Contribution of Nitric Oxide to Reactive Hyperemia
Impact of Endothelial Dysfunction


Abstract—Our objectives were to (1) test the hypothesis that nitric oxide (NO) contributes to peak reactive hyperemia (RH) in the human peripheral vasculature, (2) examine the impact of atherosclerosis and its risk factors on RH, and (3) investigate whether l-arginine will improve RH in patients with endothelial dysfunction. The endothelium contributes to shear stress–mediated vasomotion by releasing a variety of dilating factors, including NO, but the contribution of NO to peak RH in patients with and without endothelial dysfunction is unknown. Endothelium-dependent and endothelium-independent function was assessed with intrafemoral arterial acetylcholine (ACh) and sodium nitroprusside. RH was produced by occlusion of blood flow to the leg for 3 minutes. The study was repeated after N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA) in 44 subjects and L-arginine in 9 patients with atherosclerosis. There were 15 normal control subjects without risk factors for atherosclerosis and 29 patients with risk factors or angiographic atherosclerosis. Microvascular vasodilation in response to ACh, but not to sodium nitroprusside, was lower in the patients with risk factors or atherosclerosis compared with normal control subjects, \( P = 0.048 \), and the inhibition of ACh-induced microvascular dilation by L-NMMA was also greater in normal control subjects \( (P = 0.045) \). Similarly, RH, including the peak response, was inhibited by L-NMMA in normal control subjects \( (P = 0.0011) \) but not in patients with risk factors or atherosclerosis, suggesting that the contribution of NO to both ACh-induced dilation and RH was diminished in patients with risk factors or atherosclerosis. L-Arginine did not affect vasodilation in response to ACh, sodium nitroprusside, or RH. We concluded that (1) NO contributes to all phases of RH in the normal human peripheral vasculature, (2) patients with atherosclerosis or its risks have abnormal NO bioactivity in response to pharmacological and physiological stimulation, and (3) L-arginine does not improve RH in atherosclerosis. Reduced physiological vasodilation in atherosclerosis may contribute to or exacerbate hypertension and ischemia. (Hypertension. 1998;32:9-15.)

Key Words: hyperemia ■ nitric oxide ■ endothelium ■ atherosclerosis

Myogenic, neural, and local factors such as adenosine, prostaglandins, and ischemic metabolites are believed to play a critical role in the RH response that is stimulated by transient interruption of blood flow.\textsuperscript{1-11} The vascular endothelium, by releasing endothelium-derived relaxing factors\textsuperscript{12-14} or by stimulation of ATP-sensitive potassium channels, also contributes to vascular smooth muscle relaxation,\textsuperscript{10,15,16} but the role of these mediators in determining RH in humans remains controversial. The most important endothelium-derived relaxing factor, which plays a pivotal role in modulating smooth muscle tone in the human conductance and resistance vessels, is NO.\textsuperscript{12,17,18} Endothelial NO release can be stimulated by physiological changes, including hypoxia and increases in shear stress, both features of RH,\textsuperscript{15-22} and its activity is reduced in hypertension, hypercholesterolemia, diabetes, and ATH.\textsuperscript{12,18,23-29} Furthermore, L-arginine, the precursor for NO synthesis, has been shown to improve endothelium-dependent vasodilation in some patients with endothelial dysfunction.\textsuperscript{30-36}

The purpose of this study was to test the hypotheses that (1) NO contributes to the peak RH in the human peripheral vasculature, (2) its contribution is reduced in patients with ATH and its risk factors, and (3) L-arginine will improve RH in patients with endothelial dysfunction.

Methods

Patients
Femoral arterial RH was studied in 53 subjects, 45 patients and 8 healthy volunteers. Patients were undergoing cardiac catheterization for evaluation of chest pain or underlying coronary artery disease. Forty-four subjects participated in the L-NMMA study (Table), and 9 in the L-arginine study.

L-NMMA Study
Thirty-six patients and 8 healthy volunteers were recruited for the L-NMMA study. Volunteers had a mean age of 41 ± 7 years, 6 were male, and all were nonsmokers, were free of hypertension, hypercholesterolemia, diabetes, and other systemic disorders, and were not on any medication. No patient had a history of claudication, decrease in peripheral pulses, or abnormal pressures in the leg. Of the 36...
patients in the L-NMMA study, 23 had angiographic evidence of coronary and/or femoral ATH and 13 had no angiographic evidence of ATH affecting either circulation. Of the latter patients, 6 had a history of hypertension (n = 5) and/or hypercholesterolemia (cholesterol > 6.2 mmol/L; n = 1) and/or diabetes (n = 2). The remaining 7 patients without ATH were free of any risk factors (Table).

**1-Arginine Study**

Nine additional patients with either ATH (n = 6) or risk factors for ATH (n = 3) participated in the 1-arginine study. Their mean age was 58 ± 10 years, 8 were male, 4 had hypertension, 3 had diabetes, and 4 had hypercholesterolemia. All subjects refrained from caffeine-containing beverages for at least 12 hours and from aspirin or other cyclooxygenase inhibitors for at least 1 week before the study. Informed consent was obtained from all subjects, and the protocol was approved by the National Heart, Lung, and Blood Institute Institutional Review Board.

**Measurement of Femoral Blood Flow**

A 6F angiographic right Judkins or multipurpose A2 ( Cordis, Inc) catheter was introduced 1 cm beyond the end of a 7F femoral artery sheath into which drug infusions were made. A 0.018-in Doppler flow wire ( Cardiometrics, Inc) was introduced through the catheter and positioned 1 cm beyond the catheter tip to obtain an adequate flow velocity signal. Thus, the drug infusions were given downstream of the flow wire. A femoral angiogram was performed to measure femoral diameter at the level of the flow wire and to visualize atherosclerotic plaque in the femoral artery, and all patients with significant stenosis of the iliofemoral circulation were excluded. In normal volunteers, a 6F sheath and 5F catheter were used. The time-average velocity (that is, an average of the velocities during the whole cardiac cycle) with each intervention was recorded and baseline blood flow measurement computed by the formula $(\pi \times$ time-average velocity $\times 0.125 \times$ diameter)$^{39}$ Because diameter measurements were not made at the level of the Doppler wire with each intervention, we calculated FVRI as the mean arterial pressure divided by femoral blood flow velocity. To exclude any significant changes in femoral artery diameter at the site of the flow wire during conditions of increased blood flow, we used serial angiography to measure femoral artery diameter at the site of the flow wire during administration of 300 $\mu$g/min ACh and 40 $\mu$g/min SNP in 24 patients and after 64 $\mu$mol/min L-NMMA in 7 patients. There was no significant alteration in femoral arterial diameter at the site of the flow wire during these drug infusions: baseline, 5.1 ± 0.9 cm; ACh, 5.1 ± 0.9 cm; SNP, 5.1 ± 0.9 cm; and L-NMMA, 5.0 ± 0.6 cm (all P = NS compared with baseline).

**Selected Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ACH</td>
<td>acetylcholine</td>
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<tr>
<td>ATH</td>
<td>atherosclerosis</td>
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<tr>
<td>CV</td>
<td>coefficient of variation</td>
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<tr>
<td>FVRI</td>
<td>femoral vascular resistance index</td>
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<td>NO</td>
<td>nitric oxide</td>
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<td>RH</td>
<td>reactive hyperemic</td>
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<td>SNP</td>
<td>sodium nitroprusside</td>
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**Study Protocols**

**L-NMMA Study**

After 2-minute infusions of ACh at 150 and 300 $\mu$g/min, pressure and flow-velocity measurements were made (Figure 1). This was followed after a 10-minute period by RH. A sphygmomanometer cuff was placed immediately above the knee and inflated to a pressure $\geq 25$ mm Hg above the systolic blood pressure for 3 minutes in 44 subjects (36 patients and 8 volunteers). Flow velocity and mean arterial pressure were measured at 5, 15, 30, 45, 60, 75, 90, 115, 120, 150, 180, 210, 240, 270, and 300 seconds after deflation of the cuff. Ten minutes after RH, SNP was administered at 40 $\mu$g/min for 3 to 5 minutes, and the peak flow velocity and blood pressure measurements were repeated. After a 15-minute recovery period and return to baseline flow velocity, L-NMMA was infused at 32 $\mu$mol/min, followed by 64 $\mu$mol/min, for 5 minutes each. This was followed by 2-minute infusions of ACh at 150 and 300 $\mu$g/min. Repeat 3-minute occlusion and RH was produced, followed by 40 $\mu$g/min of SNP for 2 to 3 minutes.

**1-Arginine Study**

After baseline measurements of arterial blood pressure and flow velocity, ACh was administered at 150 and 300 $\mu$g/min for 2 minutes each and was followed by RH as in the L-NMMA study. A total of 11 RH measurements were made in 9 patients: in 4 patients, 3-minute occlusion followed by RH was studied; in 3 patients, 5-minute RH; and in 2 patients, both 3-minute and 5-minute RH studies were performed. This was followed by SNP infusion of 40 $\mu$g/min for 4 minutes. After a 15-minute recovery period and return to baseline flow velocity, 1-arginine was infused into the femoral artery at 160 $\mu$mol/min for 10 minutes. While the infusion of 1-arginine was continued, the 2 doses of ACh, RH, and SNP infusion were repeated as in the control study.

**Reproducibility of RH**

In a separate group of 6 patients with ATH or its risks, we performed 2 consecutive measurements (20 minutes apart) of flow velocity and blood pressure during RH after 3 minutes of ischemia to determine the reproducibility of the measurements.

**Statistical Analysis**

Data are expressed as mean ± SD in the text and mean ± SEM in the figures. Means were compared by paired or unpaired Student’s t test, as appropriate. The differences between the effects of L-NMMA in patients with and those without ATH were studied using the percent change from baseline for all parameters because of the baseline differences in flow velocity and FVRI. All P values are two-tailed. The global effect of L-NMMA on 2 doses of ACh and the effects of 2 doses of L-NMMA in normal control subjects and in patients with ATH were compared by ANOVA for repeated measures. When the effects of 2 doses of L-NMMA on hemodynamics were each compared with those at baseline, separate paired t tests were performed with a Bonferroni adjustment to α. The effect of L-NMMA on RH was studied by the repeated measures ANOVA,
which included patients, medications (L-NMMA/control), and time as main effects and also incorporated the two-factor interactions between them. Multiple stepwise regression analysis was performed to test whether the magnitude of inhibition of peak RH with L-NMMA was related to age, sex, presence of hypertension, diabetes, cigarette use, or cholesterol level. Additional regression analyses were performed to assess whether any of the individual risk factors explained the group effect.

**Results**

**Reproducibility of RH**
In 6 patients in whom RH was elicited on 2 occasions, there was no significant difference between the first and second procedures ($P=NS$). The maximal reduction in FVRI during the first test (from 6.8±0.1 to 1.8±0.2 mm Hg · cm$^{-1}$ · s$^{-1}$, −74±3%, CV=0.11) was similar to the reduction observed with the second test (from 6.1±0.3 to 1.7±0.3 mm Hg · cm$^{-1}$ · s$^{-1}$, −73±3%, CV=0.08), $P=0.6$.

**L-NMMA Study**
Patients were divided into 2 groups for analysis of RH: (1) normal control subjects, including 7 patients without risk factors and 8 healthy volunteers, and (2) patients with risk factors or ATH, including 6 with risk factors and 23 with angiographic coronary and/or peripheral ATH (Table). Resting femoral blood flow was similar: 100±43 mL/min in normal control subjects and 119±51 mL/min in the patients with risk factors or ATH.

**Effect of L-NMMA at Rest and on Endothelium-Dependent and -Independent Vasodilation in Normal Control Subjects**
L-NMMA reduced flow velocity by 18±11% (from 21.4±1.9 to 16.6±1.5 cm · s$^{-1}$) and increased FVRI by 32±21% (from 5.4±0.9 to 7.0±1 mm Hg · cm$^{-1}$ · s$^{-1}$) after the 32-μmol/min infusion ($P<0.001$) and reduced flow velocity to 14.6±1.3 cm · s$^{-1}$ (26±12%) and increased FVRI to 8±1.1 mm Hg · cm$^{-1}$ · s$^{-1}$ (50±20%) after the 64-μmol/min infusion of L-NMMA ($P<0.001$ for both). There was a 3.3% increase (from 96.2±11 to 99.6±10 mm Hg, $P=0.016$) in mean arterial pressure after the 64-μmol/min infusion of L-NMMA.

L-NMMA significantly inhibited vasodilation in response to ACh but not to SNP (Figure 2). Thus, at the peak dose of ACh, a flow velocity increase of 158±84% was reduced to an 84±93% increase after L-NMMA, $P<0.001$, whereas there was no change in SNP-induced vasodilation (115±67% to 110±59% increase in flow velocity, before versus after L-NMMA, $P=0.8$ (Figure 2).

**Effect of L-NMMA on RH in Normal Control Subjects**
The peak increase in flow velocity and thus maximal microvascular vasodilation occurred at 5 seconds in 9 patients and at 15 seconds in 6 patients before L-NMMA. Vasodilation during the entire RH response was reduced after L-NMMA in normal control subjects; this difference was present at 5 and 15 seconds ($P<0.02$) and at all time points thereafter.

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**Figure 2.** Effects of L-NMMA on maximum vasodilation due to ACh, SNP, and 3 minutes of occlusion and hyperemia in normal control subjects without risk factors for ATH. Control study (solid line) and after L-NMMA (dashed line). †$P<0.001$ control vs L-NMMA.

**Figure 3.** FVRI during hyperemia before and after L-NMMA in normal control subjects without risk factors for ATH. Control study (solid line) and after L-NMMA (dashed line). Baseline resistance index before L-NMMA at time 0 was similar, but there were significant differences throughout the hyperemic period; $P$ value calculated by ANOVA from 30 to 120 seconds. There were also significant differences at 5 and 15 seconds ($P<0.02$).
\( P = 0.001 \) by ANOVA, Figure 3). Comparison of FVRI with RH before versus after L-NMMA by ANOVA revealed a significant L-NMMA \( \times \) time interaction, suggesting that the magnitude of the effect was different at different time points. This was due to the 5- and 15-second time points, after which the curves were parallel. The \( t_{1/2} \) of FVRI (time in seconds to doubling of minimum FVRI) decreased from 31 \( \pm \) 8 seconds during the control study to 23 \( \pm \) 5 seconds after L-NMMA, \( P < 0.001 \). After L-NMMA, minimum FVRI after 3 minutes of ischemia and RH was 43% higher, increasing from 1.5 \( \pm \) 1 before to 2.2 \( \pm \) 1.3 mm Hg \( \cdot \) cm\(^{-1} \) \( \cdot \) s\(^{-1} \) (\( P = 0.0011 \), Figures 2 and 3). Similarly, peak RH flow velocity after L-NMMA was lower (72 \( \pm \) 25 before versus 57 \( \pm \) 24 cm \( \cdot \) s\(^{-1} \) after) and mean arterial pressure in the femoral artery during RH was higher (91 \( \pm \) 10 mm Hg before and 99 \( \pm \) 11 mm Hg after L-NMMA, \( P < 0.001 \) for both).

**Comparison of L-NMMA, ACh, and SNP Responses in Normal Control Subjects and Patients With ATH and Risks**

ACh-mediated but not SNP-mediated dilation was significantly greater in normal control subjects than in patients with risk factors or ATH (Figure 4). L-NMMA inhibited ACh-induced dilation in both groups, but the inhibition was greater in normal control subjects; thus, at the highest dose of ACh, flow velocity after L-NMMA and ACh was 38 \( \pm \) 20% lower than control ACh in normal subjects and 26 \( \pm \) 19% lower in patients with risk factors for ATH (\( P < 0.05 \) between groups).

**Comparison of the Effect of L-NMMA on RH in Normal Control Subjects and Patients With Risk Factors for ATH**

The peak dilator response to 3-minute occlusion and release was greater in normal control subjects than in patients with risk factors or ATH: compared with baseline, flow velocity increased by 245 \( \pm \) 91% versus 192 \( \pm \) 67% (\( P = 0.04 \)), respectively. Figure 4. With L-NMMA, peak RH was inhibited in normal control subjects (43% increase in minimum FVRI and 20% decrease in flow velocity), but the 22% increase in minimum FVRI and a 6% decrease in flow velocity in patients with risk factors or ATH was not significant (\( P = 0.05 \) between the 2 groups, Figure 4). Thus, after L-NMMA, the percent decrease in FVRI and increase in flow velocity with RH were similar in both patient groups, suggesting that the greater response in normal control subjects at baseline was due to a higher contribution of NO to RH in this group.

In addition, there was a significant correlation (\( r = 0.47, P = 0.006 \)) between the magnitude of increase in FVRI with ACh after L-NMMA and the increase in FVRI during RH after L-NMMA, indicating that patients in whom L-NMMA produced greater inhibition of ACh-induced dilation also had greater inhibition of RH and vice versa.

Simple and multiple regression models were constructed to examine whether a particular risk factor explained the reduction in contribution of NO to RH. Risk stratification into normal control subjects or patients with risk factors or ATH was predictive of the increase in minimum hyperemic FVRI after L-NMMA. None of the risk factors taken individually were predictive, either in all patients as a group or in either of the 2 risk subgroups. Furthermore, there was no remaining statistically significant residual risk subgroup effect on the response to L-NMMA during RH after all the individual risk factors studied were accounted for.

**Effect of L-Arginine on RH**

Patients undergoing the L-arginine study had reduced dilation with ACh compared with the normal control subjects in the L-NMMA study (97 \( \pm \) 64% versus 185 \( \pm \) 65% increase in flow velocity with ACh). Intra-arterial L-arginine infusion did not alter blood pressure (110 \( \pm \) 19 to 111 \( \pm \) 20 mm Hg), femoral blood flow velocity (15.9 \( \pm \) 5.7 to 15.1 \( \pm \) 5.9 cm \( \cdot \) s\(^{-1} \)), FVRI (7.4 \( \pm \) 1.6 to 8.1 \( \pm \) 3.4 mm Hg \( \cdot \) cm\(^{-1} \) \( \cdot \) s\(^{-1} \), all \( P = NS \)), or the responses to ACh and SNP (Figure 5). The responses before and after L-arginine were also similar during all phases of RH; thus, the peak increase in flow velocity (224 \( \pm \) 56%) before was similar to that after L-arginine (242 \( \pm \) 75%, \( P = 0.53 \), Figure 5). With a sample size of 9, we can exclude a pre–minus post– L-arginine difference in FVRI of \( \geq 5.5\% \) (compared with baseline FVRI) as being significant with 80% power and \( \alpha = 0.05 \).

**Discussion**

The major findings of this study are that (1) inhibition of NO production attenuated the peak, mid, and late phases and the total duration of RH; (2) compared with normal control subjects, patients with ATH or its risk factors who had an abnormal response to ACh also had reduced inhibition of RH with L-NMMA, indicating reduced contribution of NO to RH in patients with endothelial dysfunction; and (3) L-arginine did not appear to enhance RH in patients with endothelial dysfunction. These results indicate that NO contributes to RH.
of human peripheral microvessels. Furthermore, ATH and its risk factors not only result in depressed NO bioavailability in response to endothelium-dependent pharmacological vasodilators but also reduce the contribution of NO to physiological stimuli such as RH.

**Microvascular RH**

Local factors that contribute to the RH of the microvessels include changes in interstitial potassium ions, hydrogen ions, osmolality, carbon dioxide, catecholamines, prostaglandins, and adenosine. Among these, adenosine is an important player, although its contribution appears to account for only 30% of the response. Recently, the critical contribution of ATP-sensitive potassium channels to RH has been demonstrated. The crucial role of the vascular endothelium in modulating peripheral arteriolar RH in animals has recently been stressed in studies by Koller and Kaley, but the relative contribution of NO has remained controversial.

Two recent studies examining the effect of L-NMMA on RH in normal human brachial arteries have demonstrated a reduced late phase of RH, as in our study, but in contrast to our findings, they did not elicit inhibition of peak RH with L-NMMA. There may be several methodological explanations for these discrepancies. First, the doses of L-NMMA used in those studies were considerably lower and may have resulted in incomplete inhibition of NO synthase. In a previous investigation, we have shown that lower doses of L-NMMA insignificantly reduced exercise-induced forearm dilation that was suppressed only at higher doses. Second, in the study by Tagawa et al, blood flow was measured by forearm plethysmography, which does not provide continuous measurements. Continuous measurements are critical in the early phases of RH, when flow changes rapidly. Furthermore, measurement of inflow arterial blood pressure at the time of RH is important to truly determine the magnitude of vasodilation, because significant underestimation of vascular resistance can result from measurement of blood pressure in the contralateral limb. As observed in our study, the mean arterial pressure in the study femoral arteries fell by a mean of 6.2% during peak RH in the control study and by 4.9% during RH after L-NMMA. Third, we directly measured the Doppler flow velocity using an intra-arterial catheter, whereas reliance was placed on 30 continuous measurements of percutaneous Doppler flow velocity in the study by Joannides et al, which may be subject to inaccuracies.

One criticism of our methodology is that we measured flow velocity and estimated vascular resistance without correcting for changes in diameter that may occur in the femoral artery. Drugs were delivered downstream from the site of measurement of the flow velocity, and therefore, there was no direct pharmacological effect of the interventions on femoral diameter at that site. Changes in diameter may nevertheless occur as a result of flow-mediated vasodilation. However, at 5 to 15 seconds after onset of hyperemia, when flow velocity is maximal, the vessel diameter does not change significantly. The greatest conductance vessel diameter change during hyperemia occurs between 45 and 90 seconds, and flow-mediated vasodilation appears to be minimal in large conductance vessels. In addition, the peak increase in flow velocity was 3-fold before and 2.7-fold after L-NMMA, a difference that may, if anything, cause a slightly smaller flow-mediated dilation of the femoral artery at the level of the Doppler flow wire after L-NMMA. Thus, it is unlikely that the changes in hyperemic flow velocity are significantly underestimating the changes in flow. Finally, our angiographic studies excluded significant changes in femoral artery diameter at the site of the flow wire during drug infusions.

**Specificity of Action of L-NMMA**

L-NMMA infusion produced a 50% increase in resting FVRI, indicating that, as previously reported, NO modulates resting vasomotor tone in the human peripheral vasculature. It may be argued that the increased vascular resistance during hyperemia after L-NMMA is due to a nonspecific effect of L-NMMA on baseline resistance. To overcome this concern, we tested the specificity of L-NMMA with 2 vasodilators, ACh and SNP. Inhibition of ACh-induced and lack of inhibition of SNP-induced dilation by L-NMMA suggests that the attenuating effect of L-NMMA during RH is specific for its inhibition of NO. Furthermore, the correlation between inhibition of ACh-induced dilation and inhibition of RH by L-NMMA and the lack of correlation with the effect of SNP also indicate that the effect of L-NMMA is endothelium specific.

**Mechanism of NO Release During RH**

The mechanisms by which NO contributes to RH remain uncertain. First, there appears to be a pivotal role for NO in the guinea pig heart, with a 2-fold increase in NO release during RH in the coronary effluent that was inhibited with L-NAME and was accompanied by a parallel reduction in RH. Second, since hypoxia increases production of NO from the microvessels, it is possible that inhibition of NO production during ischemic hypoxia in the femoral circula-
tion would limit the earlier and peak hyperemic response.19,20 The reduction in the mid to late hyperemic dilation observed during L-NMMA is probably secondary to the inhibition of flow-mediated vasodilation of arterioles.31,42

Impact of ATH and Its Risk Factors
Consistent with previous studies, femoral microvascular dilation in response to endothelium-dependent but not endothelium-independent agents was depressed in patients with ATH and its risk factors compared with normal subjects.18,23–29,52,53 The purpose of our present study was to extend these observations by determining whether endothelial dysfunction in response to pharmacological stimulation translates into reduced dilation in response to a physiological stimulus such as RH. Our results demonstrate that the contribution of NO to RH was greater in normal subjects without risk factors. Since RH after L-NMMA was similar in both groups, the reduced dilation observed in ATH during the control study before L-NMMA was most likely due to a diminished contribution of NO to peak RH in this population. These observations are consistent with our previous study in which the contribution of NO to pacing-induced coronary vasodilation was also depressed in patients with risk factors for ATH.24 Multivariate analysis failed to reveal any individual risk factor as a determinant of the response to L-NMMA during RH and reinforced the concept that the presence of ATH or one or more of its risk factors determined the response.

L-Arginine and RH
L-Arginine is known to improve the vascular response to ACh in hypercholesterolemic animals.17,55 However, its effects in the human coronary and peripheral vasculature have been controversial30–36; some studies have shown that parental L-arginine improves the vasodilator response to ACh in the forearm and coronary vasculature of hypercholesterolemic patients, but others have failed to confirm these findings.32,56–58 One purpose of our study was to examine whether L-arginine, by increasing NO production during RH, would improve the response in patients with ATH and endothelial dysfunction. There was no appreciable enhancement of the response to ACh, SNP, or RH with intra-arterial L-arginine. Thus, although NO contributes to RH, administration of its substrate did not improve RH in endothelial dysfunction. We may nevertheless have underestimated the effect of L-arginine in this study because of the small number of patients studied.

Studies in which L-arginine was given intravenously or orally showed improvement in endothelium-derived vasomotion,31,33,59,60 whereas most studies with intra-arterial administration have failed to show improvement in peripheral endothelium-dependent vasodilation.32,56–58 This difference may be, in part, due to activation of neurohormonal pathways, such as insulin release, during parenteral L-arginine infusions.45

Limitations
Three or 5 minutes of occlusion probably did not result in maximum RH, but these were tolerable periods of ischemia in patients. However, the increase in postischemic flow was significant and reproducible for us to study the response before and after interventions.

In conclusion, our study demonstrates the pivotal role of NO during RH in humans. The reduced contribution of NO to RH in patients with risk factors or established ATH highlights the effect of endothelial dysfunction on physiological functions of the vascular endothelium in vivo. This vascular abnormality may thus contribute to hypertension and ischemia.

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33. Hospital of the University of Pennsylvania, Philadelphia, PA, USA.


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