Short-term exercise training prevents micro- and macrovascular disease following coronary stenting

Xin Long,1 Ian N. Bratz,2 Mouhamad Alloosh,1 Jason M. Edwards,1 and Michael Sturek1

1Department of Cellular and Integrative Physiology, Indiana University School of Medicine, Indianapolis, Indiana; and 2Department of Integrative Medical Sciences, Northeastern Ohio University College of Medicine and Pharmacology, Rootstown, Ohio

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Long X, Bratz IN, Alloosh M, Edwards JM, Sturek M. Short-term exercise training prevents micro- and macrovascular disease following coronary stenting. J Appl Physiol 108: 1766–1774, 2010. First published March 18, 2010; doi:10.1152/japplphysiol.01014.2009.—The purpose of this study was to determine the effects of exercise on coronary blood flow and macrovascular atherosclerosis in response to stent deployment. Male Yucatan swine were placed on a control diet (C); on a high-fat/cholesterol diet (hypercholesterolemic; H); or on a high-fat/cholesterol diet and aerobically exercise trained (HX) starting after 36 wk on the diet. All pigs underwent coronary angiography and intravascular ultrasound (IVUS) guided placement of a bare metal stent in the circumflex coronary artery after 40 wk on diets and 3 wk later pigs underwent repeat angiography and IVUS and coronary blood flow (CBF) measurement. Average peak velocity (APV) was measured under basal conditions and in response to intracoronary application of the endothelium-dependent vasodilator bradykinin. There was a similar ~8-fold increase in total cholesterol in H and HX compared with control. Baseline CBF was increased above control and H in HX (P < 0.05). At all doses adenosine-induced CBF was impaired in H, but preserved in HX. Similarly, bradykinin-induced CBF was impaired in H vs. control, yet was potentiated in HX. Microvascular density was decreased in H and preserved in HX vs. control. Native atheroma in HX was lower relative to H and control, while in-stent stenosis in HX was not different from H. Hyperlipidemia-induced microvascular dysfunction after stent deployment may be a result of reduction in microvasculature. This is the first report that short-term exercise training near the time of stenting prevents stent-induced microvascular dysfunction and attenuates native atheroma independent of changes in plasma cholesterol in this porcine model.

DEPLOYMENT of a stent in a coronary artery in the setting of flow-limiting stable coronary artery disease (CAD) results in initial improvement in functional capacity and quality of life. However, almost half of patients undergoing coronary angioplasty/stenting eventually experience recurrent angina, resulting in a decline in functional capacity, and require further medical attention (16). It has been shown that coronary stenting mechanically damages vascular cells in the target conduit artery segment, endothelium in peri-stent segments (30), and induces downstream microvascular dysfunction that persists for weeks and has been implicated in causing exertional ischemia (5, 28, 42, 59, 61). Camici and Crea (5) termed this phenomenon poststent “iatrogenic coronary microvascular dysfunction,” which may be due to enhanced alpha-adrenergic constriction (19), impaired autoregulation (28, 59), or microemboli that may cause microinfarcts (50) and perhaps more severe rarefaction (microvessel dropout). Despite enormous efforts to reduce in-stent stenosis and microvascular dysfunction, there remains significant room for improvement in addressing both of these post-stent procedure complications. This is especially important because drug-eluting stents, which are the main treatment for flow-limiting lesions, cause even greater microvascular dysfunction than bare metal stents (29, 39).

Exercise training of patients with coronary artery disease elicits beneficial effects, including improvement in exercise tolerance, left ventricular function, and reduction in the ischemic response to submaximal workload after exercise training, and increased dilation of conduit and microvessel coronary arteries (2, 7, 15, 21, 22, 38). Most of these studies have shown concomitant changes in plasma cholesterol: specifically, decreased total and low-density lipoprotein (LDL) and increased high-density lipoprotein (HDL) cholesterol (3, 26, 46). Hambrecht et al. (22) removed the confounding variable of hypercholesterolemia by excluding those patients from their study, thus implying that the beneficial effects of exercise may be due to more direct actions of exercise on the vasculature. The benefits of exercise training may also extend to the population undergoing percutaneous coronary revascularization (3, 21, 26, 46). Belardinelli et al. (3) found that progression of macrovascular CAD in arterial segments proximal and distal to the angioplasty/stented segment, i.e., peri-stent CAD, was attenuated by exercise training and accompanied by an improved thallium perfusion stress test that is indicative of increased metabolic vasodilation of the coronary microvasculature. Further, exercise training may be more beneficial and cost effective than percutaneous coronary intervention in the setting of chronic stable CAD (21). Fleenor and Bowles (17) showed that long-term exercise training of pigs with normal plasma cholesterol attenuated conduit artery neointimal proliferation elicited by overexpansion injury from balloon angioplasty; however, microvascular effects were not determined. Further, the effects of plasma cholesterol and exercise training on in-stent stenosis, peri-stent CAD, and microvascular dysfunction after coronary stenting have not been studied. We have chosen to use the hyperlipidemic swine model, which is similar to human patients undergoing coronary stenting to test several hypotheses.

The purpose of this study was first to test the hypothesis that stent deployment in coronary arteries of hypercholesterolemic swine would elicit microvascular dysfunction and whether short-term exercise training would prevent the microvascular dysfunction and progression of macrovascular CAD after stenting. A second hypothesis tested whether exercise elicits bene-
METHODS

All protocols involving animals were approved by an Institutional Animal Care and Use Committee and complied fully with standards (1, 43). Nineteen male Yucatan swine, 52–70 kg, were randomized to 43 wk of control (C, \( n = 8 \)), high fat/cholesterol (H, \( n = 8 \)), or H with short-term exercise training (HX, \( n = 3 \)). The lower number of pigs in the HX group vs. C and H groups was used in strict compliance with the principle of reducing the number of animals exposed to surgical and exercise stress to the minimum number required for statistical power (1, 6, 44). H and HX were fed a diet consisting of 46% kcal from fat (41). The feed base consisted of mini pig chow with supplemental fat kilocalories from coconut oil (<1% trans fatty acids; Research Diets, New Brunswick, NJ) and cholesterol (Research Diets). Controls were fed a diet consisting of mini pig chow base with 8% kcal from fat.

Exercise training. Animals randomized to the exercise group began treadmill training 4 wk before initial cardiac catheterization. During the first week of training, the exercise pigs ran on the treadmill at 3 mph (endurance) with 0% grade for 20–30 min and at 4 mph (sprint) for 15 min. The speed and duration of running were increased progressively over the course of the following 4 wk. During the 4th through the 7th week of training, a typical 70-min training session consisted of the following: 1) 5-min warm-up at 2.5 mph, 2) 15-min sprint at speeds of 5.5–6 mph, 3) 40- to 45-min endurance run at 3.5 mph, and 4) 5-min cool down at 2 mph. Ranges of running speeds are presented because the exercise training advanced during the course of the week. Following coronary stenting, each animal resumed symptom-limited exercise training. This regimen was maintained for 3 wk after stenting when the animals returned for repeat cardiac catheterization. Exercise protocols were compliant with guidelines from the American Physiological Society (6).

Submaximal stress test. At weeks 1, 4, and 7 of exercise training the endurance-trained animals underwent a submaximal stress test consisting of running on a treadmill at 3.1 mph, 0% grade for 15 min at which point heart rate data were collected.

Stent procedure (12, 37, 55). Each animal received antiplatelet therapy with aspirin 325 mg daily starting the day before the initial catheterization procedure and continuing through the completion of the study. Each pig was fasted overnight, and all animals arrived at the same time of day for procedure. Animals received preanesthesia with intramuscular injections of atropine (0.05 mg/kg), xylazine (2.2 mg/kg), and telazol (6.6 mg/kg). Following intubation, isoflurane (2–4%, with oxygen) was administered to maintain stable systemic hemodynamics and a stable level of anesthesia. Heart rate, blood pressure (tail cuff and aortic), respiratory rate, and electrocardiographic data were continuously monitored throughout the procedure. Under sterile conditions, the right femoral artery was exposed with surgical cut-down technique, and an 8-F vascular introducer sheath was inserted into the femoral artery following by administration of heparin 200 U/kg. An 8-F Amplatz L (sizes 0.75–2.0) guiding catheter (Cordis, Miami, FL) was inserted through the sheath and advanced to engage the left main coronary ostium (Fig. 1A). Blood pressure measurements were recorded directly from the coronary ostium via a pressure transducer.
Coronary vascular resistance (CVR, mmHg ml⁻¹ min⁻¹) was calculated as mean arterial pressure (MAP) divided by CBF. The rate-pressure product (RPP, beats/min × mmHg) was calculated as heart rate (HR, beats/min) multiplied by systolic blood pressure (SBP, mmHg). Vessel cross-sectional area was calculated offline from recorded IVUS images using the commercially available Sonos Intravascular Imaging software package. End-systolic and end-diastolic cross-sectional areas were determined for three consecutive cardiac cycles. The average of these six measurements was taken as the CSA to be used in subsequent calculations and analysis.

**Lipid analysis.** Blood was taken via the anterior vena cava and collected into vacutainers containing 0.117 ml of 15% EDTA after 18 h of fasting, centrifuged in the cold at 4°C for 20 min at 2,000 rpm in a Marathon 21,000 R Centrifuge (Fisher Scientific, Pittsburgh, PA), and frozen at −80°C. Plasma was analyzed for triglycerides, total cholesterol, and high-density lipoprotein (HDL) while low-density lipoprotein (LDL) was calculated from the Friedewald equation: LDL = total cholesterol – HDL – (triglyceride/5) (10).

For each plasma analysis, all samples for a given subject were analyzed in a single run. Plasma triglyceride and total cholesterol were assayed directly by standard enzymatic kit (Thermo Trace, Melbourne, Australia). HDL was measured by precipitating apolipoprotein B-containing lipoproteins with heparin-MnCl₂. The supernatant was used to assess HDL using the aforementioned total cholesterol kit and method.

**Quantification of atherosclerosis and neointima formation.** To determine extent of atherosclerosis in the coronary arteries, we performed IVUS catheterization on the circumflex and left anterior descending arteries. The left anterior descending was not previously formed IVUS catheterization on the circumflex and left anterior descending arteries. The left anterior descending was not previously treated with antibodies, each section was deparaffinized, and stained with hematoxylin and eosin. The adventitia, which is composed predominantly of collagen, was used as the reference color (1). The adventitia, which is composed predominantly of collagen, was used as the reference color. Immunostaining was performed on sections of stained arteries. Although technically very challenging, we obtained several sections for histology per animal per stain. In animals where more than one section was obtained, values were averaged. Neointima formation (Fig. 3B) was determined by obtaining area measurements bounded by the external elastic lamina and internal elastic lamina (tunica media), internal elastic lamina and adventitia (neointima) using commercially available software (ImagePro 3.0). The percent stenosis was calculated by dividing the area of the neointima by the area demarcated by the internal elastic lamina × 100. Collagen content in the sections of the stented arteries was determined by colorimetric analysis (9). The adventitia, which is composed predominantly of collagen, was used as the reference color.
myocardium contained a wide range of microvessels and thus were normalized to cross-sectional area. Optical density (OD) of microvessel cross sections was normalized to the average of the control values and expressed as a percentage of control as indicated in the following: (OD of each individual vessel)/(average OD of control vessels) × 100% = OD as a percentage of control. Finally, microvessel density was measured by counting the number of microvessels in randomly selected 6.5-mm² areas.

Data analysis. Statistical analysis was performed using commercially available software (SPSS version 10, SigmaStat 5.0). Coronary blood flow responses and hemodynamic parameters were compared between groups using repeated-measures ANOVA. Vessel cross-sectional area and percent degrees atheroma were compared between groups using single-factor ANOVA. The Dunnett’s T3 multiple comparison test was used for data that were not normally distributed (microvessel density). In all cases, P < 0.05 was considered significant.

RESULTS

High-fat/cholesterol diet resulted in ~8-fold increase in plasma total cholesterol, greater than 20-fold increase in LDL cholesterol, and a slight but significant increase in HDL cholesterol in H and HX relative to control (Table 1). These differences resulted in a 16-fold increase in LDL/HDL ratio in H and HX vs. control. Despite the significant cholesterol response to diet, the experimental design ensured that the body weight was not different between groups. Heart rate response was taken during a submaximal treadmill stress test with the pig running at 3.1 mph at 0% grade for 15 min. In H, heart rate response to submaximal stress testing was ~25% lower after 7 wk of training compared with performance on submaximal stress testing completed at the beginning of week 1 (Table 1), thus confirming the efficacy of the exercise training regimen. Heart weight, mean arterial pressure, and rate pressure product were not different between groups as shown in Table 1. IVUS interrogation revealed no flow-limiting stenosis in the studied vessel. Baseline conduit cross-sectional area was not different between groups and did not change with administration of vasodilators.

As shown in Fig. 2A, baseline CBF in control is ~50% in HX relative to control and ~100% relative to H (P < 0.05). The baseline CBF in H was lower vs. control but did not reach statistical significance (P = 0.08). Interestingly, bradykinin-induced CBF was ~40% lower in H relative to control at all doses (Fig. 2B, P < 0.05). Short-term exercise training potentiated the CBF responses to bradykinin relative to control. Figure 2C demonstrated adenosine-induced CBF was reduced 50% in H vs. control at all doses (P < 0.05). Similarly, exercise training prevented the impaired CBF response to adenosine.

Coronary vascular resistance is shown in Table 2. Baseline CVR was elevated in H relative to control (2.22 ± 0.23 vs. 1.46 ± 0.14 mmHg·ml⁻¹·min⁻¹), whereas short-term exercise training significant reduced baseline CVR (0.96 ± 0.05 mmHg·ml⁻¹·min⁻¹). Adenosine-induced CBF was approximately two times higher in H vs. control at all doses (i.e., 1.60 ± 0.15 vs. 0.88 ± 0.10 mmHg·ml⁻¹·min⁻¹ for adenosine 0.33 µg/kg). Short-term exercise training preserved the CVR response to adenosine (0.86 ± 0.16 mmHg·ml⁻¹·min⁻¹ at 0.33 µg/kg). Similarly, CVR in response to bradykinin administration was approximately 50–60% higher in H than control at all doses (i.e., 1.34 ± 0.05 vs. 0.82 ± 0.06 at bradykinin 4 ng/kg). Similar to adenosine, the impaired response to bradykinin was prevented with exercise training (0.80 ± 0.13 at bradykinin 4 ng/kg).

In addition to assessment of microvascular function, we investigated the effect of exercise on the macrovascular atheroma along the entire length of the artery that was interrogated by IVUS. Results of IVUS analysis are shown in Table 3. The overall macrovascular atheroma in H was almost twofold greater than control as shown as circumflex percent degrees atheroma. Remarkably, this response was completely prevented with exercise training. At death the left anterior descending (LAD), which had not been previously accessed, was also interrogated with IVUS to determine the effects of short-term exercise on native atheroma not related to coronary stenting. As shown in Table 3 there was significantly higher percent degrees atheroma in LAD of H vs. control, which was completely absent of atheroma. Importantly, exercise reduced LAD atheroma. Note that overall atheroma burden was evident.

Table 1. Phenotypic characteristics of control, H, and HX and adaptations to exercise

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C</th>
<th>H</th>
<th>HX</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>59 ± 2</td>
<td>59 ± 4</td>
<td>58 ± 3</td>
<td>None</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>52 ± 4</td>
<td>466 ± 78</td>
<td>393 ± 112</td>
<td>C &lt; H, HX</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>14 ± 3</td>
<td>408 ± 72</td>
<td>332 ± 115</td>
<td>C &lt; H, HX</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>30 ± 3</td>
<td>51 ± 4</td>
<td>50 ± 8</td>
<td>C &lt; H, HX</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>0.53 ± 0.1</td>
<td>7.97 ± 0.8</td>
<td>7.75 ± 3.4</td>
<td>C &lt; H, HX</td>
</tr>
<tr>
<td>TG, mg/dl</td>
<td>34 ± 5</td>
<td>41 ± 9</td>
<td>61 ± 34</td>
<td>None</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>77 ± 4</td>
<td>76 ± 6</td>
<td>86 ± 6</td>
<td>None</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>50 ± 4</td>
<td>52 ± 6</td>
<td>57 ± 6</td>
<td>None</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>88 ± 6</td>
<td>77 ± 9</td>
<td>105 ± 9</td>
<td>None</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>59 ± 4</td>
<td>60 ± 6</td>
<td>67 ± 6</td>
<td>None</td>
</tr>
<tr>
<td>RPP, beats/min-mmHg</td>
<td>6,730 ± 580</td>
<td>5,800 ± 820</td>
<td>9,000 ± 820</td>
<td>None</td>
</tr>
<tr>
<td>CSA, mm²</td>
<td>12 ± 3</td>
<td>12 ± 2</td>
<td>12 ± 3</td>
<td>None</td>
</tr>
<tr>
<td>Heart wt, g</td>
<td>235 ± 12</td>
<td>263 ± 18</td>
<td>236 ± 7</td>
<td>None</td>
</tr>
<tr>
<td>Submaximal stress test exercise heart rate, beats/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Start exercise training</td>
<td>200 ± 30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mid exercise training</td>
<td>160 ± 40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>End exercise training</td>
<td>160 ± 10</td>
<td></td>
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</table>

Values are means ± SE. Male Yucatan swine were on a control diet (C); on a high-fat/cholesterol diet (H); or on a high-fat/cholesterol diet and aerobically exercise trained (HX). LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride; MAP, mean arterial pressure; RPP, rate-pressure product; CSA, conduit cross-sectional area. Statistical significance at P < 0.05.

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in the CFX in the control group only because of the in-stent stenosis in controls contributed substantially to the overall atheroma burden. Finally, to address the effect of hyperlipidemia and exercise on the in-stent atherosclerotic processes, analyses for neointima formation (percent stenosis) and collagen content were performed on the stented sections of the circumflex artery (Fig. 3A). We report no statistical difference across groups with respect to percent stenosis (Fig. 3E). This is in stark contrast to the percent stenosis that was determined 5 mm (Fig. 3, A and D) and 10 mm (data not shown) proximal to the stent, as H pigs had significantly greater percent stenosis in both locations compared with the control and HX groups. In addition, we found significantly higher percent collagen in the stented sections of the H animals (21.5 ± 6.8%) compared with the control (0.9 ± 0.3%) or HX (2.1 ± 0.8%). Taken together, these data suggest that the higher amount of atheroma in the circumflex artery of the H animals was predominantly in the nonstented sections of the interrogated artery, which is strongly supported by the summary comparison data of histology in Fig. 3, D and E.

Finally, Fig. 4, A–D, represents microvessel density in the left ventricular sections obtained from all pigs. Microvessel density was significantly decreased in H compared with control (47% of C). Microvessel density in the HX group was higher than in the H group (80% of C), suggesting that exercise training partially prevented this reduction in microvessel density.

**DISCUSSION**

To our knowledge, this is the first study examining the effects of exercise training on microvascular function and macrovascular atheroma after coronary stenting in a model of hypercholesterolemia and CAD. Additionally, we provide evidence that microvascular rarefaction may underlie the blunted hyperlipidemia-induced response and normalized exercise training-induced blood flow responses to adenosine and bradykinin in the coronary microcirculation. Coronary stenting has been shown to mechanically damage vascular cells in the target conduit-artery segment, endothelium in peri-stent segments (30), and induces downstream microvascular dysfunction that persists for weeks and has been implicated in causing exertional ischemia (5, 28, 42, 59, 61). A major finding of this study is bare metal stent deployment in our hyperlipidemic swine model results in microvascular dysfunction, as assessed by the decreased CBF response to intracoronary bradykinin and adenosine administration and a reduction in baseline CBF.

| Table 2. Short-term exercise training prevents CVR derangements |
|------------------|----------------|----------------|----------------|----------------|
|                  | C              | H              | HX             | Significance   |
| CVR (mmHg/ml/min)|                |                |                |                |
| Baseline         | 1.46 ± 0.14    | 2.22 ± 0.23    | 0.96 ± 0.05    | C < H; HX < C, H |
| Adenosine, μg/kg |                |                |                |                |
| 0.167            | 0.96 ± 0.08    | 1.80 ± 0.20    | 0.83 ± 0.03    | C, HX < H      |
| 0.33             | 0.88 ± 0.10    | 1.60 ± 0.15    | 0.86 ± 0.16    | C, HX < H      |
| 1                | 0.71 ± 0.05    | 1.18 ± 0.07    | 0.67 ± 0.03    | C, HX < H      |
| 3                | 0.58 ± 0.05    | 0.91 ± 0.13    | 0.60 ± 0.03    | C, HX < H      |
| Bradykinin, ng/kg|                |                |                |                |
| 1                | 1.14 ± 0.08    | 1.72 ± 0.14    | 0.86 ± 0.11    | C < H; HX < C, H |
| 2                | 1.01 ± 0.06    | 1.71 ± 0.38    | 0.73 ± 0.15    | C < H; HX < C, H |
| 4                | 0.82 ± 0.06    | 1.34 ± 0.05    | 0.80 ± 0.13    | C, HX < H      |

Values are means ± SE. CVR, coronary vascular resistance. C, H, HX as denoted in Table 1. Statistical significance at *P < 0.05.*
intracoronary bradykinin and/or adenosine (Fig. 2, B and C). A third major finding was that hyperlipidemia decreased microvessel density (Fig. 4), thus providing a structural mechanism for the in vivo responses to adenosine and bradykinin reported herein. Interestingly, exercise training prevented the reduction in microvessel density (Fig. 4D). These data demonstrate the efficacy of short-term exercise training on preventing early pathophysiological responses to coronary stent deployment in hyperlipidemic swine. Equally remarkable is that the beneficial effects of exercise training were independent of changes in plasma lipids. Taken together, we conclude that, in addition to the well-known effect of exercise training to improve plasma cholesterol profile, exercise may act directly on the vascular wall or other plasma risk factors not measured here.

Similar impairment of endothelium-dependent microvascular function and CBF have been reported in the setting of hypercholesterolemia alone (4, 18, 47, 48, 51). Possible mechanisms for the hypercholesterolemia-induced endothelium-dependent dysfunction include an increase in endothelin-1 (ET-1) (4), a reduction in nitric oxide (NO) synthase (56), or responsiveness to NO (60). We did not measure protein levels of ET-1 or NO synthase or responsiveness to NO in the microvascular dysfunction in response to intracoronary bradykinin and/or adenosine (Fig. 2, A–C). Second, short-term exercise training prevented the microvascular dysfunction in response to intracoronary bradykinin and/or adenosine (Fig. 2, B and C). A third major finding was that hyperlipidemia decreased microvessel density (Fig. 4), thus providing a structural mechanism for the in vivo responses to adenosine and bradykinin reported herein. Interestingly, exercise training prevented the reduction in microvessel density (Fig. 4D). These data demonstrate the efficacy of short-term exercise training on preventing early pathophysiological responses to coronary stent deployment in hyperlipidemic swine. Equally remarkable is that the beneficial effects of exercise training were independent of changes in plasma lipids. Taken together, we conclude that, in addition to the well-known effect of exercise training to improve plasma cholesterol profile, exercise may act directly on the vascular wall or other plasma risk factors not measured here.

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Previous reports have shown beneficial effects of exercise training including improved myocardial perfusion and improved endothelial function (20, 36, 57). However, these studies did not involve revascularized patients. More recent studies involving patients undergoing coronary interventions report improvements in functional capacity and quality of life but report conflicting results regarding recurrent events and rehospitalizations, and these studies did not directly assess microvascular function (3, 25, 31, 40, 46). Hosokawa et al. (26) directly assessed endothelial function in vivo and reported regular exercise training improved endothelial function in non-infarct-related coronary arteries following coronary interventions for myocardial infarction. Importantly, that study did not evaluate function of the culprit artery. Finally, it is important to note that these studies involved long-term exercise training, which contrasts sharply to the short-term (7 wk) regimen in the present study.

Short-term exercise training resulted in significantly lower native atheroma burden as determined by IVUS interrogation.

<table>
<thead>
<tr>
<th>Percent Degree</th>
<th>Atheroma</th>
<th>C</th>
<th>H</th>
<th>HX</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAD</td>
<td>35.2 ± 5.5</td>
<td>59.5 ± 12.5</td>
<td>23.0 ± 11.1</td>
<td>C &lt; H; HX &lt; C; H</td>
<td></td>
</tr>
<tr>
<td>CFX</td>
<td>0</td>
<td>17.9 ± 2.4</td>
<td>3.9 ± 0.3</td>
<td>C &lt; H; HX; HX &lt; H</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. CFX, circumflex artery; LAD, left anterior descending coronary artery. C, H, HX as denoted in Table 1. Statistical significance at P < 0.05.
of the left anterior descending (nonintervened vessel) (Table 3). These data are consistent with other investigators who have shown that long-term exercise training has beneficial cardio-protective effects with some evidence suggesting that exercise training may delay the progression or even induce regression of CAD (45, 52, 53). Additionally, IVUS interrogation of the circumflex artery revealed that short-term exercise training significantly decreased the macrovascular atheroma response following stenting. This result is noteworthy in light of recent human studies involving long-term exercise training following coronary interventions that did not result in significant differences in restenosis rates (3, 25, 31, 46). Interestingly, the formation of neointima within the stent was not different across groups; thus the differences in atheroma burden across groups stem from difference in the nonstented regions. This is noteworthy as little is known regarding the progression of CAD in a stented vessel proximal and distal to the stent. Because of the increase in collagen content in the H animals in the tunica media, the mechanisms responsible for the alteration in cellular composition in each of these regions warrant additional investigation. Further punctuating these present findings is the fact that coronary stenting is clearly the most widely accepted treatment option for patients with CAD, yet restenosis still affects 15–25% of patients and often requires repeated interventions even with recent advances in therapy (35, 49). The exact mechanisms involved in the beneficial effects of short-term exercise training are still not clearly elucidated but could involve neurohumoral, mechanical, direct, or indirect effects on the vascular wall.

Revascularization of an occluded artery with percutaneous angioplasty has been shown to impair conduit and microvascular dilation (5, 19, 28, 30, 42, 50, 61). We investigated one potential underlying mechanism related to microvascular dysfunction in the present study—the reduction in microvascular density in the hypercholesterolemic swine (Fig. 4D). Since the hyperemic response to adenosine and bradykinin is a result of microvessel vasodilation, it stands to reason that one potential common explanation for a blunted response to both adenosine and bradykinin could be a reduction in number of microvessels per tissue area. As for the relative importance of different mechanisms for CBF changes, we have no definitive conclusions yet based on our current studies. Since short-term exercise training potentiated bradykinin-induced CBF in HX relative to both H and C, the endothelium-dependent factors may be more exercise sensitive in terms of CBF changes.

The smaller pig number in the HX group could be considered a limitation of our study; however, this design was strictly compliant with the principle of reducing the number of animals exposed to surgical and exercise stress to the minimum number required for statistical power (1, 6, 44). Further, evidence for the validity of the results from the small pig number in the HX group was shown by similar results in our report that exercise training significantly decreased atheroma burden in Ossabaw miniature swine with metabolic syndrome that were treated with coronary stents (13). Another limitation of our study is the animals did not receive a typical medical regimen that would be considered standard of care for human patients undergoing coronary interventions. Pigs in our study were given daily aspirin and periprocedural heparin but did not receive clopidogrel. Further, the pigs did not receive typical lipid-lowering agents, by design, which have also been shown to have an ameliorating effect on endothelial dysfunction and even atheroma burden as well as outcomes in patients with CAD (14, 27, 33, 34, 54, 58) and our model (9, 23, 32). Our rationale for translation of these results to human patients is that those patients who experience side effects of statins (e.g., liver dysfunction, rhabdomyolysis), clopidogrel (e.g., excess bleeding, gastrointestinal irritation), angiotensin converting-enzyme inhibitors and beta blockers (e.g., cough, orthostatic hypotension) might be able to decrease their dosage to avoid side effects because of the profound effects of exercise training. Additionally, by design, our study did not address how long the beneficial exercise effect was maintained, nor did it address whether the effect was preserved following cessation of exercise. Clearly, future studies should include exercise superimposed on all standard care medications and the effects of cessation of exercise.

The results of this study demonstrate that short-term exercise training preserves endothelium-independent coronary microvascular responses, potentiates endothelium-dependent coronary microvascular function, and significantly diminishes the overall macrovascular atheroma burden after coronary stenting in a porcine model of hyperlipidemia and CAD. Our results add direct evidence for microvessel dropout (rarefaction), which may explain the parallel CBF responses to endothelium-dependent (bradykinin) and endothelium-independent (adenosine) dilators. Combined with the exercise-induced decrease in macrovascular atheroma burden, these data provide a compelling basis for exercise training to ameliorate complications associated with coronary stenting in hyperlipidemic swine. To
our knowledge this is the first study to report such effects associated with peri-procedural short-term exercise training independent of favorable changes in plasma cholesterol. These phenomenal effects of exercise training, if translatable to human clinical medicine, could offer an outstanding adjunct to drug-eluting stent and pharmacological treatment for flow-limiting lesions if exercise training can ameliorate the even greater microvascular dysfunction in drug-eluting compared with bare metal stents (29, 39).

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DISCLOSURES
No conflicts of interest (financial or otherwise) are declared by the authors.

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