Effect of Long-term Exercise on Regional Myocardial Function and Coronary Collateral Development After Gradual Coronary Artery Occlusion in Pigs*

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The effect of myocardial ischemia, induced by long-term exercise, on regional myocardial function and coronary collateral development was examined in pigs after gradual occlusion of the left circumflex coronary artery (LCx) with an ameroid occluder. Thirty days after surgery, regional myocardial function and blood flow were assessed during exercise in 22 pigs separated into exercise (n=12) and sedentary groups (n=10). The exercise group trained on a treadmill for 25±1 days, 30–50 min/day, at heart rates of 210–220 beats/min. After 5 weeks, another exercise test was performed. In the exercise group, after training, we observed an improvement in systolic wall thickening, expressed as a percentage of rest, in the collateral-dependent LCx region from 64±8% to 87±6% (p<0.01) at moderate exercise levels (220 beats/min) and from 45±7% to 73±7% (p<0.01) at severe exercise levels (265 beats/min). Transmural myocardial blood flow in the LCx region expressed as a ratio of flow in the nonoccluded region of the left ventricle also increased significantly (p<0.01) during severe exercise after 5 weeks. The sedentary group showed an improvement in systolic wall thickening in the LCx region during moderate exercise compared with the initial exercise test (p<0.05) but no significant change in systolic wall thickening or myocardial blood flow ratios during severe exercise after 5 weeks. We conclude that long-term exercise after gradual LCx coronary artery occlusion in pigs improves myocardial function and coronary collateral reserve in collateral-dependent myocardium during exercise. (Circulation 1990;82:1778–1789)

Pigs possess a sparse, innate coronary collateral circulation1 consisting of a fine anastomotic network of endomural vessels similar to coronary collateral vessels in humans.2 Previous studies have shown that pigs develop coronary collateral vessels after gradual occlusion of the left circumflex coronary artery (LCx) with an ameroid occluder.3,4 Coronary collateral vessels that developed in this model are sufficient to provide normal myocardial blood flow and support myocardial function at rest within 3–4 weeks of placement of the occluder. However, collateral vessel development is limited so that myocardial perfusion is inadequate to support regional myocardial function in the collateral-dependent myocardium during exercise. The exercise-induced underperfusion of the collateral-dependent region and ensuing myocardial dysfunction in the pig model persist for at least 16 weeks after occluder placement and improve little during the 4-month period.4 Humans with coronary artery disease also demonstrate persistent regional myocardial dysfunction and ischemia in collateral-dependent myocardium during exercise.5 Thus, gradual coronary artery occlusion in the pig, characterized by persistent myocardial ischemia during stress, provides a good model to study the effects of long-term exercise training on regional myocardial function and blood flow in collateral-dependent myocardium.

Previous studies in dogs undergoing exercise training and coronary artery occlusion have used several methods to assess growth and development of the

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coronary collateral circulation and have arrived at equivocal results. Early work by Eckstein,6 who measured retrograde blood flow to evaluate collateral development, demonstrated beneficial effects of exercise on the coronary collateral circulation. However, studies using isolated heart perfusion techniques to measure coronary collateral vessels have shown enhanced collateral growth7 and little effect of exercise on coronary collateral development.8 Other investigators, measuring the coronary collateral circulation during exercise9.10 or cardiac pacing,11 have demonstrated variable degrees of improvement in collateral blood flow as a result of exercise training. Bloor et al12 previously showed that exercise training increases collateral perfusion of myocardium under resting conditions in a fixed-stenosis model in pigs. These previous studies did not examine the effects of exercise training on regional myocardial function in collateral-dependent myocardium. Such measurements are critical to demonstrate that any exercise-induced improvements in collateral blood flow have meaning with regard to cardiac function.

Therefore, the purpose of the present investigation was to evaluate, under the physiological conditions associated with exercise, the effect of long-term, intermittent myocardial ischemia induced by exercise training on regional myocardial function and coronary collateral development in pigs after gradual coronary artery occlusion. We hypothesized that long-term, repetitive, exercise-induced myocardial ischemia would enhance coronary collateral development and improve regional myocardial function and collateral perfusion of collateral-dependent myocardium during exercise.

Methods

Animal protocols used in this study were approved by the animal subjects committee at the University of California, San Diego.

Thirty-two Yucatan minipigs were used in the study. Seven pigs died suddenly during the period between surgical instrumentation and the initial exercise test, probably as a result of ventricular fibrillation. An additional pig died suddenly with ventricular fibrillation during the initial stress test. Two pigs were killed during the study, one after developing pneumonia and a second after a joint injury. Data were obtained from 22 pigs separated into an exercise group (n=12; body weight at surgery, 44±2 kg; five male, seven female) and a sedentary group (n=10; body weight at surgery, 45±4 kg; six male, four female). Before surgery, both groups underwent 1 week of treadmill familiarization consisting of daily 5-minute walks and 3–5-minute low-speed runs.

Surgical Protocol

The pigs were sedated with ketamine (25 mg/kg i.m.), atropine (0.05 mg/kg i.m.), and sodium thiamylal (20 mg/kg i.v.). They then were intubated and maintained on 1–2% halothane anesthesia throughout the remainder of the aseptic procedure. A left thoracotomy was performed through the fifth intercostal space. A Silastic catheter was placed in the left atrium for injection of radiolabeled microspheres and for blood pressure monitoring. The proximal left circumflex coronary artery was dissected free of surrounding tissue for a length of 1–1.5 cm. A metal-encased ameroid occluder with a 2.5–3.0-mm lumen (K-G Ulrich, Montreal, Quebec, Canada) was placed around the dissected artery. The occluder was chosen with the proper luminal dimensions to provide a close, but nonconstrictive, fit to the artery. Three pairs of sonomicrometer dimension gauges (5 MHz, 2.5 mm in diameter) were placed across the left ventricular free wall for the measurement of left ventricular wall thickness.13 The subendocardial crystal of the pair was held at the tip of a Teflon tube (1.75 mm o.d.) and advanced diagonally through the myocardium a tract created by an 18-gauge hypodermic needle. The second crystal of the pair was sewn on the epicardial surface of the heart using an attached Dacron patch. Two crystal pairs were placed near the center of the LCx region 1–2 cm below the LCx. One crystal pair was placed in the left anterior descending region of the myocardium between the main coronary artery and a diagonal branch. Proper location of the crystal pairs was confirmed by the presence of myocardial dysfunction in the LCx region during a 30-second occlusion of the LCx at the level of the ameroid occluder. Two copper wires were sewn to the epicardial surface of the right and left ventricles, and one wire was sewn to the latissimus dorsi muscle to monitor an electrocardiogram. In addition, in nine pigs (four pigs in the exercise group and five in the sedentary group), a high-fidelity pressure transducer (model 7, Konigsberg) was placed in the apex of the left ventricle through a stab wound in the apex of the heart. A Silastic catheter was placed in the proximal descending aorta in 14 pigs (five pigs from the exercise group and nine from the sedentary group) to monitor blood pressure and to withdraw reference blood samples for the microsphere technique. Aortic catheters were not used in seven pigs in the exercise group to minimize embolization of the hind limbs and to maximize survival throughout the exercise protocol. After instrumentation, all catheters and wires were exited from the chest, tunneled under the muscle, and exteriorized on the animal's midback. The pigs' chests were closed, and the animals were allowed to recover.

Experimental Protocol

During a 4-week recovery period, the pigs were placed on the treadmill 1 day/wk, and all instrumentation signals were monitored on an eight-channel ink recorder (model 7848-A, Hewlett-Packard, Palo Alto, Calif.). The pigs also walked on the treadmill for 5 minutes to maintain familiarization with the treadmill procedure. One month after surgery (31±2 days for the exercise group, 31±1 days for the sedentary group), a treadmill exercise stress test was performed on all pigs. The pigs were placed on the
treadmill, and aortic pressure, left atrial pressure, left ventricular pressure, first derivative of left ventricular pressure (dP/dt), electrocardiogram, and sonomicrometer dimension signals were monitored. At rest, with the animal standing still on the treadmill, myocardial blood flow was measured by injecting approximately 6×10^6, 15-μm radiolabeled microspheres (New England Nuclear, Boston, Mass.) through the left atrial catheter. Spheres were suspended in 1–2 ml 10% dextran and Tween 80 solvent and were agitated with a vortex mixer before injection. The radiolabels included scandium-46, niobium-95, ruthenium-103, tin-113, chromium-51, indium-114, and cesium-141. A reference blood sample was withdrawn with an aortic catheter at a rate of 7.75 ml/min. Sonomicrometer measurements of regional left ventricular wall thickness dimensions also were recorded at rest; and then an exercise protocol was performed. Exercise consisted of 2 minutes of running at 3 km/hr, 4 km/hr, and 5 km/hr up a 5% grade, followed by successive percent grade increases of 2.5% every 2 minutes until target heart rates were achieved. At a moderate heart rate of approximately 220 beats/min (exercise duration, 2–8 minutes) and after the heart rate was stable for 1 minute, regional ventricular wall thickening was recorded, and regional myocardial blood flow was measured by the microsphere technique. Regional ventricular wall thickening and myocardial blood flows also were assessed at a stable heart rate during severe exercise at a heart rate of approximately 265 beats/min (exercise duration, 8–18 minutes). The first five pigs to complete the initial stress test were trained as exercising animals. The remaining pigs were randomly assigned to either the exercise (n=12) or the sedentary group (n=10). Pigs in the exercise group underwent a 5-week exercise protocol. The exercise protocol consisted of treadmill running for 30 min/day, 5 days/wk during the first week. The duration of exercise was increased 5 min/day each week to a maximum of 50 min/day by the fifth week. A typical run consisted of a 5-minute warm-up period at 3 km/hr up a 5% grade followed by 25–45 minutes of running at a target heart rate of 210–220 beats/min. The pigs averaged 25±1 days of exercise for a total of 930±26 minutes of exercise during the training period. Heart rates were monitored during daily exercise by electrocardiogram. Condition of each pig's instrumentation was assessed weekly by recording signals at rest and during exercise. Pigs in the sedentary group were put on the treadmill 1 day/wk and walked for 5 minutes to maintain familiarization with the treadmill. Hemodynamic data were collected at this time.

After 5 weeks of exercise or sedentary activity (36±3 days and 33±1 days, respectively) pigs performed a final treadmill exercise stress test using a protocol similar to the initial test. Upon completion of the study, the animals were killed with T-61 euthanasia solution. The hearts were dissected carefully to free adhering tissue and excised from the thorax. Polyethylene catheters were placed in the LCx just distal to the aneroid occluder, and the proximal left anterior descending and right coronary arteries. Colored dyes were injected simultaneously at approximately equivalent pressures and flow rates into the three catheters to delineate perfusion regions of the main coronary arteries. The aneroid occluder was located, removed, and carefully examined to determine status of the vessel lumen. The lumen was occluded completely in all pigs.

**Determination of Regional Left Ventricular Wall Function**

Regional myocardial function was assessed with sonomicrometer dimension gauges (Schuessler and Associates, Cardiff, Calif.). Left ventricular wall thickening was measured over ejection phase of the systolic cycle from the onset of ventricular ejection to the end of systole.

In pigs with Konigsberg transducers, the ejection phase was defined as the time from peak left ventricular pressure rise (peak dP/dt) to 20 msec before peak negative dP/dt (Figure 1) or the corresponding points on the RT interval of the electrocardiographic trace. In a previous study from this laboratory, we established that the timing of systole could be determined by the electrocardiogram from epicardial electrocardiographic leads. This correlation between the timing of systole was determined in normal pigs undergoing daily exercise for 10 weeks. In comparing the time of systole using either dP/dt or ST interval, we found no consistent differences between the two methods. In the present study and in preparation of failures of pressure transducers or in cases where they were not implanted, we used a systematic method to ensure that the electrocardiogram would provide a precise measure of the systolic interval. Two epicardial electrocardiographic leads were sewn to the surface of the nonischemic regions of the left and right ventricles and a third lead was sutured to the latissimus dorsi muscle. These leads were color coded to ensure a consistent connection to the left atrium, right atrium, and ground. A lead II configuration was used, and the gain was adjusted so that the T wave was always evident and between 4 and 7 mm in height.

To confirm the relation between the length of systole determined by two methods, the following procedure was performed: six animals with aneroid occluders and both pressure transducers and electrocardiographic surface leads were compared during exercise at matched heart rates at the beginning of the study and after 5 weeks of exercise. Three animals were from the sedentary group, and three were from the exercise-trained group. The length of systole was determined from peak positive dP/dt to 20 msec before peak negative dP/dt using the Konigsberg left ventricular pressure trace. The equivalent length from electrocardiographic traces was from the beginning of the S wave to 30 msec after the end of the peak of the “T” wave. During the second and
fifth weeks after the first test, percent wall thickening was calculated independently from both the electrocardiogram and dP/dt systolic lengths. No statistical differences were seen in percent wall thickening calculated by either method in either group (Table 1).

Data were expressed as percent ventricular wall thickening and ventricular wall thickening excursion. Percent wall thickening was calculated as the ratio of the end-systolic wall thickness minus wall thickness at onset of ejection to the wall thickness at onset of ejection multiplied by 100. Wall thickening excursion was calculated as end-systolic wall thickness minus wall thickness at onset of ejection. Values for wall thickness were averaged for 5 cardiac cycles at rest and 10 cardiac cycles during exercise. Data in pigs with two pairs of functioning dimension gauges in the LCx region were averaged.

**Determination of Regional Myocardial Blood Flows**

The formalin-fixed hearts were cut into five rings, 1–1.5 cm thick, from base to apex. The collateral-dependent LCx region, determined by dye delinea-

| Table 1. Percent Wall Thickening Calculated From dP/dt and the Electrocardiogram |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                            | dP/dt                      |                             | ECG                        |                             |                             |                             |
|                            | Wk 1 | Wk 2 | Wk 5 | Wk 1 | Wk 2 | Wk 5 |
| Group                      |      |      |      |      |      |      |
| Sedentary                  |      |      |      |      |      |      |
| 1 1                        | 12.7 | 12.9 | 13.2 | 12.7 | 13.1 | 12.7 |
| 2 2                        | 19.3 | 20.1 | 20.2 | 19.3 | 18.4 | 19.9 |
| 3 3                        | 22.4 | 22.6 | 22.8 | 22.4 | 21.2 | 23.0 |
| Mean                       | 18.1 | 18.5 | 18.7 | 18.1 | 17.6 | 18.5 |
| SEM                        | 3.1  | 3.2  | 3.2  | 3.1  | 2.6  | 3.3  |
| Exercise                   |      |      |      |      |      |      |
| 4 4                        | 16.1 | 17.3 | 18.9 | 16.1 | 17.1 | 20.4 |
| 5 5                        | 20.4 | 22.1 | 24.4 | 20.4 | 23.1 | 25.7 |
| 6 6                        | 23.9 | 23.9 | 23.9 | 23.9 | 24   | 27.1 |
| Mean                       | 20.1 | 21.2 | 23.7 | 20.1 | 21.7 | 24.4 |
| SEM                        | 2.4  | 2.1  | 2.8  | 2.4  | 2.5  | 2.2  |

Values are mean±SEM as percent wall thickening. No significant differences were found between the dP/dt and ECG method. ECG, electrocardiogram.
tion of the fixed myocardium, was cut from the rings and weighed. The LCx region weighed 33±2 g and represented 21±2% of the left ventricle. One transmural section from the LCx region from each of the two most basal rings (3–5 g each) lying at least 10–15 mm inside the dye border of the region was removed and divided into subendocardial, midmyocardial, and subendocardial layers. Similarly, transmural sections of left ventricle perfused by the right and left anterior descending coronary arteries were removed and divided into three layers. Samples of myocardium and available reference blood samples were analyzed for the quantity and energy level of gamma radiation with an Auto-Gamma spectrometer (model 5912A, Packard Instruments, Downers Grove, Ill.). The radiation counts were corrected for background activity and overlap with solution of simultaneous equations by use of the matrix-inversion technique. Blood flow data from all pigs were reported as the ratio of radiation counts in the collateral-dependent LCx region relative to counts in the control right and left anterior descending perfused regions of myocardium. Data were analyzed in this manner to allow longitudinal, normalized comparisons of collateral blood flow. This method also allowed us to maximize data from pigs lacking patent aortic catheters at the time of the study and to minimize the effects of possible sphere loss from the myocardium over time on our longitudinal data. Previous work in our laboratory has shown that detectable preferential loss of microspheres from the collateral-dependent region does not occur.

The presence of normal exercise-induced hyperemias in the myocardium was confirmed in 11 pigs (four in the exercise group and seven in the sedentary group). Absolute regional myocardial blood flows were determined using reference sample withdrawals by the general method of Heymann et al. Myocardial blood flows during this final exercise test averaged 1.0±0.1 and 1.5±0.3 ml/min/g at rest in the subendocardium of the LCx region in the sedentary and exercise groups, respectively. At severe exercise, blood flows were 1.7±0.2 and 1.8±0.8 ml/min/g in the subendocardium of the LCx region in the sedentary and exercise groups, respectively. Resting blood flows in the subendocardium of the left anterior descending region were 1.3±0.1 and 1.6±0.1 ml/min/g in the sedentary and exercise groups, respectively. Blood flows in the subendocardium of the left anterior descending region during severe exercise were 5.3±0.3 and 4.8±0.8 ml/min/g in the sedentary and exercise groups, respectively. These measurements show exercise-induced hyperemias similar to those demonstrated previously in our model.

Postmortem Histology

The myocardial tissue samples used for blood flow analysis also were used for histological analysis and quantitative morphometric point counting to determine the percent infarction in the collateral-dependent myocardium. Transmural sections of myocardium also were removed from the fixed hearts at the site of sonomicrometer crystals with a 7.5-mm cork bore to assess extent of infarction at the crystal sites. Paraffin-embedded, transmural 5-μm sections of myocardium were stained with Masson’s trichrome stain to identify fibrous necrotic tissue. The slides were point counted from a projected image on a microfilm reader (Documat DL 2, Jenoptic, Jena, G.D.R.), and the areas of infarction were determined with an Apple Ile computer. Percent infarction at the crystal sites averaged 9.8±2.0% between crystals in the collateral-dependent LCx region and 3.4±1.8% between crystals in the left anterior descending region. Percentages of infarction in the exercise and sedentary groups are provided in “Results.”

Statistical Analysis

Data are expressed as mean±SEM. Intragroup statistical comparisons of hemodynamics, regional myocardial function, and myocardial blood flow ratios were made using Student’s t test (two tailed) for paired comparisons in a longitudinal design (initial exercise test compared with final exercise test). Intergroup comparisons of regional myocardial function and exercise and sedentary group characteristics were performed with the Student’s t test for unpaired comparisons. Multiple intragroup and intergroup comparisons of regional myocardial function were corrected with the Bonferroni’s correction for multiple comparisons. A p value less than 0.05 was considered significant.

Results

Hemodynamics

Hemodynamic parameters during measurement of myocardial function and myocardial blood flow are shown in Tables 2 and 3. Hemodynamics during function and flow determinations have been presented separately to account for missing function or flow data and data measured at different heart rates. Numbers of animals represent subsets of pigs in the exercise and sedentary groups.

Heart rates for the exercise and sedentary groups were not significantly different at rest, moderate, or severe exercise (initial compared with final test). Mean arterial blood pressure at rest tended to be higher during the final exercise test compared with that during the initial test in both groups but was significantly higher only in the sedentary group at rest during measurement of myocardial function. Mean left atrial pressures were not significantly different in either group at rest or during exercise when the initial and final exercise tests were compared.

Regional Myocardial Function

Left ventricular wall thickness parameters, percent left ventricular wall thickening, and wall thickening excursion at rest, moderate, and severe exercise are presented in Table 4. Percent systolic wall thickening data from the collateral-dependent LCx region of
myocardium of individual pigs in the exercise group at rest and during intense exercise are shown in Figure 2. Percent systolic wall thickening in the collateral-dependent region was decreased significantly at rest and increased significantly during intense exercise after the 5-week exercise protocol. Linear regression analysis of data from Figure 2 comparing the extent of regional systolic dysfunction in the collateral-dependent myocardium during intense exercise compared with rest before training to the percent improvement in regional systolic function after training correlated with an r value of 0.68.

Significant changes in percent wall thickening were not observed in the nonoccluded left anterior descending region of myocardium from pigs in the exercise or sedentary groups when the initial and final stress tests were compared (Table 4).

Figure 3 compares myocardial wall thickening during exercise, normalized to myocardial function at rest, in the LCx region of exercised and sedentary pigs. In the exercise group, during the initial exercise test before training, systolic wall thickening in the LCx region dropped to 64% and 45% of that present at rest during moderate and intense exercise, respectively. After the 5-week training protocol, regional systolic wall thickening in the collateral-dependent myocardium improved significantly (p<0.01) so that it declined to only 87% and 73% of resting function during moderate and intense exercise, respectively. In the sedentary group, wall thickening in the LCx

### Table 2. Hemodynamics During Determination of Myocardial Function

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Moderate</th>
<th>Severe</th>
<th>Rest</th>
<th>Moderate</th>
<th>Severe</th>
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</thead>
<tbody>
<tr>
<td><strong>Heart rate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(beats/min)</td>
<td>91±6</td>
<td>218±5</td>
<td>264±4</td>
<td>92±6</td>
<td>220±5</td>
<td>264±4</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
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</tr>
<tr>
<td><strong>MABP</strong> (mm Hg)</td>
<td>102±4</td>
<td>134±4</td>
<td>146±6</td>
<td>122±6</td>
<td>138±3</td>
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<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
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</tr>
<tr>
<td><strong>MLAP</strong> (mm Hg)</td>
<td>8±2</td>
<td>21±2</td>
<td>22±2</td>
<td>11±2</td>
<td>21±3</td>
<td>25±3</td>
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<td>9</td>
<td>9</td>
<td>9</td>
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</tbody>
</table>

Values are mean±SEM. n is number of pigs in group. MABP, mean arterial blood pressure; MLAP, mean left atrial pressure.

### Table 3. Hemodynamics During Determination of Myocardial Blood Flow Ratios

<table>
<thead>
<tr>
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<th>Rest</th>
<th>Moderate</th>
<th>Severe</th>
<th>Rest</th>
<th>Moderate</th>
<th>Severe</th>
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<tbody>
<tr>
<td><strong>Heart rate</strong></td>
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<td>(beats/min)</td>
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<td>4</td>
<td>4</td>
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<tr>
<td><strong>MLAP</strong> (mm Hg)</td>
<td>7±1</td>
<td>19±3</td>
<td>22±2</td>
<td>10±2</td>
<td>20±4</td>
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<td>n</td>
<td>11</td>
<td>5</td>
<td>11</td>
<td>11</td>
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Values are mean±SEM. n is number of pigs in group. MABP, mean arterial blood pressure; MLAP, mean left atrial pressure.
TABLE 4. Regional Myocardial Function Parameters

<table>
<thead>
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<th></th>
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<th>Moderate exercise</th>
<th>Severe exercise</th>
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<tbody>
<tr>
<td></td>
<td>OEWth (mm)</td>
<td>ESWth (mm)</td>
<td>%Wth</td>
</tr>
<tr>
<td>Collateral-dependent left circumflex region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretraining</td>
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<td>12.4±0.5</td>
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<td>Post-training</td>
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<td>12.3±0.4</td>
<td>33.8±4.4*</td>
</tr>
<tr>
<td>Control left anterior descending region</td>
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</tr>
<tr>
<td>Pretraining</td>
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<td>11.8±1.2</td>
<td>30.0±4.5</td>
</tr>
<tr>
<td>Post-training</td>
<td>10.2±0.9</td>
<td>13.3±1.3†</td>
<td>30.6±4.9</td>
</tr>
</tbody>
</table>

**Values are mean±SEM. n is number of pigs in group.**

OEWth, onset ejection wall thickness; ESWth, end-systolic wall thickness; %Wth, percent wall thickening; Wth ex, wall thickening excursion.

*,† p<0.01, and p<0.05, respectively, before and after the training or sedentary period.

region declined to 56% of rest at moderate exercise and 42% of rest at intense exercise during the initial exercise test. After 5 weeks of sedentary activity, regional wall thickening improved significantly (p<0.05) to 76% of rest during moderate exercise but showed no significant change during intense exercise compared with the initial exercise test.

**Regional Myocardial Blood Flow Ratios**

Ratios of myocardial blood flow in the collateral-dependent LCx region of myocardium to blood flow in the control left anterior descending and right coronary perfused regions at rest and during exercise for both the exercise and sedentary groups are presented in Table 5. In the exercise group, blood flow ratios were increased significantly in the subendocardium, midmyocardium, and subepicardium during severe exercise after the training protocol compared with pretraining levels. No significant increases in blood flow ratios were observed in the sedentary group during severe exercise when the initial and final exercise tests were compared. During moderate exercise, subendocardial blood flow ratios were increased in the exercise and sedentary groups. How-

**FIGURE 2.** Plots of percent systolic wall thickening in the collateral-dependent left circumflex region of myocardium of individual pigs from the exercise group at rest and during severe exercise. Values are shown for the pretraining exercise test (Pre Train) and the post-training exercise test (Post Train) 5 weeks later. **p<0.01 Pre Train vs. Post Train.**
ever, the magnitude of the change in the sedentary group was less than the change observed in the trained group. No significant changes in blood flow ratios were present at rest in either group when comparing the two exercise tests. Figure 4 shows subendocardial blood flow ratios for individual pigs in the exercise group at intense exercise before and after training. Linear regression analysis comparing the subendocardial blood flow ratio during intense exercise before training to the percent improvement in the subendocardial blood flow ratio after training showed no correlation (r=0.09).

**Exercise and Sedentary Group Characteristics**

In the exercise group, work loads achieved at the matched heart rates increased significantly between the two exercise tests (p<0.05) from 310±58 to 506±83 kg/m/min and from 710±75 to 867±99 kg/m/min at moderate and severe exercise, respectively. Conversely, in the sedentary group, exercise work loads were unchanged when the two exercise tests were compared. Body weights showed increases in both groups of pigs during the exercise and sedentary periods (from 50±2 to 55±2 kg in the exercise group and from 55±4 to 59±5 kg in the sedentary group). Postmortem analysis demonstrated that ratios of left ventricular weight to body weight were increased in the exercise group compared with the sedentary group, but this difference was not significant (2.9±0.2 g/kg for the exercise group and 2.6±0.2 g/kg for the sedentary group, p<0.19). Histological analysis revealed similar extents of myocardial infarct in the entire collateral-dependent LCx region (4.8±0.8% and 7.8±1.3% in the exercise and sedentary groups, respectively) (p<0.06, NS). The transmural distribution of the infarcts in the region at risk was 8.2±1.4%, 4.1±0.9%, and 1.8±0.7% in the subendocardium, midmyocardium, and subepicardium of the exercise group and 13.7±3.0%, 7.5±1.3%, and 2.3±0.8% of these regions in the sedentary group.

**Discussion**

This study examined the effects of long-term, repetitive exercise on regional myocardial function and coronary collateral development in pigs after...
gradual coronary artery occlusion. We confirmed our hypothesis by demonstrating that after a 5-week training period myocardial function and coronary blood flow are significantly improved in the collateral-dependent myocardium during exercise compared with pretraining values at matched heart rates.

In a comparison of pretraining with post-training values, our results show that during severe exercise at heart rates of approximately 260 beats/min (approximately 90% of heart rate reserve) regional myocardial function and collateral blood flow ratios improved significantly in the collateral-dependent LCX region of myocardium. These parameters did not significantly improve in the sedentary pigs at the severe exercise level. We studied the pigs at heart rates of 260 beats/min because previous studies in our laboratory showed that this exercise intensity exhausts collateral vascular reserve. At moderate exercise levels with matched heart rates of 210–220 beats/min (approximately 65% of heart rate reserve), myocardial function and subendocardial blood flow ratios in the collateral-dependent region increased significantly in both the exercise and sedentary groups. Of note, work loads at the matched heart rates in the exercise group increased by 64% during moderate and 22% during intense exercise during the 5-week training period. Sedentary pigs showed an insignificant 14% increase in work load during moderate exercise and a 9% decrease during intense exercise compared with work loads during the initial exercise test. Heaton et al previously used matched speed and grade treadmill running to assess coronary collateral development in exercised and sedentary dogs. Such a protocol may not actually match work loads because body weights of the animals can change over time and should be included in the work load calculation. Because our pigs were exercised at increased work loads after exercise training, matched–work load measurements would only tend to augment differences in regional function and flow between the initial and final exercise tests. This further supports our conclusion that long-term exercise increases myocardial function and collateral blood flow in the collateral-dependent myocardium during exercise.

Our data collected during moderate exercise show that some improvement in regional function and collateral blood flow occurs in the nonexercised group during the 5-week sedentary period. A previous study in our laboratory showed that absolute coronary collateral blood flow measured at a heart rate of 235 beats/min increased from 0.80±0.11 to 1.14±0.21 ml/min/g in sedentary pigs between 3 and 7 weeks after surgery. This change was not significant. However, data in the previous study were not paired. It is difficult to discern the extent that natural progression of coronary collateral development influenced the results observed in our exercise group. However, an exercise-related response does appear to occur over and above the nonexercise-induced collateral development previously reported.

Paszk et al showed that tachycardia and the resultant increased extravascular resistance significantly influences regional myocardial blood flow in collateral-dependent myocardium of dogs. They demonstrated that coronary resistance increases greatly in the subendocardium of the collateral-dependent region when heart rate is increased above 220 beats/min by cardiac pacing. This increased resistance limits the blood flow increase in the collateral-dependent region and increases the degree of myocardial ischemia. Our results showing a beneficial effect of long-term exercise on regional myocardial function and collateral perfusion primarily at intense exercise levels demonstrate that long-term exercise improves the ability of the collateral circulation and the collateral-dependent myocardium to withstand the increased myocardial tissue pressures associated with tachycardia.

Shaper et al have proposed that chemical factors such as hypoxia and physical factors such as tangential wall stress induce the coronary collateral circulation of dogs to expand and enlarge by an active growth process. Recent work in our laboratory using tritiated thymidine to label mitotic activity in the vasculature has shown evidence for active growth processes occurring in the collateral circulation of the pig. Our results suggest that long-term exercise alters the chemical and physical factors influencing coronary collateral development. Growth factors such as platelet-derived growth factor may be involved in the collateral development process. We could hypothesize that platelet-derived growth factor release is initiated by an inflammatory response to myocardial ischemia. Platelet-derived growth factor can be expressed by various cell types present in the blood and myocardium, such as platelets, macrophages, smooth muscle, and endothelial cells. Unfortunately, the design of our present protocol does not provide evidence of which factors may be important in enhancing collateral vessel growth. We hypothesized that myocardial ischemia induced by long-term exercise would stimulate coronary collateral development in our model. Regression analysis of the data from individual pigs in the exercise group relating the degree of myocardial ischemia during intense exercise before training with the subsequent improvement in myocardial function and flow demonstrated poor linear correlations. Similar results were reported by Heaton et al after exercise training in dogs with coronary artery occlusion. Thus, the extent of myocardial ischemia experienced by the animal during long-term exercise may not account fully for the subsequent extent of coronary collateral development. This indicates that mechanical or chemical factors may be important in the collateral development that may be associated with exercise training. In addition, because our study design included no direct measure of coronary collateral proliferation, factors that can affect vascular tone in
the absence of increased coronary collateral development, such as adrenergic nerve activity, vasoactive substances, or altered extravascular support, could be playing a role in the observed improvements in coronary collateral perfusion after exercise training.

After the long-term exercise protocol, the exercised pigs also demonstrated a significant decrease in myocardial function at rest in the collateral-dependent region. Similar decreases in regional systolic wall thickening were not observed in the control left anterior descending region of the ventricle or in the collateral-dependent region of hearts from the sedentary pigs. The decline in regional function was not associated with a decrease in the ratio of myocardial blood flow to the collateral-dependent myocardium compared with the nonoccluded myocardium and, as such, does not appear to represent ischemic dysfunction. The reduction in function at rest also was not the result of infarction of the collateral-dependent region because the exercise group did not have a greater degree of infarction compared with the sedentary group. It is not known whether the decreased regional myocardial function at rest after the long-term, exercise-induced ischemia observed in our model is similar to "stunned" myocardium described previously in hearts after acute ischemia. The significance and mechanisms underlying the reduction in myocardial function at rest after long-term exercise in our model need to be defined by further investigation.

Left ventricular wall thickness measured by sonomicrometer crystals at onset of ventricular ejection was increased significantly in the left anterior descending region at rest and at exercise after training. Even though heart weight to body weight ratios were not significantly increased compared with the sedentary group, mild hypertrophy probably occurred as a result of long-term exercise. Evidence of hypertrophy also was observed in the collateral-dependent myocardium because wall thickness at onset of ejection in this region had an increased mean value at rest and was significantly increased at both exercise levels compared with pretraining values. Sasayama et al. using sonomicrometer dimension crystals, recently showed that ischemia induced by repeated coronary occlusions can induce myocardial hypertrophy in the region at risk of ischemia during a 20-day period. Thus, myocardial ischemia, induced by repeated sessions of exercise in our model, may have induced hypertrophy in the collateral-dependent region. To account for hypertrophy in our data analysis, regional myocardial function was calculated as systolic wall thickening excursion as well as percent systolic wall thickening because the excursion calculation is independent of ventricular wall thickness at onset of ejection. Conclusions drawn from percent systolic wall thickening measurements were supported by similar findings when wall thickening excursion was analyzed.

Ameroid occlusion of the proximal LCx in the pig provides a unique model for the study of the effects of possible therapeutic modalities such as exercise on collateral-dependent myocardium. The model is characterized in the sedentary state by sufficient coronary collateral development to allow normal myocardial blood flow during resting conditions and prevent severe myocardial necrosis in the collateral-dependent region within 3-7 weeks after occluder placement. However, collateral development is limited and shows little improvement over a 4-month period after occlusion, resulting in regional myocardial ischemia and dysfunction in the collateral-dependent myocardium during exercise. Limited coronary collateral development in the pig contrasts with several studies in dog models in which blood flow in collateralized myocardium is normal even during exercise within approximately 1-3 months after coronary artery occlusion. The endomural distribution of the coronary collateral circulation in pigs compared with the primarily epicardial collateral network of dogs also provides advantages for the study of collateral development. In this regard, Schaper has speculated that the epicardial coronary collateral vessels in the dog, which are known to respond to ischemia by demonstrating cellular proliferation, may not be able to respond to primarily subendocardial exercise-induced myocardial ischemia. The fine Anastomotic network of endomural coronary collateral vessels in pigs is more like the coronary collateral circulation of humans, which also possesses subendocardial collateral vessels.

The exercise protocol used in our study consisted of 5 weeks of treadmill exercise at target heart rates of 210-220 beats/min. This level of exercise training was chosen because 1) 210-220 beats/min approximates 65% of heart rate reserve in the pig (assuming a resting heart rate of 90 beats/min and a maximum heart rate of 275 beats/min), which is an exercise level that is similar to that prescribed to promote cardiovascular fitness in humans, 2) pigs demonstrate myocardial ischemia at this heart rate as shown by regional myocardial dysfunction and relative subendocardial underperfusion in the collateral-dependent myocardium. Despite an exercise intensity sufficient to cause myocardial ischemia, no pigs were lost from the study after the start of the training period. Similar survival rates have been demonstrated in dogs during exercise training after coronary occlusion. In our study, seven pigs died suddenly within 3 weeks after surgery before exercise testing. Therefore, pigs are susceptible to myocardial ischemia during the closure period of the ameroid occluder but become resistant to ischemia-related sudden death shortly after coronary occlusion. Furthermore, exercise training did not increase the incidence of myocardial necrosis in the pigs. Percentages of myocardial infarction in collateral-dependent myocardium were not significantly different between pigs from the exercise and sedentary groups. Pigs in the exercise group showed lower mean values for percent myocardial necrosis in the collateral-dependent region compared with the sedentary group; this most likely results from pig-to-pig varia-
tion rather than a protective effect of exercise, for we have shown previously that most of the myocardial necrosis present in this model develops within the first 3 weeks after surgery during the occlusion period. Exercise training began approximately 30 days after surgery to allow the training period to occur when the coronary collateral circulation of the pig shows little further development. This protocol contrasts with previous studies in dogs and pigs in which training began within 2 weeks or 3 months after surgery. In pigs, exercise training after coronary occlusion and initial collateral development may be analogous to the situation in patients with coronary disease who are exercising in a cardiac rehabilitation program. Our pigs were exercised for 5 weeks, a time period used previously by Neill and Oxendine in a group of dogs but a shorter period than the average 6–12 weeks used by others. The effects of long-term exercise on regional myocardial function and coronary collateral development in our study were assessed during exercise in conscious animals before and after training. This longitudinal assessment of data likely provided a more sensitive means to discern changes in myocardial function and collateral flow because we were able to use a paired analysis of data control for interanimal variations in collateral blood flow and myocardial function. In addition, the present study is unique in that exercise-induced alterations in regional function in collateral-dependent myocardium were examined for the first time. Previous studies involving exercise and collateral development in dogs and pigs focused on coronary collateral blood flow and global indexes of myocardial function. Our study provides novel information concerning functional adaptation of collateral-dependent myocardium after long-term exercise.

There are several limitations to this study. One limitation was the necessity to limit the use of invasive arterial catheters and Konigsberg pressure transducers in our pigs to maximize general health and survival. In long-term studies, catheters can lead to embolization of limbs and removal of the animal from the protocol. As shown in Table 2, arterial blood pressure measurements were made in only four pigs in the exercise group during the actual exercise testing. We measured arterial pressures in an additional four pigs from the exercise group using a second surgical procedure to advance a Silastic catheter into the aorta through the internal carotid artery after completion of the final exercise protocol. Mean arterial pressures measured during rest and intense exercise in these pigs (116±10 and 157±13 mm Hg, respectively) were not significantly different from pressures measured in other pigs in the exercise or sedentary group (Table 2). These results suggest that the improvements in myocardial function and coronary collateral blood flow were not related to hemodynamic differences between the groups. Another limitation of the study was that the coronary collateral circulation was less developed in sedentary pigs than in exercised pigs before the exercise or sedentary protocols began. Because of this lesser development, sedentary pigs had lower blood flow ratios at rest and during exercise (Table 5), a greater degree of dysfunction in the collateral-dependent region during exercise (Figure 3), and ultimately a slightly higher percentage of myocardial necrosis. The increased percentage of myocardial necrosis in the sedentary group was not significantly different from that in the exercise group and in itself probably did not limit the improvement of coronary collateral blood flow and regional myocardial function during exercise observed in our sedentary group. In this regard, a previous study in our laboratory showed very similar limitations to collateral blood flow improvement in 37 sedentary pigs with LCX ameroid occlusion and infarct sizes similar to the exercise group in this study. The discrepancies in collateral blood flow between the exercise and sedentary groups in the present study limited our ability to draw conclusions from intergroup comparisons. Therefore, our conclusions were drawn primarily from intragroup longitudinal comparisons.

In conclusion, our data provide evidence that physical training in the pig after coronary artery occlusion improves regional myocardial function and coronary collateral perfusion of collateral-dependent myocardium during exercise. It is important to keep in mind the limitations of comparing the results from investigations in animals with those in humans. Therefore, we suggest that caution be used in extrapolating our results to physical training in patients with occlusive coronary heart disease.

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**KEY WORDS**
- myocardial dysfunction
- ischemia, exercise-induced
- exercise
- ameroid occlusion
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