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Redox Balance in the Aging Microcirculation: New Friends, New Foes, and New Clinical Directions

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Abstract

Cardiovascular aging is associated with a decline in the function of the vascular endothelium. Considerable evidence indicates that age-induced impairment of endothelium-dependent vasodilation results from a reduction in the availability of nitric oxide ($NO^{\bullet}$). $NO^{\bullet}$ can be scavenged by reactive oxygen species (ROS), in particular by superoxide radical ($O_2^{\bullet -}$), and age-related increases in ROS have been demonstrated to contribute to reduced endothelium-dependent vasodilation in numerous large artery preparations. In contrast, emerging data suggest that ROS may play a compensatory role in endothelial function of the aging microvasculature. The primary goal of this review is to discuss reports in the literature which indicate that ROS function as important signaling molecules in the aging microvasculature. Emphasis is placed upon discussion of the emerging roles of hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^{\bullet -}$) in the aging microcirculation. Overall, existing data in animal models suggest that maintenance in the balance of ROS is critical to successful microvascular aging. The limited work that has been performed to investigate the role of ROS in human microvascular aging is also discussed, and the need for future investigations of ROS signaling in older humans is considered.

Keywords

microvasculature; reactive oxygen species; nitric oxide; hydrogen peroxide; peroxynitrite

INTRODUCTION

Healthy aging, from the microvascular standpoint, is associated with endothelial health and redox balance [23,74], A decline in the function of the endothelium occurs with advancing age. This decline of function manifests as reduced angiogenic capacity, alteration of expression of adhesion molecules that regulate interaction with circulating factors and cells

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of the immune system, and impaired vasodilatory function. The well-documented loss of endothelium-dependent vasodilation that occurs with advancing age is present in both conduit arteries and resistance arterioles. Animal models have been used to characterize this loss of endothelium-dependent vasodilation and to define the mechanisms that underlie it. The preponderance of data obtained in animal models indicate that age-related endothelial dysfunction of the microcirculation occurs due to decreased availability of $NO^{\bullet}$ [15,60,84,89].

$NO^{\bullet}$ BIOAVAILABILITY IN THE AGED ENDOTHELIUM

Vasodilatory responses that are inhibited by NOS blockade have been reported to decline with age in arterioles from coronary [14,41,42], skeletal muscle [60,84,91,96], cerebral [55], and mesenteric [87] vascular beds. In resistance arteries of skeletal muscle, age-related reduction of $NO^{\bullet}$-dependent vasodilation is accompanied by reduced expression of eNOS [96]. In contrast, $NO^{\bullet}$-mediated dilation of soleus muscle resistance arteries declines with advancing age despite an increase in eNOS protein levels [84], Thus, the age-related decline in bioavailability of $NO^{\bullet}$ may be dependent upon numerous factors that regulate both its production and degradation. Parallel findings have been reported in studies of the human microcirculation, obtained indirectly through measures of flow-mediated vasodilation or more directly through study of the skin microcirculation [11,34,66].

The eNOS activity is regulated by availability of substrate and cofactors, by protein−protein interactions, and by coordinated phosphorylation and dephosphorylation [22,25,31]. In the absence of sufficient levels of the cofactor, tetrahydrobiopterin, uncoupled eNOS can produce $O_2^{\bullet-}$. Degradation of $NO^{\bullet}$ is heavily dependent upon the presence of cellular $O_2^{\bullet-}$, a by-product of cellular respiration, which reacts readily with $NO^{\bullet}$, eliminating its vasodilatory action [82], Increased production of superoxide ions has been reported to reduce $NO^{\bullet}$ availability in coronary, skeletal muscle, and mesenteric arterioles of aged rats [14,20,56,87,92],

$O_2^{\bullet-}$-DERIVED REACTIVE OXYGEN SPECIES: ROLE IN ENDOTHELIAL SIGNALING WITH ADVANCING AGE

Although many studies of the aging vasculature indicate that increased production of ROS contributes to endothelial dysfunction, both $O_2^{\bullet-}$ and $O_2^{\bullet-}$-derived ROS exhibit vasoactive properties. As early as 1996, Wei *et al.* [95] demonstrated that superoxide and reactive species derived from superoxide relaxed cat cerebral vessels. Cellular $O_2^{\bullet-}$ is regulated by SOD, which catalyzes the dismutation of $O_2^{\bullet-}$ into H_2O_2. H_2O_2 has also been reported to produce membrane hyperpolarization of vascular smooth muscle, leading to reduced calcium entry through voltage-gated calcium channels, and subsequent vasorelaxation of arteries in various vascular beds [54,58]. Furthermore, H_2O_2 regulates eNOS protein expression and activity [32,90]. In addition, $ONOO^{\bullet-}$, formed from the reaction of $O_2^{\bullet-}$ with $NO^{\bullet}$, may cause relaxation through two mechanisms: (1) generation of $NO^{\bullet}$ and activation of guanylate cyclase in smooth muscle [43,63,64,71], and (2) hyperpolarization of smooth muscle [43,65]. Although the vasoactive and signaling properties of these ROS have been well-documented, relatively little work has been performed to determine whether or not

these molecules can compensate for an age-related decline in $NO^{\bullet}$-mediated vasodilation. In particular, clinical studies have only begun to consider two important possibilities regarding the role of ROS in the loss and/or maintenance of endothelium-dependent vasodilation that occurs with advancing age. The first possibility that deserves consideration is that tight regulation of the balance of ROS is more critical to preservation of endothelium-dependent function in the aged vasculature than the absolute levels of any specific molecule or enzyme. The second possibility that warrants investigation is that ROS can act as vasodilatory signaling molecules that compensate for an age-induced reduction in $NO^{\bullet}$ signaling. Although such compensatory signaling may be less efficient than vasodilation mediation by authentic $NO^{\bullet}$, elimination of these compensatory pathways may prove detrimental in an aged vasculature where $NO^{\bullet}$ production is reduced.

Work performed in animal models provides limited evidence that a balance in ROS signaling is critical to successful cardiovascular aging. Although it is clear that overproduction of ROS can lead to endothelial dysfunction in the microvasculature [14], evidence also exists to indicate that regulated production of both H_2O_2 and $ONOO^{\bullet-}$ can contribute to endothelium-dependent vasodilation in the aged vasculature [39,40], which may be linked to SOD activity through at least three vasodilatory pathways. As shown in Figure 1, dismutation of $O_2^{\bullet-}$ could (1) increase levels of vasodilatory $NO^{\bullet}$, (2) increase levels of vasodilatory H_2O_2, and (3) reduce levels of vasodilatory $ONOO^{\bullet-}$. Dismutation of $O_2^{\bullet-}$ could also indirectly alter vasoactive signaling pathways by (1) increasing levels of highly reactive hydroxyl radical $HO^{\bullet}$ if the rate of dismutation of $O_2^{\bullet-}$ into H_2O_2 exceeds that rate of conversion of H_2O_2 to H_2O by catalase or glutathione peroxideases, or (2) reducing levels of $ONOO^{\bullet-}$ that act as donors of $NO^{\bullet}$. Thus, tight regulation of $0_2^{\bullet-}$ is necessary for maintenance of optimal endothelium-dependent function, and age-related alterations in the balance of activity between eNOS, SOD, and catalase are likely contributors to age-induced endothelial dysfunction. Alternatively, because age-induced changes in vascular signaling occur over an extended time course, alterations in the relative activity of SOD and catalase could compensate for reduced eNOS-mediated production of authentic $NO^{\bullet}$. For example, in coronary arterioles from old and young female rats, treatment with either the SOD mimetic, Tempol (Sigma, St. Louis, MO, USA), or with catalase reduced flow-induced vasodilation and eliminated age-related differences in the maximal response to flow [39]. Treatment with the Cu/Zn SOD inhibitor, diethyldithiocarbamate, enhanced flow-induced vasodilation in arterioles from both young and old rats but did not eliminate age-related differences in flow-induced vasodilation. These findings suggest that with age, the dependence on H_2O_2-mediated vasodilation increases in coronary arterioles, although an $ONOO^{\bullet-}$ component of the dilation persists. In contrast, in skeletal muscle arterioles from rats, H_2O_2-mediated vasodilation to flow decreases with age [40,85].

The source of ROS that act as signaling molecules in the aged microvascular endothelium has not been definitively determined; however, recent reports indicate that an imbalance of ROS is a critical contributor to age-induced endothelial dysfunction in rodents [40,78,92], Trott *et al* [92] reported that either inhibition of NAD(P)H oxidase or scavenging of $O_2^{\bullet-}$ improved endothelial function in skeletal muscle feed arteries of aged rats. These results imply that either overproduction of $0_2^{\bullet-}$ or inadequate scavenging of $O_2^{\bullet-}$ contributes to endothelial dysfunction with age. In contrast, scavenging of endogenous $O_2^{\bullet-}$ by addition of

exogenous SOD reduced endothelium-dependent vasodilation in arteries from young rats [92], Similarly, scavenging of $O_2^{\bullet-}$ with Tempol impaired flow-mediated vasodilation in coronary arterioles from young but not old rats, indicating that the contribution of this ROS to endothelium-dependent vasodilation changes with age [40]. In coronary arterioles from old rats, endogenous SOD protein increased significantly but this increase in SOD was not paralleled by a rise in catalase protein, resulting in an imbalance of these antioxidant enzymes and overproduction of H_2O_2 [40]. These results suggest that balanced activity of antioxidant enzymes is necessary for maintenance of endothelial function with advancing age.

Recent work also indicates that successful maintenance of endothelial function is critically dependent upon the ability to maintain antioxidant defense mechanisms [45,93,94], Relocation of SOD-1 to the endothelial mitochondria has been reported to function as a compensatory mechanism that counters increased ROS production in the aged aorta [45], eNOS upregulation in large arteries has also been reported to parallel the age-related increase in $0_2^{\bullet-}$ that occurs in rat aorta [46], NAD(P)H oxidase- derived ROS may act as intercellular regulators of the redox-sensitive transcription factors HIF-lα and Nrf2, and their target genes including NQOl, γ-glutamylcysteine synthetase, and HO-1 [94], In aortic endothelial cells, advanced glycation end products evoke ROS generation and activate Nrf2-dependent expression of HO-1 and NQOl, providing evidence of adaptive Nrf-2-mediated protection against oxidative stress in diabetes [33], Increased ROS production by the mitochondria, xanthine oxidase, and uncoupled eNOS may also activate these transcription factors leading to upregulation of antioxidant enzymes; however, with age the responsiveness of redox- sensitive transcription factors wanes in the aorta and carotid arteries [93,94], Together, these findings suggest that an age-related decline in the ability to activate endogenous antioxidant mechanisms contributes to increased endothelial inflammation and apoptosis in large arteries. Future work will be needed to determine whether or not the function of endogenous antioxidant defense mechanisms declines in the microvascular endothelium with advancing age. The impact of an age-related decline in endogenous antioxidant mechanisms on angiogenesis, endothelium- dependent vasodilation, and microvascular permeability remains to be assessed in the microvasculature.

SIGNALING ROLES OF H_2O_2 IN THE AGING ENDOTHELIUM

In contrast to $O_2^{\bullet-}$, H_2O_2 is not a free radical (i.e., unpaired electrons on an open shell configuration), making it less reactive, more stable and longer lasting [2], These properties and the ability of H_2O_2 to diffuse across cell membranes allow it to play an important signaling role. H_2O_2 is primarily produced by the dismutation of $O_2^{\bullet-}$ by SOD, but can also be formed by the spontaneous dismutation of $O_2^{\bullet-}$, or directly by the action of enzymes such as xanthine oxidase, glucose oxidase [7], and NADPH oxidase [17,51,72,76]. H_2O_2 is found in both physiological and pathophysiological states. In aging, H_2O_2 production is increased [13,48] possibly due to age-related increases in mitochondrial H_2O_2 generation [79–81] and eNOS dependent $O_2^{\bullet-}$ generation [4],

H_2O_2 does not inactivate $NO^{\bullet}$ and in conditions of oxidant stress, H_2O_2 may act as a compensatory mechanism to maintain $NO^{\bullet}$ bioavailability. H_2O_2 has been shown to cause a

potent dose-dependent increase in $NO^{\bullet}$ production [9], upregulate eNOS expression [8,19], and to enhance eNOS function by promoting eNOS phosphorylation and eNOS dephosphorylation at Thr-495 [90]. Recently, Martin-Garrido *et al* [50] demonstrated th–at H_2O_2 enhances vascular relaxation to NO by stabilizing sGCβ1 mRNA through HuR, increasing the expression of sGCβ1 and thus increasing cGMP formation. However, Gerassimou *et al.* [27] showed that higher concentrations of H_2O_2 downregulated sGCα1 mRNA indicating that the levels of H_2O_2 may dictate its action.

The impact of H_2O_2 on vascular function is complex depending on the species, vascular bed and experimental protocol (exogenous vs. endogenous H_2O_2, localization, and concentrations). Several studies have proposed that H_2O_2 is an EDHF [52,53,58,59,77]. H_2O_2 produces vasorelaxation in various murine, porcine, and human vessels via either endothelium-dependent or endothelium-independent mechanisms [3,5,6,24,37,44,47,75,98,99] but in some studies H_2O_2 causes vasoconstriction [26,38,47,68,73,83,100]. H_2O_2 is required for flow-induced increases of $NO^{\bullet}$ [40] and flow-mediated dilation [58]. Overexpression of NAD(P)H oxidase in transgenic mice predominately increases H_2O_2 levels and exerts beneficial effects on vasodilator function and blood pressure due to H_2O_2 production [72], In coronary ischemia/reperfiision injury endogenous H_2O_2 contributes *in vivo* to coronary vasodilation to compensate for the loss of $NO^{\bullet}$ and plays a cardioprotective role, particularly in microvessels [97].

H_2O_2 that functions in endothelial signaling may be derived from several sources, depending on physiological conditions. In skeletal muscle arterioles exposed to intraluminal flow, both age and exercise training increased eNOS- derived $O_2^{\bullet-}$ signaling; this elevation in eNOS-derived $O_2^{\bullet-}$ was accompanied by an increase in catalase-sensitive vasodilation, suggesting that eNOS-derived $O_2^{\bullet-}$ constituted the source of vasodilatory H_2O_2 [78]. In contrast, in skeletal muscle arterioles from both young and old rats, stimulation with acetylcholine produces catalase-sensitive vasodilation that is abolished by treatment with either apocynin or an inhibitor of gp91phox (Sindler, A.L., Muller-Delp, J.M, unpublished observations). In cerebral arterioles of aged rats, both p67phox and gp91phox proteins increased, with accompanying impairment of endothelial function, suggesting that NAD(P)H-derived $O_2^{\bullet-}$ is not transformed to vasodilatory H_2O_2 [55]. In the aged myocardium, H_2O_2 is generated by the electron transport chain of myocytes, and because it is freely diffusible, produces metabolic vasodilation of coronary arterioles [48]. Thus, the cellular sources of H_2O_2 vary between arterioles from distinct vascular beds. In future work, identifying the sources of ROS generation may provide insight into therapeutic targets for prevention and/or remediation of age- related vascular dysfunction.

ROLE OF CYTOTOXIC HYDROXYL RADICAL IN THE AGING ENDOTHELIUM

SOD reduces oxidant stress by dismutating $O_2^{\bullet-}$ into H_2O_2; however, in the presence of catalytic transition metals, SOD can rapidly form $HO^{\bullet}$ [67]. H_2O_2 generates $HO^{\bullet}$ through metal-catalyzed reactions, such as the Fenton reaction as follows: $H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + HO^{\bullet} + OH^{-}$. The formation of $HO^{\bullet}$ is further promoted by the presence of $O_2^{\bullet-}$, which reacts with Fe^{3+} to produce Fe^{2+} through the Haber-Weiss reaction [29,70]. The net effect of SOD is the dismutation of $O_2^{\bullet-}$ to produce either the vasodilatory H_2O_2, or in the presence of

Fe^{2+}, $HO^{\bullet}$. This production of $HO^{\bullet}$ may occur more readily if the production of H_2O_2 exceeds the enzymatic capacity of endogenous catalase or peroxidases. In coronary arterioles from both young and old rats, addition of the SOD-mimetic, Tempol, reduced flow-induced vasodilation, and addition of the iron chelating agent, deferoxamine, reversed this Tempol-mediated inhibition of endothelium-dependent dilation [40]. These results suggest that treatment with exogenous SOD may drive overproduction of H_2O_2 and promote formation of HO* in the endothelium. Deferoxamine alone reversed impairment of flow-induced vasodilation in coronary arterioles from old rats, but had no effect on arterioles from young rats [40], suggesting that flow stimulates production of $HO^{\bullet}$ in arterioles from old but not young rats. Similarly, deferoxamine reversed Tempol-induced reduction of flow- induced vasodilation in skeletal muscle of old rats [78]. Together these data suggest that although H_2O_2 may function as an important endothelium-dependent vasodilator, production of H_2O_2 that exceeds the buffering capacity of the endothelium can impair endothelial function, and this is likely due to excess production of $HO^{\bullet}$. The age-related increase in production of $HO^{\bullet}$ could result from (1) an age-associated decrease in the activities of catalase and/or peroxidases in the endothelium, (2) an age-induced increase in the activity of SOD isoforms, or (3) increased accumulation of Fe^{2+} in the aged endothelium. It is also possible that accumulation of Fe^{2+} is accompanied by a relative imbalance in the activities of SOD and catalase.

AGE-INDUCED ALTERATIONS OF $NO^{\bullet}$ BIOAVAILABILITY IN HUMANS

Several *in vivo* models have been used to study vascular aging in humans. Doppler methods for determination of cutaneous blood flow and blood flow in large/medium size upper body arteries are the most commonly employed models [1,11,28,36]. In general, these models have assessed the participation of $NO^{\bullet}$ in vascular reactivity using NOS inhibition (i.e., L-NAME or L-NNMA). Interestingly, these studies have shown conflicting results, which could be associated with differences in the vascular beds being studied and differences in the stimuli employed to trigger vasodilation, e.g., acetylcholine vs. cuff occlusion methods.

Both Green *et al.* [28] and Casey *et al.* [11] have shown an age-dependent decrease in $NO^{\bullet}$-mediated forearm blood flow during exercise. In contrast, Holowatz *et al.* [34,35] have shown an increase in $NO^{\bullet}$-dependent, cutaneous vasodilation in the elderly. Despite these conflicting results, all these studies concluded that reduced $NO^{\bullet}$ bioavailability would be the principal cause of age-related impairment of vascular reactivity [11,34,35].

Compensatory vasodilation that occurs in response to a stressor such as hypoxic exercise is blunted in aged subjects [10,11]. Casey *et al.* [11] reported that eNOS inhibition reduced the vascular response to hypoxemic exercise in young but not in old subjects, suggesting that the age- related reduction of this vasodilatory response occurred as a result of impaired $NO^{\bullet}$ signaling. The hypoxic stress produced greater vasodilation in young subjects; however, there were no differences in forearm blood flow and vascular conductance between old and young subjects [11]. These results could indicate that healthy aging involves arterial remodeling, such as increased brachial diameter [16,18,66], thereby providing a compensatory mechanism for the impairment of $NO^{\bullet}$ signaling. Similar observations have been shown using the skin blood flow model [34], Although $NO^{\bullet}$-dependent cutaneous

vasodilation was impaired in the elderly, there was no significant difference in the reflex cutaneous vasodilation threshold between old and young subjects [34], Unfortunately, due to the relative nature of Laser-Doppler probes, cutaneous raw blood flow cannot be used to assess age-related structural changes, lArginine supplementation and arginase inhibition improve thermoregulatory cutaneous vasodilation in the elderly, confirming the $NO^{\bullet}$-dependency of this age-related alteration in vascular reactivity [35].

Although the aforementioned studies suggest that $NO^{\bullet}$ availability is impaired in the elderly, a recent study [21] has shown that cellular signaling downstream of $NO^{\bullet}$, i.e., activation of cAMP and cGMP, is preserved in smooth muscle cells of older subjects. Therefore, we could speculate that $NO^{\bullet}$ production is blunted in the elderly, whereas $NO^{\bullet}$ bioavailability is not decreased. Vascular structural changes observed in the elderly [16,18,66] may also impact $NO^{\bullet}$-dependent vasodilation. Increased basal and submaximal blood flow through larger vessels may compensate for impaired reactivity and a decrease in the shear stress- induced endothelial $NO^{\bullet}$ production. This “new” healthy vascular status in the elderly could be associated with a new endothelial redox status in which $NO^{\bullet}$ production is not the primary determinant of endothelium-dependent- vasodilation.

ROLE OF H_2O_2 IN ENDOTHELIAL FUNCTION IN HUMANS

Although some reports describe H_2O_2 as an EDHF in humans [53,58], others have offered conflicting evidence regarding the role of H_2O_2 in mediating endothelium- dependent vasodilation [12,30,32,44,53,57,62,69]. Hamilton *et al.* [30] reported that $NO^{\bullet}$/prostanoid-independent relaxation of human radial arteries to carbachol was resistant to treatment with either SOD or catalase, suggesting that this EDHF-like component of the endothelium-dependent response to carbachol was not mediated by H_2O_2. It is important to note that these authors assessed only the contribution of H_2O_2 that originated from $O_2^{\bullet-}$. In contrast, Nacitarhan *et al.* [62] studied internal thoracic artery rings and found that authentic H_2O_2 produced dose-dependent relaxations that were blunted by 4-aminopyridine, a voltage-dependent potassium channel blocker. These contradictory results may reflect differences in the vascular beds and vasodilatory stimuli being studied. Using a similar approach, Conklin *et al.* [12] assessed vasoreactivity to H_2O_2 in rings from human radial arteries, internal mammary arteries, and saphenous veins. Although the responses differed between vessels, a vasorelaxant effect of H_2O_2 was observed, especially in radial arteries and internal mammary arteries. Interestingly, these authors suggested that H_2O_2 generation occurs at the vascular smooth muscle cell plasma membrane rather than in the endothelium [12].

In coronary arterioles from heart failure patients [44,58], flow-induced vasodilation is inhibited by catalase and by inhibitors of potassium channels, providing evidence that H_2O_2 functions as an EDHF in this vascular bed. Similar observations have been made in other human microvascular beds [32,53,69]. For example, Matoba *et al.* [53] found that H_2O_2 is a primary EDHF in human mesenteric resistance arteries and Phillips *et al.* [69] observed that H_2O_2 could replace $NO^{\bullet}$ as the primary vasodilatory agent in microvessels from human visceral fat. Interestingly, Hatoum *et al.* [32] observed that H_2O_2 is released by the vascular endothelium of human submucosal intestinal microvessels, but that it does not act as EDHF in these vessels; on the contrary, it produces vasoconstriction in denuded vessels. Overall

these results indicate that H_2O_2 functions as an EDHF in human arterioles; however, the net vasoactive effect of H_2O_2 may depend on the vascular bed and the health status of the patients being studied [32].

In a recent study of the human cutaneous microcirculation, Medow *et al* [57], showed that H_2O_2 scavenging with Ebselen (Sigma, St. Louis, MO, USA) reduced cutaneous vasodilation to heat in healthy young subjects. These results provide evidence that H_2O_2 contributes to control of local blood flow *in vivo* and emphasize the need for further studies to establish the mechanisms of H_2O_2 generation and action in the human microcirculation *in vivo.* Moreover, it would be interesting to use this *in vivo* model to study the role of H_2O_2 in regulation of cutaneous blood flow in elderly subjects.

Although numerous studies have now implicated a role for H_2O_2 in regulation of vascular resistance in humans, virtually nothing is known regarding the effects of age on H_2O_2 signaling in the microcirculation of humans. The work of Miura *et al.* suggests that H_2O_2 functions as a significant endothelium-dependent vasodilator in coronary arterioles from heart failure patients [57], a disease that is more prevalent in elderly populations. It is possible that H_2O_2 compensates for a loss of $NO^{\bullet}$-mediated vasodilation in elderly humans. Alternatively, if dysregulation of H_2O_2 production/degradation occurs with age, damage to either the endothelium or the vascular smooth muscle could ensue and contribute to age-induced vascular dysfunction. Further studies in human subjects are needed to assess the effects of age on (1) regulation of vascular H_2O_2 production/scavenging, and (2) H_2O_2 signaling in both the endothelium and vascular smooth muscle.

ROLE OF $ONOO^{\bullet-}$ IN ENDOTHELIAL FUNCTION OF ELDERLY PATIENTS

Although increased oxidative stress in the endothelial cell can result in increased production of $ONOO^{\bullet-}$ (Figure 1), an increase in $ONOO^{\bullet-}$ does not necessarily decrease $NO^{\bullet}$ bioavailability. In fact, $ONOO^{\bullet-}$ could become a $NO^{\bullet}$ donor when $NO^{\bullet}$ production is impaired [63,88] and an increased endothelial $ONOO^{\bullet-}$ is associated with aging [46], which could establish a “new” redox status. This is in agreement with animal studies [63,78,92] in which ROS have been reported to play a significant role as signaling molecules in this “new” healthy vascular endothelium. In their recent study, Medow *et al* [57] also showed that $O_2^{\bullet-}$ scavenging with Tempol produced a decrease in skin blood flow in healthy young subjects [57]. If these results, added to those obtained with H_2O_2, mimic those obtained in young rats [78,92], it would be interesting to determine the effects of Tempol and/or Ebselen on skin blood flow in elderly subjects.

Although these models have answered several important questions, they are not designed to study peripheral muscle or myocardial microvascular beds, which are more difficult to study *in vivo* in humans. One way to study the coronary microvasculature *in vivo* in humans is by studying refractory angina. Refractory angina is normally observed in patients with coronary artery disease that do not respond to antiangina treatment [61]. Moreover, an increase in nitrate dosage, normally a sublingual $NO^{\bullet}$ donor (e.g., nitroglycerine), does not improve chest pain. Interestingly, there is a negative association between the use of nitrates and outcomes in the elderly when compared with younger patients [86] and, although nitrates are

commonly prescribed drugs, they do not reduce mortality in aged patients [49]. There are multiple mechanisms that could explain this nitrate intolerance [61]. It is assumed that, in some patients, adding extrinsic $NO^{\bullet}$ to an oxidatively stressed vessel would increase $ONOO^{\bullet-}$ production resulting in a further decrease of $NO^{\bullet}$ bioavailability; however, in the elderly coronary artery disease patient adding extrinsic $NO^{\bullet}$ could disrupt the "new" vascular redox status, limiting $ONOO^{\bullet-}$ as an $NO^{\bullet}$ donor. Currently, these hypotheses are speculative, and there is ample opportunity for new studies investigating the role of $NO^{\bullet}$ and $ONOO^{\bullet-}$ in the coronary microcirculation of patients with refractory angina.

PERSPECTIVE

The effectiveness of therapeutic interventions in elderly patients relies upon comprehensive knowledge of the alterations in vascular control mechanisms that occur with advancing age. In the microcirculation of aged animals, increasing evidence indicates that ROS function as important signaling molecules in both the endothelium and vascular smooth muscle. Therapies directed at scavenging or removal of these reactive species could have deleterious consequences, particularly if vascular control becomes increasingly dependent upon these reactive species with advancing age. In patients, future studies need to focus on determining how age affects the balance between oxidant production and antioxidant enzymes. In addition, future studies are needed to determine whether or not ROS signaling is critical to maintenance of vascular control mechanisms in healthy, successful aging.

Biographies

Christiaan Leeuwenburgh received his PhD from the University of Illinois, Urbana-Champagne in 1995 where his doctoral work focused on the regulation of glutathione homeostasis during chronic glutathione deficiencies and/or supplementation. He became an Assistant Professor and Director of the Biochemistry of Aging Laboratory in 1998 at the University of Florida. He is currently a Professor with the Department of Aging and Geriatric Research, College of Medicine and Institute on Aging at the University of Florida and is the Chief of the Division of Biology of Aging.

His major research focus is to understand the molecular mechanism of oxidative stress and apoptosis with age. His work on assessment of oxidative damage and apoptosis with age has been increasingly recognized and appreciated by gerontologists worldwide.

Demetra Christou, Ph.D. received her doctoral training at the University of Illinois at Urbana-Champaign in the area of Exercise Physiology/Body Composition. She then trained as a Research Associate for six years in the area of Human Cardiovascular Physiology at the University of Colorado at Boulder. Prior to coming to the University of Florida, Dr. Christou was an Assistant Professor in the Department of Health and Kinesiology and the Department of Internal Medicine, Division of Cardiology at Texas A&M University and Health Science Center.

For the past 4 years Dr. Christou has directed the Integrative Cardiovascular Physiology Laboratory. Her lab performs mechanistic biomedically-relevant research in humans from an integrative perspective using whole- body measures (e.g., flow mediated dilation via ultrasonography) complemented with cellular/molecular approaches (vascular endothelial protein expression, mRNA expression in peripheral blood mononuclear cells). The general research focus of her lab is the study of alterations in cardiovascular-autonomic function in aging and related risk factors for cardiovascular disease. In addition, her group is interested in the effect of lifestyle interventions such as physical activity/exercise training and diet on cardiovascular function. Current projects investigate the mechanisms responsible for vascular endothelial dysfunction and arterial stiffness in healthy aging and in older adults with metabolic syndrome.

Alvaro Gurovich, P.T., Ph.D. received his Physical Therapy degree from Pontificia Universidad Católica de Chile in 1990 and worked as a clinician for more than 15 years. Even though Dr. Gurovich had granted tenure in the School of Kinesiology and Physical Therapy at Pontificia Universidad Católica de Valparaíso, he moved to University of Florida where he received his doctoral degree in Health and Human Performance in 2010. Once graduated, he started his tenure as post-doctoral associate at University of Florida College of Medicine, in the Department of Physiology and Functional Genomics, under Dr. Judy M. Muller-Delp training, where he is learning some *in vitro* and *in situ* techniques that will strength his translational research background.

Judy Muller-Delp received her Ph.D. in Physiology from the University of Missouri in 1992, where her work focused on coronary microvascular adaptations to exercise training. She trained as a postdoctoral research associate at Texas A&M University and at the University of Missouri. She became an Assistant Professor of Kinesiology at Texas A&M University in 2000. She is currently an Associate Professor of Physiology and Functional Genomics at the University of Florida.

Research in Dr. Muller-Delp's laboratory focuses on understanding microvascular adaptations to aging and interventional exercise training in cardiac and skeletal muscle, with a major emphasis on assessing the cellular mechanisms that underlie age- induced dysfunction of the endothelium and vascular smooth muscle in resistance arteries.

Abbreviations used:

cAMP	cyclic adenosine monophosphate
cGMP	cyclic guanosine monophosphate
EDHF	endothelium- derived hyperpolarizing factor
eNOS	endothelial nitric oxide synthase
H_2O_2	hydrogen peroxide
HIF-lα	hypoxia-inducible factor-lα
HO	hydroxyl radical
HO-1	heme oxygenase-1
L-NAME	L-nitro-arginine methyl ester
L-NNMA	L-NG- monomethyl arginine
NAD(P)H	nicotinamide adenine dinucleotide phosphate
NO	nitric oxide
NOS	nitric oxide synthase
NQOl	NAD(P)H:quinone reductase 1
Nrfi2	nuclear factor (erythroid-derived 2)-related factor-2
O_2	superoxide
ONOO	peroxynitrite
sGC(β)1	soluble guanylate cyclase (β)1 subunit
SOD	superoxide dismutase

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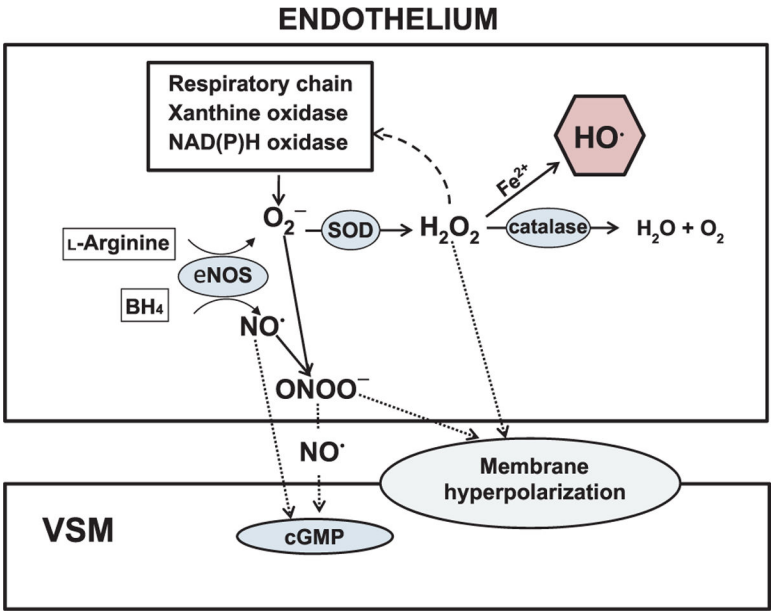

Figure 1.

Depiction of $NO^{\bullet}$- and ROS-mediated signaling in the endothelium and vascular smooth muscle. In addition to causing direct activation of cGMP in vascular smooth muscle, $NO^{\bullet}$ can react with $O_2^{\bullet-}$ to form $ONOO^{\bullet-}$. $ONOO^{\bullet-}$ can then act as a donor of $NO^{\bullet}$ to activate cGMP. $O_2^{\bullet-}$ from multiple sources and can either combine with $NO^{\bullet}$ to form $ONOO^{\bullet-}$ or can be dismutated to H_2O_2 by SOD. Cell-permeable H_2O_2 can produce hyperpolarization of vascular smooth muscle. H_2O_2 that is not enzymatically converted into H_2O and O_2 by catalase can react with Fe^{2+} to produce highly reactive $HO^{\bullet}$.