Exercise training reverses age-related decrements in endothelium-dependent dilation in skeletal muscle feed arteries

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Trott DW, Gunduz F, Laughlin MH, Woodman CR. Exercise training reverses age-related decrements in endothelium-dependent dilation in skeletal muscle feed arteries. J Appl Physiol 106: 1925–1934, 2009. First published March 19, 2009; doi:10.1152/japplphysiol.91232.2008.—We tested two hypotheses, first that exercise training reverses age-related decrements in endothelium-dependent dilation in soleus muscle feed arteries and second that this improved endothelium-dependent dilation is the result of increased nitric oxide (NO) bioavailability due to increased content and phosphorylation of endothelial NO synthase (eNOS) and/or increased antioxidant enzyme content. Young (2 mo) and old (22 mo) male Fischer 344 rats were exercise trained (Ex) or remained sedentary (Sed) for 10–12 wk, yielding four groups of rats: 1) young Sed (4–5 mo), 2) young Ex (4–5 mo), 3) old Sed (24–25 mo), and 4) old Ex (24–25 mo). Soleus muscle feed arteries (SFA) were isolated and cannulated with two glass micropipettes for examination of endothelium-dependent (ACh) and endothelium-independent [sodium nitroprusside (SNP)] vasodilator function. To determine the mechanism(s) by which exercise affected dilator responses, ACh-induced dilation was assessed in the presence of Nω-nitro-L-arginine (l-NNA; to inhibit NO synthase), indomethacin (Indo; to inhibit cyclooxygenase), and l-NNA + Indo. Results indicated that ACh-induced dilation was blunted in old Sed SFA relative to young Sed SFA. Exercise training improved ACh-induced dilation in old SFA such that vasodilator responses in old Ex SFA were similar to young Sed and young Ex SFA. Addition of l-NNA, or l-NNA + Indo, abolished the exercise effect. Immunoblot analysis revealed that extracellular superoxide dismutase (SOD) protein content was increased by training in old SFA, whereas eNOS and SOD-1 protein content were not altered. Addition of exogenous SOD, or SOD + catalase, improved ACh-induced dilation in old Sed SFA such that vasodilator responses were similar to young Sed SFA. Addition of l-NNA abolished the effect of exogenous SOD in old Sed arteries. Collectively, these results indicate that exercise training reverses age-induced endothelial dysfunction in SFA by increasing NO bioavailability and that increases in vascular antioxidant capacity may play an integral role in the improvement in endothelial function.

endothelial dysfunction; endothelial nitric oxide synthase; superoxide dismutase

AGING is associated with a decline in endothelial function characterized, in part, by impaired endothelium-dependent vasodilator responses in central and peripheral arteries (1, 4, 8, 29, 45, 53, 54). The resulting endothelial dysfunction is believed to contribute to an increased risk of cardiovascular disease in older adults. In addition, the decline in endothelial function may contribute to impaired muscle blood flow and reduced exercise tolerance in the elderly (2, 18, 25, 31, 34, 37, 49). The mechanism(s) for the age-related decrement in endothelial-dependent dilation is not fully understood; however, previously published studies indicate that a decline in the bioavailability of nitric oxide (NO) plays an integral role (1, 5, 6, 29, 44, 54).

Endurance exercise training improves endothelium-dependent dilation in some conduit arteries in young healthy subjects (7, 22, 28, 30, 32, 42, 43) and in skeletal muscle arterioles/resistance arteries (24, 27, 39, 40). Importantly, examination of the effects of training in the arteriolar tree of skeletal muscle indicates that the improvement in endothelium-dependent dilation induced by exercise training is not uniformly distributed throughout the arteriolar tree (24, 27). The beneficial effect of exercise training has also been reported to be associated with increased expression of endothelial nitric oxide synthase (eNOS) (23, 38, 52), enhanced production of NO (31), and improved NO-mediated, endothelium-dependent dilation (30). In addition, exercise training has been reported to increase expression of cystosolic and extracellular superoxide dismutases (SOD-1 and ecSOD) in the aorta of mice and pigs, which may improve endothelial function by enhancing the capacity to scavenge superoxide and prolonging the biological half-life of NO (15, 36).

Previous studies indicate that endurance exercise training is also an effective intervention for attenuating or reversing age-induced endothelial dysfunction in first-order (1A) arterioles perfusing skeletal muscle (39, 40). Specifically, Spier et al. reported that endurance exercise training improves endothelium-dependent vasodilator responses in 1A arterioles from soleus and gastrocnemius muscles of aged rats, and that the improved endothelium-dependent dilation was mediated by enhanced NO bioavailability (39, 40). In addition, exercise training appears to enhance antioxidant status and improve endothelium-dependent dilation in conduit arteries of aged human subjects (8, 12, 14).

In skeletal muscle it has been established that a primary control point for regulating total muscle blood flow during exercise is the feed artery (50). Indeed, previous research indicates that feed arteries, which lie immediately external to skeletal muscle, provide the principal site of resistance to flow through individual skeletal muscles and play an integral role in mediating increases in skeletal muscle blood flow during physical activity (21, 50, 51). Thus an exercise training-induced improvement in vasodilator responses in skeletal muscle feed arteries could potentially work in concert with enhanced endothelial function previously reported in skeletal muscle arterioles (39, 40) by increasing the capacity to augment total muscle blood flow (feed arteries) and the ability to redistribute the augmented blood flow (arterioles) to active skeletal muscle fibers. Given that exercise in young animals has been shown to exert changes in endothelium-dependent dilation in the resis-
tance artery network of skeletal muscles in a nonuniform manner and the importance of feed arteries to perfusion of skeletal muscle, it is important to know whether exercise training in aged animals has a beneficial impact on soleus muscle feed arteries. We know that exercise training has been shown to improve endothelium-dependent dilation in aged soleus muscle 1A arterioles (39, 40); however, the efficacy of endurance exercise training to improve endothelial function in senescent soleus muscle feed arteries is not known. Therefore, the purpose of this study was to test the hypothesis that exercise training reverses age-related decrements in endothelium-dependent dilation in soleus muscle feed arteries (SFA). We also hypothesized that endurance exercise training would improve endothelium-dependent dilation in senescent SFA by increasing NO bioavailability due to increased content and phosphorylation of eNOS and/or increased antioxidant enzyme content.

METHODS

Experimental Design

Endothelium-dependent dilation in response to application of ACh was examined in SFA isolated from young and old sedentary and exercise-trained rats, using standard techniques. The relative contribution of NO synthesis by NOSs was evaluated by examining vasodilator responses in the presence of Nω-nitro-l-arginine (l-NNA; 300 μM) to inhibit nitric oxide synthase (NOS). The contribution of cyclooxygenase (COX) was evaluated by examining vasodilator responses in the presence of indomethacin (Indo; 5 μM) to inhibit COX, and the role of non-NOS and non-COX pathways to the responses was determined by examining vasodilator responses in the presence of l-NNA + Indo to inhibit NOS and COX. Feed arteries were also harvested for examination of expression of eNOS, Ser 1177 phosphorylated eNOS (p-eNOS), SOD-1, and ecsOD using immunoblot analysis. When these results revealed that ecsSOD content was increased by exercise training, we did a series of experiments to determine whether the improvement in endothelium-dependent dilation could be produced simply by increasing antioxidant levels by adding exogenous antioxidants.

Animals

All of the protocols used in the present study were approved by the Animal Care and Use Committees at the University of Missouri and Texas A&M University. To determine the efficacy of exercise training to improve endothelium-dependent dilation in aged SFA, male Fischer 344 rats (2 and 22 mo of age; n = 20/age group) were purchased from a commercial dealer (Harlan Sprague-Dawley, Indianapolis, IN) and housed in the University of Missouri College of Veterinary Medicine’s Animal Care Facility. One week after arrival, rats were exercise trained (Ex) or remained sedentary (Sed) for 10–12 wk. Thus at the end of the training program, the ages of the young and old rats were 4–5 mo or 24–25 mo, respectively. The resulting experimental design consisted of four groups of rats: 1) young Sed (n = 10), 2) young Ex (n = 10), 3) old Sed (n = 10), and 4) old Ex (n = 10). To determine whether exogenous antioxidants produce exercise-like effects on aged SFA, a separate group of male Fischer 344 rats (4 and 24 mo of age) was purchased and housed at the University of Missouri Comparative Medicine Program Facility. Both animal facilities were maintained at 22°C with a 12:12-h light-dark cycle. Food and water were provided ad libitum, and the rats were examined daily by the investigators and by veterinarians affiliated with their respective institutions.

Training Program

The exercise training protocol used in the present study has been published previously in detail (39). In brief, rats were familiarized with running on a motorized treadmill and randomly assigned to an Ex or Sed group for 10–12 wk. Rats assigned to the Ex group ran 60 min/day, 5 days/wk, at 15 m/min (15° incline). Rats assigned to the Sed group were restricted to their cages and did not exercise. The efficacy of the exercise-training protocol was assessed from measurements of citrate synthase activity in the vastus lateralis muscle (41).

Isolation of Feed Arteries

Procedures used to isolate SFA have been published previously (53–56). In brief, rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (50–60 mg/kg body wt ip). Soleus muscles from the left and right hindlimb were removed and placed in MOPS-buffered physiological saline solution (PSS) containing (in mM) 145.0 NaCl, 4.7 KCl, 2.0 CaCl2, 1.17 MgSO4, 1.2 NaH2PO4, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA, 25.0 MOPS, at pH 7.4. SFA from one hindlimb were dissected free of paired veins and connective tissue under a dissection microscope, cut on both ends, and transferred to a Lucite chamber containing MOPS-PSS (4°C) for cannulation. SFA from the contralateral hindlimb were dissected free, transferred to a microcentrifuge tube, snap-frozen, and stored at −80°C for subsequent immunoblot analysis.

Determination of Vasodilator Responses

Preparation of arteries. SFA were prepared for functional analysis as described previously (19, 42). Specifically, arteries were cannulated with two resistance-matched glass micropipettes and secured with 11-0 surgical silk. The micropipettes were subsequently attached to separate pressure reservoirs filled with MOPS-PSS supplemented with albumin (1 g/100 ml). The height of each reservoir was initially adjusted to set intraluminal pressure in each SFA to 60 cmH2O (1 mmHg = 1.36 cm H2O) for 20 min. After intraluminal pressure was raised to 90 cmH2O, and the feed arteries were allowed to equilibrate for an additional 40 min at 37°C. At the end of the 60-min equilibration period, feed arteries that did not develop at least 25% spontaneous tone were constricted with phenylephrine. All experimental protocols were subsequently conducted at an intraluminal pressure of 90 cmH2O to approximate in vivo intraluminal pressure (50).

Endothelium-dependent dilation. Endothelium-dependent dilation was assessed in feed arteries by adding increasing doses of ACh to the bath solution in cumulative doses over the range of 10−9–10−4 M in whole log increments as described previously (19, 53, 54). A total of 4 SFA were studied in parallel from each rat. In SFA 1, ACh-induced vasodilator responses were assessed in the absence of enzyme inhibitors. In SFA 2, vasodilator responses were assessed in the presence of l-NNA (300 μM) to inhibit NOS. In SFA 3, vasodilator responses were assessed in the presence of Indo (5 μM) to inhibit COX. In SFA 4, vasodilator responses were assessed in the presence of l-NNA + Indo to inhibit NOS and COX.

In a separate series of experiments, endothelium-dependent dilation was assessed in SFA from young Sed and old Sed rats in the absence and presence of exogenous antioxidants. In these studies, a total of 3 SFA were studied in parallel from each rat. In SFA 1, ACh-induced vasodilator responses were assessed in the presence of exogenous antioxidants. In SFA 2, vasodilator responses were assessed in the presence of superoxide dismutase (SOD; 120 U/ml) to scavenge superoxide. In SFA 3, vasodilator responses were assessed in the presence SOD and catalase (CAT; 100 U/ml) to scavenge superoxide and hydrogen peroxide. When results revealed that exogenous antioxidants improved endothelium-dependent dilation, a subsequent set of experiments was performed to determine whether NO mediated the improvement in endothelial function. In these studies 3 SFA were studied in parallel from each rat. In SFA 1, ACh-induced vasodilator responses were assessed in the absence of exogenous antioxidants. In SFA 2, vasodilator responses were assessed in the presence of SOD.
In SFA, vasodilator responses were assessed in the presence of SOD and L-NNA.

Endothelium-independent dilation. Endothelium-independent dilation was assessed by adding increasing doses of sodium nitroprusside (SNP) to the bath solution in cumulative doses over the range of $10^{-9}$–$10^{-4}$ M in whole log increments (19, 53, 54). SNP-induced dilation was also assessed in SFA from young and old Sed rats in the presence of SOD, SOD + CAT, and SOD + L-NNA.

Passive diameter. At the end of each experiment, SFA were incubated for 30 min in Ca$^{2+}$-free PSS to determine passive diameter at an intraluminal pressure of 90 cmH$_2$O.

Solutions and Drugs

All reagents used in dose-response experiments were obtained from Sigma Chemical (St. Louis, MO). Reagents were prepared on the day of the experiment.

Quantification of eNOS, Ser1177 p-eNOS, SOD-1, and ecSOD protein content

Relative differences in eNOS, Ser1177 p-eNOS, SOD-1, and ecSOD protein contents were assessed in feed arteries using immunoblot analysis as described previously in detail (19). eNOS protein content was evaluated with a monoclonal antibody (1:1,250; catalog no. 610297, BD Transduction Laboratories). Ser1177 p-eNOS protein content was assessed with a monoclonal antibody (1:250; catalog no. 612393, BD Transduction Laboratories). SOD-1 protein content was assessed with a polyclonal antibody (1:1,000; catalog no. SOD-105, Stressgen). Immunoblots were probed with the Ser1177 p-eNOS antibody (1:1,250; catalog no. SOD-105, Stressgen). Immunoblots were probed with an eNOS antibody (1:1,000; catalog no. AB374, Millipore). To determine whether the ratio of Ser1177 p-eNOS to total eNOS protein content was altered with aging or exercise training, immunoblots were carried out in the absence and presence of SOD or L-NNA, or SOD and L-NNA, or SOD + L-NNA, or SOD + L-NNA + Indo, or SOD + L-NNA + Indo + L-NNA. SOD-1 protein content was assayed with a monoclonal antibody (1:1,000; catalog no. SOD-105, Stressgen). GAPDH protein content was assessed with a polyclonal antibody (1:1,000; catalog no. AB374, Millipore). To determine whether the ratio of Ser1177 p-eNOS to total eNOS protein content was altered with aging or exercise training, immunoblots were probed with the Ser1177 p-eNOS antibody, stripped with Restore Western Blot Stripping Buffer (Thermo catalog no. 21059), and reprobed with the eNOS antibody.

Statistical Analysis

All values are means ± SE. Between-group differences in body mass, citrate synthase activity, percent tone, relative protein content, and passive diameter were assessed using one-way ANOVA. Concentration-response curves were analyzed by two-way ANOVA with repeated measures on one factor (dose) to determine whether vasodilator responses to ACh and SNP differed by group. In addition, between-group differences in IC$_{50}$ values and maximal responses were assessed with one-way ANOVA. Concentration-response data were expressed as a percentage of maximal possible dilation. Percent possible dilation was calculated as $((D_{max} - D_{b})(D_{max} - D_{p})) \times 100$, where $D_{max}$ is measured diameter for a given dose, $D_{b}$ is baseline diameter before an intervention was started, and $D_{p}$ is maximal passive diameter. A total of 230 SFA were used in experiments to assess vasodilator function. Fifteen young (8 Sed; 7 Ex) and 21 old (11 Sed; 10 Ex) SFA required phenylephrine to achieve 25% tone. Deletion of these arteries from the statistical analyses did not alter interpretation of the results; therefore, all 230 SFA were included in the final analyses. When a significant $F$ value was obtained, post hoc analyses were performed with Duncan’s multiple-range test and Fisher’s least significant difference (LSD) test. Statistical significance was set at the $P \leq 0.05$ probability level.

Table 1. Body weight and citrate synthase activity in the vastus lateralis red muscle

<table>
<thead>
<tr>
<th></th>
<th>Young Sed</th>
<th>Young Ex</th>
<th>Old Sed</th>
<th>Old Ex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>391 ± 9</td>
<td>353 ± 8*</td>
<td>425 ± 12*</td>
<td>402 ± 12</td>
</tr>
<tr>
<td>Citrate synthase activity; mmol·min$^{-1}$·g wet wt$^{-1}$</td>
<td>29.9 ± 0.7</td>
<td>39.7 ± 3.7*</td>
<td>25.6 ± 1.8</td>
<td>38.7 ± 3.0*</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 8–10$ rats/group. Sed, sedentary; Ex, exercise trained. Significantly different from *young Sed, †young Ex, ‡old Sed, §old Ex, $P \leq 0.05$.
The decreased ACh-induced dilation observed in SFA from old Sed rats in the present study (Figs. 1, 4, and 5) is in accord with previous studies indicating that endothelium-dependent dilation is impaired in feed arteries (53, 54) as well as 1A arterioles (29) of the soleus muscle of aged rats.

Table 2. Characteristics of soleus muscle feed arteries from young and old rats used in the exercise training study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Con</th>
<th>l-NNA</th>
<th>Indo</th>
<th>l-NNA + Indo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal diameter, μm</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Young Sed</td>
<td>199±5</td>
<td>193±7</td>
<td>195±11</td>
<td>180±13</td>
</tr>
<tr>
<td>Young Ex</td>
<td>196±12</td>
<td>178±16</td>
<td>191±12</td>
<td>184±15</td>
</tr>
<tr>
<td>Old Sed</td>
<td>170±8</td>
<td>173±10</td>
<td>174±17</td>
<td>187±12</td>
</tr>
<tr>
<td>Old Ex</td>
<td>200±10</td>
<td>194±8</td>
<td>171±11</td>
<td>171±10</td>
</tr>
<tr>
<td>Initial tone, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young Sed</td>
<td>48.6±4.1</td>
<td>73.9±2.8</td>
<td>45.3±6.9</td>
<td>57.6±4.5</td>
</tr>
<tr>
<td>Young Ex</td>
<td>40.1±4.3</td>
<td>60.4±4.7</td>
<td>34.4±4.1</td>
<td>54.7±6.1</td>
</tr>
<tr>
<td>Old Sed</td>
<td>44.0±6.4</td>
<td>67.5±5.2</td>
<td>32.0±4.0</td>
<td>45.6±4.9</td>
</tr>
<tr>
<td>Old Ex</td>
<td>46.4±3.9</td>
<td>55.8±4.9</td>
<td>33.4±2.3</td>
<td>44.1±2.7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8–10 rats/group. Con, control; l-NNA, Nω-nitro-l-arginine; Indo, indomethacin. Significantly different from *Con, *l-NNA, *Indo, *l-NNA + Indo, P ≤ 0.05.

SNP-Induced Dilation

SNP elicited a concentration-dependent dilation of all arteries. Statistical analysis revealed no significant between-group differences (data not shown).

eNOS, Ser1177 p-eNOS, SOD-1, and ecSOD Protein Content

Immunoblot analysis revealed that eNOS (Fig. 6A), Ser1177 p-eNOS (Fig. 6B), and SOD-1 (Fig. 7A) protein contents were not significantly altered by age or exercise training. The Ser1177 p-eNOS-to-total eNOS protein content ratio was also not altered by age or training status (Fig. 6C). In contrast, ecSOD protein content was significantly increased by exercise training in young and old SFA such that ecSOD content was greater in young Ex and old Ex arteries than in young Sed and old Sed arteries (Fig. 7B).

DISCUSSION

The purpose of this study was to test the hypothesis that exercise training reverses age-related decrements in endothelium-dependent dilation in SFA by increasing NO bioavailability due to increased content and phosphorylation of eNOS and/or increased antioxidant enzyme content. The primary new findings of this study are that: 1) exercise training improved ACh-induced dilation in old SFA such that vasodilator responses in old Ex SFA were similar to young Sed and young Ex SFA; 2) ecSOD protein content was increased by training in old SFA, whereas the SFA protein content of eNOS, Ser1177 p-eNOS, and SOD-1 were not altered by training; and 3) exogenous SOD, and SOD + CAT, restored ACh-induced dilation in old SFA in an NO-dependent manner similar to the effects of exercise training. Results also demonstrate that treatment with l-NNA, or l-NNA + Indo, inhibited ACh-induced dilation in old Ex SFA such that the response was not greater than the response in the old Sed SFA. Also, ACh-induced dilation was not significantly inhibited by Indo alone in young or old rats. Collectively, these results indicate that exercise training reverses the detrimental effects of aging on endothelium-dependent dilation in SFA and that increased vascular ecSOD protein content may contribute to this improvement. To our knowledge, this is the first study to demonstrate that exercise training reverses age-induced endothelial dysfunction in SFA or any skeletal muscle feed artery. Given that skeletal muscle feed arteries contribute importantly to regulating total skeletal muscle blood flow during exercise (47), this exercise-induced adaptation may have substantial functional significance.

Reduced exercise capacity with age is well documented in humans and animals (2, 3, 26). The mechanism(s) for the age-related decline in exercise tolerance is not fully understood; however, an impaired ability to increase skeletal muscle blood flow during exercise may contribute to the reduction in exercise capacity (2, 18, 31, 34, 37). There are numerous potential mechanisms for the impairment in the blood flow response to exercise, including age-related increases in vasconstrictor responsiveness (9–11, 37, 47) and blunted NO-mediated endothelium-dependent dilation (37). Although NO does not appear to be obligatory for increasing muscle blood flow during exercise in young subjects (13, 35), Schrage et al. (37) have shown that the contribution of NO to exercise hyperemia is reduced in older subjects.

The decreased ACh-induced dilation observed in SFA from old Sed rats in the present study (Figs. 1, 4, and 5) is in accord with previous studies indicating that endothelium-dependent dilation is impaired in feed arteries (53, 54) as well as 1A arterioles (29) of the soleus muscle of aged rats.

Table 3. Characteristics of soleus muscle feed arteries from young and old rats used in the antioxidant studies

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Con</th>
<th>SOD</th>
<th>SOD + CAT</th>
<th>SOD + l-NNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal diameter, μm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>162.6±5.5</td>
<td>178.1±8.0</td>
<td>175.8±7.3</td>
<td>181.9±8.7</td>
</tr>
<tr>
<td>Old</td>
<td>158.3±7.6</td>
<td>183.2±9.4</td>
<td>175.3±13.9</td>
<td>169.0±14.2</td>
</tr>
<tr>
<td>Initial tone, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>41.1±2.3</td>
<td>38.3±2.9</td>
<td>38.2±3.9</td>
<td>47.4±5.2</td>
</tr>
<tr>
<td>Old</td>
<td>40.8±3.2</td>
<td>39.0±2.8</td>
<td>37.1±4.1</td>
<td>42.4±4.9</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6–21 rats/group. SOD, superoxide dismutase; CAT, catalase.
Also, previous studies indicate that exercise training attenuates the detrimental effects of aging on endothelium-dependent dilation of skeletal muscle 1A arterioles (39, 40). Present results indicate that Ex improved ACh-induced dilation in old SFA such that vasodilator responses in old Ex SFA were similar to young Sed and young Ex SFA (Fig. 1). The exercise-induced improvement in endothelial function in SFA may be functionally significant given that feed arteries serve as the primary control point for regulating total muscle blood flow to soleus muscle at rest and during exercise (50). In addition to enhancing NO-mediated dilation in aged skeletal muscle arterioles, exercise training has also been shown to attenuate vasoconstrictor responses in skeletal muscle arterioles (10, 11, 39, 40). Thus it is likely that the exercise-induced improvement in endothelium-dependent vasodilator responses in SFA observed in the present study works in concert with enhanced vasodilator, and attenuated vasoconstrictor, responses in skeletal muscle arterioles to enhance the capacity to increase skeletal muscle blood flow and to distribute blood flow to the actively contracting muscle fibers during exercise.

Mechanisms Responsible for increased Vasodilator Responses

Results of the present study indicate that the beneficial effect of exercise training on ACh-induced vasodilator function is primarily due to increases in NO bioavailability, since the exercise effect was eliminated in the presence of L-NNA (Fig. 2B). Importantly, the improvement in NO-mediated dilation could not be attributed to an exercise-induced improvement in the ability of vascular smooth muscle cells to respond to NO, since vasodilator responses to SNP (an NO donor) were similar in all groups of arteries. To determine whether the beneficial effect of exercise on endothelium-dependent dilation also involved COX products, ACh-induced dilation was assessed in the presence of Indo to block COX. ACh-induced dilation was not significantly inhibited by Indo alone in young or old rats regardless of training status. Thus the beneficial effect of exercise training on vasodilator responses in aged SFA does not appear to be mediated by altered COX signaling.

To determine whether enhancement of a NOS- and COX-independent vasodilator mechanism contributed to the beneficial effect of exercise training, ACh-induced dilation was assessed in the presence of L-NNA + Indo (double blockade). In the presence of L-NNA + Indo, residual dilation to ACh can...
be attributed to vasodilators other than NO and prostacyclin (PGI2), primarily endothelium-derived hyperpolarizing factors (EDHF). Double blockade had no statistically significant impact on old Sed arteries (Fig. 3B), suggesting that endothelium-dependent dilation in old Sed SFA is mediated entirely by non-NOS, non-COX mechanisms. Double blockade inhibited, but did not eliminate, ACh-induced dilation in old Ex SFA (Fig. 3B). Equally important was the finding that ACh-induced dilation in old Ex SFA in the presence of L-NNA/L11001/Indo was not different from the old Sed arteries in the presence of double blockade (Fig. 3B). These data indicate that a vasodilator pathway independent of NOS and COX contributed to ACh-induced dilation in young and old arteries; however, the beneficial effect of exercise training in aged arteries cannot be attributed to enhancement of this pathway. In conclusion, our pharmacological experiments indicate that exercise training reverses the detrimental effects of aging on endothelial function of SFA primarily by enhancing NO bioavailability.

Table 4. IC50 —log M values and response to 10^-4 M ACh of soleus muscle feed arteries from young and old rats used in the exercise training study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Con</th>
<th>l-NNA</th>
<th>Indo</th>
<th>l-NNA + Indo</th>
</tr>
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<tbody>
<tr>
<td>IC50, —log M ACh</td>
<td></td>
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<tr>
<td>Young Sed</td>
<td>7.52 ± 0.35</td>
<td>7.13 ± 0.26</td>
<td>7.37 ± 0.26</td>
<td>7.37 ± 0.26</td>
</tr>
<tr>
<td>Old Sed</td>
<td>7.83 ± 0.55</td>
<td>7.15 ± 0.26</td>
<td>7.20 ± 0.60</td>
<td>7.20 ± 0.60</td>
</tr>
<tr>
<td>Response to 10^-4 M ACh, % possible dilation</td>
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</tr>
<tr>
<td>Young Sed</td>
<td>87.0 ± 6.2</td>
<td>52.4 ± 8.2</td>
<td>64.6 ± 9.3</td>
<td>60.6 ± 8.8</td>
</tr>
<tr>
<td>Young Ex</td>
<td>76.9 ± 10.7</td>
<td>49.1 ± 12.6</td>
<td>73.6 ± 9.4</td>
<td>28.1 ± 8.6</td>
</tr>
<tr>
<td>Old Sed</td>
<td>54.2 ± 8.7</td>
<td>37.9 ± 6.0</td>
<td>67.4 ± 9.0</td>
<td>28.5 ± 11.1</td>
</tr>
<tr>
<td>Old Ex</td>
<td>80.9 ± 6.8</td>
<td>41.8 ± 9.5</td>
<td>61.8 ± 11.3</td>
<td>39.1 ± 8.3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8–10 rats/group. Significantly different from *Con, †L-NNA, ‡Indo, ‡L-NNA + Indo, §Sed, ¶Young, P ≤ 0.05.

Fig. 4. Values are means ± SE; n = 6–14 rats/group. ACh-induced dilation in young (A) and old (B) SFA in the absence or presence of exogenous antioxidants superoxide dismutase (SOD, 120 U/ml) or SOD + catalase (CAT, 100 U/ml). B, baseline diameter before the 1st dose of ACh. Dose-response curve significantly different from †control, P ≤ 0.05.

Fig. 5. Values are means ± SE; n = 8–21 rats/group. ACh-induced dilation in young (A) and old (B) SFA in the absence or presence of SOD or SOD + l-NNA. B, baseline diameter before the 1st dose of ACh. Dose-response curve significantly different from †control, P ≤ 0.05.
conclusion is similar to previous reports for 1A arterioles of soleus muscle of aged rats (39, 40).

Results of the present study also indicate that exercise training did not induce increases in eNOS protein content in young or old SFA (Fig. 6A), nor did exercise training increase basal levels of Ser 1177 p-eNOS (Fig. 6B) or alter the Ser 1177 p-eNOS-to-total eNOS ratio (Fig. 6C). These data suggest that enhanced NO-mediated, endothelium-dependent dilation in old Ex SFA is not due to increased eNOS protein content or eNOS phosphorylation on Ser 1177. In addition, these results indicate that while exercise training increases NO bioavailability in SFA (Fig. 6) and 1As (39, 40) of aged soleus muscle, the vascular adaptation induced by exercise in SFA is not the same as that reported in soleus 1A arterioles by Spier et al. where exercise training increased eNOS protein content (39). Thus, while exercise training improves endothelial function in SFA and 1A arterioles perfusing soleus muscle, the mechanism by which exercise improves NO-mediated endothelium-dependent dilation is different in SFA and 1A arterioles of this muscle (39, 40). Exercise training has also been shown to increase eNOS mRNA and protein content in aged rat aorta (46). The differences in aging- and exercise training-induced alterations in different vessels suggest that findings in one vessel cannot necessarily be applied to other vessels.

Another mechanism whereby exercise training could increase NO bioavailability in aged SFA is through an exercise-induced reduction in the rate of NO degradation. The primary mechanism for NO degradation in blood vessels is rapid interaction with superoxide anion, leading to the formation of peroxynitrite (16). This mechanism has been implicated in aging-induced endothelial dysfunction (16, 48). Vascular superoxide dismutases play a role in preserving the bioactivity of NO by scavenging superoxide. Consequently, an exercise-induced increase in the expression of superoxide dismutases could lead to the exercise-induced improvement in NO-mediated, endothelium-dependent dilation in aged SFA. Therefore we tested the hypothesis that exercise training increases the expression of SOD-1 and ecSOD in old SFA. The rationale for this hypothesis was based on experimental evidence indicating that exercise training increases expression of SOD-1 and ecSOD in the aorta of young mice and pigs (15, 36). Our results reveal that ecSOD (not SOD-1) content was increased by training in aged SFA (Fig. 7B). These results support the hypothesis that the beneficial effect of exercise on endothelium-dependent dilation observed in the old SFA is, in part, the result of an enhanced capacity of ecSOD to scavenge superoxide, resulting in an increase in the bioavailability of NO. Because ecSOD activity is regulated by a number of factors including protein folding, reactions with NO, and hydrogen peroxide and copper availability (15, 17, 20, 33), further study is needed to fully elucidate the role of ecSOD in the beneficial effects of exercise training in aged SFA.

In light of the observation that ecSOD protein content was increased in old SFA with exercise training, we reasoned that the beneficial effects of exercise on endothelium-dependent dilation of SFA may be mimicked by treatment with exogenous antioxidant enzymes. To test this hypothesis we chose SOD (non-cell permeable) rather than PEG-SOD (cell permeable) because the primary exercise-induced alteration in antioxidant protein content occurred with the extracellular isoform of SOD (ecSOD). Results indicated that addition of exogenous SOD, or SOD + CAT, did not alter the ACh dose response in feed arteries from young rats but resulted in greater ACh-induced dilation in old Sed SFA compared with old Sed SFA without antioxidants (Fig. 4). In addition, ACh-induced dilation was either equal to (in the presence of SOD + CAT) or greater than (in the presence of SOD alone) ACh-induced dilation in young SFA (Fig. 4). Also, l-NNA abolished the improvement in ACh-induced dilation in old Sed SFA observed in the presence of SOD (Fig. 5). These results demonstrate that exogenous antioxidant enzymes mimic the effects of exercise in old SFA, consistent with our hypothesis. Based on the observation that exercise training increased ecSOD protein content and that exogenous SOD mimicked the effects of exercise on ACh-induced dilation in senescent SFA, we conclude that exercise training-induced improvements in NO-mediated, endothelium-dependent dilation in senescent SFA may be in part the result of enhanced scavenging of superoxide by ecSOD, resulting in increased stability of NO. These results are consistent with those obtained in human subjects where older athletes exhibited enhanced endothelium-dependent dilation and antioxidant capacity compared with their sedentary counterparts (14); also, addition of exogenous antioxidants enhanced endothelium-dependent dilation in old sedentary subjects (12). Complementing these observations, our study is the first to demonstrate enhanced ecSOD protein content after exercise training in the aged vasculature.

**Conclusion**

The results of this study indicate that exercise induces improvement in endothelium-dependent dilation in soleus muscle feed arteries of aged rats. The beneficial effect of exercise training in aged feed arteries was mediated by enhanced NO bioavailability that appears to be the result of increased ecSOD protein content in the aged SFA. The improved NO bioavailability was not associated with increased content or phosphorylation of eNOS or increased SOD-1 protein content. Exogenous SOD treatment of

Table 5. **IC<sub>50</sub>** —log <i>M</i> values and response to 10<sup>-4</sup> <i>M</i> ACh of soleus muscle feed arteries from young and old rats used in the antioxidant studies

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Con</th>
<th>SOD</th>
<th>SOD + CAT</th>
<th>SOD + l-NNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt;, —log &lt;i&gt;M&lt;/i&gt; ACh</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>−6.61±0.20</td>
<td>−7.28±0.26</td>
<td>−6.72±0.20</td>
<td>−8.18±0.62&lt;sup&gt;ac&lt;/sup&gt;</td>
</tr>
<tr>
<td>Old</td>
<td>−7.01±0.18</td>
<td>−7.39±0.14</td>
<td>−7.44±0.35</td>
<td>−7.33±0.46</td>
</tr>
<tr>
<td>Response to 10&lt;sup&gt;-4&lt;/sup&gt; &lt;i&gt;M&lt;/i&gt; ACh, % possible dilation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>72.5±4.7</td>
<td>78.7±5.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>64.9±9.3</td>
<td>52.7±13.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Old</td>
<td>44.1±5.3&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>72.9±6.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>72.8±7.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>27.2±4.5&lt;sup&gt;abc&lt;/sup&gt;</td>
</tr>
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</table>

Values are means ± SE; n = 6–21 rats/group. Significantly different from *Con, <sup>b</sup>SOD, <sup>ac</sup>SOD + CAT, <sup>b</sup>SOD + l-NNA, <sup>c</sup>Young, <i>P</i> ≤ 0.05.
SFA from old Sed rats mimicked the effects of exercise training. Collectively, these results suggest that exercise training reverses the detrimental effects of aging on endothelial function in skeletal muscle feed arteries by enhancing the capacity to scavenge superoxide, increasing the bioavailability of NO. The exercise training-induced improvement in endothelial function in SFA may work in concert with enhanced endothelial function in skeletal muscle arterioles to improve skeletal muscle blood flow and increase exercise tolerance in the elderly.

Fig. 6. Values are means ± SE; n = 9–10 rats/group. Comparison of endothelial nitric oxide synthase (eNOS) (A), Ser1177 phospho-eNOS (p-eNOS; B) protein content, and Ser1177 p-eNOS-to-eNOS ratio (C) in SFA from young and old rats. A and B, insets: representative immunoblots for the target protein (top) and the same blot reprobed for GAPDH (bottom). YS, young sedentary; OS, old sedentary; YX, young exercise trained; OX, old exercise trained. Statistical analysis revealed no significant differences.

Fig. 7. Values are means ± SE; n = 8 rats/group. Comparison of Cu,Zn-dependent superoxide dismutase (SOD-1; A) and extracellular superoxide dismutase (ecSOD) (B) protein content in SFA from young and old rats. A and B, insets: representative blots for the target protein (top) and the same blot reprobed for GAPDH (bottom). *Significantly different from Sed rats, P ≤ 0.05.
REFERENCES


