Acute dietary nitrate supplementation enhances compensatory vasodilation during hypoxic exercise in older adults

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Casey DP, Treichler DP, Ganger CT 4th, Schneider AC, Ueda K. Acute dietary nitrate supplementation enhances compensatory vasodilation during hypoxic exercise in older adults. J Appl Physiol 118: 178–186, 2015. First published November 20, 2014; doi:10.1152/japplphysiol.00662.2014.—We have previously demonstrated that aging reduces the compensatory vasodilator response during hypoxic exercise due to blunted nitric oxide (NO) signaling. Recent evidence suggests that NO bioavailability can be augmented by dietary nitrate through the nitrate-nitrite pathway. Thus we tested the hypothesis that acute dietary nitrate supplementation increases the compensatory vasodilator response to hypoxic exercise, particularly in older adults. Thirteen young (25 ± 1 yr) and 12 older (64 ± 2 yr) adults performed rhythmic forearm exercise at 20% of maximum voluntary contraction during normoxia and hypoxia (~80% O2 saturation); both before (control) and 3 h after beetroot juice (BR) consumption. Forearm vascular conductance (FVC; ml·min⁻¹·100 mmHg⁻¹) was calculated from forearm blood flow (ml/min) and blood pressure (mmHg). Compensatory vasodilation was defined as the relative increase in FVC due to hypoxic exercise (i.e., % increase compared with respective normoxic exercise trial). Plasma nitrite was determined from venous blood samples obtained before the control trials and each of the exercise trials (normoxia and hypoxia) after BR. Consumption of BR increased plasma nitrite in both young and older adults (P < 0.001). During the control condition, the compensatory vasodilator response to hypoxic exercise was attenuated in older compared with young adults (3.8 ± 1.7% vs. 14.2 ± 2.1%, P < 0.001). Following BR consumption, compensatory vasodilation did not change in young (13.7 ± 3.3%, P = 0.81) adults but was substantially augmented in older adults (11.4 ± 2.1%, P < 0.001). Our data suggest that acute dietary nitrate supplementation increases the compensatory vasodilator response to hypoxic exercise in older but not young adults.

hypoxia; vasodilation; hypoxia; exercise; dietary nitrate

HYPOXIA CAN HAVE PROFOUND influences on the circulation, including vasodilation in skeletal muscle vascular beds. In humans, acute exposure to moderate hypoxia in combination with submaximal exercise produces a compensatory vasodilation and augmented blood flow in contracting skeletal muscles relative to the same level of exercise under normoxic conditions (7, 28, 54). In a series of studies we explored a number of potential mechanisms that might contribute to the compensatory vasodilation observed during hypoxic exercise. These studies have investigated the contribution of vasodilating substances such as nitric oxide (NO) and adenosine, the role of β-adrenergic receptors, and the interactions between sympathetic vasoconstriction and metabolic vasodilation in the regulation of vascular tone during hypoxic exercise (8, 10, 11, 65, 66). A synthesis of our findings clearly indicates that NO contributes to the compensatory vasodilator responses to exercise under conditions of reduced oxygen availability in young adults (8–10). However, we recently demonstrated that 1) the compensatory vasodilation during hypoxic exercise is reduced with aging and 2) this attenuated response is probably due to blunted NO signaling (13).

It is widely known that the l-arginine-NOS synthesis (NOS) pathway significantly contributes to the overall production of NO. Once synthesized, NO is rapidly oxidized to form nitrite (NO2⁻) and nitrate (NO3⁻). Until recently the inorganic anions NO2⁻ and NO3⁻ were considered inert end products of NO metabolism. However, accumulating evidence suggests that NO2⁻ reduction (i.e., nitrate-nitrite-NOS pathway) represents an alternative and differentially regulated system for NO generation that operates in parallel with the classic l-arginine-NOS pathway (5, 41–43). Interestingly, the nitrate-nitrite-NOS pathway is greatly enhanced under hypoxic conditions (61). Additionally, recent evidence in experimental animals suggests that dietary nitrate supplementation increases skeletal muscle blood flow and vasodilation during exercise (22). Therefore, increasing plasma NO2⁻ and NO3⁻ levels in humans may enhance NO bioavailability and, subsequently, the hyperemic and vasodilator responses to exercise. With this information as background we aimed to examine the effects of acute dietary nitrate supplementation on the local control of skeletal muscle blood flow and vasodilation during exercise in young and older adults. We hypothesized that acute dietary nitrate supplementation via beetroot (BR) juice would augment blood flow and vasodilation in contracting skeletal muscle, particularly under hypoxic conditions. Moreover, we hypothesized that the improvement in the compensatory vasodilator response to hypoxic exercise would be greater in older adults than in young adults.

METHODS

Subjects. A total of 13 young and 12 older healthy subjects volunteered to participate in the study. Subjects completed written informed consent and were healthy, nonobese (body mass index ≤30 kg/m²), nonsmokers, not taking any vasoactive medications, and were sedentary to moderately active. Two older subjects were taking aspirin (withheld 5 days prior to study) and seven older subjects reported taking a daily vitamin (withheld 3 days prior to study). Studies were performed after an overnight fast and refraining from exercise and caffeine for at least 24 h. Subjects were also instructed to refrain from using antibacterial mouthwash or chewing gum the morning of the study. This was to avoid any possible interference with the processing of NO2⁻ and NO3⁻ in the saliva or stomach (23, 48).

Young female subjects were studied during the early follicular phase of the menstrual cycle or the placebo phase of oral contraceptives (45, 46). All older female subjects were postmenopausal and were not...
taking any form of hormone replacement therapy. All study protocols were approved by the University of Iowa Institutional Review Board and were performed according to the Declaration of Helsinki.

**Forearm exercise.** Subjects performed rhythmic forearm exercise with a handgrip device using the nondominant arm at 20% of each subject’s maximal voluntary contraction (MVC). The weight was lifted 4 to 5 cm over a pulley at a duty cycle of 1-s contraction and 2-s relaxation (20 contractions per minute) using a metronome to ensure correct timing. The average weight used for forearm exercise was 9.7 ± 0.7 kg and 8.1 ± 0.6 kg for the young and older adults, respectively (P = 0.11).

**Heart rate and systemic blood pressure.** Heart rate was recorded via continuous three-lead electrocardiography, and systemic blood pressure was assessed (beat to beat) with a finger plethysmograph (Nexfin; Edwards Lifesciences, Irvine, CA) on the nonexercising hand.

**Forearm blood flow.** Brachial artery mean blood velocity and 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 with a probe insonation angle previously calibrated to 60°. Measured brachial velocity wave forms were synchronized to a data acquisition system (WinDaq; DATAQ Instruments, Akron, OH) via a Doppler audio transformer (26). Brachial artery diameter measurements were obtained at end diastole between contractions during steady-state conditions. Forearm blood flow (FBF) was calculated as the product of mean blood velocity (cm/s) and brachial artery cross-sectional area (cm^2^) and expressed as milliliters per minute (ml/min). Mean blood velocity measurements included the velocity profiles across the entire duty cycle (both contraction and relaxation phases).

**Systemic hypoxia.** Subjects were instrumented with a tight-fitting oronasal facemask and a self-regulating partial rebreathe system that clamps end-tidal CO2 at baseline levels despite large changes in minute ventilation and was used to help generate hypoxic conditions (4, 10, 13). The target arterial O2 saturation (assessed via pulse oximetry; SpO2), heart rate, MAP, and FBF were determined by averaging values over the final minute of rest and exercise for each trial. Forearm vascular conductance (FVC) was calculated as (FBF/arterial pressure) × 100 and expressed as ml·min^-1·100 mmHg^-1_. Plasma [NO2^-] was determined from blood samples obtained prior to the control trials (baseline) and each of the exercise trials (normoxia and hypoxia) following consumption of the BR.

All values are expressed as means ± SE. ANOVA was used to analyze baseline differences between age groups. To determine the effect of acute nitrate supplementation on skeletal muscle blood flow and vasodilation at rest and during exercise in young and older adults under normoxic and hypoxic conditions, the differences in FBF and FVC were determined via repeated measures ANOVA. All other hemodynamic variables and plasma [NO2^-] were also assessed via repeated measures ANOVA. 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. To further examine the effects of aging and nitrate supplementation on the compensatory vasodilator response to hypoxic exercise we compared via ANOVA the absolute and relative change in FVC (steady-state FVC during hypoxic exercise; steady-state FVC during normoxic exercise) between the young and older adults under each respective condition (with and without BR). Finally, the absolute and relative compensatory vasodilator responses to hypoxic exercise in the seven older adults who performed the placebo trial were compared with their respective responses during the nitrate-rich BR trials via a paired t-test.

**RESULTS**

Subject characteristics are summarized in Table 1. Although young and older subjects were of similar height and weight, older adults had a higher body mass index (P < 0.05). Forearm volume (P = 0.76) and MVC (P = 0.10) did not differ between the young and older subjects.

**Effects of BR on plasma [NO2^-].** Due to catheter-related issues, complete blood samples (all three time points; baseline and both the normoxia and hypoxia trials following BR consumption) were collected in only 20 (10 in each age group) of the 25 subjects. In both young and older adults plasma [NO2^-] was substantially greater (approximately threefold) prior to each of the exercise trials (normoxia and hypoxia) following
consumption of BR compared with baseline values (pre-BR consumption) (Fig. 1).

**Systemic hemodynamic responses.** The group data (means ± SE) for hemodynamic responses due to combined forearm exercise and hypoxia under each condition are presented in Table 2. Under normoxic conditions older adults demonstrated a lower SpO2 and end-tidal CO2 compared with their young counterparts (P < 0.05). As expected, SpO2 was decreased and heart rate increased as a consequence of systemic hypoxia and incremental forearm exercise in both the young and older adults (P < 0.05). Despite higher MAP values under resting conditions in older adults, the magnitude of change in MAP during exercise (both normoxia and hypoxia) was similar between age groups. By design, end-tidal CO2 was maintained during all conditions within each age group. Nitrate supplementation did not alter MAP, SpO2, or end-tidal CO2 at rest or during exercise under normoxic or hypoxic conditions in the young or older adults. However, resting heart rate during normoxic conditions was lower following BR consumption in both age groups (P < 0.05).

**Forearm exercise.** Table 3 shows (means ± SE) forearm hemodynamics at rest and during normoxic and hypoxic exercise before and after BR consumption. In the young adults, FBF and FVC during hypoxic exercise were higher compared with normoxic exercise under both control and post-BR conditions (main effect of hypoxia, P < 0.05, Table 3). In the older adults, FBF and FVC did not differ between normoxic and hypoxic exercise under control conditions (P = 0.08 for both). However, the FBF and FVC response to hypoxic exercise post-BR was substantially greater compared with normoxic exercise under the same condition in older adults (P < 0.001, Table 3). In turn, FBF and FVC during hypoxic exercise post-BR were greater compared with values during hypoxic exercise under control conditions (P < 0.01). Although older adults demonstrated lower hypoxic exercise FBF and FVC responses compared with their younger counterparts under control conditions (P < 0.05), these age-related differences in hypoxic exercise FBF and FVC were not observed following BR consumption (P = 0.25 and 0.17, respectively).

**Dietary nitrate and compensatory vasodilatation.** Similar to several of our previous studies (8, 12, 13), we also examined the compensatory vasodilator response (steady-state FVC during hypoxic exercise; steady-state FVC during normoxic exercise) to better understand the effect of acute dietary nitrate supplementation on muscle blood flow during hypoxic exercise in both young and older adults. Under control conditions both the absolute (9.3 ± 4.5 vs. 44.3 ± 5.3 ml·min⁻¹·100 mmHg⁻¹, P < 0.001) and relative (3.8 ± 1.7 vs. 14.2 ± 1.2%, P < 0.001) compensatory vasodilator response to hypoxic exercise was attenuated in the older compared with young adults (Fig. 2, A and B). The compensatory vasodilator responses (both absolute and relative) did not change following consumption of BR in the young adults (Fig. 2, A and B). Conversely, acute nitrate supplementation augmented the absolute (29.1 ± 5.6 ml·min⁻¹·100 mmHg⁻¹) and relative (11.4 ± 2.1%) compensatory vasodilation in the older adults (P < 0.01, Fig. 2, A and B). Moreover, the change in FVC between the two hypoxic conditions (control vs. BR) was related to the increase in plasma [NO₂⁻] in older adults (r = 0.69, P < 0.05, Fig. 3), whereas no relationship was observed in the young adults (r = 0.28, P = 0.43).

**Placebo trials.** In the seven older adults who completed the placebo trials, FBF and FVC did not differ between normoxic and hypoxic exercise under control conditions (prior to consumption of nitrate-depleted BR). Both absolute (9.7 ± 2.4 vs. 10.8 ± 2.7 ml·min⁻¹·100 mmHg⁻¹, P = 0.36) and relative (3.1 ± 0.8 vs. 3.7 ± 0.8%; P = 0.18, Fig. 4A) compensatory vasodilation were unchanged following consumption of the nitrate-depleted BR. Conversely the absolute (10.8 ± 2.7 vs. 36.3 ± 5.7 ml·min⁻¹·100 mmHg⁻¹) and relative (3.7 ± 0.8 vs. 14.1 ± 1.4%; Fig. 4, A and B) compensatory vasodilator responses to hypoxic exercise were substantially less during the nitrate-depleted BR (placebo) trials compared with the nitrate-rich BR trials in the seven older subjects who completed both study days (P < 0.01 for both).

**DISCUSSION**

In recent years, data have accumulated to suggest that dietary nitrate supplementation can have physiological and therapeutic effects in both animal models and humans [see review by Weitzberg and Lundberg (64)]. Briefly, several of the reported physiological benefits of acute oral nitrate supplementation can have physiological and clinical benefits in both animal models and humans [see review by Weitzberg and Lundberg (64)]. Briefly, several of the reported physiological benefits of acute oral nitrate supplementation include but are not limited to improvements in l) exercise efficiency and tolerance (2, 3, 30, 35, 37), 2) measures of endothelial function and 3) plasma nitrate concentration.

**FIG. 1.** Plasma levels of nitrite ([NO₂⁻]̄) before the control trials (baseline) and each of the exercise trials (normoxia and hypoxia) following consumption of beetroot juice (BR). Acute consumption of BR resulted in substantial increases in plasma [NO₂⁻] in both young and older adults. *P < 0.001 vs. baseline.

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**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>25 ± 1</td>
<td>64 ± 2*</td>
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<tr>
<td>Men/Women</td>
<td>10/3</td>
<td>9/3</td>
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<tr>
<td>Height, cm</td>
<td>179 ± 3</td>
<td>174 ± 3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>74 ± 3</td>
<td>77 ± 2</td>
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<tr>
<td>BMI, kg/m²</td>
<td>23.1 ± 0.6</td>
<td>25.5 ± 0.7*</td>
</tr>
<tr>
<td>FAV, ml</td>
<td>1,015 ± 69</td>
<td>966 ± 48</td>
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<tr>
<td>MVC, kg</td>
<td>48 ± 3</td>
<td>40 ± 3</td>
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Values are means ± SE. BMI, body mass index; FAV, forearm volume; MVC, maximal voluntary contraction. *P < 0.05 vs. young.
vascular stiffness (25, 52, 63), and 3) blood pressure (29, 30, 36, 37, 62, 63).

To our knowledge, this is the first study to investigate the effects of inorganic dietary nitrate on exercising skeletal muscle blood flow in humans. Our primary findings are that 1) acute dietary nitrate supplementation enhances skeletal muscle blood flow and vasodilation in older adults during hypoxic but not normoxic exercise, and 2) the blood flow and vasodilator responses to forearm exercise under normoxic and hypoxic conditions are unaltered in young adults following acute dietary nitrate supplementation. Taken together, the results suggest that acutely elevating plasma [NO$_2^-$] via BR consumption has a beneficial effect on the exercise hyperemic and vasodilator responses to exercise in older adults under conditions of reduced oxygen availability.

**Aging and vasodilator responses to exercise: normoxia vs. hypoxia.** It is commonly believed that the control of blood flow to dynamically contracting skeletal muscle is altered with normal aging during submaximal exercise. This belief is supported by several studies that have demonstrated that the hyperemic and vasodilator responses to exercise are attenuated in older adults during submaximal forearm and leg exercise under normoxic conditions (32, 34, 38, 47, 49–51). Additionally, the age-related differences have been attributed in part to less NO-mediated vasodilation in older adults (55). However, it should be noted that other studies have failed to identify any age-associated differences in the hyperemic and vasodilator response during or immediately following dynamic forearm exercise (19, 27, 40). In the current study we did not observe any age-related differences in FBF ($P = 0.19$) or FVC ($P = 0.11$) during normoxic exercise under control conditions. Of particular interest to the current study, there was a trend for an increase in FBF (271 ± 30 vs. 282 ± 31 ml/min, $P = 0.12$) and FVC (262 ± 29 vs. 276 ± 32 ml·min$^{-1}$·100 mmHg$^{-1}$, $P = 0.07$) response to exercise under normoxic conditions in older adults following acute dietary nitrate supplementation; however, these changes were not significant (Table 3).

Although the overall purpose of the current study was to examine the effects of dietary nitrate supplementation on muscle blood flow, we were particularly interested in the compensatory vasodilator response to hypoxic exercise in older adults. This interest was primarily driven by evidence that suggests 1) aging appears to affect the hyperemic and vasodilator response to exercise to a greater extent under hypoxic compared with normoxic conditions (13), and 2) the nitrate-nitrite-NO pathway appears to be enhanced under hypoxic conditions in humans (15, 44). Despite acute dietary nitrate having no effect on hypoxic-mediated blood flow and vasodilation during exercise in young adults (discussed below), older adults demonstrated substantial increases in compensatory vasodilation following consumption of BR. In fact, the age-related differences in the compensatory vasodilator response to hypoxic exercise observed under control conditions (pre-BR) were abolished following BR supplementation (Fig. 2). Furthermore, we found a positive correlation between the change in plasma [NO$_2^-$] following BR supplementation and the change in FVC between the two hypoxic exercise conditions (control vs. BR). That is, older adults with the largest increase in plasma [NO$_2^-$] demonstrated the greatest increase in FVC from the control to post-BR hypoxic exercise trials (Fig. 3). Finally, the inclusion of placebo (nitrate-depleted BR) trials in a subset of older adults supports the idea that nitrate, as opposed to other components of BR (i.e., antioxidants), is likely the major contributor to the enhanced compensatory vasodilation during hypoxic exercise (Fig. 3).

**Potential mechanisms for nitrite-induced compensatory vasodilation.** As mentioned previously, we have demonstrated that NO contributes to hypoxic-mediated vasodilation during...
exercise (10), and this response is attenuated with aging, likely due to alterations in NO-mediated mechanisms (13). Theoretically, the reduction of NO\textsubscript{3} and NO\textsubscript{2} following BR supplementation leads to an increased NO bioavailability, which in turn, directly contributes to the beneficial effect of dietary nitrate supplementation on muscle blood flow in older adults observed in the current study. Along these lines, acute dietary nitrate supplementation increases plasma cyclic guanosine 5\' monophosphate levels, an indicator of generation of bioactive NO (29). In addition to the direct vasodilator effects of NO, an increase in bioavailable NO may also contribute to the reduction in reactive oxygen species (56), which subsequently improves vascular function. Indeed, scavenging of reactive oxygen species with intra-arterial ascorbic acid in older adults can improve skeletal muscle blood flow during forearm exercise (34), which has been primarily attributed to an increase in bioavailable NO (16). Moreover, 3 wk of sodium nitrite supplementation decreases arterial oxidative stress in older mice and subsequently improves vascular endothelial function (57). In addition to possessing antioxidant properties, sodium nitrite also has been shown to possibly work as an anti-inflammatory agent (57). Markers of oxidative stress and inflammation were not assessed in the current study, so we are unable to determine whether the improvements in compensatory vasodilation in older adults following acute dietary nitrate consumption were influenced in part by antioxidant and anti-inflammatory effects.

**Table 3. Forearm hemodynamics at rest and with forearm exercise during normoxia and hypoxia before and after beetroot juice consumption**

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>20%</th>
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<tr>
<td><strong>Young (n = 13)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Forearm blood flow, ml/min</td>
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<td></td>
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<tr>
<td>Control, before beetroot juice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoxia</td>
<td>66±9</td>
<td>305±19</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>69±7</td>
<td>350±22*</td>
</tr>
<tr>
<td>After beetroot juice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoxia</td>
<td>62±10</td>
<td>312±21</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>67±8</td>
<td>362±28*</td>
</tr>
<tr>
<td>Forearm vascular conductance, ml·min\textsuperscript{-1}·100mmHg\textsuperscript{-1}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, before beetroot juice</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoxia</td>
<td>72±10</td>
<td>308±17</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>75±7</td>
<td>352±21*</td>
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<tr>
<td>After beetroot juice</td>
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<td></td>
</tr>
<tr>
<td>Normoxia</td>
<td>68±10</td>
<td>319±21</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>73±8</td>
<td>361±25*</td>
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<tr>
<td><strong>Older (n = 12)</strong></td>
<td></td>
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<tr>
<td>Forearm blood flow, ml/min</td>
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<tr>
<td>Control, before beetroot juice</td>
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<td></td>
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<tr>
<td>Normoxia</td>
<td>59±6</td>
<td>271±30</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>60±7</td>
<td>284±31†</td>
</tr>
<tr>
<td>After beetroot juice</td>
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<td></td>
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<tr>
<td>Normoxia</td>
<td>52±6</td>
<td>282±31</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>55±6</td>
<td>315±33*‡</td>
</tr>
<tr>
<td>Forearm vascular conductance, ml·min\textsuperscript{-1}·100mmHg\textsuperscript{-1}</td>
<td></td>
<td></td>
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<tr>
<td>Control, before beetroot juice</td>
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<tr>
<td>Normoxia</td>
<td>63±7</td>
<td>262±29</td>
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<tr>
<td>Hypoxia</td>
<td>62±7</td>
<td>271±30</td>
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<tr>
<td>After beetroot juice</td>
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<tr>
<td>Normoxia</td>
<td>55±7</td>
<td>276±32</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>57±6</td>
<td>305±34*‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Main effect of hypoxia, \(P < 0.05\); †\(P < 0.05\) vs. young; ‡\(P < 0.01\) vs. control.
Lastly, both aging and hypoxia are associated with an increased sympathetic vasoconstrictor activity directed at the skeletal muscle at rest and during exercise (24, 58). Furthermore, evidence suggests that older adults have impairments in functional sympatholysis (ability to blunt sympathetic vasoconstriction) in the vascular beds of contracting skeletal muscle during dynamic exercise (18, 33, 47). Taken together, these age-related changes likely contribute to the reduced compensatory vasodilation during hypoxic exercise in older adults. 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, 17, 59). If functional sympatholysis is indeed mediated in part by NO, then increasing NO bioavailability (via NO\(_2\) reduction) could indirectly enhance muscle blood flow and vasodilation, as was observed in the older adults during hypoxic exercise.

**Effects of BR on muscle blood flow and vasodilation in young adults.** The lack of change in exercising FBF and FVC (Table 3) following BR supplementation in young adults observed in the current study is in contrast to recent evidence in young rats (22). Ferguson and colleagues (22) demonstrated that 5 days of inorganic nitrate supplementation enhances total hindlimb muscle blood flow and vasodilation during treadmill running in rats. The discrepancy between the current findings and those of Ferguson et al. (22) could be simply due to species differences or the duration of BR supplementation (single bolus vs. 5 days). However, two other possibilities exist that may explain the discrepant findings. First, the improved muscle blood flow and vascular conductance in rats following BR supplementation was observed primarily in fast-twitch type IIb + d/x muscles of the hindlimb, which suggests a fiber-type selective effect of dietary nitrate on vascular control (22). The relevance of these findings to our data in humans is unclear because, unlike rodents, which have highly compartmentalized skeletal muscle containing predominantly fast, slow, or intermediate fiber types (1, 22), human skeletal muscle is mixed (39). Additionally, the fiber type distribution in the flexor muscles of the human hand and forearm involved with the work associated with handgrip type exercise are unknown.

Second, the lack of change in FBF and FVC following BR consumption in young adults in the current study might be related to the amount of dietary nitrate consumed. Along these lines, a high dose of dietary nitrate has been shown to be necessary to produce significant changes in exercising skeletal muscle blood flow and vasodilation in rats (21). However, the dose of BR administered in the current study was sufficient to elicit improvements in FBF and FVC in older adults and has also been shown to be effective in increasing tissue oxygenation during exercise in the lower limbs of patients with peripheral arterial disease (31). Moreover, the lack of an effect of BR on FBF and FVC (during both normoxic and hypoxic conditions) in the young adults is not likely due to issues related to the metabolism of NO\(_3\) to NO\(_2\), because the magnitude of change in plasma [NO\(_2\)] after BR were similar between age groups. Taken together, we favor the idea that the lack of change in FBF and FVC following BR supplementation in the young adults is more of a function of them having normal blood flow and vasodilator responses to exercise and/or a sufficient constitutive NO production by endothelial NOS (eNOS) for normal physiological function prior to nitrate supplementation rather than issues related to the dose. Whereas older adults demonstrate a blunted vasodilator response to hypoxic exercise, likely due to alterations in the \(\text{L-arginine-eNOS}\) pathway (13), and therefore may be more amendable to an intervention aimed at improving NO bioavailability such as dietary nitrate supplementation.

**Experimental considerations.** Aging is associated with lower plasma and arterial concentrations of NO\(_2\) in mice (57). In the
levels of NO\textsubscript{2} were not observed in humans (Fig. 1). This could be interpreted as young and older adults having similar endogenous production of NO (via NOS enzymes). However, plasma levels of NO\textsubscript{2} are strongly influenced by diet (53), which was not controlled for in our subjects (aside from fasting overnight). Therefore, the variability in dietary patterns among subjects in the days prior to each respective study day could have influenced the baseline NO\textsubscript{2} values.

In the current study, we used a single dose of a commercially available BR (500 ml), which had a measured content of 18,708 \textmu mol/liter NO\textsubscript{3}. Using a single dose did not allow us to determine whether changes in muscle blood flow and vasodilation during normoxic and hypoxic exercise in humans were possibly dose-dependent. This is particularly important because varying doses of nitrate consumption have been shown to elicit different physiological responses, including blood flow in contracting muscles of rats (21). Moreover, tissue NO production from nitrite under hypoxic conditions occurs in a dose-dependent manner (20). Therefore, it might be possible to further augment the hyperemic and vasodilator responses to exercise with either higher doses or longer durations of nitrate supplementation.

Conclusions. This study was the first to examine the effects of dietary nitrate supplementation on skeletal muscle blood flow and vasodilation during normoxic and hypoxic exercise in both young and older humans. Our results demonstrate that acute supplementation of dietary nitrate (via BR juice) has the potential to improve blood flow and vasodilation in the contracting skeletal muscle of older adults under hypoxic conditions. We believe these findings provide important information related to the potential therapeutic effects of dietary nitrate in improving vascular control and exercise hyperemia in populations that often demonstrate impairments in skeletal muscle blood flow and/or reduced NO bioavailability (i.e., diabetes, heart failure, vascular disease).

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GRANTS

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

D.P.C. conception and design of research; D.P.C., D.P.T., C.T.G., A.C.S., and K.U. performed experiments; D.P.C., D.P.T., and C.T.G. analyzed data; D.P.C., D.P.T., C.T.G., A.C.S., and K.U. interpreted results of experiments; D.P.C. prepared figures; D.P.C. drafted manuscript; D.P.C., D.P.T., and K.U. edited and revised manuscript; D.P.C., D.P.T., C.T.G., A.C.S., and K.U. approved final version of manuscript.

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