Exercise attenuates the effects of hypercholesterolemia on endothelium-dependent relaxation in coronary arteries from adult female pigs

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Woodman, Christopher R., James R. Turk, James W. E. Rush, and M. Harold Laughlin. Exercise attenuates the effects of hypercholesterolemia on endothelium-dependent relaxation in coronary arteries from adult female pigs. J Appl Physiol 96: 1105–1113, 2004.—We tested the hypothesis that exercise training (Ex) attenuates the effects of hypercholesterolemia on endothelium-dependent relaxation in left anterior descending coronary arteries. Adult female pigs were fed a normal-fat (NF) or high-fat (HF) diet for 20 wk. Four weeks after the diet was initiated, pigs were trained or remained sedentary (Sed) for 16 wk, yielding four groups of pigs: 1) NF-Sed, 2) NF-Ex, 3) HF-Sed, and 4) HF-Ex. Sensitivity (EC50) to bradykinin (BK) was impaired in HF-Sed arteries. Ex improved BK-induced relaxation such that the EC50 and maximal response to BK in HF-Ex arteries was not different from that in NF-Sed and NF-Ex. ACh-induced constriction was less in HF-Ex arteries than in HF-Sed, NF-Sed, and NF-Ex. To determine the mechanism(s) by which HF and Ex affected responses to BK and ACh, vasoactive responses were assessed in the presence of L-NAME to inhibit nitric oxide (NO) synthase, indomethacin (Indo); to inhibit cyclooxygenase, and L-NAME + Indo. L-NAME inhibited BK-induced relaxation in NF (not HF) arteries. Indo did not significantly alter relaxation to BK in NF arteries; however, relaxation was enhanced in HF-Sed arteries. Double blockade with L-NAME + Indo attenuated BK-induced relaxation in NF arteries and eliminated relaxation in HF arteries. Neither L-NAME nor Indo altered constrictor responses to ACh in NF or HF arteries; however, double blockade with L-NAME + Indo attenuated constriction to ACh in NF-Ex arteries. Endothelium-independent relaxation to sodium nitroprusside was enhanced in HF-Sed and HF-Ex arteries. Collectively, these results indicate that HF impaired endothelial function in coronary arteries by impairing production of NO and by enhancing production of a constrictor that was inhibited by Indo. Ex attenuated the effects of hypercholesterolemia by improving NO-mediated, endothelium-dependent relaxation and by reducing the influence of the Indo-sensitive constrictor.

Whereas the mechanism(s) for the detrimental effects of hypercholesterolemia on endothelial function is not completely understood; studies indicate that a reduction in the bioavailability of nitric oxide (NO) may contribute to the dysfunction (2).

Recently, the effects of hypercholesterolemia on endothelial function were studied in brachial arteries from adult female Yucatan miniature swine to assess the effects of a high-fat diet in developmentally mature animals (22). The results of this study indicated that hypercholesterolemia, induced by 20 wk on a high-fat diet, impaired endothelium-dependent relaxation in brachial arteries by impairing NO- and PGI2-mediated relaxation. In addition, the study indicated that the detrimental effects of hypercholesterolemia were attenuated, or reversed, in pigs that completed a 16-wk endurance exercise program (22).

Importantly, whereas the deleterious effect of hypercholesterolemia on endothelial function in brachial arteries was due to impairment of NO-mediated, endothelium-dependent relaxation, the protective effect of exercise was due to enhancement of a vasodilator mechanism independent of NO and prostacyclin, possibly endothelium-derived hyperpolarizing factor (22).

The purpose of the present study was to determine whether endurance exercise training also attenuates the effects of hypercholesterolemia on endothelial function in porcine coronary arteries. We hypothesized that exercise training would attenuate or reverse the detrimental effects of hypercholesterolemia on endothelial function in porcine coronary arteries by enhancing NO-mediated, endothelium-dependent relaxation.

METHODS

Experimental animals. Before this study was initiated, 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 Research Farm, Columbia, MO). 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. One-half of the pigs (n = 16) were provided a normal-fat (NF) diet (Purina Lab Mini-pig Chow) in which 8% of daily caloric intake from fat 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 chloride (0.7%). Pigs provided the HF diet derived 46% of their daily caloric intake from fat (4). Four weeks after the diet was initiated, 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-Sed (n = 8), 2) NF-Ex (n = 8), 3) HF-Sed (n = 8), and 4) HF-Ex (n = 8). It is important to note that plasma lipid and brachial artery function data from the pigs used in the present study have been reported elsewhere (20, 22). The results indicated that the HF diet significantly elevated plasma levels of cholesterol and triglycerides (20) and impaired endothelium-dependent relaxation in brachial arteries of Sed pigs (22).

Training program. The exercise training protocol used in the present study has been published previously in detail (12, 14, 20, 22). In brief, pigs were familiarized with running on a motorized treadmill and randomly assigned to an Ex group or a Sed group (n = 16 wk). Pigs assigned to the Ex group ran on a treadmill 90 min/day, 5 days/wk, for 16 wk. Pigs assigned to the Sed group were restricted to their home range (n = 16 wk). At the conclusion of the 16-wk training program, the Ex and Sed pigs performed a graded-intensity treadmill exercise test to exhaustion to assess exercise capacity (11). The training program resulted in 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 (20).

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). After an intrathoracic incision (intravenous), hearts were removed and placed in iced Krebs bicarbonate buffer solution (4°C). Segments of the left anterior descending coronary artery (LAD) 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. Procedures used to assess the length-tension relationship have been published previously in detail (22). The maximal point in 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, KCl was washed out with Krebs bicarbonate buffer solution and vascular rings were allowed 1 h to stabilize before the experimental protocols were initiated. All pharmacological studies were subsequently conducted at Lmax.

Assessment of vasorelaxation and vasoconstriction. Procedures used to assess vasoactive responses of LAD rings have been published previously in detail (22). Before dose-response curves were initiated, all arterial rings were preconstricted with PGE2 (30 μM). Endothelium-dependent vasorelaxation was assessed by using bradykinin (BK; 10^{-11}–10^{-6} M). Endothelium-independent relaxation was assessed with sodium nitroprusside (SNP; 10^{-10}–10^{-4} M). BK should be noted, however, that in porcine coronary arteries, ACh is a vasoconstrictor, not a vasodilator (7, 15). A total of four LAD rings were studied in parallel from each pig. In arterial ring 1, responses to agonist were measured by using NO-sensitive nitro-l-arginine methyl ester (l-NAME; 300 μM) to block NO synthase (NOS). In arterial ring 3, the importance of prostacyclin (PGI2) in vasoactive 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. 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 the next protocol was initiated.

Immunohistochemistry. The LAD attached to the left ventricle was sampled immediately distal to the site from which LAD rings were obtained. Endothelial NOS (eNOS), superoxide dismutase (SOD)-1, and caveolin-1 (Cav-1) protein expression in endothelial and vascular smooth-muscle cells of LAD were assessed with immunohistochemistry. All procedures used for immunohistochemistry have been published previously in detail (22). The specific antibodies and dilutions used for immunohistochemistry were as follows: eNOS (1:800; Transduction Laboratories), SOD-1 (1:1800; Stressgen), and Cav-1 (1:800; Santa Cruz Laboratories). All primary antibodies were incubated with tissue sections overnight at 4°C. Sections were examined and photographed with an Olympus BX40 photomicroscope.

Immunoblot analysis. Relative differences in eNOS, SOD-1, SOD-2, and Cav-1 protein content in LAD rings were assessed by using immunoblot analysis. LAD rings were homogenized in a buffer consisting of 50 mM Tris-HCl, pH 7.4, 6 M urea, and 2% SDS with a glass–glass homogenizer. After a 2-h incubation at 45°C and centrifugation (10,000 g, 1 min), protein concentration of the supernatant was determined by using the bicinchoninic acid assay (Pierce). Before electrophoresis, aliquots of these samples were supplemented with 150 mM dithiothreitol and boiled for 1 min. Samples containing 30 μg protein were loaded onto gels, electrophoresed, and electroblotted to polyvinylidene difluoride membranes. Membranes were probed for eNOS (Transduction Laboratories, 1:3500), Cav-1 (Transduction Laboratories, 1:2000), SOD-1 (Stressgen, 1:2000), and SOD-2 (Stressgen, 1:2000). Secondary antibodies were conjugated with horseradish peroxidase. All antibody and blocking solutions contained 5% nonfat milk and 0.1% Tween in Tris-buffered saline. Immunoblot signals were generated via chemiluminescence (Amersham) and captured on X-ray film (Amersham). Scanning densitometry (NIH Image) was used to quantify immunoblot signals. To facilitate comparisons, equal numbers of samples from the four treatment groups being compared were loaded in the same gel. 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 difference from the NF-Sed arteries.

Solutions and drugs. Krebs bicarbonate buffer solution contained (in mM) 131.5 NaCl, 5.0 KCl, 1.2 NaH2PO4, 1.2 MgCl2, 2.5 CaCl2, 11.2 glucose, 20.8 NaHCO3, 0.003 propranolol, and 0.025 EDTA. Solutions were aerated with 95% O2-5% CO2 (ph 7.4) and maintained at 37°C. All drugs and chemicals were purchased from Sigma Chemical.

Statistical analysis. All values are means ± SE. Between-group differences in arterial ring characteristics, EC50 values, and IC50 values were determined with one-way ANOVA. Means of the EC50 and IC50 values are presented as the negative log of the molar concentration. Concentration-response curves were analyzed by two-way ANOVA with repeated-measures on one factor (dose). To determine the mechanism(s) by which HF and Ex affected responses to BK and ACh, the two-way repeated-measures ANOVA was used under the following four conditions: in the absence of enzyme inhibitors, in the presence of l-NAME, in the presence of Indo, and in the presence of l-NAME + Indo. When a significant F value was obtained, post hoc analyses were performed with Duncan’s multiple-range test. Statistical significance was set at the P ≤ 0.05 probability level.

RESULTS

Vascular ring characteristics. Arterial ring characteristics are presented in Table 1. One-way ANOVA revealed that inner diameter, axial length, and resting tension were similar in all groups of arteries. Outer diameter and wall thickness were significantly greater in LAD from HF pigs than in LAD from NF pigs. In addition, percent stretch required to reach the Lmax was lower in LAD from HF pigs. The lower percent stretch to reach Lmax in HF LAD, taken together with a similar resting tension, suggests that vessel stiffness was greater in HF LAD.
BK responses. BK produced a concentration-dependent relaxation of LAD from all groups of pigs (Fig. 1). Statistical analysis of dose-response curves indicated that the EC₅₀ for BK-induced relaxation in HF-Sed arteries was significantly greater than the EC₅₀ for BK in NF-Sed and NF-Ex arteries (Table 2). Maximal relaxation to BK was not significantly lower in HF-Sed arteries than in NF-Sed and NF-Ex arteries. Exercise attenuated or reversed the effects of the HF diet such that the EC₅₀ and maximal response to BK in HF-Ex arteries was not significantly different from NF-Sed and NF-Ex arteries.

Effects of inhibitors on BK-induced relaxation. In the presence of l-NAME, BK-induced relaxation was significantly inhibited in NF-Sed and NF-Ex LAD (Fig. 2A). l-NAME did not significantly impair BK-induced relaxation in HF-Sed or HF-Ex arteries (Fig. 2B). Thus, in the presence of l-NAME, BK-induced relaxation was similar in all groups (Fig. 2C). Indo tended to impair BK-induced relaxation in HF-Sed arteries but not HF-Ex arteries (Fig. 3A). Axial length, mm 2.88 ± 0.23, 2.92 ± 0.12, 2.97 ± 0.05, 3.14 ± 0.13.

Percent stretch to Lmax, % 179 ± 3, 179 ± 2, 166 ± 2, 170 ± 2.

Values are means ± SE; n = 8 pigs per group. All data were analyzed by one-way ANOVA. NF-Sed, normal fat sedentary; NF-Ex, normal fat exercise trained. At high doses of BK, relaxation tended to be lower in HF-Sed than in NF-Sed and NF-Ex arteries (group × dose; P = 0.07). In addition, the sensitivity (EC₅₀) to BK was significantly less in HF-Sed arteries than in NF-Sed and NF-Ex arteries. Exercise improved BK-induced relaxation such that the EC₅₀ and maximal response to BK in HF-Ex arteries were not different from results in NF-Sed and NF-Ex arteries.

ACh responses. ACh elicited a concentration-dependent constriction of LAD in all groups of arteries (Fig. 5). Statistical analysis of dose-response curves indicated that the IC₅₀ for ACh-induced constriction in HF-Sed arteries was similar to that in NF-Sed and NF-Ex arteries. In contrast, the IC₅₀ for ACh-induced constriction in HF-Ex arteries was significantly lower than the IC₅₀ for ACh in all other groups (Table 2). Maximal ACh-induced constriction was similar in all groups.

Effects of inhibitors on ACh-induced constriction. l-NAME did not significantly alter ACh-induced constriction in NF-Sed and NF-Ex arteries (Fig. 6A) or HF pigs (Fig. 6B). Similarly, Indo did not significantly alter ACh-induced constriction in NF (Fig. 7A) or HF arteries (Fig. 7B). Double blockade with l-NAME + Indo attenuated constriction to ACh in NF-Ex arteries but not in NF-Sed arteries (Fig. 8A). Double blockade did not significantly alter constrictor responses to ACh in HF-Sed or HF-Ex arteries (Fig. 8B).

SNP responses. SNP elicited a concentration-dependent relaxation in LAD rings from all groups of pigs (Fig. 9). Direct smooth-muscle relaxation induced by SNP was similar in NF-Sed and NF-Ex pigs; however, relaxation induced by SNP was enhanced in LAD from HF pigs with the greatest relaxation seen in HF-Ex arteries.

Immunohistochemistry. Immunohistochemistry revealed positive immunoreactivity for eNOS in the endothelium of LAD in all groups of pigs. Faint positive staining for eNOS was also present in foam cells in HF-Sed and HF-Ex LAD (Fig. 10). Positive immunoreactivity for SOD-1 and Cav-1 was present in endothelium and vascular smooth muscle in all groups of pigs (Figs. 11 and 12). Positive staining for SOD-1 and Cav-1 was also present in foam cells in HF-Sed and HF-Ex pigs (Figs. 11 and 12). In the absence of primary antibody against eNOS, SOD-1, or Cav-1, no immunoreactivity was detected (data not shown).

Table 1. Characteristics of left anterior descending coronary arteries

<table>
<thead>
<tr>
<th>Variable</th>
<th>NF-Sed</th>
<th>NF-Ex</th>
<th>HF-Sed</th>
<th>HF-Ex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer diameter, mm</td>
<td>2.58 ± 0.09</td>
<td>2.79 ± 0.06</td>
<td>2.93 ± 0.14</td>
<td>3.26 ± 0.13†</td>
</tr>
<tr>
<td>Inner diameter, mm</td>
<td>1.61 ± 0.08</td>
<td>1.53 ± 0.07</td>
<td>1.43 ± 0.08</td>
<td>1.50 ± 0.08</td>
</tr>
<tr>
<td>Wall thickness, mm</td>
<td>0.48 ± 0.04</td>
<td>0.63 ± 0.04</td>
<td>0.75 ± 0.08*</td>
<td>0.88 ± 0.06†</td>
</tr>
<tr>
<td>Axial length, mm</td>
<td>2.88 ± 0.23</td>
<td>2.92 ± 0.12</td>
<td>2.97 ± 0.05</td>
<td>3.14 ± 0.13</td>
</tr>
<tr>
<td>Resting tension at Lmax, g</td>
<td>4.76 ± 0.37</td>
<td>4.84 ± 0.26</td>
<td>3.99 ± 0.39</td>
<td>5.85 ± 0.71</td>
</tr>
<tr>
<td>Percent stretch to Lmax, %</td>
<td>179 ± 3</td>
<td>179 ± 2</td>
<td>166 ± 2*</td>
<td>170 ± 2†</td>
</tr>
</tbody>
</table>

Table 2. EC₅₀ (−log M) values for bradykinin-induced relaxation and IC₅₀ (−log M) values for ACh-induced constriction of left anterior descending coronary arteries from normal-fat and high-fat fed pigs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NF-Sed</th>
<th>NF-Ex</th>
<th>HF-Sed</th>
<th>HF-Ex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradykinin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>−8.76 ± 0.2</td>
<td>−8.72 ± 0.1</td>
<td>−7.76 ± 0.5*‡</td>
<td>−8.69 ± 0.1</td>
</tr>
<tr>
<td>l-NAME</td>
<td>−8.66 ± 0.3</td>
<td>−8.37 ± 0.2</td>
<td>−8.42 ± 0.1</td>
<td>−8.89 ± 0.3</td>
</tr>
<tr>
<td>Indo</td>
<td>−8.38 ± 0.3</td>
<td>−8.83 ± 0.1</td>
<td>−8.35 ± 0.2</td>
<td>−8.81 ± 0.2</td>
</tr>
<tr>
<td>l-NAME +</td>
<td>−8.66 ± 0.2</td>
<td>−8.89 ± 0.3</td>
<td>−8.33 ± 0.5</td>
<td>−8.35 ± 0.3</td>
</tr>
<tr>
<td>Indo</td>
<td>−6.49 ± 0.2</td>
<td>−6.16 ± 0.2</td>
<td>−6.1 ± 0.2</td>
<td>−5.71 ± 0.2*</td>
</tr>
<tr>
<td>l-NAME</td>
<td>−6.34 ± 0.1</td>
<td>−6.12 ± 0.1</td>
<td>−5.82 ± 0.1*</td>
<td>−5.68 ± 0.1†</td>
</tr>
<tr>
<td>Indo</td>
<td>−6.36 ± 0.2</td>
<td>−6.00 ± 0.2</td>
<td>−6.15 ± 0.3</td>
<td>−5.53 ± 0.2</td>
</tr>
<tr>
<td>l-NAME +</td>
<td>−6.38 ± 0.1</td>
<td>−5.93 ± 0.2*</td>
<td>−5.57 ± 0.1*</td>
<td>−5.39 ± 0.1†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 pigs per group. l-NAME, Nω-nitro-l-arginine methyl ester. Significantly different from *NF-Sed, †NF-Ex, §HF-Ex: P ≤ 0.05.
Immunoblot analysis. The effect of exercise training and hypercholesterolemia on eNOS, Cav-1, SOD-1, and SOD-2 protein content in LAD rings is shown in Fig. 13. Immunoblot analyses revealed elevated eNOS protein levels in NF-Ex \( (P < 0.006) \) and HF-Sed \( (P < 0.015) \) arteries. In contrast, neither exercise nor diet affected the relative levels of Cav-1 or SOD-1 protein in LAD artery rings. SOD-2 protein content was lower in NF-Ex \( (P < 0.035) \) and higher in HF-Sed arteries \( (P < 0.008) \) relative to NF-Sed.

DISCUSSION

The purpose of this study was to test the hypothesis that exercise training attenuates the detrimental effects of hypercholesterolemia on endothelium-dependent relaxation in porcine coronary arteries by enhancing NO-mediated relaxation. The primary findings of the study were as follows. 1) BK-induced relaxation was impaired in LAD from HF-Sed pigs. 2) Exercise prevented or reversed the effects of the HF diet such that the dose-response curve and EC\(_{50}\) for BK in HF-Ex arteries were not significantly different from those of NF-Sed and NF-Ex arteries. 3) L-NAME significantly inhibited BK-induced relaxation in NF arteries, not HF arteries. 4) Indo did not alter BK-induced relaxation in HF arteries; however, in the presence of Indo, relaxation was greater in HF-Sed than in HF-Ex arteries. 5) Double blockade with L-NAME + Indo attenuated relaxation in NF arteries and eliminated relaxation in HF arteries. Collectively, these results indicate that the HF diet impaired
BK-induced relaxation by impairing NO-mediated, endothelium-dependent relaxation and by increasing production of a constrictor that was inhibited by Indo. In addition, these data indicate that Ex preserved endothelial function in HF arteries by enhancing NO-mediated relaxation and by reducing the influence of the Indo-sensitive constrictor.

**Influence of hypercholesterolemia on endothelium-dependent relaxation.** Impairment of NO-mediated, endothelium-dependent relaxation has been reported previously in coronary and peripheral arteries from hypercholesterolemic juvenile pigs (1, 3, 8, 17, 18, 21). In addition, hypercholesterolemia has been shown to impair endothelial function in brachial arteries from adult female pigs (22). Results from the present study indicate that consumption of the HF diet similarly impaired endothelial BK-induced relaxation by impairing NO-mediated, endothelium-dependent relaxation and by increasing production of a constrictor that was inhibited by Indo. In addition, these data indicate that Ex preserved endothelial function in HF arteries by enhancing NO-mediated relaxation and by reducing the influence of the Indo-sensitive constrictor.

**Fig. 4.** BK-induced relaxation in LAD rings in the presence or absence of L-NAME (0.3 mM) and Indo (5.0 μM) to block nitric oxide synthase and cyclooxygenase. A: NF pigs. B: HF pigs. C: all groups. Values are means ± SE; n = 8 pigs per group. Percent relaxation was calculated as percent reduction in force from PGF$_{2\alpha}$ (30 μM)-induced tension. Two-way repeated-measures ANOVA indicated that L-NAME + Indo significantly inhibited BK-induced relaxation in LAD from NF pigs (A) and HF pigs (B). In the presence of L-NAME + Indo, BK-induced relaxation was similar in all groups (C). Significantly different from 1NF-Sed and 2NF-Ex, P < 0.05.

**Fig. 5.** ACh-induced constriction in LAD rings. Values are means ± SE; n = 8 pigs per group. Percent constriction was calculated as percent increase in force from PGF$_{2\alpha}$ (30 μM)-induced tension. Statistical analysis of dose-response curves indicated that the IC$_{50}$ for ACh-induced constriction in HF-Ex arteries was significantly lower than the IC$_{50}$ for all other groups; however, maximal ACh-induced constriction was similar in all groups.

**Fig. 6.** ACh-induced constriction in LAD rings in the presence or absence of L-NAME (0.3 mM) to inhibit nitric oxide synthase. A: NF pigs. B: HF pigs. Values are means ± SE; n = 8 pigs per group. Percent constriction was calculated as percent increase in force from PGF$_{2\alpha}$ (30 μM)-induced tension. Two-way repeated-measures ANOVA indicated that L-NAME did not significantly alter ACh-induced constriction in LAD from NF pigs (A) or HF pigs (B).
constrictor. Thus, in porcine coronary arteries from adult female pigs, hypercholesterolemia-induced endothelial dysfunction is associated with decreased production of NO and increased production of an Indo-sensitive constrictor.

Influence of exercise training on endothelium-dependent relaxation. Our laboratory reported previously that endurance exercise training prevented or reversed the effects of hypercholesterolemia on endothelium-dependent relaxation of porcine brachial arteries (22). In the present study, we determined whether Ex would also preserve endothelial function in coronary arteries, and we hypothesized that exercise training would enhance NO-mediated relaxation and restore endothelial function in LAD from HF pigs. This hypothesis was based on previous studies demonstrating that NO-mediated relaxation is enhanced in coronary arteries and arterioles of Ex pigs fed a NF diet (5, 14, 16). Results of this study indicate that Ex attenuated the effects of the HF diet such that maximal relaxation and EC50 for BK in HF-Ex arteries was not significantly different from that in NF-Sed and NF-Ex arteries (Fig. 1 and Table 2). Thus the protective effects of endurance exercise training observed in porcine brachial arteries of hyperlipidemic pigs (22) also occurred in coronary arteries. Importantly, present results indicated that the protective effect of Ex on endothelium-dependent relaxation of LADs was due, in part, to enhanced production of NO, because between-group differences in dose-response curves and ED50 values for BK were eliminated in the presence of l-NAME (Table 2).

To determine whether the protective effect of Ex on endothelium-dependent relaxation also involved altered COX products, relaxation responses were assessed in the presence of Indo to block cyclooxygenase. ACh-induced constriction in LAD rings in the presence or absence of Indo (5 μM) to inhibit cyclooxygenase. A: NF pigs. B: HF pigs. Values are means ± SE; n = 8 pigs per group. Percent constriction was calculated as percent increase in force from PGF2α (30 μM)-induced tension. Two-way repeated-measures ANOVA indicated that Indo did not significantly alter ACh-induced constriction in LAD from NF arteries (A) or HF arteries (B).

Fig. 7. ACh-induced constriction in LAD rings in the presence or absence of Indo (5 μM) to inhibit cyclooxygenase. A: NF pigs. B: HF pigs. Values are means ± SE; n = 8 pigs per group. Percent constriction was calculated as percent increase in force from PGF2α (30 μM)-induced tension. Two-way repeated-measures ANOVA indicated that Indo did not significantly alter ACh-induced constriction in LAD from NF arteries (A) or HF arteries (B).

Fig. 8. ACh-induced constriction in LAD rings in the presence or absence of l-NAME (0.3 mM) and Indo (5 μM) to inhibit nitric oxide synthase and cyclooxygenase. A: NF pigs. B: HF pigs. Values are means ± SE; n = 8 pigs per group. Percent constriction was calculated as percent increase in force from PGF2α (30 μM)-induced tension. Two-way repeated-measures ANOVA indicated that double blockade with l-NAME + Indo attenuated ACh-induced constriction in LAD from NF-Ex pigs but not NF-Sed pigs (A). Double blockade with l-NAME + Indo did not significantly alter ACh-induced constriction in HF-Sed or HF-Ex arteries (B). Significantly different from 1NF-Sed, 2NF-Ex, and 3HF-Sed: P < 0.05.

Fig. 9. Sodium nitroprusside (SNP)-induced relaxation of LAD rings. Values are means ± SE; n = 8 pigs per group. Percent relaxation was calculated as percent reduction in force from PGF2α (30 μM)-induced tension. Direct smooth-muscle relaxation induced by SNP was similar in NF-Sed and NF-Ex pigs; however, relaxation induced by SNP was enhanced in LAD from HF pigs with the greatest relaxation seen in HF-Ex arteries. Significantly different from 1NF-Sed and 2NF-Ex, P < 0.05.
strictor. In addition, these data suggest that Ex suppressed production of the prostanoid constrictor contributing to the protective effect of Ex. Further study is needed to directly test this hypothesis.

To determine whether enhancement of a NOS- and COX-independent vasodilator mechanism contributed to the protective effects of Ex on endothelium-dependent relaxation, relaxation responses were assessed in the presence of L-NAME + Indo (double blockade). Importantly, double blockade eliminated BK-induced relaxation in HF-Sed and HF-Ex arteries (Fig. 4B). These data indicate that a vasodilator pathway independent of NOS and COX did not contribute to BK-induced relaxation in HF LADs and that the protective effect of exercise was not due to enhancement of a NOS- and COX-independent mechanism in the LAD. Importantly, previously published studies of HF brachial arteries indicated that Ex preserved endothelial function by enhancing relaxation by a vasodilator molecule other than NO and PGI₂ (22). Thus while pigs fed the HF diet received an important benefit from Ex, the cellular mechanisms accounting for the protective effects of Ex in coronary arteries differed from mechanisms observed in brachial arteries (22).

It has been reported previously that consumption of a HF diet impaired relaxation responses to ACh in porcine brachial
arteries and that Ex improved relaxation to ACh such that responses in HF-Ex arteries were not different from those in NF arteries (22). Therefore, in the present study, the effects of HF and Ex on vasoactive responses to ACh were assessed in coronary arteries. It is important to note, however, that, in porcine coronary arteries, ACh is a potent vasoconstrictor, not an endothelium-dependent dilator (7, 15). Interestingly, constrictor responses of vascular smooth muscle to ACh were decreased by Ex in HF arteries such that constriction to ACh in HF-Ex LAD was less than for NF-Sed, NF-Ex, and HF-Sed arteries. Hambrecht et al. (6) have shown previously that exercise training attenuates constrictor responses to ACh in

Fig. 12. Representative panels of immunohistochemistry performed for caveolin-1 (Cav-1) in the LAD branch of the coronary artery. A: NF-Sed. B: NF-Ex. C: HF-Sed. D: HF-Ex. Note staining of endothelium and smooth-muscle cells in all sections. Also, note, in the insets, staining of foam cells in the sections from HF-Sed and HF-Ex (arrows). Bars = 100 μm.

Fig. 13. Immunoblot analyses of LAD rings. A: eNOS. B: Cav-1. C: SOD-1. D: SOD-2. Top: representative immunoblot illustrating 3 different samples (animals) from each treatment group. Bottom: summary data from n = 9 animals per group, each analyzed in duplicate immunoblots. Values are means ± SE, normalized to set the mean NF-Sed group results to unity for each protein analyzed. NS, NF-Sed; NX, NF-Ex; HS, HF-Sed; HX, HF-Ex. Significant difference vs. NF-Sed: *P < 0.05; †P < 0.01.
patients with coronary artery disease. Collectively, these results suggest that one mechanism by which Ex preserves vasodilator function in HF-LAD is decreased sensitivity of vascular smooth muscle to vasoconstrictor molecules.

To further assess the effects of HF and Ex on vascular smooth-muscle function, relaxation responses to SNP were assessed in LAD rings. Importantly, SNP-induced relaxation was enhanced in LAD rings from HF–Sed and HF–Ex pigs (Fig. 3). These data indicate that NO-mediated relaxation of vascular smooth muscle was enhanced by hypercholesterolemia. Although the mechanism accounting for enhanced relaxation to SNP is not clear, enhanced SNP responses have been reported previously in coronary arteries from hypercholesterolemic mice (10). It is conceivable that enhanced smooth-muscle responses to NO reflected adaptation in vascular smooth muscle, compensating for decreased production of endothelium-derived NO in hypercholesterolemic pigs. Regardless of the mechanism, the finding that vascular smooth muscle from HF pigs was more sensitive to NO, in conjunction with the finding that 1-NAME did not significantly inhibit BK-induced relaxation in HF arteries (Fig. 2B), is consistent with decreased release of endothelium-derived NO in HF arteries.

In summary, the results of this study indicate that endothelium-dependent relaxation was impaired by hypercholesterolemia in porcine coronary arteries. The detrimental effect of the HF diet was characterized by impaired relaxation to BK due to decreased production or release of NO. In addition, consumption of the HF diet appeared to increase production of a constrictor that was inhibited by Indo. Endurance exercise training attenuated the deleterious effects of hypercholesterolemia on endothelial function by enhancing NO-mediated, endothelium-dependent relaxation and by reducing production of the Indo-sensitive constrictor.

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