This Review is part of a thematic series on Oxidant Signaling in Cardiovascular Cells, which includes the following articles:

NAD(P)H Oxidase: Role in Cardiovascular Biology and Disease
Oxidant Signaling in Vascular Cell Growth, Death, and Survival
Potential Antiatherogenic Mechanisms of Ascorbate (Vitamin C) and α-Tocopherol (Vitamin E)
Endothelial Dysfunction in Cardiovascular Diseases: The Role of Oxidant Stress
Interactions Between Nitric Oxide and Lipid Oxidation Pathways: Implications for Vascular Disease
Vascular Oxygen Species Generation

Interactions Between Nitric Oxide and Lipid Oxidation Pathways
Implications for Vascular Disease

Valerie B. O’Donnell, Bruce A. Freeman

Abstract—Nitric oxide (NO) signaling pathways and lipid oxidation reactions are of central importance in both the maintenance of vascular homeostasis and the progression of vascular disease. Because both of these pathways involve free radical species that can also react together at extremely fast rates, convergent interactions between these pathways are expected. Biochemical and cell biology studies have defined multiple interactions of NO with oxidizing lipids that could lead to either vascular protection or potentiation of inflammatory vascular injury. For example, low levels of NO generated by endothelial nitric oxide synthase can terminate propagating lipid radicals and inhibit lipoxygenases, reactions that would be protective. Alternatively, if generated at elevated levels, for example, after inducible nitric oxide synthase expression in inflammation, NO can be converted to prooxidant species, such as peroxynitrite (ONOO−) and nitrogen dioxide (NO2•), that can potentiate inflammatory injury to vascular cells. Finally, both enzymatic and nonenzymatic lipid oxidation reactions can influence NO bioactivity by directly scavenging NO or altering the induction and catalytic activity of nitric oxide synthase enzymes. In this review, we summarize the biochemical interactions between NO and lipid oxidation reactions and discuss the recognized and potential roles of these reactions in the vasculature. (Circ Res. 2001;88:12-21.)

Key Words: eicosanoid signaling | lipid | nitric oxide | oxygen | free radical

The free radical species NO is an endogenously generated mediator of smooth muscle relaxation and inhibitor of platelet/leukocyte activation that is essential for maintenance of vascular homeostasis. In many vascular pathologies, altered NO generation rates, often coupled with accelerated NO removal through poorly understood pathways, leads to impaired NO signaling and secondary generation of toxic NO-derived species.1–6 Reaction of NO with O2•−, yielding peroxynitrite (ONOO−), accounts for a major part of the accelerated NO removal2–6 but is not the only mechanism involved, because endothelium-derived relaxing factor (EDRF) activity is often incompletely restored by O2•− scavengers.7–9 This suggests that the reaction of NO with other biochemical “sinks” can also account for enhanced rates of EDRF consumption. Further evidence for alterations in NO metabolism in vascular disease is provided by observations of...
the tyrosine oxidation/nitration product 3-nitrotyrosine (NO₂-tyr) and elevations in inducible nitric oxide synthase (iNOS or NOS2) activity. For example, both NO₂-tyr and NOS2 expression are consistently elevated clinically (transplant coronary artery disease, atherosclerotic lesions, cardiac allograft rejection, and myocardial inflammation) and in animal models of vascular disease (hypercholesterolemia-induced atherosclerosis, balloon-injured arteries, ischemic heart injury, and myocardial inflammation) (reviewed in Reference 10; see also References 11–17).

Increased lipid oxidation is a characteristic feature of inflammatory vascular diseases and has been suggested to sometimes play a causative role, although this has not been conclusively proven.18–24 The candidate mechanisms that generate oxidized lipids in vivo are numerous and include metal-dependent Fenton oxidation, enzyme-catalyzed oxidation by lipoxygenase (LOX) or myeloperoxidase (MPO), reaction with hypochlorous acid (HOCI), cell-dependent oxidation via a diversity of O₂⁻ and H₂O₂-generating oxidases, and, finally, oxidation by NO-derived reactive species (eg, NO₂, nitryl chloride [NO₂Cl], and ONOO⁻).25–32 In particular, support for a pathogenic role of LOX-catalyzed lipid oxidation in vivo in atherogenesis includes the observations that functional 15-LOX and its products are present in human and rabbit lesions,21,33,34 disruption of the mouse 12/15-LOX gene diminishes atherosclerosis in apoE-deficient mice, and inhibition of 15-LOX prevents development of atherosclerosis in cholesterol-fed rabbits.35,36 In contrast, targeted overexpression of rabbit macrophage 15-LOX prevents diet-induced atherosclerosis.37

In the vasculature, nitric oxide (NO) and lipid oxidation signaling pathways can potentially interact at several levels. Because of the diversity of the biochemical pathways involved, an understanding of how these processes might impact on vascular homeostasis is important. In this review, we summarize current knowledge of how lipid oxidation pathways and NO-derived species interact at a chemical and cellular level and describe what is known about how these interactions might influence disease progression.

Reactions of NO With Purified Lipids, Lipoproteins, and Membranes

NO potently inhibits lipid oxidation in a variety of in vitro model systems, including unsaturated free fatty acid emulsions, phosphatidylcholine liposomes or LDL oxidized by Cu²⁺, azo initiators of LOO radical reaction, endothelial cells, or macrophages.30,31,38–42 This is primarily a consequence of NO reacting with lipid-derived radicals (eg, L⁻, LO, and LOO) via diffusion-limited rates (10⁹ to 10¹¹ [mol/L]⁻¹ s⁻¹) to terminate lipid peroxidation propagation reactions.38,40,43–50 During the reaction of LOO with NO, two molecules of NO are consumed as the primary organic peroxynitrite (LOONO) intermediate rapidly decomposes (t₁/₂=0.2 to 0.6 second) to secondary radical species that react further.38 For example, LOONO can decompose to caged radicals [LO⁻NO₂⁻], which can either terminate after rearrangement of LOO to an epoxide, L(O)NO₂, or dissociate and react with additional NO (Figure 1). Alternatively, LOONO can hydrolyze to LOOH and NO₂⁻. The small molecular radius and hydrophobicity of NO facilitates partitioning into hydrophobic membrane compartments,51 thus enhancing the efficacy of NO to terminate lipid peroxidation. The fast rate of reaction between LOO and NO (2×10⁹ [mol/L]⁻¹ s⁻¹), compared with α-tocopherol (1 to 5×10⁸ [mol/L]⁻¹ s⁻¹), with rate depending on the alkyl chain length and charge characteristics of the LOO species), allows NO to spare tocopherol during lipid peroxidation and predicts that steady-state NO concentrations of 30 nmol/L will outcompete endogenous α-tocopherol concentrations (20 μmol/L) for termination of LOO.38 In addition, the reduction of...
topheroxyl radical by ascorbate is less effective at preventing lipid oxidation–induced tocopherol loss than the reaction of NO with LOO\(_{2}\)\(\cdot\)\(52\). These comparisons underscore the profound capacity of NO for antioxidant reactions in the vasculature. It is emphasized that these properties of NO can be manifested only if alternative prooxidative reactions (eg, NO reaction with O\(_2^–\) to yield ONOO\(^{−}\)) do not predominate. NO-mediated termination of lipid radicals can also limit secondary lipid oxidation–mediated processes that are involved in vascular injury, including nuclear factor-κB (NF-κB) activation, the linkage of vascular cell adhesion molecule-1 (VCAM-1) gene expression with NF-κB, \(53–59\) Finally, NO can undergo reactions with \(O_2\) and/or \(O_2\)^{−} to yield oxidizing and nitrating species that also cause NO-dependent cytotoxicity in vitro through inducing lipid oxidation.

**Peroxynitrite-Induced Lipid Oxidation and Nitration**

Peroxynitrite is unique as a lipid oxidant, because it mediates peroxidation of unsaturated fatty acids in the absence of transition metal catalysts.\(28\) Peroxynitrite is more than two orders of magnitude more potent than H\(_2\)O\(_2\) in catalyzing lipid oxidation in vitro and, in contrast to transition metal catalysts, mediates LDL oxidation even in the presence of lipophilic antioxidants.\(60\) In vitro, ONOO\(^{−}\) oxidizes diverse classes of lipids (eg, purified fatty acids, neutral lipids and phospholipids, and lipophilic antioxidants and LDL lipids) forming conjugated diene, malondialdehyde, lipid peroxide, lipid hydroxide, F\(_2\)-isoprostane, and oxysterol products.\(29,44,50–63\) In the case of LDL, this results in an LDL derivative recognized by the macrophage scavenger receptor.\(64\) In addition to oxidizing lipids, ONOO\(^{−}\) mediates linoleate and LDL cholesteryl linoleate nitration to the derivative LNO\(_2\)\(\cdot\)\(65\) (also A. Bloodsworth and B.A. Freeman, unpublished data, 2001). This reaction proceeds via either hydrogen abstraction by \(NO\) or addition mechanisms involving \(NO\)\(^{−}\) (Figure 2). A role for ONOO\(^{−}\) in initiating lipid oxidation in atherosclerosis has been suggested\(66\); however, this requires that the barrier of competing reactions with thiols be overcome, which are present intracellularly at concentrations of up to 10 mmol/L \((k=5.9\times10^3\) [mol/L]\(^{−}\)s\(^{−}\) for ONOO\(^{−}\) reaction with cysteine).

**Reactions of the NO Metabolites \(\text{NO}_2\) and \(\text{NO}_2\text{Cl} \) With Unsaturated Lipids**

Several reactive nitrogen species derived from NO oxidize and nitrates unsaturated fatty acids and their methyl/ethyl esters in vitro. Nitrogen dioxide will both oxidize and nitrate unsaturated lipids, with nitration occurring by hydrogen abstraction and addition reactions.\(28,65–67,74\) These reactions result in formation of a complex mixture of products including nitrated lipid derivatives and alkylnitrites, including those shown in Figure 3.\(75\) Nitration of methyl linoleate and linolenate by NO\(_2\) proceeds via initial hydrogen abstraction to form a carbon-centered alkyl radical, which at low oxygen tensions combines with NO\(_2\) to form allylic nitro compounds (Figure 2). The yield of oxygen-containing lipid products (eg, LOOH, LOH, etc) formed by NO\(_2\) oxidation thus depends on the concentration of \(O_2\) that will facilitate peroxidation reactions.\(28\) At high \(O_2\) concentrations, for example, in lung lining fluid, \(NO\) will predominantly mediate lipid oxidation. Conversely, at low \(O_2\) tension (eg, within an inflamed hypoxic organ or microvessel), nitration reactions may preferentially occur. Somewhat analogous to NO, NO\(_2\) can also react at diffusion-limited rates with peroxyl and alkoxyl radicals, leading to inhibition of peroxidation and formation of novel N-containing lipid derivatives.\(46,76\)

The oxidation of nitrite \((\text{NO}_2\text{)}\) by MPO-derived hypochlorous acid (HOCI) will yield nitril chloride \((\text{NO}_2\text{Cl})\),\(77,78\) which, in purified LDL, depletes \(\beta\)-carotene and \(\alpha\)-tocopherol, initiates lipid oxidation, forms 3-nitrotyrosine,\(27\) and can yield an LDL particle similar to that found in foam cells. The direct oxidation of NO\(_2\) by MPO+H\(_2\)O\(_2\) yields NO\(_2\) and also oxidizes LDL lipids to a proatherogenic particle.\(79–81\) Oxidation and nitration...
of membrane lipids by MPO may be operative in atherogenesis, because products of MPO activity are found in vascular lesions, although this has not been conclusively proven. Finally, acidification of NO\textsubscript{2} forms nitrous acid (HONO), which decomposes to nitrosating and nitrating species including N\textsubscript{2}O and NO\textsubscript{2}. Reaction of ethyl linoleate with HONO yields several nitrated lipids, including nitroalkenes and nitroalcohols, whereas reaction of lipid hydroperoxides (LOOH) with HONO forms nitroepoxylinoleate\textsuperscript{65,75} (Figure 4). Formation of HONO is favored at pH <4; therefore, if these reactions are to occur in the vasculature, they will require acidic microenvironments, for example, in the phagolysosomes of neutrophils or macrophages.

### NO-Derived Reactive Species Modulate the Activity and Expression of Lipid Oxidation Enzymes

Enzymes such as LOX, prostaglandin endoperoxide H synthase (PGHS), and cytochrome P450 (CYP) that oxidize lipids to bioactive eicosanoids play critical signaling roles in the regulation of vascular cell function and inflammatory responses and are ubiquitously expressed by virtually all vascular cells under both physiological and inflammatory conditions (Figure 5). Generally, lipid oxidation by these enzymes involves formation of enzyme-bound radical intermediates, including lipid alkyl (L) and peroxy (LOO) radical species. Free peroxyl or alkyl radicals react with NO at diffusion-limited rates. Thus, reaction of NO with enzyme-bound lipid radicals will modulate rates of eicosanoid product formation and decrease bioavailable concentrations of NO, as discussed below.\textsuperscript{46–49} In addition to lipid radicals, these enzymes form several other intermediates that can react with NO during turnover, including amino acid or porphyrin radicals and various redox states of iron. In the next sections, the known interactions of NO with the various enzyme intermediates formed during catalysis are discussed.

### Prostaglandin Endoperoxide H Synthase (PGHS)

Prostaglandins are generated via arachidonate oxygenation by PGHS, of which there are both constitutive (PGHS-1: stomach, gut, kidney, and platelets) and inducible (PGHS-2: fibroblasts and macrophages) isotypes. Under inflammatory conditions, both NOS2 and PGHS-2 expression is upregulated in tandem by proinflammatory cytokines including interleukin-1 (IL-1) and tumor necrosis factor, indicating that high levels of both prostaglandin and NO will be produced in concert in vivo.\textsuperscript{91–92}

Reactive nitrogen species have multiple effects on PGHS activity. Purified PGHS-1 is not significantly inhibited by NO; however, in several cell types, including endothelial cells and platelets, NO highly stimulates prostaglandin production.\textsuperscript{93–96} In other cell types, NO suppresses lipopolysaccharide (LPS)-induced PGHS-2 expression, resulting in apparent enzyme inhibition.\textsuperscript{97,98} Finally, in NOS2 knockout mice, less urinary prostaglandin E\textsubscript{2} is found, although platelets from these animals generate more thromboxane B\textsubscript{2} in vitro.\textsuperscript{99}

NO can interact in several ways with PGHS, by forming an Fe-nitrosyl complex, acting as a peroxidase-reducing substrate, directly terminating the catalytic tyrosyl radical in the enzyme active site, and theoretically, by termination of NO proceeds at essentially diffusion-limited rates, it is intriguing that PGHS is not more readily inhibited in vitro by NO. Possible explanations are that NO-tyrosyl radical reactions are readily reversible, or by acting as a peroxidase-reducing substrate, NO alternatively contributes to enzyme activation.\textsuperscript{93,103} Peroxynitrite is also an

---

**Figure 4.** Products of nitrous acid reaction with ethyl linoleate and linoleate hydroperoxide. A, Nitrosation/nitration of ethyl linoleate by nitrous acid proceeds via similar pathways to nitrogen dioxide–dependent reactions. However, as a result of the low pH required for these reactions (typically <4.0), alkyl nitrite compounds are not stable and either hydrolyze to alcohols or lose HNO\textsubscript{2} via acid catalysis, forming the species shown. B, Nitrosation of lipid hydroperoxides by HONO forms nitroepoxy derivatives and likely proceeds via the LOONO intermediate shown in Figure 1. The two possible rearrangements are shown.

**Figure 5.** Localization of lipid oxidation enzymes in the vasculature. LOX indicates lipoxygenase; PGHS, prostaglandin endoperoxide H synthase; TXS, thromboxane synthase; PGIS, prostacyclin synthase; and CYP, cytochrome P450.
Lipoxygenases (LOX)

Lipoxygenases are non-heme iron–containing enzymes that catalyze oxidation of arachidonate or linoleate to bioactive lipid hydroperoxides. In mammalian cells, at least three isozymes are known with the best characterized, 5-LOX, found mainly in leukocytes. 12-LOX isoforms are present in platelets and monocytes. 15-LOX is expressed in reticulocytes during maturation into erythrocytes, where it plays a central role in intracellular membrane degradation. In human monocytes, expression of 15-LOX is induced by IL-4 and IL-13. A role for 15-LOX in the initiation and progression of atherosclerosis has also been suggested by the observation of 15-LOX products at elevated levels in atherosclerotic lesions. 21,22,34,111

Lipoxygenases contain a single non-heme iron that alternates between Fe$^{2+}$ and Fe$^{3+}$ during catalysis. Resting enzyme predominantly exists as the reduced form, requiring oxidation by hydroperoxides before dioxygenation can occur. Inhibition of LOX (soybean, rabbit and human 15-LOX, and human platelet 12-LOX) by NO has been reported 43,112–115 and was proposed to result from formation of an Fe-nitrosyl complex with the ferrous enzyme. However, metal center reaction only occurs at high and nonphysiological NO concentrations, 116–119 making this pathway of LOX inhibition unlikely. Rather, tissue LOX inhibition results from a termination reaction between NO and the enzyme-bound lipid peroxyl radical (EradLOO$^-$), 43 which would be expected to occur at nanomolar concentrations of NO present in vivo. After this reaction, dissociation and hydrolysis of the organic peroxynitrite (LOONO) gives LOOH and NO$_3^-$ as products (Figure 7). Because the LOX catalytic cycle is not completed, reoxidation of the enzyme-bound iron is required. 43

Thus, because NO reaction occurs after O$_2^-$ insertion into the fatty acid substrate, the LOX product profile is unchanged, the rate of product generation is suppressed, and NO is consumed.

**Cytochrome P450 (CYP)**

CYP enzymes are a ubiquitously expressed family of heme proteins that play major roles in xenobiotic metabolism and lipid oxidation. Nonhepatic CYP arachidonate metabolites also act as intracellular signaling molecules in vascular tissue. For example, the CYP4A product 20-hydroxyicosatetraenoic acid (20-HETE) is a potent vasoconstrictor whose generation in vascular smooth muscle cells is inhibited by NO. 120 A second product, 11,12-EET, is produced by endothelial cells, avidly esterified into endothelial phospholipid pools, and mediates vascular relaxation, possibly accounting for a component of the presently undescribed endothelium-derived hyperpolarizing factor activity. 121–123 Preformed EETs in endothelial membranes can influence vascular function by altering membrane characteristics, ion transport, or lipid-dependent signaling pathways. 124 For example, one isomer, 5,6-EET, mediates vasodilation by either increasing NO production through stimulating Ca$^{2+}$ influx into endothelial cells 125 or by directly activating smooth muscle K$_a$ channels. 121,126 NO has been shown to inhibit the CYP enzymes thromboxane synthase and prostacyclin synthase in vitro. This can have a significant effect on vascular function, in that these enzymes generate thromboxane and prostacyclin, eicosanoid mediators that are central in regulation of platelet aggregation and smooth muscle tone in vivo. 127 Formation of nitrosyl complexes has been observed for some CYP isoforms; however, the detailed mechanisms by which NO interacts with CYP have not been elucidated.

**Catalytic NO Consumption by Lipid Oxidation Enzymes**

PGHS and LOX catalyze NO consumption via reaction with intermediates formed during enzyme turnover, a reaction first confirmed using purified enzymes and isolated vascular cells, including platelets and monocytes 43,103 (also M.J. Coffey and V.B. O’Donnell, unpublished data, 2001). In these cell models, the rates and amounts of NO consumed are high, relative to expected rates and amounts of NO generated, suggesting that these reactions might play a role in both physiological and pathological NO removal in vascular cells.
Lipid Oxidation Products Regulate NO Bioactivity

Although NO production regulates the induction and activity of lipid oxidation enzymes, their eicosanoid products can conversely modulate rates of cellular NO production. In platelets, activation of nitric oxide synthase is inhibited by aspirin or indomethacin, an effect that is overcome by addition of thromboxane A₂. In the murine macrophage cell line J774, induction of NOS2 by LPS is inhibited by indomethacin, inferring involvement of PGHS products. Activation of LOX also leads to increases in NOS2 expression. For example, the nonspecific LOX inhibitor nordihydroguaiaretic acid prevents induction of NOS2 in myocytes or smooth muscle by IL-1 or LPS, respectively. In addition, isolated peritoneal macrophages from 12-LOX knockout mice display 50% less NO₂⁻ generation after interferon-γ/LPS challenge. Finally, oxidized LDL can have opposing effects on NO bioactivity, either through lysolecithin-dependent impairment of endothelium-dependent arterial relaxation or by causing induction of NOS2.

Conclusions: Implications for Vascular Disease

NO and NO-derived reactive species interact with lipid oxidation pathways via multiple mechanisms in vitro that are only recently being revealed. Because both processes are central to vascular regulation, an understanding of the particular interactions that are involved in pathogenesis of vascular disease in vivo is important. A role for NO acting as an antioxidant in vivo by inhibiting proatherogenic lipid oxidation is suggested, because increasing NO bioactivity through l-arginine supplementation has been successful in attenuating vascular dysfunction in hypercholesterolemic rabbits. In humans, results have been mixed, with intravenous infusion of l-arginine acutely improving coronary vasodilation but having no effect on microvascular endothelial function in patients with hypercholesterolemia. An alternative successful strategy has been to lower steady-state concentrations of NO-inactivating reactive oxygen species in animal models, via supplementation with antioxidant enzymes and oxidant scavengers. Several isoforms of PGHS and LOX are upregulated in both clinical and experimental cases of vascular disease, with inhibition of these enzymes normalizing blood pressure in some cases.

Studies of soybean LOX-1, purified rabbit reticulocyte 15-LOX, human 15-LOX in murine fibroblast PA317 cells, and porcine leukocyte 12-LOX in monocytes have shown that the reaction of NO with E₄₆₅₅ LOO⁺ results in turnover-dependent NO consumption (also M.J. Coffey and V.B. O’Donnell, unpublished data, 2001). This scavenging of NO effectively prevents activation of purified or monocyte soluble guanylyl cyclase (sGC), indicating that enzyme-bound lipid radicals can compete with the heme of sGC for NO binding and thus attenuate the bioactivity of NO in mammalian cells (Figure 8A).

Through acting as a peroxidase-reducing substrate, NO is also consumed rapidly by both purified PGHS-1 plus arachidonate and by the A23187 or thrombin-activated PGHS-1 activity of human platelets. Rates of NO removal by platelets are fast enough to deplete micromolar NO levels and potentely prevent NO-dependent activation of platelet sGC, thus causing platelets to overcome the antiaggregatory effects of NO (Figure 8B). This reveals a second novel proaggregatory function for PGHS-1 in addition to its generation of proaggregatory eicosanoids—specifically, catalytic consumption of the antiaggregatory species NO.

O’Donnell and Freeman

Vascular Nitric Oxide and Eicosanoid Signaling

This work was supported by the British Heart Foundation (V.B.O’D.) and National Institutes of Health Grants RO1-HL64937, RO1-HL58115, and P6-HL8418 (B.A.F). V.B.O’D. is a Wellcome Trust RCD Fellow.
References


Interactions Between Nitric Oxide and Lipid Oxidation Pathways: Implications for Vascular Disease
Valerie B. O'Donnell and Bruce A. Freeman

Circ Res. 2001;88:12-21
doi: 10.1161/01.RES.88.1.12

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/88/1/12

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/