Proximal coronary vasomotor reactivity after exercise training in dogs

ALFRED A. BOVE, M.D., PH.D., AND JERRY D. DEWEY, B.S.

ABSTRACT  The effects of exercise on large coronary vasoreactivity were determined in eight dogs trained by treadmill running for 8 weeks. Six nontrained dogs comprised the control group. The trained group showed a significant reduction in heart rate during graded submaximal exercise testing when compared with the controls, and resting plasma levels of norepinephrine (nontrained group, 331 ± 99 pg/ml; trained group, 142 ± 30 pg/ml; p < .05) and epinephrine (nontrained, 424 ± 105; trained, 258 ± 45 pg/ml; p < .05) were reduced significantly in the trained group. Epicardial coronary responses to intracoronary infusion of serotonin and phenylephrine were evaluated by quantitative coronary angiography, and myocardial blood flow was measured with 15 μm radioactive microspheres. Left ventricular/body weight ratio was similar in the trained (4.81 ± 0.24 g/kg) and nontrained groups (4.79 ± 0.17), and no differences were noted in resting myocardial oxygen consumption or coronary arteriovenous oxygen difference. The constriction of the proximal left anterior descending artery (LAD) in response to serotonin infusion was not different in the two groups, but the LAD and circumflex artery constrictor responses to phenylephrine were attenuated in the trained when compared with the nontrained dogs. The data indicate that endurance exercise diminishes the large epicardial coronary vasoconstrictor response to α-adrenergic stimulation, but not to serotonin. The blunted constrictor response in the trained animals suggests that exercise may be useful in reducing epicardial coronary vasoconstriction, which is thought to be important in some patients with coronary artery disease.


BECAUSE OF the now well-established role of coronary vasomotion in patients with coronary artery disease, interest in regulation of the proximal coronary artery has grown. Over 70 years ago, Osler1 suggested that large coronary vasoconstriction was an important component of coronary disease, but only recently has the significance of large coronary regulation become evident.2-4 We have previously shown that large coronary arteries can be regulated independently of coronary resistance vessels,5 and data from other laboratories have also demonstrated that large coronary arteries can be constricted by both α-adrenergic influence and by serotonin.6-7 Since exercise is used commonly in the rehabilitation of patients with heart disease, improved understanding of effects of exercise training on large coronary arteries would be useful. Studies of long-term effects of exercise on large coronary arteries have, to date, demonstrated a change in size of the arteries after training,8,9 but no studies have described their vasomotor characteristics. One might expect endurance exercise to affect vasomotor reactivity based on several findings. First, it is known that resting levels of plasma catecholamines are lowered by endurance training,10,11 and second, it is known that adrenergic receptors can be “down regulated” by long-term stimulation.12-14 Since short-term exercise produces an increase in catecholamines,15,16 the repetitive exposure to short-term exercise associated with exercise training might cause a reduction in α-receptors. Both observations suggest that coronary vasomotor reactivity, as well as resting coronary caliber, will be affected by endurance exercise. In this study, using an intact, closed-chest dog preparation, we tested the hypothesis that long-term exposure to endurance exercise alters the vasomotor reactivity of large coronary arteries.

Methods

The study was conducted in 14 male mongrel dogs ranging from 16 to 26 kg in weight. The animals were divided into two groups (eight exercise and six controls) and were familiarized with the treadmill for 2 to 3 days. The animals in the exercise group were then given a standard submaximal exercise test17 to document their heart rate responses before training. Three of the
dogs also had venous blood samples drawn for catecholamine analysis both while they were at rest and immediately after running for 7 min at a work level of 4 mph, 20% grade.

Exercise training of the eight dogs was begun the week after the completion of the exercise tests, and was carried out 5 days per week for 8 weeks on a motorized treadmill (Collins P3800-AB) at progressively increasing workloads until all animals were exercising at a peak level of 6 mph, 20% grade, for 30 min, followed by 30 min at 7.5 mph, 12% grade. At the end of the training period, the animals were retested with the graded submaximal exercise test and blood samples were again obtained while the dogs were at rest and after exercise for catecholamine analysis, which was used to document a response to training.

The six control animals remained sedentary and were confined to cages during the 8 week training period. They then underwent familiarization with the exercise test for 2 days followed by retesting and blood sampling identical to that performed in the exercise group.

Blood samples used in the catecholamine analysis consisted of 10 ml of heparinized blood drawn via direct venipuncture of an external jugular vein while the dog sat on the treadmill. The sampling procedure required less than 20 sec and was well tolerated by the animals. Epinephrine and norepinephrine plasma levels were determined by high-performance liquid chromatography and electrochemical detection. Studies of coronary arteries in vivo were performed within 4 days of the final exercise test for all dogs, and within 2 days of the final training session for the exercise animals.

Coronary studies. The animals were sedated with 0.25 ml/kg Innovar (trentalyl citrate, 0.4 mg/ml; droperidol, 20 mg/ml) and lightly anesthetized by the addition of nitrous oxide (65%) in oxygen administered through an endotracheal tube with a volume respirator (Harvard Apparatus Co.). Catheters were placed in the coronary sinus via the right jugular vein for blood sampling and pacing, into the left atrium via transapical puncture for microsphere injection, and into the femoral artery for blood sampling. A specially designed double catheter was inserted into the left coronary artery via the left carotid artery. This catheter consisted of a large bore-guide catheter that was positioned at the left main coronary artery orifice and a small (1.1 mm diameter) selective coronary catheter that was advanced through the large catheter into the left anterior descending coronary artery (LAD). The tip of this small catheter, identified by an opaque marker, was located in the midportion of the LAD, just distal to the bifurcation of the first diagonal branch. After placement of the catheters all pressure gauges (Statham P23 DB) were balanced and calibrated against a mercury manometer. The heart of each dog was paced at 80 to 90 beats/min for all measurements.

Coronary reactivity. To test the vasomotor characteristics of the large coronary arteries serotonin and phenylephrine were infused into the midportion of the LAD of each dog. The infusion protocol for each vasoconstrictor included a control and two doses of the mediator (serotonin, 10 and 50 μg/min; phenylephrine, 10 and 50 μg/min) in an infusion volume of 0.1 or 0.5 ml/min. In the control state and after 7 min of infusion of each dose, pressures and heart rate were measured, blood was sampled from the aorta and coronary sinus to allow determination of oxygen content (Instrumentation Laboratories, oxygen analyzer), and a coronary arteriogram was recorded on high-resolution x-ray film during injection of 4 to 6 ml of meglumine diatrizoate into the guide catheter located at the left main coronary orifice. To ensure well-defined coronary images, we used a specially designed x-ray trigger that timed the 35 msec x-ray exposure from the R wave of the electrocardiogram. Timing was set to expose the angiogram in mid-diastole.

Quantitative analysis of the angiogram was done with a computer-based image analysis system. Diameters of arteries were measured at 1 mm intervals and converted to cross-sectional area with use of the formula for area of a circle. Our quantitative angiographic system has been tested both in vitro and in vivo and was found to produce accurate measurements of dimension. Measurements of myocardial blood flow. Blood flow was determined with 15 μCi radioactive microspheres (New England Nuclear) labeled with 57Co, 113Sn, or 46Sc in dogs in the control state and after infusion of the highest dose of each mediator. The microspheres (1.4 million in 2 ml) were injected into the left atrium while blood was sampled at 7.6 ml/min from the femoral artery with the use of a constant-rate withdrawal pump (Harvard Apparatus). After the final dose of drug was given, 3 ml of α-zurine blue dye (10 mg/ml) was injected through the selective coronary catheter into the LAD to delineate the myocardial tissue perfused by the artery. The dogs were then killed with KCl and the heart of each was excised.

The atria and great vessels were separated from the ventricles, which were then weighed, placed in 10% buffered formalin for 3 to 4 days, and sectioned into 36 left and 18 right ventricular pieces according to a standard map. Each piece was weighed and then placed in a counting vial. All tissue samples and the reference blood were counted in a three-channel automatic gamma counter, and the data were corrected for energy overlap with standard linearly equation technique. Flow from the LAD distribution was determined from the blue-stained tissue samples.

Assessment of coronary vasomotor response. Determination of the coronary response was based on comparison with that in other intact dog, isolated vascular ring, and isolated muscle preparations. Since absolute length changes in smooth muscle depend on the initial length, it is necessary to express the shortening response to vasoconstrictor stimulation as a strain (ΔU/ūo). We therefore determined the shortening response as a fraction of initial length and converted the results to percent change. Because flow is dependent on cross-sectional area, the shortening responses to phenylephrine and serotonin were expressed in area units (ΔA/Ao, where Ao is the unstimulated cross-sectional area).

Statistical evaluation. Exercise heart rate response and dose–response curves between trained and nontrained animals were compared by two-way analysis of variance. Single measurements were compared by Student's t test.

Results

Heart and body weights. The two groups of animals had similar heart to body weight ratios (table 1), although the exercise group had a lower average body weight.

Hemodynamic data. Heart rates and aortic pressures

TABLE 1

<table>
<thead>
<tr>
<th>Mean ± SEM heart and body weights in the two groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Body wt (kg)</td>
</tr>
<tr>
<td>Heart wt (g)</td>
</tr>
<tr>
<td>LV wt (g)</td>
</tr>
<tr>
<td>LV/BW (g/kg)</td>
</tr>
</tbody>
</table>

T = trained; NT = nontrained; wt = weight; LV = left ventricular; LV/BW = ratio of left ventricular to body weight.
are listed in table 2. Heart rate was held constant by pacing for all hemodynamic measurements. No changes in mean aortic pressure were noted after either mediator (table 2).

Myocardial blood flow in the region perfused by the LAD (table 3) showed a significant increase with serotonin, but no significant changes were noted with phenylephrine. Responses of blood flow in the total left ventricular myocardium were less evident because the unaffected circumflex flow was averaged with the LAD flow. Coronary arteriovenous oxygen differences were the same under all conditions, but small changes followed the same trend as the flow data. No changes in myocardial oxygen consumption occurred in either group (table 3).

Exercise training. Figure 1 shows the heart rate response to the standard submaximal exercise test in the trained and nontrained animals. There was a significant reduction of heart rate (p < .01 by two-way analysis of variance) at all levels of exercise. This change constitutes a training effect.

large coronary effects. Cross-sectional area of the proximal LAD and the circumflex artery are given in table 4. Area change is listed in the right column. Both phenylephrine and serotonin caused constriction in both groups and in both arteries. Reduced constriction was found in the trained animals.

Changes in cross-sectional area of the proximal LAD and circumflex artery, expressed as percent changes from control (100 · ΔA/Ao), are shown in figures 2 and 3. In nontrained animals, 50 μg/min of either serotonin or phenylephrine produced a 35% reduction in cross-sectional area in the LAD, with a smaller effect in the circumflex artery. In the exercise group the effect of phenylephrine was diminished in both arteries (figure 3). Evaluation of the responses in trained vs nontrained animals for the LAD and circumflex artery by two-way analysis of variance showed a significant (p < .05) training-induced reduction in the vasoconstrictor response to phenylephrine, but no training effect on serotonin-induced vasoconstriction.

To compare resting coronary dimensions in the two groups, we expressed the control coronary cross-sectional area of trained and nontrained animals in terms of the amount of myocardium supplied by the artery. Thus, the cross-sectional area of the LAD was expressed as area/gram, with the myocardial region that was stained blue by direct injection of blue dye into the LAD considered to represent the total perfused area of the artery. Figure 4 compares the normalized coronary areas for the LAD and the total LAD/circumflex distribution. There was a significant training-induced increase in normalized coronary cross-sectional area (p < .05).

Catecholamines. We measured free plasma levels of epinephrine and norepinephrine to determine if changes in these hormones contributed to the re-

### TABLE 2
Mean ± SEM hemodynamic data from trained and nontrained groups: invasive catheterization study

<table>
<thead>
<tr>
<th></th>
<th>Heart rate (beats/min)</th>
<th>Mean aortic pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nontrained</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>87 ± 1</td>
<td>84 ± 5</td>
</tr>
<tr>
<td>After 5 HT</td>
<td>84 ± 2</td>
<td>82 ± 4</td>
</tr>
<tr>
<td>After PHE</td>
<td>82 ± 4</td>
<td>93 ± 3</td>
</tr>
<tr>
<td>Trained</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>84 ± 2</td>
<td>82 ± 4</td>
</tr>
<tr>
<td>After 5HT</td>
<td>84 ± 2</td>
<td>76 ± 5</td>
</tr>
<tr>
<td>After PHE</td>
<td>86 ± 3</td>
<td>87 ± 5</td>
</tr>
</tbody>
</table>

5HT = serotonin; PHE = phenylephrine.

### TABLE 3
Mean ± SEM myocardial blood flow and oxygen consumption in trained and nontrained groups

<table>
<thead>
<tr>
<th></th>
<th>LAD flow (ml/100 g min)</th>
<th>LV flow (ml/100 g min)</th>
<th>AVO₂ (ml O₂/dl)</th>
<th>MVO₂ (ml O₂/100 g min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nontrained</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>92 ± 12</td>
<td>88 ± 11</td>
<td>7.4 ± 0.6</td>
<td>6.6 ± 1.0</td>
</tr>
<tr>
<td>5HT</td>
<td>121 ± 19*</td>
<td>96 ± 14</td>
<td>6.7 ± 0.5</td>
<td>6.4 ± 1.1</td>
</tr>
<tr>
<td>PHE</td>
<td>84 ± 7</td>
<td>78 ± 7</td>
<td>7.0 ± 0.2</td>
<td>5.5 ± 0.4</td>
</tr>
<tr>
<td>Trained</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100 ± 7</td>
<td>97 ± 6</td>
<td>6.3 ± 0.6</td>
<td>6.4 ± 0.7</td>
</tr>
<tr>
<td>5HT</td>
<td>159 ± 29*</td>
<td>128 ± 24</td>
<td>5.4 ± 3.0</td>
<td>6.8 ± 1.4</td>
</tr>
<tr>
<td>PHE</td>
<td>101 ± 11</td>
<td>97 ± 10</td>
<td>6.3 ± 0.6</td>
<td>6.3 ± 1.2</td>
</tr>
</tbody>
</table>

AVO₂ = arterial venous oxygen difference; MVO₂ = myocardial oxygen consumption; other abbreviations are as in tables 1 and 2.

*Effect of 5HT significant at p < .05.
sponses of the coronary artery. Both epinephrine and norepinephrine levels were reduced in the trained group (table 5).

Discussion

The effects of endurance training on the coronary circulation are not well understood. Previous studies have shown no change in distal coronary vascular resistance and an increase in proximal coronary artery dimensions with exercise training. The postmortem observations by Currens and White on Clarence DeMar, a lifelong marathon runner, suggested that prolonged endurance exercise training was associated with enlarged epicardial coronary arteries. Wyatt and Mitchell, using coronary angiography in a longitudinal study involving a single group of animals, suggested that small increases in circumflex area occurred in dogs after 12 weeks of training. Kramsch et al. examined monkeys on an exercise program and a high-cholesterol diet and found larger coronary arteries and less atherosclerosis in the trained animals than in a similar control group not exposed to the exercise regimen. Increased coronary tree size in ducks was reported by Tepperman and Pearson using a corrosion-cast technique, and similar findings in exercising rats were reported by Stevenson et al. Our results concur with these previous studies and suggest that epicardial coronary arteries enlarge under the intermittent high-flow stimulus resulting from long-term endurance exercise. No studies to date, however, have examined the vasoconstrictor responses of the large coronary arteries after training.

Vasomotion of large coronary arteries has been shown to contribute significantly to the clinical manifestations of coronary artery disease. Recent reports of myocardial infarction, angina pectoris, and exercise-induced ischemia resulting from large coronary constriction (spasm) document the significance of coronary vasomotion in coronary disease. Since exercise is known to improve symptoms of coronary disease, we hypothesized that exercise may contribute to improvements in symptoms by reducing large coronary vasoconstriction. To produce coronary vasoconstriction we used phenylephrine and serotonin, both of which are considered to be important in large coronary regulation.

In a recent study from our laboratory, both demonstrated a vasoconstrictor action on epicardial coronary arteries. With this preparation we found that the vasoconstrictor effect of phenylephrine was reduced after exercise training, while the effect of serotonin was unchanged. In addition to this reduced

![FIGURE 1. Heart rate response to the graded submaximal exercise test described by Tipton et al. The nontrained animals (NT) have a higher heart rate response at all levels of rest and exercise than the trained (T) animals. Heart rate in beats/min.](http://circ.ahajournals.org/)

**TABLE 4**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>10 µg/min</th>
<th>50 µg/min</th>
<th>ΔA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylephrine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD-NT</td>
<td>4.50 ± 0.46</td>
<td>3.12 ± 0.43</td>
<td>2.82 ± 0.44*</td>
<td>1.68 ± 0.33*</td>
</tr>
<tr>
<td>LAD-T</td>
<td>4.81 ± 0.26</td>
<td>3.79 ± 0.29</td>
<td>3.50 ± 0.31*</td>
<td>1.31 ± 0.18</td>
</tr>
<tr>
<td>CX-NT</td>
<td>6.73 ± 0.58</td>
<td>4.95 ± 0.55</td>
<td>4.95 ± 0.66*</td>
<td>1.78 ± 0.24*</td>
</tr>
<tr>
<td>CX-T</td>
<td>5.59 ± 0.39</td>
<td>4.69 ± 0.65</td>
<td>4.74 ± 0.68*</td>
<td>0.85 ± 0.50</td>
</tr>
</tbody>
</table>

Serotonin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>10 µg/min</th>
<th>50 µg/min</th>
<th>ΔA</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAD-NT</td>
<td>4.67 ± 0.40</td>
<td>3.97 ± 0.35</td>
<td>2.88 ± 0.29*</td>
<td>1.78 ± 0.64</td>
</tr>
<tr>
<td>LAD-T</td>
<td>4.81 ± 0.26</td>
<td>3.64 ± 0.36</td>
<td>3.46 ± 0.30*</td>
<td>1.35 ± 0.39</td>
</tr>
<tr>
<td>CX-NT</td>
<td>6.38 ± 0.52</td>
<td>5.97 ± 0.63</td>
<td>5.05 ± 0.62*</td>
<td>1.33 ± 0.46</td>
</tr>
<tr>
<td>CX-T</td>
<td>5.59 ± 0.39</td>
<td>5.30 ± 0.45</td>
<td>3.83 ± 0.32*</td>
<td>1.76 ± 0.26</td>
</tr>
</tbody>
</table>

CX = circumflex; T = trained; NT = nontrained.

*Significant change from control, p < .05.

**T vs NT, p < .10.**
α-adrenergic sensitivity, there was also a reduction in the concentration of circulating catecholamines. Decreased α-adrenergic sensitivity and decreased plasma levels of α-agonists should result in less large coronary vasconstriction at rest and during short-term exercise. Lower blood pressure, which could result from reduced peripheral adrenergic sensitivity after exercise training, as reported by Paulik and Frenkle,37 may contribute to the reduction of anginal symptoms after exercise training in patients with heart disease. The combination of reductions in proximal coronary constriction, peripheral adrenergic sensitivity, heart rate, and blood pressure after training supports the use of exercise training these patients. Exercise-induced changes in catecholamine metabolism, which we have also reported,38 could also benefit patients with coronary disease.

In this study, we did not test the mechanism for reduced large coronary sensitivity to α-adrenergic stimulation. An important mechanism that remains to be demonstrated is a reduction in α-receptor density induced by the frequent increases in levels of catecholamines associated with repetitive exposure to short-term exercise. Colucci et al.12 demonstrated a reduction in α-receptor affinity and number when levels of catecholamines were elevated. Similar data were reported by Carrier et al.13 in studies of aortic strips from rabbits, and the same phenomenon can be demonstrated in isolated vascular smooth muscle cells.14 Our data in intact animals could be explained by a reduction in α-receptors, but this response remains to be proven. Although reduced resting catecholamine levels could reduce resting tone of the large coronary arteries and result in a larger resting diameter, decreased α-adrenergic sensitivity is not explained by this finding.

Our preparation for examining proximal coronary regulation also provides data on distal coronary regulation in the same animal. We found no significant changes in coronary flow or arteriovenous oxygen difference after exercise training with either phenylephrine or serotonin, which suggests that no significant changes in distal coronary responsiveness occurred. A recent study by Gwirtz and Stone,39 however, suggested that distal vascular sensitivity may be increased. They injected several drugs, including phenylephrine, as an intracoronary bolus in awake animals, while we gave a continuous intracoronary infusion in anesthe-

### TABLE 5
Mean ± SEM plasma norepinephrine and epinephrine levels in exercise-trained and sedentary dogs

<table>
<thead>
<tr>
<th></th>
<th>Norepinephrine (pg/ml)</th>
<th>Epinephrine (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontrained (n = 6)</td>
<td>331 ± 99</td>
<td>158 ± 26</td>
</tr>
<tr>
<td>Trained (n = 8)</td>
<td>142 ± 30</td>
<td>76 ± 14</td>
</tr>
</tbody>
</table>

*Training effect significant at p < .05 by two-way analysis of variance.
tized dogs. These differences in technique may explain our differing results.

Our findings indicate that long-term aerobic exercise training exerts an effect on large coronary vasoreactivity, and in combination with reduced plasma catecholamine levels, the training effect results in reduced proximal coronary vasoconstriction. If similar changes occur in exercising man, then trained subjects would have less \( \alpha \)-adrenergic–induced coronary vasoconstriction.

We thank Dr. G. M. Tyce for performing the catecholamine assay, Mr. C. Grabau and Mr. M. B. Mock for their efforts in training the animals, and Mrs. Lynn Schwieder for assistance in preparing the manuscript.

References

33. Wengel NK, Bilbert CA, Skora MP: Cardiac conditioning after myocardial infarction. Cardiac Rehab 2: 17, 1971
Proximal coronary vasomotor reactivity after exercise training in dogs.
A A Bove and J D Dewey

Circulation. 1985;71:620-625
doi: 10.1161/01.CIR.71.3.620
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1985 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/71/3/620

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/