A study by Jiang et al1 in this issue of Hypertension reports evidence suggesting that NO has a novel antioxidant-type effect in cultured human microvascular endothelial cells associated with an inhibition of Nox oxidase activation, which seems to be mediated by increasing the expression and activity of heme oxygenase-1 (HO-1). The induction of HO-1 was observed to occur when the cultured endothelium was exposed to a long-acting NO donor for ≥6 hours. Because freshly isolated vascular tissue from normal animals is generally thought to contain low levels of HO-1, the levels of NO seen in endothelium under physiological conditions do not seem to be a stimulus for maintaining an elevated level of this enzyme.

Physiological stresses associated with oxidant production were initially observed to increase HO-1 expression in multiple tissue preparations.2 The formation of reactive NO-derived species (NOx) from the reaction of NO with superoxide, such as peroxynitrite, were initially observed to promote HO-1 expression in endothelial cells exposed to NO donors in a manner that was modulated by free thiol availability.3 Although the precise mechanism involved in how NO induces HO-1 expression is currently not known, the gene for HO-1 has many sites in its promoter region for redox regulation by intracellular and nuclear signaling events that lead to transcriptional activation.2 Thus, increases in HO-1 expression by elevated levels of NO are likely to occur when endothelium is exposed to physiological stress or pathophysiological conditions associated with vascular disease processes, such as hypertension.

The decreased detection of superoxide associated with induction of HO-1 by NO in endothelium was investigated to assess whether it was related to alterations in the expression of Nox oxidase subunits or Cu,Zn-superoxide dismutase (SOD) or a pattern of effects that could be equated with the increased activity of HO-1.1 Inhibition of HO activity or prevention of increased HO-1 expression with small interfering RNA attenuated the effects of exposure to NO, suggesting that the catalytic activity of HO-1 and not an alternative effect of NO on superoxide scavenging is responsible for the antioxidant effects of NO that were detected. The heme-depleting activity resulting from increased HO-1 expression was observed in a previous study4 to decrease NADPH oxidase activity in macrophages through processes thought to involve impairment of protein maturation and increased degradation in its heme-containing catalytic component, the gp91phox (Nox2) subunit. However, in the present study it was observed that cellular levels of the key heme-containing Nox subunits present in endothelium, other subunits thought to regulate the Nox oxidases investigated, and Cu,Zn-SOD were not altered by the increased HO-1 expression elicited by exposure to NO.1 These data on the absence of detectible changes in Nox oxidase expression also rule out another recently reported mechanism5 involving NO donors inhibiting the expression of mRNA for Nox1. Thus, the superoxide-depressing effects of exposure of endothelium to NO seemed to be associated with an increased HO-1 activity and not with an alternative antioxidant action of NO or a change in the expression of Cu,Zn-SOD or key components of Nox oxidases present in endothelium.

The product of the metabolism of heme by the HO-1 reaction, bilirubin, was observed to have an action potentially associated with decreasing Nox oxidase activation by a p47phox-dependent mechanism,1 which could contribute to the antioxidant effects of increased HO-1 expression by NO. An early observation made in studies characterizing modulation of the activation of Nox oxidase in neutrophils was that bilirubin, a key metabolic product of increased heme metabolism by HO, interacted with a cytosolic component in a manner that inhibited the activation of superoxide production by this oxidase.6 Bilirubin has also been observed to inhibit what seems to be a growth factor activation of Nox oxidases in airway smooth muscle cells.7 Although the mechanism through which bilirubin inhibits Nox oxidase activation is not known, data in the study of Jiang et al1 suggest that this agent may interfere with the membrane binding of p47phox associated with Nox oxidase activation or possibly the protein kinase C–p47phox is likely to activate Nox oxidases in neutrophils containing Nox1 or Nox2 but not the Nox4 subunits8 of the oxidases, which are observed to be present in the endothelial cells studied.1

Overall, the results of the study of Jiang et al1 support a mechanism shown in the Figure according to which prolonged exposure to NO elicits increased expression of HO-1, and the generation of bilirubin, resulting from the metabolism of heme by this enzyme, attenuates Nox oxidase–derived superoxide production by preventing oxidase activation by p47phox-dependent pathways. Based on previous work in the literature demonstrating the potential importance of NO, in increasing the expression of HO-1 and the redox stress-associated regulation of transcriptional activation of this gene,2,3 NO derived from the reaction of superoxide with NO or the oxidation of NO are likely to mediate the increase in HO-1 expression caused by prolonged exposure of endothelium to NO.
Known effects of the HO-1 reaction, including removing the oxidant effects of heme, and additional oxidant scavenging and signaling actions of bilirubin and carbon monoxide² probably also contribute to preventing endothelial dysfunction when NO is forming as a result of exposure to NO. Thus, endothelial HO-1 induction by NO may function as a feedback protective mechanism to preserve NO-mediated endothelial regulation of vascular function. It has been observed that increasing the expression of HO-1 and processes regulated by the metabolism of heme and generation of bilirubin.

Model highlighting how a prolonged exposure of endothelium to increased levels of NO could have antioxidant effects potentially preventing endothelial dysfunction through attenuating Nox oxidase activation as a result of increased expression of HO-1 and processes regulated by the metabolism of heme and generation of bilirubin.

Sources of Funding
Our laboratories are funded by National Institutes of Health grants HL31069, HL43023, and HL66331 (M.S.W.) and HL34300 and HL55601 (N.G.A.).

References
Heme Oxygenase-1 Inhibition of Nox Oxidase Activation Is a Microvascular Endothelial Antioxidant Effect of NO
Michael S. Wolin and Nader G. Abraham

Hypertension. 2006;48:826-827; originally published online September 18, 2006;
doi: 10.1161/01.HYP.0000242338.42238.d6
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/48/5/826

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension 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 Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/