Chronic exercise training improves ACh-induced vasorelaxation in pulmonary arteries of pigs

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Chronic exercise training improves ACh-induced vasorelaxation in pulmonary arteries of pigs. J. Appl. Physiol. 88: 443–451, 2000.—We hypothesized that exercise training would lead to enhanced endothelium-dependent vasodilation in porcine pulmonary arteries. Pulmonary artery rings (2- to 3-mm OD) were obtained from female Yucatan miniature swine with surgically induced coronary artery occlusion (ameroid occl.1) or exercise training was performed for 16 wk, and vascular responses were studied using standard isometric techniques. Contractile responses to 80 mM KCl, isosomotic KCl (10–100 mM), and norepinephrine (10–8 to 10–4 M) did not differ between sedentary (Sed) and exercise-trained (Ex) pigs. Relaxation was assessed to endothelium-dependent and endothelium-independent vasodilators after norepinephrine contraction. Pulmonary arteries of Ex pigs exhibited greater maximal relaxation to ACh (61.9 ± 3.5%) than did those of Sed pigs (52.3 ± 3.9%; P < 0.05). Endothelium-independent relaxation to sodium nitroprusside did not differ. Inhibition of nitric oxide synthase significantly decreased acetylcholine-induced relaxation, with greater inhibition in arteries from Ex pigs (P < 0.05). Inhibition of cyclooxygenase enhanced relaxation to acetylcholine in arteries from Sed pigs. We conclude that exercise training enhances endothelium-dependent (ACh-mediated) vasorelaxation in pulmonary arteries by mechanisms of increased reliance on nitric oxide and reduced production of a prostanooid constrictor.

The effects of chronic exercise training on the peripheral circulation have been extensively evaluated in a variety of vascular beds in different species. Considerable evidence documents vascular endothelium as an important site of functional adaptation to exercise training in both coronary (16, 18, 22, 28) and peripheral circulations (4–6, 17). For example, Delp et al. (6) showed that the abdominal aorta of exercise-trained rats demonstrated greater ACh-induced maximal relaxation than did sedentary controls. Relaxation to endothelium-independent agonists [sodium nitroprusside (SNP) and forskolin] did not differ between sedentary and exercise-trained rats, suggesting that endothelial cell adaptations were responsible for the training effects observed. In many studies, exercise training has been shown to result in specific endothelium-mediated adaptations, and there is evidence that increased endothelial cell nitric oxide synthase (NOS) contributes to these adaptations (18, 28, 30). Chronic increases in blood flow augment NOS gene expression, perhaps signaled by increased shear stress (19, 24).

Pulmonary blood flow increases during exercise, and the role of endothelium-derived mediators in the acute reduction of pulmonary vascular resistance during bouts of exercise has been investigated (14, 21). However, fewer reports exist on pulmonary vascular adaptations to chronic exercise training (4, 13). Chen and Li (4) demonstrated enhanced ACh-induced relaxation in pulmonary arterial rings from exercise-trained rabbits, suggesting that exercise training resulted in increased release of nitric oxide in response to ACh.

The purpose of our study was to assess functional adaptations in endothelium-mediated responses of pulmonary arteries from chronically exercise-trained pigs. Experiments were conducted on arteries isolated from pigs that had chronic occlusion of the left circumflex coronary artery. The coronary arteries of these pigs were used for other research projects (11).

METHODS

Animal Instrumentation for Coronary Occlusion

Female, adult Yucatan miniature swine weighing 25–45 kg were sedated with ketamine (20 mg/kg im), midazolam (0.5 mg/kg im), and glycopyrrolate (0.004 mg/kg im). Pigs were intubated and maintained on isoflurane anesthesia. The animals were prepared for aseptic surgery, a left lateral thoracotomy was performed, and the pericardium was incised. The left circumflex coronary artery was bluntly dissected free of surrounding connective tissue, and the artery was encircled with an ameroid occluder (2.5- to 3.5-mm ID) to provide gradual obstruction of the artery. Buprenorphine (0.01 mg/kg iv) was administered intraoperatively for pain control. A standard thoracotomy closure was performed in layers. In the immediate postoperative period, intrapleural bupivacaine was instilled for pain relief. Penicillin (30,000 U im) was administered perioperatively, and trimethoprim sulfa (480 mg/50 lb per os) was given for 5 days after surgery. Surgical procedures described were in compliance with the Position of the American Heart Association on Research Animal Use and were approved by the Animal Care and Use Committee of the University of Missouri.
Exercise Training

Eight weeks after surgery, pigs were divided into sedentary (Sed) and exercise-trained (Ex) groups. Ex pigs were acclimated to a low-speed motorized treadmill (Quinton) to initiate an exercise training protocol of progressive intensity adapted from a treadmill training program formulated by Tipton et al. (27) and used extensively by our laboratory (5, 6, 11, 16, 22, 27, 28, 30). During the first week of training, pigs ran on the treadmill at 3 miles/h (mph) and 0% grade for 20–30 min followed by a 15-min sprint at 5 mph. Length of training and treadmill speed were increased over time in accordance with the tolerance of individual pigs. From the fourth week of training, the exercise protocol consisted of an 85-min workout with a 5-min warm-up at 2.5 mph, a 15-min sprint at 5–8 mph, a 60-min endurance run at 4–5 mph, and a 5-min cooldown run at 2 mph. Sedentary pigs remained in pens during the same time period.

The efficacy of training was assessed by comparing heart-to-body weight ratios and skeletal muscle oxidative capacity of Sed and Ex pigs (6, 16–18, 22). Muscle samples were taken from the triceps brachii, frozen in liquid nitrogen, and stored at −70°C until processed for citrate synthase activity as described by Srere (26). Increased citrate synthase activity, as measured by spectrophotometry, was considered indicative of successful exercise training.

Isolation and Preparation of Pulmonary Arteries

At the end of training period, a Sed or Ex pig was sedated with ketamine (35 mg/kg im) and xylazine (2 mg/kg im), anesthetized with pentobarbital (30 mg/kg iv), and administered heparin (2,000 U/kg iv). The heart was removed via a left lateral thoracotomy by transection of the pulmonary artery and ascending aorta. Lungs were immediately placed in ice-cold Krebs solution containing (in mM) 131.5 NaCl, 5.0 glucose, and 20.8 NaHCO₃ for vessel isolation.

Pulmonary arteries (2- to 3-mm OD) were located by their position medial to the bronchi. Lobar pulmonary arteries to the right caudal lung lobe were identified, and the first ventral branch from this artery was carefully dissected from associated bronchial and adventitial tissue. This careful isolation procedure ensured that precise, reproducible, and essentially identical artery samples within the pulmonary circulation were studied on successive days. Vessels were dissected in ice-cold Krebs solution, by using minimal manipulation to clean adventitia from the vessel surface. Adjacent segments of pulmonary artery were cut into four ring segments, each 2–3 mm in length. Inner and outer diameters and axial length were recorded in millimeters for each vascular ring, and these measurements were used to calculate vessel area.

Pulmonary artery rings were mounted on two stainless steel wires in individual 20-ml baths containing Krebs solution equilibrated at 37°C and continuously bubbled with 95% O₂:5% CO₂. One wire was connected to a force transducer (ETH-200/400 series, CB Science, Dover, NH) for measurement of tension. The other wire was attached to a micrometer microdrive, allowing stretch of the vessel by known increments. Tension measurements were performed as previously described by McAllister et al. (17). Isometric tension (in g) was continuously recorded on a polygraph connected to a computer acquisition system (MacLab). Arterial rings were passively stretched to a resting tension of 1.0 g for 1 h. Individual length-tension curves were generated for each vessel ring through repeated exposure to 30 mM KCl. Arterial rings were progressively stretched by 10% of the outer diameter for successive tension determinations. The optimal circumferential length for a given vessel (Lₘₐₓ) was defined as the length at which the contractile force evoked by KCl failed to increase by >10% of the previous measurement. Arterial rings were stabilized at Lₘₐₓ for 30 min before experimentation.

Contractile Responses

Maximum contraction to 80 mM KCl was determined in all arterial rings. Contractile responses to activation of voltage-gated calcium channels and receptor-mediated contractions were assessed in some pigs. A concentration-response relationship to KCl was generated by using osmotically balanced solutions containing increasing concentrations of KCl (10–100 mM) as replacement for NaCl. KCl solutions were made fresh weekly in modified Krebs buffer. Response to contractile agonists was expressed as the developed change in tension (g) from baseline resting tension. A concentration-response curve was constructed by plotting developed tension against millimolar K⁺ concentration, and the concentration at which half-maximal contraction occurred (EC₅₀) was determined for Sed and Ex pigs. In some pigs, receptor-mediated contraction was measured by cumulative addition of norepinephrine (NE; 10⁻⁶ to 10⁻⁴ M) to the vessel bath. Krebs solution contained propranolol (3 × 10⁻⁶ M) to oppose β₂-adrenergic-receptor-mediated vasorelaxation. For NE, the EC₅₀ was determined from the semilog concentration-response curve. Vessels were rinsed to stable resting tension levels between interventions.

Relaxation Responses

After contractile responses, vessels were precontracted with the predetermined EC₅₀ of NE (5.75 × 10⁻⁷ M) and stabilized for 20 min. Preliminary experiments indicated that NE resulted in stable contraction for 150 min in porcine pulmonary arteries. The presence of endothelium was confirmed by observing >60% relaxation to bradykinin (10⁻⁶ M). Vessels were rinsed, restabilized at baseline resting tension, precontracted with 5.75 × 10⁻⁷ M NE, and endothelium-dependent relaxation was assessed through cumulative addition of half-log doses of ACH (10⁻¹⁰ to 10⁻⁴ M). Less than 0.5 ml total volume was added to each 20-ml bath. Relaxation of each arterial ring was determined by measuring reduction in tension in response to each cumulative addition of ACh and was expressed as percent relaxation from tension developed after precontraction with NE. A concentration-response curve was constructed by plotting percent relaxation at increasing ACh concentrations (log M concentration). The concentration at which half-maximal relaxation occurred (IC₅₀) was determined from the semilog concentration-response curve. Vessels were restabilized at resting tension, precontracted with 5.75 × 10⁻⁷ M NE, and endothelium-independent relaxation to half-log doses of SNP (10⁻¹⁰ to 10⁻⁴ M) was determined in similar fashion. At the end of experimentation, vessels were incubated in zero-calcium Krebs solution containing EGTA (2 mM) for 30–60 min to determine maximal relaxation.

Role of Endothelium-Derived Mediators

To determine the role of endothelium-derived mediators in relaxation, arterial rings were placed in one of four treatment groups: 1) control vessels, 2) rings denuded of endothelium, 3) rings treated with indomethacin (10⁻⁵ M) to block cyclooxygenase activity, and 4) rings treated with 300 μM L-NAME for inhibition of NOS. Each arterial ring received only one treatment throughout the experiment. Vessels were denuded of endothelium by gently rubbing the internal surface of each ring with the edge of
stainless steel forceps. Vessels were recontracted with \(5.75 \times 10^{-7}\) M NE, and response to \(10^{-6}\) M bradykinin was re-assessed. Denudation was considered successful when <5% relaxation was observed after reexposure to \(10^{-6}\) M bradykinin. Denudation was confirmed by histological examination.

Basal release of factors. In one group of pigs (n = 8), vessels were precontracted with the EC\(_{50}\) of norepinephrine (\(5.75 \times 10^{-7}\) M), and tension was stabilized for 20 min. L-NAME or indomethacin was added to the appropriate vessel bath, and tension was restabilized for 20 min. Response to addition of inhibitors was calculated by dividing developed tension (in g) after addition of inhibitors by developed tension (in g) after NE and was expressed as percentage of NE-induced tension. In another group of pigs (n = 9), pharmacological inhibitors were added to the vessel bath before NE was given to assess the effect of L-NAME or indomethacin on baseline resting tension. Developed tension was calculated by subtracting resting tension from tension found after 20 min of incubation with the inhibitor.

Vasorelaxation. When stable contraction was obtained, response to \(10^{-6}\) M bradykinin was reevaluated to determine the contribution of nitric oxide and prostaglandin mediators to single-dose bradykinin-induced relaxation. Vessels were then washed to resting tension, and NE and inhibitors were added simultaneously. Preliminary experiments showed that repeat exposure to L-NAME or indomethacin did not lead to a further change in tension. After tension had stabilized, ACh-induced relaxation (\(10^{-10}\) to \(10^{-4}\) M) was determined through cumulative addition of stock solutions to the vessel bath. Percent relaxation was determined as described in Relaxation Responses. After exposure to ACh, vessels were rinsed and restabilized at resting tension, and then \(5.75 \times 10^{-7}\) M NE and appropriate inhibitors were added. Tension was stabilized for 20 min, and relaxation to SNP (\(10^{-10}\) to \(10^{-4}\) M) was determined to assess endothelium-independent relaxation. After exposure to SNP, vessels were rinsed and incubated in zero-calcium Krebs solution containing 2 mM EGTA to determine maximal relaxation.

Control experiments were performed in the presence of 300 \(\mu\)M N\(^{-}\)-nitro-L-arginine methyl ester (L-NAME), the inactive isomer of L-NAME, to confirm that inhibition of NOS was responsible for the observed effects. In some vessels, control experiments were performed in the presence of the diluent for indomethacin (0.07% ethanol) to confirm that the observed effect was related to pharmacological inhibition of cyclooxygenase.

Solutions

All chemicals were obtained from Fisher Chemicals (Fair Lawn, NJ) unless otherwise noted. Krebs solution was made fresh daily, equilibrated at 37°C, bubbled with 95% O\(_2\)-5% CO\(_2\) for 20 min, and adjusted to pH 7.4 before use. Stock solutions of ACh and NE dissolved in distilled, deionized water were made in a single batch and stored at 4°C until use. Dilutions used in experiments were made fresh daily by using Krebs solution as a diluent. NE was kept covered and on ice throughout the experiment to prevent oxidative degradation. SNP was made fresh daily and was stored in the dark immediately before addition to the bath. L-NAME (Sigma Chemical) or \(\alpha\)-NAME (Sigma Chemical) was dissolved in distilled, deionized water and was stored at 4°C as a stock solution of 300 mM. Indomethacin was prepared in 70% ethanol as a stock solution of \(10^{-2}\) M and stored at 4°C before use.

Data Analysis

Data are presented as means \(\pm\) SE. Citrate synthase activities and heart-to-body weight ratios were compared by using the Student's t-test. When more than one arterial ring from an animal was used in a single treatment group, values were averaged before statistical analysis such that each animal represented one observation. Contractile response was expressed as developed tension (in g) from resting tension. EC\(_{50}\) values were individually determined for each arterial ring with a linear regression computer program (Basica IC\(_{50}\)).

Relaxation was expressed as percent relaxation from precontracted tension. IC\(_{50}\) was determined by the Basica IC\(_{50}\) computer program. Cumulative concentration-response curves for ACh and SNP were analyzed by using repeated-measures analysis of variance (SuperANOVA, Abacus Concepts). Comparisons were made between Sed and Ex animals in the presence and absence of indomethacin, L-NAME, and endothelial denudation for each vasoactive drug. The contribution of nitric oxide to ACh-mediated vasorelaxation was calculated by subtracting relaxation obtained in the presence of L-NAME from control relaxation and dividing by control relaxation. Percentage of ACh-induced relaxation due to nitric oxide was compared between Sed and Ex pigs to assess the relative contribution of nitric oxide to relaxation in each group. ACh-induced relaxation in the presence and absence of indomethacin was compared in arteries from Sed and Ex pigs. When indicated by a significant F test, planned post hoc comparisons were performed to detect differences between individual means. EC\(_{50}\) and IC\(_{50}\) concentrations determined for vasoactive responses of arteries from Sed and Ex pigs were compared by using the Student's t-test. For all analyses, \(P < 0.05\) was considered significant.

RESULTS

Animals

In all pigs, the left cranial lung lobe was adhered to the internal thoracic wall where thoracotomy had been performed for placement of the ameroid occluder. Grossly, the left cranial lobe appeared collapsed and hemorrhagic and was similarly affected in Sed and Ex pigs. Histopathology of the left cranial lung lobe revealed focal alveolar hemorrhage, collapse of alveoli, collections of lymphocytes around bronchioles, and focal alveolar hyperplasia consistent with a mild interstitial pneumatic process. Importantly, the right caudal lung lobe, which was the site of pulmonary artery isolation for this study, was not involved in adhesion formation and exhibited no gross pathology in any pig. Right pulmonary arteries from Sed and Ex pigs had similar outer and inner diameters, vessel axial length, and resting tension (Table 1). Passive tension required...
sensitivity to ACh did not differ between Sed and Ex pigs. Pulmonary arteries from Ex pigs showed greater relaxation to ACh throughout the concentration-response curve (Fig. 1), ACh-induced relaxation in pulmonary arteries of sedentary (Sed; ○) and exercise-trained (Ex; □) pigs. Values are means ± SE from 17 Sed and 14 Ex pigs. Percent relaxation was calculated as percent reduction from norepinephrine-induced (5.75 ± 0.19 g) and Ex pigs (1.59 ± 0.12 g; P = 0.17). Addition of indomethacin after stable contraction with NE resulted in no significant change in developed tension, and precontracted tension did not differ in arteries from Sed (1.50 ± 0.21 g) and Ex (1.31 ± 0.25 g) pigs.

Vasorelaxation. Relaxation to bradykinin in the presence of L-NAME was equally inhibited in arteries from Sed (44.5%) and Ex (42.1%) pigs. There were no differences between responses of Sed and Ex pulmonary arteries to bradykinin in the presence of indomethacin. Denudation abolished vasorelaxation in response to ACh in both Sed and Ex pigs (Fig. 1). Responses to ACh to reach the apex of the length-tension curve (Lmax), reported as the percent stretch to Lmax, did not differ between Sed and Ex pigs.

Indexes of Exercise Training

Exercise training resulted in a significant increase in citrate synthase activity of locomotory muscles. In Ex pigs, citrate synthase activity in the long head of the triceps was 132% of activity in Sed pigs and was 142% of Sed activity in the deltoid and lateral head of the triceps (P < 0.03). Ex pigs had a significantly increased heart-to-body weight ratio (6.28 ± 0.09 g) compared with Sed pigs (5.62 ± 0.16; P < 0.002).

Pulmonary Artery Contractile Responses

Response to 80 mM KCl did not differ between Sed and Ex groups (Table 2). Isosmotic KCl and NE resulted in concentration-dependent increases in tension in pulmonary arteries from both Sed and Ex pigs. Concentration-response curves, maximal developed tension, and EC50 values did not differ between Sed and Ex groups for any contractile stimulation (Table 2). Precontracted tension induced by 5.75 × 10⁻⁷ M NE (EC50 levels) did not differ in pulmonary arteries from Sed (1.62 ± 0.14 g) and Ex (1.74 ± 0.16 g) pigs.

Relaxation Responses

Single-dose bradykinin (10⁻⁶ M) resulted in 81.4 ± 2.7% relaxation in Sed (n = 10) and 76.9 ± 2.1% in Ex (n = 9) groups (P = 0.22). As shown in Fig. 1, ACh produced concentration-related relaxation of arteries from both Sed and Ex pigs. Pulmonary arteries from Ex pigs showed greater relaxation to ACh throughout the concentration-response curve (P < 0.05). However, sensitivity to ACh did not differ between Sed and Ex pigs, as indicated by similar IC50 values (Sed = 9.70 ± 1.91 × 10⁻⁸ M and Ex = 6.20 ± 1.28 × 10⁻⁸ M; P = 0.16).

Direct smooth muscle relaxation mediated by SNP did not differ in arteries from Sed and Ex pigs (Fig. 2). Sensitivity to SNP also did not differ between arteries from Sed and Ex pigs, as indicated by similar IC50 values for arteries from Sed (8.54 ± 1.8 × 10⁻⁸ M) and Ex (11.22 ± 1.5 × 10⁻⁸ M) pigs (P = 0.27).

Incubation in zero-calcium Krebs solution containing 2 mM EGTA resulted in further relaxation in both Sed (111%) and Ex pigs (115%). No significant difference was present between the two groups (P = 0.38).

Role of Endothelium-Derived Mediators

Basal release of factors. Addition of L-NAME at resting tension caused no increase in tension in arteries from Sed or Ex pigs. Developed tension after NE and L-NAME did not differ in pulmonary artery rings from Sed (2.04 ± 0.19 g) and Ex pigs (1.59 ± 0.12 g; P = 0.17). Addition of indomethacin after stable contraction with NE resulted in no significant change in developed tension, and precontracted tension did not differ in arteries from Sed (1.50 ± 0.21 g) and Ex (1.31 ± 0.25 g) pigs.

Vasorelaxation. Relaxation to bradykinin in the presence of L-NAME was equally inhibited in arteries from Sed (44.5%) and Ex (42.1%) pigs. There were no differences between responses of Sed and Ex pulmonary arteries to bradykinin in the presence of indomethacin. Denudation abolished vasorelaxation in response to ACh in both Sed and Ex pigs (Fig. 1). Responses to ACh

Table 2. Maximal developed tension and EC50 values for contractile responses in Sed and Ex pigs

<table>
<thead>
<tr>
<th>Constrictor</th>
<th>Sed</th>
<th>EC50</th>
<th>Ex</th>
<th>EC50</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Max tension, g</td>
<td>NA</td>
<td>Max tension, g</td>
<td>NA</td>
</tr>
<tr>
<td>KCl 80 mM (n = 17, 14)</td>
<td>3.64 ± 0.19</td>
<td></td>
<td>3.52 ± 0.26</td>
<td></td>
</tr>
<tr>
<td>Isosmotic KCl (n = 6, 6)</td>
<td>4.01 ± 0.30</td>
<td>31.8 ± 1.0 mM</td>
<td>3.42 ± 0.66</td>
<td>31.5 ± 0.9 mM</td>
</tr>
<tr>
<td>NE (n = 6, 7)</td>
<td>3.00 ± 0.40</td>
<td>6.65 ± 1.36 × 10⁻⁷ M</td>
<td>3.44 ± 0.47</td>
<td>4.69 ± 1.08 × 10⁻⁷ M</td>
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Values are means ± SE; n, no. of pigs in Sed (first no.) and Ex (second no.) groups. Max tension, maximal developed tension; EC50, concentration at which half-maximal contraction occurred; NE, norepinephrine; NA, not applicable. No significant differences were detected between Sed and Ex pigs for any contractile parameter.
in denuded arteries did not differ between Sed and Ex pigs.

In the presence of L-NAME, ACh-induced relaxation decreased in arteries from Sed (maximal relaxation decreased from 52.3 to 44.8%) and Ex pigs (maximal relaxation decreased from 61.9 to 33.2%); L-NAME effectively reversed the exercise-induced enhancement of relaxation to ACh. After L-NAME, ACh-induced relaxation was significantly less in arteries from Ex compared with Sed animals (P < 0.05; Fig. 3A). The percentage of ACh relaxation due to nitric oxide was calculated from 10^{-8} M to 10^{-4} M as described in METHODS. This analysis (Fig. 3B) suggested that arteries from Ex pigs had greater reliance on nitric oxide for ACh-mediated relaxation than did arteries from Sed pigs (P < 0.05). D-NAME did not inhibit ACh-mediated vasorelaxation in either group of pigs.

In the presence of indomethacin, concentration-response curves to ACh did not differ in arteries from Sed and Ex pigs (P = 0.62). Maximal relaxation in arteries from Sed (71.8 ± 3.6%) did not differ from that of arteries from Ex (64.6 ± 3.6%) pigs (P = 0.39). Arteries from Sed pigs showed significantly enhanced relaxation in response to ACh when indomethacin was added to the vessel bath (P < 0.05; Fig. 4A). In contrast, arteries from Ex pigs exhibited similar ACh-mediated relaxation in the presence and absence of cyclooxygenase inhibition (Fig. 4B). Relaxation responses were not enhanced in Sed or Ex pigs by the presence of 0.07% ethanol, the diluent used for the indomethacin stock solution.

Endothelium-independent vasorelaxation in arteries from Sed or Ex pigs was enhanced by NOS inhibition with L-NAME (P < 0.05; Fig. 5). Arteries from both Sed and Ex pigs showed increased sensitivity to SNP in the presence of L-NAME as indicated by a lower IC_{50} compared with control (P < 0.05; Table 3). Endothelial denudation slightly increased sensitivity to SNP-mediated relaxation in arteries from Ex pigs (P < 0.05), but the change was not significant in Sed arteries. There were no other significant differences in SNP responses between arteries from Sed and Ex pigs in the presence of pharmacological inhibitors (Table 3).

DISCUSSION

Vascular adaptations to chronic exercise training have been demonstrated in many different laboratories; however, the mechanism(s) by which exercise alters endothelial function has not been fully elucidated. Increases in blood flow might alter receptor subtypes within the vasculature, change the amount or efficiency of vasoactive mediator release from endothelial cells, or modify the sensitivity of vascular smooth muscle cells to endothelium-derived factors. In other vascular beds in the pig, increased biosynthesis of nitric oxide has been clearly implicated in exercise-
induced enhancement of endothelial function (11, 18, 30). Our data are the first to show that exercise training leads to endothelial adaptations in pulmonary arteries of pigs. The data generated here suggest that at least two signaling pathways within endothelial cells of the pulmonary circulation are altered by exercise training in this model.

Training Effects on Contractile Responses in Pulmonary Arteries

Contractile responses of vascular smooth muscle to activation of voltage-gated calcium channels (KCl mediated) and receptor-mediated contraction (NE induced) did not differ between arteries from Sed and Ex pigs in this model. The endothelium-intact abdominal aorta of exercise-trained rats had significantly less pressor response to hypoxic and angiotensin II-induced pulmonary vasoconstriction.

In the present study, contractile tension developed in response to EC50 levels of norepinephrine did not differ in arteries from Sed and Ex pigs. Pharmacological inhibitors had no effect on resting tension in pulmonary arteries, and developed tension to NE in the presence of pharmacological inhibitors did not differ between Sed and Ex pigs. Taken together, our data show that exercise training did not alter contractile responses of porcine pulmonary arteries used in this study.

Training Effects on Relaxation Responses in Pulmonary Arteries

Pulmonary arteries from Ex pigs exhibited enhanced response to ACh, and relaxation in Sed and Ex groups did not differ in arteries denuded of endothelium (Fig. 1), indicating that the enhanced response was due to an adaptation in endothelial cells. Potential mechanisms for the increased relaxation response include 1) increased sensitivity of vascular smooth muscle to endothelium-derived mediators, 2) modifications in production of endothelium-derived vasoactive mediators, or 3) alterations in cholinergic-muscarinic receptors on endothelial cells. We will consider each of these potential mechanisms.

Relaxation to SNP did not differ between pulmonary arteries from Sed and Ex pigs (Fig. 2). SNP is thought to act by providing a source of nitric oxide that directly stimulates guanylate cyclase in smooth muscle cells, resulting in increased cGMP and smooth muscle relaxation. The lack of a difference in relaxation to SNP between pulmonary arteries from Sed and Ex pigs

![Fig. 4. Effect of indomethacin (Indo) on ACh-induced relaxation. Values are means ± SE.](image)

![Fig. 5. Effect of nitric oxide synthase inhibition (300 µM L-NAME) on SNP relaxation in pulmonary arteries of Sed and Ex pigs. Values are means ± SE from same 17 Sed and 13 Ex control arteries as in Fig. 2. L-NAME increased sensitivity to SNP compared with control in arteries from both Sed (○; n = 9) and Ex (●; n = 8) pigs (P < 0.05); however, response to SNP did not differ between Sed and Ex groups.](image)
indicates that exercise training does not alter nitric oxide/cGMP-dependent intracellular signaling pathways or second-messenger systems within vascular smooth muscle cells.

In this study, use of pharmacological inhibitors of endothelial enzyme systems suggested that vasomotor adaptations were due to alterations in at least two signaling pathways involved in production of endothelium-derived vasoactive mediators. Acetylcholine binds to muscarinic receptors on endothelial cells and causes vasorelaxation through activation of NOS to produce nitric oxide and stimulation of arachidonic acid metabolism to produce prostacyclin (9, 10). Arachidonic acid metabolism can also result in production of prostaglandin vasoconstrictors and endothelium-derived hyperpolarizing factor (3, 20, 23). Our data show that pulmonary arteries of Ex pigs have enhanced reliance on nitric oxide for ACh-induced relaxation. Figure 3B suggests that ~46% of ACh-induced relaxation in arteries from Ex pigs was due to nitric oxide, whereas, in Sed pigs, only 19% of relaxation could be ascribed to nitric oxide. Exercise training in healthy animals has been shown to upregulate endothelial cell NOS mRNA in systemic arteries (6, 20, 30), and a similar response might explain the results obtained in this study in the pulmonary circulation.

Regulation of NOS within the pulmonary circulation is complex, and chronic training adaptations in pulmonary vessels of large mammals have not been investigated previously. Increased blood flow during exercise is thought to result in increased endothelium-mediated dilation in peripheral vascular beds through shear stress-induced changes in gene expression and protein production (6, 20, 30). However, chronic increases in pulmonary blood flow alone may not alter endothelial cell NOS expression. Everett et al. (8) induced chronic, sustained increases in lung blood flow in the rat through the creation of an aortocaval shunt. Although medial hypertrophy was seen in the pulmonary circulation of these animals, pulmonary pressures were not elevated and NOS protein levels and mRNA expression were not altered (8). Pulmonary blood flow increases sixfold during exercise in swine (1) and mild pulmonary arterial hypertension develops (12). Therefore, it is reasonable to propose that pulmonary endothelial cells experience temporary increases in shear stress during bouts of exercise. Shear stress applied to isolated arterioles and cultured endothelial cells upregulates endothelial cell NOS expression (19, 31), and this is a likely mechanism for the results noted here. Chen and Li (4) found that basal nitric oxide production did not differ between sedentary and exercise-trained rabbits but surmised that ACh-stimulated nitric oxide production was enhanced in exercise-trained subjects, as evidenced by an increased sensitivity to endothelium-mediated vasodilation. Our data indicate that Ex pigs have increased dependence on nitric oxide for pulmonary vasorelaxation induced by ACh.

Alterations in NOS activity did not appear to explain fully the enhanced relaxation noted in pulmonary arteries from Ex pigs. Figure 4A suggests that control arteries from Sed pigs produced a vasoconstrictor factor that suppressed ACh-stimulated relaxation. In the presence of the cyclooxygenase inhibitor indomethacin, ACh-induced relaxation in arteries from Sed pigs increased and was no longer different from relaxation found in arteries from Ex pigs. Thus it appears that indomethacin inhibited cyclooxygenase-dependent production of a constrictor agent in arteries from Sed pigs. On the other hand, indomethacin did not alter ACh-mediated relaxation in pulmonary arteries from Ex pigs (Fig. 4B), indicating lack of production of this vasoconstrictor in response to ACh after training. Potential mediators of vasoconstriction would include thromboxane A2, prostaglandin H2, or other metabolites of arachidonic acid (20). These data indicate that pulmonary endothelium of Sed pigs produces a prostaglandin constrictor in response to ACh. The combined pharmacological data suggest that exercise training results in adaptations in at least two endothelium-dependent signaling pathways in this model: with exercise training, nitric oxide plays a greater role in inducing pulmonary vasorelaxation and endothelial production of a prostaglandin constrictor is decreased.

Chen and Li (4) showed that pulmonary arteries from exercise-trained rabbits had enhanced relaxation to ACh but responded similarly to pulmonary arteries from sedentary rabbits in response to the receptor-independent calcium ionophore A-23187. The authors surmised that the enhanced response noted in trained subjects was related to enhanced receptor-dependent stimulated NO release. Muscarinic receptor numbers or subtypes and binding efficiency were not evaluated in the present study.

**Caveats**

The animals available for this study had been instrumented for use in a model of surgically induced coro-
nary occlusion. This model has been well established for the study of coronary collateral development (2) and has been used to assess effects of exercise training (11). All animals developed coronary collaterals in response to aortocoronary occlusion but showed no signs of congestive heart failure, and pigs in the exercise group were able to complete a rigorous protocol of exercise training. Importantly, the lung lobes and pulmonary arteries used in this study exhibited no gross pathology related to the surgical procedure or coronary occlusion. However, inflammation due to adhesion formation at the left cranial lung lobe may have lead to some reduction in functional lung volume. The surgical procedure or induction of coronary artery occlusion could have resulted in altered lung blood flow during exercise and/or caused occult lung dysfunction. Surgical manipulation may have influenced local or systemic production of inflammatory mediators that could modify vascular function (20). Our experiments were not designed to define potential effects of surgical manipulation or coronary occlusion alone on pulmonary vascular function, and these results must be interpreted carefully relative to the effects of exercise training in normal pigs. However, animals were randomly divided into sedentary and exercise-training groups; no gross or histological differences were observed between groups. Also, the training protocol used here resulted in similar increases in muscle oxidative enzymes and heart-to-body weight ratios in pigs with coronary occlusion as have been reported for normal pigs (those not subjected to occlusion) studied previously (16–18, 22), making it likely that the effects observed were related to chronic exercise training. We believe that the results presented here accurately represent direct effects of exercise training on pulmonary vasorelaxation in this model. Importantly, our studies may reveal beneficial effects of exercise training on pulmonary vascular function in experimental models and human patients with coronary artery disease and/or coronary artery occlusion. Improved matching of ventilation and perfusion has been proposed to contribute to training-induced enhancement of aerobic performance in patients with cardiac and coronary disease (25). Our studies implicate a potential role of enhanced pulmonary endothelial cell function after exercise training, a new aspect of the mechanisms underlying beneficial effects of chronic exercise.

Chronic hypoxia has been reported to increase NOS expression in pulmonary resistance arteries from rats and to increase NOS activity in lung homogenates (32). However, in some endothelial cell cultures, NOS gene expression and mRNA half-life is reduced by hypoxic conditions (15). Healthy pigs develop an increase in ventilation-perfusion inequality during exercise; however, arterial oxygenation does not decline (12). Thus NOS activity and expression may be variably regulated by hypoxic conditions; however, it is unlikely that hypoxia-induced alterations in NOS activity or expression played a role in pigs studied here.

Summary

Benefits of exercise on the cardiopulmonary system have long been recognized; however, the mechanisms by which these adaptive responses in the vasculature improve the quantity and quality of life remain elusive. Exercise is beneficial in preventing cardiovascular disease, and some form of exercise training could maximize vascular responses in a variety of systemic and cardiopulmonary diseases, thus resulting in improved clinical condition. Many disease conditions, including congestive heart failure, mitral stenosis, mitral regurgitation, and chronic pulmonary disease, lead to structural and functional changes in the pulmonary vasculature (7, 29). Our data indicate that exercise training results in an increased vasodilator role of nitric oxide and reduced contribution of a prostanoid constrictor in the pulmonary vasculature of pigs studied in the present work, leading to enhanced endothelium-mediated vasorelaxation in the absence of changes in smooth muscle contractile function. Understanding the effects of exercise on endothelium-mediated responses in the pulmonary vasculature is important and could result in improved therapeutic strategies in patients with vascular dysfunction.

This work was supported by National Institutes of Health Grants HL-03856, HL-36088, and HL-52490. Preliminary data from this study were presented at the 1998 American Physiological Society Conference in Augusta, GA, in September 1998.

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Received 3 August 1999; accepted in final form 1 October 1999.

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