Acute superoxide scavenging reduces sympathetic vasoconstrictor responsiveness in short-term exercise-trained rats

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Jendzowsky NG, DeLorey DS. Acute superoxide scavenging reduces sympathetic vasoconstrictor responsiveness in short-term exercise-trained rats. J Appl Physiol 114: 1511–1518, 2013. First published April 4, 2013; doi:10.1152/japplphysiol.00131.2013.—We hypothesized that acute superoxide (O₂⁻) scavenging would attenuate sympathetic vasoconstrictor responsiveness by augmenting nitric oxide (NO)–mediated inhibition of sympathetic vasoconstriction in exercise-trained rats. Sprague-Dawley rats were randomly assigned to sedentary time control (S; n = 7) or mild- (M: 20 min/min, 5° grade; n = 7) or heavy-intensity (H: 40 min/min, 5° grade; n = 7) exercise training (ET) groups and trained 5 days/wk for 4 wk with matched training volume. Following ET, rats were anesthetized and instrumented for lumbar sympathetic chain stimulation and measurement of femoral vascular conductance. In resting skeletal muscle, the percentage change of femoral vascular conductance in response to continuous (2 Hz) and patterned (20 and 40 Hz) sympathetic stimulation was determined during control conditions, O₂⁻ scavenging (TIRON, 1 g·kg⁻¹·h⁻¹ iv) and combined O₂⁻ scavenging + nitric oxide synthase blockade (Nω-nitro-l-arginine methyl ester, 5 mg·kg⁻¹ iv). ET augmented the vasoconstrictor response to sympathetic stimulation in a training intensity-dependent manner (P < 0.05) (S: 2 Hz: −26 ± 7.1%; 20 Hz: −26.9 ± 7.3%; 40 Hz: −27.7 ± 7.0%; M: 2 Hz: −37.4 ± 8.3%; 20 Hz: −35.9 ± 7.4%; 40 Hz: −38.2 ± 9.4%; H: 2 Hz: −46.9 ± 7.8%; 20 Hz: −48.5 ± 7.2%; 40 Hz: −51.2 ± 7.3%). O₂⁻ scavenging did not alter (P > 0.05) in ET rats (S: 2 Hz: −23.9 ± 7.6%; 20 Hz: −26.1 ± 9.1%; 40 Hz: −27.5 ± 7.2%), whereas the response in ET rats was diminished (M: 2 Hz: −26.3 ± 5.1%; 20 Hz: −28.7 ± 5.3%; 40 Hz: −28.5 ± 5.6%; H: 2 Hz: −35.5 ± 10.3%; 20 Hz: −38.6 ± 6.8%; 40 Hz: −43.9 ± 5.9%, P < 0.05). TIRON + Nω-nitro-l-arginine methyl ester increased vasoconstrictor responsiveness (P < 0.05) in ET rats (M: 2 Hz: −47.7 ± 9.8%; 20 Hz: −41.2 ± 7.2%; 40 Hz: −50.5 ± 7.9%; H: 2 Hz: −55.8 ± 7.6%; 20 Hz: −55.7 ± 7.8%; 40 Hz: −58.7 ± 6.2%), whereas, in S rats, the response was unchanged (2 Hz: −29.4 ± 8.7%; 20 Hz: −30.0 ± 7.4%; 40 Hz: −35.2 ± 10.3%; P > 0.05). These data indicate that the augmented sympathetic vasoconstrictor responsiveness in ET rats was related to increased oxidative stress and altered nitric oxide-mediated inhibition of vasoconstriction.

reactive oxygen species; oxidative stress; antioxidant; sympathetic nervous system; exercise-intensity; vascular conductance

METHODS

Animals and animal care. Male Sprague-Dawley rats (~8 wk old) were obtained from the institutional animal colony. Rats were housed in pairs in a 12:12-h light-dark cycle, environmentally controlled (22–24°C, 40–70% humidity) room. Water and rat chow (Lab Diet 5001, PMI Nutrition, Brentwood, MO) were provided ad libitum. All experiments were conducted in accordance with the Canadian Council on Animal Care Guidelines and Policies with approval from the

IN ADDITION TO BEING A POTENT vasodilator, nitric oxide (NO) has also been shown to inhibit sympathetic vasoconstriction (11, 18, 33, 34). NO is biologically inactivated by reactive oxygen species (ROS), in particular superoxide anion (O₂⁻) (20). An imbalance between ROS and antioxidant defense mechanisms is defined as oxidative stress and may lead to reduced NO bioavailability (7). This reduced NO bioavailability may decrease the capacity to inhibit sympathetic vasoconstriction (5, 51, 57). During an acute bout of exercise, the production of ROS increases in an exercise-intensity-dependent manner in concert with mitochondrial respiration (1, 4). Endurance exercise training has also been shown to increase the production of O₂⁻ and other oxygen free radicals; however, this often occurs in conjunction with an upregulation of antioxidant enzymes (14, 24). Thus, whether exercise training is associated with increased or decreased oxidative stress is unclear (3, 14, 37, 38). However, it appears that the intensity of exercise training may influence the production of ROS and antioxidants, as evidence of oxidative stress has been documented after chronic heavy-intensity endurance exercise training (2, 9, 38, 58).

We recently reported that the skeletal muscle vasoconstrictor response to sympathetic stimulation was augmented in a training-intensity-dependent manner following 4 wk of exercise training (17). We wondered if the increased sympathetic vasoconstrictor responsiveness following exercise training may be due to a reduction of NO-mediated inhibition of sympathetic vasoconstriction, secondary to a training-induced increase in O₂⁻. If training were associated with increased O₂⁻ levels, acute O₂⁻ scavenging would be expected to attenuate sympathetic vasoconstrictor responsiveness by reducing ROS, liberating NO and augmenting NO-mediated inhibition of sympathetic vasoconstriction in trained compared with control rats. If the effects of O₂⁻ scavenging were mediated by an NO-dependent mechanism, subsequent NO synthase inhibition would result in a larger increase in the vascular response to sympathetic stimulation in exercise-trained compared with control rats.

Therefore, the purpose of this investigation was to determine the effects of acute O₂⁻ scavenging on sympathetic vasoconstrictor responsiveness and NO-mediated inhibition of sympathetic vasoconstriction in sedentary and mild- and heavy-intensity exercise-trained rats. It was hypothesized that acute O₂⁻ scavenging would decrease the vasoconstrictor response to sympathetic stimulation in trained compared with sedentary rats by improving NO-mediated inhibition of sympathetic vasoconstriction. We also hypothesized that these effects would occur in an exercise training-intensity-dependent manner.

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Animal Care and Use Committee: Health Sciences for the University of Alberta.

**Exercise training.** All rats were habituated to the lab and treadmill (Panlab LE8710, Barcelona, Spain) by walking 10 min/day at 10 m/min, 0° grade, for 5 days. Rats were then randomly assigned to one of three groups: 1) sedentary time control (S; n = 7); 2) mild-intensity exercise training (M; 20 m/min, 5° grade, n = 7); or 3) heavy-intensity exercise training (H; 40 m/min, 5° grade, n = 7). Exercise training was completed 5 days/ wk for 4 wk. The total volume of training was matched between groups by having both M and H groups run the same distance (600 m) during each training bout. S rats were handled and weighed daily. The M rats ran at 20 m/min, 5° grade, for 600 m immediately following familiarization and maintained this intensity during all training sessions. The H group completed fifteen 1-min running intervals at 40 m/min, 5° grade, on the first day of training. Each interval was separated by 1 min of rest, and, on each subsequent day, interval run time was increased while rest time was maintained. This training progression allowed the H group to run continuously at 40 m/min, 5° grade, for 600 m, 10 ± 1 days following the onset of training, consistent with previous studies from our laboratory (16–18).

**Instrumentation.** Approximately 24 h after the last training session (22, 44, 48), rats were anesthetized with inhalation of isoflurane (3–3.5%, balance O2) and surgically instrumented for study. The right jugular vein was cannulated, and anesthesia was maintained by infusion of α-chloralose (8–16 mg·kg$^{-1}$·h$^{-1}$) and urethane (50–100 mg·kg$^{-1}$·h$^{-1}$). The depth of anesthesia was assessed by the stability of arterial blood pressure, heart rate (HR), and the absence of a withdrawal reflex in response to a painful stimulus (i.e., paw pinch). A tracheotomy was performed to allow the spontaneous breathing of room air. We have previously demonstrated that arterial blood gases and acid base balance are maintained in this preparation (18). Thus arterial blood gases were only checked periodically to confirm the maintenance of blood gases and acid base balance in this experiment. The left brachial artery was cannulated and connected to a solid-state pressure transducer (Abbott, North Chicago, IL) for continuous measurement of arterial blood pressure. The left femoral artery and vein were cannulated for the delivery of pharmacology. Blood flow was measured from the right femoral artery using a transit-time flow probe (0.7 V; Transonic Systems T107, Ithaca, NY) connected to a flow meter (T106 Transonic Systems). Core temperature was monitored by rectal probe and maintained at 36–37°C by external heating pad (Physitemp TCAT-2, Clifton, NJ).

**Lumbar sympathetic chain stimulation.** Following a laparotomy, the aorta and vena cava were temporarily retracted, and the lumbar chain was exposed by dissection with a blunt glass pipette. A bipolar silver-wire stimulating electrode was attached to the lumbar sympathetic chain at the level of L3 and L4. The electrodes were embedded and electrically isolated in a rapidly curing nontoxic silicone elastomer (Kwiksil, WI, Sarasota, FL). The electrodes delivered constant-current stimulations from an isolated stimulator (Digitimer DS3, Welwyn City, UK).

**Effect of acute O2 scavenging on sympathetic vasoconstrictor responsiveness and NO-mediated inhibition of sympathetic vasoconstriction.** Sympathetic nerve activity is characterized by random bursts of activity, followed by periods of quiescence. In humans and animals, there is a continuous low-frequency nerve discharge under basal conditions, with an average firing frequency of ~1 Hz (29). However, sympathetic excitation increases sympathetic nerve discharge frequency, and single intraburst nerve discharge frequencies between 20 and 50 Hz have been recorded (15, 29). Thus, in the present study, the lumbar sympathetic chain was stimulated with patterns designed to reflect low resting levels of muscle sympathetic nerve activity and moderate- to high-frequency bursts of muscle sympathetic nerve activity, which have been recorded in response to physiological stress, such as exercise (32). Stimulation patterns included 1) continuous stimulation at 2 Hz; 2) 20-Hz bursts for 1 s repeated every 10 s; and 3) 40-Hz bursts for 0.5 s repeated every 10 s. During each 1-min stimulation period, a total of 120 1-ms impulses were delivered at 1 mA. Following a 20-min recovery from the surgical instrumentation, sympathetic stimulations were delivered under control conditions in random order and were separated by 5 min to allow restoration of baseline hemodynamics between stimulations.

After a short recovery period, the membrane-permeable O2 scavenger 4,5-dihydroxy-1,3-benzene-disulfonic acid (TIRON) was continuously infused intravenously with a syringe pump at a rate of 1 g·kg$^{-1}$·h$^{-1}$. Following a 20-min stabilization period, sympathetic stimulations were repeated in random order. Following a brief recovery period, TIRON infusion was maintained, and the NO synthase inhibitor, N$^\text{ω}$-nitro-L-arginine methyl ester hydrochloride (l-NAME), was injected (5 mg/kg iv). Approximately 20 min after the bolus injection of l-NAME (47, 57), sympathetic stimulations were again delivered in random order.

On completion of experiments, animals were euthanized by anesthetic overdose, and the heart was dissected free. Heart mass and the heart mass-to-body mass ratio were used as indicators of the efficacy of exercise training.

**Drugs.** Drugs were purchased from Sigma-Aldrich (Oakville, ON, Canada) and dissolved in 0.9% physiological saline.

**Data analysis.** Data were recorded using Chart 7.2 data acquisition software (AD Instruments, Colorado Springs, CO). Arterial blood pressure and femoral artery blood flow (FFB) were recorded continuously at 100 Hz. HR was derived from the arterial blood pressure waveform, mean arterial blood pressure (MAP) was calculated, and femoral vascular conductance (FVC) was calculated as FBF + MAP (ml/min$^{-1}$·mmHg$^{-1}$). The magnitude of vasocostriction in response to sympathetic stimulation was calculated as the difference between the integral of FBF and FVC during the 1-min lumbar sympathetic chain stimulation period and a 1-min integral of FBF and FVC immediately before the stimulation and expressed as a percent change from FBF and FVC preceding the stimulation. The attenuation of sympathetic vasoconstrictor responsiveness due to O2 scavenging was calculated as the difference between the magnitude of sympathetic vasocostriction during control and TIRON conditions. The magnitude of NO-mediated inhibition of sympathetic vasocostriction during acute O2 scavenging was calculated as the difference between the magnitude of sympathetic vasocostriction during the TIRON and combined TIRON + l-NAME conditions. All data were expressed as means ± SD.

**Statistics.** The effects of exercise training and drug treatment on sympathetic vasoconstrictor responsiveness were determined by two-way repeated-measures ANOVA (group × drug condition; SigmaPlot 12.3 Systat, Richmond, CA). A one-way ANOVA was used to assess group differences in the magnitude of the change in sympathetic vasoconstrictor responsiveness induced by TIRON and combined TIRON + l-NAME treatment. The effects of exercise training on body mass, cardiac mass, and the heart mass-to-body mass ratio was determined by one-way ANOVA. When significant F-ratios were found, Student Newman Keuls post hoc analysis was performed. A P value <0.05 was considered statistically significant.

**RESULTS**

All exercise-trained rats completed the prescribed training regimen. Body mass was not different (P > 0.05) between groups, whereas heart mass was greater (P < 0.05) in exercise-trained compared with S rats (Table 1). The heart mass-to-body mass ratio was also increased (P < 0.05) in a training intensity-dependent manner (Table 1).

**Basal hemodynamics.** Resting HR and MAP were not different (P > 0.05) between S, M, and H rats (Table 2). Resting FBF was greater (P < 0.05; main effect) in S compared with M rats, but was not different from H rats (Table 2), whereas
FVC was not different ($P > 0.05$) between groups (Table 2). HR was not altered ($P > 0.05$) by TIRON (Table 2). MAP and FBF were significantly elevated ($P < 0.05$) by TIRON, and FVC was unchanged ($P > 0.05$; Table 2). The subsequent bolus injection of l-NAME further increased ($P < 0.05$) MAP and significantly reduced ($P < 0.05$) HR, FBF, and FVC in all groups (Table 2).

**Effect of acute $O_2^-$ scavenging on sympathetic vasoconstrictor responsiveness and NO-mediated inhibition of sympathetic vasoconstriction.** The vascular response to lumbar sympathetic stimulation delivered in a bursting pattern at 20 Hz during control, TIRON infusion, and during combined TIRON + l-NAME treatment in a representative rat is illustrated in Fig. 1. Within each group, all stimulation patterns produced a similar ($P > 0.05$) decrease in FBF and FVC. However, sympathetic vasoconstrictor responsiveness was augmented ($P < 0.05$) in exercise-trained compared with S rats at all stimulation frequencies and was increased as a function of training intensity (Fig. 2).

$O_2^-$ scavenging with TIRON did not change ($P > 0.05$) the vascular response to sympathetic stimulation at 2, 20, and 40 Hz in S rats (Fig. 2). However, $O_2^-$ scavenging diminished ($P < 0.05$) the vasoconstrictor response to sympathetic stimulation in M and H rats at all stimulation frequencies (Fig. 2). The difference in the vasoconstrictor response between control and TIRON conditions was greater ($P < 0.05$) in M (2 Hz: 10.7 ± 3.7%; 20 Hz: 7.2 ± 4.1%; 40 Hz: 8.0 ± 4.8%) and H (2 Hz: 11.4 ± 5.5%; 20 Hz: 10.6 ± 4.4%; 40 Hz: 8.9 ± 4.8%) compared with S (2 Hz: 0.8 ± 7.8%; 20 Hz: 0.8 ± 10.1%; 40 Hz: 0.2 ± 7.7%) rats at all frequencies of sympathetic stimulation.

Combined $O_2^-$ scavenging with TIRON and NO synthase blockade with l-NAME did not alter ($P < 0.05$) the vascular response to sympathetic stimulation delivered at 2 and 20 Hz, but did increase the response to sympathetic chain stimulation at 40 Hz in S rats (Fig. 2). In contrast, sympathetic vasoconstrictor responsiveness was increased by combined NO synthase blockade and $O_2^-$ scavenging in M and H rats at all stimulation frequencies (Fig. 2). The difference in the vasoconstrictor response between the combined TIRON + l-NAME condition and the TIRON condition was greater ($P < 0.05$) in M (2 Hz: 21.4 ± 7.1%; 20 Hz: 12.5 ± 4.2%; 40 Hz: 19.8 ± 6.0%) and H (2 Hz: 20.4 ± 6.3%; 20 Hz: 17.1 ± 7.8%; 40 Hz: 16.3 ± 3.4%) compared with S rats (2 Hz: 3.0 ± 14.4%; 20 Hz: 2.9 ± 5.0%; 40 Hz: 7.7 ± 4.4%) at all frequencies of sympathetic stimulation.

**DISCUSSION**

The purpose of this study was to investigate the effect of acute $O_2^-$ scavenging on skeletal muscle sympathetic vasoconstrictor responsiveness and NO-mediated inhibition of sympathetic vasoconstriction in S, M, and H rats. Consistent with previous findings from our laboratory (17), short-term exercise training augmented sympathetic vasoconstrictor responsiveness in a training-intensity-dependent manner. Acute $O_2^-$ scavenging did not alter sympathetic vasoconstriction in S rats, but reduced sympathetic vasoconstrictor responsiveness in exercise-trained rats. Subsequent NO synthase blockade had no effect in S rats, whereas the constrictor response to sympathetic stimulation was significantly increased in trained rats. These data suggest that the augmented sympathetic vasoconstrictor responsiveness in exercise-trained rats was related to increased $O_2^-$ levels, and that acute $O_2^-$ scavenging increased NO-mediated blunting of sympathetic vasoconstriction. In contrast to our hypothesis, these effects were not dependent on the intensity of exercise training.

An acute bout of exercise has been shown to increase vascular and intramuscular oxidative stress with no discernible increase in antioxidant defense mechanisms (26, 38, 40). Prolonged exercise training has also been shown to increase oxidant enzyme expression (3, 37); however, a significant upregulation of antioxidant enzyme expression and capacity (8, 38) has also been reported following exercise training. Indeed, several studies have reported increased $O_2^-$ dismutase (7, 14, 25, 35, 43), glutathione (27, 30), and glutathione peroxidase levels (3, 13, 19, 27, 37) following exercise training. However, some studies have reported that, following exercise training, the increase in oxidant enzyme capacity may be greater than the increase in antioxidant capacity, suggesting that training may increase ROS to a level that surpasses the skeletal mus-

### Table 1. **Indicators of training efficacy**

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Mass, g</th>
<th>Heart Mass, g</th>
<th>Heart-to-Body Mass Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary time control</td>
<td>431 ± 43</td>
<td>1.29 ± 0.09</td>
<td>0.30 ± 0.03</td>
</tr>
<tr>
<td>Mild-intensity trained</td>
<td>423 ± 48</td>
<td>1.65 ± 0.20*</td>
<td>0.38 ± 0.05*</td>
</tr>
<tr>
<td>Heavy-intensity trained</td>
<td>387 ± 28</td>
<td>1.60 ± 0.13*</td>
<td>0.40 ± 0.03*</td>
</tr>
</tbody>
</table>

Values are means ± SD. *Difference from the sedentary time control group. A $P$ value <0.05 was considered statistically significantly.

### Table 2. **Basal hemodynamics**

<table>
<thead>
<tr>
<th>Group</th>
<th>Condition</th>
<th>HR, beats/min</th>
<th>MAP, mmHg</th>
<th>FBF, ml/min</th>
<th>FVC, ml·min⁻¹·mmHg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary time control</td>
<td>Control</td>
<td>380 ± 27</td>
<td>92 ± 14‡</td>
<td>3.7 ± 0.9‡</td>
<td>0.041 ± 0.008</td>
</tr>
<tr>
<td></td>
<td>TIRON</td>
<td>378 ± 20</td>
<td>105 ± 18‡</td>
<td>4.4 ± 1.0*‡</td>
<td>0.043 ± 0.013</td>
</tr>
<tr>
<td></td>
<td>TIRON + l-NAME</td>
<td>352 ± 18†</td>
<td>135 ± 18‡</td>
<td>2.6 ± 0.4*‡</td>
<td>0.019 ± 0.004‡</td>
</tr>
<tr>
<td>Mild-intensity trained</td>
<td>Control</td>
<td>357 ± 29</td>
<td>97 ± 10‡</td>
<td>3.1 ± 0.4‡</td>
<td>0.033 ± 0.007</td>
</tr>
<tr>
<td></td>
<td>TIRON</td>
<td>365 ± 28</td>
<td>111 ± 7e</td>
<td>3.4 ± 0.6‡</td>
<td>0.031 ± 0.006</td>
</tr>
<tr>
<td></td>
<td>TIRON + l-NAME</td>
<td>339 ± 23†</td>
<td>130 ± 18‡</td>
<td>2.1 ± 0.4‡</td>
<td>0.018 ± 0.007‡</td>
</tr>
<tr>
<td>Heavy-intensity trained</td>
<td>Control</td>
<td>358 ± 29</td>
<td>95 ± 12‡</td>
<td>3.0 ± 0.5‡</td>
<td>0.032 ± 0.009</td>
</tr>
<tr>
<td></td>
<td>TIRON</td>
<td>355 ± 33</td>
<td>107 ± 5‡</td>
<td>4.1 ± 0.8‡</td>
<td>0.038 ± 0.009</td>
</tr>
<tr>
<td></td>
<td>TIRON + l-NAME</td>
<td>325 ± 39†</td>
<td>141 ± 10‡</td>
<td>2.2 ± 0.9‡</td>
<td>0.016 ± 0.006†</td>
</tr>
</tbody>
</table>

Values are means ± SD. HR, heart rate; MAP, mean arterial blood pressure; FBF, femoral blood flow; FVC, femoral vascular conductance. ‡Main effect of drug condition [4,5-dihydroxy-1,3-benzene-disulfonic acid (TIRON) + Nω-nitro-l-arginine methyl ester (l-NAME) different from control and TIRON]. †Main effect of drug (all conditions are different from one another). *Main effect of group (sedentary time control rats different from mild-intensity-trained rats). A $P$ value <0.05 was considered statistically significant.
cle’s capability to maintain an oxidant/antioxidant balance (3, 12, 37). The available literature suggests that the intensity of exercise training may particularly influence the balance between oxidant and antioxidant production (3, 12, 37). For example, 3 wk of strenuous exercise generated greater plasma markers of oxidant stress than 5 wk of moderate-intensity exercise (58). Similarly, Goto et al. (9) reported increased levels of ROS following heavy-intensity exercise training, whereas moderate-intensity exercise training was not associated with elevated ROS levels. Bergholm et al. (2) have also shown that heavy-intensity exercise training decreased circulating antioxidants. Collectively, the results from these studies suggest that heavy-intensity exercise training may be associated with oxidative stress.

Oxidative stress is known to alter vascular smooth muscle function/signaling (28). One of the primary targets of O$_2^-$ appears to be the vasoactive molecule NO (28). NO readily reacts with O$_2^-$ to produce peroxynitrite (ONOO$^-$), effectively reducing NO bioavailability. O$_2^-$ may also oxidize the necessary NO synthase cofactor tetrahydrobiopterin and uncouple NO synthase (produce O$_2$ instead of NO) (6). Reduced NO bioavailability, uncoupling of NO synthase, as well as the decline in soluble guanylyl cyclase activity have all been mechanistically linked to decrements in endothelium-dependent vasodilation (EDD) in aged humans and animals and in hypertension (21, 41, 56).

Following heavy-intensity exercise training, decreased circulating antioxidant levels and attenuated EDD have been reported in young, healthy males, suggesting that intense exercise training was associated with oxidative stress and decreased NO-mediated vasodilation (2). Similarly, Goto et al. (9) have reported increased levels of circulating oxidants following heavy- but not moderate-intensity exercise training. Moderate-intensity exercise training improved NO-mediated vasodilation; whereas heavy-intensity training did not alter EDD, suggesting that the increased ROS production in response to intense training inhibited an increase in NO bioavailability and improvements in EDD (9). However, our laboratory recently reported a training-intensity-dependent increase in EDD following short-term exercise training (17), and others have shown that O$_2^-$ production contributes to training-induced improvements in EDD (44). Thus the effects of exercise training on EDD appear to be multifactorial and involve a complex balance between NO and O$_2^-$ and ONOO$^-$ levels.
Further investigation will be required to determine how exercise training (including the individual components of the training paradigm) influences the net balance of NO and O$_2$/H$_2$O$_2$ and how the relative levels of these signal molecules result in improvements or decrements to EDD following chronic aerobic exercise training.

In addition to being a potent vasodilator, NO has been shown to inhibit sympathetic vasoconstriction (11, 33, 34, 36, 50). An augmented vasoconstrictor response to sympathetic activation and reduced NO-mediated inhibition of sympathetic vasoconstriction has been reported in humans and animals with oxidative stress (5, 51, 57). The present data suggest that exercise training was associated with increased ROS concentrations as O$_2$/H$_2$O$_2$ scavenging reduced the vasoconstrictor response to sympathetic stimulation in resting skeletal muscle of exercise-trained rats. Thus the larger vasoconstrictor response to sympathetic stimulation in exercise-trained rats under control conditions appears to be related to a reduced ability to inhibit sympathetic vasoconstriction, secondary to increased oxidative stress. The reduction in sympathetic vasoconstrictor responsiveness during treatment with TIRON was similar in M and H rats, suggesting that the training-induced increase in O$_2$ was not a function of training intensity. Interestingly, TIRON treatment normalized sympathetic vasoconstrictor responsiveness in M rats to levels observed in S rats (Fig. 2), suggesting that O$_2$/H$_2$O$_2$ scavenging with TIRON was sufficient to offset the training-induced increase in sympathetic vasoconstrictor responsiveness in M rats. However, while treatment with TIRON did reduce sympathetic vasoconstrictor responsiveness in the H rats, the vasoconstrictor response to sympathetic stimulation remained significantly elevated in H compared with M and S rats. The maintenance of a heightened response to sympathetic stimulation in H rats suggests that ROS other than O$_2$ may have also been elevated in H rats and were not altered by treatment with TIRON; or that factors beyond oxidative stress contributed to the augmented constriction in H rats.

In S rats, combined treatment with TIRON + l-NAME did not alter the constrictor response to sympathetic stimulation at 2 and 20 Hz, whereas the constrictor response to stimulation at 40 Hz was increased. In contrast, TIRON + l-NAME aug-

Fig. 2. Percentage change of FBF and FVC in resting skeletal muscle, in response to sympathetic stimulation delivered at 2, 20, and 40 Hz during control conditions (open bars), acute O$_2$/H$_2$O$_2$ scavenging (TIRON, 1 g·kg$^{-1}$·h$^{-1}$ iv; shaded bars), and combined O$_2$/H$_2$O$_2$ scavenging and NO synthase blockade (l-NAME, 5 mg/kg iv; solid bars) in sedentary time control (S) and mild- (M) and heavy-intensity-trained (H) rats. Values are means ± SD. Top: significant main effect of group: ^H different from S, ‡H different from M and S, #all groups different; significant main effect of drug: †TIRON different from control, ‡TIRON + l-NAME different from TIRON; main effect of drug: Δ all conditions different. Bottom: #significant difference between all groups within the control condition; †significant difference of trained groups (M and H) from S rats within the TIRON condition; ‡significant difference of H rats from M and S rats within the TIRON + l-NAME condition; #significant difference between all groups within the TIRON + l-NAME condition; γ significant decrease in sympathetic vasoconstriction attributed to TIRON within the specified training group; δ significant increase in sympathetic vasoconstriction attributed to l-NAME within the specified training group. A P value < 0.05 was considered statistically significantly.
mented the vasoconstrictor response to all stimulation frequencies in trained rats. The lack of a consistent effect of combined treatment with TIRON and L-NAME at all stimulation frequencies in S rats is puzzling. It could be argued that NO may preferentially inhibit vasoconstriction at high frequencies of sympathetic stimulation in S rats; however, we have previously reported NO-mediated inhibition of sympathetic stimulation delivered at 2 Hz in S rats (18), suggesting that a frequency-dependent inhibition of vasoconstriction is unlikely.

In exercise-trained rats, the data suggest that the reduced constrictor response during $O_2^-$ scavenging was likely mediated by an increased NO-mediated inhibition of sympathetic vasoconstriction in trained rats. Other studies have also demonstrated that oxidative stress reduced NO-mediated blunting of sympathetic vasoconstriction. For example, in rats with angiotensin II-dependent hypertension, or myocardial infarction, oxidative stress increased sympathetic vasoconstriction and acute scavenging of $O_2^-$ with TEMPOL and/or TIRON abolished the heightened sympathetic vasoconstrictor responsiveness during muscular contraction (51, 57). Subsequent treatment with L-NAME suggested that the effect of $O_2^-$ scavenging was mediated by changes in NO bioavailability, in agreement with the present findings in exercise-trained rats.

**Experimental considerations and limitations.** The present study utilized a short-term training stimulus and an integrated whole animal preparation to further investigate the mechanism responsible for the training-intensity-dependent increase in sympathetic vasoconstrictor responsiveness in resting skeletal muscle previously reported by our laboratory (17). The lack of real-time measures of $O_2^-$, NO, and antioxidants is a limitation of this study. However, measurement of $O_2^-$, NO, and antioxidants is typically performed in isolated vascular segments and/or muscle cross sections and homogenates. Due to the heterogeneous distribution of ROS and antioxidant enzymes throughout the vascular tree and within skeletal muscle, these measures may not be reflective of the entire hindlimb (3, 23, 25, 37) and are difficult to align with the functional changes in sympathetic vasoconstrictor responsiveness of the intact hindlimb.

Our current understanding of the combined effects of exercise training intensity, duration and frequency, and the overall duration of a training program on the temporal adaptations of oxidant and antioxidant pathways is incomplete (42). Thus it is possible that the present findings may be unique to the short-term training stimulus. However, 1 wk of mild-intensity training (25 m/min, 0% grade, 40 min) (52) and 10–12 wk of moderate- to high-intensity training (30 m/min, 9–20% grade, 30–90 min) (3, 37) appear to produce similar changes in skeletal muscle $O_2^-$ dismutase protein concentration (3, 37, 52). Other studies have reported a greater succinate dehydrogenase to $O_2^-$ dismutase or glutathione peroxidase ratio following 10–12 wk of training, suggesting that the increase in expression of oxidative enzymes may exceed any increase in antioxidant enzyme capacity during longer duration training as well (3, 37). Thus the relationships between exercise training, oxidative stress, and vascular function remain incompletely defined, and further investigation in this area is warranted. Finally, the superoxide dismutase mimetic TIRON was chosen as the $O_2^-$ scavenger in the present study, as it has been shown to effectively scavenge $O_2^-$ in cultured myotubes at rest and during contraction, in single skeletal muscle fibers from mice and in the rat hindlimb (31, 39, 55). Additionally, TIRON does not produce the sympatho-inhibition that has been documented with TEMPOL (53, 54).

**Perspectives.** A decline in the ability to inhibit sympathetic vasoconstriction may reduce skeletal muscle perfusion. Thus the present findings could be interpreted as a deleterious adaptation to exercise training. However, a heightened sympathetic vasoconstrictor responsiveness may be an adaptive response to training-mediated improvements in EDD and potentially lower effluent sympathetic nerve activity at rest (10) or in response to physiological stress (i.e., exercise) (45, 46) to ensure the maintenance of arterial blood pressure and the distribution of cardiac output. Consistent with this notion, we have shown concurrent improvements in sympathetic vasoconstrictor responsiveness and EDD following short-term training (17). Similarly, Sugawara et al. (49) reported that 3 mo of moderate-intensity exercise training increased both tonic sympathetic vasoconstriction and NO bioavailability, while basal leg blood flow was unchanged.

**Conclusion.** The present study demonstrated that acute $O_2^-$ scavenging attenuated the vascular response to sympathetic stimulation in exercise-trained rats. Subsequent NO synthase inhibition indicated that the attenuation of sympathetic vasoconstrictor responsiveness in trained rats was mediated by improved NO-mediated blunting of sympathetic vasoconstriction. Our findings indicate that short-term exercise training was associated with increased $O_2^-$ levels, and that the increased $O_2^-$ decreased NO-mediated inhibition of sympathetic vasoconstriction in resting skeletal muscle.

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**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**

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

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