Time- and order-dependent changes in functional and NO-mediated dilation during exercise training

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Lash, Julia M., and H. Glenn Bohlen. Time- and order-dependent changes in functional and NO-mediated dilation during exercise training. J. Appl. Physiol. 82(2): 460–468, 1997.—Arterial vessel responses to sodium nitroprusside (SNP) and acetylcholine (ACh) were measured in the spinotrapezius muscle of sedentary (Sed) and treadmill-trained (Tr) rats to determine whether these endothelium-dependent (ACh) and -independent (SNP) mechanisms contribute to the training-induced increase in functional vasodilation previously observed. Control and maximal vessel diameters were similar between Sed and Tr. After 8 wk of training, functional dilation (2-, 4-, and 8-Hz contractions) was enhanced in all orders of vessels studied [terminal feed artery (FA), largest arterioles (1A), and intermediate-sized arterioles (2A)], but responses to SNP were increased only in FA. Responses to ACh were not significantly increased in any vessel order. After 16 wk of training, functional dilation had regressed in Tr such that only the FA response to 4 Hz was significantly elevated relative to Sed. However, the FA and 1A responses to SNP were significantly greater in Tr than in Sed, as were the 1A and 2A responses to ACh. These results show a dissociation of functional dilation and SNP- or ACh-mediated responses, as well as age-dependent interactions, a time-dependent progression, and vessel order specificity in the adaptations to training.

AEROBIC EXERCISE TRAINING enhances the blood flow to active skeletal muscles during acute bouts of exercise (1, 18, 21). This increase in blood flow is likely due to a combination of functional and anatomical vascular adaptations to the training stimulus. During low- to moderate-intensity contractions, the relative dilation of skeletal muscle arterioles is 25–100% greater in trained (Tr) than sedentary (Sed) animals (14, 16). However, the precise vascular regulatory adaptations responsible for increased functional dilation in Tr animals are relatively unknown. Some studies have suggested that the endothelium-mediated dilation is enhanced by aerobic training (3, 12, 29, 32), whereas others have found no change (6, 22, 23).

Historically, it was assumed that blood flow during skeletal muscle contractions was regulated by dilation of the microvessels within the metabolically active tissue. However, more recent studies demonstrate that the small feed arteries, upstream from the microcirculation and outside of the tissue proper, contribute significantly to the total flow response during muscle activity (2, 14, 33). Because these vessels are not directly exposed to the chemical environment of the active tissue, dilatory mechanisms other than direct metabolic control must be responsible for functional dilation of these vessels. One possible mechanism for the transduction of the dilator stimulus is flow-mediated vasodilation (26). Theoretically, metabolic dilation of the microvascular arterioles results in an increase in muscle blood flow and a concurrent increase in flow through the arterial and arteriolar feed vessels. This enhanced flow may, in turn, elicit additional dilation of the larger feed arteries through flow-mediated release of endothelium-derived relaxing factors (EDRF; 12, 13, 26). In Sed animals, ~50% of the functional dilation of the largest arterioles can be attributed to the action of nitric oxide (NO), one of the EDRF (8). Exercise training has been shown to increase the dilation of the terminal feed artery (FA) and the largest arterioles (1A) of the rat spinotrapezius muscle during muscle contractions (14, 16), and flow-mediated dilation is enhanced in intermediate-sized arterioles (2A) from the gracilis muscle of exercise-trained rats (12). These results suggest that flow-mediated vasodilation may play an important role in the enhanced functional hyperemia resulting from aerobic exercise training.

Based on this rationale, the purpose of the present study was to determine whether the vascular responses to acetylcholine (ACH) or sodium nitroprusside (SNP) are altered as a result of aerobic exercise training. In many vessel types, local application of ACh results in the endothelium release of NO (11), one of the identified EDRF and a potential mediator of flow-induced vasodilation (13). In contrast, SNP serves as an exogenous NO donor (20). Therefore, both ACh and SNP elicit vasodilation through the NO pathway, with the ACh effect being dependent on endothelium function. By comparing the vascular responses to these two drugs, it is possible to distinguish between endothelial and vascular smooth muscle adaptations in the NO response. The resistance vessels studied included the terminal FA and 1A and 2A of the rat spinotrapezius muscle to evaluate both parenchymal and extraparenchymal vessels. This particular muscle preparation was chosen because of its suitability for microvascular observations and the fact that exercise training increases functional dilation in both the macro- and microvessels perfusing this tissue (14, 16). Experiments were performed after 8 and 16 wk of exercise training to determine whether the vascular adaptations to aerobic exercise training evolve over time.

METHODS

Animals. All procedures were approved by the Animal Care and Use Committee of Indiana University. Male 4- to 5-wk-old Sprague-Dawley rats were received in shipments of eight each from Harlan Laboratories (Indianapolis, IN). Animals

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were exposed to a 12:12-h light-dark cycle and received food and water ad libitum. Three to four days after arrival, animals were randomly assigned to Sed or Tr groups, and animals in the Tr group began exercising daily on a rodent treadmill (Columbus Instruments, Columbus, OH). Exercise intensity was increased from 15 m/min, 0° incline, for 30 min to 30 m/min, 1.5° incline, for 90 min during the first 5 wk of training, and intensity was then maintained for the duration of the training regimen. Experiments were performed after 8–10 or 16–18 wk of training, at ages 12–15 and 20–23 wk, respectively. The 8-wk training regimen has previously been shown to result in a 40% increase in plantaris citrate synthase activity and to enhance functional vasodilation and hyperemia in the rat spinotrapezius muscle (14, 16). Animals in the Sed and Tr groups were housed together, and experiments were performed at comparable ages. Data were compared between Sed and Tr groups and between 8- and 16-wk training durations to determine the effects of training over time.

Surgical preparation. Animals were prepared for experimental observations at least 24 h after their last training session to minimize the residual effects of acute exercise. Anesthesia was induced with thiopental sodium (initial dose 100 mg/kg ip, supplemental dose 10 mg sc, as needed; Pentotal, Abbott Laboratories, North Chicago, IL). Body temperature was maintained near 37°C by placing the animal on a flow-through heating pad. Atropine was administered to minimize respiratory secretions (0.10–0.15 mg sc; American Reagent Laboratory, Shirley, NY). The trachea was intubated (PE-240) to ensure a patent airway, and the left femoral artery was cannulated (PE-50) for the subsequent monitoring of blood pressure.

The right spinotrapezius muscle was prepared for experimental observation, as previously described (16, 17). Care was taken to maintain in situ length and width, and the rostral region of the muscle was carefully isolated to allow observation of the terminal FA as it entered the muscle tissue (14). During surgical preparation, drying of the tissue was prevented by intermittent superfusion with a heated physiological saline solution (36°C; Plasma-Lyte, Baxter Healthcare, Deerfield, IL). During experimental observations, the tissue was continuously superfused with a bicarbonate-based physiological solution (in mM: 119 NaCl, 6 KCl, 3.3 CaCl₂, and 25 NaHCO₃), equilibrated with 5% CO₂-95% N₂, and warmed to 35°C. Superfusion flow rate was maintained at 4–5 ml/min. Needle-style stimulating electrodes were placed at the rostral and caudal ends of the muscle and attached to a Grass SD-9 stimulator to induce muscle contractions. Stimulation parameters were maintained below the threshold for direct vascular effects (0.2-ms duration, <6-V amplitude) (17).

In vivo measurements. During in vivo analyses, systemic arterial pressure was continuously monitored, by using a Statham 23 Db pressure transducer and Gould 2400S chart recorder. The microvasculature of the spinotrapezius muscle was transilluminated with a 100-W quartz iodine direct-current light source and was observed through an Olympus BH2-MJ intravital microscope (New Hyde Park, NY) by using Nikon water-immersion objectives (X10, numerical aperture 0.22; X20, numerical aperture 0.33). Images of the vessels were obtained by using a closed-circuit video system (CCD-200-R video camera, VideoScope International, Washington, DC; VC-405 video counter-timer, Thalner Electronic Laboratories; PVM-1342Q video monitor, Sony, Ichinomiya, J apan) and were digitized and stored for analysis by using the Image-1 acquisition and analysis software (Universal Imaging, West Chester, PA; model 2000, 486-33 MHz computer, Gateway, North Sioux City, SD; Tahuee-130 optical disk drive, Pinnacle Micro, Irvine, CA; or Mountain tape drive, Mountain Computer, Campbell, CA). Measurements of vessel luminal diameters were obtained from the digitized images by using the caliper-measuring function of the Image-1 software.

In a few experiments, measurements of venular (2 and 3 V) blood hemoglobin oxygen saturation (SO₂) were obtained under various experimental conditions to provide an index of tissue oxygen extraction. These measurements were performed using the spectrophotometric technique described by Pittman and Dulung (24), modified for use with digital image acquisition as previously described (15, 28). Briefly, optical narrow-band-pass filters (Oriel) of 520, 546, and 555 nm were introduced into the incident light path of the closed-circuit video system via an eight-position filter wheel (Metaltek Instruments, Raleigh, NC). Filter wheel positions were determined by computer control, images of the visual field were automatically acquired, and intensity measurements were performed by using the area brightness function of the Image-1 system. The corrected 555–546-nm light-intensity ratios were used to determine the SO₂ of blood in vitro by using the calibration technique previously described (15, 28).

Data collection. The animals were allowed to stabilize for 15–30 min after completion of the surgical procedure. The presence of vascular tone was verified by dilation in response to 8-Hz muscle contractions, and a 10-min recovery period was allowed before data collection was initiated. All animals that maintained a normal mean arterial pressure (MAP; >85 mmHg) also demonstrated vascular tone in the spinotrapezius muscle. A segment of the rostral terminal FA and specific locations along a downstream 1A and 2A were selected for analyses. In most cases, the order of data collection was 1) terminal FA, 1A, and 2A responses to iontophoretically applied SNP; 2) 2A, 1A, and terminal FA responses to iontophoretically applied ACh; and 3) simultaneous dilator responses to muscle contractions. Data collection was discontinued if MAP decreased below 85 mmHg, local vasodistraction or vasodilation was observed, the vessel failed to respond to a maximal dose of either drug, or resting diameter was not restored to within 15% of the original measurement after all stimuli were discontinued. Iontophoresis pipettes were sharpened to a luminal tip diameter of 10–15 μm and were filled with the appropriate drug (SNP or ACh, both from Sigma Chemical, St. Louis, MO). A retaining current of 40–60 nA was used to prevent release of drug from the pipette during control conditions. The pipette was placed as close to the outer edge of the vessel wall as possible without dimpling the surface or causing a local vascular response. The position of the pipette tip was adjusted as needed to maintain close proximity with the vessel wall. Incremental increases in pressure were applied in sequence, and images were acquired for measurement of diameter responses after 90–120 s to allow for development of the steady-state response. Pipette drug concentrations and application currents were as follows: ACh, 5 × 10⁻⁴ M at 10, 25, 50, 100, and 200 nA; SNP, 2 × 10⁻⁴ M at 10, 25, 50, 100, 200, and 500 nA. These parameters were determined in preliminary experiments and were chosen to elicit the widest possible range of responses for the three orders of vessels studied. Adequate recovery time (>2 min) was allowed during the repositioning of the pipette to ensure the complete restoration of normal resting diameter between experimental manipulations, and post hoc statistical analyses revealed no significant differences between repeated measurements of resting diameter at any vessel location.

After completion of data acquisition for the drug dose-response relationships, images of each of the three vessels were obtained in the resting muscle and immediately after 2 min of muscle contractions at frequencies of 2, 4, or 8 Hz.
Presented as means. All results are responses to drug application and muscle contractions, diameters were considered the largest absolute diameters for any optical distortions inherent to the system. Maximal vessel diameter was achieved within 90 s after the onset of muscle contraction and is maintained for >5 min of total contraction time (17), and 8-Hz contractions elicit maximal functional dilation of the spinotrapezius muscle arterioles in Sed and Tr rats (14, 16). Contractions frequencies were presented in random order, and a minimum of 10-min recovery time was allowed after the cessation of muscle contractions. Resting diameters were consistently restored between stimulation periods.

In some experiments, the final manipulation was to retest the response of the terminal FA to a submaximal (25 nA) dose of ACh. An inhibitor of NO synthase, N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA; Sigma Chemical), was then added to the superfusion solution (0.1 mM). After ~30 min, the vascular response to a 25-nA present dose of ACh was again recorded for the terminal FA to determine the extent to which this response was mediated by NO. Subsequently, the dilator responses to 2-, 4-, and 8-Hz muscle contractions were determined in the presence of L-NMMA in a manner similar to that previously described. In a few animals, venular SO\textsubscript{2} were determined in the resting and contracting muscle before and after application of L-NMMA, as previously described in detail (15).

Citrate synthase activity. At the end of the experimental procedures, the right spinotrapezius and plantaris muscles were removed and stored at -20°C until enzymatic analyses were performed. Homogenized solutions (10% spinotrapezius, 5% plantaris) of each muscle were prepared in 0.1 M tris(hydroxymethyl)aminomethane buffer containing 0.1% Triton X-100, and citrate synthase activity was determined by using the method described by Srere (27). Samples were analyzed at 30°C in duplicate or triplicate by using a Spectronic 401 spectrophotometer (Milton Roy). The average coefficients of variation for repeated samples were 3.6% for the spinotrapezius and 4.2% for the plantaris. Citrate synthase activity was expressed as micromoles per minute per gram of muscle tissue.

Data analysis. Measurements of luminal vessel diameters were determined from the digitized images by using the image-analysis software previously described. The system was calibrated by using the image of a stage micrometer and a stage micrometer (Soft, Tulsa, OK). Repeated-measures analyses of variance were performed across drug doses and contraction frequencies to evaluate group differences due to training status and training duration (Sed or Tr × 8 or 16 wk × dose or frequency). Post hoc tests were performed by using least significant difference analyses with statistical significance established at P < 0.05.

## RESULTS

### Descriptive characteristics

Descriptive physical characteristics of the four groups studied are presented in Table 1. Tr animals of both ages had lower body weights than their Sed counterparts. MAP values in anesthetized animals were similar between Sed and Tr groups. Citrate synthase activity, which reflects the oxidative capacity of skeletal muscle tissue, was ~40 and 16% higher in the plantaris and spinotrapezius muscles of Tr compared with Sed rats. There was no age group effect on the citrate synthase activity of either muscle.

Resting and maximal arterial diameters. Exercise training had no significant effect on resting or maximal diameters of the 1A through 2A vessels evaluated in this study (Fig. 1), with the exception that the resting diameters of 1A were smaller in Tr than Sed after 8 wk of training. However, resting and maximal vessel diameters tended to increase with age in both Sed and Tr groups. In most cases, maximal vessel diameter was more than twice the resting diameter in both the younger and older age groups. Resting tone, as represented by the relative dilatory capacity (maximal diameter/resting diameter), tended to be greater in Tr than Sed (P = 0.026) and was significantly greater in the 1A of Tr than Sed after 8 wk of training (2.63 ± 0.101 vs. 2.33 ± 0.073; P = 0.0253, 1-tailed t-value). This enhanced tone may account for the smaller resting diameters of these vessels.

Functional vasodilation. There was no age-dependent change in functional vasodilation of the three vessel types studied in Sed animals (Fig. 2). As previously shown (14, 16), after 8 wk of training, functional vasodilation was enhanced in Tr animals for all vessel types studied. However, the magnitude of the functional dilation appeared to decrease between the 8th and 16th wk of training, and comparable vasodilation was found between the 1A and 2A of Sed and Tr animals at the older age. Functional dilation of the terminal FA remained slightly greater in Tr than Sed animals after 16 wk of training.

### Table 1. Body weight, mean arterial blood pressure, and citrate synthase activities in sedentary and treadmill-trained rats

<table>
<thead>
<tr>
<th></th>
<th>Sedentary, 8 wk</th>
<th>Trained, 8 wk</th>
<th>Sedentary, 16 wk</th>
<th>Trained, 16 wk</th>
</tr>
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<tr>
<td>n</td>
<td>11</td>
<td>12</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>394 ± 12.3</td>
<td>366 ± 8.6*</td>
<td>509 ± 12.5†</td>
<td>424 ± 14.0‡</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>109 ± 3.5</td>
<td>111 ± 1.6</td>
<td>101 ± 3.5</td>
<td>99 ± 4.1</td>
</tr>
<tr>
<td>Citrate synthase, µm·g\textsuperscript{-1}·min\textsuperscript{-1}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spinotrapezius</td>
<td>15.4 ± 0.94</td>
<td>18.5 ± 0.71*</td>
<td>15.7 ± 1.32</td>
<td>18.1 ± 0.78*</td>
</tr>
<tr>
<td>Plantaris</td>
<td>24.4 ± 1.98</td>
<td>34.6 ± 3.06*</td>
<td>27.1 ± 5.30</td>
<td>35.6 ± 3.29*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. Training periods were 8 or 16 wk in duration. MAP, mean arterial pressure. *P < 0.05 vs. sedentary of same age group (1-tailed test); †P < 0.05 vs. 8-wk group of same training status (2-tailed test).
Pharmacological dilation. For each vessel order studied, the dilatory responses to SNP and ACh are presented in Figs. 3–5, A and B, respectively. After 8 wk of training, the responses of the terminal FA to SNP were significantly enhanced in Tr, relative to Sed, animals. After 16 wk of training, this adaptation was further enhanced in the FA, and a similar adaptation was then evident in the 1A. The adaptation in 1A can be attributed to both an increase in the dilatory response of vessels in Tr animals and a regression of the response in Sed animals between the 8th and 16th wk. The vascular responses to ACh were enhanced in the 1A and 2A of Tr animals after 16 wk, but not after 8 wk, of training. In fact, after 8 wk of training, the responses to ACh tended to be suppressed in these vessels. The FA responses to ACh were not affected by training. In summary, the larger vessels of Tr animals, the terminal FA, demonstrated an increased response to SNP within the first 8 wk of training, whereas the smaller 2A vessels demonstrated an increased response to ACh, which developed between the 8th and 16th wk of training.
training. The larger 1A arterioles exhibited an increased response to both drugs, but only after 16 wk of training.

Effects of L-NMMA. Superfusion of the tissue with 0.1 mM L-NMMA reduced the terminal FA dilatory response to a 25-nA present dose of ACh by 40 and 30% in the younger and older age groups, respectively; there was no apparent effect of training status on the L-NMMA suppression of this response. These results suggest that L-NMMA suppressed, but did not eliminate, the release of NO in response to ACh. Superfusion with this concentration of L-NMMA had no effect on MAP, as was seen in preliminary experiments with slightly higher doses. The presence of L-NMMA tended to reduce, but did not significantly change (P > 0.25), resting diameters of the terminal FA in both age groups studied (Figs. 6 and 7, Rest). However, smaller vessels tended to dilate in the younger animals and constrict in the older animals (Table 2). In all animals, blood flow velocity appeared to decrease during the application of L-NMMA. Measurements of venular blood SO2 in the resting muscle were reduced from 48 to 24% SO2 with the application of L-NMMA (Fig. 6B), providing circumstantial evidence that blood flow was in fact reduced. In both age groups, the presence of L-NMMA was associated with smaller terminal FA diameters during submaximal functional vasodilation (Figs. 6 and 7), but there was no such effect on 1A and 2A diameters (Table 2). In a small subgroup of the younger animals, measurements of venular blood SO2 were shown to decrease only slightly during muscle contractions under control conditions, remaining well above 30% SO2 (Fig. 6B). However, during perfusion with L-NMMA, venular blood SO2 were reduced to ~25% SO2 in the resting muscle and fell to below 10–15% SO2 during contractions.
DISCUSSION

The results of this study indicate that the in vivo regulation of functional vasodilation and vascular reactivity to SNP and ACh are affected by the age of the animals, the location of a vessel in the branching pattern, and the duration of exercise training. Whereas functional vasodilation remained relatively constant in Sed animals between the ages of 12 and 20 wk, the vascular responses to SNP tended to decrease, and those to ACh tended to increase. In contrast, the enhanced functional dilation that has been observed in Tr animals after 8 wk of treadmill running (14, 16) appeared to regress in the following weeks so that functional dilation was similar between Sed and Tr animals after 16 wk of training (Fig. 2). In addition, compared with Sed animals, the terminal FA of Tr animals demonstrated an increased response to SNP within the first 8 wk of training (Fig. 3), whereas 2A
demonstrated an increased response to ACh which developed between the 8th and 16th wk of training (Fig. 4). The observed regression of functional vasodilation with prolonged exercise training (Fig. 2) was not an expected result. We anticipated a maintenance of enhanced functional dilation to support the increased aerobic capacity of Tr skeletal muscle. However, without direct measurements of blood flow, we cannot conclude that the magnitude of functional hyperemia also regressed during this time. It is important to note

Fig. 5. Responses of 2A to iontophoric applications of SNP (A) and ACh (B). Dilation in response to SNP was similar between Tr and Sed rats at both time points. Responses in Sed rats increased slightly with time at the lower doses, whereas those in Tr rats tended to increase across entire dose range. Dilation in response to ACh was similar between Tr and Sed rats after 8 wk of training but were significantly greater in Tr rats after 16 wk of training. Responses in both Sed and Tr rats tended to increase over time. No. of observations per group: after 8 wk, Sed = 10 (9 for ACh) and Tr = 10; after 16 wk, Sed = 6 and Tr = 8 (7 for ACh). *Significant differences between similar groups of different ages; #significant differences between different training status at similar ages.

Fig. 6. Terminal FA diameter (A) and venular blood hemoglobin oxygen saturation (B) responses to muscle contractions before and after 30-min application of N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA) to block production of nitric oxide (NO). Data are pooled for Sed and Tr rats from the 8-wk groups. Application of L-NMMA significantly reduced terminal FA dilation in response to 4-Hz contractions (A), but this effect was not observed in 1A and 2A vessels (see Table 2, 8 wk). Application of L-NMMA significantly reduced venular blood hemoglobin oxygen saturation in the resting and contracting muscle (B), supporting the visual observation of reduction in red cell velocity despite presence of near normal arteriolar diametters. Diameter data are from 2 Sed rats and 1 Tr rat; saturation data are from 6 vessels in 3 Sed rats and 1 Tr rat. *Significant differences between pre- and postconditions.
that, although the 1A and 2A responses to muscle contraction were similar between Sed and Tr rats, and those trained for 16 wk, functional dilation of the arterioles remained somewhat enhanced, relative to Sed animals, after 16 wk of training (Fig. 2), and the possibility exists that an enhanced functional hyperemia was maintained due to increased vasodilation of these vessels and a potential recruitment of even larger feed vessels. Despite this possibility with regard to local vascular reactivity, we propose that the responses observed in animals after 16 wk of training are most representative of steady-state vascular changes with chronic aerobic training, and those observed after 8 wk of training reflect intermediate or transitional changes.

The training-induced changes in vascular reactivity to SNP and ACh appear to be somewhat dissociated from the changes in functional dilation. It is possible that the increased terminal FA response to SNP reflects a mechanism contributing to the enhanced functional dilation observed after 8 wk of training (Figs. 2 and 3). However, in these vessels, functional dilation tended to regress between the 8th and 16th wk of training, whereas the response to SNP was further enhanced, suggesting that the vascular response to other mediators of functional dilation were also changing during this time period. A similar argument can be made regarding the 1A responses to SNP and muscle contractions (Figs. 2 and 4).

In contrast, the 1A and 2A responses to ACh were significantly enhanced after 16 wk but not after 8 wk of training (Figs. 4 and 5). Therefore, changes in the endothelium response to ACh do not appear to contribute to the enhanced functional dilation observed after 8 wk of training (Fig. 2). The increased reactivity to ACh in the absence of enhanced functional dilation after 16 wk of training further suggests that the endothelium release of NO may play only a minor role in the functional dilation of these vessels. These conclusions are based on the assumption that ACh produces vasodilation primarily through the endothelial release of NO (11), although other mechanisms are proposed to be contributing factors (7, 10, 31). A minor role for NO in the functional dilation of 1A and 2A vessels is also suggested by the absence of a significant effect of L-NMMA on functional dilation of these vessels (Table 2). However, it should be noted that L-NMMA suppressed, but did not eliminate, the dilatory effects of ACh (see RESULTS), and it is possible that an adequate amount of NO was released during muscle contractions in the presence of L-NMMA to elicit the full contribution to functional dilation. There are also indications that additional mechanisms of vasodilation may have been active during L-NMMA application. Although the change in venular SO\textsubscript{2} from rest to muscle contractions was similar before and after L-NMMA application, the absolute values were substantially lower in the presence of L-NMMA, falling to ~10% SO\textsubscript{2} during contractions (Fig. 6B). These numbers correspond roughly to a local PO\textsubscript{2} of ~10 Torr, a level that has been associated with vasodilation (4, 5). It is likely that other metabolic conditions were also compromised, presumably due to the lower tissue blood flow. Therefore, metabolic vasodilation of these smaller vessels may have been enhanced in the presence of L-NMMA to the extent that it compensated for any suppression of NO formation. Considering the SO\textsubscript{2} data, the present data suggest that NO plays a major role in the regulation of blood flow in both the resting and contracting spinotrapezius muscle, with the primary effects being on the FA. These results are consistent with those of others who have studied the effects of NO blockade on resting and exercise blood flow.

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**Table 2.** Arteriolar diameters during muscle contractions before and after addition of L-NMMA to bathing medium to block release of nitric oxide.

<table>
<thead>
<tr>
<th>Group</th>
<th>Block</th>
<th>Control</th>
<th>2 Hz</th>
<th>4 Hz</th>
<th>8 Hz</th>
<th>n</th>
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<tbody>
<tr>
<td>Sed, 8 wk</td>
<td>Pre</td>
<td>1.00 ± 0.00</td>
<td>1.38 ± 0.12</td>
<td>1.96 ± 0.27</td>
<td>2.30 ± 0.35</td>
<td>3</td>
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<tr>
<td></td>
<td>Post</td>
<td>1.35 ± 0.31</td>
<td>1.89 ± 0.14*</td>
<td>1.81 ± 0.24</td>
<td>1.86 ± 0.36</td>
<td>3</td>
</tr>
<tr>
<td>Sed, 16 wk</td>
<td>Pre</td>
<td>1.00 ± 0.00</td>
<td>1.82 ± 0.21</td>
<td>2.30 ± 0.12</td>
<td>2.32 ± 0.08</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>0.79 ± 0.09</td>
<td>1.56 ± 0.24</td>
<td>2.16 ± 0.14</td>
<td>2.25 ± 0.13</td>
<td>4</td>
</tr>
<tr>
<td>Tr, 16 wk</td>
<td>Pre</td>
<td>1.00 ± 0.00</td>
<td>1.40 ± 0.24</td>
<td>2.16 ± 0.17</td>
<td>2.42 ± 0.28</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>0.74 ± 0.17</td>
<td>1.50 ± 0.28</td>
<td>2.04 ± 0.19</td>
<td>2.24 ± 0.24</td>
<td>4</td>
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</table>

**Values are means ± SE.** Diameters are expressed relative to resting diameter during preblock conditions (Pre) and reflect differences in absolute diameters between pre- and postblock (Post) conditions. Comparable data for terminal feed arteries are shown in Figs. 6 and 7. L-NMMA, N\textsuperscript{G}-monomethyl-L-arginine; Sed, sedentary; Tr, trained n, no. of rats. *P < 0.05 vs. preblock, 2-tailed test.
flows in the dog (25) and rat (9) hindlimbs and the rat cremaster muscle (8). However, the present results also suggest that when NO production is suppressed, other vasodilatory mechanisms can elicit near normal functional vasodilation in the arteriolar vessels. Our results were not adequate to fully explore the potential differential effects of L-NMMA between Sed and Tr animals.

The training-induced increase in the 2A reactivity to ACh observed in the present study (Fig. 5) is similar to that observed in 2A isolated from the rat gracilis muscle (12, 29). Sun et al. (29) found that as little as 3 wk of treadmill training enhanced the endothelium-dependent dilator responses of the 2A to ACh and L-arginine by 20–40%; the endothelium-independent responses to SNP were similar between Sed and Tr animals. Koller et al. (12) have found a similar enhancement of flow-dependent dilation in these vessels after 3 wk of training, and this dilation was attributed to endothelial release of both prostaglandins and NO.

The primary difference between the present and past studies is the time required for the expression of the adaptation, that is, 16 vs. 3 wk of training. It is likely that the degree to which a muscle is recruited during a training bout plays a role in determining the nature, time course, and magnitude of any vascular adaptations. The primary function of the rat spinotrapezius muscle is to stabilize the shoulder girdle during standing and locomotion (30), and treadmill training has minimal effects on the oxidative capacity of this tissue (14, 16) (Table 1). In contrast, the gracilis muscle contributes to leg adduction and experiences at least transient increases in blood flow during treadmill running (1). We suspect that the stimulus for endothelium adaptations, possibly increases in luminal blood flow (19), is more intense in the gracilis than in the spinotrapezius muscle during treadmill running. Therefore, we would expect the adaptations to occur more rapidly in the gracilis muscle. Similarly, the minimal training-induced changes in spinotrapezius muscle oxidative capacity suggest that the vascular adaptations observed in the present study may be representative of other inactive tissues and may be very different from those found in highly recruited skeletal muscles.

The adaptations of the 1A vessels appear to be a compilation of those of the terminal FA and the 2A (Fig. 4). As in the terminal FA, the 1A response to SNP was enhanced by training, and this adaptation was apparent to some extent after only 8 wk of training. After 16 wk of training, the response to ACh was also enhanced relative to Sed animals, as seen in the 2A. Although ACh may produce vasodilation through NO-independent mechanisms (7, 10, 31), the endothelium release of NO is thought to be a principal component of the response (11). Therefore, it is likely that at least a portion of the increased 1A response to ACh was secondary to the increased vascular smooth muscle response to NO. Unfortunately, we cannot determine the extent to which this is the case. With the use of a similar line of reasoning, it is somewhat surprising that the increased reactivity of the terminal FA to SNP was not also apparent during ACh application (Fig. 3), opening the possibility that the response of the terminal FA endothelium to ACh was actually suppressed as a result of exercise training.

As previously noted, the vascular responses in Sed animals were different between 12- and 20-wk-old animals (Figs. 3–5). In general, the terminal FA and 1A responses to SNP decreased with time and age, whereas the 2A responses to ACh increased. Thus exercise training essentially reversed or inhibited the “natural” decline in the terminal FA and 1A responses to SNP, magnifying the “natural” increase in the 2A response to ACh, and enhanced the 1A response to ACh. Therefore, a portion of the training effect may be attributed to a slowing or reversal of the “natural” effects of aging in Sed animals, and a potential interaction of aging and training effects may be important to consider in future work.

This study is the first to evaluate simultaneously the in vivo regulation of functional and pharmacological vasodilation at various levels of the skeletal muscle microcirculation in exercise-trained animals. For the largest vessels observed in this study, the terminal FA of the rat spinotrapezius muscle, the primary vascular adaptation to exercise training was an enhanced vascular smooth muscle response to SNP, a nitrosamine compound. For the smallest vessels studied, the 2A, the primary adaptation was an enhanced endothelium-mediated response to ACh. Adaptations of both types were observed in the intermediate-sized 1A. For all sizes of vessels studied, functional vasodilation tended to regress between the 8th and 16th wk of training, and there was an apparent dissociation between the enhancement of vasodilation elicited by muscle contractions and that elicited by SNP and ACh. A comparable dissociation was apparent in Sed animals over time, as functional dilation was similar between 12- and 20-wk-old animals, but during the same period of time, dilation in response to SNP tended to decrease and dilation in response to ACh tended to increase. Therefore, age, training duration, vascular branching order, and the specificity of the vasoactive stimulus must all be considered when evaluating the effects of exercise training on microvascular function.

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