Chronic Exercise Enhances Endothelium-Mediated Dilation of Epicardial Coronary Artery in Conscious Dogs

Jie Wang, Michael S. Wolin, Thomas H. Hintze

Whether endothelium-derived relaxing factor (EDRF)/nitric oxide (NO) plays a role in the dilation of the left circumflex coronary artery during acute exercise and whether endothelium-mediated dilation of this artery is altered after chronic exercise training have not been determined previously. Nine dogs were chronically instrumented for measurements of systemic hemodynamics, left circumflex coronary artery diameter, and blood flow. Acute treadmill exercise (10.9 km/h) caused dilation of the circumflex coronary artery by 4.33±0.84% and an increase in coronary blood flow by 32±5.2 mL/min. After the administration of intravenous nitro-L-arginine, the dilation of the circumflex coronary artery was converted to vasoconstriction (−4.13±1.58%), whereas the increase in coronary blood flow was not altered (24±3.6 mL/min). Chronic exercise training (2 hours each day at a speed of 10.9 km/h for 7 days) enhanced acetylcholine-induced dilation and reactive dilation (following release of a brief coronary artery occlusion) of the large coronary artery (P<.05), whereas the coronary blood flow responses were not changed. These enhanced acetylcholine-induced and reactive dilations of the circumflex coronary artery were due to a greater release of EDRF/NO since they were eliminated by nitro-L-arginine. Thus, in the circumflex coronary artery, EDRF/NO-dependent dilation was enhanced after 7 days of exercise training. This may represent the mechanism responsible for the perception that chronic exercise induces cardiovascular "well being." (Circ Res. 1993;73:829-838.)

Key Words • exercise • endothelium-derived relaxing factor • epicardial coronary artery • acetylcholine • reactive dilation

The benefits of exercise on the cardiovascular system have been well documented. These benefits include improved cardiac function,1,2 increased coronary reserve,3 lower incidence of coronary disorders,4 and more tolerance to cardiovascular diseases.5 However, the mechanisms responsible for these benefits have not been defined. In 1980, Furchgott and Zawadzki6 first described that vascular endothelial cells release a vasodilator named endothelium-derived relaxing factor (EDRF) in response to acetylcholine. The release of EDRF is triggered either by agents such as acetylcholine and bradykinin6,7 or by mechanical stimuli such as blood flow and shear stress.8

During exercise, blood flow is elevated, particularly in the coronary and the skeletal muscle circulations, because of increases in metabolic demand.9 The increases in coronary blood flow cause the dilation of the epicardial coronary artery since, if coronary blood flow was held constant by a stenosis during exercise, the dilation of the epicardial coronary artery was eliminated.10 In the coronary circulation, we11 and other investigators12,13 have shown that an acute increase in flow velocity following release of a transient coronary artery occlusion results in the dilation of epicardial coronary arteries via a mechanism involving EDRF/nitric oxide (NO). Studies from our laboratory and other laboratories also have documented that chronic increases in coronary blood flow by chronic cardiac pacing14 and a chronic increase in femoral blood flow following opening an arterial venous fistula15 caused an elevated production of EDRF from vascular endothelium. The characteristic high coronary blood flow during exercise and the potential positive feedback between blood flow velocity and the production of EDRF led us to hypothesize that the dilation of the circumflex coronary artery during acute exercise was EDRF/NO-mediated and that the endothelium-mediated dilation of the circumflex coronary artery would be enhanced after chronic exercise training. Perhaps one of the mechanisms responsible for the benefits of exercise is through enhancing endothelium-mediated control of the large coronary artery. Controversial results have been provided by previous studies in vitro. For instance, endothelium-mediated relaxations of canine coronary arteries to the α2-adrenergic agonists norepinephrine and UK14304 were not altered by chronic exercise.16 Furthermore, in porcine coronary arteries taken from animals with chronic exercise training, norepinephrine caused less maximal tension development compared with control arteries; this appeared to be an endothelium-independent phenomenon.17 Therefore, the goals of this study were to determine (1) which mediator was responsible...
for the dilation of epicardial coronary arteries during acute treadmill exercise and (2) whether endothelium-mediated control of epicardial coronary arteries was enhanced after brief exercise training.

**Materials and Methods**

**Surgical Preparation and Measurements**

Before surgery, dogs were screened to run on our treadmill (Telbot Carlson, Inc, Audubon, Iowa), and surgery was only carried out on dogs who ran well at speeds of 10.9 km/h. This study used nine dogs of either sex (eight males and one female), weighing 25 to 29 kg. Dogs were sedated using acepromazine (0.3 mg/kg IM, Ayerst Laboratories, New York, NY) and anesthetized with pentobarbital sodium (25 mg/kg IV). These dogs were prepared for sterile surgery, and a thoracotomy was performed in the left fifth intercostal space using sterile surgical technique. A Tygon catheter was placed in the descending thoracic aorta. A solid pressure gauge (model P6.5, Konigsberg Instruments, Pasadena, Calif) was placed in the apex of the left ventricle (LV). A flow-cuff transducer (3.5 to 5 mm in diameter) was implanted on the left circumflex coronary artery. Sonomicrometer crystals (7 MHz, 1×2 mm) were sutured on opposing surfaces of the same artery. A hydraulic occluder was placed around the same artery distal to the flow transducer and sonomicrometer crystals.

The catheter and wires were run subcutaneously to the intracapsular region. The chest was closed in layers, and the pneumothorax was reduced. The dogs were allowed to fully recover. Antibiotics were given after surgery as necessary. After 10 days, dogs were trained to lie quietly on the laboratory table.

On the day of the first experiment, the dog was brought to the laboratory and placed on the table. A 19-gauge intravenous catheter was inserted in a peripheral vein and attached to extension tubing for injections of drugs without disturbing the dogs. All interventions including drug injections and brief coronary artery occlusions were performed in a random order. Between drug injections or coronary artery occlusions, enough time was allowed so that systemic and coronary hemodynamics returned to baseline, and then the next injection or coronary artery occlusion was performed.

**Hemodynamic Recordings**

With the dog lying on the laboratory table or running on the treadmill, the following recording techniques were used in both sets of experiments. The previously implanted aortic catheter was attached to a P23ID strain gauge transducer (Statham Instruments, Inc) to measure arterial pressure. LV systolic pressure was measured using the previously implanted solid-state pressure gauge. The LV pressure signal was differentialed to measure LV dp/dt. Left circumflex coronary artery diameter and coronary blood flow were measured, respectively, using the previously implanted sonomicrometer crystals and Doppler flow probe with a pulsed Doppler system (System 6, Triton Technology, Inc, San Diego, Calif). The data were recorded on a 14-channel tape recorder (model 3700B, Bell and Howell) and played back on a direct-writing oscillograph (model 3800s, Gould). Mean values were derived for aortic pressure, circumflex coronary artery diameter, and blood flow using 2-Hz resistance-capacitor filters. Heart rate was measured using a cardiograph (model 9857B, Beckman Instruments), which was triggered by the LV pressure signal. Late diastolic coronary vascular resistance (LDCR) was calculated as an index of changes in the diameter of coronary resistance vessels according to the following equation: LDCR = late diastolic aortic pressure/diastolic coronary blood flow. Any drift in the amplifiers, the tape recorder, and oscillograph was eliminated by frequent calibration during experiments.

**Experimental Design**

The dogs were trained to stand on the treadmill and to run on the treadmill at 3.7, 5.8, 7.4, 9.5, and 10.9 km/h for at least 5 minutes at each speed (to create multiple steady states for hemodynamic measurements) and to run on the treadmill at a speed of 10.9 km/h for 1 hour.

Experiments were divided into four steps: (1) Table experiments were performed. (2) The following day, the dog started to run on the treadmill. Chronic exercise consisted of 2 hours a day at a speed of 10.9 km/h (1 hour in the morning and 1 hour in the afternoon) for 7 days. After 2 days, hemodynamics were recorded with the dog standing on the treadmill and exercising at speeds of 3.7, 5.8, 7.4, 9.5, and 10.9 km/h. (3) After 7 days of the exercise training program, the table experiments were repeated. To determine whether NO was responsible for the alteration of endothelium-mediated control of the epicardial coronary artery after chronic exercise, acetylcholine at dose of 10 µg/kg, release of 15- and 30-second coronary artery occlusion, and nitroglycerin at a dose of 25 µg/kg were studied before and after the administration of nitro-L-arginine. (4) On the following day, hemodynamics were recorded with the dog standing on the treadmill and then running on the treadmill after nitro-L-arginine only. This was done because nitro-L-arginine has long-lasting effects on vascular function even when arginine is given prophylactically after each experiment.

**Effects of Acute Exercise on Epicardial Coronary Artery**

We postulated that increases in coronary blood flow that would occur during treadmill exercise training should lead to a flow-dependent dilation of the epicardial coronary artery and that this dilation was mediated by EDRF. To test this hypothesis, all nine dogs were trained to stand quietly on the treadmill for the measurement of control values and then to run on the treadmill at speeds of 3.7, 5.8, 7.4, 9.5, and 10.9 km/h. Circumflex coronary artery diameter, coronary blood flow, LV pressure, LV dp/dt, and mean arterial pressure (MAP) were simultaneously recorded during each steady state. On a separate day, the same protocols were repeated after nitro-L-arginine (Aldrich Chemical Company, Inc, Milwaukee, Wis) was given as a bolus. During the initial table experiment, we determined for each dog the precise dose of nitro-L-arginine that abolished reactive dilation and acetylcholine-induced dilation of the left circumflex coronary artery.

**Effects of Chronic Exercise on the Circumflex Coronary Artery**

We also hypothesized that chronic exercise may be a powerful stimulus to enhance the release or production
of EDRF from the endothelium of epicardial coronary arteries caused by the increase in coronary blood flow that occurred during exercise. To test this hypothesis, we have examined the responses of the circumflex coronary artery to the release of a transient coronary artery occlusion and to acetylcholine and nitroglycerin before and after 7 days of exercise training.

Acetylcholine and Endothelium-Mediated Dilation of the Circumflex Coronary Artery

Multiple doses of acetylcholine (0.25, 0.5, 1, 5, 10, and 20 µg/kg) were injected as a bolus intravenously. The peak responses of epicardial coronary artery diameter, coronary blood flow, MAP, heart rate, LV pressure, and LV dP/dt were recorded. The dose-response curve of circumflex coronary artery diameter and blood flow were constructed. Acetylcholine may dilate epicardial coronary arteries by both a receptor-mediated component and a flow-mediated component. To distinguish which of these two mechanisms was responsible for the alteration of acetylcholine-induced dilation of circumflex coronary artery after chronic exercise, acetylcholine (5 and 20 µg/kg) was administered in five dogs with coronary blood flow held constant by partial inflation of the previously implanted hydraulic occluder.

Transient Coronary Artery Occlusion and Flow-Dependent Endothelium-Mediated Dilation of the Circumflex Coronary Artery

Multiple coronary artery occlusions (5, 10, 15, 20, and 30 seconds) were performed. The dilation of the circumflex coronary artery (reactive dilation) and the peak response of coronary blood flow (reactive hyperemia) were recorded. Other systemic hemodynamics were also monitored.

Nitroglycerin and Endothelium-Independent Dilation of the Circumflex Coronary Artery

The responses of circumflex coronary artery diameter and coronary blood flow to multiple doses of nitroglycerin (0.1, 0.8, 5, and 25 µg/kg IV) were examined.

Effects of Nitro-L-Arginine on Endothelium-Mediated Dilation of the Circumflex Coronary Artery After Chronic Exercise

After 7 days of exercise with the dog lying on the laboratory table, nitro-L-arginine was administered intravenously as a bolus. The inhibition by nitro-L-arginine of endothelium-mediated circumflex coronary artery dilation was tested using acetylcholine (5 µg/kg IV) injection. Once the acetylcholine-induced dilation of circumflex coronary artery was abolished by nitro-L-arginine, 15- and 30-second coronary artery occlusions and acetylcholine (10 µg/kg) and nitroglycerin (25 µg/kg) injections were performed. If the blockade was not adequate, then an additional dose of nitro-L-arginine was administrated. The averaged total dose of nitro-L-arginine was 48±14 mg/kg.

Data Analysis

Data were expressed as mean±SEM. The response of the circumflex coronary artery diameter to any one of the interventions was expressed as percent change from baseline, and the responses of coronary blood flow to those interventions were expressed as absolute values of flow, since we believe that the increases in coronary blood flow were the stimuli responsible for the dilation of the circumflex coronary artery. On the day of the experiment, significant differences from baseline were determined using one-way analysis of variance (ANOVA). The changes of coronary and systemic hemodynamics to an intervention on the control day were compared with the changes in the same intervention after chronic exercise using a two-way ANOVA. A post hoc Scheffé’s test was used to convert the ratio of F values to a r distribution. There were no missing data for any intervention for statistical processing before or after chronic exercise. After chronic exercise, the responses of the circumflex coronary artery to acetylcholine, release of a brief coronary artery occlusion, and nitroglycerin after nitro-L-arginine were analyzed using a paired t test. Statistical significance was determined at P<.05.

The protocols were approved by the Institutional Animal Care and Use Committee of New York Medical College and conform to the guiding principles for the use and care of laboratory animals of the American Physiological Society and the National Institutes of Health.

Results

Effects of Acute Exercise on the Circumflex Coronary Artery

Fig 1 shows recordings from one dog standing on the treadmill and running at 10.9 km/h. There were increases in LV systolic pressure, LV dP/dt, MAP, heart rate, circumflex coronary artery diameter, and blood flow. There was also a fall in calculated late diastolic coronary vascular resistance during exercise (Table 1). Table 1 summarizes the hemodynamic changes during exercise before and after nitro-L-arginine. The increase in circumflex coronary artery diameter was eliminated after the administration of nitro-L-arginine. A significant constriction of the large coronary artery was observed during exercise after nitro-L-arginine (Fig 2). The increases in LV systolic pressure, LV dP/dt, MAP, heart rate, and coronary blood flow during exercise were not affected by the dose of nitro-L-arginine used in the present study. Late diastolic coronary vascular resistance still fell during exercise after nitro-L-arginine; however, this was significantly attenuated (Table 1).

Effects of Chronic Exercise on the Circumflex Coronary Artery

LV systolic pressure, LV dP/dt, MAP, and heart rate were not altered by the 7 days of exercise. There was a tendency for increases in the baseline large coronary artery diameter and coronary blood flow and for a decrease in late diastolic coronary vascular resistance after 7 days of exercise training, but none of these were statistically significant from before chronic exercise. Table 2 summarizes those data.

Acetylcholine and Endothelium-Mediated Dilation of the Left Circumflex Coronary Artery

Acetylcholine caused dose-dependent decreases in LV systolic pressure and MAP and increases in LV dP/dt and heart rate. These hemodynamic measurements in response to the 20 µg/kg dose before and after 7 days of exercise training are summarized in Table 3. This was the highest dose tested, and hemodynamic
Fig 1. Recordings from one dog standing and running on the treadmill. There are increases in left ventricular (LV) pressure (LVP), LV dP/dt, mean arterial pressure (AP), coronary blood flow (CBF), circumflex coronary artery diameter (CD), mean CD (CD), and heart rate (HR) during exercise.

responses to acetylcholine were not different before and after 7 days of exercise.

Fig 3 (top) shows the circumflex coronary artery dilation to increasing doses of acetylcholine. There was a significant enhancement of the response of circumflex coronary artery diameter to each dose of acetylcholine after chronic exercise. The peak responses of coronary blood flow to acetylcholine were unchanged (Fig 3, bottom).

In five dogs, the dilations of the circumflex coronary artery following injections of acetylcholine at doses of 5 and 10 μg/kg with coronary blood flow held constant by partial inflation of the previously implanted hydraulic occluder were also significantly enhanced after chronic exercise (Fig 4).

Transcutaneous Coronary Artery Occlusion and Flow-Dependent Endothelium-Mediated Dilation of the Circumflex Coronary Artery

The reactive dilation was significantly enhanced after chronic exercise (Fig 5, top). However, the peak reactive hyperemia was not altered (Fig 5, bottom) in the present study. LV pressure, MAP, and heart rate were not influenced by the transient coronary artery occlu-
**Table 1. Effects of Acute Exercise on Hemodynamics Before and After the Administration of Nitro-L-Arginine**

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>3.7 km/h</th>
<th>5.8 km/h</th>
<th>7.4 km/h</th>
<th>9.5 km/h</th>
<th>10.9 km/h</th>
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<tbody>
<tr>
<td><strong>Left ventricular pressure, mm Hg</strong></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>139±5.0</td>
<td>172±6.2†</td>
<td>175±9.0†</td>
<td>184±12†</td>
<td>199±16†</td>
<td>208±15†</td>
</tr>
<tr>
<td>NLA</td>
<td>148±12</td>
<td>173±10†</td>
<td>179±7.0†</td>
<td>182±6.1†</td>
<td>182±7.6†</td>
<td>198±12†</td>
</tr>
<tr>
<td><strong>Left ventricular dP/dt, mm Hg/s</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>3339±275</td>
<td>4604±430†</td>
<td>4852±484†</td>
<td>5116±516†</td>
<td>5552±455†</td>
<td>5768±502†</td>
</tr>
<tr>
<td>NLA</td>
<td>3134±190</td>
<td>3856±297†</td>
<td>3993±239†</td>
<td>4064±226†</td>
<td>4119±161†</td>
<td>4593±378†</td>
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<tr>
<td><strong>Mean arterial pressure, mm Hg</strong></td>
<td></td>
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<tr>
<td>Control</td>
<td>105±3.7</td>
<td>123±6.1†</td>
<td>125±5.8†</td>
<td>127±5.2†</td>
<td>126±3.8†</td>
<td>133±6.4†</td>
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<tr>
<td>NLA</td>
<td>115±5.8*</td>
<td>140±6.7†</td>
<td>145±7.5†</td>
<td>148±5.9†</td>
<td>145±7.1†</td>
<td>152±9.4†</td>
</tr>
<tr>
<td><strong>Heart rate, bpm</strong></td>
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</tr>
<tr>
<td>Control</td>
<td>99±5.8</td>
<td>163±6.9†</td>
<td>181±7.6†</td>
<td>190±7.2†</td>
<td>201±8.9†</td>
<td>217±12†</td>
</tr>
<tr>
<td>NLA</td>
<td>82±6.3*</td>
<td>132±17†</td>
<td>144±18†</td>
<td>151±17†</td>
<td>160±15†</td>
<td>177±14†</td>
</tr>
<tr>
<td><strong>Peak coronary blood flow, mL/min</strong></td>
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<tr>
<td>Control</td>
<td>37±2.8</td>
<td>55±6.7†</td>
<td>62±6.0†</td>
<td>65±6.6†</td>
<td>65±6.4†</td>
<td>69±7†</td>
</tr>
<tr>
<td>NLA</td>
<td>38±5.8</td>
<td>55±6.6†</td>
<td>55±6.3†</td>
<td>56±6.8†</td>
<td>58±7.5†</td>
<td>62±8.8†</td>
</tr>
<tr>
<td><strong>Late diastolic coronary resistance, mm Hg · mL⁻¹ · min⁻¹</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.60±3.1</td>
<td>1.98±0.26†</td>
<td>1.84±0.27†</td>
<td>1.63±0.27†</td>
<td>1.62±0.22†</td>
<td>1.64±0.18†</td>
</tr>
<tr>
<td>NLA</td>
<td>3.61±0.3*</td>
<td>3.13±0.37†</td>
<td>3.02±0.41†</td>
<td>2.98±0.42†</td>
<td>2.85±0.37†</td>
<td>2.78±0.38†</td>
</tr>
</tbody>
</table>

NLA indicates nitro-L-arginine; bpm, beats per minute. Values are mean±SEM (n=8).
*P<.05 vs corresponding control value.
†P<.05 vs corresponding rest value.

Nitroglycerin and Endothelium-Independent Dilation of the Circumflex Coronary Artery

The dose-response curves of circumflex coronary artery diameter and blood flow to nitroglycerin were constructed. Neither the coronary artery diameter nor the coronary blood flow response to this endothelium-independent vasodilator was altered after 7 days of exercise (Fig 6). The hypotension, tachycardia, and increases in LV dP/dt in response to nitroglycerin were not altered after 7 days of exercise.

**Table 2. Baseline Hemodynamics Before and After Chronic Exercise**

<table>
<thead>
<tr>
<th></th>
<th>Before Exercise</th>
<th>After Exercise</th>
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<tbody>
<tr>
<td><strong>Left ventricular pressure, mm Hg</strong></td>
<td>131±1.9</td>
<td>136±4.0</td>
</tr>
<tr>
<td><strong>Left ventricular dP/dt, mm Hg/s</strong></td>
<td>3014±127</td>
<td>3021±114</td>
</tr>
<tr>
<td><strong>Mean arterial pressure, mm Hg</strong></td>
<td>102±2.1</td>
<td>107±2.5</td>
</tr>
<tr>
<td><strong>Heart rate, bpm</strong></td>
<td>80±3.1</td>
<td>89±4.4</td>
</tr>
<tr>
<td><strong>Epicardial coronary artery diameter, mm</strong></td>
<td>3.65±0.1</td>
<td>3.72±0.09</td>
</tr>
<tr>
<td><strong>Coronary blood flow, mL/min</strong></td>
<td>32.3±2.3</td>
<td>40.1±4.5</td>
</tr>
<tr>
<td><strong>Late diastolic coronary resistance, mm Hg · mL⁻¹ · min⁻¹</strong></td>
<td>2.72±0.21</td>
<td>2.53±0.31</td>
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</tbody>
</table>

Values are mean±SEM (n=7).
Effects of Nitro-L-Arginine on Endothelium-Mediated Dilation of the Left Circumflex Coronary Artery After Chronic Exercise

After 7 days of exercise, the enhanced endothelium-mediated dilations were abolished by nitro-L-arginine. After nitro-L-arginine, reactive dilation following release of 15- and 30-second occlusions and acetylcholine-induced dilation at a dose of 10 μg/kg were eliminated, whereas the nitroglycerin-induced dilation of the circumflex coronary artery remained unchanged (Fig 7).

Discussion

The most significant findings of the current study are as follows: (1) The dilation of the circumflex coronary artery during exercise was not only completely abolished, but to our surprise, vasoconstriction was actually observed after the administration of nitro-L-arginine. (2) After 7 days of exercise, the endothelium-mediated dilation of left circumflex coronary artery following the injection of acetylcholine and the release of a brief coronary artery occlusion was enhanced. These dilations were also eliminated by nitro-L-arginine.

Two recent studies have reported dilation of the epicardial coronary artery during exercise. These authors concluded that the dilation of the epicardial coronary artery was due to increases in coronary blood flow, but the role of EDRF in this exercise-induced dilation was not determined. Berdeaux et al recently reported that the dilation of epicardial coronary artery during exercise was eliminated by β-adrenergic receptor blockade. They concluded that β-adrenergic receptors were essential for the dilation of the epicardial coronary artery during exercise. On the other hand, Schwartz et al have shown that the dilation of the epicardial coronary artery during exercise was via a flow-dependent mechanism, since the dilation of the epicardial coronary artery was prevented by a flow-limiting stenosis. Since it has been shown previously that β-adrenergic receptors and α-adrenergic receptors do not play a role in flow-induced (by hyperemia) dilation of the epicardial coronary artery, the report from Berdeaux et al left the role of flow-dependent dilation of the epicardial coronary artery open to question. They hypothesized that other factors might be involved and have also recently shown that removal of the endothelium elimi-
Fig 5. Graphs showing response to occlusion. After chronic exercise, the dilation of epicardial coronary artery following release of a brief coronary artery occlusion, the reactive dilation, was enhanced (top). The reactive hyperemia, which is the coronary blood flow (CBF, bottom) response to release of a brief coronary artery occlusion, was not different before and after chronic exercise. CD indicates epicardial coronary artery diameter. *P<.05 vs control (n=7).

Fig 6. Graphs showing the endothelium-independent dilation of epicardial coronary artery examined using multiple doses of nitroglycerin (NTG) before and after chronic exercise (n=7). Neither epicardial coronary artery dilation (CD, top) nor coronary blood flow response (CBF, bottom) to this vasodilator was altered.

The results clearly demonstrate that the dilation of the circumflex coronary artery during treadmill exercise was completely abolished by nitro-L-arginine at a dose sufficient to block acetylcholine-induced dilation and reactive dilation. Very recently, Buxton et al21 showed that acetylcholine released from parasympathetic nerve endings caused coronary vasodilation using EDRF/NO as a mediator. Because of the potential changes in autonomic tone during treadmill exercise, it is possible that the release of acetylcholine from nerve endings may initiate endothelium-mediated dilation; however, in general, there is vagal withdrawal to the heart during exercise. Buxton et al22 reported that arginine analogues with alkyl or aryl ester groups such as N\textsuperscript{5}-nitro L-arginine methyl ester, L-arginine methyl ester, and L-arginine ethyl ester eliminated endothelium-mediated re-

<table>
<thead>
<tr>
<th>TABLE 4. Responses of Systemic Hemodynamics to 30-Second Coronary Artery Occlusion Before and After 7 Days of Exercise Training</th>
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<tbody>
<tr>
<td><strong>Before Exercise</strong></td>
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<tr>
<td>---------------------</td>
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<tr>
<td>Left ventricular systolic pressure, mm Hg</td>
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<tr>
<td>Left ventricular dP/dt, mm Hg/s</td>
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<tr>
<td>Mean arterial pressure, mm Hg</td>
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<tr>
<td>Heart rate, bpm</td>
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bpm indicates beats per minute. Values are mean±SEM (n=7).

*P<.05 vs corresponding baseline value.
sponses through blockade of muscarinic receptors. Nitro-L-arginine, which does not have an alkyl or aryl ester modification at the carboxyl end, was used in the present study. Fortunately, these kinds of arginine analogue compounds were not muscarinic antagonists. Therefore, our data indicate that EDRF/NO is the mediator responsible for the dilation of the circumflex coronary artery during exercise.

We believe that the increases in coronary blood flow and, consequently, the increases in shear stress that occurred during exercise were the stimuli for production of EDRF/NO. Furthermore, this EDRF/NO mechanism may buffer adrenergic vasoconstriction of epicardial coronary arteries during exercise. In the current study, we did observe vasoconstriction of the circumflex coronary artery during exercise when the production of EDRF/NO was blocked. An interaction between sympathetic or parasympathetic nerve activity and EDRF has been reported. For instance, the vasoconstriction of rabbit carotid artery to adrenergic nerve stimulation was inhibited by EDRF. In an isolated perfused rabbit carotid artery preparation, Tesfamariam and Cohen showed that adrenergic vasoconstriction that was triggered by transmural electrical stimulation was depressed by the increased shear stress acting on endothelial cells and that this adrenergic vasoconstriction was augmented by methylene blue, an inhibitor of guanylate cyclase. During exercise, sympathetic tone is increased in order to provide a greater cardiac output and redistribute peripheral blood flow, and coronary blood flow is also increased because of a marked increase in myocardial oxygen demand. Perhaps the control of large coronary artery vasomotor tone is controlled by the interaction between neural factors and flow-dependent endothelium-mediated mechanisms. Data from the present study demonstrate that the release of EDRF/NO from the endothelium during increases in coronary blood flow is essential for the dilation of the circumflex coronary artery during exercise. Vasoconstrictor mechanisms of epicardial coronary arteries might be unmasked if the endothelium-mediated EDRF/NO mechanisms disappear as a result of nitro-L-arginine administration or the pathological events that reduce EDRF/NO synthesis in vascular endothelium. The fall in late diastolic coronary vascular resistance during exercise was attenuated by nitro-L-arginine. This increase in late diastolic coronary vascular resistance may suggest a possible role of EDRF/NO in the regulation of coronary vascular resistance during exercise. Several studies have reported recently that EDRF/NO might play such role in the coronary circulation. Although the changes in coronary blood flow during exercise were not depressed at the dose of nitro-L-arginine used in the present study, our data do not imply that EDRF/NO has no role in the control of coronary blood flow during exercise, because the dose of nitro-L-arginine used in the present study was selected specifically to eliminate circumflex coronary artery dilation in response to acetylcholine and acute increase in flow velocity and not to eliminate blood flow responses to these agents. This experimental design allowed us to distinguish whether an altered coronary blood flow response or an altered EDRF/NO mechanism was responsible for elimination of epicardial coronary artery dilation after nitro-L-arginine administration during exercise. Therefore, the role of EDRF/NO in coronary resistance vessels during exercise remains uncertain.

We11 and other14 investigators have demonstrated that chronic increases in shear stress resulting from an increased flow have led to functional and histological alterations of the vascular endothelium. We have reported that in conscious dogs coronary blood flow was elevated by rapid cardiac pacing for 3 weeks, and endothelium-mediated dilation of the circumflex coronary artery was enhanced. Dewey and Bussoleti6 showed that the shape and structure of endothelial cells were modified by shear stress in cell culture. If the endothelium of the epicardial coronary artery was repeatedly exposed to an elevated blood flow—induced shear-stress stimulus (eg, the exercise regimen used in our study), an alteration of endothelium-mediated control of the circumflex coronary artery was also observed.

Acetylcholine is the classic stimulus for the release of EDRF and dilates epicardial coronary arteries via a receptor component and a flow component, both of which are mediated by EDRF/NO in conscious dogs. Acetylcholine-induced dilation of epicardial coronary arteries was not due to the altered systemic hemodynamic effects in conscious dogs, since Cox et al19 showed that this dilation still occurred after autonomic blockade. The dilation of the epicardial coronary artery following the release of a brief coronary artery occlusion is flow-dependent and endothelium-mediated16,30,31 and is also accomplished via an EDRF/NO mechanism.11,12,29 Seven days of exercise training did not alter the coronary blood flow or other systemic hemodynamic responses to acetylcholine or occlusion of the coronary artery. Furthermore, when the response of circumflex coronary artery to acetylcholine was examined with coronary blood flow held constant by partial inflation of the occluder, the dilation of this coronary artery was still significantly greater after chronic exercise. Therefore, the possibility that an enhanced cor-

![Fig 7. Bar graph showing that the acetylcholine (Ach)– induced dilation and the reactive dilation of epicardial coronary artery following release of 15-second (15s) and 30-second (30s) coronary artery occlusions were abolished by nitro-L-arginine (NLA) after chronic exercise but that the nitroglycerin (NTG)–induced dilation remained unchanged. This indicates that endothelium-derived relaxing factor is responsible for the enhanced endothelium-mediated dilation of epicardial coronary artery after chronic exercise. CD indicates epicardial coronary artery diameter; C, control. *P<.05 vs control (n=6).](http://circres.ahajournals.org/doi/10.1161/01.RES.73.5.836)
ony blood flow response or an altered systemic hemodynamic response after 7 days of exercise training was responsible for the enhanced dilation of the circumflex coronary artery seems unlikely.

Our data with nitroglycerin also suggest that the enhanced responses of the left circumflex coronary artery to acetylcholine and to release of a brief coronary artery occlusion were not due to an increase in the sensitivity of vascular smooth muscle, since neither the large vessel dilation nor the coronary blood flow response to nitroglycerin was altered. Nitroglycerin stimulates guanylate cyclase and dilates the epicardial coronary artery through an endothelium-independent flow-independent manner that is not dependent on its systemic hemodynamic effects. Moreover, the enhanced dilation of the circumflex coronary artery following injection of acetylcholine or release of a brief coronary artery occlusion was completely eliminated by nitro-L-arginine, a competitive inhibitor of EDRF/NO synthesis, after 7 days of exercise. Thus, we concluded that an enhanced release of EDRF contributed to the augmented endothelium-mediated dilation of the circumflex coronary artery in response to acetylcholine and acute increases in coronary blood flow after chronic exercise.

The chronic exercise protocols used in our study did not lead to increases in the coronary blood flow response to acetylcholine and to release of a brief coronary artery occlusion. This result is consistent with a study by Stone, who observed no difference in the peak reactive hyperemia between his untrained and trained awake dogs. The chronic exercise protocols used in our study were not long enough by design to produce alterations of coronary blood flow. A greater peak reactive hyperemia following release of a 15-second coronary artery occlusion was observed in anesthetized dogs after chronic exercise, but this only occurred after 10 weeks of training. It is generally agreed that significant increases in the diameter of epicardial coronary artery, left ventricular mass, myocardial capillary density, and maximal coronary reserve in dogs take 4 to 12 weeks of training. In the present study, dogs were only trained for 7 days with no sign of cardiovascular conditioning such as a resting bradycardia. Consequently, structural changes of the myocardial vasculature were not expected. This design allowed us to exclude the potential role of enhanced flow stimuli in the endothelium-mediated regulation of circumflex coronary artery, since the coronary blood flow responses to acetylcholine and release of a brief coronary artery occlusion remained unchanged after 7 days of exercise training. Our results also suggest that 7 days of exercise training was sufficient to cause enhanced endothelium-mediated dilation of the circumflex coronary artery in conscious dogs.

In summary, acute treadmill exercise caused increases in coronary blood flow, resulting in an increase in the diameter of the circumflex coronary artery. This dilation was via an EDRF/NO–dependent mechanism, since it was eliminated by nitro-L-arginine. Vasocostriction of the circumflex coronary artery was observed during exercise after nitro-L-arginine. Chronic exercise for 7 days potentiated endothelium-mediated dilation of the large coronary artery, and EDRF/NO was responsible for this alteration. Thus, the benefits of exercise training, ie, during hypertension, on the coronary circulation may be associated with altered EDRF/NO–dependent mechanisms.

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