Abstract
Lipoprotein oxidation is a key early stage in the development of atherosclerosis. Oxidation of low-density lipoprotein (LDL) is initiated by both enzyme-mediated and non-enzymic mechanisms in vivo, and oxidized LDL has many atherogenic properties. Oxidation of LDL in vivo is likely to be influenced by local environmental factors, such as pH. The composition of LDL is also important, including such factors as antioxidant content, fatty acid composition and particle size.

Introduction
Elevated serum cholesterol, particularly in the form of apolipoprotein B (apo B)-containing lipoproteins, is an important aetiological factor in the pathogenesis of atherosclerosis. However, at any level of serum cholesterol, there is a wide variation in the incidence of coronary heart disease. It is therefore evident that there are other factors impacting on the lipoprotein–arterial-wall interaction, which exacerbate or retard the atherogenic process. Among these factors, it is believed that modification of low-density lipoprotein (LDL) in the arterial wall, particularly by oxidation, is crucial to the cellular uptake of LDL in the first stages of atherosclerotic plaque development [1].

A key early step in atherogenesis is the formation of the fatty streak, consisting of a subendothelial collection of foam cells, which are cholesterol-laden macrophages or smooth muscle cells. Under normal circumstances, uptake of LDL cholesterol via the native LDL receptor is down-regulated with increasing intracellular cholesterol content, and internalization of cholesterol by this route does not result in foam cell formation. The existence of an alternative pathway for cellular uptake of LDL is supported by the fact that macrophages completely lacking LDL receptors can still become foam cells [2]. The existence of scavenger receptor A was first demonstrated when it was shown that LDL modified in vitro by acetylation was taken up avidly when incubated with macrophages [2]. Uptake was not down-regulated by increasing intracellular cholesterol concentrations, so that the macrophages became lipid-laden, resembling foam cells. However, significant acetylation of LDL does not occur in vivo, and it is now clear that there are other more physiologically relevant modifications of LDL that can result in its cellular uptake via one of several types of scavenger receptor [2]. Incubation of LDL with endothelial cells or smooth muscle cells results in such a modification of LDL, and this modification was subsequently shown to be oxidative in nature. Although there are other candidate modifications that can enhance LDL uptake by macrophages in vitro, including glycation, self-aggregation, immune-complex formation, complex-formation with proteoglycans and hydrolysis, most interest to date has focused on the oxidation of LDL.

Atherogenic properties of oxidized LDL (oxLDL)
OxLDL has many characteristics that potentially promote atherogenesis, in addition to the ability to be taken up rapidly by macrophages to form foam cells. It is a chemoattractant for circulating monocytes [3], both directly and also via stimulation of the release of monocyte chemoattractant protein-1 from endothelial cells [4]. The chemoattractant activity of LDL resides in its lipid moiety, and is attributable to lysophosphatidylcholine generation during the conversion of LDL into its oxidized form. OxLDL promotes the differentiation of monocytes into tissue macrophages by enhancing the release of macrophage colony-stimulating factor from endothelial cells [5], and inhibits the motility of resident macrophages [3]. It is a chemoattractant for T cells [6], although not for B cells, and consequently the atherosclerotic plaque contains primarily monocytes and T cells. Unlike native LDL, oxLDL is immunogenic [7], and it is also cytotoxic to various cell types, including endothelial cells [8], resulting in loss of endothelial integrity. It inhibits tumour necrosis...
factor expression [9], stimulates release of interleukin-1β [10] from monocyte/macrophages, and can inhibit endothelial cell-dependent arterial relaxation [11]. OxLDL also activates matrix-digesting enzymes, which may play a role in plaque instability [12]. However, some cellular responses to peroxidation products appear to be protective, and it is possible that peroxidation is an essential intermediary in an effective response to an oxidative insult [13].

Evidence supporting the occurrence of LDL oxidation in vivo

The occurrence of LDL oxidation in vivo is supported by several strands of evidence. (1) Oxidized apo B-100 epitopes and increased levels of lipid peroxidation products can be detected in LDL gently extracted from both rabbit and human atherosclerotic lesions [14]. (2) Immunohistochemical staining of atherosclerotic lesions with specific monoclonal antibodies has demonstrated the presence of oxLDL [7]. (3) Circulating anti-oxLDL antibodies have been demonstrated in serum, and titres correlate with the progression of atherosclerotic lesions [7]. (4) Several studies in different animal models of atherosclerosis strongly suggest that progression of the lesions can be delayed by intervention with antioxidants (for a review, see [1]). The fact that several different antioxidants have been used (probucol, vitamin E, butylated hydroxytoluene and diphenylphenylene-diamine) supports the implication that the anti-atherogenic effect is due to the antioxidant properties of these drugs, rather than any other biological effect. (5) Epidemiological evidence, including ecological, case-control and prospective studies, indicates that low antioxidant consumption is associated with an increased risk of cardiovascular disease [15,16]. The evidence is strongest in the case of vitamin E, with less consistent support for the protective roles of vitamin C, carotenoids and flavonoids. (6) Several trials of antioxidant supplementation in patients at risk of vascular events have demonstrated a reduction in cardiovascular end points [17–21], while other studies have been negative [22–25]. The discrepancies in these trials may reflect differences in the dose and mix of antioxidants used as supplements, the different dietary backgrounds of the subjects, and intervention at a relatively advanced stage of disease. It seems clear, however, that β-carotene supplementation is ineffective in preventing vascular disease, while conclusive evidence one way or the other is lacking for vitamin C and vitamin E.

Mechanisms of LDL oxidation in vivo

Many cell types are capable of oxidizing LDL, including monocytes, macrophages, neutrophils, endothelial cells, smooth muscle cells and fibroblasts. However, cell types that are involved in the atherosclerotic lesion in which oxLDL is found, i.e. macrophages, endothelial cells and smooth muscle cells, would seem to be the most likely to contribute to LDL oxidation in vivo. It appears likely that LDL is oxidized in microdomains in the arterial wall, sequestered by proteoglycans and other extracellular matrix constituents, where it is protected from plasma antioxidants [26]. It is still unclear which oxidative mechanisms or radical species are involved; potential candidates to date include NADPH oxidase, myeloperoxidase, cytochrome P450, the mitochondrial electron transport chain, peroxynitrite, xanthine oxidase, caeruloplasmin and lipoxygenase. The last enzyme has received much attention with the discovery that not only does lipoxygenase modify LDL in vitro to a form taken up by the scavenger receptor [27], but disruption of the lipoxygenase gene diminishes atherosclerosis in transgenic mice [28]. Detailed chemical studies of plaque composition using reaction products specific for different modes of oxidation suggest that different mechanisms of LDL oxidation may predominate at different stages of plaque development [29]. In particular, transition-metal-mediated oxidation seems only to occur in advanced lesions, while oxidation mediated by myeloperoxidase or reactive nitrogen species occurs throughout plaque development.

Once initiated, oxidation of LDL is a free-radical-driven lipid peroxidation chain reaction. Lipid peroxidation is initiated by free radical attack on a double bond associated with a poly-unsaturated fatty acid (PUFA). This results in the removal of a hydrogen atom from a methylene (CH₂) group, the rate of which determines the rate of initiation, a key step. Molecular rearrangement of the resulting unstable carbon radical results in a more stable configuration, a conjugated diene. The conjugated diene reacts very quickly with molecular oxygen, and the peroxyl radical thus formed is a crucial intermediate [30] (Scheme 1). A PUFA peroxyl radical in LDL may abstract a hydrogen atom from an adjacent PUFA to form a hydroperoxide and another lipid radical, a reaction which results in chain propagation. Removal of hydrogen atoms by the peroxyl radical from other
lipids, including cholesterol, eventually yields oxysterols. Lipid hydroperoxides fragment to shorter-chain aldehydes, including malondialdehyde and 4-hydroxynonenal. These reactive aldehydes in turn may bind to ε-amino groups of apo B-100, giving the protein an increased net negative charge. The classical LDL receptor recognizes a specific domain of positive charges from lysine, arginine and histidine residues on apo B. Alteration of this domain results in failure of binding by the apo B/E receptor, and an increase in negative surface charge on apo B-100 results in increased recognition by the scavenger receptor.

In the presence of a lipid phase chain-breaking antioxidant such as α-tocopherol, the peroxyl radical may be scavenged. The tocopheroxyl radical thus formed has very low reactivity and will generally result in chain termination. LDL exposed to oxidative stress in vitro will not form significant amounts of hydroperoxides until it becomes depleted of chain-breaking antioxidants. In vitro studies of LDL oxidation have verified the existence of a 'lag phase', during which significant oxidation of LDL cannot be detected, prior to the onset of the 'propagation phase', presumably after the endogenous antioxidants have been consumed (Figure 1). There follows a steady increase in the detectable by-products of oxidation, until the substrate, i.e. the PUFAs, has been depleted, and a plateau phase is reached. Assessment of resistance of LDL to oxidation has generally in-

**Figure 1**

Typical absorption profile at 234 nm (conjugated diene formation) produced during oxidation of isolated LDL by copper *in vivo*.

**Scheme I**

Basic reaction sequence of lipid peroxidation

- Fatty acid with three double bonds
- Hydrogen abstraction by Hydroxyl radical
- Unstable carbon radical
- Molecular Rearrangement
- Conjugated diene
- Oxygen uptake
- Peroxyl radical
- Hydrogen abstraction $\Rightarrow$ Chain reaction
- Lipid hydroperoxide
  - malondialdehyde
  - 4-hydroxynonenal
  - ethane/pentane
  - etc.
involved measurement of the duration of the lag phase, although other variables, such as the rate of propagation, are also noted.

**Factors influencing susceptibility of LDL to oxidation**

The susceptibility of LDL to oxidation *in vitro* is influenced by both LDL composition (intrinsic factors) and the microenvironment in which the LDL is found (extrinsic factors). Among the intrinsic factors, the fatty acid composition of LDL is of prime importance; a high proportion of PUFAs confers greater susceptibility to oxidation, while a high proportion of monounsaturated fatty acids protects against oxidation [31–33]. Since the propagation phase of LDL oxidation begins after the endogenous antioxidants have been consumed, susceptibility to oxidation is also highly dependent on the antioxidant content, which in lipoproteins is mainly α-tocopherol, although ubiquinol-10 and carotenoids are also important [34]. The molar ratio of PUFA to total antioxidants in LDL is approx. 150:1, and just as there is considerable between-subject variation in fatty acid content depending on diet, so lipophilic antioxidant intake also varies significantly from individual to individual [35]. Dietary supplementation with vitamin E results in increased LDL resistance to copper-induced oxidation *in vitro* [36–38], an effect that is dose-dependent [39]. LDL size is another factor that has been shown to affect oxidative susceptibility [40]. The small, dense subfractions of LDL are more susceptible to oxidation than large, less dense LDL particles, which may to a large extent be due to differences in antioxidant content [41]. A preponderance of small dense LDL particles is found in subjects with moderately elevated triacylglycerols (1.5 mmol/l), and is due to the metabolism of relatively large lipid-rich very-low-density lipoprotein particles. The concentration of pre-existing fatty acid peroxides in LDL also exerts an effect on the oxidative susceptibility of the particle, with higher levels of these ‘seeding’ peroxides being associated with a shorter lag time when transition metals are used to initiate oxidation [42].

The *in vitro* assessment of the susceptibility of LDL to oxidation takes into account only those factors intrinsic to LDL, as discussed above. It is important to bear in mind that LDL susceptibility to oxidation *in vivo* is likely to be heavily influenced by the particle’s immediate microenvironment, including local antioxidant concentrations, transition metal availability, pH and the presence of specific enzyme systems, among other factors. Understanding of the importance of these factors is limited, but in the case of pH it is likely that, within atherosclerotic lesions, the extracellular environment is acidic. Atherosclerotic lesions resemble sites of chronic inflammation, and cellular elements are relatively distant from capillaries [43]. Furthermore, both activated inflammatory cells and the process of LDL oxidation itself may generate protons. An acidic environment will promote, in particular, LDL oxidation by myeloperoxidase and caeruloplasmin [44], and may also affect the interactions of LDL with arterial wall components [45].

**Conclusions**

Oxidation of LDL is an important process in atherogenesis. Understanding of the mechanisms by which LDL oxidation occurs and its consequences *in vivo* have improved greatly in recent years. However, the relative importance of enzyme-mediated and non-enzyme-mediated LDL oxidation remains unclear, as does the significance of tocopherol-mediated oxidation, arterial wall pH and interactions of LDL with arterial wall components. While the harmful effects of LDL oxidation are well recognized, increased appreciation of the protective responses which oxidation initiates may help in the development of new strategies to prevent atherosclerosis.

**References**


