Exercise training improves functional sympatholysis in spontaneously hypertensive rats through a nitric oxide-dependent mechanism

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1Department of Health Care Sciences, University of Texas Southwestern Medical Center, Dallas, Texas; 2Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas, Texas; 3Department of Hypertension Section University of Texas Southwestern Medical Center, Dallas, Texas; and 4Division of Cardiology, University of Texas Southwestern Medical Center, Dallas, Texas

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Mizuno M, Iwamoto GA, Vongpatanasin W, Mitchell JH, Smith SA. Exercise training improves functional sympatholysis in spontaneously hypertensive rats through a nitric oxide-dependent mechanism. Am J Physiol Heart Circ Physiol 307: H242–H251, 2014. First published May 9, 2014; doi:10.1152/ajpheart.00103.2014.—Functional sympatholysis is impaired in hypertensive animals and patients. Exercise training (ET) improves functional sympatholysis through a nitric oxide (NO)-dependent mechanism in normotensive rats. However, whether ET has similar physiological benefits in hypertension remains to be elucidated. Thus we tested the hypothesis that the impairment in functional sympatholysis in hypertension is reversed by ET through a NO-dependent mechanism. In untrained normotensive Wistar-Kyoto rats (WKYUT; n = 13), untrained spontaneously hypertensive rats (SHRUT; n = 13), and exercise-trained SHR (SHRET; n = 6), changes in femoral vascular conductance (FVC) were examined during lumbar sympathetic nerve stimulation (1, 2.5, and 5 Hz) at rest and during muscle contraction. The magnitude of functional sympatholysis (Δ%FVC = Δ%FVC muscle contraction − Δ%FVC rest) in SHRET was significantly lower than WKYUT (1 Hz: 2 ± 2 vs. 13 ± 3%; 2.5 Hz: 9 ± 3 vs. 21 ± 3%; and 5 Hz: 12 ± 3 vs. 26 ± 3%, respectively; P < 0.05). Three months of voluntary wheel running significantly increased maximal oxygen uptake in SHRET compared with non-trained SHRUT (78 ± 6 vs. 62 ± 4 ml·kg−1·min−1, respectively; P < 0.05) and restored the magnitude of functional sympatholysis in SHRUT (1 Hz: 9 ± 2%; 2.5 Hz: 20 ± 4%; and 5 Hz: 34 ± 5%). Blockade of NO synthase (NOS) by N[G-nitro-L-arginine methyl ester attenuated sympatholysis in WKYUT but not SHRUT. Furthermore, NOS inhibition significantly diminished the improvements in functional sympatholysis in SHRET. These data demonstrate that impairments in functional sympatholysis are normalized via a NO mechanism by voluntary wheel running in hypertensive rats.

blood flow; blood pressure; muscle contraction; vascular conductance; voluntary running

THE SYMPATHETIC NERVOUS SYSTEM plays a critical role in regulating the cardiovascular system during exercise producing increases in cardiac output (3, 34) and augmentations in vascular resistance in visceral organs (14, 35) and nonactive skeletal muscle (22). Increases in sympathetic outputs to nonexercising muscle help to redistribute cardiac output to the working muscle and maintain blood pressure. In contrast, the vasoconstrictor response in the exercising muscle is blunted, at least in part, by the production of local metabolic by-products (1, 7, 10, 21, 56). The attenuated sympathetic vasoconstriction in exercising muscle, termed functional sympatholysis (50), is thought to be a physiological mechanism that optimizes blood flow to the working muscles and prevents an excessive increase in blood pressure during exercise.

Evidence suggests that functional sympatholysis is impaired in hypertensive animals (61) and patients (49, 58). Additionally, the sympathetically mediated blood pressure response to exercise is also abnormally elevated in hypertensive animals (32, 41–44, 52) and patients (11, 12, 36, 58). The impairments in functional sympatholysis might be a mechanism by which the blood pressure response to physical exercise is exaggerated in this disease. For example, insufficient oxygen delivery to working muscle due to abnormal functional sympatholysis might generate a mismatch between oxygen supply and demand. This could supra-stimulate afferent nerves in skeletal muscle causing augmented increases in sympathetic nerve activity and blood pressure during exercise (31, 38, 39).

In addition to ATP (51), nitric oxide (NO) has been shown to contribute importantly to the normal process of functional sympatholysis (8, 10, 15, 17, 56). Moreover, it has been recently demonstrated that 4 wk of endurance training augments functional sympatholysis in an intensity-dependent manner in normotensive rats (29). Interestingly, NO synthase (NOS) inhibition was shown to diminish the training-induced augmentations in functional sympatholysis. The findings suggest that improvements in functional sympatholysis evoked by exercise training are mediated by elevations in NO-mediated inhibition of sympathetic vasoconstriction in healthy animals (29). Whether exercise training has similar beneficial effects on abnormal functional sympatholysis in hypertension remains to be elucidated.

Given this background, we hypothesized that impairments in functional sympatholysis in hypertension can be reversed by dynamic exercise training through a NO-dependent mechanism. To test this hypothesis, we examined the following in untrained normotensive Wistar-Kyoto (WKYUT), untrained spontaneously hypertensive (SHRUT), and exercise-trained spontaneously hypertensive rats (SHRET): 1) vasoconstrictor responses to electrical sympathetic nerve stimulation at rest and during muscle contraction; 2) magnitude of functional sympatholysis during muscle contraction; and 3) the magnitude of functional sympatholysis before and after inhibiting NOS with N[G-nitro-L-arginine methyl ester (L-NAME).
METHODS

Ethical Approval

Acute sympatholysis experiments were performed in age-matched (14–29 wk) male WKY (n = 13) and SHR (n = 19) rats. Animals were maintained in a temperature-controlled environment, fed ad libitum, and kept on a 12-h light-dark cycle. The procedures outlined were approved by the Institutional Animal Care and Use Committee of the University of Texas Southwestern Medical Center at Dallas. All studies were conducted in accordance with the US Department of Health and Human Services, National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Exercise Training

Male SHR (n = 19) were kept in individual cages and randomly divided into untrained (n = 13; SHRUT) and exercise-trained (n = 6; SHRET) groups at 21 days of age. SHRUT were provided with a running wheel of 106 cm in circumference (Tecniplast) in their cage. These animals were allowed to exercise spontaneously. The total number of wheel rotations was recorded daily for the duration of the training period. The total distance run by each animal was then calculated from the total number of wheel rotations. The training period was 85–95 days in total duration.

Measurement of Peak Oxygen Uptake

Peak oxygen uptake (VO₂) was assessed in both the control groups (WKYUT and SHRUT) and the trained group (SHRET) after 68–70 days of ET). Four to six days before beginning oxygen uptake testing, animals were given two familiarization trials on the treadmill apparatus to allow adaptation to the testing environment (4). Each familiarization trial lasted 7 min, and the trials were conducted on nonconsecutive days. The speed during the first familiarization trial increased progressively from 10 to 15 m/min throughout the 7-min duration. The speed during the second familiarization trial increased progressively from 10 to 20 m/min. Peak VO₂ was determined for WKYUT (n = 3), SHRUT (n = 6), and SHRET (n = 6) according to previously established methods (23). Briefly, the test began with a 3-min warm-up at a treadmill grade and speed of 0% and 15 m/min, respectively. The treadmill speed and/or grade was increased every 3 min. Peak VO₂ was defined as the point at which the VO₂ did not increase with further increases in workload or when the rat was unable or unwilling to continue running. Confirmation that peak VO₂ was truly attained in each animal was demonstrated by having each rat perform a subsequent maximal exercise test after 48 h of recovery from the initial test.

General Acute Surgical Procedures

Rats were initially anesthetized with isoflurane gas (2–4% in 100% oxygen) and intubated for mechanical ventilation (model 683; Harvard). A pressure transducer (MLT0380/D; ADInstruments) connected to a left carotid arterial catheter was used to measure arterial blood pressure continuously. Fluid-filled catheters were placed within the right jugular vein for the administration of solutions. The gastrocnemius and soleus muscle of the right hindlimb were isolated. The calcaneal bone of the right hindlimb was cut, and the Achilles tendon was connected to a force transducer (FT-10; Grass Instruments) for the measurement of muscle tension. Blood flow was measured using a probe (MA1PRB; Transonic Systems) placed around the right femoral artery and connected to a flowmeter (T402; Transonic Systems). To experimentally stimulate sympathetic nerves, the right lumbar sympathetic chain was exposed and attached to a pair of stainless steel wire electrodes (Bioflex wire AS633; Cooner Wire) through a laparotomy procedure. To evoke muscle contraction, the right tibial nerve was exposed and instrumented with bipolar electrodes. The nerve and electrodes were covered with silicone glue (Kwik-Sil; World Precision Instruments) for insulation and fixation. Once all surgeries were finished, rats were anaesthetized with an intravenous injection (1.5 ml/kg) of a mixture of urethane (250 mg/ml) and α-chloralose (40 mg/ml). To stabilize fluid balance and maintain baseline arterial blood pressure, a continuous infusion (2 ml of 1 M NaHCO₃ and 10 ml of 5% dextrose in 38 ml of Ringer solution) was established via the jugular vein (3–5 ml·h⁻¹·kg⁻¹). A minimum recovery period of 1.5 h was employed after all surgeries before the beginning of any experimental protocol.

Experimental Protocols

Protocol 1: assessment of vasoconstriction in response to sympathetic stimulation at rest and during muscle contraction. In WKYUT (n = 13), SHRUT (n = 13), and SHRET (n = 6), the skeletal muscle vascular response evoked by lumbar nerve stimulation (1-ms pulses of ~1.5 V at randomized frequencies of 1, 2.5, and 5 Hz for 1 min) was examined at rest and during muscle contraction. Muscle contraction was produced by tibial nerve stimulation at approximately five times motor threshold (the minimum current required to produce a muscle twitch) with 100-ms pulsed trains at a rate of 30 trains/min. In contraction trials, the lumbar sympathetic chain was stimulated after stabilization of hemodynamic parameters (~6 min after the onset of muscle contraction).

Protocol 2: the effect of NOS inhibition on vasoconstriction in response to sympathetic stimulation at rest and during muscle contraction. Following completion of protocol 1 and a 60-min recovery period, a bolus injection of the nonselective NOS inhibitor 1-NAME (5 mg/kg iv) was administered in WKYUT (n = 4), SHRUT (n = 4), and SHRET (n = 4). To maintain consistency with and enhance comparisons to previously published works (29, 57, 61), 1-NAME was administered systemically. After stabilization of hemodynamic parameters (~15 min), stimulation of the lumbar sympathetic chain was repeated at rest and during contraction in the same manner as described in protocol 1.

At the conclusion of all experiments, animals were humanely killed by anesthetic overdose (mixture of urethane and α-chloralose) followed by intravenous injection of saturated potassium chloride (4 M, 2 ml/kg iv). The heart and lungs were excised and weighed. Additionally, the tibia was harvested, weighed, and measured.

Data Acquisition and Statistical Analyses

Mean arterial pressure (MAP), femoral blood flow (FBF), and contractile force data were acquired, recorded, and analyzed using data acquisition software (LabChart; ADInstruments) for the Powerlab analog-to-digital converter (Powerlab8/30; ADInstruments) at a 1-kHz sampling rate. Femoral vascular conductance (FVC) was calculated as FBF/MAP (ml·min⁻¹·mmHg⁻¹). Data sets of 1-s averages for MAP, FBF, and FVC were analyzed. Basal measurements were obtained by taking the mean value of 30 s of baseline data immediately before lumbar nerve stimulation. The magnitude of the vasoconstrictor response to lumbar sympathetic nerve stimulation was determined by calculating the peak of the FBF and FVC response to lumbar nerve stimulation and expressing it as a percent change from baseline. The magnitude of functional sympatholysis was calculated as the difference between the percent change in FBF and FVC in response to sympathetic stimulation at rest and the percent change in FBF and FVC in response to sympathetic stimulation during muscular contraction (%ΔFBF = ΔFBF muscle contraction − ΔFBF rest; %ΔFVC = ΔFVC muscle contraction − ΔFVC rest) (27, 29). The peak tension was defined as the greatest change from baseline elicited by muscle contraction.

Data were analyzed using paired one-way ANOVA (rat group), two-way ANOVA (rat group × stimulation frequency, rat group × muscle contraction, stimulation frequency × muscle contraction, and rat group × drug effect), and three-way ANOVA (rat group × stimulation frequency × muscle contraction). If significant interaction...
and main effects were observed with ANOVA, a post hoc Fisher’s protected least significant difference test was used to identify differences between specific group means. The significance level was set at \( P < 0.05 \). Results are presented as means ± SE.

## RESULTS

Morphometric characteristics, peak tension during induced muscle contraction, tension reduction from peak over time, and peak \( \dot{V}O_2 \) for WKYUT, SHRUT, and SHRET are presented in Table 1. There were no significant differences in body weight, lung weight, or peak tension development among groups. Likewise, the percent reduction in tension from peak over the period of contraction was not different between groups. Heart weight-to-body weight ratios as well as heart weight-to-tibia length ratios were significantly greater in SHRET than WKYUT as previously reported (42, 43, 52). Furthermore, these ratios were significantly larger in SHRET compared with SHRUT. Three months of voluntary wheel running significantly increased peak \( \dot{V}O_2 \) in SHRET compared with WKYUT and SHRUT. There was no statistical significant difference in peak \( \dot{V}O_2 \) between the untrained groups. Daily running distance in SHRET significantly increased week by week through the 7th wk of training (1st wk: 2,325 ± 250 m; 4th wk: 3,061 ± 128 m; 7th wk: 9,589 ± 691 m; and 10th wk: 6,089 ± 1,200 m).

### Protocol 1

Baseline hemodynamic parameters for WKYUT, SHRUT, and SHRET at rest and during muscle contraction are summarized in Table 2. Baseline MAP in SHRET as well as SHRET was significantly higher than WKYUT at rest and during muscle contraction (rat group: \( P < 0.0001 \); muscle contraction: \( P = 0.2291 \); rat group \( \times \) muscle contraction: \( P = 0.1881 \)). Although FBF during muscle contraction was significantly greater than at rest in all animal groups, there were no significant differences among groups (rat group: \( P = 0.1841 \); muscle contraction: \( P < 0.0001 \); rat group \( \times \) muscle contraction: \( P = 0.9273 \)). Consequently, FVC in both SHRUT and SHRET at rest and during muscular contraction was significantly lower compared with WKYUT (rat group: \( P < 0.0001 \); muscle contraction: \( P < 0.0001 \); rat group \( \times \) muscle contraction: \( P = 0.5954 \)). No effect of stimulation frequency was observed in any baseline hemodynamics. Furthermore, there were no differences between SHRUT and SHRET.

Representative tracings of MAP, FBF, FVC, and percent change of FVC responses to lumbar sympathetic nerve stimulation at 5 Hz are presented in Fig. 1. Figure 2 summarizes the percent changes in FBF and FVC in response to lumbar sympathetic stimulation. Additionally, absolute changes in FBF and FVC responses to lumbar nerve stimulation are summarized in Table 3. FBF responses were associated with stimulation frequency in all animal groups (stimulation frequency: \( P < 0.0001 \); rat group \( \times \) stimulation frequency: \( P = 0.8864 \)). As previously reported (27–29, 54–57, 61), muscle contraction significantly attenuated FBF responses to lumbar nerve stimulation associated with stimulation frequency (muscle contraction: \( P < 0.0001 \); muscle contraction \( \times \) stimulation frequency: \( P = 0.0285 \)). More importantly, the extent of attenuation was not similar among groups (rat group \( \times \) muscle contraction: \( P = 0.0086 \)). At rest, the percent decrease in FBF to lumbar nerve stimulation in WKYUT was significantly greater than in both SHRUT (\( P = 0.0004 \)) and SHRET (\( P = 0.0201 \); Fig. 2A); the vasoconstrictor response to lumbar nerve stimulation in SHRET did not differ from SHRUT (\( P = 0.5596 \)). During muscle contraction, the percent decrease in FBF to lumbar nerve stimulation in SHRET was significantly less than...

### Table 1. Morphometric characteristics, peak tension, and peak \( \dot{V}O_2 \)

<table>
<thead>
<tr>
<th></th>
<th>WKYUT</th>
<th>SHRUT</th>
<th>SHRET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>325 ± 6 (13)</td>
<td>333 ± 8 (13)</td>
<td>343 ± 13 (6)</td>
</tr>
<tr>
<td>Heart weight/body weight ratio, mg/g</td>
<td>3.20 ± 0.05 (9)</td>
<td>3.53 ± 0.07* (9)</td>
<td>4.29 ± 0.15** (4)</td>
</tr>
<tr>
<td>Heart weight/tibia length ratio, mg/mm</td>
<td>28.0 ± 0.6 (9)</td>
<td>32.0 ± 1.3* (9)</td>
<td>38.5 ± 1.0** (4)</td>
</tr>
<tr>
<td>Lung weight/body weight ratio, mg/g</td>
<td>6.1 ± 0.3 (9)</td>
<td>6.3 ± 0.3 (9)</td>
<td>5.0 ± 0.3 (4)</td>
</tr>
<tr>
<td>Peak tension, g</td>
<td>579 ± 31 (13)</td>
<td>562 ± 62 (13)</td>
<td>532 ± 54 (6)</td>
</tr>
<tr>
<td>Tension reduction, % peak tension</td>
<td>57 ± 4 (13)</td>
<td>50 ± 6 (13)</td>
<td>53 ± 6 (6)</td>
</tr>
<tr>
<td>Peak ( \dot{V}O_2 ), ml·kg⁻¹·min⁻¹</td>
<td>52 ± 6 (3)</td>
<td>62 ± 4 (6)</td>
<td>78 ± 6** (6)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Numbers in parentheses indicate group size. \( \dot{V}O_2 \), oxygen uptake; WKYUT, untrained normotensive Wistar-Kyoto rats; SHRUT, untrained spontaneously hypertensive rats; SHRET, exercise-trained SHR. *\( P < 0.05 \), compared with WKYUT. †\( P < 0.05 \), compared with SHRUT.

### Table 2. Baseline hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>WKY (( n = 13 ))</th>
<th>SHR (( n = 13 ))</th>
<th>SHRET (( n = 6 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 Hz</td>
<td>2.5 Hz</td>
<td>5 Hz</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>99 ± 3</td>
<td>98 ± 4</td>
<td>101 ± 4</td>
</tr>
<tr>
<td>FBF, ml/min</td>
<td>2.6 ± 0.4</td>
<td>2.6 ± 0.3</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>FVC, ml·min⁻¹·mmHg⁻¹</td>
<td>0.027 ± 0.004</td>
<td>0.027 ± 0.004</td>
<td>0.028 ± 0.004</td>
</tr>
<tr>
<td>Muscle contraction</td>
<td>100 ± 4</td>
<td>94 ± 8</td>
<td>103 ± 4</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>4.7 ± 0.71</td>
<td>5.0 ± 0.81</td>
<td>5.2 ± 0.71</td>
</tr>
<tr>
<td>FVC, ml·min⁻¹·mmHg⁻¹</td>
<td>0.047 ± 0.007†</td>
<td>0.049 ± 0.007†</td>
<td>0.050 ± 0.007†</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; FBF, femoral blood flow; FVC, femoral vascular conductance. *\( P < 0.05 \), compared with WKY rats. †\( P < 0.05 \), compared with rest.
both WKY\textsubscript{UT} \((P = 0.0004)\) and SHR\textsubscript{UT} \((P = 0.0010); \text{Fig. 2A} \). FVC responses were likewise associated with stimulation frequency in all animal groups \((\text{stimulation frequency: } P = 0.0001; \text{rat group} \times \text{stimulation frequency: } P = 0.8946\). Muscle contraction significantly attenuated FVC responses to lumbar nerve stimulation associated with stimulation frequency \((\text{muscle contraction: } P = 0.0001; \text{muscle contraction} \times \text{stimulation frequency: } P = 0.0067)\). Notably, the extent of attenuation was different among groups \((\text{rat group} \times \text{muscle contraction: } P = 0.0046)\). At rest, the percent decrease in FVC to lumbar nerve stimulation in WKY\textsubscript{UT} was significantly greater than both SHR\textsubscript{UT} \((P < 0.0001)\) and SHR\textsubscript{ET} \((P = 0.0020); \text{Fig. 2B} \). At rest, the percent decrease in FVC to lumbar nerve stimulation in WKY\textsubscript{UT} was significantly greater than both SHR\textsubscript{UT} \((P < 0.0001)\) and SHR\textsubscript{ET} \((P = 0.0020); \text{Fig. 2B} \). The vasoconstrictor response to lumbar nerve stimulation in SHR\textsubscript{ET} was similar to SHR\textsubscript{UT} \((P = 0.6328)\). During muscle contraction, the percent decrease in FVC to lumbar nerve stimulation in SHR\textsubscript{ET} was significantly less than both WKY\textsubscript{UT} \((P = 0.0001)\) and SHR\textsubscript{UT} \((P = 0.0009); \text{Fig. 2B} \).

Figure 3 summarizes the magnitude of functional sympatholysis expressed as the absolute difference between the \(\Delta\%\text{FBF} \) and \(\Delta\%\text{FVC} \) to lumbar nerve stimulation during contraction and rest. With regard to the \(\Delta\%\text{FBF} \), the magnitude of functional sympatholysis in SHR\textsubscript{ET} was significantly lower than WKY\textsubscript{UT} \((P = 0.1341)\). With regard to the \(\Delta\%\text{FVC} \), the magnitude of functional sympatholysis in SHR\textsubscript{ET} was significantly greater than SHR\textsubscript{UT} \((P < 0.0001)\). There was no difference in the magnitude of functional sympatholysis between WKY\textsubscript{UT} and SHR\textsubscript{ET} \((P = 0.0001)\).

Protocol 2

Table 4 summarizes the effect of L-NAME administration on hemodynamic parameters at baseline and during 5-Hz lumbar nerve stimulation for WKY\textsubscript{UT}, SHR\textsubscript{UT}, and SHR\textsubscript{ET} at rest and during muscle contraction. L-NAME administration significantly increased MAP and significantly decreased FVC in all groups. In contrast, blockade of NOS did not affect baseline FBF \((\text{drug effect: } P = 0.1783; \text{rat group} \times \text{drug effect: } P = 0.7343)\).

L-NAME administration attenuated the FBF response to 5-Hz lumbar nerve stimulation at rest \((\text{drug effect: } P = 0.0016; \text{rat group} \times \text{drug effect: } P = 0.1122; \text{WKY}\textsubscript{UT}: -74 \pm 5 \text{ vs.} -75 \pm 5\% \text{; SHR}\textsubscript{UT}: -75 \pm 6 \text{ vs.} -44 \pm 7\% \text{; SHR}\textsubscript{ET}: -70 \pm 5 \text{ vs.} -47 \pm 5\% \text{, before and after L-NAME, respectively)}\). Although NOS inhibition demonstrated a strong tendency to reduce the magnitude of sympatholysis with regard to FBF, especially in SHR\textsubscript{ET} \((P < 0.05 \text{ by paired } t\text{-test})\), statistical
significance was not reached by two-way ANOVA (Fig. 4; drug effect: $P = 0.0692$; rat group × drug effect: $P = 0.1918$).

L-NAME administration did not significantly affect the FVC response to 5-Hz lumbar nerve stimulation at rest (drug effect: $P = 0.2617$; rat group × drug effect: $P = 0.3801$; WKY UT: $72 \pm 7$ vs. $75 \pm 5\%$; SHR UT: $54 \pm 7$ vs. $46 \pm 6\%$; SHR ET: $60 \pm 6$ vs. $46 \pm 6\%$, before and after L-NAME, respectively). In contrast, blockade of NOS significantly attenuated the magnitude of functional sympatholysis with regard to FVC in WKY UT as well as SHRET but not SHR UT (Fig. 4B; drug effect: $P = 0.0135$; rat group × drug effect: $P = 0.0377$).

**DISCUSSION**

The major findings from this investigation were 1) the magnitude of functional sympatholysis in untrained hypertensive rats was significantly lower than untrained normotensive rats; 2) dynamic exercise training normalized the impairments in functional sympatholysis in hypertensive animals; and 3) blockade of NOS significantly diminished the improvements in functional sympatholysis in exercise-trained hypertensive rats. Collectively, these findings support the contention that functional sympatholysis is impaired in hypertension. Moreover, this investigation provides the first evidence that impairments in functional sympatholysis in hypertensive animals are ameliorated, via a NO-dependent mechanism, by dynamic exercise training.

**Impairments in Functional Sympatholysis in SHR**

Consistent with a previous study using a different animal model of hypertension (61) and an investigation in humans (58), the magnitude of functional sympatholysis in SHR UT was significantly lower than WKY UT. In contrast, the magnitude of functional sympatholysis was similar in WKY UT and SHRET. Further, NOS inhibition significantly attenuated the magnitude of functional sympatholysis in WKY UT as well as SHRET but not SHR UT. These findings suggest that impairments in functional sympatholysis in SHR are mediated, at least in part, by a decrease in the availability of NO. This is not entirely surprising as it has been reported that functional sympatholysis is mediated by NO in normotensive humans and animals (8, 10, 15, 17, 56). Recent evidence suggests that protein levels for endothelial NOS in the soleus muscle of SHR are significantly lower compared with WKY (18). Decreases in NOS expression could mediate reductions in the availability of NO within skeletal muscle in hypertension and may potentially account for the blunting of functional sympatholysis in hypertensive animals.

**Exercise Training Enhances Functional Sympatholysis in SHR**

In hypertensive animals, 3 mo of voluntary wheel running augmented the magnitude of functional sympatholysis compared with untrained SHR. This finding supports a previous
report that short-term (4-wk) endurance training augments functional sympatholysis in a training intensity-dependent manner in normotensive rats (29). In human studies, it remains controversial as to whether exercise training alters functional sympatholysis. Exercise training has been reported to increase (45, 46), decrease (60), and have no effect (59) on the magnitude of functional sympatholysis in normotensive humans. Studies demonstrating training-induced enhancements in functional sympatholysis were performed in nonathlete young (45) and elderly men (46), while other studies showing either a decrease or no effect on functional sympatholysis used athletes (59) or a different study design (e.g., cross-sectional) (60). It has also been reported that short-term (2 wk) muscle immobilization (i.e., de-training) impairs functional sympatholysis in healthy young men (45). Collectively, the effects of exercise training on functional sympatholysis may be dependent on multiple factors such as training history, physical activity levels, and health status.

NO-Dependent Mechanisms Underlie Training-Induced Improvements in Functional Sympatholysis

L-NAME administration significantly attenuated the improvements in functional sympatholysis in SHRET, suggesting that NO is involved in the training-induced restoration of functional sympatholysis in hypertensive animals. This is consistent with a previous study demonstrating similar results in healthy young men (45).

Table 3. Absolute changes in blood flow and vascular conductance during lumbar nerve stimulation

<table>
<thead>
<tr>
<th></th>
<th>WKY (n = 13)</th>
<th>SHR (n = 13)</th>
<th>SHRet (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 Hz</td>
<td>2.5 Hz</td>
<td>5 Hz</td>
</tr>
<tr>
<td>Rest</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBF, ml/min</td>
<td>-1.13 ± 0.24</td>
<td>-1.50 ± 0.28</td>
<td>-1.94 ± 0.29</td>
</tr>
<tr>
<td>FVC, ml·min⁻¹·mmHg⁻¹</td>
<td>-0.0102 ± 0.0024</td>
<td>-0.0152 ± 0.0033</td>
<td>-0.0191 ± 0.0035</td>
</tr>
<tr>
<td>Muscle contraction</td>
<td>-1.20 ± 0.23</td>
<td>-1.97 ± 0.42</td>
<td>-2.39 ± 0.46</td>
</tr>
<tr>
<td>FVC, ml·min⁻¹·mmHg⁻¹</td>
<td>-0.0093 ± 0.0018</td>
<td>-0.0161 ± 0.0036</td>
<td>-0.0207 ± 0.0004</td>
</tr>
</tbody>
</table>

Values are means ± SE. Magnitude of changes in FBF and FVC are significantly related with stimulation intensity in all groups (P < 0.0001). *P < 0.05, compared with WKY rats.

Fig. 3. Magnitude of functional sympatholysis calculated as the difference between the percent change in FBF (A) and FVC (B) in response to lumbar nerve stimulation (1, 2.5, and 5 Hz) at rest and during muscle contraction in WKY (white bars), SHR (black bars), and SHRet (grey bars). *P < 0.05, compared with WKY rats.

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Table 4. Hemodynamics before and after L-NAME administration at baseline and during 5-Hz lumbar nerve stimulation

<table>
<thead>
<tr>
<th></th>
<th>WKYUT (n = 4)</th>
<th>SHRUT (n = 4)</th>
<th>SHRET (n = 4)</th>
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<tbody>
<tr>
<td><strong>Baseline Before</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>95 ± 5</td>
<td>107 ± 5</td>
<td>108 ± 5</td>
</tr>
<tr>
<td>FBF, ml/min</td>
<td>4.0 ± 0.3</td>
<td>8.0 ± 1.3</td>
<td>6.0 ± 1.3</td>
</tr>
<tr>
<td><strong>Baseline After</strong></td>
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<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>144 ± 7</td>
<td>147 ± 7</td>
<td>156 ± 6</td>
</tr>
<tr>
<td>FBF, ml/min</td>
<td>4.5 ± 0.3</td>
<td>10.2 ± 1.1</td>
<td>8.0 ± 0.9</td>
</tr>
<tr>
<td><strong>During LNS Before</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>105 ± 6</td>
<td>134 ± 5</td>
<td>156 ± 6</td>
</tr>
<tr>
<td>FBF, ml/min</td>
<td>6.2 ± 0.6</td>
<td>13.8 ± 1.2</td>
<td>11.9 ± 0.8</td>
</tr>
<tr>
<td><strong>During LNS After</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>170 ± 6</td>
<td>178 ± 5</td>
<td>185 ± 6</td>
</tr>
<tr>
<td>FBF, ml/min</td>
<td>11.0 ± 0.5</td>
<td>21.8 ± 1.5</td>
<td>19.6 ± 1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. L-NAME, Nω-nitro-L-arginine methyl ester. LNS, lumbar nerve stimulation. *P < 0.05, compared with WKYUT rats. †P < 0.05, compared with before. §P < 0.05, compared with WKYUT rats after L-NAME administration.

Fig. 4. Effect of Nω-nitro-L-arginine methyl ester (L-NAME) administration on the magnitude of functional sympatholysis calculated as the difference between the percent change in FBF (A) and FVC (B) in response to lumbar nerve stimulation (5 Hz) at rest and during muscle contraction in WKYUT (white bars), SHRUT (black bars), and SHRET (grey bars) before (solid bars) and after L-NAME administration (hatched bars). §P < 0.05, compared with before L-NAME administration.
strictor responses to lumbar nerve stimulation in WKYUt was quite similar to that reported earlier in normotensive Sprague-Dawley rats (17, 25–29, 54–57, 61). Thus the reason for the attenuated FBF and FVC responses to nerve stimulation in SHRUT remains unclear. Compared with WKY, it has been documented that vascular reactivity to adrenergic stimulation in SHR is generally augmented. For example, the developed wall tension induced by adrenergic vasoconstriction in isolated femoral arteries in SHR is greater than that in WKY (2). Thus we anticipated that changes in FBF and FVC to sympathetic stimulation would be the same if not larger in SHR. It is possible, however, that information on vascular reactivity obtained in isolated vessel preparations is not entirely physiological. To this point, it has been shown that the renal vascular response to intra-arterial norepinephrine administration in SHR is smaller than WKY in an in situ auto-perfused preparation (19). In addition, a previous study has suggested that a reciprocal relationship exists between sympathetic nerve activity and vascular adrenergic sensitivity in humans (9). Albeit pure speculation, it is possible that chronically higher sympathetic nerve traffic in untrained SHR, a common feature of hypertension, might have mediated a decrease in vascular sensitivity manifesting as a reduced responsiveness to experimental stimulation of the lumbar sympathetic chain.

Inconsistent with previous studies demonstrating that short-term exercise training augments sympathetic vasoconstrictor responsiveness in normotensive rats (27–29), wheel running did not affect the magnitude of the vasoconstrictor responses to lumbar nerve stimulation in SHRut compared with SHRut at rest in the present study (Fig. 2). In a different experimental setting, vascular responsiveness to adrenergic stimulation has been reported to be increased (33, 37) or decreased (13, 16) by exercise training. The inconsistency between investigations may have arisen from differences in animal species, exercise paradigms, or the experimental preparation utilized. Additional studies are warranted to address these important questions.

Collectively, it is possible that the observed impairments in functional sympatholysis in SHRut might have resulted from a reduced ability to vasoconstrict at rest in response to lumbar nerve stimulation in SHRut compared with WKYut. This is especially noteworthy given that there were no differences between the two groups in the degree of vasoconstriction during muscle contraction (Fig. 2). In a human study demonstrating impaired functional sympatholysis in hypertensive patients, vasoconstrictor responses at rest (assessed by quantification of tissue oxygenation using near-infrared spectroscopy as well as changes in sympathetic nerve activity during lower body negative pressure) were similar in normotensive and hypertensive patients (58). Despite this discrepancy between human and animal studies, the current investigation clearly demonstrated that dynamic exercise training improved functional sympatholysis in hypertensive animals; animals in which vasoconstrictor responses at rest were similar regardless of training status.

**Analytical and Methodological Considerations**

By design, exercise training was not performed in WKY in the current investigation. The rationale for not performing training studies in this group of animals was twofold: 1) the ability of dynamic exercise training to enhance functional sympatholysis in normotensive rats has been reported previously; and 2) it has likewise been established that training-induced enhancements in normotensive rats are mediated, at least in part, by a NO-dependent mechanism (29). As a result, the reported research was focused on elucidating the effects of training on functional sympatholysis in hypertension as well as determining the mechanisms underlying any beneficial effects derived from such exercise training.

It should also be noted that baseline vascular conductance differed at rest and during muscle contraction in all groups (Table 2). Additionally, the administration of L-NAME decreased baseline vascular conductance (Table 4). These differences in FVC could potentially influence the results obtained. However, it is generally accepted that direct comparisons can be made when using percent changes in vascular conductance since a given percent reduction will reflect a predictable percent decrease in the radius of the vessel (6). As such, it is suggested that the differences noted impacted the findings and conclusions of the investigation minimally. With regard to L-NAME, this agent was administered systemically rather than locally in the contracting hindlimb. It is acknowledged that injection of L-NAME in this manner potentially evokes unintended reflex responses (e.g., baroreflex-mediated cardiovascular adjustments) that could influence the results reported. This possibility should be considered when interpreting findings.

Consistent with earlier studies (47), 3 mo of voluntary wheel running significantly increased peak VO2 in SHRET. In addition, running distance was comparable to that reported previously in SHR (20, 30, 47). Interestingly, daily wheel running in SHRET decreased dramatically between the 7th and 10th wk of training. This is, however, consistent with previously reported training patterns in SHR animals (47). Since average daily distance is negatively correlated to body mass at the onset of exercise (53), the decline in daily running may be attributable to the increases in body mass associated with normal growth over the time period of training. Baseline MAP under anesthesia in SHRET did not differ from SHRUt (Table 2). That being stated, it has been clearly demonstrated that this type of exercise training significantly lowers, but does not normalize, resting blood pressure (47). Most likely, the reason a similar decrease was not documented in the current study was that blood pressure was not assessed in the conscious state but only under inhalant anesthesia; a known autonomic depressor. Supporting this speculation, Brum et al. (5) previously demonstrated that training-induced decreases in baseline blood pressure discerned in the conscious state of SHR are no longer observable after anesthetization.

**Perspectives and Clinical Significance**

The blood pressure response to exercise is often exaggerated in hypertension (11, 12, 32, 36, 41–44, 52, 58). This augmented pressor response to physical activity is associated with an increased risk for adverse cardiovascular and/or cerebrovascular events during or immediately following a bout of exercise (24, 40). Disease-induced impairments in functional sympatholysis may contribute significantly to the generation of this abnormally large pressor response. That being stated, a moderate level of exercise training has been recommended as a beneficial nonpharmacological treatment for hypertension (48). To this end, the current study has demonstrated that impair-
ments in functional sympatholysis can be largely reversed by a relatively short period of exercise training in hypertensive animals. Speculatively, it is quite possible that such a training-induced restoration of functional sympatholysis may prevent and/or correct the exaggerated pressor response to physical activity reducing the risks associated with the performance of exercise in this disease. Reductions of such risks may allow the implementation of exercise paradigms of progressively higher intensity and duration maximizing the benefits of physical training. Understanding the mechanisms underlying the beneficial effects of exercise training, like those reported in the current investigation, may prove advantageous in the development of novel therapeutic strategies for the treatment of hypertension.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


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