# Oxidized LDL and HDL: antagonists in atherothrombosis

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ABSTRACT **Increased LDL oxidation is associated with coronary artery disease. The predictive value of circulating oxidized LDL is additive to the Global Risk Assessment Score for cardiovascular risk prediction based on age, gender, total and HDL cholesterol, diabetes, hypertension, and smoking. Circulating oxidized LDL does not originate from extensive metal ion-induced oxidation in the blood but from mild oxidation in the arterial wall by cell-associated lipoxygenase and/or myeloperoxidase. Oxidized LDL induces atherosclerosis by stimulating monocyte infiltration and smooth muscle cell migration and proliferation. It contributes to atherothrombosis by inducing endothelial cell apoptosis, and thus plaque erosion, by impairing the anticoagulant balance in endothelium, stimulating tissue factor production by smooth muscle cells, and inducing apoptosis in macrophages. HDL cholesterol levels are inversely related to risk of coronary artery disease. HDL prevents atherosclerosis by reverting the stimulatory effect of oxidized LDL on monocyte infiltration. The HDL-associated enzyme paraoxonase inhibits the oxidation of LDL. PAF-acetyl hydrolase, which circulates in association with HDL and is produced in the arterial wall by macrophages, degrades bioactive oxidized phospholipids. Both enzymes actively protect hypercholesterolemic mice against atherosclerosis. Oxidized LDL inhibits these enzymes. Thus, oxidized LDL and HDL are indeed antagonists in the development of cardiovascular disease.—Mertens, A., Hobvoet, P. Oxidized LDL and HDL: antagonists in atherothrombosis. *FASEB J.* 15, 2073–2084 (2001)**

*Key Words: atherosclerosis · oxidation · lipoproteins · coronary artery disease*

IN THIS REVIEW we focus on the presence of different forms of oxidatively modified LDL in the circulation and their relation to cardiovascular disease. We discuss cellular mechanisms of oxidative modification of LDL, the effects of oxidized low density lipoproteins (LDL) on endothelial cells, and their interaction with monocytes/leukocytes and platelets in vitro and in vivo.

The inverse relation between high density lipoprotein (HDL) cholesterol and risk of cardiovascular disease is well established. The protective effect of HDL has been attributed to its role in reverse cholesterol transport. We focus here on the anti-inflammatory and antioxidative effects of HDL. The relationship between HDL-associated antioxidative enzymes (paraoxonase and PAF-acetylhydrolase) and the oxidation of lipoproteins in vivo is discussed.

## LDL OXIDATION

### Biochemical composition of LDL

The central core of LDL particles contains 1600 molecules of cholesterol ester and 170 molecules of triglycerides. It is surrounded by a monolayer of 700 phospholipid molecules, consisting primarily of lecithin, small amounts of sphingomyelin and lysolecithin, and 600 molecules of cholesterol (1). Embedded in the outer layer is one apoB-100 molecule. About half of the fatty acids in LDL are polyunsaturated fatty acids (PUFAs), mainly linoleic acid with minor amounts of arachidonic acid and docosahexaenoic acid. These PUFAs are protected against free radical attack and oxidation by antioxidants, primarily α-tocopherol (~six molecules per LDL particle), with minor amounts of γ-tocopherol, carotenoids, cryptoxanthin, and ubiquinol-10 (2). The amount of PUFAs and antioxidants varies significantly within individuals, resulting in a great variation in LDL oxidation susceptibility.

### Mechanisms of LDL oxidation

LDL can be oxidized by metal ions, lipoxygenases, myeloperoxidase, and reactive nitrogen species (**Fig. 1**).

### Metal ions

In vitro oxidation of LDL by metal ions (e.g., $Cu^{2+}$) occurs in three phases: an initial lag phase (consumption of endogenous antioxidants), a propagation phase (rapid oxidation of unsaturated fatty acids to lipid hydroperoxides), and a decomposition phase (hydroperoxides are converted to reactive aldehydes, e.g., malondialdehyde, 4-hydroxynonenal). Interaction of these aldehydes with positively charged ε-amino groups

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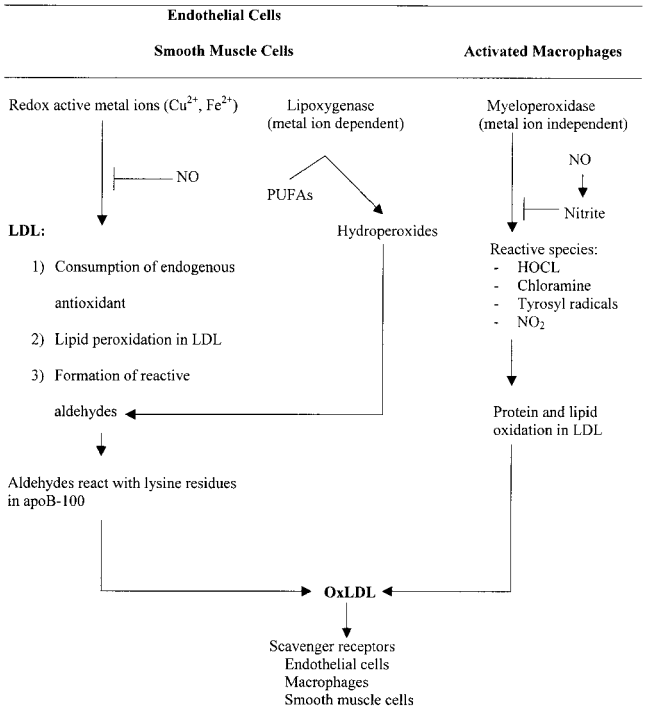


**Figure 1.** Mechanisms of LDL oxidation. In vitro oxidation of LDL by metal ions occurs in three phases: an initial lag phase (consumption of endogenous antioxidant), a propagation phase (rapid oxidation of unsaturated fatty acids to lipid hydroperoxides), and a decomposition phase (formation of reactive aldehydes). These aldehydes react with lysine residues in apoB-100, resulting in oxidized LDL. NO inhibits copper-mediated oxidation. The metal ion-dependent enzyme lipoxygenase converts polyunsaturated fatty acids into lipid hydroperoxides and thereby oxidizes LDL. Activated macrophages secrete myeloperoxidase, which generates reactive species, thereby oxidizing protein and lipid moieties of LDL. NO is converted under aerobic conditions to nitrite. Nitrite inhibits the myeloperoxidase-mediated oxidation of LDL. Finally, oxidized LDL is interacting with scavenger receptors present on endothelial cells, macrophages, and smooth muscle cells.

of lysine residues renders the LDL more negatively charged, resulting in decreased affinity for the LDL receptor and increased affinity for scavenger receptors (Fig. 1).

It is unlikely that free metal ions are responsible for in vivo LDL oxidation. There is no convincing evidence for free metal ion in plasma or the arterial wall. There is also no significant accumulation of o-tyrosine and m-tyrosine, typical oxidation products of free metal ion, in fatty streaks or intermediate atherosclerotic lesions (3).

## Lipoxygenases

15-Lipoxygenase, produced by endothelial cells and monocytes/macrophages, converts polyunsaturated fatty acids into lipid hydroperoxides and thereby oxidizes LDL (Fig. 1). Lipoxygenase inhibitors block in vitro oxidation of LDL by these cells (4–6). Overexpression of 15-lipoxygenase in vascular endothelium accelerates early atherosclerosis in LDL receptor-deficient mice (7). Disruption of the 12/15-lipoxygenase genes diminishes atherosclerosis in apoE knockout mice (8) in the absence of changes in cholesterol, triglyceride, and lipoprotein levels.

Hyperglycemia causes up-regulation of 12-lipoxygenase (9). Increased production of 12-hydroxyeicosatetraenoic acid, the lipoxygenase metabolite of arachidonic acid, results in enhanced adhesion of monocytes to endothelium, a key early event in atherogenesis.

## Myeloperoxidase

Activated phagocytes secrete myeloperoxidase that generates reactive species including hypochlorous acid (HOCl), chloramines, tyrosyl radicals, and nitrogen dioxide ($NO_2$). These reactive species oxidize antioxidants, lipids, and protein of LDL (10) (Fig. 1). Reactive nitrogen species generated by the myeloperoxidase-$H_2O_2$-$NO_2$ system of monocytes convert LDL into an atherogenic form that is avidly taken up and degraded by macrophages, leading to foam cell formation (11).

Monoclonal antibodies raised against HOCl-modified LDL that do not cross react with other LDL modifications (copper-oxidized LDL, 4-hydroxynonenal-LDL, malondialdehyde-LDL, and glycated LDL) detect HOCl-modified LDL in the neighborhood of endothelial cells and monocyte/macrophages in human atherosclerotic lesions (12).

Activated human neutrophils generate *p*-hydroxyphenyl acetaldehyde (pHA), the major product of L-tyrosine oxidation by the myeloperoxidase-HOCl-$H_2O_2$ system. The concentration of pHA-modified phospholipid in LDL isolated from human atherosclerotic lesions is markedly increased compared with circulating LDL (13). Sugiyama et al. (14) recently identified granulocyte macrophage colony-stimulating factor as an endogenous regulator of myeloperoxidase expression in human atherosclerosis. Furthermore, increased numbers of myeloperoxidase-expressing macrophages were demonstrated in eroded or ruptured plaques causing acute coronary syndromes, suggesting a role for myeloperoxidase-expressing macrophages in human atheroma complications. In contrast, disruption of the myeloperoxidase gene in LDL receptor-deficient mice resulted in increased atherosclerosis (15). This study thus suggested an important distinction between mouse and human atherosclerosis with regard to the potential involvement of myeloperoxidase in protein oxidation.

## Reactive nitrogen species

Nitric oxide (NO) is a free radical released by various vascular cells (16). It inhibits copper-mediated oxidation (17) as well as cell-mediated oxidation of LDL (18) (Fig. 1). NO is converted under aerobic conditions to nitrite, and low concentrations of nitrite (12 μM compared with physiological concentrations of up to 200 μM) inhibit myeloperoxidase-mediated oxidation of

LDL (19). NO also acts as an antioxidant by scavenging alkoxyl and peroxyl radicals.

The NO radical interacts with superoxide anion to form the peroxynitrite anion ($ONOO^-$) that decomposes into the hydroxyl radical $OH^{\cdot}$, which oxidizes LDL (20). Peroxynitrite also oxidizes tetrahydrobiopterin, a critical cofactor for NO synthase (NOS), and thereby decreases NO production (21). Expression of inducible NOS, associated with increased peroxynitrite production, resulted in increased apoptotic cell death in atheromatous plaques of human coronary arteries (22). Thus, when NO is in excess of surrounding oxidants, lipid oxidation and monocyte margination into the vascular wall are attenuated, producing antiatherogenic effects. However, when oxidant defenses become depleted or endogenous tissue rates of oxidant production are accelerated, NO gives rise to secondary oxidizing species that increase membrane and lipoprotein lipid oxidation as well as foam cell formation in the vasculature (23).

### Biochemical composition of oxidized LDL

We have isolated oxidized LDL from the plasma of patients with coronary artery disease. It was characterized by a 1.3-fold higher electrophoretic mobility on agarose gels compared with native LDL fractions of the same patients, a 75% reduction of the arachidonate levels and an 80% reduction of the linoleic acid levels. The fractions of aldehyde-substitution of lysine residues was ~30–40% of that in standard preparations of in vitro oxidized LDL, indicating that between 60 and 90 lysine residues in the apoB-100 moiety of oxidized LDL were substituted. These characteristics suggested that in vivo oxidized LDL does not originate from extensive metal ion-induced oxidation of LDL, but that it is most likely generated by cell-associated oxidative enzymatic activity in the arterial wall (24, 25).

## OXIDIZED LDL IN CARDIOVASCULAR DISEASE

Until recently, methods for direct measurement of oxidized LDL in blood were lacking. Therefore, indirect methods have been used to study the association between oxidized LDL and cardiovascular disease. An indirect indication of in vivo oxidation of LDL is the increase of the titer of autoantibodies against neoepitopes in oxidized LDL. Salonen et al. (26) reported a correlation between titers of autoantibodies against oxidized LDL and the progression of carotid atherosclerosis. However, data on the relation between autoantibody titer and coronary or carotid atherosclerosis are not consistent (27–31), possibly due to assay divergences in the different studies. For example, there is no agreement about how the oxidized LDL used in the different immunoassays should be generated in vitro and what its composition should be. Furthermore, most of the studies evaluating the clinical significance of antibodies against oxidized LDL are cross-sectional and only a few follow-up studies are published. The role of humoral immune reactions in atherogenesis is not clear, and may be different in patient groups as well as at different stages of the disease. Thus, there is need for well-controlled follow-up studies with standardized assays to determine the prognostic value of antibodies against oxidized LDL.

Sensitive assays to measure circulating LDL are now available. We have raised the monoclonal antibody mAb-4E6 against a neo-epitope in the aldehyde-substituted apolipoproteinB-100 moiety of oxidized LDL (32). A mAb-4E6-based competition ELISA has been used to study the association between circulating oxidized LDL and coronary artery disease (CAD) (24, 25). At a cutoff value of 2.30 mg/dl, the sensitivity of our assay for CAD was 76% with a specificity of 90%. The American Heart Association and the American College of Cardiology have introduced the Global Risk Assessment Score for Cardiovascular Risk prediction (GRAS) (33). It is based on the Framingham risk factors age, total cholesterol, HDL cholesterol, systolic blood pressure, diabetes mellitus, and smoking. The predictive value of circulating oxidized LDL was additive to that of GRAS; 94% of subjects with high circulating oxidized LDL and high GRAS had CAD (34). These data indicate that circulating oxidized LDL is a useful marker for identifying patients with CAD. Meanwhile, two other groups have confirmed our results using an immunoassay for oxidized LDL based on a monoclonal antibody raised against oxidized products of phosphatidylcholine (35, 36). We recently demonstrated that plasma levels of circulating oxidized LDL predict the development of transplant coronary artery disease in heart transplant patients (37), suggesting that oxidized LDL plays an active role in the development of CAD.

Studies with stable isotopes have suggested that oxidation of apolipoprotein B-100 (apoB-100) in circulating LDL is related to LDL residence time (38, 39). The longer the residence time of LDL is, the longer the exposure of its apoB-100 moiety to the attack of reactive oxygen species. Especially small, dense LDL ($LDL_2$ and $LDL_3$) has a reduced affinity for the hepatic LDL receptor (40), a longer residence time, and therefore greater susceptibility to oxidation (41). Four recent prospective reports provided further support for a critical role of small, dense LDL particles in the etiology of atherosclerosis (42–45).

## REGULATION OF EXPRESSION OF SCAVENGER RECEPTORS FOR OXIDIZED LDL

Interaction of modified LDL with scavenger receptors (class A, class B, LOX-1) induces rapid and unregulated uptake of the modified LDL leading to massive cholesterol accumulation (46). Scavenger receptor class A (SR-A) regulates the development of atherosclerotic lesions through uptake of oxidized LDL by macrophages. The expression of SR-A in vitro is related to the state of cell differentiation. Freshly isolated human

monocytes have minimal SR-A activity, but differentiated macrophages express SR-A activity. Expression of SR-A in human monocytes is induced by macrophage colony-stimulating factor (M-CSF) in human monocytes (47), and inhibited by interferon gamma (IFN-γ) (48), transforming growth factor beta (TGF-β) (49), tumor necrosis factor alpha (TNF-α) (50), and peroxisome proliferator-activated receptor gamma ligands (51), such as fatty acids and prostaglandin D2 metabolites. IFN-γ and TNF-α increase SR-A expression in smooth muscle cells (52, 53). The reason for the opposite effects of these cytokines on SR-A expression in smooth muscle cells vs. macrophages is unclear.

CD36 (a class B scavenger receptor) is a second oxidized LDL receptor present on platelets, monocytes, endothelial cells, and adipocytes. The affinity of oxidized LDL for CD36 is about threefold greater than that of native LDL (54). CD36 is the major receptor responsible for high-affinity recognition of oxidized LDL by macrophages (55). Macrophages of CD36-apoE double knockout mice accumulate 60% less copper-oxidized LDL and LDL modified by monocyte-derived reactive nitrogen species than macrophages from apoE knockout mice (56). These results suggest that blockade of CD36 may be protective even in more extreme proatherogenic circumstances.

Mouse macrosialin or its human homologue CD68 is a third oxidized LDL receptor that is expressed only in macrophages and dendrite cells. Yoshida et al. (57) showed that oxidized LDL induces expression of macrosialin and other scavenger receptors. Extensively oxidized LDL induced more macrosialin expression than mildly oxidized LDL, but the effect of pretreatment with mildly oxidized LDL on internalization and degradation of oxidized LDL is much greater than with extensively oxidized LDL. The generation of macrosialin knockout mice will teach us more about the role of the macrosialin receptor in atherosclerosis.

The expression of the lectin-like oxidized LDL receptor or LOX-1 in vascular endothelial cells (58) is induced by TNF-α and shear stress (59, 60). Uptake of oxidized LDL via LOX-1 in vascular endothelial cells in vivo may not result in massive lipid accumulation, but may cause endothelial activation and/or dysfunction. Superoxide anions, hydrogen peroxide, and homocystein also increase LOX-1 expression (61). Different groups have shown that expression of LOX-1 can also be induced in smooth muscle cells and macrophages (62, 63). TGF-β appears to be one of the key regulators of scavenger receptor expression (SR-A, CD36 and LOX-1) (64).

## BIOLOGICAL ACTIVITIES OF OXIDIZED LDL

### Effect of oxidized LDL on monocyte infiltration

Adhesion and infiltration of macrophages into the arterial wall contributes to fatty streak formation. A triggering event in the development of fatty streaks is accumulation, retention and oxidation of LDL into minimally oxidized LDL (MM-LDL). MM-LDL induces endothelium to express adhesion molecules for monocytes, intercellular adhesion molecule 1 (ICAM-1) and vascular adhesion molecule (VCAM-1) (65) (**Fig. 2*A***).

Active compounds in MM-LDL are phospholipid autooxidation products such as 1-palmitoyl-2-(5-oxovaleroyl)-*sn*-glycero-3-phosphocholine (POVPC), 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphocholine (PGPC), and 1-palmitoyl-2-epoxyisoprostane E(2)-*sn*-glycero-3-phosphocholine (PEIPC) (66, 67). Pretreatment of endothelial cells with platelet-activating factor (PAF) receptor antagonists prevented their activation with POVPC and PEIPC but not with PGPC, suggesting a role of the PAF receptor in POVPC and PEIPC but not in PGPC-induced activation. Whereas POVPC induces only monocyte binding, PGPC induces both monocyte and neutrophil adhesion. At concentrations equal to those present in MM-LDL, POVPC inhibits PGPC-induced adhesion of neutrophils (68). MM-LDL also promotes monocyte proliferation and differentiation into macrophages by inducing M-CSF expression by endothelial cells (69).

MM-LDL becomes extensively oxidized LDL because of the action of sphingomyelinase, which induces LDL retention and aggregation (70), and of myeloperoxidase and secretory phospholipase $A_2$ (71). Polyunsaturated free fatty acids liberated from LDL and HDL by secretory phospholipase $A_2$ ($sPLA_2$) increase the formation of oxidized phospholipids that stimulate monocyte–endothelial interactions and atherosclerosis in transgenic mice (72). Upston et al. (73) demonstrated that phospholipase $A_2$ significantly enhanced the accumulation of cholesterol ester hydroperoxides induced by 15-lipoxygenase. Furthermore, MM-LDL induces expression of $sPLA_2$ in monocyte-derived macrophages (74) (Fig. 2*A*).

Oxidized LDL stimulates endothelium to secrete monocyte chemotactic protein 1 (MCP-1), which induces the infiltration of monocytes into the subendothelial space (75). Li and Mehta (76) demonstrated that interaction of oxidized LDL with LOX-1 is required for the oxidized LDL-mediated up-regulation of MCP-1 by human coronary artery endothelial cells (Fig. 2*A*). Because oxidized LDL is a potent inhibitor of macrophage motility, it may promote macrophage retention in the arterial wall.

### Effect of oxidized LDL on smooth muscle cell migration and proliferation

During atherogenesis, smooth muscle cells undergo a phenotypic modification to a synthetic state, allowing them to migrate from the intima to the media where they are proliferating and secreting growth factor, extracellular matrix glycoprotein, and metalloproteinases (77, 78). This leads to fibrous plaque formation. Oxidized LDL induces migration of smooth muscle cells by increasing the expression of platelet-derived growth factor (PDGF) by endothelial cells, smooth muscle cells, and macrophages (79–81). Oxidized LDL

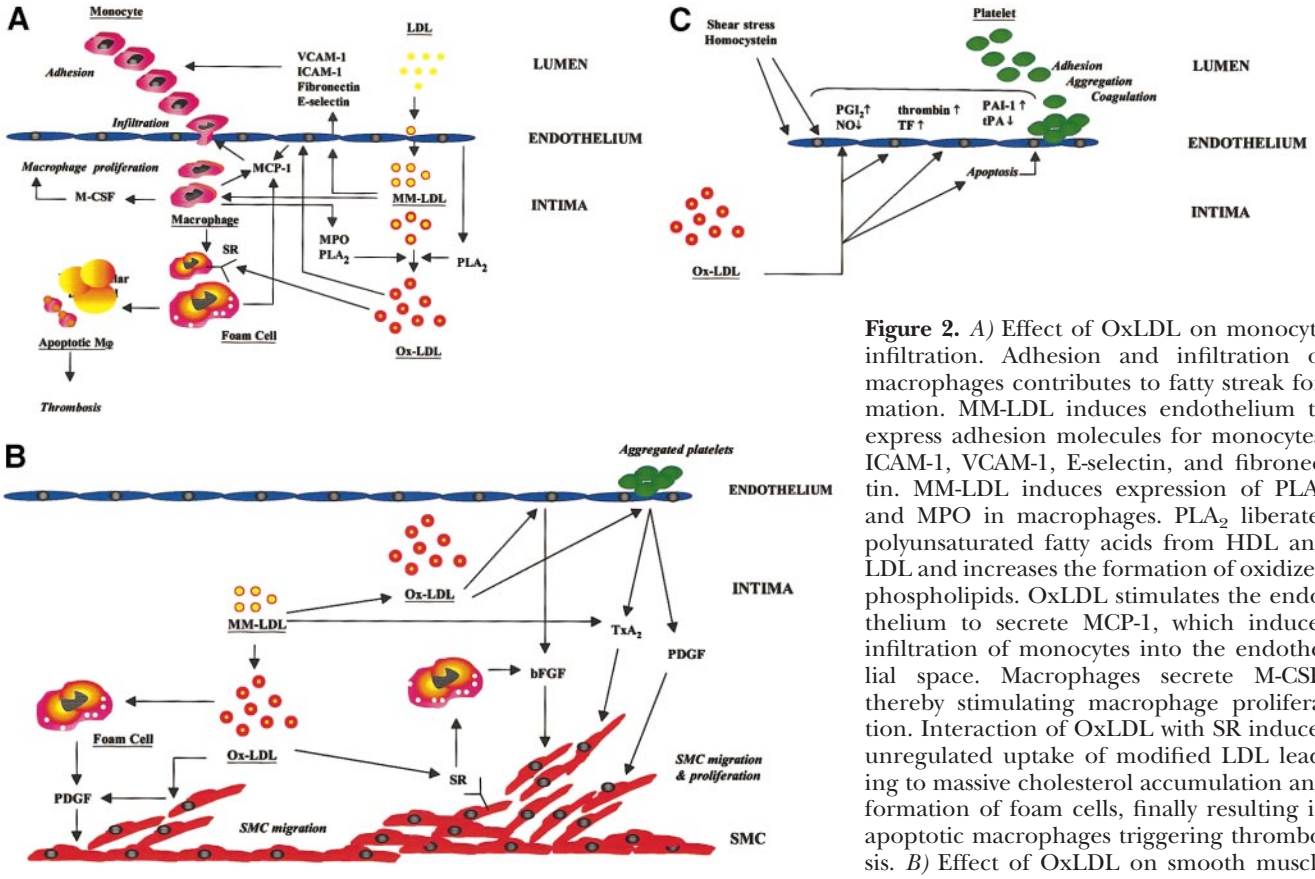


**Figure 2.** *A)* Effect of OxLDL on monocyte infiltration. Adhesion and infiltration of macrophages contributes to fatty streak formation. MM-LDL induces endothelium to express adhesion molecules for monocytes, ICAM-1, VCAM-1, E-selectin, and fibronectin. MM-LDL induces expression of $PLA_2$ and MPO in macrophages. $PLA_2$ liberates polyunsaturated fatty acids from HDL and LDL and increases the formation of oxidized phospholipids. OxLDL stimulates the endothelium to secrete MCP-1, which induces infiltration of monocytes into the endothelial space. Macrophages secrete M-CSF, thereby stimulating macrophage proliferation. Interaction of OxLDL with SR induces unregulated uptake of modified LDL leading to massive cholesterol accumulation and formation of foam cells, finally resulting in apoptotic macrophages triggering thrombosis. *B)* Effect of OxLDL on smooth muscle cell migration and proliferation. OxLDL induces migration of smooth muscle cells by increasing the expression of PDGF by endothelial cells, smooth muscle cells, and macrophages. OxLDL stimulates smooth muscle cell proliferation by inducing expression of bFGF by endothelial cells and smooth muscle cells. MM-LDL and $TxA_2$ released by aggregating platelets have a synergistic interaction on smooth muscle cell proliferation. *C)* Effect of OxLDL on thrombosis. OxLDL stimulates platelet adhesion and aggregation by decreasing endothelial production of NO, increasing $PGI_2$ production, and stimulating the synthesis of prostaglandins and prostaglandin precursors. OxLDL enhances the procoagulant activity of endothelium by inducing the release of TF by endothelial cells and smooth muscle cells. TF is a cofactor of factor VIIa that activates factors IX and X, resulting in thrombin formation. OxLDL reduces the fibrinolytic activity of endothelium by decreasing secretion of tPA and increasing the release of PAI-1. OxLDL contributes to apoptosis in endothelial cells, smooth muscle cells, and foam cells and thereby induces thrombosis. Increased shear stress and increased plasma levels of homocystein also induce endothelial dysfunction.

also stimulates smooth muscle cell proliferation by inducing expression of basic fibroblast growth factor (bFGF) by endothelial cells and smooth muscle cells (82). A synergistic interaction between MM-LDL and thromboxane $A_2$ on smooth muscle cell proliferation has been demonstrated (83). Thromboxane $A_2$ is released by aggregating platelets at sites of endothelial injury (Fig. 2*B*).

### Effect of oxidized LDL on vasoreactivity

Intimal thickening is caused by accumulation of foam cells and by smooth muscle cell migration and proliferation. It results in reduction of arterial lumen, which is exacerbated by impairment of the vasodilator capacity of the artery. Oxidized LDL may induce vasoconstriction through inhibition of NO production (84, 85) and stimulation of expression of endothelin (86) (Fig. 2*C*).

### Effect of oxidized LDL on apoptosis

Apoptosis in endothelial and smooth muscle cells is contributing to plaque rupture (87). The apoptotic effect of oxidized LDL on endothelial cells could be attributed to oxidation products of phosphatidylcholine (88) or to oxysterols. Oxysterol-mediated apoptosis induces activation of MAP and Jun/SAP kinases and increased p53 production (89, 90). An irreversible caspase inhibitor decreased oxidized LDL-induced apoptosis in endothelial cells (91).

### Effect of oxidized LDL on thrombosis

Endothelial dysfunction is associated with enhanced platelet adhesion, increased procoagulant activity, and impaired fibrinolysis (92). Oxidized LDL stimulates platelet adhesion and aggregation by decreasing endothelial production of NO, increasing prostacyclin ($PGI_2$) production (93, 94), and stimulating the synthe-

sis of prostaglandins and prostaglandin precursors (95) (Fig. 2*C*).

Oxidized LDL enhances the procoagulant activity of endothelium by inducing the release of tissue factor (TF) by endothelial cells and smooth muscle cells (96, 97). TF is a cofactor of factor VIIa that activates factors IX and X, resulting in thrombin formation. Oxidized LDL also stimulates coagulation by reducing thrombomodulin transcription (TM) (98), suppressing protein C activation (99), and modulating tissue factor pathway inhibitor (100).

Oxidized LDL reduces the fibrinolytic activity of endothelium by decreasing secretion of tissue-type plasminogen activator (tPA) and increasing release of plasminogen activator inhibitor 1 (PAI-1) (101–103).

Oxidized LDL may also contribute to plaque disruption and/or vascular remodeling by increasing expression of metalloproteinase 9 (MMP-9) and decreasing expression of its tissue inhibitor TIMP-1 by monocytes/macrophages (104).

## HDL IN CARDIOVASCULAR DISEASE

The Framingham Heart Study (105) demonstrated that high density lipoprotein cholesterol (HDL-C) is the most potent lipid predictor of coronary artery disease risk in men and women $>$ 49 years of age. Every 1 mg/dl increment in HDL-C is associated with a 2% decreased risk of coronary artery disease in men and a 3% decreased risk in women.

In the Veterans Affairs HDL Intervention Trial (VA-HIT) (106), subjects were randomized to gemfibrozil or placebo. HDL-C increased by 6% on treatment. Total cholesterol and triglycerides decreased by 4% and 31%, respectively. There was no change in LDL-C levels. This change in lipid was associated with a cumulative reduction in the trial primary end points: all cause mortality and nonfatal myocardial infarction. A significant reduction in secondary end points, including death from coronary artery disease, nonfatal myocardial infarction, stroke, transient ischemic attack, and carotid endarterectomy, was associated with the increase in HDL-C. In VA-HIT, for every 1% increase in HDL-C, there was a 3% reduction in death or myocardial infarction, a therapeutic benefit that eclipses the benefit associated with LDL-C reduction. Triglyceride levels were not predictive of clinical events.

HDL-C is also an important predictor of survival in men after coronary artery bypass graft surgery. One-third fewer patients survive at 15 years if their HDL-C levels are $\leq$ 35 mg/dl (107).

## EFFECT OF HDL ON LDL OXIDATION

Navab et al. (108, 109) have proposed that biologically active lipids in LDL are formed in a series of three steps. The first step is the seeding of LDL with products of the metabolism of linoleic acid and arachidonic acid as well as with hydroperoxides. The second step is trapping LDL in the subendothelial space and the accumulation in LDL of additional reactive oxygen species derived from artery wall cells. The third step is the nonenzymatic oxidation of LDL phospholipids that occurs when a certain threshold of reactive oxygen species is reached, resulting in the formation of specific oxidized phospholipids that induce monocyte binding, chemotaxis, and differentiation into macrophages.

Normal HDL and its major protein, apolipoprotein AI (apoAI), inhibit all three steps in the formation of MM-LDL. Pretreatment of LDL with apoAI renders LDL resistant to oxidation and reduces the chemotactic activity of LDL. ApoAI also renders LDL resistant to in vivo oxidation. It removes 13(S)-hydroxyperoxyoctadecadienoic acid [(13(S)-HPODE] and 15(S)-hydroxyperoxyeicosatetraenoic acid [(15(S)-HPETE] from LDL. [(13(S)-HPODE] and [(15(S)-HPETE] enhance the nonenzymatic oxidation of both 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine and cholesterol linoleate (108, 109). This results in the formation of three biologically active oxidized phospholipids: POVPC, PGPC, and PEIPC.

Paraoxonase, a HDL-associated enzyme, prevents LDL oxidation by hydrolyzing lipid peroxides, cholesterol linoleate hydroperoxides, and hydrogen peroxide (110–112). Paraoxonase also renders HDL resistant to oxidation, thereby maintaining the capacity of HDL to induce reverse cholesterol transport. MM-LDL inhibits paraoxonase expression (113). When fed a fat-rich diet, C57BL/6 mice susceptible to atherosclerosis display decreased levels of paraoxonase mRNA. However, mice resistant to atherosclerosis (C3H/HeJ) display a decrease in hepatic paraoxonase mRNA levels (114). Shih and collaborators (115) recently demonstrated that paraoxonase/apoE double knockout mice exhibit increased lipoprotein oxidation and atherosclerosis compared with apoE knockout mice.

Recent investigations suggest that another HDL-associated enzyme lecithin:cholesterol acyltransferase (LCAT) also prevents the accumulation of oxidized lipids in LDL (116). Once MM-LDL is present, it inhibits plasma LCAT activity and thereby impairs HDL metabolism and reverse cholesterol transport (117, 118).

The early inflammatory phase of atherosclerosis (119) involves the generation of PAF and oxidized phospholipids with PAF-like bioactivity in LDL (67). PAF is a potent lipid mediator that stimulates macrophages to produce superoxide anions, thus contributing to progression of atherosclerosis (120, 121). PAF and PAF-like oxidized phospholipids are inactivated by PAF-acetylhydrolase (PAF-AH), a $Ca^{2+}$-independent enzyme that hydrolyzes the *sn*-2 group of PAF, converting it into lyso-PAF (122). PAF-AH is released by monocytes and macrophages, platelets, erythrocytes, spleen and liver cells (123), and has anti-inflammatory properties (124). Human PAF-AH is mainly associated with both LDL and HDL (125). In mice, PAF-AH is predominantly associated with HDL (126).

Transgenic apoE knockout overexpressing apoAI mice

display increased plasma PAF-AH activity and reduced oxidative stress (127). Human-like HDL, generated by adenovirus-mediated apoAI gene transfer, protects apoE knockout mice against neointima formation (128).

Adenovirus-mediated gene transfer of PAF-AH in apoE knockout mice results in a decrease in oxidative stress, deposition of oxidized LDL, and accumulation of smooth muscle cells and macrophages in the arterial wall. This finally results in reduction of injury-induced neointima formation and prevention of spontaneous atherosclerosis, suggesting a direct anti-atherogenic effect of PAF-AH (127, 129). These protective effects of HDL are summarized in **Fig. 3**.

### Protective effect of HDL on endothelial function

Low HDL-C concentrations relate to lipoprotein oxidation and endothelial dysfunction (130). α-Tocopherol, the most potent antioxidant form of vitamin E, is bound mainly to lipoproteins in plasma; its incorporation into the vascular wall prevents the endothelial dysfunction at an early stage of atherosclerosis (131). The plasma phospholipid transfer protein (PLTP) promotes the net mass transfer of α-tocopherol from HDL to endothelial cells. This transfer has two beneficial roles in preventing endothelium damage: the antioxidant protection of membrane-bound phospholipids and the preservation of the normal relaxing function of vascular endothelial cells (Fig. 3).

Preservation of the relaxing function can be explained by preservation of NO production. Oxidized LDL depletes caveolae of cholesterol, resulting in the displacement of endothelial NOS (eNOS) from caveolae and impaired eNOS activation (132). CD36-blocking antibodies prevented oxidized LDL-induced redistribution of eNOS, indicating that oxidized LDL–scavenger receptor interaction is required. HDL binding to its specific receptor, scavenger receptor class BI maintained the concentration of caveolae-associated cholesterol by promoting the uptake of cholesterol esters, thereby preventing oxidized LDL-induced depletion of caveola cholesterol (Fig. 3).

HDL has a direct inhibitory effect on the oxidized LDL-induced overexpression of both ICAM-1 and VCAM-1 at the surface of endothelial cells (127, 133, 134; Fig. 3).

### Effect of oxidized LDL on reverse cholesterol transport

The role of HDL in reverse cholesterol transport has been emphasized during the past decade. However, in vitro experiments as well as genetic family and population studies and investigations of transgenic animals have revealed that HDL cholesterol levels do not necessarily reflect the efficacy and the anti-atherogenicity of the reverse cholesterol transport. Important determinants of HDL metabolism and reverse cholesterol transport are the HDL-associated enzymes LCAT and paraoxonase, which are inactivated by mild and extensive oxidized LDL, resulting in an impairment of reverse cholesterol transport. It has been demonstrated that cholesterol delivered to macrophages by oxidized LDL is primarily sequestered in lysosomes and fails to efflux normally (135). Thus, prevention of oxidation of

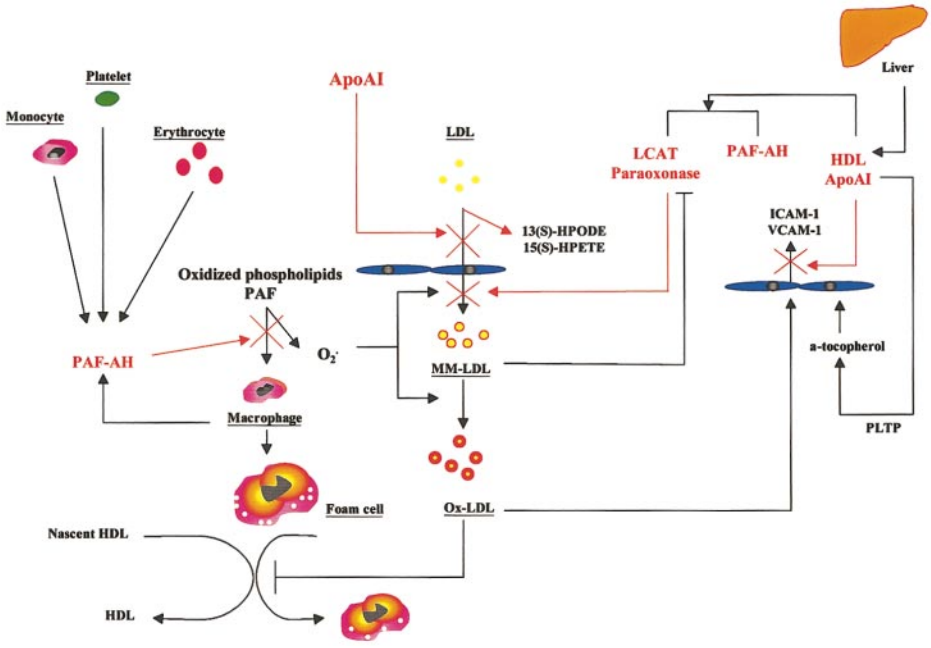


**Figure 3.** Effect of HDL and HDL-associated enzymes on LDL oxidation. ApoAI inhibits all three steps in the formation of MM-LDL. Paraoxonase prevents LDL oxidation by hydrolyzing lipid peroxides, cholesterol linoleate hydroperoxides, and hydrogen peroxide. MM-LDL inhibits paraoxonase expression. LCAT prevents accumulation of oxidized lipids in LDL. MM-LDL inhibits plasma LCAT activity and thereby impairs HDL metabolism and reverse cholesterol transport. PAF and oxidized phospholipids with PAF-like bioactivity in LDL stimulate macrophages to produce superoxide anions, and thus stimulate the generation of MM-LDL and OxLDL. PAF-AH is released by monocytes, macrophages, platelets, erythrocytes, spleen and liver cells, and hydrolyzes the *sn*-2 group of PAF, converting it to lyso-PAF. Plasma PLTP promotes the net mass transfer of α-tocopherol from HDL to endothelial cells. Vitamin E prevents the endothelial dysfunction at an early stage of atherosclerosis. HDL has a direct inhibitory effect on the oxidized LDL-induced overexpression of ICAM-1 and VCAM-1 at the surface of endothelial cells. Cholesterol delivered to macrophages by oxidized LDL is primarily sequestered in lysosomes and thereby inhibits normal cholesterol efflux.

LDL by HDL is also beneficial for the active role of HDL in reverse cholesterol transport and the regression of atherosclerotic plaques. FJ

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