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Review

# Formation of methionine sulfoxide-containing specific forms of oxidized high-density lipoproteins

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**Abstract**

Atherosclerosis is characterized by the accumulation of both lipoprotein-derived lipids and inflammatory cells in the affected vascular wall that results in a state of heightened oxidative stress and that is reflected by the accumulation of oxidized lipoproteins. Circulating oxidized low-density lipoprotein (oxLDL) is used as a surrogate marker for coronary artery disease, although the 'escape' of oxLDL from the vessel wall is hindered by the large size of this lipoprotein and its specific retention by the extracellular matrix. Also, the oxidation of lipoproteins in human atherosclerotic lesions is not limited to LDL. In fact, the lipids of all classes of lipoproteins are oxidized to a comparable extent. Examining the fate of lipid hydroperoxides, the primary lipid peroxidation products, in high-density lipoproteins (HDL) undergoing oxidation, revealed that they become reduced to the corresponding alcohols by specific Met residues of apolipoprotein A-I (apoA-I) and apoA-II. As a consequence, Met residues in apoA-I and apoA-II become selectively and consecutively oxidized to their respective Met sulfoxide (MetO) forms that can be separated by HPLC. This review describes the characterization of specifically oxidized HDL with an emphasis on MetO formation, the structural and functional consequences of such oxidation, and the potential utility of specifically oxidized HDL as a surrogate marker of atherosclerosis.


*Keywords:* Lipoprotein; Oxidation; Atherosclerosis; Surrogate marker; Protein oxidation

## 1. Introduction—relevance of early stage oxidized HDL in atherosclerosis

Oxidation of low-density lipoprotein (LDL) is generally thought to be critical to atherogenesis [1]. During the initial stage of LDL oxidation ubiquinol-10 disappears [2] and this is followed by the loss of α-tocopherol (α-TOH) [2,3] and the concomitant accumulation of lipid hydroperoxides (LOOH) [2,4] that in turn may then lead to oxidation of apolipoprotein B-100 (apoB-100) [5]. Oxidized LDL (oxLDL) is present in human atherosclerotic lesions [6], and its detection in the circulation by immunological assays is used as a surrogate marker of atherosclerotic disease [7,8]. However, the use of measurement of oxLDL as a diagnostic for cardiovascular disease is limited due to several reasons. First, the term oxLDL refers to a mixture of modified lipoproteins that remain chemically uncharacterized [9]. Second, there are caveats concerning the accuracy of these measurements, including the extent of LDL oxidation, reproducibility of reference oxLDL, and a better characterization of the epitope recognized by the antibody used [10]. Third, the 'escape' of oxLDL from the vessel wall is hindered by the large size of this lipoprotein and its specific retention by extracellular matrix [11,12].

In contrast to LDL, plasma levels of high-density lipoprotein (HDL)-cholesterol and apolipoprotein A-I (apoA-I) inversely correlate with the risk of developing coronary artery disease [13]. This antiatherosclerotic activity is thought to be due to the ability of HDL to

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remove cholesterol from extrahepatic tissues and transport it to the liver for excretion, a process termed reverse cholesterol transport [14,15]. In addition, HDL has anti-inflammatory and other protective activities (for a recent review, see, e.g., Ref. [16]), and it has been proposed to aid the removal and detoxification of pro-atherogenic oxidized lipids [17–19]. Thus, cholesterylester transfer protein transfers oxidized lipids from LDL to HDL [19], and HDL carries the majority of hydroperoxides of cholesterylesters (CE-OOH, the first and major products formed during lipoprotein oxidation) in human plasma [17]. In addition, CE-OOH and their corresponding hydroxides (CE-OH) are removed rapidly from HDL but not LDL via selective uptake by liver parenchymal cells in vitro [18] and by perfused liver in situ [20]. This selective uptake is associated with cellular detoxification of CE-OOH [18] and is more rapid than that of the corresponding non-oxidized cholesterylesters [18,20]. Furthermore, CE-OH are also removed rapidly from HDL via hepatic clearance in vivo, and this is associated with biliary secretion of the oxidized lipid-derived cholesterol [21], indicating that oxidation of the fatty acid moiety of cholesterylesters may aid the elimination of cholesterol from the body.

There is evidence suggesting that HDL is at least as susceptible to oxidative modification as LDL, since lipids in HDL become oxidized before those in LDL when plasma is exposed to peroxyl radicals [17]. Also, lipids in HDL and LDL isolated from human atherosclerotic lesions are oxidized to a comparable extent [22] that increases with increasing severity of disease [23]. Importantly, these lesion lipoproteins appear to retain normal concentrations of α-TOH [22–25], and most of their major lipoprotein lipid oxidation products accumulate in the presence of the vitamin [26]. These findings indicate that for a majority of lipoproteins present in human atherosclerotic lesions, oxidation remains within the early stage and does not proceed beyond consumption of significant amounts of vitamin E, raising the possibility that mildly oxidized HDL could be present in plasma of subjects with atherosclerotic disease.

Several considerations favor the potential use of oxidized HDL or their component(s) over oxidized LDL as a potential marker of atherosclerotic disease. First, being substantially smaller and interacting less strongly with extracellular proteoglycans, vascular wall HDL is expected to re-enter the circulation more readily than LDL [27]. Second, compared to apoB-100, HDL's apolipoproteins dissociate readily from the lipoprotein particle (reviewed in Ref. [28]), increasing the likelihood of their existence in circulation. Third, given their physical properties and smaller molecular size, oxidized forms of apoA-I and apoA-II are simpler to work with and to chemically characterize than apoB-100. Mechanistic studies on the oxidation of HDL lipids and its relationship to limited apolipoprotein oxidation and functional consequences are therefore of potential physiological significance.

## 2. Characterization of native and oxidized forms of apoA-I and apoA-II in HDL

Human apoA-I is a polypeptide consisting of highly homologous 11- and 22-residue amphipathic α-helices. Three of the 243 amino acid residues of apoA-I are Met residues, present at positions 86, 112, and 148. Apolipoprotein A-II is a dimer consisting of two identical peptide monomers connected by a disulfide bound and one Met residue per monomer at position 26 from the N-terminus. Apolipoprotein A-I comprises about 70% of total HDL protein, with apoA-II accounting for ~20–25% and several minor proteins comprising the remainder [28]. Anantharamaiah et al. [29] were the first to report the presence of oxidized Met residues in human apoA-I and apoA-II. These authors separated apolipoprotein isoforms from HDL isolated from plasma, and purified apoA-I by reversed phase HPLC [30]. Characterization by amino acid analysis, N-terminal analysis, SDS-PAGE, and CNBr digestion (that specifically cleaves peptides at Met residues unless Met is oxidized) revealed one and two oxidation products of apoA-I and apoA-II, respectively, containing oxidized Met residues. More direct evidence for the presence of Met sulfoxide (MetO) was obtained using reversed phase HPLC of proteolytic peptides of apoA-I combined with mass spectrometry [31]. In contrast to these studies, and using a similar HPLC method, Sattler et al. [32] did not detect oxidized forms of apoA-I and apoA-II in fresh HDL isolated rapidly from healthy human plasma [33] (see below and Fig. 1A), a finding since confirmed [34–37].

During atherogenesis, different oxidants contribute to different extents and at different stages to the modification of lipoproteins [38]. For this reason, different in vitro studies on HDL oxidation have utilized different oxidants that modify apolipoproteins in different ways and to varying extents. For example, when hypochlorite, a strong oxidant produced by the phagocyte heme enzyme myeloperoxidase (MPO), is used at large molar excess over HDL particles, gross changes occur with oxidation of amino acids in addition to Met residues [39]. In contrast, when hypochlorite is used at a comparatively lower molar oxidant-to-lipoprotein ratio (up to 6:1), oxidation is limited to MetO formation [40].

Recent reports document that HDL isolated from plasma of patients with established coronary artery disease contains increased levels of 3-chlorotyrosine [41], a specific and stable end product of the reaction of hypochlorite and tyrosine residues of proteins, and of 3-nitrotyrosine [42,43], a product that may be generated through MPO-dependent generation of reactive nitrogen species [44]. These findings indicate that it is possible to