Short-term exercise training increases ACh-induced relaxation and eNOS protein in porcine pulmonary arteries

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Johnson, Lynelle R., James W. E. Rush, James R. Turk, Elmer M. Price, and M. Harold Laughlin. Short-term exercise training increases ACh-induced relaxation and eNOS protein in porcine pulmonary arteries. J Appl Physiol 90: 1102–1110, 2001.—We tested the hypothesis that short-term exercise (STEx) training and the associated increase in pulmonary blood flow during bouts of exercise cause enhanced endothelium-dependent vasorelaxation in porcine pulmonary arteries and increased expression of endothelial cell nitric oxide synthase (eNOS) and superoxide dismutase-1 (SOD-1) protein. Mature, female Yucatan miniature swine exercised 1 h twice daily on a motorized treadmill for 1 wk (STEx group, n = 7); control pigs (Sed, n = 6) were kept in pens. Pulmonary arteries were isolated from the left caudal lung lobe, and vasomotor responses were determined in vitro.

Arterial tissue from the distal portion of this pulmonary artery was processed for immunoblot analysis. Maximal endothelium-dependent (ACh-stimulated) relaxation was greater in STEx (71 ± 5%) than in Sed (44 ± 6%) arteries (P < 0.05), and endothelium-independent (sodium nitroprusside-mediated) responses did not differ. Sensitivity to ACh was not altered by STEx training. Immunoblot analysis indicated a 3.9-fold increase in eNOS protein in pulmonary artery tissue from STEx pigs (P < 0.05) with no change in SOD-1 or glyceraldehyde-3-phosphate dehydrogenase protein levels. We conclude that STEx training enhances ACh-stimulated vasorelaxation in pulmonary arterial tissue and that this adaptation is associated with increased expression of eNOS protein.

pulmonary circulation; nitric oxide synthase; superoxide dismutase; acetylcholine; endothelium

EXERCISE TRAINING IS ASSOCIATED with endothelial adaptations in different circulatory beds of several species (4, 10, 19). Many studies have focused on the effects of exercise on coronary vasomotion because exercise has been demonstrated to reduce the risk of death due to coronary artery disease or stroke (20). Exercise training for 7 days enhanced ACh-induced dilation of canine coronary arteries via increased release of nitric oxide (NO) (19), and ACh-stimulated nitrite production from coronary vessels was increased after 10 days of training (16). In addition, mRNA expression of endothelial cell nitric oxide synthase (eNOS) was increased in aortic endothelial cells of dogs exercised for 10 days (16), indicating an adaptation in gene expression after exercise training. Thus in vivo studies have confirmed enhanced endothelial function in coronary vessels and increased production of endothelium-derived NO following short-term exercise (STEx) training.

Exercise is also beneficial in rehabilitation of patients with chronic obstructive pulmonary disease (COPD), although the physiological mechanisms responsible for reduced symptomatology have not been identified. In COPD patients, exercise-induced improvements in the clinical disease state have been ascribed to alterations in skeletal muscle energetics that result in a reduction in symptoms and clinical signs (7, 8) or to improvements in ventilatory capacity (18) or efficiency (3). Although pulmonary vascular dysfunction may also contribute to clinical signs in patients with interstitial lung disease (5), few experimental studies have analyzed the effects of exercise training on the pulmonary circulation.

In the present study, we tested the hypothesis that STEx would enhance endothelium-dependent dilation and increase eNOS protein content in pulmonary arteries of miniature swine. Enhanced endothelium-dependent relaxation was theorized to represent a beneficial adaptation to exercise. We have previously shown that endothelium-dependent vasorelaxation of pulmonary arterial rings was not altered by 16 wk of exercise training (chronic training) in normal pigs (6). However, other investigations indicate that the length of the training protocol can impact the development of adaptations in vascular function. In the miniature swine model used here, exercise training for 1 wk resulted in enhanced relaxation to bradykinin in brachial artery rings (10), whereas chronic training for 16–20 wk did not (9). Therefore, we tested the hypothesis that STEx training would also be associated with augmented endothelium-dependent relaxation in pulmonary arteries of pigs.

Increased eNOS in response to training has been demonstrated in various species (4, 16, 19), although...
the effect of exercise training on eNOS protein content has not been previously investigated in the pulmonary circulation. We hypothesized that STEX and the associated increase in pulmonary blood flow during exercise bouts would increase eNOS protein in pulmonary arterial tissue. Because the efficacy of NO can be reduced by the presence of superoxide anion (O\textsuperscript{2-}) in the vascular environment, we also hypothesized that STEX would increase superoxide dismutase-1 (SOD-1), thus increasing the availability of NO by decreasing O\textsuperscript{2-} concentrations. Previous work in coronary arteries has demonstrated that increased flow upregulates eNOS and SOD-1 mRNA expression (22). Therefore, in addition to physiological studies, we tested the hypothesis that increased blood flow associated with STEX would increase the relative expression of SOD-1 protein levels in pulmonary artery tissue from STEX pigs relative to sedentary controls (Sed).

METHODS

Exercise training. Mature, female Yucatan miniature swine were divided into Sed and STEX groups. STEX pigs were acclimated to a low-speed motorized treadmill (Quinton); Sed pigs remained in pens. Each day for 7 days, pigs in the STEX group ran two bouts of exercise on the treadmill, each consisting of 1 h at 3.5 mph, 0% grade. The second bout of exercise occurred at least 4 h after completion of the first bout. Routine biochemical profiles and complete blood counts were performed on each group of pigs to investigate potential differences in systemic health.

The efficacy of training was assessed by comparing heart-to-body weight ratios and skeletal muscle oxidative capacity of Sed and STEX pigs (4, 9, 14). Muscle samples were taken from the triceps brachii and deltoid, frozen in liquid nitrogen, and stored at −70°C until processed for spectrophotometric determination of citrate synthase activity (17).

Isolation and preparation of pulmonary arteries. Pigs were sedated with ketamine (25 mg/kg im) and xylazine (2 mg/kg im), anesthetized with pentobarbital sodium (30 mg/kg iv), and administered heparin (2,000 U/kg iv). The heart was removed by transection of the great vessels, and lungs were immediately placed in ice-cold Krebs solution containing (in mM) 131.5 NaCl, 5.0 KCl, 1.2 MgCl\textsubscript{2}, 11.2 glucose, and 20.8 NaHCO\textsubscript{3}, for vessel isolation.

Pulmonary arteries (2- to 3-mm outer diameter) were located by their position medial to the bronchi. Lobar pulmonary arteries to the left caudal lung lobe were identified, and the first ventrally oriented artery was carefully isolated. Arteries were washed until they returned to resting tension; and then were precontracted with the EC\textsubscript{50} of NE (5.75 × 10\textsuperscript{-7} M) and stabilized for 20 min. Preliminary experiments indicated that NE resulted in stable contraction for >150 min in these porcine pulmonary arteries. The presence of endothelium was confirmed by observing relaxation to a single dose of bradykinin (10\textsuperscript{-6} M) after NE precontraction. Arterial rings that did not exhibit relaxation to bradykinin were discarded.

Arterial rings were washed until they returned to resting tension; 5.75 × 10\textsuperscript{-7} M NE was then added to vessel baths. Endothelium-dependent relaxation was determined through cumulative addition of Ach stock solutions to the vessel bath (final concentration of Ach: 10\textsuperscript{-10} to 10\textsuperscript{-4} M, in half-log doses). Percent relaxation for each dose of Ach was determined as percent reduction in NE-induced tension. Arterial rings were washed until they returned to resting tension; 5.75 × 10\textsuperscript{-7} M NE was then added to each vessel bath. Tension was stabilized for 20 min, and relaxation to sodium nitroprusside (SNP: bath concentration of SNP: 10\textsuperscript{-10} to 10\textsuperscript{-4} M, in half-log doses) was determined to assess endothelium-dependent relaxation. Maximal relaxation was identified through incubation in modified Krebs solution containing no calcium and 2 mM EGTA.

**Immunoblot analysis.** Pulmonary arterial tissue distal to the rings used in physiology studies was processed for Western blotting. Preliminary studies indicated that these two adjacent segments of pulmonary artery did not express different amounts of eNOS protein (data not shown). Pulmonary artery tissue (50–100 mg) that had been cleaned of adventitia was placed in 0.5 ml of ice-cold Tris-buffered extraction buffer containing 50 mM Tris, 0.1 mM EDTA, 0.1 mM EGTA, 0.1% β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 1 mM pepstatin A, 2 mM leupeptin, 20 mM CHAPS, and 10% vol/vol glycerol at pH 7.4. Tissue was homogenized while on ice and then ultracentrifuged at 100,000 g for 30 min. Protein content of the supernatant was determined with the Bradford protein assay using bovine serum albumin as the standard (2); the supernatant was maintained at −70°C until analyzed by Western blotting.

For Western blotting, 10 μg of protein per sample were aliquoted and diluted 2–5 parts (vol/vol) in Laemml buffer, containing 2% SDS, 6 M urea, 62.5 M Tris, and 160 mM dithiothreitol, pH 6.8. Preliminary studies indicated that loading 10 μg of protein produced bands for eNOS or SOD-1 in the linear portion of the reference curve for analysis. Samples were boiled for 5 min and then loaded in individual
lanes of 4–20% gradient SDS-polyacrylamide gels (Bio-Rad Laboratories, Hercules, CA). Equal numbers of Sed and STEx samples were loaded on each gel. A broad-range protein marker (New England Bio-Labs, Beverly, MA) was run on each gel as a molecular weight standard. For membranes analyzed for eNOS, an aliquot of bovine aortic endothelial cells was loaded in one lane as a positive control.

SDS-PAGE was performed in buffer containing 25 mM Tris base, 100 mM glycine, and 1% (3.5 mM) SDS (200 V, 45–50 min). Proteins were electrophoretically transferred to polyvinylidene difluoride membrane (100 V for 1 h) in Tris-glycine transfer buffer. Membranes were blocked in 5% nonfat dried milk in Tris-buffered saline with 0.1% Tween (TBS-Tween) for 4 h. Separate membranes were incubated on a shaker platform overnight at room temperature with 1:2,000 monoclonal mouse anti-human eNOS antibody (Transduction Laboratories N30020, San Diego, CA), 1:2,500 polyclonal anti-SOD-1 antibody (Stressgen Biotechnology SOD-100E, Colleageville, PA), or 1:5,000 anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Chemicon International, Temecula, CA); all antibodies were in 5% nonfat dried milk-TBS-Tween. After exposure to primary antibody, the membrane was washed six times for 10 min in TBS-Tween and then incubated for 1 h at room temperature on a shaker platform with 1:3,000 dilution of appropriate secondary antibody complexed to horseradish peroxidase (Sigma Chemical, St. Louis, MO). The membrane was washed six times for 10 min in TBS-Tween, and protein bands were visualized using enhanced chemiluminescence (ECL Western blotting detection, Amersham Pharmacia Biotech, Piscataway, NJ) and ECL hyperfilm (Amersham Pharmacia Biotech). Bands for eNOS, SOD-1, and GAPDH were quantitated using National Institutes of Health (NIH) Image analysis software.

**Immunohistochemistry.** To confirm the localization of eNOS to the endothelium, some pulmonary arterial rings were fixed in zinc paraformaldehyde (Anatech LTD, Battle Creek, MI) for 24 h and imbedded in paraffin blocks. For processing, blocks were deparaffinized; 5-μm sections were then cut with an automated microtome, and sections were floated onto positively charged slides (Fisher, St. Louis, MO). Slides were steamed in citrate buffer at pH 6.0 (Dako target retrieval solution S1699, Carpintiera, CA) for 20 min to achieve antigen retrieval and then cooled for 20 min. The Dako auto stainer (Dako DC3400) was used for immunohistochemical staining. Sequential Tris buffer and water wash steps were performed after each protocol in the automated staining process. Sections were incubated in avidin biotin two-step blocking solution (Dako X590) to inhibit background staining and in 3% hydrogen peroxide to inhibit endogenous peroxidase. Nonserum protein block (Dako X909) was applied to prohibit nonspecific protein binding, and slides were incubated in primary mouse monoclonal IgG1 anti-eNOS antibody (Transduction Laboratories N30020) at 1:800 dilution for 60 min. After the appropriate washing steps were completed, slides were incubated in biotinylated anti-mouse link secondary antibody in PBS containing 15 mM sodium azide and peroxidase-labeled streptavidin (Dako LSAB+ kit, peroxidase, K0690). Diaminobenzidine (Dako) applied for 5 min allowed visualization of eNOS antibody staining. Slides were counterstained in Mayer’s hematoxylin stain for 1 min, dehydrated, and coverslipped.

Histological sections of brachial artery were used as positive controls for eNOS staining of the endothelium. For negative controls, histological sections of lung tissue were prepared as described above, but incubation in primary antibody was excluded from the protocol.

**Data analysis.** Data are presented as means ± SE. Contractile tension was calculated by subtracting resting tension from tension measured after each addition of drug. A concentration-response curve was constructed by plotting developed tension against log NE concentration, and the concentration that resulted in half-maximal contraction was defined as the EC_{50}. This value was interpolated from regression on points taken from the linear portion of the log concentration-response curve and was expressed as a percentage of the maximal response (using Basica IC_{50}). Relaxation was expressed as percent reduction from precontracted tension induced by 5.75 × 10^{-7} M NE (EC_{50}) at each dose added. IC_{50} was defined as the drug concentration resulting in half-maximal relaxation. This value was determined by interpolation on each log concentration-response curve by using the same computer regression program (Basica IC_{50}).

Maximal responses and IC_{50} concentrations were compared between Sed and STEx groups by using the Student’s t-test. Cumulative concentration-response curves for ACh and SNP were analyzed using repeated measures analysis of variance (SuperANOVA, Abacus Concepts). Comparisons between Sed and STEx were made for ACh and SNP concentration-response curves. When indicated by a significant F test, planned contrasts were performed to detect differences between individual means.

Densitometry was performed using NIH Image analysis software. Samples for Western blot analysis were run in triplicate, and an average densitometry measurement was determined for each sample. Twenty-one gels were analyzed to assess short-term training effects on enzyme levels. Samples that failed to show immunoreactivity to eNOS (2 STEx pigs and 1 Sed pig) or GAPDH (1 STEx pig) were not included in the analysis. Optical density units were averaged across all samples in each group, and mean values were compared between Sed and STEx groups by using the Student’s t-test. A STEx-to-Sed ratio was then constructed for each protein by using absolute densitometry measurements. For all analyses, significance was set at P < 0.05.

**RESULTS**

**Indexes of exercise training.** STEx pigs had a significantly greater heart-to-body weight ratio (4.84 ± 0.14 mg/kg) than did Sed (4.24 ± 0.51 mg/kg) (P < 0.05). Body weight did not differ between Sed (37.4 ± 1.4 kg) and STEx (35.8 ± 8.1 kg) pigs (P = 0.64). In pigs that had undergone exercise training, there was a trend toward increased citrate synthase activity in the lateral and long heads of the triceps (P = 0.06–0.08) (Table 1).

**Characteristics of pulmonary arteries.** Arterial rings from Sed and STEx pigs did not differ in physical dimensions or percent stretch required to reach L_{max}. Outer diameter of pulmonary arterial rings was 2.54 ± 0.07 mm in Sed pigs and 2.51 ± 0.07 mm in STEx pigs.
Table 1. Citrate synthase activity in Sed and STEx pigs

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Citrate Synthase Activity, μmol min⁻¹ g⁻¹</th>
<th>P Value</th>
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<tbody>
<tr>
<td>Long head of the triceps</td>
<td>11.54 ± 1.00</td>
<td>0.08</td>
</tr>
<tr>
<td>Lateral head of the triceps</td>
<td>11.17 ± 1.00</td>
<td>0.06</td>
</tr>
<tr>
<td>Deltoid</td>
<td>14.95 ± 1.26</td>
<td>0.23</td>
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Values are means ± SE; n = 8 each for the sedentary group (Sed) and the short-term exercise group (STEx).

Passive tension necessary to attain $L_{max}$ also did not differ between arteries of Sed and STEx pigs.

Contraction and relaxation responses. Response to 80 mM KCl did not differ in arteries from Sed (3.85 ± 0.50 g) and STEx (3.40 ± 0.42 g) pigs (n = 7, P = 0.50). The concentration-response relationship to NE did not differ between arteries from Sed and STEx pigs (P = 0.32), and contractile tension to EC₅₀ concentration of NE (5.75 × 10⁻⁷ M) was similar in pulmonary arteries from Sed (2.11 ± 0.28 g) and STEx (1.55 ± 0.29 g) pigs (n = 7, P = 0.19).

Acetylcholine resulted in a dose-dependent relaxation from NE-induced precontraction in arteries from both Sed and STEx pigs. Pulmonary arteries from STEx pigs demonstrated significantly greater relaxation at each concentration ≥10⁻⁷ M ACh than arteries from Sed pigs (P < 0.05) (Fig. 1), but sensitivity to ACh did not differ between groups, as indicated by similar IC₅₀ concentrations for Sed (7.23 ± 0.65 × 10⁻⁸ M) and STEx (8.41 ± 1.8 × 10⁻⁸ M) arteries (P = 0.58). Previous experiments on arteries denuded of endothelium confirmed that the response to ACh was endothelium dependent (6).

Relaxation to SNP was equivalent in arteries from Sed and STEx pigs (Fig. 2). Sensitivity to SNP also did not differ between Sed (IC₅₀ = 8.16 ± 3.01 × 10⁻⁸ M) and STEx (IC₅₀ = 15.00 ± 5.95 × 10⁻⁸ M) arteries (P = 0.33).

Immunoblot analysis of short-term training effects. Immunoblot analysis for eNOS protein content was performed on samples from six Sed and six STEx pigs, and immunoreactive eNOS was detected in tissue from five Sed and four STEx pigs. Densitometry readings were similar for samples run in triplicate. Figure 3A displays protein expression for eNOS in pulmonary arterial tissue from STEx samples. Densitometry readings were similar for samples run in triplicate. Figure 3A illustrates a 3.9-fold increase in eNOS protein in samples obtained from STEx pigs compared with Sed controls (P = 0.01).

Immunoblot analysis was performed on samples from six Sed and seven STEx pigs, and immunoreactivity to SOD-1 was detected in five Sed and seven STEx samples. Densitometry readings were similar for samples run in triplicate. Immunoblot detection of SOD-1 in Sed (lanes 2–5) and STEx (lanes 6–9) samples is illustrated in Fig. 4A. Although a 58% increase in mean SOD-1 protein content was detected in pulmonary artery tissue from STEx pigs, response was variable among pigs (see lanes 6–9), and the combined results showed no statistically significant difference between Sed and STEx (P = 0.64) (Fig. 4, B and C).

GAPDH expression was assessed in pulmonary arterial samples from all pigs; adequate immunoreactivity was not detected in one STEx sample, and this
sample was excluded from all analyses. Densitometry values for GAPDH bands were similar for Sed and STEx samples ($P = 0.72$) (10 mg total protein load), confirming equal loading conditions on each gel and lack of an effect of exercise training on GAPDH expression in pulmonary artery tissue (Fig. 5).

**Immunohistochemistry.** Immunohistochemistry revealed that eNOS staining was confined to the endothelium in the pulmonary arteries of Yucatan swine (Fig. 6). Nonspecific staining was absent, and eNOS reactivity was absent from other cellular layers of these arteries. In the absence of incubation with pri-
mary antibody to eNOS, no immunoreactivity was detected.

**DISCUSSION**

We have previously reported that chronic exercise training (16 wk) does not alter vasorelaxation in pulmonary arteries of normal pigs (6). The primary purpose of this study was to test the hypothesis that STEx training results in enhanced endothelium-dependent vasorelaxation and increased expression of eNOS and SOD-1 protein in pulmonary arteries. Our hypothesis was supported by the finding that ACh-induced relaxation is enhanced and eNOS protein levels are increased 3.9-fold in pulmonary arterial tissue of pigs that have completed a short-term training protocol. Although levels of SOD-1 protein are 58% higher in pulmonary arterial tissue from STEx pigs relative to Sed controls, the difference is not statistically significant. GAPDH protein content is also unaltered by exercise, indicating that exercise training has a selective effect on eNOS protein production in these pulmonary arteries.

STEx training did not alter contractile responses to activation of voltage-gated calcium channels. This contrasts with findings in chronically trained pigs, in which increased contraction to 80 mM KCl was noted in pulmonary arteries of exercised pigs compared with Sed controls (6). In addition, contraction to EC50 levels of NE and relaxation to SNP in pulmonary arteries from Sed and STEx pigs were similar, indicating that STEx training had no effect on measured vascular smooth muscle functional responses within the pulmonary circulation. The sole difference in vascular reactivity of pulmonary artery rings between Sed and STEx pigs was enhanced endothelium-dependent relaxation in pigs that completed STEx training.

STEx training produces similar functional adaptations in the coronary circulation of the dog, in which 7–10 days of exercise training resulted in enhanced epicardial coronary artery dilation associated with greater release of NO (19). Sessa et al. (16) also reported increased eNOS gene expression in the aorta of
dogs trained for 10 days, indicating that STEx training is associated with endothelial adaptations in conduit-sized arteries. Our experiments provide support for enhanced endothelium-dependent pulmonary arterial vasorelaxation and increased eNOS protein content following short-term training in the pulmonary arteries examined here.

Previous work by other investigators has implicated a role for altered expression of eNOS in the time dependence of training-induced improvements in endothelium-dependent vasorelaxation. Delp and Laughlin (4) studied the time course of exercise-induced adaptations in aortic tissue of the rat and found enhanced ACh-induced relaxation after 4 wk of endurance training. Aortic tissue from exercise-trained rats displayed increased eNOS protein levels coincident with improved relaxation. In our model of exercise training, exercise-induced adaptations in endothelium-dependent relaxation of pulmonary arteries appear to mimic the response reported in brachial arteries, in which relaxation was increased with the same short-term training program used in the present study (10) but was unchanged after 16 wk of exercise training (9). The different time course of endothelial adaptations in rats and pigs may be related to differences between species or vascular beds in response to exercise training.

Increased expression of eNOS protein has been illustrated in cultured endothelial cells exposed to increased shear stress (13), and increased blood flow associated with exercise is thought to impact endothelial cell function by exerting shear stress on the endothelial lining of the vessel. Pulmonary blood flow increases five- to sixfold in the exercising pig (1) and likely increases shear stress within pulmonary arteries; however, shear stress has not been measured directly in the pulmonary arteries studied here. Interestingly, short-term training augments endothelial cell production of eNOS protein and endothelium-dependent relaxation in pulmonary arteries, whereas this adaptation is not present following chronic exercise training (6). If shear stress is the stimulus for the adaptation noted, our results suggest an alteration in the stimulus-response relationship as training becomes long-term. Potential mechanisms that might alter the response to blood flow and/or shear stress would include structural remodeling of the vasculature, a decrease in the shear stress gradient sensed by endothelial cells, or decreased response to the shear stress stimulus. In the coronary circulation, conduit arteries from pigs used in this model fail to demonstrate enhanced endothelium-dependent relaxation following chronic exercise training (14); however, coronary arterioles display augmented relaxation mediated by NO (11). These resistance arterioles also have increased eNOS mRNA (21) and SOD-1 protein and mRNA content (15). In the coronary circulation, it is possible that long-term training enhances vasorelaxation and endothelium-dependent function in the microcirculation, leading to a reduction in the shear stress signal for endothelial adaptation in conduit-sized arteries. This potential mechanism has not been investigated in the pulmonary circulation.

In a previous study of the effects of chronic exercise (16 wk) training, pulmonary arterial rings from sedentary and chronically trained pigs displayed equal endothelium-dependent relaxation and relied equally on NO for ACh-mediated relaxation (6). To determine whether chronic training altered eNOS protein content, we performed Western blot analysis on pulmonary arterial tissue taken from seven sedentary and seven chronically trained pigs from that study and evaluated expression of eNOS, SOD-1, and GAPDH protein levels utilizing similar methods to those described here. No significant differences in protein expression of SOD-1, GAPDH, or eNOS were detected in conduit pulmonary arterial tissue of Sed pigs and pigs that had completed 16 wk of exercise training (Fig. 7), 39 supporting a time-dependent alteration in the effects of exercise training on levels of eNOS protein that correlates with endothelium-dependent changes in vasoreactivity. Thus both eNOS protein levels and ACh-mediated relaxation are increased in pulmonary arteries of pigs that have completed short-term exercise but are not different from Sed controls following chronic exercise training. Further investigation into the mechanisms responsible for an alteration in this adaptation with chronic training will provide insight into the impact of exercise training in the pulmonary circulation.

Increased eNOS protein following STEx training could reflect increased protein production and/or decreased protein breakdown. Short-term training could augment gene expression of eNOS, or the half-life of mRNA for eNOS may be prolonged by STEx training. Evidence in the literature supports upregulation of eNOS gene expression coincident with increased eNOS protein content in response to chronic increases in blood flow (12) and increased shear stress (13). Woodward et al. (22) reported upregulation of eNOS mRNA...
following 2 and 4 h of high-flow conditions in perfused coronary arterioles isolated from Sed pigs. Gene expression for eNOS and SOD-1 has not currently been investigated in the pulmonary circulation of trained swine.

Pigs in this study completed a strenuous exercise protocol designed to provide maximal stimulus for exercise-induced effects on the vasculature within a short period of time. Heart-to-body weight ratio increased by 14% in STEx pigs in the absence of a change in body weight, indicating a degree of myocardial hypertrophy following STEx training similar to that seen after chronic training (9, 15, 21). However, citrate synthase activity showed only a trend toward increased activity in certain muscles, indicating that the stable adaptation of skeletal muscle to training that occurs with more protracted training protocols in this pig model (6, 21) had not been achieved. With the use of available methods, conduit-sized pulmonary arteries of STEx pigs displayed no gross changes in physical characteristics or in passive properties compared with Sed controls, suggesting that structural remodeling had not occurred in these vessels.

The level of exercise performed in these studies was carefully controlled, and application of these results to the response to exercise in patients with cardiopulmonary disease is difficult to predict. The exercise capacity achieved by individuals with heart or lung disease depends on multiple factors, and, just as exercise limitation results from a number of variables including vascular dysfunction, the effects of exercise on health status may be impacted by numerous factors including improvements in endothelial function. STEx training improves endothelial function in the pulmonary circulation, and this represents a previously unrecognized effect of exercise on cardiopulmonary function.

In conclusion, our data indicate that STEx training (1 h twice daily for 1 wk) results in increased expression of eNOS protein and enhanced ACh-mediated relaxation in the pulmonary arteries studied here. This adaptation was endothelium dependent because relaxation to SNP, a direct donor of NO to vascular smooth muscle cells, did not differ between arteries from Sed and STEx pigs. These studies complement previous findings in the pulmonary circulation of normal pigs, which demonstrate the lack of an adaptation after chronic (16 wk) exercise training (6). Together, these studies suggest that the length of training impacts the development of adaptations within the pulmonary circulation. After short-term training, eNOS protein is increased and endothelium-dependent relaxation is enhanced. With chronic training, eNOS protein is no longer increased and a functional endothelial adaptation is no longer present in these conduit-type pulmonary arteries (6), perhaps due to ultrastructural adaptations in the vasculature, adaptations in other pulmonary arteries, reduced response to shear stress, or a decrease in the gradient of shear stress encountered. Further studies are required to analyze mechanisms contributing to the time course of training effects in the pulmonary circulation and to examine induction of training-related adaptations throughout different sites within the pulmonary vasculature.

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REFERENCES

