Contribution of nitric oxide in the contraction-induced rapid vasodilation in young and older adults

Darren P. Casey, Branton G. Walker, Sushant M. Ranadive, Jennifer L. Taylor, and Michael J. Joyner

1Department of Physical Therapy and Rehabilitation Science, Carver College of Medicine, University of Iowa, Iowa City, Iowa; and 2Department of Anesthesiology, Mayo Clinic, Rochester, Minnesota

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Casey DP, Walker BG, Ranadive SM, Taylor JL, Joyner MJ. Contribution of nitric oxide in the contraction-induced rapid vasodilation in young and older adults. J Appl Physiol 115: 446–455, 2013. First published June 20, 2013; doi:10.1152/japplphysiol.00446.2013.—We tested the hypothesis that reduced nitric oxide (NO) bioavailability contributes to the attenuated peak and total vasodilation following single-muscle contractions in older adults. Young (n = 10; 24 ± 2 yr) and older (n = 10; 67 ± 2 yr) adults performed single forearm contractions at 10, 20, and 40% of maximum during saline infusion (control) and NO synthase (NOS) inhibition by Nω-monomethyl-L-arginine. Blood flow (ml/min) and blood pressure (mmHg) were calculated from blood flow (ml/min) and blood pressure (mmHg). Peak and total vasodilator responses [change (Δ) in FVC from baseline] were attenuated in older adults at all intensities (P < 0.05). NOS inhibition reduced the peak ΔFVC at 10% (88 ± 12 vs. 52 ± 9 ml/min–1·100 mmHg–1), 20% (125 ± 13 vs. 83 ± 13 ml/min–1·100 mmHg–1), and 40% (207 ± 26 vs. 133 ± 20 ml/min–1·100 mmHg–1) in young subjects, (P < 0.05 for all) and in older adults at 10% (59 ± 5 vs. 47 ± 7 ml/min–1·100 mmHg–1, P < 0.05) and 20% (88 ± 9 vs. 68 ± 9 ml/min–1·100 mmHg–1, P < 0.05), but not 40% (128 ± 12 vs. 105 ± 11 ml/min–1·100 mmHg–1, P = 0.11). The relative (%) reduction in peak ΔFVC due to NOS inhibition was greater in young vs. older adults at 20% (−36 ± 5 vs. −23 ± 5%, P < 0.05) and 40% (−35 ± 6 vs. −16 ± 7%, P < 0.05). The reduction in the total vasodilator response (area under the curve) with NOS inhibition was also greater in young vs. older adults at all intensities. Our data suggest that contraction-induced rapid vasodilation is mediated in part by NO, and that the contribution of NO is greater in young adults.

Evidence from both humans (8, 9, 38) and experimental animals (2, 3, 34) suggests that rapid vasodilation and increases in blood flow after a muscle contraction are attenuated with aging. Interestingly, aging is also associated with a reduction in NO bioavailability and decreased endothelial-dependent (NO-mediated) vasodilation (12, 19, 33, 54, 55). Moreover, alterations in NO bioavailability have been implicated in the age-related reductions in skeletal muscle blood flow during dynamic exercise (11, 17, 52). However, whether alterations in NO-mediated mechanisms contribute to the attenuated rapid vasodilator response after a single-muscle contraction in older adults is unknown.

With this information as background, we tested the hypothesis that the contribution of NO to the rapid vasodilator response would be reduced in older adults. Specifically, we examined whether the reduction in the rapid vasodilator response after a single forearm contraction during NOS inhibition was greater in young compared with older adults. Additionally, we used bolus intra-arterial infusions of acetylcholine (ACh) and sodium nitroprusside (NTP) to mimic single forearm contractions and determined how aging affects the rapid endothelial and vascular smooth muscle vasodilator responses, respectively.

METHODS

Subjects. A total of 10 young (age range 18–32 yr) and 10 older (age range 61–73 yr) healthy subjects volunteered to participate in the study. Subjects completed written, informed consent, underwent a standard screening, and were healthy, nonobese (body mass index ≤ 30 kg/m²), nonsmokers, not taking any vasoactive medications, and were sedentary to moderately active. One young subject was taking Pantoprazole (proton pump inhibitor) to treat gastroesophageal reflux (withheld for a minimum of 3 days before study), and two older subjects were taking Synthroid to treat hypothyroidism (withheld 3 days before study). Six subjects (one young and five older) reported...
taking a daily vitamin. Studies were performed after an overnight fast and refraining from exercise and caffeine for at least 24 h. Young female subjects were studied during the early follicular phase of the menstrual cycle or the placebo phase of oral contraceptives (43). All older female subjects were postmenopausal and were not taking any form of hormone replacement therapy. All study protocols were approved by the Institutional Review Board and were performed according to the Declaration of Helsinki.

**Arterial catheterization.** A 20-gauge, 5-cm (model RA-04020, Arrow International, Reading, PA) catheter was placed in the brachial artery of the experimental arm under aseptic conditions after local anesthesia (2% lidocaine) for administration of study drugs. The catheter was connected to a three-port connector in series, as previously described in detail (21). One port was linked to a pressure transducer positioned at heart level (model PX600F, Edwards Lifescience, Irvine, CA) to allow measurement of arterial pressure and was continuously flushed (3 ml/h) with saline with a stop-cock system to enable arterial blood sampling. The remaining two ports allowed arterial drug administration.

**Heart rate and systemic blood pressure.** Heart rate was recorded via continuous three-lead ECG. A pressure transducer connected to the arterial catheter measured beat-to-beat blood pressure (Cardiocap5, Datex-Ohmeda, Louisville, CO).

**Forearm blood flow.** Brachial artery mean blood velocity and brachial artery diameter were determined with a 12-MHz linear-array Doppler probe (model M12L, Vivid 7, General Electric, Milwaukee, WI). Brachial artery blood velocity was measured throughout each condition with a probe insonation angle previously calibrated to 60°. Brachial artery diameter measurements were obtained at end diastole at rest (before contraction) and 45 s post-contraction. Forearm blood flow (FBF) was calculated as the product of mean blood velocity (cm/s) and brachial artery cross-sectional area (cm²) and expressed as milliliters per minute (ml/min).

**Single-muscle contractions.** For the experimental trials, brief forearm contractions were performed with a handgrip device at 10, 20, and 40% of the subject’s maximal voluntary contraction (MVC), determined at the beginning of each experiment. The weight was lifted 4–5 cm over a pulley for a single, 1-s forearm muscle contraction. Subjects were instructed to contract and relax on verbal command issued from laboratory personnel. Each contraction was visually observed by laboratory personnel to ensure the proper timing of contraction. Two minutes of relaxation were given between each contraction to allow continuous measures of forearm hemodynamics post-contraction. Workload intensity was randomized within each condition, and each contraction intensity was performed in duplicate to calculate an average response for each subject for a given condition. The average weight used for the young subjects was 4.1 ± 0.5, 8.2 ± 1.1, and 16.5 ± 2.2 kg for 10, 20, and 40% MVC, respectively. The average weight used for the older subjects was 3.4 ± 0.3, 6.8 ± 0.7, and 13.6 ± 1.4 kg for 10, 20, and 40% MVC, respectively (P = 0.22–0.27 compared with young subjects).

**Pharmacological infusions.** N⁵-monomethyl-L-arginine (l-NMMA; NOS inhibitor; Bachem, Switzerland) was infused at a loading dose of 5 mg/min for 5 min and then at a maintenance dose of 1 mg/min for the remainder of the study. This dose of l-NMMA has been shown to effectively attenuate the forearm vasodilatory response to exogenous ACh (10). The immediate peak vasodilator responsiveness to the endothelium-dependent and -independent vasodilators ACh (4 μg/dl of limb volume) and NTP (10 μg/dl of limb volume) were assessed using 2-ml intra-arterial bolus infusions of each respective drug.

**Experimental protocol.** Each subject completed a single study day, which consisted of single forearm contractions during saline (control) and l-NMMA administration. Contraction intensity (10, 20, and 40% MVC) was performed in duplicate and randomized within each condition. Thus each subject performed a total of 12 single forearm contraction trials during the study [6 contractions (2 at each intensity) × 2 conditions]. Each trial consisted of 2 min of rest, followed by a single forearm contraction. Brachial artery velocity and hemodynamics were measured during the rest period and for 45-s post-contraction. Additionally, intra-arterial bolus infusions of ACh and NTP were performed. This pharmacological approach was used as follows: 1) to confirm effective NOS inhibition; and 2) in an attempt to create a rapid vasodilator response that resembled what is observed following a single-muscle contraction. That is, we tried to create a brief stimulus to elicit a vasodilator response that mimicked the transient rapid nature that occurs following a brief forearm contraction. Furthermore, the bolus infusions of ACh and NTP were used to gain insight into the endothelial and vascular smooth muscle contributions to the rapid vasodilator responses. Each bolus infusion was completed in duplicate and consisted of 2 min of rest, followed by a rapid infusion of either ACh or NTP. Brachial artery velocity and hemodynamics were measured during the rest period and for 90-s post-rapid infusion; Bolus infusions were performed during saline (control) and l-NMMA administration. Due to the long half-life of l-NMMA, NOS inhibition trials were always performed last. A rest period of 15 min was allowed between conditions.

**Data analysis and statistics.** Data were collected at 250 Hz, stored on a computer, and analyzed off-line with signal processing software (WinDaq, DATAQ Instruments, Akron, OH). Mean arterial pressure (MAP) was determined from the brachial artery pressure waveform, and heart rate was determined from the electrocardiogram. Baseline FBF and MAP represent an average of the last 30-s of the resting time period before each muscle contraction and were used to quantify the hyperemic response. Forearm vascular conductance (FVC) was calculated as (FBF/MAP) × 100 and expressed as ml-min⁻¹100 mmHg⁻¹. To account for baseline changes (Δ) in FBF and FVC with l-NMMA, the flow and vasodilator responses following muscle contraction or bolus drug infusion (ACh and NTP) were adjusted (i.e., postcontraction or infusion value − baseline value) and expressed as ΔFBF and ΔFVC. Of particular interest to this study, the peak and total FBF and FVC responses were analyzed between conditions and age groups. Total FBF and FVC were defined as the area under the curve after respective baseline values were subtracted for a given flow or conductance curve (28, 42). To further investigate the contribution of NO in the age-related changes in contraction-induced rapid vasodilator responses (ΔFVC), we compared the relative (%) reductions during l-NMMA administration between age groups.

All values are expressed as means ± SE. ANOVA was used to analyze baseline differences between age groups. Baseline MAP, FBF, and FVC were compared via repeated-measures ANOVA to detect differences between age groups and across conditions. To determine the effect of age (group) and NOS inhibition (condition) on the hyperemic and vasodilator response to single forearm contractions, differences in the peak ΔFBF and ΔFVC and total FBF and FVC at each contraction intensity (10, 20, and 40% MVC) were determined via repeated-measures ANOVA. Additional two-way repeated-measures ANOVA were performed to examine rapid endothelial and vascular smooth muscle vasodilator responses to ACh and NTP within each condition (saline vs. l-NMMA) and between age groups. Appropriate post hoc analysis determined where statistical differences occurred. When significance was detected, Tukey’s post hoc analysis was used to identify differences between groups. Statistical difference was set a priori at P < 0.05.

**RESULTS**

Subject characteristics are summarized in Table 1. Young and older subjects were of similar height, weight, and body mass index (P > 0.05 for all). Additionally, young and older subjects exhibited similar forearm volume and MVC (P > 0.05 for both). Older subjects demonstrated a greater MAP, total cholesterol, and high- and low-density lipoprotein than their younger counterparts (P < 0.05).
Utilizing this approach revealed single-muscle contractions observed in older adults persisted hyperemic and vasodilator responses (FBF and intensity between groups, we examined whether the attenuated MVC or differences in the weight used for each relative workload (Fig. 1). Older adults demonstrated reduced peak hyperemic and vasodilator responses at all workloads (P < 0.05; Fig. 2). Moreover, total (area under the curve for 30 cardiac cycles postcontraction) hyperemic and vasodilator responses were attenuated in older adults at all workloads (P < 0.05; Fig. 3).

Although there were no significant age-related reductions in MVC or differences in the weight used for each relative intensity between groups, we examined whether the attenuated hyperemic and vasodilator responses (ΔFBF and ΔFVC) to single-muscle contractions observed in older adults persisted after normalizing for workload (i.e., flow and/or conductance per 100 g of weight lifted). Utilizing this approach revealed similar results as those reported above. Under control conditions, older adults still demonstrated reduced peak hyperemic (1.86 ± 0.21 vs. 2.26 ± 0.19, 1.37 ± 0.15 vs. 1.57 ± 0.12, and 1.01 ± 0.09 vs. 1.29 ± 0.16 ml·min⁻¹·100 g of weight lifted⁻¹) and vasodilator (1.79 ± 0.21 vs. 2.26 ± 0.19, 1.29 ± 0.14 vs. 1.60 ± 0.12, and 0.98 ± 0.10 vs. 1.32 ± 0.15 ml·min⁻¹·100 mmHg⁻¹·100 g of weight lifted⁻¹) responses at 10, 20, and 40% MVC, respectively, compared with their young counterparts (P < 0.05). Additionally, the total hyperemic (0.24 ± 0.03 vs. 0.37 ± 0.02, 0.19 ± 0.03 vs. 0.28 ± 0.04, and 0.20 ± 0.02 vs. 0.31 ± 0.06 ml/100 g of weight lifted) and vasodilator (0.27 ± 0.03 vs. 0.39 ± 0.06, 0.20 ± 0.02 vs. 0.28 ± 0.04, and 0.20 ± 0.02 vs. 0.32 ± 0.06 ml/100 mmHg⁻¹·100 g of weight lifted⁻¹) responses remained lower in older compared with young adults at 10, 20, and 40% MVC, respectively, after correcting for workload (P < 0.05).

**Effect of NOS inhibition (via l-NMMA) on hyperemic and vasodilator responses to single forearm contractions.** l-NMMA reduced baseline FBF and FVC and increased MAP in both young and older adults (P < 0.05; Table 2). The time to reach a peak vasodilator response was achieved at the fourth cardiac cycle postcontraction in both young and older adults for each relative workload. At 40% MVC, NOS inhibition resulted in a faster time to reach a peak vasodilator response in all subjects (P < 0.05; main effect of l-NMMA). In young adults, NOS inhibition reduced the peak and total hyperemic and vasodilator responses at all contraction intensities (P < 0.01; Figs. 2 and 3, respectively). Peak hyperemic and vasodilator responses were reduced during l-NMMA at 10 and 20% MVC in the older adults (P < 0.01; Fig. 2). Furthermore, the total hyperemic response (Fig. 3A) at 10% MVC and the total vasodilator response (Fig. 3B) at 10 and 20% MVC were reduced in the older adults during the l-NMMA trials (P < 0.05). The age-associated differences observed under control conditions for peak and total responses were no longer present during NOS inhibition (Figs. 1–3). The relative reduction in peak vasodilator responses during the NOS inhibition trials (via l-NMMA) were substantially greater in young compared with older adults at 20 and 40% MVC (P < 0.05; Fig. 4A). Additionally, NOS inhibition resulted in a greater relative reduction of the total vasodilator responses in young compared with older adults at all contraction intensities (P < 0.05; Fig. 4B).

**Vasodilator responses to rapid infusion of ACh in young and older adults.** Figure 5A demonstrates the rapid vasodilator response over 60 cardiac cycles to bolus infusions of ACh in young and older adults under control (saline) and NOS inhibition (l-NMMA) conditions. Under control conditions, peak vasodilator responses to ACh were observed at the 16th cardiac cycle following the infusion of ACh in both young and older adults. Older adults demonstrated a blunted peak and total vasodilator response to ACh compared with their young counterparts (P < 0.05; Fig. 5, B and C). NOS inhibition did not alter the timing of peak vasodilatation in either age group. However, the peak and total vasodilator responses to ACh

### Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young</th>
<th>Older</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>24 ± 2</td>
<td>67 ± 2*</td>
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<tr>
<td>Men/women</td>
<td>5:5</td>
<td>5:5</td>
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<td>Height, cm</td>
<td>174 ± 3</td>
<td>170 ± 3</td>
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<td>Weight, kg</td>
<td>72 ± 5</td>
<td>72 ± 6</td>
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<td>BMI, kg/m²</td>
<td>23.5 ± 1.1</td>
<td>24.7 ± 1.1</td>
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<tr>
<td>FAV, ml</td>
<td>879 ± 71</td>
<td>841 ± 96</td>
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<td>MVC, kg</td>
<td>41 ± 5</td>
<td>34 ± 4</td>
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<td>MAP, mmHg</td>
<td>97 ± 2</td>
<td>106 ± 2*</td>
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<tr>
<td>Total cholesterol, mmol/l</td>
<td>3.8 ± 0.2</td>
<td>4.9 ± 0.2*</td>
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<tr>
<td>LDL, mmol/l</td>
<td>2.1 ± 0.2</td>
<td>2.9 ± 0.2*</td>
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<tr>
<td>HDL, mmol/l</td>
<td>1.2 ± 0.1</td>
<td>1.7 ± 0.2*</td>
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<tr>
<td>Triglycerides, mmol/l</td>
<td>1.0 ± 0.2</td>
<td>1.1 ± 0.1</td>
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</table>

Values are means ± SE. BMI, body mass index; FAV, forearm volume; MVC, maximal voluntary contraction; MAP, mean arterial pressure; LDL, low-density lipoprotein, HDL, high-density lipoprotein. *P < 0.05 vs. young.

### Table 2. Baseline (resting) hemodynamics under each condition

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Older</th>
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<tbody>
<tr>
<td></td>
<td>FBF, ml/min</td>
<td>FVC, ml/min</td>
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<tr>
<td>Control</td>
<td>10% MVC</td>
<td>66 ± 10</td>
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<td></td>
<td>20% MVC</td>
<td>66 ± 10</td>
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<tr>
<td></td>
<td>40% MVC</td>
<td>66 ± 8</td>
</tr>
<tr>
<td>l-NMMA</td>
<td>10% MVC</td>
<td>47 ± 8†</td>
</tr>
<tr>
<td></td>
<td>20% MVC</td>
<td>41 ± 7†</td>
</tr>
<tr>
<td></td>
<td>40% MVC</td>
<td>45 ± 8†</td>
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</tbody>
</table>

Values are means ± SE. l-NMMA, N⁵-monomethyl-l-arginine; FBF, forearm blood flow; FVC, forearm vascular conductance. *P < 0.05 vs. young. †P < 0.05 vs. control at respective MVC.
after a single forearm contraction in young adults is in agreement with previous work by Brock et al. (6). However, when comparing the relative changes in flow during NOS inhibition between the two studies at moderate contraction intensities (~20% MVC), we observed approximately a twofold greater reduction in the peak (~36 vs. ~17%) and total (~41 vs. ~26%) hyperemic response, thus suggesting the contribution of NO to contraction-induced rapid hyperemia and vasodilation might be greater in young adults than previously thought.

**NO and age-related reductions in contraction-induced rapid vasodilation.** The primary novel finding of the present study is that, although NO appears to contribute to the rapid hyperemic and vasodilator responses after a single-muscle contraction at mild to moderate intensities (10–20% MVC) in older adults, the contribution is substantially less than that observed in young adults (Fig. 4). The diminished role of NO in the contraction-induced vasodilation is likely due to less bioavailable NO in older adults. However, the age-related impairment in the vasodilator response to a muscle contraction might also be due in part to a diminished responsiveness to NO, as evidenced by the attenuated and delayed vasodilation to bolus infusions of NTP in older adults (Fig. 6). Theoretically, age-related reductions in NO bioavailability can be a result of changes in endothelial NOS (eNOS) expression and/or activity of the enzyme. To date, evidence to support this idea is unclear as eNOS expression and/or activation from arterial tissue in experimental animals has shown to be decreased, increased, or unchanged with aging (13, 26, 49, 59). Moreover, recent evidence from vascular endothelial cells obtained from the brachial artery of humans suggests that eNOS protein expression and/or activation from arterial tissue might be greater in young adults than previously thought.
The increased activation of eNOS with age has been postulated to be a compensatory mechanism for low NO bioavailability in older adults (24).

Age-related reductions in NO bioavailability may also be a result of increased oxidative stress and an enhanced scavenging of NO. Along these lines, measures of endothelial function are inversely related to circulating markers of oxidative stress (29, 31). Additionally, acute administration of antioxidants (e.g., ascorbic acid) improves endothelial-dependent vasodilation (30, 54, 60) and more recently has been shown to increase muscle blood flow during continuous dynamic forearm exercise in older adults (38). However, acute administration of intra-arterial ascorbic acid failed to improve the rapid hyperemic and vasodilator response to single-muscle contractions in older adults (38). Therefore, it is unclear if the reductions in NO-mediated vasodilation following single-muscle contractions observed in the older adults of the present study are due to oxidative stress related mechanisms.

**Alternative role of NO in the rapid vasodilation.** In a recent study, we demonstrated that α-adrenergic blockade via phentolamine augments hyperemic and vasodilator responses after a single-muscle contraction in older adults (9). This finding could be interpreted that an enhanced α-adrenergic tone contributes to the age-related reductions in contraction-induced rapid vasodilation. However, the improvement in rapid vasodilation observed in older adults during phentolamine administration could also suggest the age-related differences are a result of impairments in functional sympatholysis in the vascular beds of contracting skeletal muscle, as observed during dynamic exercise (22). Evidence in young adults suggests that functional sympatholysis occurs after a single-muscle contraction (18). Although the mechanisms for functional sympatholysis have not been fully elucidated, NO has been shown to inhibit sympathetic vasoconstriction in contracting skeletal muscle of experimental animals and humans (14, 51, 56, 57). Taken together with our present data, a decreased NO bioavailability with aging might be linked to a decreased ability to blunt sympathetic vasoconstriction in older adults and thus contribute to the attenuated rapid vasodilator responses to muscle contractions in older adults.

**Single vs. dynamic muscle contractions and the influence of aging.** To date, the majority of studies comparing muscle blood flow and vascular control in exercising young and older humans have demonstrated that the control of blood flow to dynamically contracting skeletal muscle is altered with normal aging during submaximal forearm and leg exercise (36, 38, 39, 46–48), which has been attributed in part to less NO-mediated vasodilation in older adults (52). However, it should be noted that other studies have failed to identify any age-associated differences in the hyperemic and vasodilator response during

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**Fig. 2.** Peak hyperemic (ΔFBF; A) and peak vasodilator (ΔFVC; B) responses to single-muscle contractions at 10, 20, and 40% MVC during saline (control) and L-NMMA administration in young and older adults. Under control conditions, peak hyperemic and vasodilator responses were attenuated in older adults at all contraction intensities. NO synthase inhibition (L-NMMA) blunted the peak hyperemic and vasodilator responses at all contraction intensities in young adults and at 10 and 20% MVC in older adults. *P < 0.05 vs. young. †P < 0.01 vs. control.
or immediately following dynamic forearm exercise (25, 35, 40). If blood flow under steady-state conditions is maintained with aging, it could be argued that the impairment in NO-mediated vasodilation following a single-muscle contraction is compensated for by other vasodilator mechanisms as exercise continues. Thus the physiological importance of an impaired rapid vasodilator response might appear insignificant. However, we would argue that daily activities with which older adults often have difficulty are commonly characterized by short bursts of activity that require rapid adjustments in blood flow, and, therefore, any impairment in rapid vasodilation can be considered physiologically relevant.

Pharmacological induced rapid vasodilation: effect of aging. In the present study, we used rapid bolus infusions (2 ml intra-arterial push within 1–2 s) of ACh and NTP as a novel approach to help pharmacodissect and further examine whether the blunted contraction-induced responses with aging were due to altered endothelial (ACh) or vascular smooth muscle (NTP) responsiveness. The magnitude of endothelial-dependent vasodilation via continuous ACh administration has consistently been shown to be blunted with aging (1, 11, 19, 38, 54, 55). Furthermore, evidence in isolated skeletal muscle resistance arterioles from rats indicates that, in addition to a reduced magnitude of vasodilation in response to ACh, aging also impairs the dynamics and timing of vasodilation, in part, through the endothelium (4). In the present study, rapid infusion of ACh produced a relatively rapid rise in flow that promptly decays over time following infusion. Of particular interest to the current study, the rapid and total vasodilator responses to ACh were

\[ \begin{align*}
1) \text{ significantly less in older adults} \\
2) \text{ only sensitive to NOS inhibition in young adults (Fig. 5).}
\end{align*} \]

These observations related to age-related differences and the impact of NOS inhibition are very similar to what was observed during single-muscle contractions and supports the idea that age-related endothelial dysfunction and/or decreased NO bioavailability contribute in part to the reduced contraction-induced rapid vasodilation.

The older subjects in the present study also demonstrated a blunted and delayed vasodilator response to bolus infusions of the endothelium-independent agonist NTP (Fig. 6). The substantially lower vasodilator responses to NTP observed in the present study were somewhat surprising to us, as a large majority of the studies in humans indicate that the steady-state vascular smooth muscle responsiveness to continuous infusions of NTP is preserved with aging (19, 20, 23, 27, 37, 55). However, it should be noted that others have reported some
degree of impairment in NTP-induced vasodilation with aging (1, 38, 45). Irrespective of these discrepancies in the literature, this is the first study to quantify the magnitude and timing of vasodilation in response to a rapid bolus infusion of NTP in humans. As seen in Fig. 6, the responses to NTP were slower and more prolonged than those observed during the ACh infusion (Fig. 5). Additionally, the vasodilator responses to NTP in young and older adults were unaffected during NOS inhibition. When considering the timing of the vasodilator response as well as the lack of change with NOS inhibition, the attenuated vascular smooth muscle responsiveness to NTP observed in the older subjects might not be related to the age-associated reductions in the rapid vasodilator responses to single-muscle contractions. However, since some degree of NTP-mediated vasodilation occurs within the timeframe in which contraction-induced peak responses appear (i.e., 4–5 cardiac cycles), it may be possible that alterations in smooth muscle responsiveness contribute to some extent to the age-related reductions in vasodilation following a single-muscle contraction. Lastly, we cannot rule out the possibility that some of the age-related differences in the responses to pharmacological stimulation or muscle contraction may be attributed to structural adaptations in the vascular smooth muscle of older adults. Along these lines, remodeling of resistance arteries that is associated with aging and certain pathologies (e.g., hyper-

Fig. 4. Relative reduction (%) in peak (A) and total (B) vasodilator responses to single forearm contractions at 10, 20, and 40% MVC during l-NMMA administration compared with saline (control) condition in young and older adults. NO synthase inhibition (l-NMMA) reduced peak vasodilator responses to a greater extent in young compared with older adults at 20 and 40% MVC. NO synthase inhibition reduced total vasodilator responses to a greater extent in young compared with older adults at all contraction intensities. *P < 0.05 vs. young.

Fig. 5. Vasodilator responses to bolus infusions of acetylcholine (ACh) during saline (control) and l-NMMA administration in young and older adults. A: rapid vasodilator responses (ΔFVC) over 60 cardiac cycles after bolus infusion of ACh. Peak (B) and total (C) vasodilator responses to ACh were attenuated in older adults under control conditions. NO synthase inhibition (l-NMMA) blunted the peak and total vasodilator responses to ACh in young adults (B and C). *P < 0.05 vs. young. †P < 0.05 vs. control.
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Fig. 6. Vasodilator responses to bolus infusions of sodium nitroprusside (NTP) during saline (control) and l-NMMA administration in young and older adults. A: rapid vasodilator responses (ΔFVC) over 60 cardiac cycles after bolus infusion of NTP. Peak (B) and total (C) vasodilator responses to NTP were attenuated in older adults under control and l-NMMA conditions. Peak and total vasodilator responses to NTP were unaffected by l-NMMA in both age groups. *P < 0.05 vs. young.

Experimental considerations. Basal leg blood flow has been shown to be positively related to leg muscle mass and inversely related to age (44). Additionally, aging is often associated with decreases in skeletal muscle mass and strength (53), both of which might contribute to lower exercise blood flows in older adults. Lean muscle mass was not measured in this study, and we cannot be certain that potential differences in muscle mass contributed to the attenuated vasodilator responses in the older adults. However, lean muscle mass in the forearm has been shown to be similar between young and older adults (8). Moreover, the forearm volume, MVC, and relative contraction intensities were not different between the age groups in the present study. Taken together, the age-related attenuation in contraction-induced rapid vasodilation is not likely explained by differences in forearm muscle mass.

In the present study, we quantified the hyperemic and vasodilator responses to single-muscle contractions as an absolute change from baseline (resting) values. This approach is similar to our previous study, which examined the influence of α-adrenergic vasoconstriction in the contraction-induced rapid vasodilation (9). However, prior studies from other groups examining the rapid vasodilator response to single-muscle contractions have expressed the magnitude of the response as a relative (%) change from baseline (5, 8, 38). Our approach of expressing the flow and vasodilator response to a single-muscle contraction as an absolute change above baseline (i.e., postcontraction value − baseline value) supports the findings that age-related decrements in rapid vasodilation exist.

It has been argued that expressing data as a percent change in flow or conductance is the most appropriate approach when differences in resting vascular tone exist (7). When the present data are expressed as a relative (%) change in FVC from baseline, there is no difference between control (saline) and NOS inhibition (l-NMMA) trials in the young subjects. However, using the percent change in FVC in older adults would suggest that the rapid vasodilation is significantly enhanced during l-NMMA infusions compared with saline trials at 20 and 40% MVC (189 ± 12 vs. 131 ± 11% and 279 ± 11 vs. 312 ± 19%, respectively; P < 0.01). The paradoxical increase in percent change vasodilation during l-NMMA in the older adults might simply be explained by the lower denominator used in the equation of percent differences. Using the absolute changes to quantify the hyperemic and vasodilator responses (ΔFBF and ΔFVC) in the present study accounts for the baseline (resting) values and minimizes the potential confound that a smaller denominator might present. Furthermore, changes in absolute flow closely reflect the metabolic activity of the contracting muscle (50). Expressing the absolute rapid vasodilator responses normalized to the metabolic activity (i.e., workload performed) in the present study supports the idea of age-related impairments in the contraction-induced rapid vasodilation, as well as the role of NO in the response. Of particular interest to the present study, when we examined the changes in the peak and total ΔFBF and ΔFVC during the l-NMMA trials (compared with saline conditions), both the absolute and relative (Fig. 4) reductions were substantially greater in the young compared with older adults, which suggests a decreased role of NO in the rapid vasodilator response following a single forearm contraction in older adults. Therefore, we chose to report the absolute change in muscle blood flow and conductance from baseline and believe these values most represent the rapid vasodilator responses to single-muscle contractions.
As stated above, we attempted to pharmacologically mimic the rapid vasodilator response to single-muscle contractions and assess the endothelium-dependent and -independent components of the response. However, the time to peak vasodilation occurred substantially later during both bolus ACh (~16 cardiac cycles) and NTP (~25 and 30 cardiac cycles for young and older adults, respectively) infusions compared with single-muscle contractions (~4–5 cardiac cycles). The differences in the temporal pattern of each response are likely due to the drugs being infused upstream from the resistance arterioles of the forearm (site of action), whereas the vasodilator response originates immediately within the forearm during a muscle contraction. Additionally, the delayed vasodilator responses to ACh and NTP may also be due to issues related to drug distribution within the forearm. Nonetheless, the age-related impairments in the dynamics of vasodilation during bolus ACh infusion were similar to single-muscle contractions, and NOS inhibition attenuated rapid vasodilation to a similar degree between conditions for each respective age group.

Conclusions. To our knowledge, this is the first study to demonstrate that the age-related impairments in contraction-induced rapid vasodilation are, in part, due to alterations in endothelial function and blunted NO signaling in healthy older adults. When considered with our previous data (9), a decreased NO bioavailability with aging may contribute to the blunted rapid vasodilation through one of two ways: 1) less direct vasodilation via cGMP-induced smooth muscle cell relaxation; and/or 2) decreased ability to blunt sympathetic vasoconstriction (e.g., functional sympatholysis). Impairments in either of these mechanisms can limit blood flow distribution and oxygen delivery within the active muscle and ultimately lead to functional limitations. Regardless of the exact role of NO in the rapid vasodilator response to muscle contraction, interventions aimed at increasing NO bioavailability might prove to be useful in reversing the age-related decline in muscle blood flow during exercise, particularly at the onset of muscle contractions.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: D.P.C. and M.J.J. conception and design of research; D.P.C., B.G.W., S.M.R., J.L.T., and M.J.J. performed experiments; D.P.C., B.G.W., and J.L.T. analyzed data; D.P.C., B.G.W., S.M.R., J.L.T., and M.J.J. interpreted results of experiments; D.P.C. prepared figures; D.P.C. drafted manuscript; D.P.C., B.G.W., M.J.J., and M.J.J. edited and revised manuscript; D.P.C., B.G.W., S.M.R., J.L.T., and M.J.J. approved final version of manuscript.

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