Inhibition of Nitric Oxide Production Aggravates Myocardial Hypoperfusion During Exercise in the Presence of a Coronary Artery Stenosis

Dirk J. Duncker, Robert J. Bache

Abstract  Regulation of coronary vasomotor tone during myocardial hypoperfusion is incompletely understood. The present study was performed to test the hypothesis that endogenous production of nitric oxide contributes to resistance vessel dilation distal to a coronary artery stenosis that results in myocardial ischemia during exercise. Seven dogs instrumented with a Doppler velocity probe, hydraulic occluder, and indwelling microcatheter in the left anterior descending coronary artery (LAD) were studied during treadmill exercise in the presence of a coronary artery stenosis before and after intracoronary infusion of N\textsuperscript{G}-nitro-L-arginine (LNNA, 20 mg/kg). This dose of LNNA inhibited the maximal increase in LAD flow produced by intracoronary acetylcholine by 82±5% but did not alter the response to intracoronary nitroglycerin. Coronary pressure distal to the stenosis was maintained constant during the control period and after administration of LNNA. LNNA increased aortic and left ventricular systolic and end-diastolic pressures at rest and during exercise. During control in the absence of a stenosis, LNNA had no effect on coronary blood flow. In the presence of a stenosis that decreased distal coronary pressure to 55±2 mm Hg, mean myocardial blood flow measured with microspheres was 1.09±0.13 mL · min\(^{-1}\) · g\(^{-1}\) in the LAD-dependent and 2.57±0.50 mL · min\(^{-1}\) · g\(^{-1}\) in the posterior control region, respectively. With no change in distal coronary pressure, LNNA decreased mean myocardial blood flow in the LAD region to 0.68±0.11 mL · min\(^{-1}\) · g\(^{-1}\) (P<.01). To avoid systemic hemodynamic effects, LNNA was administered in a dose of 1.5 mg/kg IC to four additional dogs. This low dose inhibited the coronary blood flow increases produced by acetylcholine by 61±5% but was devoid of systemic hemodynamic effects. During exercise in the presence of a coronary stenosis that decreased coronary pressure to 52±1 mm Hg, this dose of LNNA decreased mean myocardial blood flow from 0.89±0.23 to 0.66±0.21 mL · min\(^{-1}\) · g\(^{-1}\) (P<.02). These data demonstrate that nitric oxide contributes to the maintenance of myocardial perfusion distal to a flow-limiting coronary artery stenosis during exercise. (Circ Res. 1994;74:629–640.)

Key Words:  coronary blood flow • coronary vasodilation • endothelium • microspheres • myocardial ischemia • N\textsuperscript{G}-nitro-L-arginine

The mechanism of coronary vasodilation in response to myocardial hypoperfusion remains incompletely understood but may include liberation of adenosine and activation of ATP-dependent K\(^+\) channels. Recently, it has become evident that endothelium-derived relaxing factor, which is likely nitric oxide (NO) or a nitrosyl compound produced from L-arginine, can influence vasomotor tone of coronary resistance vessels.\(^{1,6}\) However, it is unclear whether NO-dependent mechanisms contribute to coronary vasodilation during myocardial ischemia. This question is important since, contrary to the classically held view that ischemia results in maximal coronary vasodilation, recent evidence indicates that even during ischemia the resistance vessels retain some degree of vasomotor tone\(^7,12\) and can respond to vasoconstrictor stimuli.\(^{13,17}\) Conditions that impair the ability of the resistance vessels to dilate during ischemia might therefore further jeopardize perfusion of ischemic myocardium.

Recent evidence demonstrates that endothelial abnormalities resulting from atherosclerosis extend into the coronary microcirculation.\(^{18}\) Consequently, if NO-dependent mechanisms contribute to resistance vessel dilation during ischemia, interruption of these mechanisms could aggravate myocardial hypoperfusion. Furthermore, NO production has been demonstrated to increase during hypoxia, suggesting that NO-dependent vasodilator mechanisms might have increased importance during ischemia.\(^{19,21}\) The purpose of the present study was to assess the contribution of NO production to the vasodilation of coronary resistance vessels that occurs during myocardial hypoperfusion produced by exercise in the presence of a coronary artery stenosis. NO production was inhibited with N\textsuperscript{G}-nitro-L-arginine (LNNA) administered directly into the coronary artery of chronically instrumented dogs. To avoid passive changes in myocardial perfusion resulting from changes in perfusion pressure, a variable stenosis was used to maintain constant coronary pressure.\(^{12,15,16}\)

Materials and Methods

Studies were performed in 18 adult mongrel dogs weighing 20 to 27 kg and trained to run on a motor-driven treadmill. All experiments were performed in accordance with the "Guiding Principles in the Care and Use of Laboratory Animals" as approved by the Council of the American Physiological Society and under the supervision of the Animal Care Committee of the University of Minnesota.
Surgical Preparation

After sedation with fentanyl (0.4 mg IM) and droperidol (20 mg IM), dogs were anesthetized with sodium pentobarbital (30 to 35 mg/kg IV), intubated, and ventilated with a mixture of oxygen (30%) and room air (70%). Respiratory rate and tidal volume were set to keep arterial blood gases within physiological limits. A left thoracotomy was performed through the fifth intercostal space, and the heart was suspended in a pericardial cradle. A polyvinyl chloride catheter (outer diameter, 3.0 mm) filled with heparinized saline was inserted into the left internal thoracic artery and advanced into the ascending aorta. Similar catheters were introduced into the left atrium through the atrial appendage and the left ventricle through the apical dimple. A solid-state micromanometer (model P5, Konigsberg Instrument Co, Pasadena, Calif) was also introduced into the left ventricle at the apex. Pacing leads were sutured onto the right atrial appendage. Approximately 1.5 cm of the proximal left anterior descending coronary artery (LAD) was dissected free, and a Doppler flow probe (Craig Hartley, Houston, Tex) was positioned around the artery. Immediately distal to the flow probe, a hydraulic occluder (outer diameter, 3.0 mm) was placed around the vessel. A silicone catheter (internal diameter, 0.3 mm) bonded to a larger catheter (internal diameter, 1.6 mm) was introduced into the LAD immediately distal to the hydraulic occluder for measurement of coronary pressure and infusion of drugs. The pericardium was then loosely closed, and the catheters and electrical leads were tunnelled subcutaneously and exteriorized at the base of the neck. The chest was closed in layers, and the pneumothorax was evacuated. Catheters were flushed daily with heparinized saline.

Hemodynamic Measurements

Studies were performed 2 to 3 weeks after surgery with animals exercising on a motor-driven treadmill. Recordings of phasic and mean aortic pressure and coronary perfusion pressure were measured with Gould P23XL pressure transducers positioned at midchest level. Left ventricular pressure was measured with the micromanometer calibrated with the signal from the fluid-filled left ventricular catheter. Coronary blood flow velocity was measured with a Doppler flowmeter system (Craig Hartley). Data were recorded on an eight-channel direct-writing oscillograph (Coulbourn Instruments, Lehigh Valley, Pa).

Myocardial Blood Flow Measurements

Myocardial blood flow was measured with microspheres 15 μm in diameter and labeled with 141Ce, 51Cr, 85Sr, 86Nb, or 99Sc (DuPont NEN, Boston, Mass). Approximately 3×10⁶ microspheres were injected into the left atrial catheter and flushed with 8 mL of normal saline for each measurement. Before injection, microspheres were agitated for at least 10 minutes in an ultrasonic bath. An arterial blood reference sample was withdrawn from the aortic catheter at a constant rate of 15 mL/min, starting 5 seconds before injection and continuing for 90 seconds.

At the end of each study, the region perfused by the LAD was identified by injecting 10 mL of Evans blue dye into the coronary catheter. Immediately thereafter, animals were killed with a lethal dose of sodium pentobarbital. Hearts were excised and fixed in 10% buffered formalin, and the atria, aorta, right ventricular free wall, and large epicardial blood vessels were dissected from the left ventricle and discarded. The left ventricle was divided into four transverse rings from base to apex. Myocardial samples were obtained from the center of the blue-stained region perfused by the LAD and divided into four transmural layers of equal thickness from epicardium to endocardium. The resulting samples were weighed on an analytic balance and placed into vials for counting of gamma activity. Similar samples were obtained from the posterior left ventricular wall perfused by the left circumflex coronary artery to serve as the control area. Myocardial and blood reference samples were counted in a gamma spectrometer with a multichannel analyzer (model 5912, Packard Instrument Co, Downers Grove, Ill). The counts per minute and the corresponding sample weights were entered into a digital computer programmed to correct for background activity and contaminant activity contributed by the associated nuclides and to calculate the corrected counts per minute per gram of myocardial tissue. Blood flow to the myocardial specimen (Qm, in milliliters per minute per gram of myocardium) was computed as

\[ Q_m = Q_i \cdot C_m/C_i \]

where \( Q_i \) is the rate of withdrawal of reference blood sample (in milliliters per minute), \( C_m \) is counts per minute per gram of the myocardial specimen, and \( C_i \) is counts per minute of the reference blood sample.

Experimental Protocols

**LNNA Group**

**Magnitude and selectivity of inhibition of NO production by LNNA.** To assess the degree of inhibition of NO production caused by LNNA (20 mg/kg IC), systemic hemodynamic and coronary blood flow responses to intracoronary infusions of acetylcholine were studied in eight resting dogs. Acetylcholine was infused into the coronary artery catheter in doses of 1.5 to 75 μg/min. Acetylcholine was dissolved in saline so that the desired doses were infused at rates of 0.3 to 3 mL/min. After completion of the acetylcholine infusions, LNNA was infused into the coronary artery catheter at a dose of 20 mg/kg for 30 minutes. LNNA was dissolved in deionized water so that the desired dose was infused at a rate of 3.0 mL/min. After completion of the LNNA infusion, intracoronary acetylcholine infusions were repeated, and systemic hemodynamic and coronary blood flow responses were again recorded.

To assess the selectivity of inhibition of NO production by LNNA, we studied the effects of LNNA on the coronary blood flow responses to the endothelium-independent vasodilator nitroprusside in five dogs (four of which also received acetylcholine infusions). Nitroprusside was infused into the coronary artery catheter in doses of 6 to 60 μg/min. Sodium nitroprusside was dissolved in 5% dextrose solution so that the desired doses were infused at rates of 0.3 to 3 mL/min. After intracoronary infusion of 20 mg/kg LNNA, nitroprusside infusions were repeated, and systemic hemodynamic and coronary blood flow responses were again recorded.

**Exercise protocol.** Seven dogs (two of which also received acetylcholine infusions) underwent a 5-minute period of warm-up exercise, during which the speed and grade of the treadmill were gradually increased until a heart rate of 200 to 220 beats per minute was achieved. Dogs were subsequently allowed to rest on the treadmill for 10 to 15 minutes, and exercise was then restarted at the predetermined level. After 2 to 3 minutes of exercise, when hemodynamic variables had reached a steady state, the occluder was inflated with saline using a micrometer-driven syringe to produce progressively increasing severity of coronary artery stenosis until a degree of stenosis was reached that resulted in an 10% reduction of coronary blood flow. At each level of stenosis, systemic and coronary hemodynamics were measured. The degree of stenosis that resulted in an 10% flow reduction was associated with a distal coronary pressure in the range of 45 to 60 mm Hg. After coronary pressure had been maintained at a stable level for 1 minute, microspheres were injected, and exercise was continued at this level of coronary stenosis for an additional 90 seconds. Thereafter, the stenosis was gradually further inflated, and coronary pressure and blood flow measurements were made until the LAD was totally occluded. Then the occluder was deflated, exercise was discontinued, and the animals were allowed to rest for 90 minutes. A total of 10 to 25 coronary pressure-flow data points were obtained under conditions varying from no stenosis to total coronary artery occlusion.
After 60 minutes of rest, LNNA was infused into the coronary artery catheter in a dose of 20 mg/kg. After completion of the LNNA infusion, resting measurements were obtained, and exercise was started at the previous level. In three animals, hearts were paced to achieve rates equal to those during the control period. In the four other animals, spontaneous heart rates were not different from the values observed during the control period. After hemodynamic measurements during exercise with normal coronary arterial inflow, the coronary occluder was gradually inflated, and coronary pressure and blood flow measurements were obtained until the same level of coronary pressure as during the control stenosis was reached. In four animals, left ventricular end-diastolic pressure was 5 to 10 mm Hg higher in the presence of LNNA than during the control stenosis. In these animals, the coronary stenosis severity was adjusted to increase coronary pressure so that the coronary pressure–left ventricular end-diastolic pressure difference was similar to that measured during the control stenosis. When coronary perfusion pressure had remained stable for 1 minute, microspheres were again injected for measurement of myocardial blood flow. Thereafter, the stenosis was gradually further inflated, and coronary pressure and blood flow measurements were made until the LAD was totally occluded. Then the occluder was deflated, and exercise was discontinued. This procedure allowed determination of the effect of LNNA on coronary artery inflow over a range of reduced distal coronary pressures and of the effect of LNNA on the transmural distribution of blood flow at one level of coronary perfusion pressure.

Control Group

Reproducibility of hemodynamic and myocardial blood flow measurements during two consecutive control periods of exercise in the presence of progressive severity of coronary artery stenosis was assessed in seven animals. The two exercise periods were repeated as described above but without administration of LNNA. Because of an error in the collection of an arterial blood reference sample during microsphere injection, myocardial blood flow data were obtained for six of the seven dogs in the control group.

Low-Dose LNNA Group

Because of concern that the systemic hemodynamic changes caused by LNNA in a dose of 20 mg/kg might have influenced the results, four additional animals were studied while using LNNA at a dose of 1.5 mg/kg IC. This dose was devoid of systemic hemodynamic effects.

Magnitude and selectivity of inhibition of NO production by LNNA. To assess the degree and selectivity of inhibition of NO production caused by this dose of LNNA, systemic hemodynamic and coronary blood flow responses to intracoronary infusions of acetylcholine and sodium nitroprusside were studied in five resting dogs (four of which were also studied in the high-dose LNNA, acetylcholine, and nitroprusside protocol). After completion of the measurements, an intracoronary infusion of LNNA was started into the coronary artery catheter at a dose of 1.5 mg/kg infused for 12 minutes. LNNA was dissolved in deionized water so that the desired dose was infused at a rate of 0.6 ml/min. Intracoronary acetylcholine and nitroprusside infusions were repeated, and systemic hemodynamic and coronary blood flow responses were again recorded.

Exercise protocol. The effects of low-dose LNNA on myocardial blood flow distal to a coronary stenosis were studied in four dogs (one of which was also studied during the low-dose LNNA, acetylcholine, and nitroprusside protocol). Two exercise periods were repeated as described above, but now an infusion of LNNA was started into the coronary artery catheter in a dose of 1.5 mg/kg at a rate of 0.6 ml/min starting 10 minutes before the second run and continuing during exercise in the presence of normal arterial inflow. During exercise, the stopcock was switched for 3 to 4 seconds to allow measurement of coronary pressure. Two to 3 minutes after exercise had started (12 to 13 min after the start of the infusion), the infusion was discontinued, and the occluder was inflated to produce a stenosis that resulted in a coronary pressure equal to that in the first run.

Data Analysis

Heart rate, left ventricular, aortic, and coronary pressures, and coronary Doppler shift were measured from the strip-chart recordings. Coronary blood flow was computed using the following equation: \( Q = 2.5 \cdot \Delta f \cdot d \), where \( Q \) is the coronary blood flow (in milliliters per minute), \( \Delta f \) is the Doppler shift (in kilohertz), and \( d \) is the internal diameter of the coronary artery (in millimeters) within the flow probe. The factor 2.5 is a constant derived from the speed of sound \((C=1.5\times10^5 \text{ cm/s})\), the ultrasonic frequency of the sound beam emitted \((f_d=10 \text{ MHz})\), the cosine of the angle at which the sound beam is emitted \((45^\circ)\), and unit conversion factors: \((2f_d \cos 45^\circ)\). Since in the chronically instrumented animals the flow probe adheres to the coronary artery, the computation of the coronary internal diameter would affect control and intervention conditions equally. Optimal curve fitting of the coronary pressure-flow data was obtained with a fourth-order polynomial \((y=a+bx+cx^2+dx^3+ex^4)\), and coronary flows were computed for four coronary pressures in the range of the descending limb of the coronary pressure-flow relation for each animal. Hemodynamic data were compared using one-way ANOVA for repeated measures. Myocardial blood flow data in the four different layers were analyzed by two-way (treatment and layer) ANOVA for repeated measures. When a significant effect was observed, individual comparisons were made by the Wilcoxon signed-rank test or the paired \( t \) test. Statistical significance was accepted at \( P<.05 \) (two tailed). All data are presented as mean±SEM.

Results

LNNA Group

Magnitude and Selectivity of Inhibition of NO Production by LNNA

The effects of LNNA (20 mg/kg) on coronary vasodilation produced by the endothelium-dependent vasodilator acetylcholine were studied in eight resting dogs. Intracoronary infusion of acetylcholine in doses of 1.5 to 75 \( \mu \)g/min had no effect on mean arterial blood pressure (90±5 mm Hg) or heart rate (121±7 beats per minute). Coronary blood flow increased from 54±4 ml/min at baseline to 135±14 ml/min during infusion of 75 \( \mu \)g/min (Fig 1). After LNNA administration, acetylcholine infusions slightly decreased mean arterial blood pressure from 111±5 mm Hg at baseline to 105±4 mm Hg (\( P<.05 \)), with no effect on heart rate (102±8 beats per minute). Coronary blood flow increased from 56±3 ml/min at baseline to 69±4 ml/min during infusion of 75 \( \mu \)g/min. Thus, LNNA caused an 82±5% inhibition of the increase of coronary blood flow produced by the highest dose of acetylcholine.

The effects of LNNA on coronary vasodilation produced by the endothelium-independent vasodilator nitroprusside were studied in five resting dogs. Intracoronary infusion of sodium nitroprusside in doses of 6 to 60 \( \mu \)g/min decreased mean aortic blood pressure from 96±7 mm Hg at baseline to 86±7 mm Hg during the highest dose (\( P<.05 \)), with a tendency for heart rate to increase from 111±11 to 129±10 beats per minute (\( P=\text{NS} \)). After LNNA administration, nitroprusside infusions caused similar changes in mean aortic pressure and heart rate. The increases in coronary blood flow produced by nitroprusside were not altered by LNNA (Fig 1).
Systemic and Coronary Hemodynamic Responses to Exercise

In the absence of LNNA, exercise increased heart rate, left ventricular systolic pressure, and maximal left ventricular dP/dt, whereas mean aortic pressure and left ventricular filling pressure were not significantly affected (Table 1). Coronary blood flow increased from 52±7 mL/min at rest to 91±13 mL/min during exercise (P<.01). With inflation of the occluder to produce a coronary stenosis, left ventricular end-diastolic pressure increased, accompanied by a slight decrease of maximal left ventricular dP/dt. The coronary stenosis that resulted in a coronary pressure of 55±2 mm Hg was associated with a decrease of coronary blood flow to 42±5 mL/min (P<.01). LNNA increased resting values of mean aortic and coronary blood pressure and left ventricular systolic and end-diastolic pressure (Table 1). These differences were maintained during exercise with normal coronary arterial inflow and in the presence of a stenosis, although coronary pressure and left ventricular end-diastolic pressure were no longer statistically significantly higher. An example of the effects of LNNA on systemic and coronary hemodynamics during exercise with normal and restricted arterial inflow are shown in Figs 2 and 3, respectively. During exercise with normal arterial inflow, LNNA tended to slightly increase coronary blood flow, which paralleled the increase in left ventricular systolic pressure. In contrast, in the presence of a stenosis, LNNA decreased coronary blood flow from 42±5 to 28±5 mL/min (P<.01) (Table 1).

To determine whether NO inhibition contributed to coronary vasodilation over a range of coronary pressures, we obtained pressure-flow relations in six dogs. A typical example of a coronary pressure-flow relation in the presence of intact coronary vasomotor tone and the effects of LNNA on the relation are shown in Fig 4A. For all six dogs, we computed coronary blood flows for four coronary pressures on the descending limb of the relation (Fig 5A). LNNA significantly decreased coronary blood flow at each of the four levels of coronary pressure.

Myocardial Tissue Blood Flow

During exercise, mean myocardial blood flow in the anterior region perfused by the stenotic LAD was 1.09±0.13 mL·min⁻¹·g⁻¹ as compared with 2.57±0.50 mL·min⁻¹·g⁻¹ in the posterior control region. Hypoperfusion in the anterior region was most pronounced in the subendocardium, reflected by the endocardial-to-epicardial ratio of 0.49±0.07, as compared with 1.50±0.07 in the control region (Table 2). With coronary pressure kept constant, LNNA caused a further decrease of mean myocardial blood flow in the anterior region to 0.68±0.11 mL·min⁻¹·g⁻¹ (P<.01 versus con-

Table 1. Effects of Inhibition of Nitric Oxide Production on Systemic and Coronary Hemodynamics During Exercise in the Absence and Presence of a Coronary Artery Stenosis in Seven Dogs

<table>
<thead>
<tr>
<th></th>
<th>Control Rest</th>
<th>Control Exercise</th>
<th>Exercise Stenosis Rest</th>
<th>Exercise Stenosis</th>
<th>20 mg/kg IC LNNA Rest</th>
<th>Exercise Stenosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>131±6</td>
<td>213±5*</td>
<td>214±6*</td>
<td>120±5</td>
<td>214±5*</td>
<td>216±6*</td>
</tr>
<tr>
<td>Mean aortic pressure, mm Hg</td>
<td>91±5</td>
<td>99±6</td>
<td>100±6</td>
<td>109±4†</td>
<td>118±5†</td>
<td>120±6†</td>
</tr>
<tr>
<td>LV systolic pressure, mm Hg</td>
<td>111±5</td>
<td>131±6*</td>
<td>127±6*</td>
<td>126±5‡</td>
<td>140±7*‡</td>
<td>139±8*‡</td>
</tr>
<tr>
<td>LV dP/dt max, mm Hg/s</td>
<td>2410±180</td>
<td>4250±390*</td>
<td>3900±390*</td>
<td>2220±150</td>
<td>3970±370*</td>
<td>3700±430*</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mm Hg</td>
<td>7.5±1.4</td>
<td>9.1±2.4</td>
<td>16.7±3.1†</td>
<td>12.2±1.5†</td>
<td>14.0±3.0</td>
<td>20.8±3.9†</td>
</tr>
<tr>
<td>Coronary pressure, mm Hg</td>
<td>91±3</td>
<td>88±3</td>
<td>55±2*†</td>
<td>101±3‡</td>
<td>95±5</td>
<td>59±3*</td>
</tr>
<tr>
<td>Coronary blood flow, mL/min</td>
<td>52±7</td>
<td>91±13*</td>
<td>42±5†</td>
<td>70±13</td>
<td>103±14*</td>
<td>28±5†‡</td>
</tr>
</tbody>
</table>

LNNA indicates N⁰-nitro-L-arginine; bpm, beats per minute; LV, left ventricular; and LV dP/dt max, maximum rate of rise of LV pressure. Values are mean±SEM.

*P<.05 vs rest.
†P<.05 vs exercise.
‡P<.05 vs corresponding control value.
§n=6.
control stenosis), with significant flow reductions in the innermost three layers (Fig 6A), whereas blood flow in the normally perfused posterior region did not change significantly after LNNA (Fig 7A). In four of the animals, LNNA increased left ventricular end-diastolic pressure by 5 to 10 mm Hg during exercise in the presence of a coronary artery stenosis (Table 3). In these animals, the coronary stenosis severity was adjusted to increase coronary pressure so that the coronary pressure–left ventricular end-diastolic pressure difference was similar to that measured during the control stenosis. As can be seen from Table 3, the decreases of myocardial blood flow in dogs 1 through 3, in which end-diastolic pressure was not altered by LNNA, were similar to the decreases of myocardial flow in dogs 4 through 7, in which this variable was increased. Stepwise regression analysis revealed that subendocardial blood flow was positively correlated with coronary driving pressure (coronary pressure–left ventricular end-diastolic pressure) \( P<.02 \), whereas the relation was shifted downward by LNNA (Fig 8).

**Control Group**

**Systemic and Coronary Hemodynamic Responses to Exercise**

During consecutive control exercise periods, no significant differences were observed between the systemic and coronary hemodynamic variables in seven dogs (Table 4). No differences were noted between the descending limb of the coronary pressure-flow relation obtained during consecutive control periods (Figs 4B and 5B).

**Myocardial Tissue Blood Flow**

Excellent reproducibility of mean myocardial blood flow measurements was obtained during consecutive control periods of exercise in the presence of a LAD stenosis: 0.98 ± 0.16 and 1.05 ± 0.18 mL/min⁻¹·g⁻¹, respectively. Similarly, blood flow to the individual transmural layers of the hypoperfused left ventricular anterior wall (Fig 6B) and the posterior control region (Fig 7B) were nearly identical during the two consecutive control periods.

**Low-Dose LNNA Group**

**Magnitude and Selectivity of Inhibition of NO Production by LNNA**

Systemic hemodynamic and coronary blood flow responses to intracoronary infusions of acetylcholine and sodium nitroprusside were studied in five resting dogs. LNNA (1.5 mg/kg IC) attenuated the maximum increase in coronary blood flow produced by acetylcholine by 61 ± 5% without altering the flow responses to sodium nitroprusside (Fig 9).

**Systemic and Coronary Hemodynamic Responses to Exercise**

LNNA did not significantly change systemic and coronary hemodynamics at rest or during exercise, although left ventricular pressure tended to be lower after LNNA (Table 5). During exercise in the presence of a coronary stenosis, resulting in a coronary pressure of 52 ± 1 mm Hg, LNNA decreased LAD blood flow by 28 ± 8% \( P<.05 \) (Table 5).
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Myocardial Blood Flow

Myocardial blood flow, measured with microspheres, in the normally perfused control area was not different between the two exercise periods (not shown). However, LNNA decreased myocardial blood flow distal to the stenosis in all layers (Fig 10), with a decrease in mean myocardial blood flow from 0.89±0.23 mL·min⁻¹·g⁻¹ during control stenosis to 0.66±0.21 mL·min⁻¹·g⁻¹ (P<.02). These results with the low dose of LNNA further demonstrate that NO production contributed to vasodilation of coronary resistance vessels distal to a coronary artery stenosis during exercise.

Discussion

The most important finding of the present study was that inhibition of NO production exacerbated myocardial hypoperfusion produced by exercise in the presence of a coronary artery stenosis but had no effect on blood flow to myocardium with normal coronary arterial in-

![Graphs showing examples of the coronary pressure-flow relation in the presence of intact vasomotor tone in an exercising dog. A, Coronary pressure-flow relation during control conditions (○) and after intracoronary administration of 20 mg/kg \(N^\text{O}\)-nitro-L-arginine (●). B, Coronary pressure-flow relation during consecutive control periods (control 1 [○] and control 2 [●]).](http://circres.ahajournals.org/)

![Fig 3. Strip-chart recordings from a dog during exercise in the presence of a coronary artery stenosis under control conditions and after intracoronary infusion of 20 mg/kg \(N^\text{O}\)-nitro-L-arginine (LNNA). Fast tracings were obtained at 100 mm/s, and slow tracings were obtained at 10 mm/s. Each grid unit is 1 mm. Shown are mean aortic pressure (AoP), left ventricular pressure (LVP), and its first derivative (LVdP/dt), mean coronary pressure (CP), phasic coronary blood flow (CBF), and mean coronary blood flow (CBF). The slow tracings were obtained during the injection of microspheres. Consequently, aortic blood pressure was not recorded.](http://circres.ahajournals.org/)
flow. The implications of these findings will be discussed in detail.

**Methodological Considerations**

In the present study, we used LNNA to inhibit NO production. LNNA in a dose of 20 mg/kg IC caused 80% inhibition of the vasodilator response to the endothelium-dependent vasodilator acetylcholine, exceeding the degree of blockade in previous studies in anesthetized and awake dogs in which 35% to 70% reductions of the response to acetylcholine were observed. Using intravital microscopy with stroboscopic epi-illumination synchronized to the cardiac cycle, Komaru et al. observed that N²-monomethyl-L-arginine (LNMA) abolished the acetylcholine-induced vasodilation of large coronary arterioles (>120 μm), whereas it only partially inhibited the vasodilator response of smaller arterioles (<120 μm). Thus, the degree of inhibition by LNNA of the vasodilator response to acetylcholine in the present study is in agreement with other in vivo studies and is consistent with the findings of Komaru et al., who reported that LNMA did not completely inhibit the effect of acetylcholine in arterioles <120 μm, where most of the coronary resistance resides. In the present study, LNNA did not attenuate the vasodilator response to the endothelium-independent vasodilator nitroprusside. Our findings confirm previous reports that LNNA did not alter coronary vasodilator responses to the endothelium-independent vasodilators nitroglycerin in awake dogs and sodium nitroprusside in isolated perfused rabbit hearts nor to adenosine in anesthetized dogs. Thus, the effects of LNNA are specific to the endothelium-dependent vasodilation produced by acetylcholine.

The present study was designed to evaluate the effects of inhibition of NO production on the vasodilator response of coronary resistance vessels distal to a flow-limiting coronary artery stenosis during exercise. To study the effects of LNNA on vasomotor tone, it is mandatory that extravascular determinants of myocardial blood flow be taken into account. First, LNNA had no effect on maximal left ventricular dP/dt, indicating minimal effects on systolic myocardial compressive forces. Second, diastolic perfusion time was kept constant by right atrial pacing of those animals in which the high dose of LNNA caused a decrease in heart rate during exercise. Third, to prevent a passive decrease in myocardial blood flow due to an increase of diastolic myocardial compressive forces that act on the vasculature, poststenotic coronary pressure was adjusted when an increase in left ventricular end-diastolic pressure occurred (Table 4). Therefore, the observed decrease of myocardial blood flow produced by LNNA cannot be explained by a change in extravascular compressive forces. This is further supported by the observations with low-dose LNNA, which had no effect on systemic hemodynamics but did decrease myocardial blood flow distal to the stenosis.

To determine the effect of LNNA on blood flow over a range of stenosis severity, blood flows were examined at a series of coronary pressures. Although complete pressure-flow relations were obtained in each animal, we did not present flows for mean coronary artery pressure below 50 mm Hg, since Messina et al. have shown that coronary collateral blood flow can contribute significantly to total myocardial tissue blood flow when the pressure gradient between coronary arteries exceeds 70 mm Hg. Since LNNA increased aortic blood pressure, the contribution of collateral flow at coronary pressures below 50 mm Hg could have been more significant in the presence of LNNA. Therefore, we examined flows at distal coronary pressures that would result in negligible collateral blood flow. That collateral blood flow did not contribute significantly to total myocardial blood flow is further supported by the finding that the magnitude of the LNNA-induced decrease of myocardial tissue blood flow measured with micrometers (35±4%) was not different from the decrease of coronary arterial inflow (34±3%).

To determine whether exercise in the presence of a coronary artery stenosis might influence the response to LNNA during a second period of exercise performed 90 minutes later, we performed control experiments comparing two exercise periods separated by 90 minutes without LNNA treatment. Although there was substantial interanimal variability, the results from the control group showed that intra-animal reproducibility of both the descending limb of the coronary pressure-flow relation and the distribution of myocardial blood flow in the area perfused by the stenotic LAD were excellent.

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**Table 2. Effect of Blockade of Nitric Oxide Production on Myocardial Blood Flow in Seven Exercising Dogs With a Left Anterior Descending Coronary Artery Stenosis**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>20 mg/kg IC LNNA</th>
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<tr>
<td>LAD-dependent segment</td>
<td></td>
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<tr>
<td>Mean myocardial blood flow, mL·min⁻¹·g⁻¹</td>
<td>1.09±0.13*</td>
<td>0.68±0.11†</td>
</tr>
<tr>
<td>Endo/Epi</td>
<td>0.49±0.07</td>
<td>0.26±0.05*</td>
</tr>
<tr>
<td>Normal segment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean myocardial blood flow, mL·min⁻¹·g⁻¹</td>
<td>2.57±0.50</td>
<td>2.80±0.35</td>
</tr>
<tr>
<td>Endo/Epi</td>
<td>1.50±0.07</td>
<td>1.64±0.11</td>
</tr>
</tbody>
</table>

LNNA indicates N²-nitro-L-arginine; Endo, endocardial layer; and Epi, epicardial layer. Values are mean±SEM. *P<.05 vs normal segment at corresponding time point. †P<.05 vs control.
changes in blood flow were not due to the effects of the previous exercise period.

Effect of NO Synthase Inhibition on Myocardial Blood Flow During Normal Arterial Inflow

In the present study, LNNA did not significantly change LAD blood flow at rest or during exercise in the presence of normal arterial inflow. In addition, during exercise in the presence of a LAD stenosis, myocardial blood flow to the posterior left ventricular wall (control area) did not change despite the systemic presence of the drug, as evidenced by the increase in aortic blood pressure. The most important determinant of coronary blood flow under conditions of unimpeded arterial inflow is myocardial oxygen demand. In view of the near-maximal oxygen extraction during baseline conditions, any increase in oxygen demand must be met by a parallel increase in coronary blood flow. At rest and during exercise with normal coronary arterial inflow, myocardial oxygen demand was slightly higher after LNNA, as reflected by the increased product of heart rate and left ventricular systolic pressure. This was accompanied by a similar trend for an increase in coronary blood flow, suggesting that the close coupling between oxygen demand and coronary vascular tone was intact. These findings in awake dogs are in contrast to previous observations in isolated non-blood-perfused hearts from the rabbit,24,26,27 rat,28 and guinea pig,20,21,29 which reported an increase in basal coronary vasomotor tone after administration of LNNA,21,24,28,29 LNMMA,20,26,27 or N-O-nitro-l-arginine methyl ester (L-NAME).27 In support of our findings, previous studies in anesthetized2,3,23,30 and awake dogs31 failed to show a decrease of basal coronary blood flow after intracoronary administration of LNNA,2,5 LNMMA,2,23,30 or L-NAME.31 Only one study of four awake dogs found a decrease in basal coronary blood velocity after intravenous administration of LNMMA.32 In that study, LNMMA decreased heart rate by >30%, which may have contributed to the 18% decrease of blood velocity. The discrepancy between in vivo blood-perfused canine hearts and isolated buffer-perfused rodent hearts could be due to differences in species or experimental conditions. In isolated perfused hearts, myocardial perfusion is very high at baseline (=6 mL·min⁻¹·g⁻¹), favoring the release of NO under basal conditions. In addition, the absence of blood and therefore hemoglobin may have increased the biologic half-life of NO in the isolated perfused hearts, as hemoglobin is an NO scavenger.33

Our data fail to support an obligatory role for NO in the normal regulation of coronary vasomotor tone at rest or during exercise. However, it is likely that the NO pathway exerted a vasodilator influence on the coronary vessels in response to the exercise-induced increase in shear stress.34 It is possible that LNNA blunted the flow-mediated vasodilator response in coronary arteries that are not under metabolic control (>100 μm) but that compensatory vasodilation of the smallest coronary arterioles (<100 μm) allowed escape from hypoperfusion.35 Alternatively, blockade of NO production in the smallest arterioles may have led to activation of other compensatory vasodilator mechanisms in these resistance vessels. These vasodilator mechanisms could either be metabolic in origin or be endothelium-derived substances. That other endothelium-derived substances could be involved is supported by earlier findings that LNMMA did not completely inhibit acetylcholine-induced vasodilation of the smallest coronary resistance vessels4 and that resistance vessel dilation in response to bradykinin only partly involves NO production.36,37 Other endothelium-derived substances might include prostacyclin37 or endothelium-derived hyperpolarizing factor.38,39

Response of Coronary Resistance Vessels to Myocardial Hypoperfusion

Myocardial ischemia has classically been thought to cause a maximal vasodilation of the coronary resistance
vessels that would override competing vasoconstrictor influences. However, recent studies in swine and dogs have documented vasodilator reserve in ischemic myocardium, indicating that vasodilation is not maximal.8-12 Furthermore, a2-adrenergic receptor13 or a1-adrenergic receptor14 stimulation during exercise can produce vasoconstriction in ischemic myocardium and aggravate contractile dysfunction. Interestingly, we observed in exercising dogs that the relative increase in coronary blood flow produced by a1-adrenergic receptor blockade was greater in the presence of myocardial ischemia15 than under conditions of unimpeded arterial blood flow.68 In the present study, inhibition of NO production had no effect on myocardial blood flow during normal coronary arterial inflow, but a vasoconstrictor response was elicited when NO production was inhibited during myocardial hypoperfusion. A similar observation was made with the thromboxane mimetic U46619, which caused a flow reduction only in the presence of myocardial hypoperfusion16 but not during normal arterial inflow.16 On the basis of these observations, it is clear that myocardial hypoperfusion does not result in maximal vasodilation and may actually render the coronary bed more susceptible to vasoconstrictor influences.

In this study, the presence of myocardial ischemia could be explained by vasoconstriction in arterial segments that are not under metabolic control but that contribute to total coronary resistance. Chilian et al42 showed that 25% of total coronary resistance resides in vessels >150 μm in diameter, whereas metabolic vasodilation occurs predominantly in vessels <100 μm.43,44 Under normal conditions, vasoconstriction of the larger arterial segments can be counterbalanced by vasodilation of vessels <100 μm. However, when hypoperfusion has already caused metabolic vasodilation of the vessels <100 μm, the ability to compensate for vasoconstriction of larger segments is lost. In this situation, vasoconstriction of the larger arterial vessels can aggravate hypoperfusion.

### TABLE 3. Coronary Hemodynamic Data for Seven Individual Dogs During Exercise in the Presence of a Coronary Artery Stenosis

<table>
<thead>
<tr>
<th>Dog</th>
<th>Control</th>
<th>LNA</th>
<th>Control</th>
<th>LNA</th>
<th>Control</th>
<th>LNA</th>
<th>Control</th>
<th>LNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>60</td>
<td>7</td>
<td>4</td>
<td>53</td>
<td>56</td>
<td>1.32</td>
<td>1.15</td>
</tr>
<tr>
<td>2</td>
<td>51</td>
<td>49</td>
<td>19</td>
<td>17</td>
<td>32</td>
<td>32</td>
<td>0.75</td>
<td>0.55</td>
</tr>
<tr>
<td>3</td>
<td>45</td>
<td>45</td>
<td>20</td>
<td>20</td>
<td>25</td>
<td>25</td>
<td>1.19</td>
<td>0.78</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>65</td>
<td>20</td>
<td>25</td>
<td>40</td>
<td>40</td>
<td>1.21</td>
<td>0.86</td>
</tr>
<tr>
<td>5</td>
<td>52</td>
<td>66</td>
<td>30</td>
<td>38</td>
<td>22</td>
<td>28</td>
<td>0.78</td>
<td>0.30</td>
</tr>
<tr>
<td>6</td>
<td>58</td>
<td>61</td>
<td>15</td>
<td>24</td>
<td>43</td>
<td>37</td>
<td>1.62</td>
<td>0.67</td>
</tr>
<tr>
<td>7</td>
<td>60</td>
<td>64</td>
<td>7</td>
<td>17</td>
<td>53</td>
<td>47</td>
<td>0.74</td>
<td>0.42</td>
</tr>
</tbody>
</table>

**Mean±SEM**

- **Dogs 1-3**: 52±4, 51±4, 15±4, 14±5, 37±8, 38±9, 1.09±0.17, 0.83±0.17, 0.70±0.18, 0.35±0.12
- **Dogs 4-7**: 58±2, 64±1, 18±5, 26±4, 40±6, 38±4, 0.70±0.21, 0.56±0.13, 0.70±0.19, 0.28±0.11
- **Dogs 1-7**: 55±2, 59±3, 17±3, 21±4, 38±5, 38±5, 0.70±0.12, 0.68±0.11, 0.70±0.12, 0.31±0.08

LVEDP indicates left ventricular end-diastolic pressure; LNA, NG-nitro-L-arginine (20 mg/kg IC).

Note that the myocardial blood flow response in dogs 1 through 3 was similar to that in dogs 4 through 7, particularly in the subendocardium.

### TABLE 4. Systemic and Coronary Hemodynamics in Seven Exercising Dogs in the Absence and Presence of a Coronary Artery Stenosis During Consecutive Control Periods

<table>
<thead>
<tr>
<th></th>
<th>Control 1</th>
<th></th>
<th>Control 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Exercise</td>
<td>Exercise</td>
<td>Rest</td>
</tr>
<tr>
<td><strong>Heart rate, bpm</strong></td>
<td>120±8</td>
<td>208±5*</td>
<td>217±4*</td>
<td>126±3</td>
</tr>
<tr>
<td><strong>Mean aortic pressure, mm Hg</strong></td>
<td>101±4</td>
<td>112±8</td>
<td>111±7</td>
<td>97±5</td>
</tr>
<tr>
<td><strong>LV systolic pressure, mm Hg</strong></td>
<td>119±4</td>
<td>138±8*</td>
<td>135±7*</td>
<td>114±4</td>
</tr>
<tr>
<td><strong>LV dP/dt max, mm Hg/s</strong></td>
<td>2400±190</td>
<td>4180±270*</td>
<td>3940±370*</td>
<td>2370±170</td>
</tr>
<tr>
<td><strong>LV end-diastolic pressure, mm Hg</strong></td>
<td>3.8±0.9</td>
<td>4.2±0.7</td>
<td>10.0±2.1*</td>
<td>3.4±2.1</td>
</tr>
<tr>
<td><strong>Coronary pressure, mm Hg</strong></td>
<td>101±4</td>
<td>105±9</td>
<td>58±4*†</td>
<td>95±4†</td>
</tr>
<tr>
<td><strong>Coronary blood flow, mL/min</strong></td>
<td>51±6</td>
<td>78±10*</td>
<td>39±4†</td>
<td>53±9</td>
</tr>
</tbody>
</table>

bpm indicates beats per minute; LV, left ventricular; and LV dP/dt max, maximum rate of rise of LV pressure. Values are mean±SEM.

*P<.05 vs rest.
†P<.05 vs exercise.
‡P<.05 vs corresponding control 1 value.
Recent evidence suggests that in ischemic myocardium vasomotor tone exists not only in vessels that are independent of metabolic control but also in vessels that are under metabolic control. Thus, Chilian and Layne\(^45\) observed that even during severe hypoperfusion, exogenous adenosine caused further vasodilation in vessels \(<150\,\mu m\) in diameter. Furthermore, Chilian\(^46\) observed that although \(\alpha_1\)- and \(\alpha_2\)-adrenergic receptor stimulation had no effect on vessels \(<100\,\mu m\) during normal arterial inflow, myocardial hypoperfusion resulted in unmasking of both \(\alpha_1\)- and \(\alpha_2\)-adrenergic receptor-mediated vasoconstriction in vessels of this size. These findings suggest that the capacity of the coronary resistance vessels to escape from vasoconstrictor influences becomes impaired at decreased intravascular pressures distal to a coronary artery stenosis.

**Effect of NO Synthase Inhibition on the Coronary Resistance Vessels During Myocardial Hypoperfusion**

The present study indicates an important role for NO production in the coronary vasodilator response during myocardial hypoperfusion distal to a coronary artery stenosis during exercise, extending recent observations in resting dogs.\(^31\) The flow reduction in response to LNNA could be due to vasoconstriction either in arterial segments that are not under metabolic control but that contribute significantly to total coronary resistance (\(>120\,\mu m\) in diameter) or in vessels that are under metabolic control (\(<120\,\mu m\)). Early work in the microcirculation of the in situ rabbit ear suggested that under basal conditions NO production is greatest in arterial microvessels with a diameter of \(>140\,\mu m\).\(^37\) In support of those observations, small isolated porcine coronary arteries (\(=300\,\mu m\) in diameter) responded to LNMMMA with an endothelium-dependent increase of vasomotor tone, indicating basal release of NO in large arterial microvessels even in the absence of flow.\(^36\) Studies in isolated canine coronary arterioles (40 to 80 \(\mu m\)) have suggested that basal release of NO also occurs in the smallest resistance vessels but only in the presence of flow.\(^1,6\) In the in vivo canine preparation, basal release of NO occurred in small arterioles (\(<100\,\mu m\)), whereas the results obtained in large arterioles (\(>100\,\mu m\)) were equivocal\(^4,8,9\); vessels of both sizes vasodilated in response to acetylcholine.\(^4\) Differences in species, vascular beds, and experimental conditions do not allow a final conclusion concerning a preferential microvascular site for basal release of NO, but it is clear that NO production can be stimulated in canine coronary arterial microvessels of all sizes.

From the present study it cannot be determined whether the effect of LNNA was due to unmasking of normal (ie, basal and/or flow-mediated) NO release because other vasodilator mechanisms had been exhausted or whether it was due to an actual increase of NO production in response to myocardial hypoperfusion during exercise. An increase in NO production in response to impaired tissue oxygenation has been suggested by a study of Pohl and Busse.\(^19\) While studying feline mesenteric conduit vessels, they observed that hypoxia led to the release of an endothelium-derived relaxing factor. Recently, their observations have been extended to the coronary circulation, where myocardial hypoxia resulted in enhanced production of NO in coronary resistance vessels of isolated guinea pig hearts.\(^20,21\) Although oxygen tension in most parts of the coronary arterial tree is likely to be lower during hypoxia than during limited arterial inflow of well-oxygenated blood, there is evidence that even during normal arterial inflow, oxygen diffusion occurs so rapidly that in arterioles and even small arteries oxygen tensions are lower than in the central aorta.\(^49\) This suggests that the vascular endothelium could serve as a tissue oxygen sensor.\(^50\) Finally, there is evidence that \(\alpha_1\) - and \(\alpha_2\)-adrenergic receptor stimulation is enhanced in

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**Fig. 8.** Graph showing the relation between coronary pressure—left ventricular end-diastolic pressure (LVEDP) and subendocardial blood flow distal to a coronary artery stenosis during exercise under control conditions (○) and after intracoronary administration of 20 mg/kg N\(^5\)-nitro-L-arginine (LNNA, ●). Step-wise regression analysis revealed that coronary pressure-LVEDP was significantly correlated with subendocardial blood flow \((P<.02)\), whereas LNNA significantly shifted the relation downward \((P<.01)\).

**Fig. 9.** Graphs showing increases in coronary blood flow produced by intracoronary infusions of acetylcholine (A, \(n=5\)) and sodium nitroprusside (B, \(n=5\)) under control conditions (open symbols) and after intracoronary administration of 1.5 mg/kg N\(^5\)-nitro-L-arginine (closed symbols). Data are presented as mean±SEM.
TABLE 5. Effects of Inhibition of Nitric Oxide Production With a Low Dose of N\textsuperscript{\textalpha}-Nitro-L-Arginine on Systemic and Coronary Hemodynamics During Exercise in the Absence and Presence of a Coronary Artery Stenosis in Four Dogs

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1.5 mg/kg IC LNNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Exercise</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>112±9</td>
<td>198±8*</td>
</tr>
<tr>
<td>Mean aortic pressure, mm Hg</td>
<td>93±4</td>
<td>108±8*</td>
</tr>
<tr>
<td>LV systolic pressure, mm Hg</td>
<td>118±2</td>
<td>147±3*</td>
</tr>
<tr>
<td>LV dP/dt_{max}, mm Hg/s</td>
<td>2330±330</td>
<td>4910±460*</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mm Hg</td>
<td>7.3±0.9</td>
<td>7.7±2.4</td>
</tr>
<tr>
<td>Coronary pressure, mm Hg</td>
<td>93±4</td>
<td>105±10</td>
</tr>
<tr>
<td>Coronary blood flow, mL/min</td>
<td>37±5</td>
<td>64±8*</td>
</tr>
</tbody>
</table>

LNNA indicates N\textsuperscript{\textalpha}-nitro-L-arginine; LV, left ventricular; and LV dP/dt_{max}, maximum rate of rise of LV pressure. Values are mean±SEM. 
*P<.05 vs rest.  
†P<.05 vs exercise.  
‡P<.05 vs corresponding control value.

hypoperfused myocardium distal to a coronary artery stenosis during exercise.\textsuperscript{14,15} Since endothelial \textalpha\textsubscript{2}-adrenergic receptors are capable of releasing NO, an increase in \textalpha\textsubscript{2}-adrenergic activity could have enhanced NO production distal to the stenosis in the present study. Inhibition of NO production by LNNA may have aggravated hypoperfusion by leaving direct \textalpha\textsubscript{2}- and \textalpha\textsubscript{2}-adrenergic coronary vasoconstriction unopposed. In support of this possibility, a recent study showed that after LNNA administration, the vasoconstriction of coronary arterial microvessels in response to norepinephrine was enhanced.\textsuperscript{48}

Clinical Significance
The present study showed that impairment of endothelial NO production can compromise myocardial perfusion distal to a coronary artery stenosis that resulted in myocardial hypoperfusion during exercise. A decrease in NO-dependent vasodilator responses in the microcirculation has been observed in patients with atherosclerosis and/or hyperlipidemia\textsuperscript{51} or hypertension.\textsuperscript{52,53} The present findings suggest that blunted NO-dependent vasodilation of coronary resistance vessels can render such patients more vulnerable to hypoperfusion distal to a coronary artery stenosis.

Conclusions
Blockade of NO production resulted in aggravation of myocardial hypoperfusion produced by exercise in the presence of a coronary artery stenosis. This finding suggests an important role for the endothelium in the regulation of vasomotor tone in coronary resistance vessels during myocardial ischemia.

Acknowledgments
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Inhibition of nitric oxide production aggravates myocardial hypoperfusion during exercise in the presence of a coronary artery stenosis.

D J Duncker and R J Bache

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