Exercise training preserves endothelium-dependent relaxation in brachial arteries from hyperlipidemic pigs

Christopher R. Woodman, James R. Turk, Daniel P. Williams, and M. Harold Laughlin

Department of Biomedical Sciences, and The Dalton Cardiovascular Research Center, University of Missouri, Columbia, Missouri 65211; and Department of Exercise and Sport Science, College of Health, The University of Utah, Salt Lake City, Utah 84112

Submitted 7 November 2002; accepted in final form 8 January 2003

Woodman, Christopher R., James R. Turk, Daniel P. Williams, and M. Harold Laughlin. Exercise training preserves endothelium-dependent relaxation in brachial arteries from hyperlipidemic pigs. J Appl Physiol 94: 2017–2026, 2003; 10.1152/japplphysiol.01025.2002.—We tested the hypothesis that exercise training (Ex) attenuates the effects of hyperlipidemia on endothelial function by enhancing NO-mediated vasorelaxation in porcine brachial (Br) arteries. Adult female pigs were fed a normal-fat (NF) or high-fat (HF) diet for 20 wk. Four weeks after initiation of the diet, pigs underwent Ex or remained sedentary (Sed) for 16 wk. Relaxation to ACh was impaired by HF (P = 0.03). The combination of HF and Sed impaired ACh-induced relaxation more than HF or Sed alone (P = 0.0002). Relaxation to high doses of bradykinin (BK) was impaired by HF (P = 0.0002). Ex significantly improved ACh-induced relaxation (P = 0.01) and tended to improve relaxation to BK (P = 0.38). To determine the mechanism(s) by which HF and Ex affected relaxation to ACh and BK, relaxation was assessed in the presence of L-NAME (to inhibit NO synthase), indomethacin (Indo; to inhibit cyclooxygenase), or L-NAME + Indo. In the presence of L-NAME, Indo, or L-NAME + Indo, ACh-induced relaxation was no longer different between HF and NF arteries; however, relaxation remained greater in Ex than in Sed arteries. In the presence of L-NAME or Indo, BK-induced relaxation was no longer altered by HF but was enhanced by Ex. In the presence of L-NAME + Indo, BK-induced relaxation was enhanced by HF and Ex. These data indicate that hyperlipidemia impairs ACh- and BK-induced relaxation by impairing NO- and PGI2-mediated relaxation. Ex attenuates the effects of HF by enhancing a vasodilator mechanism independent of NO and PGI2.

RESULTS FROM SEVERAL STUDIES indicate that endothelial function in coronary and peripheral arteries is impaired by hyperlipidemia (2, 3, 10, 12, 14, 26, 27). The vascular dysfunction induced by hyperlipidemia is associated with blunted vasodilator responses to several endothelium-dependent agonists, including acetylcholine (ACh; Refs. 2, 14, 18, 28), 5-hydroxytryptamine (3, 14), substance P (3), aggregating platelets (10, 26, 27), and increases in intraluminal flow (12, 18). The mechanisms for the detrimental effects of hyperlipidemia on endothelial function are not completely understood; however, previously published studies indicate that a reduction in the bioavailability of NO may contribute to the dysfunction (3). This speculation is supported by experimental evidence indicating that vasorelaxation in response to NO-dependent vasodilators is impaired in arteries from hyperlipidemic subjects (12, 18, 28), whereas dilation to NO donors, such as sodium nitroprusside, is not compromised (2, 3, 18, 28). This hypothesis is also supported by the finding that ACh-induced dilation is similar in hypercholesterolemic patients and normal subjects after administration of an arginine analog to inhibit NO production (2).

It is well documented that exercise training can improve endothelial function in both coronary (22, 23, 32) and peripheral arteries (15, 17, 29, 30). The improvement in vasodilator function is associated with enhanced NO-dependent dilation (22), increased NO production (25), and increased expression of endothelial NO synthase (eNOS) in arteries and arterioles (16, 25, 34). In addition, exercise training increases the expression and activity of superoxide dismutase (SOD; Refs. 6, 24), possibly prolonging the biological half-life of NO. Because hyperlipidemia-induced endothelial dysfunction is due in part to decreased NO bioavailability, it is reasonable to predict that the effects of hyperlipidemia on endothelial function will be attenuated by a program of exercise training that is known to enhance the production and stability of NO. Therefore, the purpose of this study was to test the hypothesis that endurance exercise training attenuates or reverses the detrimental effects of hyperlipidemia on endothelial function by enhancing NO-mediated vasorelaxation in porcine brachial (Br) arteries.

METHODS

Experimental animals. Before initiation of this study, approval was received from the Animal Care and Use Committee at the University of Missouri. The experimental animals were adult female Yucatan miniature swine (n = 32) that were purchased from a commercial breeder (Sinclair Re
search Farm, Columbia, MO). The pigs used in the present study were a subgroup of pigs used in a previously published study of the effects of training on blood lipids (31). The pigs were 8–12 mo of age and weighed 25–40 kg. All of the pigs were housed in the animal care facility in the Department of Biomedical Sciences in a room maintained at 20–23°C with a 12:12-h light-dark cycle. Half of the pigs (n = 16) were provided a normal-fat (NF) diet (Purina Lab Mini-pig Chow) in which 2.5% of daily caloric intake was derived from fat. The remaining pigs (n = 16) were provided a high-fat (HF) diet consisting of pig chow supplemented with cholesterol (2.0%), coconut oil (17.1%), corn oil (2.3%), and sodium cholate (0.7%). Pigs provided the HF diet derived 46% of their daily caloric intake from fat (4). Four weeks after initiation of the diet, pigs were exercise trained (Ex) or remained sedentary (Sed) for 16 wk. During this 16-wk time period, pigs continued to consume the HF or NF diet. The resulting experimental design consisted of four groups of pigs: 1) NF sedentary (NF-Sed; n = 8), 2) NF exercise-trained (NF-Ex; n = 8), 3) HF sedentary (HF-Sed; n = 8), and 4) HF exercise-trained (HF-Ex; n = 8). Plasma lipid data from the pigs used in the present study have been reported previously (31). The results indicated that the HF diet induced significantly elevated plasma cholesterol (76 vs. 637 mg/dl), triglyceride (45 vs. 64 mg/dl), high-density lipoprotein-C (37 vs. 76 mg/dl), and low-density lipoprotein-C (31 vs. 318 mg/dl) concentrations in HF pigs (31).

Training program. All of the pigs in the study were familiarized with running on a motorized treadmill and randomly assigned to an Ex or control Sed group for 16 wk. Pigs assigned to the Ex group completed a 16-wk endurance training program consisting of 5 exercise bouts/wk using a previously published protocol (22). The intensity and duration of the exercise bouts were increased weekly to maximize the training stimulus (22). Pigs assigned to the Sed group were restricted to their enclosures (2–4 m pens) and did not exercise. At the conclusion of the 16-wk training program, the Ex and Sed pigs performed a graded-intensity treadmill exercise test to exhaustion (15). The efficacy of the training protocol was confirmed by significant increases in run time to exhaustion, heart weight-to-body weight ratio, and citrate synthase activity measured in the deltoid muscle. These data have been reported previously (31).

Vascular ring preparation. At the end of the 16-wk training period, Ex and Sed pigs were sedated with ketamine (30 mg/kg im) and anesthetized with pentobarbital sodium (35 mg/kg iv). Segments of the Br artery were removed and trimmed of connective tissue and fat. Vessel segments were taken from the same sites in all pigs. A filar calibrated micrometer eye piece was used to measure axial length, inside diameter, and outside diameter of each vascular ring.

Length-tension relationship. Vascular ring segments from the Br artery were mounted on two stainless steel wires passed through the lumen of the vessel. One wire was connected to a force transducer (model FT03, Grass). The second wire was attached to a micrometer microdrive (Stoeling), which was used to stretch the arterial ring by known increments. Arterial rings were subsequently submerged in a 20-ml tissue bath containing Krebs bicarbonate buffer solution that was maintained at 37°C and equilibrated with a 95% O2-5% CO2 gas mixture. The maximal point from the length-tension relationship (Lmax) was determined for each arterial ring by repeatedly exposing the ring to KCl (30 mM) and measuring contractile tension developed at increasing vessel diameters. Once Lmax was determined, the Krebs bicarbonate buffer solution was replaced to wash out the KCl and the vascular ring was allowed 1 h to stabilize before initiation of the experimental protocols. All pharmacological studies were subsequently conducted at Lmax.

Assessment of vasorelaxation. Before initiation of dose-response curves, all arterial rings were preconstricted with PGF2α (30 μM). Endothelium-dependent vasorelaxation was assessed in arterial rings by using ACh (10−10–10−4 M) and bradykinin (BK; 10−11–10−6 M). Endothelium-independent relaxation was assessed with sodium nitroprusside (SNP; 10−10–10−4 M). A total of four Br rings were studied from each pig. In arterial ring 1, vasorelaxation to agonist alone was measured by adding cumulatively increasing doses of the selected drug to the organ bath while measuring changes in force. In arterial ring 2, the role of NO in relaxation responses was assessed in the presence of Nω-nitro-l-arginine methyl ester (l-NAME; 300 μM) to block NOS. In arterial ring 3, the importance of prostacyclin (PGI2) in relaxation responses was assessed in the presence of indomethacin (Indo; 5 μM) to block cyclooxygenase (COX). In arterial ring 4, double blockade with l-NAME + Indo was used to assess the importance of NOS- and COX-independent mechanisms of vasorelaxation. The experimental protocol was designed such that ACh was always the first agonist administered, followed by BK and SNP. At the end of each dose-response protocol, bicarbonate buffer solution was replaced to wash out the drug, and the arterial segments were allowed 1 h to stabilize before initiation of the next protocol.

Immunohistochemistry. Samples of Br artery were dissected and immersed in 10% formalin for a minimum of 24 h. Three-millimeter rings were processed routinely to paraffin embedment. Five-micrometer sections were cut with an automated microtome (Microm), floated onto positively charged slides (Fischer), and deparaffinized. The slides were steamed in citrate buffer at pH 6.0 (Dako target retrieval solution S1699) for 30 min to achieve antigen retrieval and then cooled for 30 min. The slides were stained manually with sequential Tris buffer, and water wash steps were performed after each protocol step. Sections were incubated with avidin-biotin two-step blocking solution (Vector SP-2001) to inhibit background staining and in 3% hydrogen peroxide to inhibit endogenous peroxidase. Nonserum protein block (Dako X090) was applied to inhibit nonspecific protein binding. The primary antibodies utilized were mouse monoclonal anti-eNOS (BD Transduction Laboratories), rabbit polyclonal anti-SOD-1 (Stressgen Biotechnology), and rabbit polyclonal anticaveolin-1 (CAY-1; Santa Cruz Laboratories). All primary antibodies were diluted 1:800 and incubated with the tissue sections overnight at 4°C. After appropriate washing steps were completed, the sections were incubated with biotinylated anti-mouse or rabbit link secondary antibody in PBS containing 15 mM sodium azide and peroxidase-labeled streptavidin (Dako LSAB+ kit, peroxidase, K0690). Diamobenzidine (Dako) applied for 5 min allowed visualization of primary antibody staining. Sections were counterstained with Mayer’s hematoxylin stain for 1 min, dehydrated, and coverslipped. For negative controls, histological sections were prepared as described above, but incubation in primary antibody was excluded from the protocol. Sections were examined and photographed by use of an Olympus BX40 photomicroscope.

Immunoblot analysis. Relative differences in eNOS, SOD-1, and CAV-1 protein expression in Br rings were assessed by using immunoblot analysis as described previously in detail (16). Briefly, Br arteries were solubilized in 20 μl Laemmli buffer (13), boiled for 2 min, sonicated, and spun for 60 s in a microcentrifuge (14,000 g) to remove any insoluble material. The boiling and sonication steps were repeated five
times to ensure that the vessel was solubilized. Total protein content in individual Br rings was measured by use of the NanoOrange protein assay (Molecular Probes, Eugene, OR). Protein samples (8 μg/lane) were loaded onto 5–12% NuPage Bis Tris gradient gels (Invitrogen), electrophoresed under reducing conditions, and transferred to polyvinylidene difluoride membrane (Hybond-ECL, Amersham). The membranes were blocked for 1 h at room temperature with 5% nonfat milk in TBS-Tween (20 mmol/l Tris-HCl, 137 mmol/l NaCl, and 0.1% Tween 20) and incubated overnight at room temperature with primary antibody against eNOS (1:1,600; Transduction Laboratories). Blots were subsequently incubated for 1 h with secondary antibody (1:2,500; horseradish peroxidase-conjugated anti-rabbit; Sigma Chemical). Specific eNOS protein was detected by enhanced chemiluminescence (Amersham) and evaluated by densitometry by use of NIH Image software (National Institutes of Health, Bethesda, MD). All blots were reblocked for 1 h at room temperature and incubated overnight with a polyclonal antibody against SOD-1 (1:1,600, Stressgen). Blots were then incubated for 1 h with secondary antibody (1:2,500; horseradish peroxidase-conjugated anti-rabbit; Sigma Chemical). Lastly, blots were reprobed with monoclonal antibody against CAV-1 (1:250, Transduction Laboratories). Data were standardized such that the mean value of the NF-Sed arteries was set to 1.0, whereas NF-Ex, HF-Sed, and HF-Ex data were expressed as fold increase relative to the NF-Sed arteries.

**Vascular ring characteristics.** The effects of HF and Ex on vessel ring characteristics are presented in Table 1. Statistical analyses indicated that arterial ring characteristics (outer diameter, inner diameter, wall thickness, axial length, resting tension at Lmax, percent stretch to Lmax) were not significantly altered by diet, exercise, or the diet × exercise interaction.

**ACh responses.** ACh elicited a dose-dependent relaxation of Br rings from all groups of pigs (Fig. 1A). Mixed-factor repeated-measures ANOVA revealed a significant main effect of diet, indicating that ACh-induced relaxation was impaired in Br rings from pigs fed the HF diet (P = 0.03). In addition, statistical analysis revealed a significant diet × exercise interaction such that the combination of the HF diet and Sed lifestyle impaired ACh-induced relaxation more than HF or Sed alone (P = 0.0002). Exercise training improved ACh-induced relaxation (P = 0.01) such that relaxation in HF-Ex arteries was not significantly different from NF-Sed or NF-Ex arteries (P = 0.01). In the presence of L-NAME (Fig. 1B), relaxation to ACh was no longer different in rings from HF and NF pigs (P = 0.67), whereas relaxation remained greater in rings from Ex pigs (P = 0.0007). In the presence of Indo (Fig. 1C), relaxation to ACh was no longer altered by HF (P = 0.49) but remained improved by Ex (P = 0.01). Likewise, in the presence of L-NAME + Indo (Fig. 1D), relaxation to ACh was no longer different between rings from HF and NF pigs (P = 0.56), whereas relaxation remained greater in rings from Ex pigs (P = 0.01).

To further assess the effect of hyperlipidemia on ACh-induced relaxation, maximal responses and ED50 values were compared (Table 2). In the absence of enzyme inhibitors, maximal ACh-induced relaxation was impaired by the HF diet (P = 0.02). Ex improved maximal ACh-induced relaxation (P = 0.03) such that maximal relaxation in HF-Ex arteries was not significantly different from that in NF-Sed and NF-Ex arteries. In the presence of L-NAME, Indo, or L-NAME + Indo, maximal ACh-induced relaxation was no longer different between NF and HF arteries; however, max-

---

**Table 1. Characteristics of brachial arteries from normal-fat- and high-fat-fed pigs**

<table>
<thead>
<tr>
<th>Variable</th>
<th>NF-Sed (n = 8)</th>
<th>NF-Ex (n = 8)</th>
<th>HF-Sed (n = 8)</th>
<th>HF-Ex (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer diameter, mm</td>
<td>2.19 ± 0.10</td>
<td>2.41 ± 0.10</td>
<td>2.24 ± 0.04</td>
<td>2.36 ± 0.09</td>
</tr>
<tr>
<td>Inner diameter, mm</td>
<td>1.17 ± 0.12</td>
<td>1.26 ± 0.09</td>
<td>1.19 ± 0.11</td>
<td>1.27 ± 0.06</td>
</tr>
<tr>
<td>Wall thickness, mm</td>
<td>0.51 ± 0.04</td>
<td>0.56 ± 0.02</td>
<td>0.52 ± 0.04</td>
<td>0.55 ± 0.04</td>
</tr>
<tr>
<td>Axial length, mm</td>
<td>2.73 ± 0.06</td>
<td>2.88 ± 0.08</td>
<td>2.88 ± 0.08</td>
<td>2.82 ± 0.07</td>
</tr>
<tr>
<td>Resting tension at Lmax, g</td>
<td>5.22 ± 0.62</td>
<td>6.07 ± 0.36</td>
<td>5.30 ± 0.87</td>
<td>3.75 ± 0.45</td>
</tr>
<tr>
<td>Percent stretch to Lmax, %</td>
<td>173 ± 2</td>
<td>176 ± 2</td>
<td>176 ± 2</td>
<td>173 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of pigs. NF-Sed, normal-fat sedentary; NF-Ex, normal-fat exercise trained; HF-Sed, high-fat sedentary; HF-Ex, high-fat exercise trained; Lmax, optimal circumferential length. Statistical analysis indicated that arterial ring characteristics were not significantly altered by diet, exercise, or the diet × exercise interaction.
imal relaxation remained greater in Ex arteries than in Sed arteries. ED50 values for ACh-induced relaxation were not altered by diet or exercise in the absence or presence of enzyme inhibitors.

BK responses. BK produced a concentration-dependent relaxation of Br rings from all groups of pigs (Fig. 2A). ANOVA revealed a significant diet \times dose interaction ($P = 0.0002$), indicating that BK-induced relax-

Table 2. Maximal relaxation and ED50 values for ACh-induced relaxation of brachial arteries from normal-fat- and high-fat-fed pigs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NF-Sed (n = 8)</th>
<th>NF-Ex (n = 8)</th>
<th>HF-Sed (n = 8)</th>
<th>HF-Ex (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum relaxation, %</td>
<td>75.1 ± 5.9</td>
<td>83.5 ± 2.1</td>
<td>50.5 ± 10.5*</td>
<td>75.0 ± 6.6</td>
</tr>
<tr>
<td>ED50, (-\log M)</td>
<td>(-7.74 ± 0.2)</td>
<td>(-8.38 ± 0.2)</td>
<td>(-7.63 ± 0.6)</td>
<td>(-8.24 ± 0.1)</td>
</tr>
<tr>
<td>Maximum relaxation, %</td>
<td>39.6 ± 7.5</td>
<td>62.0 ± 3.6†</td>
<td>36.2 ± 6.1</td>
<td>63.0 ± 9.3†</td>
</tr>
<tr>
<td>ED50, (-\log M)</td>
<td>(-6.65 ± 0.5)</td>
<td>(-8.06 ± 0.2)</td>
<td>(-7.60 ± 0.4)</td>
<td>(-7.71 ± 0.5)</td>
</tr>
<tr>
<td>Indo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum relaxation, %</td>
<td>63.3 ± 5.7</td>
<td>72.6 ± 5.4</td>
<td>60.3 ± 5.2</td>
<td>83.7 ± 4.6†</td>
</tr>
<tr>
<td>ED50, (-\log M)</td>
<td>(-7.88 ± 0.2)</td>
<td>(-7.77 ± 0.4)</td>
<td>(-8.13 ± 0.1)</td>
<td>(-8.27 ± 0.1)</td>
</tr>
<tr>
<td>Maximum relaxation, %</td>
<td>25.73 ± 5.4</td>
<td>43.36 ± 6.4</td>
<td>27.48 ± 5.8</td>
<td>53.49 ± 11.5†</td>
</tr>
<tr>
<td>ED50, (-\log M)</td>
<td>(-6.38 ± 0.3)</td>
<td>(-7.29 ± 0.4)</td>
<td>(-7.09 ± 0.5)</td>
<td>(-7.79 ± 0.5)</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of pigs. ED50, half-maximal effective dose; l-NAME, N\textsuperscript{G}-nitro-l-arginine methyl ester; Indo, indomethacin. Percent relaxation was calculated as percent reduction in force from prostaglandin F\textsubscript{20} (30 \mu M)-induced tension. *Significantly different from all other groups ($P \leq 0.05$). †Significantly different from NF-Sed and HF-Sed ($P \leq 0.05$).
Relaxation was impaired by the HF diet at high doses of BK. Statistical analysis did not reveal a significant diet × ex interaction (P = 0.49), indicating that the combination of the HF diet and Sed lifestyle did not impair BK-induced relaxation more than HF alone. BK-induced relaxation was not significantly improved by Ex (P = 0.38). In the presence of L-NAME (Fig. 2B), relaxation to BK was no longer different in Br rings from HF and NF pigs (P = 0.57); however, a beneficial effect of Ex to increase BK responses became apparent during treatment with L-NAME (P = 0.03). In the presence of Indo (Fig. 2C), relaxation to BK was no longer altered by HF (P = 0.12) but was improved by Ex at high doses of BK (P = 0.01). In the presence of L-NAME + Indo (Fig. 2D), relaxation to BK was no longer altered by HF (P = 0.08) but was improved by Ex (P = 0.01).

To further assess the effect of hyperlipidemia on BK-induced relaxation, maximal responses and ED50 values were compared (Table 3). In the absence of enzyme inhibitors, maximal BK-induced relaxation was impaired by the HF diet (P = 0.04). Ex tended to improve maximal BK-induced relaxation (P = 0.17); consequently, maximal relaxation in HF-Ex arteries was not significantly different from NF-Sed and NF-Ex arteries. In the presence of L-NAME, Indo, or L-NAME + Indo, maximal BK-induced relaxation was no longer different between NF and HF arteries. ED50 values for BK-induced relaxation were not altered by diet or exercise in the absence or presence of enzyme inhibitors.

**SNP responses.** Direct smooth muscle relaxation induced by SNP was similar in Br arteries from all groups of pigs (Fig. 3).

### Table 3. Maximal relaxation and ED50 values for BK-induced relaxation of brachial arteries from normal-fat- and high-fat-fed pigs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NF-Sed (n = 8)</th>
<th>NF-Ex (n = 8)</th>
<th>HF-Sed (n = 8)</th>
<th>HF-Ex (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum relaxation, %</td>
<td>81.2 ± 3.2</td>
<td>85.2 ± 3.8</td>
<td>59.8 ± 11.1*</td>
<td>75.8 ± 7.1</td>
</tr>
<tr>
<td>ED50, log M</td>
<td>−8.93 ± 0.1</td>
<td>−9.17 ± 0.2</td>
<td>−8.97 ± 0.1</td>
<td>−8.91 ± 0.1</td>
</tr>
<tr>
<td>L-NAME</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum relaxation, %</td>
<td>54.9 ± 6.9</td>
<td>73.1 ± 2.1</td>
<td>63.4 ± 4.1</td>
<td>65.4 ± 10.4</td>
</tr>
<tr>
<td>ED50, log M</td>
<td>−8.53 ± 0.1</td>
<td>−8.98 ± 0.1</td>
<td>−8.43 ± 0.2</td>
<td>−8.93 ± 0.3</td>
</tr>
<tr>
<td>Indo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum relaxation, %</td>
<td>61.1 ± 8.8</td>
<td>73.1 ± 6.6</td>
<td>72.1 ± 8.0</td>
<td>81.9 ± 4.8</td>
</tr>
<tr>
<td>ED50, log M</td>
<td>−8.90 ± 0.1</td>
<td>−9.11 ± 0.1</td>
<td>−8.98 ± 0.1</td>
<td>−9.14 ± 0.1</td>
</tr>
<tr>
<td>L-NAME + Indo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum relaxation, %</td>
<td>31.5 ± 7.4</td>
<td>39.9 ± 7.0</td>
<td>43.4 ± 8.6</td>
<td>56.7 ± 11.2</td>
</tr>
<tr>
<td>ED50, log M</td>
<td>−8.74 ± 0.2</td>
<td>−8.88 ± 0.2</td>
<td>−8.85 ± 0.2</td>
<td>−8.93 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of pigs. Percent relaxation was calculated as percent reduction in force from prostaglandin E2 (30 μM)-induced tension. *Significantly different from all other groups (P ≤ 0.05).
Immunohistochemistry. Immunohistochemistry revealed staining for eNOS, SOD-1, and CAV-1 in Br arteries from all groups of pigs (Figs. 4–6). eNOS and CAV-1 staining was confined to the endothelium of Br arteries in all groups of pigs. In the absence of primary antibody against eNOS, SOD-1, or CAV-1, no immunoreactivity was detected (data not shown).

Immunoblot analysis. The effect of exercise training and hyperlipidemia on eNOS, SOD-1, and CAV-1 protein expression in Br arteries is shown in Fig. 7. eNOS protein expression was not altered by the HF diet (P = 0.29) but was significantly increased by exercise training (P = 0.02). SOD-1 and CAV-1 protein expression were not altered by the HF diet or Ex.

DISCUSSION

The purpose of this study was to test the hypothesis that exercise training attenuates or reverses the detrimental effects of hyperlipidemia on endothelial function by enhancing NO-mediated vasorelaxation in porcine Br arteries. The primary findings of this study were as follows. 1) ACh-induced relaxation was impaired in Br arteries isolated from adult pigs fed the HF diet. 2) The combination of a HF diet and Sed lifestyle produced greater impairment of ACh-induced relaxation than the HF diet or Sed lifestyle alone. 3) In the presence of l-NAME, Indo, or l-NAME + Indo, ACh-induced relaxation was similar in Br arteries from HF and NF pigs. 4) Relaxation to high doses of BK was impaired in Br arteries from HF pigs. 5) In the presence of l-NAME, Indo, or l-NAME + Indo, impaired responses to BK were no longer apparent in arteries from HF pigs. 6) Ex significantly improved ACh-induced relaxation and tended to improve BK-induced relaxation. 7) The beneficial effect of Ex on ACh-induced relaxation remained significant even in the presence of l-NAME, Indo, and l-NAME + Indo. Collectively, these data indicate that hyperlipidemia impaired endothelium-dependent relaxation by impairing NO- and PGI2-mediated relaxation. In addition, these data indicate that Ex attenuated the effects of hyperlipidemia by enhancing a vasodilator mechanism other than NO or PGI2.

Influence of hyperlipidemia on endothelium-dependent relaxation. A number of porcine models of hyperlipidemia have been used to study mechanisms of endothelial dysfunction in coronary and peripheral arteries from juvenile pigs (1, 3, 10, 26, 27, 33). In each of these studies, endothelial dysfunction was attributed...
in part to decreased bioavailability of NO. In the present study, the effects of hyperlipidemia were assessed in Br arteries from adult Yucatan miniature swine to minimize the effects of growth and development. The results indicated that endothelium-dependent relaxation was significantly blunted in Br arteries from pigs fed the HF diet (Figs. 1 and 2), indicating that hyperlipidemia impaired endothelial function. Importantly, impaired responses to ACh and BK were not apparent in the presence of L-NAME, Indo, or L-NAME + Indo, indicating that hyperlipidemia specifically impaired NO- and PGI₂-mediated vasodilator mechanisms. Thus hyperlipidemia impaired endothelial function in Br arteries from adult pigs, and the mechanism accounting for the dysfunction was similar to changes reported in juvenile pigs fed a HF diet (1, 3, 10, 26, 27, 33).

Influence of exercise training on endothelium-dependent relaxation. A primary aim of this study was to determine whether endurance exercise training would attenuate or reverse the effects of hyperlipidemia on endothelial function in porcine Br arteries. We hypothe-

Fig. 5. Immunohistochemistry on porcine Br artery rings. Cross section of Br arteries stained for superoxide dismutase (SOD)-1 protein.

Fig. 6. Immunohistochemistry on porcine Br artery rings. Cross section of Br arteries stained for caveolin-1 (CAV-1) protein. Staining was confined to the endothelium (arrow).
esized that exercise training would abrogate the effects of hyperlipidemia by enhancing NO-mediated relaxation in Br arteries. This hypothesis was based on previous studies demonstrating that endurance exercise training improves NO-mediated, endothelium-dependent relaxation in peripheral and coronary arteries of NF-fed pigs (7, 17, 22, 23, 32). The results of this study indicated that exercise training improved ACh-induced relaxation (Fig. 1A) and tended to improve BK-induced relaxation (Fig. 2A), such that endothelium-dependent relaxation of arteries from HF-Ex pigs was not significantly different from that of Br arteries from NF-Sed or NF-Ex pigs.

It could be argued that the NF diet used in the present study was actually low fat, resulting in an exaggeration of the deleterious effects of diet on endothelial function. Indeed, the diet was low fat relative to the typical American diet (11). However, it is important to emphasize that endothelium-dependent relaxation in arteries from pigs fed the HF diet was significantly improved by exercise. Thus the HF pigs received an important benefit from exercise training.

To determine whether the protective effects of exercise training were mediated by NO, ACh- and BK-induced relaxation was assessed in the presence of L-NAME to inhibit NOS. Importantly, the beneficial effect of Ex on ACh-induced relaxation persisted in HF-Ex arteries in the presence of L-NAME (Fig. 1B), indicating that the protective effect of exercise was not due to enhanced NO-mediated relaxation.

Interestingly, eNOS protein expression was increased by exercise training in HF arteries (Fig. 7A). The reason why exercise training increased eNOS expression yet did not enhance NO-mediated relaxation in arteries from hyperlipidemic pigs is not known. It is known, however, that superoxide anion can rapidly degrade NO produced by eNOS (8). Therefore, it is conceivable that exercise training increased eNOS activity and that free radical inactivation of NO prevented enhancement of NO-mediated relaxation. Indeed, increased production of superoxide anion has been reported in atherosclerotic arteries from cholesterol-fed rabbits (20, 21). It is also important to note that in previous studies indicating that exercise training enhanced NO-mediated relaxation in porcine coronary arterioles, both eNOS and SOD-1 expression were increased (22, 24, 34). In the present study, exercise did not increase SOD-1 protein expression in Br arteries (Fig. 7B). Therefore, it is possible that the lack of increase in SOD-1 expression and associated increase in the capacity to scavenge superoxide contributed to the lack of enhanced NO-mediated relaxation in arteries from HF-Ex pigs.

Alternatively, the lack of enhanced NO-mediated relaxation in Br arteries from HF-Ex pigs may have involved enhanced inactivation of eNOS by caveolin. Therefore, on the basis of experimental evidence indicating that protein-protein interaction between eNOS and CAV-1 reduces eNOS activity (9, 19) and evidence that hypercholesterolemia increases CAV-1 expression in cultured endothelial cells (5), we tested the hypothesis that CAV-1 protein expression was greater in Br arteries from the HF pigs. Importantly, immunoblot analysis revealed that CAV-1 protein expression was not altered by hyperlipidemia or exercise training (Fig. 7C).
7C). Although these results indicate that consumption of the HF diet did not increase expression of CAV-1 protein, the possibility that hyperlipidemia enhanced inhibitory CAV-1-eNOS complex formation, resulting in attenuated NO production, cannot be ruled out.

To determine whether improved endothelium-dependent relaxation in Br arteries from hyperlipidemic pigs was due to enhanced PGI2-mediated vasodilation, relaxation responses were assessed in the presence of Indo to block cyclooxygenase. Results indicated that enhanced ACh-induced relaxation of Br arteries from exercise trained pigs persisted in the presence of Indo (Fig. 1C). Therefore, although the detrimental effects of hyperlipidemia were mediated in part by impaired PGI2-meditation of endothelium-dependent relaxation, the protective effect of exercise was not due to enhancement of this pathway.

To determine whether improved vasorelaxation responses in arteries from HF-Ex pigs were due to enhancement of a NOS- and COX-independent vasodilator mechanism, relaxation responses were assessed in the presence of L-NAME + Indo (double blockade). In the presence of double blockade, residual relaxation in response to ACh and BK can be attributed to a vasodilator molecule other than NO and PGI2. Importantly, ACh-induced relaxation of Br arteries from Ex pigs persisted in the presence of double blockade (Fig. 1D). These data indicate that exercise training preserved endothelial function in HF-Ex arteries by enhancing vasodilation by a vasodilator molecule other than NO and PGI2, possibly endothelium-derived hyperpolarizing factor. Further study is needed to directly test this hypothesis.

In the present study, SNP was used to assess the effects of hyperlipidemia on vascular smooth muscle function. SNP-induced relaxation was similar in Br rings from all groups of pigs (Fig. 3), indicating that the ability of vascular smooth muscle to relax to endothelial derived NO was not impaired in Br arteries of HF-fed pigs and that hyperlipidemia selectively impaired endothelium-dependent vasodilator mechanisms.

In summary, the results of this study indicate that endothelium-dependent relaxation was impaired by hyperlipidemia in porcine Br arteries. The detrimental effects of hyperlipidemia were characterized by blunted relaxation responses to ACh and BK as well as by decreased reliance on NO and PGI2 to mediate ACh- and BK-induced relaxation. In addition, these data indicate that endurance exercise training prevented or reversed the deleterious effects of hyperlipidemia on endothelial function by enhancing a vasodilator mechanism independent of NO and PGI2. Further study is needed to determine whether the protective effects of endurance exercise training observed in the Br occur in other vascular beds.

We gratefully acknowledge the expert technical assistance of Pam Thorne, Denise Holiman, Jennifer Casati, and Tommy Strawn. This work was supported by National Heart, Lung, and Blood Institute Grants HL-36088 and HL-52490 (to M. H. Laughlin).

REFERENCES


J Appl Physiol • VOL 94 • MAY 2003 • www.jap.org


