Role of Nitric Oxide in Exercise-Induced Vasodilation of the Forearm

Toyonari Endo, MD; Tsutomu Imaizumi, MD; Tatsuya Tagawa, MD; Masanari Shiramoto, MD; Shin-ichi Ando, MD; Akira Takeshita, MD

Background We wished to determine the role of NO in exercise-induced metabolic forearm vasodilation.

Methods and Results Young healthy volunteers (n=11) underwent static handgrip exercise (4 to 5 kg, 3 minutes). Forearm blood flow (FBF) measured by strain plethysmography increased from 4.1±0.7 mL·min⁻¹·100 mL⁻¹ at rest to 9.8±1.2 mL·min⁻¹·100 mL⁻¹ immediately after exercise and gradually decreased thereafter. Exercise was repeated after intrabrachial artery infusion of N⁰-monomethyl-L-arginine (L-NMMA) at 4.0 μmol/min for 5 minutes. L-NMMA did not alter blood pressure and heart rate. L-NMMA decreased FBF at rest to 2.9±0.4 mL·min⁻¹·100 mL⁻¹ (P<.01), peak FBF immediately after exercise to 7.2±0.7 mL·min⁻¹·100 mL⁻¹ (P<.01), and FBF during the mid to late phase of metabolic vasodilation (P<.01). Calculated oxygen consumption during peak exercise was comparable before and after L-NMMA. Intra-arterially infused L-arginine (10 mg/min, 5 minutes) reversed the inhibitory effect of L-NMMA. To determine the effect of the decrease in resting FBF on exercise-induced hyperemia, we normalized FBF after exercise by resting FBF. The percent increases in FBF after exercise from resting FBF were similar before and after L-NMMA. Furthermore, we examined the effect of intra-arterially infused angiotensin II on FBF at rest and after exercise (n=7). Angiotensin II decreased FBF at rest from 3.1±0.3 to 1.8±0.3 mL·min⁻¹·100 mL⁻¹ (P<.01), peak FBF after exercise from 8.1±0.5 to 5.6±0.5 mL·min⁻¹·100 mL⁻¹ (P<.01), and FBF during the mid to late phase of metabolic vasodilation. The effects of L-NMMA and angiotensin II on FBF at rest and exercise were similar.

Conclusions Our results suggest that L-NMMA decreased FBF after exercise largely by decreasing resting FBF. These results suggest that NO may not play a significant role in exercise-induced metabolic arteriolar vasodilation in the forearm of healthy humans. (Circulation. 1994;90:2886-2890.)

Key Words: • blood flow • endothelium-derived factors • exercise

Skeletal muscle arterioles dilate in response to exercise. The mechanisms of metabolic vasodilation remain unclear. Prostaglandins have been shown to play a role in metabolic vasodilation during exercise in humans. However, the reductions in the flow by prostaglandin synthesis inhibitors during exercise were on the order of only 10% to 20%. Therefore, other factors must contribute to exercise-induced vasodilation in humans.

Nitric oxide is a potent vasodilator released from the endothelium that plays a very important role in control of vascular tone in animals as well as in humans. Several investigators have electrically stimulated the skeletal muscle of animals before and after administration of nitric oxide synthesis inhibitors and thus examined the role of nitric oxide in exercise-induced vasodilation. Two of them reported a minor role of nitric oxide, and one reported a significant role in exercise-induced vasodilation. However, it is still not known whether nitric oxide plays a role in exercise-induced vasodilation in humans. Accordingly, we determined changes in forearm blood flow (FBF) in response to static exercise before and after administration of N⁰-monomethyl-L-arginine (L-NMMA), a blocker of nitric oxide synthesis, to investigate the role of nitric oxide in exercise-induced forearm vasodilation in humans.

Methods

General Procedure

 Eleven healthy young men (age, 19 to 28 years) participated in this study. All subjects were free of any signs or symptoms of disease. The study protocol was explained, and informed consent was obtained from each subject. The study was approved by the Ethical Committee for Human Study in our institution. The study was done with subjects in a supine position and in an air-conditioned room with room temperature of about 25°C to 26°C. Under local anesthesia with 2% procaine, the left brachial artery was cannulated with a 20-gauge intravascular over-the-needle poly(tetrafluoroethylene) catheter (Quick-Cath, Travenol Laboratories, Inc, Baxter Healthcare Corp) for drug infusion. The catheter was connected by a three-way stopcock to a pressure transducer (Viggo-Spectramed) for direct measurement of arterial pressure. The arterial line was kept open by infusing heparinized saline (0.1 mL/min) when no drug was being administered. Heart rate was obtained by counting pulse rate for a few minutes on arterial pressure recordings.

 An 18-gauge intravascular over-the-needle poly(tetrafluoroethylene) cannula (Surflow, Terumo Corp) was inserted into the antecubital vein of the arm to be exercised and was advanced to the midbrachial vein for sampling mixed forearm venous blood for measurements of oxygen saturation.

Measurements of FBF

 FBF was measured with a mercury-in-Silastic strain-gauge plethysmograph with the venous occlusion technique. The
strain gauge was placed approximately 5 cm below the antecubital crease. FBF (milliliters per minute per 100 milliliters of forearm) was calculated from the rate of increase in forearm volume while venous return from the forearm was prevented by a cuff inflated on the upper arm. The pressure in the venous occlusion or congesting cuff on the upper arm was 40 mm Hg. Circulation to the hand was arrested by a cuff inflated around the wrist. The wrist cuff was inflated before the determination of FBF and continuously throughout the measurements. Forearm vascular resistance (FVR) was calculated by dividing the mean arterial pressure (diastolic pressure plus one third of the pulse pressure in mm Hg) by the FBF. These values are expressed as units throughout this report. An average of four flow measurements made at 15-second intervals, calculated by two authors (T.E. and T.T.) independently, was used for later analysis.

Protocols

Experiment 1

After stable FBF and arterial pressure were obtained, subjects performed static handgrip exercise by pulling a weight (4 to 5 kg) for 3 minutes. FBF was measured immediately after the cessation of exercise and for 3 minutes thereafter (n=11). At least 30 minutes after exercise, when FBF had returned to the baseline level, L-NMMA was infused intra-arterially at a dose of 4 μmol/min for 5 minutes, and hemodynamics were measured. After infusion of L-NMMA was stopped, exercise was repeated and hemodynamics were measured as described above (n=11). In 6 of 11 subjects, we examined whether L-arginine reversed the effect of L-NMMA. At least 30 minutes after the second exercise, L-NMMA (4 μmol/min) and L-arginine (40 μmol/min) were infused intra-arterially and simultaneously for 5 minutes, and hemodynamics were measured. After infusion of L-NMMA plus L-arginine was stopped, exercise was repeated, and hemodynamics were measured as described above. In 6 subjects, to measure O2 saturation we sampled 1 mL of arterial and venous blood at rest and at 2 minutes during exercise before and after L-NMMA. Forearm oxygen consumption (FVO2) was calculated by the Fick principle (FVO2=forearm arterial-venous oxygen difference \[ \text{[vol%]} \times 1.34 \times \text{FBF [mL/min]} \times 100 \text{mL/g (g/dL)} + 100)$. 

Experiment 2

In another group of subjects (n=7), control FBF and arterial pressure at rest and after exercise were measured in the same way as in experiment 1. Subjects performed the same exercise as in experiment 1. After they had completely recovered from exercise, we infused angiotensin II at a dose of 20 nmol/min for 5 minutes, and hemodynamics were measured. After infusion of angiotensin II was stopped, exercise was repeated, and hemodynamics were measured.

Drugs

L-NMMA was obtained from Clinalfa AG. It was dissolved in physiological saline immediately before use. For the infusion of L-arginine, commercially available L-arginine solution (0.1 g of L-arginine/mL, Morishita Pharmaceutical) was used. Angiotensin II was purchased from Sigma Chemical Co and diluted with saline immediately before use.

Statistical Analysis

Resting forearm hemodynamic values were compared by paired t test. The forearm hemodynamic values after exercise were compared by two-way ANOVA on both factors. When they were significantly different, the value at each time was compared by a post hoc t test. Arteriovenous O2 difference and O2 consumption were compared by paired t test. All values are expressed as mean±SEM. A value of P<.05 was considered statistically significant.

Results

Responses to Exercise

FBF increased to the peak value immediately after exercise and gradually declined thereafter (Fig 1). FVR decreased to the lowest value immediately after exercise (Table 1) and gradually increased thereafter.

Effects of L-NMMA

Intra-arterial infusion of L-NMMA decreased resting FBF (P<.01) and increased resting FVR (P<.01) (Table 1 and Fig 1, left) without changes in arterial pressure or heart rate (Table 1). L-NMMA attenuated increases in FBF and decreases in FVR (P<.01) after exercise (Table 1 and Fig 1, left). However, percent increases in FBF after exercise were similar before and after L-NMMA (Fig 1, right). Arteriovenous O2 saturation was constant throughout the study. Arteriovenous O2 difference was greater after L-NMMA than before L-NMMA at rest and during exercise (Table 2). Calculated O2 consumption was similar before and after L-NMMA at rest and during exercise. L-Arginine reversed the inhibitory effects of L-NMMA (Table 1 and Fig 2).

Effects of Angiotensin II

Intra-arterial infusion of angiotensin II decreased resting FBF (P<.01) and increased FVR (P<.01) (Table 3 and Fig 3) without changes in arterial pressure or heart rate (Table 3). Angiotensin II attenuated increases in FBF and decreases in FVR (P<.01) (Table 3 and Fig 3) after exercise. However, percent increases in
FBF after exercise were similar before and after angiotensin II (Fig 3, right).

Discussion

We demonstrated that intra-arterially infused L-NMMA, a blocker of synthesis of nitric oxide, decreased FBF at rest and attenuated the increases in FBF after static handgrip exercise. However, the percent increases in FBF after exercise were similar before and after L-NMMA; intra-arterial infusion of angiotensin II decreased FBF at rest and attenuated the increases in FBF after exercise as well. The decreases in FBF at rest and after exercise were comparable between L-NMMA and angiotensin II. These results may suggest that nitric oxide plays a major role in control of vascular tone at rest but does not during metabolic arteriolar vasodilation after exercise. It is likely that L-NMMA decreased FBF after exercise by decreasing resting FBF.

Oxygen Consumption During Static Exercise

In this study, forearm oxygen consumption was measured at rest and during static exercise. Forearm oxygen consumption was similar before and after L-NMMA at rest and during static exercise. To evaluate the quantity of static exercise by forearm oxygen consumption, several aspects deserve consideration. First, in this study, arterial O₂ saturation was obtained only one time to calculate forearm oxygen consumption. If arterial desaturation occurred with static handgrip exercise, calculated forearm oxygen consumption may have been erroneously low during exercise. However, this possibility is unlikely. It has been shown that exercise by bicycle ergometer, which is more intense than static handgrip exercise, did not alter arterial oxygen saturation. Second, we introduced a venous catheter from the antebrachial vein to a deep vein 10 cm proximal to the antebrachial fossa. It is possible that oxygen-rich cutaneous blood might have been shunted to deep vein sampling sites. This possibility was not likely, however, since Wahren showed complete separation of forearm muscle and cutaneous circulation during strenuous forearm exercise. Thus, forearm oxygen consumption in this study represented the quantity of static handgrip exercise.

Metabolic Vasodilation After Exercise

The arterioles of the skeletal muscle dilate and oxygen uptake increases in response to exercise to meet oxygen demand. In this study, we applied static handgrip exercise and measured FBF after exercise but not during exercise to obtain stable FBF. We demonstrated that static handgrip exercise increased FBF and arteriovenous O₂ difference, resulting in increased oxygen consumption. Several mechanisms are postulated for metabolic vasodilation during exercise, including prostaglandins, adenosine, ATP-sensitive potassium channels, and nitric oxide. In humans the contribution of prostaglandin to metabolic vasodilation during exercise is small. The contribution of other mechanisms, such as adenosine, ATP-K channel, and nitric oxide, to metabolic vasodilation during exercise in hu-

| Table 1. Forearm Hemodynamics Before L-NMMA, After L-NMMA, and After L-NMMA Plus L-Arginine |
|-----------------------------------------|-----------------------------------------|-----------------------------------------|
|                                         | At Rest                                 | During Static Exercise                  |
|                                         | HR, bpm       | MBP, mm Hg | Max FBF, mL·min⁻¹·100 mL⁻¹ | FVR, U | HR, bpm       | MBP, mm Hg | FBF, mL·min⁻¹·100 mL⁻¹ | Min FVR, U |
| Saline (n=11)                           | 69.6±3.9     | 89.2±1.6   | 4.1±0.7               | 28.3±5.6   | 67.3±3.4     | 85.0±2.4   | 9.8±1.2             | 9.9±1.4   |
| L-NMMA (n=11)                           | 69.6±3.5     | 88.1±1.6   | 2.8±0.4*              | 37.6±7.0*  | 76.0±3.2     | 82.5±3.0   | 8.0±0.7             | 11.0±0.9  |
| L-NMMA (n=6)                            | 73.3±3.7     | 86.2±1.5   | 3.1±0.4               | 31.1±3.8   | 76.0±3.2     | 82.5±3.0   | 8.0±0.7             | 11.0±0.9  |
| L-NMMA+L-arginine (n=6)                 | 72.2±1.7     | 89.0±2.4   | 4.1±0.6†              | 23.3±2.8†  | 70.3±3.9     | 84.3±2.9   | 9.5±1.1†             | 9.4±1.0†  |

L-NMMA indicates N⁵-monomethyl-L-arginine; HR, heart rate; bpm, beats per minute; MBP, mean blood pressure; Max FBF, maximal forearm blood flow; Min FVR, minimal forearm vascular resistance; Saline, during infusion of saline; L-NMMA, after infusion of L-NMMA; and L-NMMA+L-arginine, after simultaneous infusion of L-NMMA and L-arginine.

*P<.05 vs Saline.

†P<.05 vs L-NMMA.

* Table 2. O₂ Saturation and Oxygen Consumption Before and After L-NMMA

<table>
<thead>
<tr>
<th></th>
<th>Arterial O₂ Saturation, %</th>
<th>Venous O₂ Saturation, %</th>
<th>A-V O₂ Difference, %</th>
<th>O₂ Consumption, mL·min⁻¹·100 mL⁻¹ ×10⁻²</th>
<th>Arterial O₂ Saturation, %</th>
<th>Venous O₂ Saturation, %</th>
<th>A-V O₂ Difference, %</th>
<th>O₂ Consumption, mL·min⁻¹·100 mL⁻¹ ×10⁻²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (n=6)</td>
<td>97.1±0.8</td>
<td>79.0±6.0</td>
<td>18.1±6.0</td>
<td>14.2±4.5</td>
<td>97.1±0.8</td>
<td>69.1±6.6</td>
<td>27.1±6.6</td>
<td>47.7±8.1</td>
</tr>
<tr>
<td>L-NMMA (n=6)</td>
<td>98.0±0.2</td>
<td>78.2±5.9</td>
<td>22.5±5.9*</td>
<td>14.1±3.7</td>
<td>98.0±0.2</td>
<td>68.4±7.4</td>
<td>31.1±7.2*</td>
<td>53.0±16.3</td>
</tr>
</tbody>
</table>

L-NMMA indicates N⁵-monomethyl-L-arginine; A-V, arteriovenous; Saline, during infusion of saline; and L-NMMA, after infusion of L-NMMA.

*P<.05 vs Saline.
mans is unknown. Recently, it is well appreciated that nitric oxide released from the endothelium plays a very important role in control of vascular tone not only at rest but during the high-flow state.\textsuperscript{15,16} In animals, two previous studies reported an insignificant role of nitric oxide,\textsuperscript{7,14} and one reported an important role\textsuperscript{12} during exercise-induced vasodilation in the skeletal muscle contracted by electrical stimulation. In humans, no data on the role of nitric oxide in metabolic vasodilation during exercise are available.

To determine the role of nitric oxide in metabolic vasodilation after exercise, we infused L-NMMA intra-arterially and exercise was repeated. L-NMMA decreased FBF at rest, and the inhibitory effects of L-NMMA on FBF were reversed by L-arginine, a substrate of nitric oxide, indicating a significant role of nitric oxide in control of vascular tone at rest. The magnitude of decreases in FBF by L-NMMA was similar to that of our previous study\textsuperscript{17} and of others.\textsuperscript{13} The decreases in FBF by L-NMMA were not caused by the systemic effects of L-NMMA, because arterial pressure and heart rate were not altered.

L-NMMA attenuated increases in FBF after exercise, and the inhibitory effects of L-NMMA were reversed by supplementation of L-arginine. FVR after exercise was significantly greater after than before L-NMMA. Calculated $O_2$ consumption during peak exercise was similar before and after L-NMMA, indicating that the same intensity of exercise was applied before and after L-NMMA. These results may suggest a significant role of nitric oxide in control of vascular tone during exercise-induced vasodilation. However, because L-NMMA decreased resting FBF, the interpretation of attenuated increases in FBF after exercise needs great caution. To solve this problem, we expressed FBF after exercise as percent changes and performed another experiment in which angiotensin II was infused instead of L-NMMA to decrease resting FBF to the same level as L-NMMA. The percent increases in FBF after exercise were similar before and after L-NMMA when FBF after exercise was normalized by resting FBF. Moreover, the effects of intra-arterially infused angiotensin II on FBF after exercise were similar to those of L-NMMA. These results may suggest that nitric oxide plays a minimal role in metabolic arteriolar vasodilation induced by exercise. In other words, the attenuated increases in FBF after exercise by L-NMMA were due to the decrease in resting FBF.

It could be considered that flow-mediated release of nitric oxide may contribute to increase in FBF after exercise, even though nitric oxide is not involved in exercise-induced metabolic arteriolar vasodilation per se. Our results are not consistent with this consideration, since the percent increases in FBF after exercise were similar before and after L-NMMA. However, it is possible that flow-mediated release of nitric oxide might contribute to exercise-induced hyperemia when more strenuous exercise is applied.

It is also possible that the amount of L-NMMA used in this study may have been insufficient to block the production of nitric oxide during exercise. This possibility is unlikely, however, because we previously have demonstrated that L-NMMA at 4 $\mu$mol/L was effective in totally inhibiting acetylcholine-induced increases in FBF to 24 mL$\cdot$min$^{-1}$$\cdot$100 mL$^{-1}$\textsuperscript{13,17}

In summary, our study suggests that nitric oxide may not play a significant role in exercise-induced metabolic

**TABLE 3. Forearm Hemodynamics Before and After Angiotensin II**

<table>
<thead>
<tr>
<th>HR, bpm</th>
<th>MBP, mm Hg</th>
<th>Max FBF, mL$\cdot$min$^{-1}$$\cdot$100 mL$^{-1}$</th>
<th>Min FVR, U</th>
<th>After Static Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At Rest</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline (n=7)</td>
<td>59.5±2.5</td>
<td>86.4±0.9</td>
<td>3.1±0.3</td>
<td>28.4±3.5</td>
</tr>
<tr>
<td>Angiotensin II (n=7)</td>
<td>55.8±2.5</td>
<td>88.6±0.4</td>
<td>1.8±0.3*</td>
<td>60.0±3.2*</td>
</tr>
</tbody>
</table>

HR indicates heart rate; bpm, beats per minute; MBP, mean blood pressure; Max FBF, maximal forearm blood flow; Min FVR, minimal forearm vascular resistance; Saline, during infusion of saline; and Angiotensin II, after infusion of angiotensin II. *P<.01 vs Saline.
arteriolar vasodilation in the forearm of healthy humans.

Acknowledgments

This study was supported by a Grant-in-Aid for General Scientific Research and by a Grant-in-Aid for Scientific Research on Priority Areas from the Japanese Ministry of Education, Science, and Culture. We thank Fumiko Amano for her technical assistance. We appreciate Drs. Daisuke Teshima and Osamu Fujishita at the pharmacological section for preparing drugs.

References
Role of nitric oxide in exercise-induced vasodilation of the forearm.
T Endo, T Imaizumi, T Tagawa, M Shiramoto, S Ando and A Takeshita

Circulation. 1994;90:2886-2890
doi: 10.1161/01.CIR.90.6.2886

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 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/90/6/2886