Exercise training enhances flow-induced vasodilation in skeletal muscle resistance arteries of aged rats: role of PGI₂ and nitric oxide

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Abstract

Exercise training enhances flow-induced vasodilation in skeletal muscle resistance arteries of aged rats: role of PGI₂ and nitric oxide. Am J Physiol Heart Circ Physiol 292: H3119–H3127, 2007. First published March 2, 2007; doi:10.1152/ajpheart.00588.2006.—Flow-induced vasodilation is attenuated with old age in rat skeletal muscle arterioles. The purpose of this study was to determine whether diminished cyclooxygenase (COX) signaling contributes to the age-induced attenuation of flow-induced vasodilation in gastrocnemius muscle arterioles and to determine whether, and through which mechanism(s), exercise training restores this deficit in old rats. Fischer 344 rats (3 and 22 mo old) were assigned to a sedentary or exercise-trained group. First-order arterioles were isolated from the gastrocnemius muscles, cannulated, and pressurized to 70 cmH₂O. Diameter changes were determined in response to graded increases in intraluminal flow in the presence and absence of nitric oxide synthase (NOS) inhibition (10−5 M Nω-nitro-L-arginine methyl ester (L-NAME)), COX inhibition (10−5 M indomethacin), or combination NOS (10−5 M L-NAME) plus COX (10−5 M indomethacin) inhibition. Aging reduced flow-induced vasodilation in gastrocnemius muscle arterioles. Exercise training restored responsiveness to flow in arterioles of aged rats and enhanced flow-induced vasodilation in arterioles from young rats. L-NAME inhibition of flow-induced vasodilation was greater in arterioles from old rats compared with those from young rats and was increased after exercise training in arterioles from both young and old rats. Although the indomethacin-sensitive portion of flow-induced dilation was not altered by age or training, both COX-1 mRNA expression and PGI₂ production increased with training in arterioles from old rats. These data demonstrate that aging-associated differences in flow-induced vasodilation in gastrocnemius muscle 1A arterioles are eliminated by simultaneous inhibition of both nitric oxide synthase (NOS) and cyclooxygenase (COX); however, the effects of aging on COX-1 mRNA expression or PGI₂ production have not been reported in skeletal muscle resistance arteries.

Exercise training ameliorates aging-associated reductions in endothelium-dependent vasodilation (8, 32, 37, 40) in a number of vascular beds. In soleus muscle arterioles from old rats, exercise training restores endothelium-dependent dilation to ACh through an increase in endothelial NOS (eNOS) expression and NO-mediated signaling (32); however, the effects of exercise training on flow-induced dilation of skeletal muscle arterioles of aged rats remain to be determined. Therefore, the purposes of this study were to determine 1) whether the aging-associated reduction in flow-induced vasodilation in 1A arterioles from gastrocnemius muscle is mediated by a reduction in COX signaling and 2) whether exercise training restores flow-induced vasodilation in gastrocnemius muscle 1A arterioles from aged rats.

METHODS

The methods employed in this study were approved by the Texas A&M University Institutional Animal Care and Use Committee and confirmed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Research Council, Washington, DC, revised 1996).
Animals. Fischer 344 male rats [3 mo (n = 66) and 22 mo (n = 57)] were obtained from Harlan (Indianapolis, IN), housed under a 12:12-h light-dark cycle, and given food and water ad libitum. This particular strain was chosen because cardiovascular function decreases with age in these rats, without the development of atherosclerosis or hypertension (17).

Exercise training. All rats were habituated to treadmill exercise, during which each rat walked on a motor-driven treadmill at 5 m/min (0° incline), 5 min/day for 3 days. After habituation, young and old rats were randomly assigned to either a control sedentary (SED) group (young SED, n = 27, and old SED, n = 33) or an exercise-trained (ET) group (young ET, n = 36, and old ET, n = 27). ET rats performed treadmill running at 15 m/min (15° incline), 5 days/wk, for 12 wk. The duration of running was gradually increased in the first 3 wk until a 60-min duration was reached. The rats continued to run 5 days/wk for 60 min/day for the remainder of the 12-wk training period. Vascular responses were determined at least 24 h after the last exercise bout in ET rats.

Microvessel preparation. The rats were anesthetized with pentobarbital sodium (60 mg/kg IP) and euthanized by decapitation. The gastrocnemius-plantaris-solius muscle group was dissected free from both hindlimbs and placed in a cold (4°C), filtered physiological saline solution (PSS) containing 145.0 mM NaCl, 4.7 mM KCl, 2.0 mM CaCl₂, 1.17 mM MgSO₄, 1.2 mM NaH₂PO₄, 5.0 mM glucose, 2.0 mM pyruvate, 0.02 mM EDTA, 3.0 mM MOPS buffer, and 1 g/100 ml BSA, pH 7.4. With the aid of a dissecting microscope (Olympus SVH10), we isolated feed arteries and first-order (1A) arterioles and dissected them free from the superficial portion of the gastrocnemius muscle as previously described (21–23, 32). The arterioles were then transferred to a Lucite chamber containing PSS with 1% albumin (pH 7.4) equilibrated with room air. Each end of the arteriole was cannulated with micropipettes filled with PSS-albumin solution and secured with nylon suture. The sizes and resistances of arterioles were then checked for leaks by introducing a 20-min incubation with one of the following: 1) NOS blocker N²-nitro-L-arginine methyl ester (L-NAME; 10⁻⁵ M (23)), 2) COX blocker indomethacin (10⁻⁵ M), or 3) combination treatment of L-NAME (10⁻⁵ M) plus indomethacin (10⁻⁵ M). In the continuous presence of these blockers, diameter measurements were determined in response to pressure difference of 4, 10, 20, 40, and 60 cmH₂O.

Evaluation of inhibitory effects of N²-nitro-L-arginine methyl ester and indomethacin. In a second series of experiments, the contribution of NO and prostaglandins to flow-induced vasodilation was determined. After assessment of flow-induced vasodilation under control conditions, arterioles underwent a series of washes and were then allowed to develop steady-state tone during a 30-min equilibration period. Arteriolar diameter was recorded immediately before and after a 20-min incubation with one of the following: 1) NOS blocker N²-nitro-L-arginine methyl ester (L-NAME; 10⁻⁵ M (23)), 2) COX blocker indomethacin (10⁻⁵ M), or 3) combination treatment of L-NAME (10⁻⁵ M) plus indomethacin (10⁻⁵ M). In the continuous presence of these blockers, diameter measurements were determined in response to pressure difference of 4, 10, 20, 40, and 60 cmH₂O.

Evaluation of COX-1 expression. Arterioles dissected from gastrocnemius muscle were snap frozen and stored at −80°C in 0.5-ml microcentrifuge tubes. Arterioles were pulverized in lysate buffer, and total RNA was extracted with the RNAqueous filter system (Ambion). Five microliters of total RNA were used to perform quantitative real-time PCR with TaqMan probes designed with the use of Primer Express from the published sequence for rat COX-1 (forward primer: CTA CTC GGG CCC CAA CTG T; reverse primer: TGG GCC GCA GGA AAC T) and a TaqMan oligonucleotide probe (probe: ACT CCT GAG ATC TGG ACC TGG CTT CGT) labeled with a fluorescent reporter dye (VIC) and a quencher dye ( TAMRA). Five microliters of total RNA were also used to simultaneously amplify 18S ribosomal RNA. Reverse transcription and PCR were performed in 50-μl volumes using GenAmp 96-well optical reaction plates. Each reaction well contained the following: 5 μl of total RNA, 25 μl of Universal PCR Master Mix, 1.25 μl (75 U) of Multiscribe reverse transcriptase, 1.0 μl (300 nM) of forward primer, 1.0 μl (300 nM) of reverse primer, 1.0 μl (100 nM) of labeled probe, and 15.75 μl of diethyl pyrocarbonate-treated water. Reactions were performed in duplicate, with all samples contained in the same reaction plate. Reverse transcription was carried out for 30 min at 48°C. PCR was initiated by a 10-min step at 95°C followed by 40 two-step cycles of 15 s at 95°C and then 1 min at 60°C. The fluorescence intensities of the dyes from each probe were measured by the ABI Prism 7700 sequence detection system at every temperature step and cycle during the reaction. The number of cycles required for the fluorescence signal from each tube to reach a fixed threshold is defined as the cycle threshold (Ct). The fluorescence signals for 18S ribosomal RNA served as controls for differences in total RNA loading in the wells. Levels of the target sequence were quantified by calculating the difference between the Ct for the target sequence and coamplified 18S ribosomal RNA (ΔCt). To ensure that the efficiency of the amplification was similar for the target sequence and the 18S ribosomal RNA, a validation reaction was performed with serial dilutions of the same RNA sample. ΔCt values were plotted vs. the log of the RNA concentrations in the serial dilutions. The slope of the line for this plot was <0.03, indicating that the consistency of amplification reaction was similar for 18S and the target sequence, independent of the starting concentration of total RNA.
Evaluation of basal and agonist-stimulated PG12 production. Paired adjacent segments of feed arteries (2–3 mm long each) were cleansed of all connective tissue and fat and were then placed into chilled (4°C), gassed (95% O2-5% CO2) Krebs-Henseleit bicarbonate buffer (KHB) solution; arteries were allowed to rest for at least 30–45 min. The arteries were then transferred into 450-μL polyethylene microcentrifuge tubes with 300 μL KHB, gassed continuously, and gradually warmed up to 37°C. After preincubation for 30 min, the KHB solution was carefully aspirated, and then 300 μL of either KHB alone (basal) or KHB with ACh (10^-6 M) was added to the tissues and incubated for 45 min at 37°C and gassed continuously. After incubation, the KHB was collected and stored in −70°C until RIA of 6-keto-prostaglandin F1α (6-keto-PGF1α).

RIA of 6-keto-PGF1α. Basal and ACh-stimulated PG1 release levels into the incubation medium were measured with a specific RIA for the stable metabolite 6-keto-PGF1α, as reported previously (34). Prostanoid standards (1.95–1,000 pg) or unknown samples were incubated with 6-[3H]keto-PGF1α and with the prostanoid antiserum overnight at 4°C. The charcoal-dextran method was used to separate bound and free fractions of 6-[3H]keto-PGF1α. Bound radioactivity was counted by liquid scintillation spectroscopy. The limit of detection of the RIA is 3.90 pg/tube for 6-keto-PGF1α; the cross-reactivity of the antiserum to other prostanoids is <0.1%, and the intra-assay and interassay coefficients of variation are 5.0% and 7.6%, respectively (34).

Solutions and drugs. Albumin was purchased from USB Chemicals (Cleveland, OH). All other drugs were purchased from Sigma (St. Louis, MO). Stock solutions were prepared with distilled water and frozen. Fresh dilutions of stock solutions were prepared on the day of the experiment. 6-keto-PGF1α for RIA standards was purchased from Cayman Chemical (Ann Arbor, MI). 6-[3H]keto-PGF1α was purchased from Amersham Biosciences. 6-keto-PGF1α antiserum was a gift from Dr. Charles Leffler (Department of Physiology and Biophysics, University of Tennessee, Memphis, TN).

Statistical analysis. To control for variations in vessel size, changes in vessel diameter in response to flow and SNP were expressed as a percentage of maximal vasodilation and calculated as follows:

\[
\text{Relaxation} \% = \frac{(D_s - D_o)/(D_M - D_s)}{100}
\]

where \(D_s\) = steady-state inner diameter recorded after each step increase in flow or dose of SNP, \(D_o\) = initial baseline inner diameter, and \(D_M\) = maximal inner diameter recorded at 60 cmH2O in calcium-free PSS. Flow-diameter and concentration-diameter curves were evaluated by repeated-measures ANOVA to detect differences within (flow rate or dose) and between (experimental groups) factors. Pairwise comparisons between specific levels were made through post hoc analysis (Scheffé’s) when a significant main effect was found. One-way ANOVA was used to determine differences between COX-1 mRNA expression, PG12 release, citrate synthase activities, body weight, muscle weight, spontaneous tone, and maximal diameters. All values are presented as means ± SE. Significance was defined as \(P \leq 0.05\).

RESULTS

Animals. Characteristics of the young and old SED and ET rats are shown in Table 1. Body weight increased significantly with age, whereas exercise training resulted in decreased body weight in both young and old animals. Gastrocnemius muscle weight was reduced with age and with exercise training in the young rats; however, exercise training did not affect gastrocnemius muscle weight in the aged rats. The efficacy of the training protocol was confirmed in that citrate synthase activity in gastrocnemius muscles was higher in both young and old ET rats relative to their SED counterparts.

Characteristics of isolated vessels. Characteristics of isolated arterioles from all groups are listed in Table 2. Consistent with our previous study (32), maximal intraluminal diameters of gastrocnemius muscle arterioles were greater in the old SED than in young SED animals, and exercise training increased maximal diameter of gastrocnemius muscle arterioles in young and old rats.

Initial spontaneous tone developed was not different among groups. Treatment with l-NAME increased tone in arterioles from all groups. In contrast, treatment with indomethacin only increased tone in arterioles from young ET and old SED rats. The combined treatment of l-NAME and indomethacin increased tone in arterioles from all groups.

Vasodilator responses to flow and SNP. Vasodilation to intraluminal flow was diminished in gastrocnemius arterioles from old SED rats (Fig. 1). Exercise training restored flow-induced vasodilation in gastrocnemius muscle arterioles from old rats to a level equivalent to that in young SED rats and enhanced arteriolar responsiveness to flow in gastrocnemius muscle arterioles from young rats (Fig. 1).

Vasodilator responses to the exogenous NO donor SNP were not altered by aging or exercise training in gastrocnemius muscle arterioles (data not shown).

Effect of NOS inhibition. NOS inhibition did not alter flow-induced vasodilation in gastrocnemius muscle arterioles from young SED rats (Fig. 2A) but virtually eliminated flow-induced vasodilation in gastrocnemius muscle arterioles from old SED rats (Fig. 2A), as well as young and old ET rats (Figs. 3A and 4A). Thus it appears that there is a shift to greater dependence on NO with aging and that exercise training increases flow-induced dilation in both young and old rats through enhancement of NO signaling.

Effect of COX inhibition. Indomethacin did not significantly alter vasodilation to flow in gastrocnemius muscle arterioles in any group (Figs. 2B, 3B, and 4B); however, differences in vasodilation to flow between young and old SED rats were no

Table 1. Muscle characteristics of young and old SED and ET rats

<table>
<thead>
<tr>
<th></th>
<th>Young SED</th>
<th>Young ET</th>
<th>Old SED</th>
<th>Old ET</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>27</td>
<td>36</td>
<td>33</td>
<td>27</td>
</tr>
<tr>
<td>Age, mo</td>
<td>5.8±0.2</td>
<td>6.3±0.1</td>
<td>24.6±0.2*</td>
<td>24.8±0.1*</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>375.8</td>
<td>359.4†</td>
<td>401±6*</td>
<td>367±6†</td>
</tr>
<tr>
<td>Gastrocnemius muscle weight, g</td>
<td>1.94±0.03</td>
<td>1.82±0.03†</td>
<td>1.56±0.4*</td>
<td>1.54±0.03*</td>
</tr>
<tr>
<td>Gastrocnemius muscle wt/body wt, mg/g</td>
<td>5.12±0.09</td>
<td>5.09±0.09</td>
<td>3.90±0.08*</td>
<td>4.18±0.08†</td>
</tr>
<tr>
<td>Gastrocnemius muscle citrate synthase activity, μmol·min⁻¹·g wet wt⁻¹</td>
<td>16.2±1.13</td>
<td>20.8±0.85†</td>
<td>14.0±1.24</td>
<td>25.4±1.49†</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significantly different from young sedentary (SED) group (\(P \leq 0.05\)). †Significant difference between respective SED and exercise-trained (ET) groups (\(P \leq 0.05\)).
longer apparent after treatment with indomethacin (Fig. 2B). Differences in flow-induced dilation remained between arterioles from SED and ET groups in both young (Fig. 3B) and old (Fig. 4B) rats after treatment with indomethacin.

Effect of combined NOS and COX inhibition. Combined NOS and COX inhibition reduced flow-induced vasodilation in gastrocnemius muscle arterioles from all groups (Figs. 2C, 3C, and 4C). The combined treatment with L-NAME and indomethacin eliminated all differences between young and old SED and ET groups.

Vasodilator responses to isocarbocyclin. Vasodilator responses to the PGI₂ mimetic isocarbocyclin are shown in Fig. 5. Responses did not differ between groups.

Table 2. Characteristics of first-order arterioles from soleus and the superficial portion of gastrocnemius muscles

<table>
<thead>
<tr>
<th></th>
<th>Young SED</th>
<th>Young ET</th>
<th>Old SED</th>
<th>Old ET</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>31</td>
<td>32</td>
<td>31</td>
<td>27</td>
</tr>
<tr>
<td>Maximal diameter, μm</td>
<td>159±4</td>
<td>201±4†‡</td>
<td>177±4*</td>
<td>191±5†‡</td>
</tr>
<tr>
<td>Spontaneous tone</td>
<td>38±4</td>
<td>35±3</td>
<td>34±2</td>
<td>38±3</td>
</tr>
<tr>
<td>Before L-NAME treatment</td>
<td>36±6</td>
<td>18±6</td>
<td>20±3</td>
<td>18±6</td>
</tr>
<tr>
<td>After L-NAME treatment</td>
<td>51±7‡</td>
<td>43±5‡</td>
<td>41±5‡</td>
<td>49±8‡</td>
</tr>
<tr>
<td>Before indomethacin treatment</td>
<td>37±4</td>
<td>20±6</td>
<td>30±4</td>
<td>28±5</td>
</tr>
<tr>
<td>After indomethacin treatment</td>
<td>46±7</td>
<td>35±4‡</td>
<td>40±5‡</td>
<td>24±1</td>
</tr>
<tr>
<td>Before l-NAME + indomethacin treatment</td>
<td>35±3</td>
<td>23±7</td>
<td>28±7</td>
<td>41±8</td>
</tr>
<tr>
<td>After l-NAME + indomethacin treatment</td>
<td>53±5‡</td>
<td>64±7‡</td>
<td>59±6‡</td>
<td>65±6‡</td>
</tr>
</tbody>
</table>

Values are means ± SE. Maximal diameter was recorded in Ca²⁺-free physiological saline solution with 100 μM sodium nitroprusside. Tone (%) = [(maximal diameter – diameter with tone)/maximal diameter] × 100. *Significantly different from young SED group (P ≤ 0.05). †Significant difference between respective SED and ET groups (P ≤ 0.05). ‡Significant difference from pretreatment level of spontaneous tone (P ≤ 0.05). L-NAME + indomethacin tended to increase tone in gastrocnemius muscle arterioles from old ET animals (P = 0.06).
COX-1 mRNA expression. Aging diminished COX-1 mRNA expression in gastrocnemius muscle 1A arterioles (Fig. 6). Exercise training reduced COX-1 expression in young animals but tended to increase COX-1 expression in arterioles from old animals ($P = 0.11$).

*Fig. 3. Effects of NOS and COX inhibition on flow-response relationships of gastrocnemius muscle arterioles from young SED and young ET rats. A: L-NAME had no effect on young SED rats but completely abolished dilation in young ET rats. B: indomethacin had no effect on either young SED or ET rats. C: combination of L-NAME and indomethacin significantly inhibited responses to flow in both young SED and ET rats and abolished the training-induced differences between young SED and ET rats. Values are means ± SE. *$P ≤ 0.05$."

*Fig. 4. Effects of NOS and COX inhibition on flow-response relationships of gastrocnemius muscle arterioles from old SED and old ET rats. A: L-NAME significantly reduced responses to flow in both old SED and ET rats and abolished the training-induced differences between them. B: indomethacin (INDO) had no effect on either old SED or ET rats. C: combination of L-NAME and indomethacin significantly reduced flow-induced vasodilation in old ET rats and abolished the training-induced differences between old SED and ET rats. Values are means ± SE. *$P ≤ 0.05$.*
Basal and stimulated PGI₂ production. Aging had no effect on either basal or ACh-stimulated PGI₂ production in gastrocnemius muscle feed arteries (Fig. 7). Similarly, exercise training did not alter either basal or ACh-stimulated PGI₂ production in gastrocnemius feed arteries from young animals. In contrast, both basal levels and stimulated PGI₂ production were increased in gastrocnemius muscle feed arteries from old ET rats.

DISCUSSION

The purposes of this study were 1) to determine whether the aging-associated reduction in flow-induced vasodilation in 1A arterioles from gastrocnemius muscle is mediated by impairment of COX signaling and 2) to determine whether, and by what mechanism(s), exercise training restores flow-induced vasodilation in gastrocnemius muscle 1A arterioles from aged rats. Several new findings regarding the effects of age and exercise training on flow-induced vasodilation and endothelium-dependent signaling in skeletal muscle arterioles emerge from this study. First, the reduction of flow-induced vasodilation that occurs in gastrocnemius 1A arterioles of old rats (Fig. 1) is accompanied by a decrease in COX-1 mRNA expression (Fig. 6) and by an increase in NO-mediated signaling (Fig. 2A). Second, exercise training reverses the age-related reduction of flow-induced vasodilation in 1A arterioles from gastrocnemius muscle (Fig. 1), and this improvement of endothelium-dependent vasodilation is associated with an increase in NOS (Fig. 4A) and COX signaling (Fig. 7). In gastrocnemius muscle arterioles of young rats, enhancement of flow-induced dilation appears linked to an increase in NOS signaling (Fig. 3A) with no change in COX signaling mechanisms.

Age and NOS signaling. Several studies have reported old age-associated impairment of endothelium-dependent vasodilation (6, 8, 23, 32, 40). Results of this study confirm our group’s (23) previous finding of impaired flow-induced vasodilation in gastrocnemius muscle arterioles of aged rats. The present results also corroborate our group’s previous observations that, although vasodilation to flow is decreased in gastrocnemius arterioles from old rats, the contribution of NO signaling to flow-induced dilation increases in arterioles from old rats compared with those from young rats. Thus it appears that, although endothelial function declines in gastrocnemius muscle arterioles with age, this impairment is accompanied by increased dependence on NOS signaling.

Age and COX signaling. In contrast to the increase in NOS signaling (Fig. 2A) and the increase in eNOS expression (32) that occurs in gastrocnemius muscle arterioles from aged rats, COX-1 mRNA expression declined with age. In coronary arterioles, Csiszár et al. (1) reported that reduced flow-induced vasodilation in coronary arterioles of old rats was associated
with diminished COX-1 protein. Treatment with indomethacin did not significantly inhibit flow-induced vasodilation in gastrocnemius muscle arterioles from any group, and PGI$_2$ production was not altered by age in gastrocnemius muscle feed arteries; however, differences in flow-induced dilation present between young and old rats were eliminated by treatment with indomethacin, suggesting that age does alter COX signaling mechanisms. It is possible that, although PGI$_2$ release is similar in arterioles from young and old rats, upregulation of prostanooid vasoconstrictors results in overall diminution of flow-induced vasodilation in gastrocnemius muscle arterioles from old rats. Although the contribution of prostanooid vasoconstrictors to endothelium-dependent vasodilation has been reported to decrease with age (31, 43), an age-related increase in prostanooid constrictor activity has also been shown in some vascular beds (13, 15). Similarly, Prostaglandin H Synthase-2 (PGHS-2) protein and PGHS-2-induced constriction increased significantly in mesenteric arteries of middle-aged rats compared with their young counterparts (35). Our present data do not indicate whether age alters production of prostanooid constrictors through a COX pathway; further studies are needed to determine the effects of age on the generation of prostanooid constrictors in skeletal muscle resistance arterioles.

Both NO and reactive oxygen species have been shown to alter the activity of constitutive COX-1 and inducible COX-2 (3, 26). Evidence exists to indicate that NO increases COX-1 activity (28), thereby causing greater production of PGI$_2$. The present data and previous results (23) indicate that NO-mediated signaling increases with age in gastrocnemius muscle 1A arterioles; thus it is conceivable that greater NO production in arterioles from aged rats restores COX-1 activity to the level of young rats, resulting in no change in PGI$_2$ production, despite reduced COX-1 mRNA expression (28). Woodman et al. (42) reported a decrease in superoxide dismutase expression in soleus muscle feed arteries of aged rats. Age-related increases in superoxide could contribute to greater generation of peroxynitrite and lipid peroxidation, potentially increasing PGHS-2 activity (2). In gastrocnemius muscle arterioles from young rats, separate blockade of NOS and COX had no significant effect on vasodilation, yet combination blockade of NOS and COX drastically reduced vasodilation. Other investigators have reported that the effects of NOS and COX blockade are not additive in skeletal muscle arterioles, suggesting that these vasodilator pathways are both redundant and interactive (30, 43). It is possible that, in young rats, blockade of one of these pathways initiated a compensatory response in the other pathway, resulting in no net inhibition of vasodilation when either was applied alone (29). In arterioles from old rats, inhibition of flow-induced dilation by NOS blockade alone was greater than the inhibition produced by simultaneous NOS and COX blockade, and blockade of COX alone did not significantly alter the dilation to flow (Fig. 4). These results raise the possibility that endothelium-dependent responses of skeletal muscle arterioles to flow involve NO, PGI$_2$ and thromboxane A$_2$ and further suggest that, when both NOS and COX pathways are blocked, a compensatory vasodilatory mechanism is activated, perhaps through activation of EDHF. It has been reported in other vascular beds that inhibition of NOS signaling results in upregulation of EDHF-induced vasodilation (12, 24, 25). Together, the present data suggest that the interaction between NOS and COX pathways differs in arterioles from young and old rats; however, direct measures of NO and both vasoconstrictor and vasodilator prostanoids will be necessary to accurately determine how age alters the relationship between NOS and COX signaling in skeletal muscle arterioles.

Despite an age-related decrease in COX-1 mRNA expression in gastrocnemius muscle arterioles, PGI$_2$ release from gastrocnemius feed arteries was not different between young and old SED animals (Fig. 7). However, the present data do not indicate whether age alters the expression of COX-2. It is conceivable that increased expression of COX-2 could compensate for the age-induced decrease in COX-1 expression, resulting in normal PGI$_2$ production. In mesenteric arterioles of middle-aged rats, inhibition of PGHS-1 had no effect on endothelium-dependent dilation, whereas PGHS-2 inhibition significantly enhanced dilation to methacholine (35). The presence of inflammatory mediators and oxidant stress have been shown to increase in vascular tissue with age (41, 44), potentially increasing COX-2 expression in skeletal muscle arterioles from aged rats. The possibility that age stimulates COX-2 expression and activity merits further investigation.

**Exercise training and endothelial function.** Recent studies have indicated that the old age-associated reductions in endothelium-dependent vasodilation are reversed by exercise training. Endothelium-dependent vasodilation of the forearm is greater in aged endurance-trained athletes than in their sedentary counterparts (8, 40). Furthermore, moderate-intensity daily aerobic exercise increased endothelium-dependent vasodilation in previously sedentary aged men to levels of young sedentary men and aged endurance-trained men (8). The results of the present study indicate that exercise training restores flow-induced vasodilation in arterioles isolated from the gastrocnemius muscle of old rats to levels equivalent to young SED rats (Fig. 1) and extends previous findings demonstrating that exercise training improves endothelium-dependent vasodilation in arterioles from highly oxidative soleus muscle (32) as well as low oxidative muscle (present study).

Exercise training has been reported to enhance vasodilator capacity and endothelium-dependent vasodilation in skeletal muscle of young humans (10, 11) and animals (4, 5, 19). Daily exercise augments endothelium-dependent vasodilation to ACh (39), L-arginine (39), and intraluminal flow (14, 36, 38, 39) in gracilis muscle arterioles and to electrical stimulation (18, 19) in spinotrapezius muscle arterioles from young rats. Likewise, longer term exercise resulted in enhanced endothelium-dependent vasodilation to ACh in first- and second-order arterioles from spinotrapezius muscles (19) and to flow in gracilis muscle arterioles (38). The present data indicate that a program of endurance exercise training sufficient to reverse the impairment of endothelial function in gastrocnemius muscle arterioles from old rats also results in significant improvement of endothelium-mediated vasodilation in arterioles from young, healthy rats.

**Exercise training and NOS signaling.** We sought to determine whether increases in NO- and PGI$_2$-mediated signaling mechanisms contribute to the enhancement of flow-induced vasodilation that occurred with exercise training in gastrocnemius muscle arterioles. We found that exercise training restores flow-induced vasodilation in gastrocnemius muscle arterioles primarily through increased NO signaling. Treatment with L-NAME did not affect flow-induced vasodilation in arterioles from young SED rats but completely abolished the
dilation to flow in young ET rats, suggesting that enhancement of the response to flow is mediated exclusively through up-regulation of NO-mediated dilation. This increase in NO-mediated, flow-induced vasodilation in young ET animals in the present study is consistent with our group’s (32) previous findings of increased eNOS mRNA and protein expression in gastrocnemius muscle 1A arterioles from young animals. In old rats, NOS inhibition eliminated the enhancement of flow-induced dilation that occurred with exercise training; in the presence of l-NAME, flow-induced dilation was similar in gastrocnemius muscle arterioles from old SED and old ET rats (Fig. 4A). These data suggest that exercise training increases NOS signaling in skeletal muscle arterioles from both young and old rats.

**Exercise training and COX signaling.** Exercise training also enhanced PGI2 release in gastrocnemius muscle resistance arterioles from old rats (Fig. 7). In contrast, exercise training did not alter PGI2 release in gastrocnemius muscle arterioles from young rats. Furthermore, the present study shows that neither COX-1 mRNA expression in gastrocnemius muscle arterioles nor PGI2 production in gastrocnemius feed arteries is altered in young ET rats (Figs. 6 and 7).

We have previously shown that exercise training increased eNOS mRNA expression but did not alter eNOS protein levels in gastrocnemius muscle arterioles from old rats (32). In contrast, the present results indicate exercise training did not alter COX-1 mRNA expression in gastrocnemius muscle arterioles but enhanced PGI2 release from feed arteries of old rats (Fig. 7). The present data do not indicate the mechanism whereby exercise training increases PGI2 production in the absence of a change in COX-1 mRNA expression (Fig. 6); however, it is possible that exercise training reduces oxidant stress (9, 20, 27) in skeletal muscle arterioles from aged rats, thus enhancing COX-1 activity without altering its expression. NO has been shown to increase activity of COX-1, thereby increasing production of PGI2, regardless of changes in COX-1 mRNA expression (28). Our present data suggest that the relative contribution of NO to flow-mediated dilation is greatest in gastrocnemius muscle arterioles from old SED rats (Fig. 4A). Thus the age-related increase in NOS expression (32) and the exercise training-induced increase in NOS signaling may also contribute to greater PGI2 release in the absence of changes in COX-1 expression. Together, the finding that exercise training increases NO-mediated dilation in gastrocnemius muscle arterioles from young rats, but augments both NO-mediated dilation and PGI2 release in gastrocnemius muscle arterioles from old rats, indicates that, although exercise improves endothelial function in skeletal muscle arterioles from both young and old rats, the mechanisms through which the enhancement of endothelial responses occur are age specific.

In conclusion, the results of this study confirm previous findings (23) that flow-induced vasodilation is reduced with old age in arterioles from low-oxidative, glycolytic muscle (e.g., white portion of gastrocnemius muscle). The present study suggests that old-age-associated reductions in flow-induced vasodilation in gastrocnemius muscle arterioles occur, in part, through a reduction of COX signaling. Exercise training restores flow-induced vasodilation of gastrocnemius muscle arterioles in old rats to the level found in arterioles of young rats and enhances flow-induced vasodilation in young SED rats. Exercise training-induced enhancement of flow-induced dilation in gastrocnemius muscle arterioles of young rats is mediates primarily through increased NOS signaling, whereas exercise training increases flow-induced vasodilation of arterioles from old rats through increases in both NOS and COX signaling.

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