Impairment of endothelium-dependent responses is an early landmark of endothelial dysfunction in blood vessels with aging and/or cardiovascular diseases. A critical manifestation of endothelial dysfunction is the reduced bioavailability of NO, a key vascular protective molecule and an independent predictor of cardiovascular events. Hence, stimuli decreasing vascular NO bioavailability manifest impaired endothelium-dependent relaxation.

Aging is associated with marked changes in the cardiovascular system, especially at the level of the vascular wall. Both structural and functional changes can take place at the level of the endothelium. Vascular smooth muscle cell (VSMC), and the extracellular matrix of blood vessels. Endothelial thickening and altered endothelium-dependent responses have been reported in aged animals. Clinical studies have also shown that endothelium-dependent relaxation of both conduit and resistance vessels declines progressively with age. In contrast, endothelium-independent relaxation to sodium nitroprusside is unaffected by aging, suggesting that the impaired relaxation with age is primarily because of the dysfunctional endothelium, rather than VSMC contraction.

Although the mechanisms underlying the aging-induced endothelial dysfunction are complex and incompletely understood, oxidative stress is a key contributor. Exposure of endothelial cells to increased levels of reactive oxygen species (ROS) during the aging process makes them a prime target for oxidative stress. As the first and initiating oxygen free radical in the ROS chain, superoxide (O$_2^-$) consumption of NO is one of the most important mechanisms of endothelial dysfunction. Vascular cells possess multifaceted protective mechanisms against the toxic effects of ROS. Among them, the 3 superoxide dismutase (SOD) isoforms, the cytosolic copper zinc SOD (CuZnSOD, SOD-1), mitochondrial manganese SOD (MnSOD, SOD-2), and extracellular SOD (EC-SOD, SOD-3), have evolved as the key enzymatic defense system for converting O$_2^-$ to hydrogen peroxide (H$_2$O$_2$) and molecular oxygen (O$_2$). The importance of SOD on endothelial function is highlighted by studies of SOD-deficient mice that manifest profound endothelial dysfunction (Table). These different isoforms, characterized by their prosthetic metal ion and cellular localization, play distinctive roles. MnSOD knockout mice exhibit neonatal lethality because the vast majority of cellular ROS (estimated at $\approx$90%) emanate from the mitochondria. EC-SOD, the only extracellular isoform, is a copper/zinc-containing enzyme mainly secreted by VSMCs and binds to the endothelial surface via its heparin-binding domain in the extracellular matrix. MnSOD and EC-SOD play pivotal roles in the regulation of the oxidant status in the mitochondria and vascular interstitium, respectively. Consistent with this notion, recent studies have shown that gene transfer of MnSOD or EC-SOD significantly suppresses elevated vascular O$_2^-$ levels in hypertension and diabetes, respectively. The CuZn-SOD, also a copper/zinc-containing dimer SOD, is the predominant form of SOD in blood vessels, because it accounts for 50% to 80% of total SOD activity. The normal activity of CuZnSOD is necessary to limit increases in superoxide, allowing release of NO from endothelium and normal endothelium-dependent relaxation. Deficiency in CuZnSOD results in increased levels of vascular O$_2^-$ and peroxynitrite (ONOO$^-$) and subsequently impaired endothelium-dependent relaxation in large arteries and microvessels, as well as hypertrophy of cerebral arterioles. In contrast, overexpression of CuZnSOD decreases vascular O$_2^-$ in some models of cardiovascular diseases and improves endothelial function. However, in the context of the vascular aging process, very little is known about the precise role of CuZn-SOD.

In the current issue of Hypertension, Didion et al report that total SOD activity is significantly lower in aorta of young heterozygous CuZnSOD-deficient mice (ie, CuZnSOD$^{+/}$) compared with that of age-matched wild-type mice (ie, 7 month old) and aged CuZnSOD$^{+/}$ mice (ie, 22 to 24 months old), accompanied by increased O$_2^-$ levels. Endothelium-dependent relaxation of the carotid artery is markedly impaired in CuZnSOD$^{+/}$ but not wild-type mice with aging. This is the first time that striking vascular phenotype in aging heterozygous CuZnSOD-deficient mice has been described, which adds new and important insights into the mechanisms underlying CuZnSOD protection of the endothelial cell from dysfunction and cell loss. In addition, tempol (an O$_2^-$ scavenger) restores endothelium-dependent responses in CuZnSOD$^{+/}$ mice with aging, providing strong evidence that the impaired response to acetylcholine in old CuZnSOD$^{+/}$ mice is mediated by O$_2^-$ in a reversible manner. These findings suggest that normal CuZnSOD expression protects endothelial function and that deficiency in a single copy of the gene accelerates endothelial dysfunction with aging.
It is also interesting to note that serotonin-induced contractile response, but not the thromboxane analog 9,11-dideoxy-11a,9a-epoxy-methanoprostaglandin-F2a (U46619), is enhanced with aging in heterozygous CuZnSOD mice. The selectively enhanced contractile response may reflect a reduction in NO bioavailability associated with CuZnSOD deficiency and aging, because it is consistent with the similarly observed response in endothelial NO synthase–deficient mice. However, the impact of changes in expression of CuZn-SOD in an aging vessel can be more complex than simply affecting NO bioavailability. For instance, overexpression of CuZnSOD protects VSMCs from oxidized low-density lipoprotein–induced DNA fragmentation and caspase activation, resulting in decreased oxidized low-density lipoprotein–induced apoptosis. These data imply that DNA damage may be an important mediator of endothelial dysfunction. To provide further insight into the mechanism underlying the impaired endothelial-dependent response that occurs with the combination of aging and reductions in CuZnSOD, the authors then investigated the role of poly-ADP-ribose polymerase (PARP) in endothelial dysfunction in CuZnSOD−/− mice with aging. PARP, abundant in the eukaryotic nucleus, can be activated by oxidant-induced DNA injury. ROS, including O$_2^-$, ONOO$^-$, and H$_2$O$_2$, are endogenous inducers of single-strand DNA, which is the trigger of PARP activation. When single-strand DNA breaks, PARP initiates an energy-consuming cycle by transferring ADP ribose units from NAD$^+$ to form ONOO$^-$, which is a potent oxidant that can inactivate EC-SOD in extracellular space. These changes result in oxidative stress, which activates the PARP. The activated nuclear enzyme initiates an energy-consuming cycle by transferring ADP ribose units from NAD$^+$ and ATP pools, resulting in increased hypoxia sensitivity and hyperhomocysteinemia.

Possible mechanisms of partial CuZnSOD deficiency on age-induced endothelial dysfunction. The levels of O$_2^-$ are increased during aging, and the subsequent energetic failure induces endothelial cell apoptosis or necrosis, which leads to the loss and dysfunction of endothelial cells (Figure).
attenuated during the aging process. Augmented release of 
O$_2^\cdot$ and subsequent reaction with NO yielding the highly reactive oxidant ONOO$^-$ could be a key mechanism for age-associated endothelial dysfunction, which characterizes the vascular aging process. Indeed, the increased formation of reactive oxidant ONOO$^-$ during aging could inactivate antioxidative enzymes, such as MnSOD, in the mitochondria. The dismutation product of O$_2^\cdot$, H$_2$O$_2$, could attenuate the EC-SOD in extra-cellular space (Figure). The present study indicates that the impairment of vascular responses with aging in heterozygous CuZnSOD deficiency is because of aging but not the presence of potentially confounding disease states, such as atherosclerotic lesions, hypertension, and hyperglycemia. This suggests that normal CuZnSOD plays a critical role in protecting blood vessels in response to oxidative stress during aging. Future studies are needed to determine the possible mechanisms of loss of CuZnSOD with advanced aging.

**Sources of Funding**
Supported by grants from the National Institutes of Health R01 GM077352 and the American Heart Association Grant-in-Aid 0655642Z.

**Disclosures**
None.

**References**
CuZn Superoxide Dismutase Deficiency: Culprit of Accelerated Vascular Aging Process

Dan-Dan Chen and Alex F. Chen

Hypertension. 2006;48:1026-1028; originally published online October 16, 2006;
doi: 10.1161/01.HYP.0000247304.56192.ce

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/48/6/1026