) 187–192.
[7] C. Rice-Evans, K.R. Bruckdorfer, Mol. Aspects Med. 13 (1992) 1–111.
[8] G. Dee, C. Rice-Evans, S. Obeyesekera, S. Meraji, M. Jacobs, K. Bruckdorfer, FEBS Lett. 294 (1991) 38–42.
[9] E.S. Green, H. Evans, P. Rice-Evans, M.J. Davies, N. Salah, C. Rice-Evans, Biochem. Pharmacol. 45 (1993) 357–366.
[10] C. Rice-Evans, E. Green, G. Paganga, C. Cooper, J. Wrigglesworth, FEBS Lett. 326 (1993) 177–182.
[11] N. Hogg, C. Rice-Evans, V. Darley-Usmar, M.T. Wilson, G. Paganga, L. Bourne, Arch. Biochem. Biophys. 314 (1994) 39–44.
[12] J.M.C. Gutteridge, D.A. Rowley, B. Halliwell, Biochem. J. 206 (1982) 605–609.
[13] W.A. Pryor, Ann. NY Acad. Sci. 393 (1982) 1–22.
[14] B. Halliwell, J.M.C. Gutteridge, Biochem. J. 219 (1984) 1–14.
[15] B. Halliwell, J.M.C. Gutteridge, Methods Enzymol. 186 (1990) 1–14.
[16] U.P. Steinbrecher, H. Zhang, M. Lougheed, Free Radical Biol. Med. 9 (1990) 155–168.
[17] V.M. Darley-Usmar, N. Hogg, V.J. O'Leary, M.T. Wilson, S. Moncada, Free Rad. Res. Commun. 17 (1992) 9–20.
[18] M.S. Baker, J.M. Gebicki, Arch. Biochem. Biophys. 234 (1984) 258–264.
[19] S. Bedwell, R.T. Dean, W. Jessup, Biochem. J. 262 (1989) 707–712.
[20] P.J. O'Brien, Can. J. Biochem. 47 (1969) 485–492.
[21] R. Labeque, L.J. Marnett, Biochemistry 27 (1988) 7060–7070.
[22] Leake, D.S. (1997) Atherosclerosis (in press).
[23] B.H. Chung, T. Wilkinson, J. Geer, J.P. Segrest, J. Lipid Res. 21 (1980) 284–291.
[24] M.A. Markwell, S.M. Haas, L.L. Bieber, N.E. Tobert, Anal. Biochem. 87 (1978) 206–210.
[25] N.J. Miller, C. Rice-Evans, M.J. Davies, V. Gopinathan, A. Milner, Clin. Sci. 84 (1993) 407–412.
[26] K.D. Whitburn, J.J. Shieh, R.M. Sellers, M.Z. Hoffman, J. Biol. Chem. 257 (1982) 1860–1869.

[27] C.A. Rice-Evans, D.S. Leake, K.R. Bruckdorfer, A.T. Diplock, Free Rad. Res. 25 (1996) 285–311.
[28] H. Esterbauer, J. Gebicki, H. Puhl, G. Jürgens, Free Rad. Biol. Med. 13 (1992) 341–390.
[29] J. Siedel, E.O. Hagele, J. Ziegenhorn, A.W. Wahlefeld, Clin. Chem. 29 (1983) 1075–1080.
[30] J. Nourooz-Zadeh, J. Tajaddini-Samardi, S.P. Wolff, Anal. Biochem. 220 (1994) 403–409.
[31] R. Walls, K.S. Kumar, P. Hochstein, Arch. Biochem. Biophys. 174 (1975) 463–468.
[32] G. Paganga, C. Rice-Evans, R. Rule, D. Leake, FEBS Lett. 303 (1992) 154–158.
[33] J. Morgan, D.S. Leake, J. Lipid Res. 36 (1995) 2504–2512.
[34] B. Kalyanamaran, W.E. Artholine, S. Parthasarathy, Biochim. Biophys. Acta. 1035 (1990) 286–292.
[35] N. Kalant, S. McCormick, K. Parmak, Arterioscler. Thromb. 11 (1991) 1322–1329.
[36] J. Morgan, D.S. Leake, FEBS Lett. 333 (1993) 275–279.
[37] D.J. Lamb, D.S. Leake, FEBS Lett. 338 (1994) 122–126.
[38] D.J. Lamb, D.S. Leake, FEBS Lett. 352 (1994) 15–18.
[39] H. Esterbauer, G. Jürgens, O. Quehenberger, Basic Life. Sci. 49 (1988) 369–373.
[40] J.P. Reyftmann, R. Santus, J.C. Maziere, P. Morliere, S. Salmon, C. Candide, C. Maziere, J. Haigle, Biochim. Biophys. Acta. 1042 (1990) 159–167.
[41] I. Jialal, D.A. Freeman, S.M. Grundy, Arterioscler. Thromb. 11 (1991) 482–488.
[42] S.L. Hazen, F.F. Hsu, K. Duffin, J.W. Heinecke, J. Biol. Chem. 271 (1996) 23080–23088.
[43] J. Fantone, S. Jester, T. Loomis, J. Biol. Chem. 264 (1989) 9408–9411.
[44] J. Kanner, S. Harel, Arch. Biochem. Biophys. 237 (1985) 314–321.
[45] G.J. Chang, P. Woo, H.M. Honda, L.J. Ignarro, L. Young, J.A. Berliner, L.L. Demer, Arterioscler. Thromb. 14 (1994) 1808–1814.
[46] D. Bonnefont-Rousselot, M. Gardes-Albert, S. Lepage, J. Delattre, C. Ferradini, J. Chem. Phys. 90 (1993) 917–930.