Contribution of Endothelium-Derived Nitric Oxide to Exercise-Induced Vasodilation

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Background Endothelium-derived nitric oxide is an important modulator of resting vascular tone in animals and humans. However, the contribution of nitric oxide to exercise-induced vasodilation is unknown.

Methods and Results The effect of N^G-monomethyl-L-arginine (L-NMMA), an inhibitor of nitric oxide synthesis, on exercise-induced vasodilation was studied in 18 healthy subjects (mean±SD, 40±10 years; 10 women). Acetylcholine was used to test the efficacy of L-NMMA in inhibiting stimulation of nitric oxide synthesis and sodium nitroprusside to test the specificity of L-NMMA in inhibiting endothelium-dependent vasodilation. Intermittent handgrip exercise and infusions of acetylcholine and sodium nitroprusside were performed during intra-arterial infusion of 5% dextrose (control) and L-NMMA (4 to 16 μmol/min). Forearm blood flow was determined by strain-gauge plethysmography. Forearm oxygen extraction was measured from arterial and venous oxygen saturations. In a separate study, 10 subjects performed exercise during infusions of 5% dextrose, L-arginine (the substrate for nitric oxide production), and D-arginine (the stereoisomer that is not a substrate for nitric oxide production). L-NMMA reduced exercise blood flow by 7±13% (P=.04), increased exercise resistance by 18±20% (P=.02), and increased exercise oxygen extraction by 16±17% (P<.001). The degree of inhibition of acetylcholine-induced vasodilation with L-NMMA correlated positively with the degree of reduction in exercise blood flow (r=.55, P=.02). The highest dose of L-NMMA (16 μmol/min) produced the greatest effect; exercise blood flow was reduced by 11±14% (P=.03), and vascular resistance increased by 26±23% (P=.005). L-NMMA did not affect the forearm vasodilation produced by sodium nitroprusside. Exercise blood flow, resistance, and oxygen extraction were not significantly modified by infusions of either L- or D-arginine.

Conclusions Inhibition of nitric oxide synthesis reduces exercise-induced vasodilation in the human forearm, indicating that nitric oxide plays a role in exercise-induced vasodilation. Increased availability of nitric oxide substrate does not enhance exercise-induced vasodilation in healthy subjects. These findings have important implications for disease states in which endothelium-derived nitric oxide production is impaired. (***Circulation*. 1994;90:2853-2858.**)

Key Words • endothelium-derived relaxing factor • L-arginine • exercise • vascular tone

In the last decade, many studies have demonstrated that the endothelium regulates vascular tone through the release of different constrictor and dilator substances that act on the underlying smooth muscle.[1-3] One of the most important endothelium-derived relaxing factors has been identified as nitric oxide.[4,5] Nitric oxide, produced in the endothelial cell from the amino acid L-arginine, diffuses locally to the smooth muscle, where it increases intracellular levels of cyclic GMP and causes vascular relaxation.[6] Analogues of L-arginine, such as N^G-monomethyl-L-arginine (L-NMMA), competitively inhibit nitric oxide production and have been used to investigate the importance of this pathway in animal and human studies.[7-9] Moreover, nitric oxide production can be enhanced by the administration of L-arginine in some settings, suggesting that the availability of L-arginine may be a rate-limiting factor in nitric oxide synthesis.[10-13]

Previous studies have shown that nitric oxide production contributes importantly to resting vascular tone in the human forearm[9,14] and that its production is impaired in several cardiovascular diseases.[15-21] However, the contribution of nitric oxide to exercise-induced vasodilation has not been examined. The purpose of this investigation, therefore, was to determine the role of nitric oxide in exercise-induced vasodilation and to determine whether exercise vasodilation can be enhanced by increased availability of the nitric oxide substrate L-arginine.

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Methods

Study Population

Twenty-seven healthy volunteers were enrolled. Clinical history, physical examination, ECG, chest radiography, and routine laboratory tests showed that these subjects had no evidence of present or past hypertension, hypercholesterolemia, cardiovascular disease, or any other systemic condition. None of the volunteers were taking any medication. The study was approved by the National Heart, Lung, and Blood Institute Review Board. All subjects gave written informed consent.

Twenty subjects participated in the first study in which the effect of L-NMMA on exercise was examined. Two of these subjects were subsequently excluded because of technically unsatisfactory blood flow recordings during exercise. Therefore, the final study group consisted of 18 subjects, 8 men and 10 women, with a mean age of 40±10 years (range, 27 to 63 years). In a separate study, the effect of L-arginine on exercise-induced vasodilation was examined in 10 subjects (3 of whom also participated in the first study). There were 5 men and 5

women, and their mean age was 41±9 years (range, 24 to 51 years).

Protocol

Studies were performed in the morning, in a quiet room, with the temperature maintained at approximately 22°C (72°F). Subjects were asked to refrain from drinking alcohol or beverages containing caffeine and from smoking for at least 24 hours before the study, and they came to the laboratory after a light breakfast.

While the subjects were supine, a cannula (1¾-in, 20 gauge; Arrow) was inserted into the brachial artery of the dominant arm (right, in most cases). A second cannula (1-in, 20 gauge, Jelco) was inserted into a deep antecubital vein of the same arm (venous cannulation was unsuccessful in 1 subject in the L-NMMA study and in 3 subjects in the L-arginine study). The arm was slightly elevated above the level of the right atrium, and a mercury-filled Silastic strain gauge was placed around the widest part of the forearm. The strain gauge was connected to a plethysmograph (model EC4, DE Hokanson) calibrated to measure the percent change in volume. The plethysmograph, in turn, was connected to a chart recorder (Pharmacia LKB). For each measurement, a cuff placed on the upper arm was inflated to 40 mm Hg with a rapid cuff inflator (model E10, DE Hokanson) to occlude venous outflow from the extremity, and a wrist cuff was inflated to 50 mm Hg above systolic pressure 1 minute before each measurement to exclude the hand circulation. Flow measurements were recorded for approximately 7 seconds, every 15 seconds. Seven readings were obtained for each mean value at rest and during drug infusions. Forearm blood flow is expressed as $mL \cdot min^{-1} \cdot 100\ mL^{-1}$ of forearm volume. Brachial artery pressure was measured directly from the intra-arterial catheter using a Spacelabs monitor (model 90308). Forearm vascular resistance was calculated as the mean arterial pressure divided by the forearm blood flow and is expressed as mm Hg per $mL \cdot min^{-1} \cdot 100\ mL^{-1}$ volume. Blood samples for oxygen (O_2) saturations were taken from the artery and vein. O_2 saturation was measured using an oximeter (AO Unistat, American Optical). Oxygen content was derived in mL/100 mL of blood by the formula: O_2 content=O_2 saturation %×1.36×hemoglobin in mg/dL.[22] Forearm arteriovenous oxygen difference or O_2 extraction (mL/100 mL of blood) and O_2 consumption (O_2 extraction×blood flow expressed in mL/min/100 mL tissue) were calculated.

Basal measurements were obtained after a 3-minute infusion of 5% dextrose solution at 1 mL/min. Forearm blood flow was then measured after the infusion of acetylcholine chloride (Sigma Chemical) at 7.5, 15, and 30 mg/min (infusion rate of 0.25, 0.5, and 1 mL/min). Acetylcholine was infused for 5 minutes at each dose, and forearm blood flow was measured during the last 2 minutes of each infusion.

The forearm was exercised by intermittent handgrip with a handgrip dynamometer (squeeze dynamometer/exerciser, Technical Products Co). The protocol used was originally described by Zelis et al[23] and modified by Arnold et al.[24] Exercise was performed at 15%, 30%, and 45% of maximum grip strength, which was determined in each subject at the start of the study. Each contraction lasted for 5 seconds followed by relaxation for 15 seconds. On relaxation, the wrist cuff was rapidly inflated to suprasystolic pressure followed immediately by rapid inflation of the upper arm cuff, and a blood flow measurement was obtained. The contraction/relaxation sequence was repeated for a total of 5 minutes at each workload (total exercise time, 15 minutes). A blood flow value for each workload was calculated as the mean of eight measurements recorded during the last 3 minutes of each stage. Preliminary studies in our laboratory demonstrated that blood flow remained constant after 2 minutes of exercise.

Forearm exercise and the acetylcholine dose-response curve were performed in random order with a 30-minute rest period between each intervention. After an additional 30-minute rest period, an intra-arterial infusion of L-NMMA (Sigma) was begun. Because the dose of L-NMMA required to maximally inhibit nitric oxide synthesis in the forearm is unknown, three doses were used: 4 μmol/min in 5 subjects, 8 μmol/min in 5, and 16 μmol/min in 8. The acetylcholine dose-response curve and forearm exercise were repeated during infusion of L-NMMA, using the same methodology and in the same order as described above.

To determine whether the forearm vasoconstriction produced by L-NMMA at rest could lead to a nonspecific reduction in subsequent forearm vasodilator responses, the 8 subjects who received the 16-μmol/min dose of L-NMMA returned for a second study. In this study, the forearm vascular responses to the endothelium-independent vasodilator sodium nitroprusside were measured during concomitant infusions of 5% dextrose and L-NMMA at 16 μmol/min. Sodium nitroprusside was infused intra-arterially at doses of 0.8, 1.6, and 3.2 mg/min for 5 minutes each (infusion rate, 0.25, 0.5, and 1 mL/min) with measurements in the last 2 minutes of each infusion.

To investigate the effect of increased availability of the nitric oxide precursor on exercise-induced vasodilation, l0 subjects performed three consecutive exercise tests (as described above) with intervening 30-minute rest periods. The first exercise test was performed during intra-arterial infusion of 5% dextrose, and the second and third tests were performed during intra-arterial infusion of either L-arginine (40 μmol/min) or D-arginine (40 μmol/min), in random order. L-Arginine is the substrate for nitric oxide synthesis, and D-arginine is the stereoisomer that cannot be metabolized by nitric oxide synthase and therefore serves as a control for the nonspecific vascular effects of L-arginine.[7,13,25]

Statistical Analysis

Student's *t* test for paired data was used to compare resting measurements between tests. The dose-response curves to acetylcholine and the results of exercise testing were compared by repeated-measures ANOVA allowing for test×stage interaction. If ANOVA indicated a significant difference between tests, pairwise differences between tests were compared using the Bonferroni multiple-comparison procedure. To compare the effect of L-NMMA on acetylcholine with its effect on exercise, linear regression analysis was performed, and a correlation coefficient was calculated. To estimate the magnitude of any L-arginine effect that could be excluded by the study, the 95% confidence interval of the mean difference in vascular resistance between the D-arginine and L-arginine tests was calculated. Data are expressed as mean±1 SD. Error bars on the figures represent ±1 SEM.

Results

Effect of L-NMMA on Forearm Vascular Tone at Rest

Five minutes after L-NMMA infusion, resting blood flow decreased by 25±19% (from 3.4±1.2 to 2.5±0.9 $mL \cdot min^{-1} \cdot 100\ mL^{-1}$, $P<.001$), vascular resistance increased by 47±43% (from 27±9 to 38±12 mm Hg per $mL \cdot min^{-1} \cdot 100\ mL^{-1}$ of volume, $P<.001$), and mean arterial pressure remained unchanged (83±8 to 85±9 mm Hg, $P=.14$). These hemodynamic changes were associated with a 55±71% increase in oxygen extraction (from 5.2±2.0 to 7.3±2.4 mL/100 mL, $P<.001$).

Effect of L-NMMA on Forearm Vascular Response to Acetylcholine

L-NMMA substantially attenuated the vascular response to all doses of acetylcholine (Fig 1). Mean blood flow with acetylcholine was reduced by 31±21% ($P<.001$), and mean vascular resistance was 86±70%

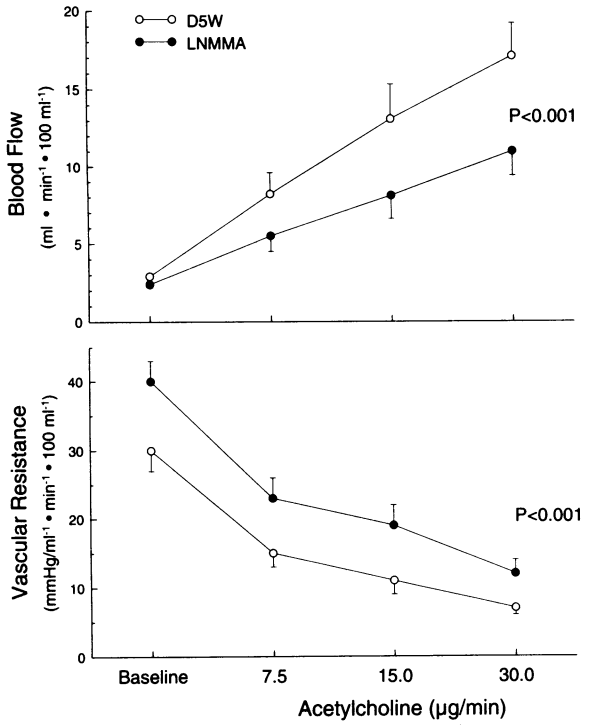

FIG 1. Plots of the effect of 4 to 16 μmol/min N^G-monomethyl-L-arginine (L-NMMA) on the vascular response to acetylcholine in 18 subjects. *P* values denote the result of ANOVA for repeated measures at the three doses of acetylcholine. D5W indicates 5% dextrose.

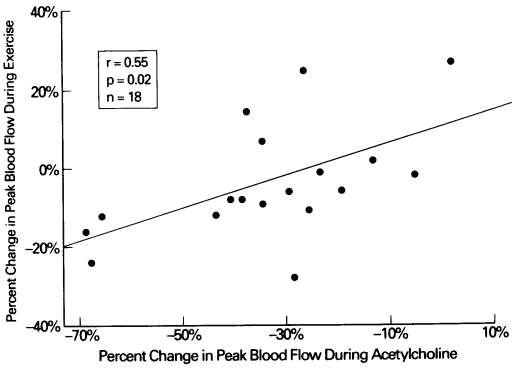

FIG 2. Plot of the relationship between the inhibition of vasodilation with N^G-monomethyl-L-arginine (L-NMMA) during exercise and with acetylcholine. Percent change in the peak acetylcholine blood flow (30-mg/min dose) after L-NMMA is compared with the percent change in peak exercise blood flow (45% maximum handgrip) after L-NMMA.

higher ($P<.001$). Mean arterial pressure was higher during the L-NMMA test by 6±7 mm Hg ($P<.01$). O_2 extraction, measured in 7 subjects, decreased markedly during acetylcholine-induced vasodilation, to a mean of 1.5±1.2 mL/100 mL, and this decrease was attenuated by L-NMMA (mean, 2.5±1.1 mL/100 mL, $P<.01$).

Effect of L-NMMA on Exercise-Induced Vasodilation

The exercise protocol produced a stepwise increase in both blood flow and O_2 extraction and a decrease in forearm vascular resistance without any significant change in heart rate or blood pressure (Table). Exercise-induced vasodilation was reduced by L-NMMA; thus, mean blood flow was 7±13% lower ($P=.04$), vascular resistance was 18±20% higher ($P=.02$), and O_2 extraction was 16±17% higher ($P<.001$) during L-NMMA infusion compared with control (Table). Mean arterial pressure was also higher during L-NMMA exercise (6±5 mm Hg, $P<0.01$), and heart rate was slightly lower (Table). O_2 consumption was similar in both tests (Table), indicating that equivalent work was performed at each stage in both tests. The degree to which L-NMMA inhibited acetylcholine-induced vasodilation correlated positively with the degree to which L-NMMA reduced exercise blood flow ($r=.55$, $P=.02$; Fig 2). The effect L-NMMA on exercise hemodynamics did not differ significantly between the three stages ($P=.43$).

ANOVA demonstrated that the effect of L-NMMA on exercise hemodynamics was dose dependent, with 16 μmol/min producing the greatest effect. The effect of 16 μmol/min L-NMMA on exercise hemodynamics is shown in Fig 3. At this dose, L-NMMA reduced exer-

Effect of 4 to 16 μmol/min L-NMMA on Forearm Exercise in 18 Healthy Subjects

	Exercise During 5% Dextrose				Exercise During L-NMMA				ANOVA
	Rest	15%	30%	45%	Rest	15%	30%	45%	*P*
Forearm blood flow (ml/min/100 ml)	3.2±1.0	6.4±2.5	11±4.2	17±5.9	2.4±0.8†	5.5±1.7	10±3.5*	16±5.3	.04
Forearm vascular resistance units	28.1±11.5	15.0±6.8	8.7±3.7	5.4±1.9	38.1±11.0†	17.4±6.5*	9.9±3.6*	6.1±2.3*	.02
Mean arterial pressure, mm Hg	80±8	82±10	83±10	83±10	85±8†	87±7*	89±10*	87±10	<.001
Heart rate, beats per minute	64±9	66±8	66±10	68±9	64±7	62±8†	66±7	65±7*	.002
Forearm O_2 extraction, mL/100 mL‡	5.6±1.9	8.7±1.9	9.8±1.9	9.9±1.9	7.4±2.4†	10.1±1.4†	10.9±1.7†	11.2±1.7†	<.001
Forearm O_2 consumption, mL · min^{-1} · 100 mL^{-1}‡	0.18±0.07	0.56±0.23	1.1±0.46	1.73±0.73	0.18±0.08	0.57±0.23	1.11±0.43	1.84±0.66	.48

L-NMMA indicates N^G-monomethyl-L-arginine. ANOVA for repeated measures on exercise stages.
*$P<.05$, †$P<.01$ for paired *t* test of L-NMMA stage vs corresponding 5% dextrose stage.
‡Measured in 17 subjects.

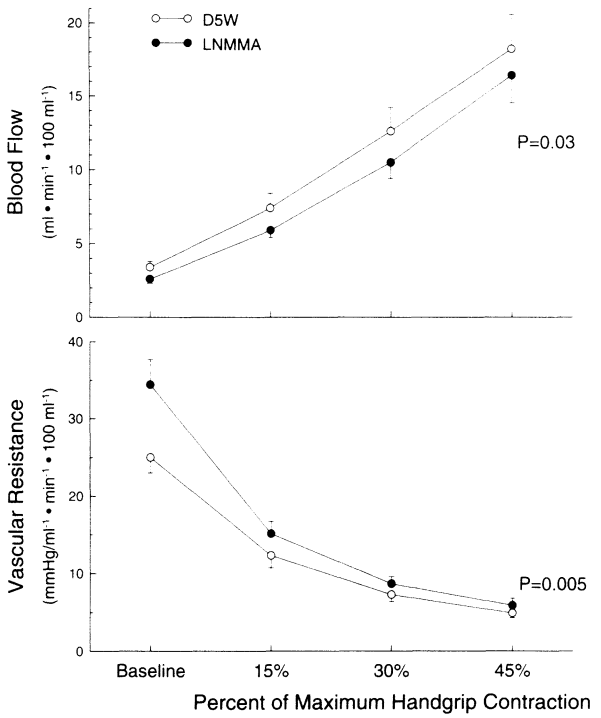

FIG 3. Plots of the effect of 16 μmol/min N^G-monomethyl-L-arginine (L-NMMA) on exercise-induced vasodilation in eight subjects. *P* values denote the result of ANOVA for repeated measures at the three exercise stages. D5W indicates 5% dextrose.

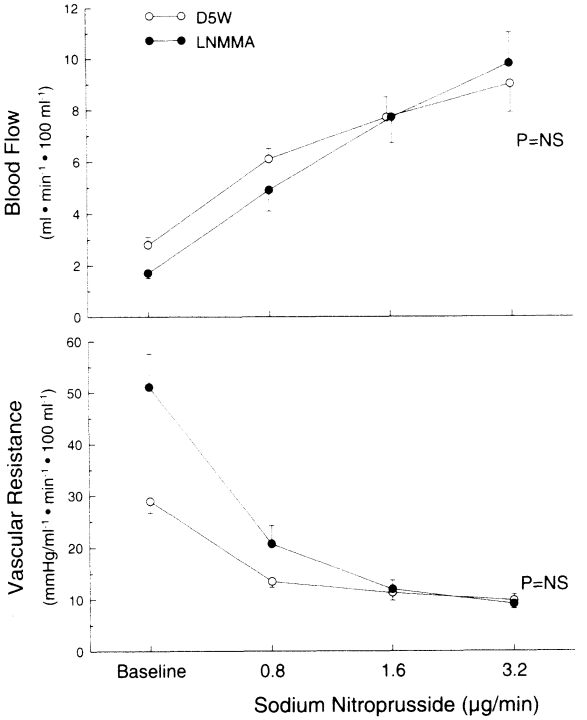

FIG 4. Plots of the effect of 16 μmol/min N^G-monomethyl-L-arginine (L-NMMA) on the vascular response to sodium nitroprusside in eight subjects. *P* values denote the result of ANOVA for repeated measures at the three doses of sodium nitroprusside. D5W indicates 5% dextrose.

cise blood flow by 11±14% (P=.03) and increased exercise vascular resistance by 26±23% (P=.005).

Effect of L-NMMA on Forearm Vascular Response to Sodium Nitroprusside

In the 8 subjects who received L-NMMA at a rate of 16 mmol/min during exercise, a second study assessed the effect of this dose of L-NMMA on the response to sodium nitroprusside. In contrast to the effect on exercise-induced vasodilation in these subjects, L-NMMA did not affect the forearm vasodilation produced by serial doses of sodium nitroprusside (Fig 4). Thus, the resting forearm vasoconstriction associated with L-NMMA was overcome by the endothelium-independent vasodilator sodium nitroprusside (Fig 4) but not by either the endothelium-dependent dilator acetylcholine (Fig 1) or by exercise (Fig 3).

Effect of L-Arginine on Exercise-Induced Vasodilation

Neither L- nor D-arginine significantly affected forearm blood flow or vascular resistance at rest. Exercise-induced changes in blood flow and vascular resistance were similar during infusions of 5% dextrose, D-arginine, and L-arginine (Fig 5). The mean oxygen extraction during exercise was also similar in the three tests; 7.7±0.9 during 5% dextrose, 7.7±1.0 during D-arginine, and 8.0±1.2 mL/100 mL during L-arginine. The difference in mean vascular resistance between the D-arginine and L-arginine tests was 0.05±1.28 mm Hg per $mL \cdot min^{-1} \cdot 100\ mL^{-1}$ of volume (95% confidence interval, −0.87 to 0.97 [11% to 12%]).

Discussion

Nitric Oxide and Vascular Tone

Since the original description of endothelium-derived relaxing factor,[1] there has been a large body of experimental and clinical investigation related to the regulatory action of the endothelium on vascular tone.[26] However, the physiological importance of nitric oxide in humans remains incompletely understood. Recently, studies using L-NMMA to block nitric oxide production have demonstrated that basal nitric oxide synthesis contributes substantially to resting blood flow in the human forearm.[9,14] The present study confirms these findings, showing a 47% increase in forearm vascular resistance after L-NMMA.

Physiological studies have identified a number of mechanisms, including release of metabolites, withdrawal of autonomic nervous control, and mechanical factors, that combine and contribute to the relaxation of smooth muscle in peripheral resistance vessels during exercise.[27-29] The results of the present study demonstrate that endothelium-derived nitric oxide is also a determinant of exercise-induced vasodilation in humans. Thus, the increase in blood flow and reduction in vascular resistance during exercise were blunted after administration of L-NMMA and were associated with an increased extraction of oxygen by the forearm. The effect was greatest with the highest dose of L-NMMA, and the greater the inhibition of the endothelium-dependent vasodilation by L-NMMA, the greater was its effect on exercise (Fig 2). L-NMMA did not affect the forearm vasorelaxation to the selective endothelium-independent vasodilator sodium nitroprusside.

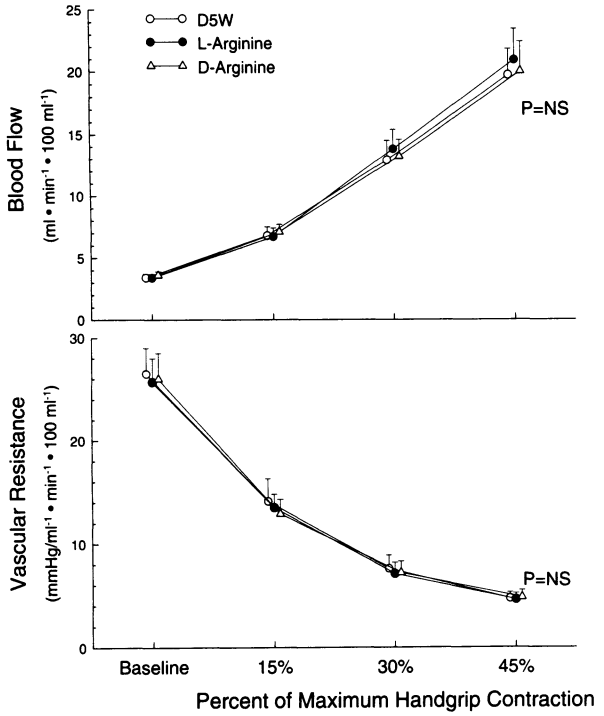

FIG 5. Plots of the effect of L-arginine and D-arginine on exercise-induced vasodilation in 10 subjects. *P* values denote the result of ANOVA for repeated measures at the three exercise stages. D5W indicates 5% dextrose.

Thus, nitric oxide production plays a significant role in human exercise-induced vasodilation. The magnitude of the effect of L-NMMA at the high dose suggests that nitric oxide production is responsible for at least 26% of forearm vascular resistance during exercise.

L-Arginine and Exercise-Induced Vasodilation

We have previously demonstrated that the vascular response to acetylcholine can be enhanced by 35% in healthy individuals after administration of L-arginine, the substrate for nitric oxide production.[13] Having discovered that nitric oxide contributed to exercise-induced vasodilation, we performed additional studies to determine whether administration of L-arginine would enhance exercise blood flow. L-Arginine did not improve exercise-induced vasodilation, suggesting that the activity of the nitric oxide pathway during this form of exercise is not significantly limited by substrate availability. Although a small effect cannot be ruled out, a ≥11% increase in vasodilation with L-arginine can be excluded, based on the 95% confidence intervals in this model. It is apparent from the relative magnitude of resistance changes after L-NMMA that nitric oxide production is stimulated to a much greater extent by acetylcholine than by this type of exercise. Because L-arginine enhances the acetylcholine response but not the exercise response, it appears that substrate limitation only occurs during conditions in which nitric oxide production is markedly stimulated.[11,13]

Pathophysiological Implications

Studies using pharmacological stimulants have demonstrated that endothelium-mediated vasodilation is impaired in several diseases, including hypercholesterolemia,[15,16] hypertension,[13,14,17] atherosclerosis,[18,19] and cardiac failure.[20,21] However, the clinical significance of this abnormality remains unclear. By demonstrating that decreased production of nitric oxide may impair the healthy physiological response to exercise, the results of the present study provide a link between endothelial dysfunction and the response to exercise in these vascular diseases. Thus, endothelial dysfunction in hypertension[17] may contribute to the failure of peripheral vascular resistance to decrease appropriately during exercise and thereby contribute to elevated blood pressure during exercise in these patients.[30] Similarly, impaired endothelial function in cardiac failure[20,21] may contribute to the reduced exercise-induced vasodilation characteristic of this condition.[31,32] Abnormal release of nitric oxide may also be the mechanism for the depressed coronary flow response to atrial pacing previously shown in patients with chest pain, normal coronary arteries, and endothelial dysfunction.[33]

Study Limitations

L-NMMA is a competitive inhibitor of nitric oxide synthesis and therefore does not block nitric oxide production entirely.[7] Moreover, there are several known mediators of exercise-induced vasodilation[27,29] that might exert compensatory effects when nitric oxide production is inhibited. Therefore, the results of the present study may underestimate the true physiological contribution of nitric oxide to blood flow during exercise. In addition, this study used only one form of exercise, an intermittent isometric exercise without systemic hemodynamic changes. It is possible that the contribution of nitric oxide to vasodilation is different in other forms of exercise, especially those in which there are greater increases in blood flow and pressure and thus greater shear stress on endothelial cells (a stimulus for nitric oxide production[34-36]).

The small increase in mean arterial pressure observed during L-NMMA exercise is compatible with a mild systemic effect of L-NMMA associated with a reflex decrease in heart rate.[8] A systemic effect was not anticipated at the low dose of L-NMMA used in this study, and its occurrence suggests that nitric oxide production has an important role in the control of blood pressure in humans as in animals.[8] Such a systemic effect of L-NMMA could have led to reflex withdrawal of α sympathetic tone from the forearm and thus lessened the vasoconstrictive effect of L-NMMA during exercise.

Because L-NMMA affects resting hemodynamics, it could be argued that the observed effect of L-NMMA during exercise is due to the higher resting vascular resistance at the beginning of exercise. However, the effect of L-NMMA at rest was entirely overcome by the endothelium-independent vasodilator sodium nitroprusside. If exercise produced vasodilation by an entirely endothelium-independent stimulus, then as with sodium nitroprusside, exercise would also be expected to overcome the effect of L-NMMA on resting vascular resistance. Therefore, the reduction in exercise-induced vasodilation with L-NMMA demonstrated in the present study indicates that exercise-induced vasodilation is partially and specifically endothelium dependent and is due to nitric oxide production.

Conclusions

The present study identifies nitric oxide as a contributor to exercise-induced vasodilation in humans. The findings may provide a link between endothelial dysfunction and abnormal exercise hemodynamics in vascular diseases such as hypertension and heart failure. Further studies are needed to determine the role of nitric oxide on exercise in disease states.

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