Effect of Aging, MnSOD Deficiency, and Genetic Background on Endothelial Function
Evidence for MnSOD Haploinsufficiency

Kathryn A. Brown, Sean P. Didion, Jon J. Andresen, Frank M. Faraci

Objective—The goal of this study was to compare vascular function, superoxide levels, and MnSOD protein expression in young (4 to 7 months) and old (22 to 24 months) MnSOD+/+ and MnSOD-deficient (MnSOD−/−) mice.

Methods and Results—Relaxation of aorta in vitro to the endothelium-dependent dilator acetylcholine (ACh) was similar in young MnSOD+/+ (n=9) and young MnSOD−/− (n=6) mice. This response was impaired in old MnSOD+/+ (n=8) mice and old MnSOD−/− mice (n=14), with dysfunction being greater in old MnSOD-deficient mice (eg, 100 μmol/L ACh produced 77±3% [mean±SE], 77±3%, 70±4%, and 57±4% relaxation in young MnSOD+/+, young MnSOD−/−, old MnSOD+/+, and old MnSOD−/− mice, respectively). The endothelial dysfunction was similar in mice on both C57BL/6 and CD-1 genetic backgrounds. In contrast to ACh, responses to the endothelium-independent dilator sodium nitroprusside were enhanced in old MnSOD+/+ and MnSOD−/− mice compared with both groups of young mice (P<0.05). Superoxide levels, as measured using lucigenin-enhanced chemiluminescence, were increased more than 2-fold in old MnSOD−/− mice compared with old MnSOD+/+ and young mice (P<0.05).

Conclusions—These data provide the first direct evidence that MnSOD haploinsufficiency results in increased vascular oxidative stress and endothelial dysfunction with aging. (Arterioscler Thromb Vasc Biol. 2007;27:1941-1946.)

Key Words: aging • oxidative stress • endothelium • mitochondria • mice

Endothelial function decreases with age in both experimental animals and humans.1–5 However, the mechanisms producing this dysfunction have yet to be fully elucidated.1–5 Although vascular dysfunction can encompass a variety of defects in multiple homeostatic mechanisms, it is believed that decreased bioavailability of nitric oxide (NO), a major endothelium-derived relaxing factor, is a primary cause of endothelial dysfunction in disease.6

The bioactivity of NO depends, in part, on its interaction with reactive oxygen species, particularly that of superoxide anion.6 NO reacts with superoxide to form peroxynitrite at a rate 3 times faster than the dismutation of superoxide by superoxide dismutases (SODs).7 Local steady-state levels of superoxide are dependent in large part on activity of endogenous SODs, of which there are 3 isoforms: cystolic CuZn-SOD (SOD1), mitochondrial or MnSOD (SOD2), and extracellular CuZnSOD (EC-SOD, SOD3).8 Although recent studies suggest that different isoforms of SOD have distinctive roles and the effects of superoxide may be compartmentalized, little is known regarding the functional importance of individual SODs within the vessel wall in aging.5,9

MnSOD has been implicated as a key regulator of oxidative stress because homozygous MnSOD-deficient mice exhibit neonatal lethality.10,11 Although mice heterozygous for MnSOD (MnSOD+/−) appear normal, they are more susceptible to at least some forms of oxidative stress.11–13 We and others have found similar vascular responses under normal conditions in aorta in young wild-type (MnSOD+/+) and MnSOD−/− mice compared with age-matched MnSOD+/+ mice.14,15 Old MnSOD−/− mice exhibit increased oxidative stress and reduced mitochondrial function compared with age-matched MnSOD+/+ mice.11,13 Thus, vascular dysfunction may arise in old MnSOD−/− mice as the result of increased superoxide attributable to mitochondrial dysfunction combined with a decrease in antioxidant capacity.

Thus, the goals of this study were (1) to examine the hypothesis that aged mice exhibit increased vascular dysfunction and superoxide levels compared with young animals, (2) to determine whether such changes are greater in MnSOD−/− mice than in MnSOD+/+ mice, and (3) to determine whether vascular changes with aging and MnSOD status were influenced by genetic background (C57BL/6 versus CD1).

Methods

Animals
Young (4 to 7 months), old (22 to 24 months), and very old (>25 months) MnSOD−/− were used in experiments designed to charac-

1941
terize vascular responses in mouse aorta with aging. Because genetic background influences vascular function,16 young and old MnSOD−/− mice on a C57BL/6 or CD1 background were used in studies designed to determine the effect of MnSOD deficiency on vascular responses with aging. Breeding pairs of MnSOD−/− mice on a CD-1 background were kindly provided by Dr Pak Chan of Stanford University (Palo Alto, Calif); MnSOD+/+ mice on a C57BL/6 background were obtained from the Jackson Laboratories (Bar Harbor, Maine).10,17 The animals were housed under a 12-hour light-dark cycle with free access to water and food. All experimental protocols were in accordance with the Guide for the Care and Use of Laboratory Animals (NIH) and approved by the Animal Care and Use Committee of the University of Iowa. Genotyping was performed by polymerase chain reaction (PCR) of DNA from tail biopsies.14

Vasomotor Function
Mice were euthanized by intraperitoneal injection of pentobarbital sodium (150 mg/kg). The thoracic aorta was quickly removed and placed in cold Krebs buffer. After careful removal of fat and excess connective tissue, aortas were cut into rings (3 to 4 mm in length) and mounted on stainless-steel hooks at a resting tension of 0.5 g. Vascular rings were suspended in an organ bath containing oxygenated (95% O2/5% CO2) Krebs solution maintained at 37°C. Resting tension was increased gradually over a period of approximately 1 hour to reach the optimal level. Vessels were precontracted submaximally (50% to 70% of maximum) with prostaglandin F2α (PGF2α). After reaching a contractile plateau, relaxation curves were generated by cumulative addition of acetylcholine (10−7 to 10−4 mol/L) or sodium nitroprusside (10−7 to 10−4 mol/L). Acetylcholine was used to assess endothelial function, whereas nitroprusside was used to examine endothelium-independent relaxation. To determine whether aging has other vascular effects, responses to serotonin (10−4 to 10−3 mol/L) and PGF2α (3×10−7 to 10−4 mol/L) were also measured. Vessels were rinsed before and after each concentration-response curve and every 30 minutes between curves. We have used these methods in previous studies using mice.9,14,18,19

One functional consequence of increases in superoxide is activation of the nuclear enzyme poly (ADP-ribose) polymerase (PARP).20 PARP activation results in depletion of intracellular NAD+ and ATP leading to cellular dysfunction.20 In separate experiments, we examined whether PARP contributed to endothelial dysfunction observed in vessels from old MnSOD−/− mice. Responses to acetylcholine were examined in aortas from old MnSOD−/− mice incubated with vehicle or PJ34 (a selective PARP inhibitor, 1 mM). Vessels were treated for 1 hour with either vehicle or PJ34 before and during generation of concentration-response curves to acetylcholine.

Measurement of Superoxide
Measurements of superoxide were made using lucigenin-enhanced chemiluminescence, as described previously.5,8,14 Briefly, aortic rings were placed in a polypropylene tube containing 0.5 mL of PBS and lucigenin (5 μmol/L). The tube was placed in a luminometer that reported relative light units emitted. Background counts were determined from vessel-free preparations and were subtracted from the readings obtained with vessels. To normalize superoxide levels, surface area of the vessel was imaged with a video camera and calculated using the Image J program (version 1.34s, NIH), as previously described.14 To determine whether the lucigenin signal was attributable to superoxide, experiments were also performed in the presence of the superoxide dismutase mimetic, Tiron.

Immunoblotting
Aortas were homogenized in extraction buffer consisting of 1% SDS and 10 mM EDTA plus a protease inhibitor cocktail (Sigma) and boiled, with the protein extract being collected as supernatant after centrifugation. Protein concentrations were determined using a Lowry assay, and then adjusted to 2.5 mg/mL with 2× Laemmli buffer. Twenty micrograms of protein were loaded onto 4% to 20% gradient SDS-PAGE gels and electrophoresed. Proteins were then transferred to a nitrocellulose membrane using a semi-dry cell. The nitrocellulose blot was blocked with PBS containing 5% non-fat milk and 1% BSA and was incubated with rabbit anti-MnSOD antibody (1:1000) or mouse anti-eNOS antibody (1: 2500) at 4°C overnight. An HRP-conjugated secondary antibody was added (1:10 000) and incubated at room temperature for 1 hour followed by rinses with PBS. Blots were incubated with chemiluminescent substrate for 1 minute, exposed to X-ray film, and developed. Intensity of bands was determined by calculating peak area (arbitrary units) using densitometry with expression in young MnSOD+/+ considered to be 100% (1.0 arbitrary unit).

Statistical Analysis
All data are expressed as means ± SE. Data obtained from rings were averaged so that each mouse represents an “n” of 1 for statistical comparisons. Two-factor ANOVA followed by Bonferroni multiple-comparisons post-test and nonlinear regression were used to compare groups. Statistical calculations were done using GraphPad Prism 4. A probability value ≤0.05 was considered significant.

Results
Vasomotor Responses
Vascular responses to acetylcholine in old MnSOD−/− mice was impaired modestly compared with young MnSOD+/+ mice, whereas very old MnSOD−/− mice exhibited greater endothelial dysfunction compared with young and old MnSOD+/+ mice (Figure 1). Because we hypothesized that old MnSOD−/− mice would have impaired vascular function compared with age-matched MnSOD+/+ mice, 22- to 24-month-old mice were used for all subsequent experiments. In MnSOD mice on a C57BL/6 background, relaxation to acetylcholine was similar in aorta from young MnSOD+/+ and MnSOD−/− mice, consistent with previous reports.14,15 Responses of aorta from old MnSOD−/− mice to acetylcholine were impaired compared with young mice, and endothelial dysfunction was significantly greater in old MnSOD−/− mice (Figure 2, left). A similar pattern was observed in MnSOD mice on a CD1 background (Figure 2, right), suggesting that the effect of MnSOD deficiency with aging is independent of the influence of genetic background. Treatment with PJ34 had no effect on responses to acetylcholine in old MnSOD−/−
mice (supplemental Figure I, available online at http://atvb.ahajournals.org), suggesting that endothelial dysfunction in old MnSOD+/− mice may involve mechanisms other than PARP activation or that the dysfunction cannot be reversed acutely.

Responses to sodium nitroprusside (SNP) were similar in aortas from young MnSOD+/+ and MnSOD+/− mice on both genetic backgrounds (Figure 3). Old C57BL/6 and CD1 MnSOD+/+ and MnSOD+/− mice exhibited enhanced responses to submaximal doses of nitroprusside that were significantly greater when compared with the young mice (Figure 3).

Contractile responses to serotonin were significantly augmented in old MnSOD−/− mice on a C57BL/6 background and a further increase in contraction in the old mice heterozygous for MnSOD was observed (Figure 4). A similar trend was observed with the vasoconstrictor PGF2α, aorta from old MnSOD+/+ mice on a C57BL/6 background had an increase in contraction to PGF2α compared with young mice, whereas aorta from old MnSOD+/− mice contracted significantly more (Figure 5).

**Superoxide Levels**

Superoxide levels were similar in aorta from young MnSOD+/+ and young MnSOD+/− mice on a C57BL/6 background (Figure 6). In contrast, measurements of superoxide in aorta from old MnSOD−/− mice were significantly higher than in old MnSOD+/+ and young mice (Figure 6).

**Protein Expression**

We have shown previously that the level of MnSOD protein in the vasculature is approximately 50% of normal in young MnSOD+/− mice.14 In this study, we examined MnSOD levels in aorta from old mice and found that MnSOD protein expression was decreased by 50% to 60% in aortas from old MnSOD−/− mice compared with old MnSOD+/+ mice (Figure 6).

Levels of eNOS protein in aorta were similar in young wild-type and MnSOD−/− mice (Figure 6). In agreement with
The degree of endothelial dysfunction increased further in blood vessels with aging. The impairment in NO-mediated responses appears to be attributable to an increase in superoxide and subsequent reduction in NO bioavailability, as previous studies suggest a causal relationship between vascular dysfunction and an increase in ROS levels.6,9,10,28 Our data support this hypothesis, as superoxide levels were significantly increased in old MnSOD−/− mice and this increase could be inhibited with a scavenger of superoxide.

Multiple pathways may be affected by chronic oxidative stress and may account for the impairment of vascular function in old MnSOD−/− mice.1,20 To address one such possibility, we examined the role of PARP. Previously, we found that treatment of carotid arteries with a PARP inhibitor improved endothelial function in old CuZnSOD−/− mice.9 In contrast, treatment of blood vessels from MnSOD−/− mice with the same PARP inhibitor did not improve endothelial function in the present study. The reason for this difference is not clear. There is increasing evidence that ROS and their effects are compartmentalized, such that the cellular impact of CuZnSOD deficiency is not the same as that produced by MnSOD deficiency. Although the present results were negative, there may be an acceleration of vascular dysfunction in latter life as the animal ages. Such a decline is consistent with the frequency of cardiovascular events known to occur in humans. For example, stroke and cerebral vascular events occur at a relatively low rate until approximately age 60.23 However, over the next 2 decades, the rate of stroke increases markedly.23

Consistent with previous work in aorta, we observed no differences in vascular function or superoxide levels between young MnSOD−/+ and MnSOD−/− mice.1,14,15 Endothelial dysfunction is a predominant feature of aging, and emerging evidence suggests that endothelial dysfunction during aging may be related to the formation of reactive oxygen and nitrogen species.9,21,24,25 Because mice heterozygous for MnSOD maybe more susceptible to some forms of oxidative damage (evidenced by markers such as increased tumor formation and apoptosis),11,26,27 we expected to see an age-related reduction of vascular function that would be exacerbated by MnSOD deficiency. Based on data from 22- to 24-month-old MnSOD−/+ mice, we hypothesized that superoxide levels and vascular dysfunction would be greater in MnSOD−/− mice at that age. Using this design, we found that old MnSOD−/− mice exhibited a larger increase in vascular superoxide and a greater reduction in the response to the endothelium-dependent vasodilator acetylcholine compared with wild-type. We did not examine vascular changes in very old MnSOD−/− mice (>25 months of age). However, based on available data, we would expect that differences in vascular superoxide and functional responses between MnSOD−/+ and MnSOD−/− mice would be maintained, or perhaps become even greater.

Previous studies have shown that endothelial function varies depending on the strain of mice studied.1,19 As there continues to be increasing use of genetically-altered mice in studies of vascular biology and vascular disease, the observation that endothelial function is present in old MnSOD−/− mice on 2 different genetic backgrounds strengthens the overall findings. The impairment in NO-mediated responses appears to be attributable to an increase in superoxide and subsequent reduction in NO bioavailability, as previous studies suggest a causal relationship between vascular dysfunction and an increase in ROS levels.6,9,10,28 Our data support this hypothesis, as superoxide levels were significantly increased in old MnSOD−/− mice and this increase could be inhibited with a scavenger of superoxide.

Multiple pathways may be affected by chronic oxidative stress and may account for the impairment of vascular function in old MnSOD−/− mice.1,20 To address one such possibility, we examined the role of PARP. Previously, we found that treatment of carotid arteries with a PARP inhibitor improved endothelial function in old CuZnSOD−/− mice.9 In contrast, treatment of blood vessels from MnSOD−/− mice with the same PARP inhibitor did not improve endothelial function in the present study. The reason for this difference is not clear. There is increasing evidence that ROS and their effects are compartmentalized, such that the cellular impact of CuZnSOD deficiency is not the same as that produced by MnSOD deficiency. Although the present results were negative, there may be an acceleration of vascular dysfunction in latter life as the animal ages. Such a decline is consistent with the frequency of cardiovascular events known to occur in humans. For example, stroke and cerebral vascular events occur at a relatively low rate until approximately age 60.23 However, over the next 2 decades, the rate of stroke increases markedly.23

Consistent with previous work in aorta, we observed no differences in vascular function or superoxide levels between young MnSOD−/+ and MnSOD−/− mice.1,14,15 Endothelial dysfunction is a predominant feature of aging, and emerging evidence suggests that endothelial dysfunction during aging may be related to the formation of reactive oxygen and nitrogen species.9,21,24,25 Because mice heterozygous for MnSOD maybe more susceptible to some forms of oxidative damage (evidenced by markers such as increased tumor formation and apoptosis),11,26,27 we expected to see an age-related reduction of vascular function that would be exacerbated by MnSOD deficiency. Based on data from 22- to 24-month-old MnSOD−/+ mice, we hypothesized that superoxide levels and vascular dysfunction would be greater in MnSOD−/− mice at that age. Using this design, we found that old MnSOD−/− mice exhibited a larger increase in vascular superoxide and a greater reduction in the response to the endothelium-dependent vasodilator acetylcholine compared with wild-type. We did not examine vascular changes in very old MnSOD−/− mice (>25 months of age). However, based on available data, we would expect that differences in vascular superoxide and functional responses between MnSOD−/+ and MnSOD−/− mice would be maintained, or perhaps become even greater.

Previous studies have shown that endothelial function varies depending on the strain of mice studied.1,19 As there continues to be increasing use of genetically-altered mice in studies of vascular biology and vascular disease, the observation that endothelial function is present in old MnSOD−/− mice on 2 different genetic backgrounds strengthens the overall findings. The impairment in NO-mediated responses appears to be attributable to an increase in superoxide and subsequent reduction in NO bioavailability, as previous studies suggest a causal relationship between vascular dysfunction and an increase in ROS levels.6,9,10,28 Our data support this hypothesis, as superoxide levels were significantly increased in old MnSOD−/− mice and this increase could be inhibited with a scavenger of superoxide.

Multiple pathways may be affected by chronic oxidative stress and may account for the impairment of vascular function in old MnSOD−/− mice.1,20 To address one such possibility, we examined the role of PARP. Previously, we found that treatment of carotid arteries with a PARP inhibitor improved endothelial function in old CuZnSOD−/− mice.9 In contrast, treatment of blood vessels from MnSOD−/− mice with the same PARP inhibitor did not improve endothelial function in the present study. The reason for this difference is not clear. There is increasing evidence that ROS and their effects are compartmentalized, such that the cellular impact of CuZnSOD deficiency is not the same as that produced by MnSOD deficiency. Although the present results were negative, there may be an acceleration of vascular dysfunction in latter life as the animal ages. Such a decline is consistent with the frequency of cardiovascular events known to occur in humans. For example, stroke and cerebral vascular events occur at a relatively low rate until approximately age 60.23 However, over the next 2 decades, the rate of stroke increases markedly.23

Consistent with previous work in aorta, we observed no differences in vascular function or superoxide levels between young MnSOD−/+ and MnSOD−/− mice.1,14,15 Endothelial dysfunction is a predominant feature of aging, and emerging evidence suggests that endothelial dysfunction during aging may be related to the formation of reactive oxygen and nitrogen species.9,21,24,25 Because mice heterozygous for MnSOD maybe more susceptible to some forms of oxidative damage (evidenced by markers such as increased tumor formation and apoptosis),11,26,27 we expected to see an age-related reduction of vascular function that would be exacerbated by MnSOD deficiency. Based on data from 22- to 24-month-old MnSOD−/+ mice, we hypothesized that superoxide levels and vascular dysfunction would be greater in MnSOD−/− mice at that age. Using this design, we found that old MnSOD−/− mice exhibited a larger increase in vascular superoxide and a greater reduction in the response to the endothelium-dependent vasodilator acetylcholine compared with wild-type. We did not examine vascular changes in very old MnSOD−/− mice (>25 months of age). However, based on available data, we would expect that differences in vascular superoxide and functional responses between MnSOD−/+ and MnSOD−/− mice would be maintained, or perhaps become even greater.

Previous studies have shown that endothelial function varies depending on the strain of mice studied.1,19 As there continues to be increasing use of genetically-altered mice in studies of vascular biology and vascular disease, the observation that endothelial function is present in old MnSOD−/− mice on 2 different genetic backgrounds strengthens the overall findings. The impairment in NO-mediated responses appears to be attributable to an increase in superoxide and subsequent reduction in NO bioavailability, as previous studies suggest a causal relationship between vascular dysfunction and an increase in ROS levels.6,9,10,28 Our data support this hypothesis, as superoxide levels were significantly increased in old MnSOD−/− mice and this increase could be inhibited with a scavenger of superoxide.

Multiple pathways may be affected by chronic oxidative stress and may account for the impairment of vascular function in old MnSOD−/− mice.1,20 To address one such possibility, we examined the role of PARP. Previously, we found that treatment of carotid arteries with a PARP inhibitor improved endothelial function in old CuZnSOD−/− mice.9 In contrast, treatment of blood vessels from MnSOD−/− mice with the same PARP inhibitor did not improve endothelial function in the present study. The reason for this difference is not clear. There is increasing evidence that ROS and their effects are compartmentalized, such that the cellular impact of CuZnSOD deficiency is not the same as that produced by MnSOD deficiency. Although the present results were negative, there may be an acceleration of vascular dysfunction in latter life as the animal ages. Such a decline is consistent with the frequency of cardiovascular events known to occur in humans. For example, stroke and cerebral vascular events occur at a relatively low rate until approximately age 60.23 However, over the next 2 decades, the rate of stroke increases markedly.23
erozygous for MnSOD have approximately half normal is lethal in mice provides some support for this concept.8 complete deficiency in MnSOD, but not CuZnSOD or ECSOD, during aging than deficiency in other SODs. The fact that involvement, and deficiency in MnSOD may be more detrimental acutely. Alternatively, mechanisms other than PARP may be involved, and deficiency in MnSOD may be more detrimental during aging than deficiency in other SODs. The fact that complete deficiency in MnSOD, but not CuZnSOD or ECSOD, is lethal in mice provides some support for this concept.8 Our finding that eNOS protein expression in the vasculature is normal or tended to increase with age is consistent with previous findings.21,22 The fact that eNOS expression was not reduced is consistent with the concept that endothelial dysfunction with aging is attributable to increased superoxide and inactivation of NO rather than reduced production of NO by eNOS. However, our findings do not exclude the possibility that reductions in eNOS activity may have occurred or the possibility that eNOS uncoupling was present such that eNOS became a source of superoxide. Previous studies of aging using aorta suggest that levels of MnSOD are comparable to or slightly higher in old compared with young animals.3,21,22 Much of the ROS production in normal cells is a byproduct of the mitochondrial energy production.29 Mitochondrial respiratory function declines with age, and defects in the respiratory chain increase the production of ROS in mitochondria.11,30 Because mice heterozygous for MnSOD have approximately half normal MnSOD protein,14 it is conceivable that antioxidant capability decreases more dramatically with age in MnSOD+/− mice because of a subsequent rise in superoxide and overall oxidative damage. In contrast to endothelial-dependent relaxation, old MnSOD+/− and MnSOD+/− mice had similar responses to the endothelial-independent vasodilator nitroprusside, and this response was augmented compared with young mice. The mechanism(s) that might account for this increased sensitivity is not certain at this point. Alterations in the expression or activity of soluble guanylate cyclase (or responsiveness of the enzyme to NO) may occur with aging as a compensatory mechanism related to reduced NO bioavailability. Though we did not examine this possibility directly, previous studies found that responses to NO are enhanced under conditions of reduced NO bioavailability.18,31,32 Contractile responses of aorta to serotonin were greater in old mice than in young mice, with aorta from old MnSOD+/− mice constricting to a greater extent than old MnSOD+/− mice. Such a difference could potentially be attributable to altered expression of receptors or loss of the inhibitory effect of basal NO or expression of other vasoconstrictor mechanisms, such as production of an endothelium-derived contracting factor(s).33,34 In addition, responses to PGF2α or serotonin may reflect enhanced signaling mechanisms within vascular muscle such as Rho kinase or protein kinase C.35
In conclusion, we have shown that MnSOD protects against increased superoxide and vascular dysfunction with aging in mice on 2 different genetic backgrounds. While endothelial dysfunction was present in old mice lacking one copy of the MnSOD gene (an example of haploinsufficiency), superoxide levels and vasomotor dysfunction were less in age-matched MnSOD—/— mice. These data suggest an important protective role for MnSOD during aging. Previous findings have shown that heterozygous MnSOD deficiency increases oxidative stress and atherosclerosis in hyperlipidemic mice. The present study extends this concept by providing proof of concept that MnSOD deficiency increases vascular oxidative stress and dysfunction with age. Our results may have implications for disease states or genetic polymorphisms that result in decreased expression and/or activity of MnSOD.

Sources of Funding

K.A.B. was supported by a Predoctoral Fellowship from the American Heart Association (AHA; 0410063Z). Studies were supported by NIH grants HL-38901, HL-62984, and NS-24621, as well as a Beginning Grant-in-Aid (0565486Z) and a Bugher Foundation Award in Stroke (0575092N) from the AHA.

Disclosures

F.M.F. has a pending research grant exceeding a value of $10,000.

References

Effect of Aging, MnSOD Deficiency, and Genetic Background on Endothelial Function: Evidence for MnSOD Haploinsufficiency
Kathryn A. Brown, Sean P. Didion, Jon J. Andresen and Frank M. Faraci

Arterioscler Thromb Vasc Biol. 2007;27:1941-1946; originally published online June 7, 2007;
doi: 10.1161/ATVBAHA.107.146852
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272
Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://atvb.ahajournals.org/content/27/9/1941

Data Supplement (unedited) at:
http://atvb.ahajournals.org/content/suppl/2007/06/11/ATVBAHA.107.146852.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology 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 Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/
Supplemental Figure.

Figure Legend. Responses to acetylcholine in aorta from old MnSOD +/- mice in the absence and presence of PJ34. Data are expressed as means ± SE.