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Aging alters reactivity of microvascular resistance networks in mouse gluteus maximus muscle

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Sinkler SY, Segal SS. Aging alters reactivity of microvascular resistance networks in mouse gluteus maximus muscle. Am J Physiol Heart Circ Physiol 307: H830–H839, 2014. First published July 11, 2014; doi:10.1152/ajpheart.00368.2014.—Aging occurs with enhanced sympathetic nerve activity and endothelial dysfunction; however, little is known of how successive branches of microvascular resistance networks are affected in vivo. We questioned whether vascular reactivity is altered differentially along resistance networks with advanced age. The left gluteus maximus muscle of anesthetized 4-mo-old and 24-mo-old male C57BL/6 mice (Young and Old, respectively) was exposed for intravital microscopy and superfused with physiological salt solution (3 ml/min; pH 7.4, 34°C). Spontaneous vasomotor tone increased progressively from proximal feed arteries (FA) and first-order (1A) arterioles through distal second-order (2A) and third-order (3A) arterioles and was ~15% greater in 2A and 3A of Old versus Young. Vasoconstriction during elevated superfusion Po2 increased with branch order and to a greater extent in Young. Peak constrictions to phenylephrine [α1 adrenoreceptor (α1AR) agonist] were similar for FA and 1A of both ages and ~20% greater for 2A and 3A of Young. Across arterioles (but not FA), constrictions to UK 14304 (α2AR agonist) were depressed ~30% in Old versus Young. Thus advanced age attenuated vasoconstriction to O2 throughout networks while blunting vasoconstriction to α1AR and α2AR activation in arterioles. With ACh, endothelium-dependent dilatation (EDD) was ~20% greater in FA of Young yet was approximately twofold greater for 2A and 3A of Old. Sodium nitroprusside evoked maximal dilations similar to ACh. Thus, with advanced age, EDD was attenuated in FA while robust in distal arterioles having enhanced vasomotor tone. We conclude that advanced age differentially alters reactivity among branches of microvascular resistance networks.

adrenoreceptors; arteriole; blood flow control; endothelium-dependent dilatation; feed artery; microcirculation; skeletal muscle

ADVANCED AGE IS A MAJOR RISK factor for cardiovascular disease (25, 36, 37, 44). Although impaired endothelium-dependent dilatation (EDD) has been well-characterized in large arteries of older human subjects (9, 12, 17, 25, 44), how aging affects reactivity in the microvessels that govern skeletal muscle perfusion is less well-defined. Early studies in the cremaster muscle of male rats found impaired dilatation of first-order (1A) and second-order (2A) arterioles to adenosine (6), whereas constrictions to norepinephrine was maintained. However, the cremaster muscle is not a true skeletal muscle because it neither attaches to the skeleton nor is it involved in locomotion. Consistent with reduced blood flow to skeletal muscle with aging in humans (8, 10, 39), the blood flow response to contraction of the plantar flexor muscles was impaired in senescent rats (20). Contributing to the restriction of skeletal muscle blood flow is that 1A isolated from rat soleus and gastrocnemius muscles demonstrated impairment in EDD with aging (2, 35), as did feed arteries (FA) isolated from the soleus muscle (50, 55). In the gluteus maximus muscle (GM) of mice, which also has FA that give rise to branching arteriolar networks (1, 33), impaired dilation and perfusion of 2A in response to muscle contraction was attributed to the enhanced activation of α-adrenoreceptors (αARs) (21). These findings in rodents are consistent with restricted muscle blood flow and enhanced sympathetic nerve activity (SNA) in older humans (5, 8). Whereas studies in humans have shown impaired α-adrenergic vasoconstriction in the forearm (7) and leg (10, 47), the actual site(s) of such responses within the vascular supply remain obscure.

In response to increased oxygen demand, the volume of blood flowing to a muscle is governed by FAs and proximal (1A) arterioles upstream, whereas the distribution of blood flow within a muscle is regulated by the smaller daughter 2A and third-order (3A) arteriolar branches downstream (45). A key feature of resistance networks is their ability to coordinate vasomotor responses among vessel branches. For example, ascending vasodilation of FAs arises from signals originating from arterioles embedded within the muscle fibers (46). The wall of arterioles and their proximal FA is comprises primarily a single layer of smooth muscle cells (SMCs) surrounding the endothelial cell monolayer in contact with the blood. In turn, all branches of the resistance network are surrounded by sympathetic nerve fibers coursing through the adventitia. During blood flow regulation, changes in vessel diameter reflect the interaction between signaling events generated in SMCs, endothelial cells, and perivascular nerves. Sympathetic vasoconstriction is mediated through the activation of αARs on vascular SMCs, of which there are two major subtypes: α1 and α2 (13). Regional variability in the functional distribution of α1ARs and α2ARs has been demonstrated along arteriolar networks in rat and mouse cremaster muscles (33, 38) and the mouse GM (33). Remarkably, little is known of how or where respective adrenergic signaling pathways are affected by ad-
AGING ALTERS MICROVASCULAR NETWORK REACTIVITY

Fig. 1. Experimental protocol for evaluating diameters and reactivity in microvascular resistance networks of mouse gluteus maximus muscle (GM). Once anesthesia was induced, surgery required ~1 h, followed by 30 min equilibration (E30”). Evaluation of constriction in respective vessel branch orders (feed artery (FA), first-order arteriole (1A), second-order arteriole (2A), third-order arteriole (3A)) during equilibration with 21% oxygen (O2) in the superfusion solution required ~15 min. The superfusion was then re-equilibrated with 0% O2 for another 20 min before proceeding (E20”). Cumulative concentration-response relationships (10⁻⁹ to 10⁻⁴ M) required ~40 min to evaluate each agonist (phenylephrine (PE), UK 14304, ACh) across vessel branch orders followed by 30 min washout and equilibration (E30”) with control physiological salt solution to restore spontaneous vasomotor tone. Each preparation experienced all stimuli with the order of agonist treatment and the sequence in which vessel branch orders were studied randomized across preparations. An entire experiment required 5 to 6 h to complete. ID, internal diameter; IDrest, resting ID; IDmax, maximal ID to 10⁻⁴ M sodium nitroprusside (SNP) or 10⁻⁵ M ACh, whichever is greater; IDO₂, ID during 21% O2; IDssr, steady-state response ID.

Table 1. Maximal internal diameters (in μm) for FA and arterioles in young and old mice

<table>
<thead>
<tr>
<th>Vessel</th>
<th>Young</th>
<th>Old</th>
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<tr>
<td>SNP</td>
<td>ACh</td>
<td>SNP</td>
</tr>
<tr>
<td>FA</td>
<td>57 ± 2</td>
<td>60 ± 2*</td>
</tr>
<tr>
<td>1A</td>
<td>50 ± 2</td>
<td>51 ± 2</td>
</tr>
<tr>
<td>2A</td>
<td>35 ± 3</td>
<td>35 ± 2</td>
</tr>
<tr>
<td>3A</td>
<td>23 ± 2</td>
<td>23 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 per group. One vessel from each branch order was studied in each gluteus maximus muscle (GM) preparation. Maximal internal diameters were obtained during superfusion with 10⁻⁵ M sodium nitroprusside (SNP) and with 10⁻⁵ M ACh. Young, 4-mo-old male C57BL/6 mice; Old, 24-mo-old male C57BL/6 mice; FA; feed arteries; 1A, first-order arteriole; 2A, second-order arteriole; 3A, third-order arteriole. *P < 0.05, ACh vs. SNP.
analyses have shown that the architecture of arteriolar networks supplying the GM of male C57BL/6 mice is conserved during aging (1, 33), substantiating direct comparisons between respective branch orders between Old and Young.

Experimental protocol. Observation sites for each network were defined during a 30-min equilibration following surgery (Fig. 1) and maintained throughout the experimental protocol. Extensive preliminary experiments defined our protocol and confirmed that completed

Fig. 2. Diameters, vasomotor tone, and O2 response in FA and arterioles of 4-mo-old and 24-mo-old male C57BL/6 mice (Young and Old, respectively). A: resting diameters decreased from proximal to distal vessels as branch order increased from FA to 3A in both age groups. B: maximal diameters were decreased as in A and were slightly but consistently greater in Old versus Young. C: spontaneous vasomotor tone (percentage; calculated as $[(\text{ID}_{\text{max}} - \text{ID}_{\text{rest}})/\text{ID}_{\text{max}}] \times 100\%$) of 2A and 3A was greater in Old versus Young. D: vasoconstriction (in percentage) to 21% O2 in the superfusion solution (calculated as $[(\text{ID}_{\text{rest}} - \text{ID}_{02})/\text{ID}_{\text{rest}}] \times 100\%$) was reduced throughout network branches in Old compared with Young. Summary data are means ± SE; $n = 5$ per group. #P < 0.05, main effect of age; ΔP < 0.05, main effect of vessel branch order; *P < 0.05, Old vs. Young.

Fig. 3. Response curves to PE in FA and arterioles of Young and Old. Advanced age attenuated peak vasoconstriction to $\alpha_1$ adrenoreceptor ($\alpha_1$AR) activation, and this effect increased with vessel branch order. Vasoconstriction (in percentage) was calculated for each branch order as $[(\text{ID}_{\text{rest}} - \text{ID}_{\text{PE}})/\text{ID}_{\text{rest}}] \times 100\%$. Summary data are means ± SE; $n = 5$ per group. #P < 0.05, main effect of age group; *P < 0.05, Old vs. Young.
GM preparations remained stable with reproducible responses for at least 5 h (33).

**Oxygen reactivity.** To evaluate the vasomotor response to a rise in PO₂, the O₂ content of the superfusion solution was increased by equilibrating the PSS with 21% O₂, 5% CO₂, and 74% N₂ for ~5 min and ID values were recorded (Fig. 1). The PSS was then re-equilibrated with 5% CO₂ and 95% N₂ for the remainder of the protocol. Empirically, reactivity to changes in PO₂ is a sensitive index of the viability of a preparation for intravital microscopy (11, 23, 33).

Concentration-response relationships. Respective agonists (PE, UK 14304, ACh) were added (≤500 μl) to the 50-ml chamber containing PSS in a cumulative fashion to achieve final agonist concentrations that started at 10⁻⁹ M and increased to 10⁻⁷ M in 0.5 log increments; steady state IDs were recorded during minutes 2–8 at each concentration. The order of superfusion with respective agonists was randomized across experiments, with a 30-min equilibration following each agonist to restore resting ID (Fig. 1). At the end of each protocol, the GM preparation was superfused with 10⁻⁴ M sodium nitroprusside (SNP) for 5 min to obtain values for maximal internal diameter (IDₘₐₓ) (33). In some cases (irrespective of age) the maximal diameter in response to 10⁻⁴ M ACh was slightly greater than that evoked by SNP (Table 1). Therefore, the IDₘₐₓ (Fig. 2) was defined as the maximal value for ID obtained with either 10⁻⁵ M ACh or 10⁻⁴ M SNP.

**Data analyses and statistics.** For each vessel branch order, spontaneous vasomotor tone was calculated as the difference between resting and maximal ID and expressed relative to maximal ID. Thus, vasomotor tone (%) = [(IDₘₐₓ - IDₖₑₜ)/(IDₘₐₓ × 100)], where IDₖₑₜ = resting baseline (control) ID. The response to elevated O₂ was calculated as the magnitude of vasoconstriction during equilibration of the superfusion solution with 21% O₂ and expressed relative to resting ID. Thus, O₂ response (%) = [(IDₖₑₜ - ID₇₅)/(ID₇₅ × 100)], where ID₇₅ = ID during superfusion with 21% O₂. To evaluate reductions in ID from control during AR activation, vasoconstriction was expressed relative to respective resting IDs. Thus, vasoconstriction (%) = [(IDₖₑₜ - IDₖₑₜ)/(IDₖₑₜ × 100)], where IDₖₑₜ = ID of the steady-state response to a given agonist concentration and 100% indicates closure of the vessel lumen.

To evaluate the sensitivity of EDD for respective vessel branches in response to ACh, vasodilation was normalized to the respective maximal change in ID. Thus, “vasodilator capacity” (%) = [(IDₖₑₜ - IDₖₑₜ)/(IDₘₐₓ - IDₖₑₜ) × 100]. This definition spans from 0% to 100% for all vessels irrespective of actual diameter values and thereby enables relative differences in sensitivity to be evaluated for a given agonist. Thus EC₅₀ values can be determined for each vessel on the same relative scale. However, this normalization does not account for changes in diameter as they pertain to vascular conductance and blood flow regulation. Therefore to evaluate the functional increase in diameter relative to control conditions at rest, vasodilation was normalized to IDₖₑₜ. Thus, “functional vasodilation” (%) = [(IDₖₑₜ - IDₖₑₜ)/(IDₖₑₜ × 100)]. For example, a 100% increase indicates a doubling of ID from the resting baseline and predicts (according Poiseuille’s law) a 16-fold increase in blood flow through the vessel at constant perfusion pressure.

<table>
<thead>
<tr>
<th>Vessel Branch</th>
<th>PE</th>
<th>UK 14304</th>
<th>ACh</th>
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<tbody>
<tr>
<td>FA</td>
<td>−6.4 ± 0.1</td>
<td>−6.4 ± 0.1</td>
<td>−7.5 ± 0.1</td>
</tr>
<tr>
<td>1A</td>
<td>−6.3 ± 0.1</td>
<td>−6.3 ± 0.1</td>
<td>−7.0 ± 0.1</td>
</tr>
<tr>
<td>2A</td>
<td>−5.9 ± 0.1</td>
<td>−6.4 ± 0.1</td>
<td>−6.4 ± 0.1</td>
</tr>
<tr>
<td>3A</td>
<td>−6.4 ± 0.1</td>
<td>−6.5 ± 0.1</td>
<td>−6.2 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5 per group. One vessel from each branch order was studied in each GM preparation. With EC₅₀ values as an index, vascular sensitivity to phenylephrine (PE), UK 14304, and ACh was not significantly different between age groups as determined by using 2-way ANOVA.
greater constriction than did activation of α2ARs with UK 14304 (Figs. 3 and 4). Whereas α2AR-mediated constriction was attenuated in all arteriolar branch orders of Old compared with Young (Fig. 4), α1AR responses were maintained in Old although 2A and 3A were resistant to closure (Fig. 3).

**Endothelium-dependent dilation.** ACh evoked concentration-dependent vasodilation in all vessel branches of Old and Young GM (Figs. 5 and 6). When expressed relative to vasodilator capacity for respective branches to evaluate sensitivity, FA and 1A responses were not different between Young and Old, whereas EDD of 2A and 3A in Old were shifted slightly (<0.5 log unit) but significantly to the left when compared with Young (Table 2 and Fig. 5). When expressed relative to respective resting control diameters, the magnitude of EDD of FA was less ($P < 0.05$) in Old compared with Young (Fig. 6).
In contrast, EDD of 2A and 3A was approximately twofold greater (P < 0.05) in Old compared with Young (Fig. 6).

**Branch order differences.** In addition to differences between Old and Young within individual branch orders, vasomotor responses to respective agonists exhibited differences between branch orders within age groups. In Young, [PE] > 10^-7 M induced greater α1-AR-mediated constriction in distal versus proximal branches such that 3A > 2A > 1A > FA (Fig. 7). In Old, this effect of branch order was maintained though less robust. For [UK 14304] > 10^-8 M, α2-AR-mediated constriction also increased with vessel branch order with 3A > 2A > 1A > FA, and this relationship was also maintained (although less robust) in Old (Fig. 7). For EDD, the trend was reversed from that seen during vasoconstriction such that the sensitivity to ACh was greatest in proximal vessels with FA > 1A > 2A > 3A. As seen with vasoconstriction, differences between branch orders were more robust in Young than in Old (Fig. 7). For each agonist, there was a significant effect (P < 0.05) of vessel branch order as well as concentration.

**Maximal responses to agonists.** To evaluate how advanced age influenced the dynamic range of vasoconstriction and vasodilation in respective vessel branch orders, maximal responses to the activation of α1-ARs (PE), α2-ARs (UK 14304), and muscarinic receptors (ACh) are presented for respective agonists in Fig. 8. Across vessel branch orders, PE consistently evoked approximately twofold greater vasoconstriction than did UK 14304. Maximal vasoconstriction to the activation of either α1-ARs or α2-ARs was reduced in Old versus Young (P < 0.05), particularly in arterioles. EDD increased with branch order in arterioles of Old but not in arterioles of Young. Irrespective of age group, maximal diameters with ACh were similar to those with SNP (Table 1).

**DISCUSSION**

The present study has defined microvascular reactivity along resistance networks of skeletal muscle in Young and Old. Using intravital microscopy to study the mouse GM, we evaluated internal vessel diameters, vasomotor tone, and responses to defined vasoactive stimuli. Under resting conditions, Young and Old exhibited similar diameters for FA and respective arteriolar branches. During maximal dilation, diameters of distal arterioles (i.e., 2A and 3A) tended to be larger in Old compared with Young, reflecting greater spontaneous vasomotor tone. Nevertheless, vasoconstriction in response to elevated O2 was attenuated throughout the networks of Old compared with Young. Furthermore, although α1-ARs were twice as effective as α2-ARs in evoking constrictions, advanced age attenuated responses to both AR subtypes, particularly in distal arterioles. Remarkably, with similar resting diameters, vasodilation to ACh (i.e., EDD) was greater in distal arterioles of Old compared with Young. Thus the effect of advanced age on the reactivity of microvessels supplying skeletal muscle varies with branch order and nature of the vasoactive stimulus.

**Vessel diameters.** In accord with Poiseuilles’ law, the IDs of resistance arteries and arterioles are principal determinants of both tissue perfusion and peripheral resistance. Consistent with previous studies of microvascular resistance networks (4, 31, 33), vessel diameter decreased as branch order increased with FA > 1A > 2A > 3A in Young and Old (Fig. 2). At rest under control conditions, the IDs of respective branch orders were not different between age groups (Fig. 2A). Although these hierarchical relationships were maintained during maximal dilation, arteriolar diameters tended to be larger in Old compared with Young as branch order increased (Fig. 2B). As a consequence, and despite no difference in resting diameters, vasomotor tone increased with vessel branch order, particularly...
in 2A and 3A of Old when compared with those in Young (Fig. 2C). This scenario contrasts with hypertension and diabetes, where thickening, narrowing, and rarefaction of arterioles (with tissue ischemia) are found (18, 19, 41, 42). Recent findings have shown no difference in systolic blood pressure in male C57BL/6 mice at 3 vs. 24 mo (49); thus the present data appear most applicable to “healthy” aging in contrast with conditions associated with vascular disease.

**Oxygen response.** As O₂ delivery increases relative to demand, resistance microvessels of skeletal muscle constrict as a mechanism of negative feedback. Raising the O₂ content of the superfusion solution provides a source of O₂ in addition to that carried in the bloodstream (11, 29, 51). In turn, constriction of arterioles in response to elevating superfusion PO₂ is an exquisitely sensitive index of the functional integrity of exposed microvessels (11, 23, 33). Typically, O₂ responses are evaluated in a single arteriole within a preparation before beginning experiments to assess viability of the preparation (1, 21, 33). Consistent with such criteria, constriction during equilibration with 21% O₂ confirmed the integrity of our preparations irrespective of age group. Because the O₂ sensor may be located within the tissue (22), our finding that constriction during elevation of superfusion PO₂ encompassed FAs external to the muscle is consistent with the ability of O₂ to depolarize arteriolar SMCs (54) and evoke conducted vasoconstriction (22). Through evaluating entire resistance networks, we demonstrate that the relative magnitude of constriction in response to elevated PO₂ increased with vessel branch order, from FA to 3A (Fig. 2D). Consistent with earlier findings focused on 2A of the GM (1, 21), the O₂ response was depressed in all branch orders in Old versus Young (Fig. 2D). Thus the ability of O₂ to evoke constriction is attenuated throughout the resistance vasculature with aging. Given the similarities in vessel IDs at rest between age groups (Fig. 2A), greater spontaneous vasomotor tone in 2A and 3A of Old versus Young (Fig. 2C) suggests that the role of PO₂ in governing SMC activation is reduced in advanced age. In light of tissue oxygenation being integral to capillary perfusion and blood flow regulation (11, 29, 51), attenuated O₂ responses in Old (Fig. 2D) imply alterations in the regulation of muscle blood flow during advanced age, e.g., as manifested through a greater role for reactive oxygen species (3, 34, 44).

**Adrenergic reactivity.** In human subjects, femoral arterial blood flow and vascular conductance were 30–40% lower in older (≥63 years) compared with young (≥28 years) males in association with tonic elevation of muscle SNA and the activation of αARs (5, 8). Although comparable recordings of SNA with aging are lacking in mice, the activities of key enzymes governing catecholamine synthesis (e.g., tyrosine hydroxylase) were approximately twofold higher in the adrenal glands of Old (28 mo) compared with Young (4 mo) male mice and rats (40). Independent studies found norepinephrine re-
lease from sympathetic nerves to be greater at rest and during stress in Old (24 mo) versus Young (3 mo) male Fischer-344 rats (32). In light of enhanced constitutive αAR activation in GM of Old (21), findings collectively suggest a greater level of SNA in Old versus Young. Adrenergic vasoconstriction in the GM microcirculation, α₁ARs consistently displayed approximately twofold greater efficacy when compared with α₂ARs. Furthermore, although the ability of α₁ARs and of α₂ARs to evoke constriction increased with vessel branch order (Fig. 8), there were no consistent differences between age groups in their sensitivity (i.e., EC₅₀ values) to respective agonists (Table 2).

In humans, reductions in forearm blood flow during α₁AR activation (via intra-arterial infusion of PE) were blunted in older (~65 years) compared with younger (~26 years) males, whereas blood flow reductions to α₂AR activation with clonidine infusion were similar between age groups (7). Thus attenuated reductions in forearm blood flow in older versus younger men during tyramine infusion (to evoke release of endogenous norepinephrine) were attributed to reduced responsiveness of α₁ARs (7). During exposure to PE, we observed that 2A and 3A of Old were unable to constrict to the same extent observed in Young (Fig. 3). This new finding illustrates an important functional limitation that may help to explain the reduced response to activation of α₁ARs in the human forearm with aging. In turn, the inability to fully constrict 2A and 3A of Old during maximal activation of α₁ARs (Fig. 3) may reflect tissue remodeling with aging (57). For example, an increase in the amount, orientation, and/or stiffness of the extracellular matrix (24, 27) may resist lumen closure of smaller arterioles in Old that are otherwise able to do so in younger animals. Further studies at the ultrastructural level are required to provide greater insight into the nature of this adaptation to advanced age.

During tyramine infusion into the femoral artery, reductions in leg blood flow were also attenuated in older (~62 years) versus younger (~24 years) men and attributed to the attenuation of both α₁AR- and α₂AR-mediated responses with aging (47). The effect of aging on reducing maximal constriction was greater for α₂AR versus α₁AR activation in the mouse GM (Fig. 8). The activation of α₁ARs was more efficacious than that of α₂ARs in evoking vasoconstriction and was relatively less affected by advanced age, particularly in FA and 1A (Fig. 8). Thus AR activation was still able to restrict muscle blood flow in Old. Indeed, relative to attenuated α₂AR-mediated constriction of arterioles with aging, α₁AR-mediated constriction was maintained at all but the highest PE concentrations (Fig. 3), which may help to ensure the maintenance of peripheral resistance and arterial blood pressure during exercise (48).

Endothelium-dependent dilation. As demonstrated in humans (9, 17, 44) and in resistance vessels isolated from rats (2, 35, 55, 56), advanced aging is associated with attenuated EDD, typically characterized by activating muscarinic receptors on the endothelium. Nevertheless, intravital studies of the mouse GM had indicated no difference in the ability of Ach to dilate 2A of Young versus Old in vivo (1). To investigate the effect of advanced age on EDD along resistance networks, we quantified responses to ACh in two ways. First, to address the sensitivity of EDD, changes in ID at each ACh concentration were normalized to the corresponding maximal change in ID. The activation of both α₁ARs and of α₂ARs with UK 14304 (33). In both Young and Old studied here, the activation of α₁ARs with PE constricted all vessel branches to a greater extent than did activation of α₂ARs with UK 14304 (Figs. 3 and 4). Thus although both receptor subtypes mediate...
indicates that the endothelium lining resistance networks of Old were fully able to drive vascular smooth muscle relaxation. In contrast, as an index for the dynamic range of blood flow control, normalizing ID changes to respective resting diameters (i.e., functional vasodilation) revealed that EDD was depressed significantly in FA of Old while enhanced in 2A and 3A of the same networks (Fig. 6). Whereas reduced EDD in FA of Old versus Young is consistent with impaired EDD of conduit and resistance arteries with advanced age (12, 44, 53), enhanced EDD of the smaller downstream arterioles in Old suggests that the effect of advanced age on EDD varies with microvessel branch order in vivo. Such regional differences within the microcirculation have not been documented previously. In part this is attributable to the focus on FA and 1A in previous studies (2, 35, 50, 55). Furthermore, in vivo studies evaluating ACh-induced EDD in human skeletal muscle with aging were based on changes in limb blood flow (9, 10, 17). In such cases, specific branch orders within the microcirculation as emphasized here could not be accounted for.

Summary and Perspective

Studies in humans have illustrated that skeletal muscle blood flow is reduced with advanced age in association with augmented SNA (8, 14) along with impaired EDD (9, 44). However, such studies are based primarily upon evaluating diameter and velocity in conduit (e.g., brachial and femoral) arteries, where changes in blood flow reflect regulatory events occurring further downstream, i.e., in the microcirculation. Such measurements in humans are thereby limited by the inability to directly observe those vessels actually responsible for controlling tissue perfusion and peripheral resistance. Thus using rodents as a model system provides an opportunity to extend direct observations of blood flow control into the mammalian microcirculation. The effect of aging on resistance microvessels of rats has focused on FAs (50, 55, 56) and the large arterioles (e.g., 1A) (2, 35). These proximal microvessels are most readily isolated and studied in vitro because they are of sufficient diameter and length to enable microdissection and cannulation for evaluation using pressure myography. Where the effect of aging on arterioles has been studied in vivo, observations have focused on 1A and 2A in muscles of rats and mice (1, 6). In contrast, this study is the first to examine the effects of aging on vascular reactivity throughout microvascular resistance networks of skeletal muscle in vivo, from proximal FA through 3A further downstream.

Using the GM preparation in male C57BL/6 mice, we report that advanced age (i.e., 24 vs. 4 mo) blunted the sensitivity to O2 throughout networks with the greatest effect in distal (2A, 3A) arterioles. Advanced age also prevented closure 2A and 3A arterioles during maximal activation of α1ARs, which may be explained by tissue remodeling and associated changes in the extracellular matrix. Nearly all constrictions to α2AR activation were attenuated throughout arteriolar networks but not in FAs, highlighting regional differences in microvascular adaptations to aging. We speculate that the loss of arteriolar α2AR reactivity with aging may result from a tonic increase in receptor activation (21, 40), consistent with findings in humans (5, 8). Remarkably, whereas constriction of the smaller (2A, 3A) arterioles was impaired consistently in Old versus Young, these same arteriolar branches in Old exhibited enhanced EDD and greater spontaneous vasomotor tone. Thus distal arterioles exhibiting the greatest attenuation of constrictor responses simultaneously exhibited the greatest dilator responses. Nevertheless, maintained constriction (or impaired EDD) of proximal FA can restrict muscle blood flow even when distal arterioles are dilated maximally (21, 52). Unlike studies in humans, which have relied on indirect measurements of limb blood flow, intravital studies in mice enable direct observations of respective microvessels that control flow magnitude (FA, 1A) as well as its distribution within the tissue (2A, 3A).

The consistency of our present findings in the mouse GM with those from earlier studies of human subjects suggests that the GM is a viable model for investigating how aging affects the microcirculation of skeletal muscle. As typical of human skeletal muscles (28, 43), the mouse GM is of mixed fiber type (26, 30). Thus the insight gained from understanding where and how advanced age affects adrenergic vasoconstriction, EDD, and blood flow control in respective microvascular branch orders of the mouse GM in vivo may well be applied toward developing selective therapeutic strategies for promoting muscle blood flow in aging humans.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Author contributions: S.Y.S. and S.S.S. conception and design of research; S.Y.S. performed experiments; S.Y.S. and S.S.S. interpreted results of experiments; S.Y.S. prepared figures; S.Y.S. and S.S.S. analyzed data; S.Y.S. and S.S.S. drafted manuscript; S.Y.S. and S.S.S. edited and revised manuscript; S.Y.S. and S.S.S. approved final version of manuscript.

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AGING ALTERS MICROVASCULAR NETWORK REACTIVITY