Effects of nitric oxide synthase inhibitor on decrease in peripheral arterial stiffness with acute low-intensity aerobic exercise

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Sugawara, Jun, Seiji Maeda, Takeshi Otsuki, Takumi Tanabe, Ryuichi Ajisaka, and Mitsuo Matsuda. Effects of nitric oxide synthase inhibitor on decrease in peripheral arterial stiffness with acute low-intensity aerobic exercise. Am J Physiol Heart Circ Physiol 287: H2666–H2669, 2004. First published July 29, 2004; doi:10.1152/ajpheart.00077.2004.—We previously reported that even low-intensity, short-duration acute aerobic exercise decreases arterial stiffness. We aimed to test the hypothesis that the exercise-induced decrease in arterial stiffness is caused by the increased production of NO in vascular endothelium with exercise. Nine healthy men (age: ∼22–28 yr) performed a 5-min single-leg cycling exercise (30 W) in the supine position under an intravenous infusion of NO3-monomethyl-L-arginine (L-NMMA; 3 mg/kg during the initial 5 min and subsequent continuous infusion of 50 μg·kg−1·min−1 in saline) or vehicle (saline) in random order on separate days. The pulse wave velocity (PWV) from the femoral to posterior tibial artery was measured on both legs before and after the infusion at rest and 2 min after exercise. Under the control condition, exercised leg PWV significantly decreased after exercise (P < 0.05), whereas nonexercised leg PWV did not show a significant change throughout the experiment. Under L-NMMA administration, exercised leg PWV was increased significantly by the infusion (P < 0.05) but decreased significantly after the exercise (P < 0.05). Nonexercised leg PWV increased with L-NMMA administration and maintained a significantly higher level during the administration compared with baseline (before the infusion, all P < 0.05). The NO synthase blockade × time interaction on exercised leg PWV was not significant (P = 0.706). These results suggest that increased production of NO is not a major factor in the decrease of regional arterial stiffness with low-intensity, short-duration aerobic exercise.

femoral artery; single-leg exercise; pulse wave velocity

LARGE ELASTIC ARTERIES in the central region (e.g., aorta) and middle-sized muscular arteries (e.g., femoral artery) play two roles as a low-resistance conduit and as a cushion (or buffer) of flow pulsations at their input (18). An artery with lower stiffness and a higher buffering capacity can efficiently absorb energy during the systolic component of pulsatile blood flow and reduce energy loss by making the blood flow smoothly. During exercise, arterial buffering capacity may be increased by a decrease of arterial stiffness, because blood flow should be markedly increased to meet oxygen demand in active muscle. It has been reported that moderate (11) and maximum (16) aerobic exercise induce an acute decrease in conduit arterial stiffness. Kingwell et al. (11) showed that a 30-min bout of moderate cycling with both legs induced decreases in central (aorta) and peripheral (femoral to dorsal pedis arteries) arterial stiffness [which was assessed by pulse wave velocity (PWV)] at 30 min after the exercise. Naka et al. (16) examined the time course of acute changes in upper- and lower-limb PWV immediately and for 60 min after maximum treadmill exercise and demonstrated that lower-limb PWV declined to a nadir ∼23% below baseline 10 min after the exercise and then gradually increased to a near steady level of ∼10% below baseline by 60 min of recovery. Arterial stiffness is determined by both the properties of the arterial wall matrix and the vascular smooth muscle tone. An acute change in arterial stiffness is probably mediated by an alteration of vascular muscle tone with exercise. Systemic (e.g., sympathetic nervous activity, circulating hormones) and regional (e.g., endothelium-derived vasoactive substances, exercised muscle-derived metabolites) factors can alter the smooth muscle tone (11, 16), but conclusive evidence about whether these are major factors affecting arterial stiffness in this case has not been reported. We previously demonstrated (29) that low-intensity, short-duration single-leg exercise (∼20–30 W, 5 min) in healthy subjects induced a significant decrease in the PWV of the exercised leg but not that of the nonexercised leg. These results suggest that the decrease of peripheral arterial stiffness with exercise may be induced mainly by exercise-related regional factors.

It is well known that an increase in blood flow stimulates vascular endothelial cells and advances the production of various vasodilatory substances, e.g., NO (2, 9, 12, 23, 24), prostacyclin (1, 8), and endothelium-derived hyperpolarizing factor (EDHF) (15). In particular, NO is a potent endothelium-dependent vasodilator that, moreover, reduces the vasoconstrictor response to α-adrenergic receptor stimulation (21). Recent studies demonstrated that NO modulates conduit arterial stiffness (or distensibility) in animals (5, 30) and humans (10). NO is increased with increased cyclic wall stress associated with increased pulsatile blood flow, e.g., during acute exercise (9).

We hypothesized that the exercise-induced decrease in peripheral arterial stiffness is caused by the increased production of NO in vascular endothelium with exercise, and we tested this hypothesis by examining the effects of systemic NO synthase (NOS) inhibition on changes of PWV in both leg arteries with low-intensity, single-leg aerobic exercise.
METHODS

Subjects. Nine young men, 25 ± 1 (22–28) yr of age, 171.4 ± 1.6 (165–178) cm in height, 68.0 ± 2.3 (62.1–81.3) kg in body weight, and with body mass index (BMI) of 23.1 ± 0.5 (21.1–26.0), participated in this study. Written informed consent was obtained from all subjects, and the study was approved by the institutional review board of the University of Tsukuba. Because risk factors such as hypercholesterolemia and insulin resistance have been shown to correlate with abnormal blood pressure responses to exercise (4) and they also adversely affect endothelial function, we selected apparently healthy men [i.e., normotensive (<140/90 mmHg), nonobese (BMI < 30), and free of overt chronic diseases as assessed by medical history]. None of the subjects was taking medications or smoking. The subjects were either sedentary or recreationally active. Peak oxygen uptake determined with an incremental maximal exercise test was 41.6 ± 1.0 (38.0 – 47.4) ml·kg⁻¹·min⁻¹.

Experimental protocol and measurements. Each subject underwent two experiments with a Portapres 2.0 (TNO-Biomedical Instrumentation, Amsterdam, The Netherlands). Before and >5 min after the start of the constant infusion, when the heart rate and blood pressure were in the steady state, PWV in both legs was measured with an automatic PWV measurement system (form-PWV/ABI, Colin, Komaki, Japan). After these measurements, each subject performed 5 min of single-leg (left) cycling at 30-W workload on a cycle ergometer (232C-EX, Combi, Tokyo, Japan). The measurements of PWV were repeated at 2 min after the cessation of the exercise. The automatic PWV measurement system consists of an applanation tonometry probe, cuffs connected to a plethysmographic sensor, and an automatic waveform analyzer. The applanation tonometry probe was placed at the right inguinal region to record pressure waveforms of the right common femoral artery. Cuffs were wrapped over both ankles to record pressure waveforms of the posterior tibial arteries. These pressure waveforms were simultaneously recorded at 1,200 Hz (common femoral artery) or 240 Hz (posterior tibial arteries). The delay times between the sharp systolic upstroke starts of the right femoral and both posterior tibial arterial pulse waves were determined by the automatic waveform analyzer (Fig. 1). We assumed that the sharp systolic upstroke starts of the right and left femoral arteries occurred at the same time and obtained the delay times of both legs for the same cardiac cycles. The sharp systolic upstroke start was determined based on the phase velocity theory. As mean phase velocity >2.5 Hz is constant and coincides with the wave-front velocity (14), the high-frequency components of the arterial wave could be used as a marker of phase shift. The high-frequency components of the arterial wave are derived mainly from the sharp systolic upstroke start and are ~30 Hz. Accordingly, to extract the high-frequency components a band-pass filter with a lower cut-off frequency of 5 Hz and a higher cut-off frequency at ~30 Hz was used in this system (17). Additionally, the R wave from the simultaneously recorded electrocardiogram was used as a reference to identify the sharp systolic upstroke starts.

The distance between the point of placement of the applanation tonometry sensor on the femoral artery and the top of the medial malleolus was measured manually in duplicate with a tape measure, and the mean value was calculated. The PWV was determined from the distance between the two recording sites of the arterial pressure pulse wave and the delay time of wave travel. The day-to-day coefficient of variation for leg PWV in our laboratory was 2.3 ± 0.6%.

Heart rate and blood pressure were continuously monitored at the finger during the experiments with a Portapres 2.0 (TNO-Biomedical Instrumentation, Amsterdam, The Netherlands). Before and >5 min after the start of the constant infusion, when the heart rate and blood pressure were in the steady state, PWV in both legs was measured with an automatic PWV measurement system (form-PWV/ABI, Colin, Komaki, Japan). After these measurements, each subject performed 5 min of single-leg (left) cycling at 30-W workload on a cycle ergometer (232C-EX, Combi, Tokyo, Japan). The measurements of PWV were repeated at 2 min after the cessation of the exercise. The automatic PWV measurement system consists of an applanation tonometry probe, cuffs connected to a plethysmographic sensor, and an automatic waveform analyzer. The applanation tonometry probe was placed at the right inguinal region to record pressure waveforms of the right common femoral artery. Cuffs were wrapped over both ankles to record pressure waveforms of the posterior tibial arteries. These pressure waveforms were simultaneously recorded at 1,200 Hz (common femoral artery) or 240 Hz (posterior tibial arteries). The delay times between the sharp systolic upstroke starts of the right femoral and both posterior tibial arterial pulse waves were determined by the automatic waveform analyzer (Fig. 1). We assumed that the sharp systolic upstroke starts of the right and left femoral arteries occurred at the same time and obtained the delay times of both legs for the same cardiac cycles. The sharp systolic upstroke start was determined based on the phase velocity theory. As mean phase velocity >2.5 Hz is constant and coincides with the wave-front velocity (14), the high-frequency components of the arterial wave could be used as a marker of phase shift. The high-frequency components of the arterial wave are derived mainly from the sharp systolic upstroke start and are ~30 Hz. Accordingly, to extract the high-frequency components a band-pass filter with a lower cut-off frequency of 5 Hz and a higher cut-off frequency at ~30 Hz was used in this system (17). Additionally, the R wave from the simultaneously recorded electrocardiogram was used as a reference to identify the sharp systolic upstroke starts.

Diastolic blood pressure significantly increased with L-NMMA then showed significant increase with exercise (P < 0.05). Under the NOS inhibition condition, systolic blood pressure progressively and significantly increased with L-NMMA administration (P < 0.05) and with exercise (P < 0.05). Under the control condition, systolic blood pressure was not affected by saline administration and then significantly increased after exercise (P < 0.05). Under the NOS inhibition condition, systolic blood pressure progressively and significantly increased with L-NMMA administration (P < 0.05) and with exercise (P < 0.05). Under the control condition, systolic blood pressure was not affected by saline administration and then showed significant increase with exercise (P < 0.05).

Results were analyzed by repeated-measures ANOVA (leg × NOS inhibition status × time course). With regard to significant F-values, Fisher’s least significant difference post hoc test was used to identify significant differences among mean values. Statistical significance was set at P < 0.05 for all comparisons.

RESULTS

Table 1 shows the responses of heart rate and blood pressure during the experiments. Under the NOS inhibition condition, heart rate significantly decreased with L-NMMA administration (P < 0.05) and returned to baseline (before the infusion) after exercise. Under the control condition, heart rate was not affected by saline administration and then significantly increased after exercise (P < 0.05). Under the NOS inhibition condition, systolic blood pressure progressively and significantly increased with L-NMMA administration (P < 0.05) and with exercise (P < 0.05). Under the control condition, systolic blood pressure was not affected by saline administration and then showed significant increase with exercise (P < 0.05). Diastolic blood pressure significantly increased with L-NMMA and saline administrations (both P < 0.05). After the exercise, diastolic blood pressure returned and had no significant differences from baseline (before the infusion) under both conditions. Mean arterial pressure significantly increased with L-NMMA administration (P < 0.05) and maintained a significantly higher level after exercise (P < 0.05). Under the control condition, mean arterial pressure did not show a significant change throughout the experiment.

Table 2 shows the responses of PWV in each leg during the experiments. There were no significant differences among the baseline PWV values in both legs before the infusions of L-NMMA. Under the control condition (i.e., vehicle infusion), exercised leg PWV decreased 8.3% after exercise (P < 0.05) from the preexercise level, whereas nonexercised leg PWV did not show a significant change throughout the experiment (+0.1% from preexercise level). Under the NOS inhibition condition (i.e., L-NMMA infusion), exercised leg PWV increased 7.9% with L-NMMA administration (P < 0.05) but decreased 7.5% after exercise (P < 0.05) from the preexercise level. Nonexercised leg PWV increased 7.4% with L-NMMA administration (P < 0.05) but did not change significantly after
exercise from the preexercise level (+0.4% from preexercise level). The NOS blockade × time interaction on exercised leg PWV was not significant ($P = 0.706$).

**DISCUSSION**

The primary findings of the present study were as follows. Irrespective of whether systemic NOS inhibition by intravenous administration of L-NMMA was carried out, low-intensity, short-duration single-leg exercise induced a decrease in femoral arterial stiffness in the exercised leg but not in the nonexercised leg. Thus systemic NOS inhibition appeared to have no effect on the decrease in middle-sized muscular arterial stiffness with exercise, at least under the present protocol conditions, although the decrease in arterial stiffness in the exercised leg was induced mainly by exercise-related regional factors.

We induced systemic NOS inhibition by intravenous L-NMMA infusion. The dose and method of L-NMMA infusion in this study were similar to those in the previous studies by Stamler et al. (27) and Mayer et al. (13). Mayer et al. (13) reported that bolus infusion of 3 mg/kg L-NMMA resulted in a maximal plasma concentration of ~13 μg/ml with an ~1-h elimination half-time and caused a small hypertensive response, decreased cardiac output, and increased systemic vasculature resistance. Mayer et al. (13) also reported that continuous infusion of 50 μg·kg$^{-1}·$min$^{-1}$ L-NMMA after the bolus infusion reduced exhaled NO by 69% without significant alterations of blood pressure and heart rate. Stamler et al. (27) reported a 65% reduction of serum NO level by an intravenous bolus infusion of 3 mg/kg L-NMMA, with a significant decrease of heart rate and significant increases of systolic, diastolic, and mean blood pressures. Although the production of NO was not evaluated in the present study, the changes of heart rate and blood pressures caused by infusion of L-NMMA were similar to those in the study of Stamler et al. (27). Additionally, the significant elevation of mean blood pressure lasted beyond the exercise. Several previous studies indicated that the basal production of endothelium-derived NO affects basal arterial stiffness via the regulation of smooth muscle tone of the arterial wall and/or via increase in systemic arterial pressure. The aortic PWV (aortic arch-abdominal artery) in rats was increased independent of concomitant increase in blood pressure when an NOS inhibitor, $N^{	ext{mon}}$-nitro-L-arginine methyl ester, was infused into the jugular vein (5). In a human study, however, the aortic PWV (carotid artery-femoral artery) was suggested to be increased mainly via the increase in mean arterial pressure when basal NO release was systemically inhibited by L-NMMA (28). PWV measured in the common iliac artery of sheep was increased by intra-arterial (iliaic artery) L-NMMA infusion (30). In humans, compliance of the brachial artery was decreased and PWV was increased by intra-arterial (brachial artery) infusion of L-NMMA (10). Together, changes in peripheral arterial stiffness could be produced by a NOS inhibitor because of the depression of NO production per se. In the present study, L-NMMA administration induced significant increase in PWV of both exercised and nonexercised legs. PWV after exercise was also higher than that before L-NMMA administration in the nonexercised leg. Thus it seems likely that the systemic L-NMMA administration in the present study could reduce at least the basal production of NO in systemic vascular endothelial cells.

Kingwell et al. (11) showed that the PWV of the femoral arteries in young men significantly decreased 30 min after moderate-intensity exercise (30 min, 65% of maximal oxygen uptake). They pointed out that shear stress-induced release of NO was one of the mechanisms associated with the decreased arterial stiffness. In the present study, we hypothesized that the exercise-induced decrease in peripheral arterial stiffness is caused by the increased production of NO in vascular endothelium with exercise and, accordingly, that L-NMMA administration would interfere with the exercise-induced decrease of PWV. It was suggested that the production of NO might be increased in nonexercised limbs during exercise at moderate to high intensity (e.g., ~60–160 W) but not at lower intensity (e.g., 40 W) (7). Under the condition without L-NMMA administration, PWV was decreased with low-intensity, short-duration exercise in the exercised leg but not in the nonexercised leg. These results were identical with those of our previous study (29). The decrease of arterial stiffness in the exercised leg might have been induced mainly by exercise-related regional factors. The administration of L-NMMA, however, had no effect on the exercise-induced change of PWV. It cannot be ruled out that the L-NMMA administration in the present study could not perfectly inhibit the increased production of NO from the exercising muscle bed. It might have been interesting to infuse L-arginine in the current series of experiments to ascertain whether NO had any effect on exercise-induced changes in arterial stiffness (3). Alternatively, the decrease of arterial stiffness in the exercised leg might have been induced by some regional factors other than NO, because multiple redundant mechanisms may substitute to regulate vascular tone under the condition when NOS is inhibited.

**Table 1. Changes in heart rate and blood pressure**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before Infusion</th>
<th>Before Exercise</th>
<th>2 min After Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>56±1</td>
<td>58±2</td>
<td>61±2†</td>
</tr>
<tr>
<td>L-NMMA</td>
<td>54±1</td>
<td>50±2*</td>
<td>54±2†</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>118±3</td>
<td>121±2</td>
<td>123±2*</td>
</tr>
<tr>
<td>L-NMMA</td>
<td>116±2</td>
<td>123±3*</td>
<td>127±4*†</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>71±2</td>
<td>75±2*</td>
<td>74±1</td>
</tr>
<tr>
<td>L-NMMA</td>
<td>71±2</td>
<td>77±3*</td>
<td>77±2</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>88±2</td>
<td>91±3</td>
<td>92±2</td>
</tr>
<tr>
<td>L-NMMA</td>
<td>88±2</td>
<td>95±3*</td>
<td>94±2*</td>
</tr>
</tbody>
</table>

Data are means ± SE. L-NMMA, $N^{	ext{mon}}$-monomethyl-L-arginine. *$P < 0.05$ vs. before infusion; †$P < 0.05$ vs. before exercise.

**Table 2. Changes in pulse wave velocity**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before Infusion</th>
<th>Before Exercise</th>
<th>2 min After Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonexercised leg PWV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>895±17</td>
<td>937±23</td>
<td>936±19</td>
</tr>
<tr>
<td>L-NMMA</td>
<td>889±33</td>
<td>948±23*</td>
<td>953±22*</td>
</tr>
<tr>
<td>Exercised leg-PWV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>915±22</td>
<td>958±23</td>
<td>876±25†</td>
</tr>
<tr>
<td>L-NMMA</td>
<td>908±35</td>
<td>972±24*</td>
<td>897±18†</td>
</tr>
</tbody>
</table>

Data (in cm/s) are means ± SE. PWV, pulse wave velocity. *$P < 0.05$ vs. before infusion; †$P < 0.05$ vs. before exercise.
NOS inhibition does not affect hemodynamics during exercise (3, 22) but reduces postexercise hyperemic flow (6, 22, 26). The reduced hyperemic flow is presumably because of the impaired vasodilation and consequent inhibition of the decrease in vascular resistance due to the reduction in NO production. The inhibition of the decrease in vascular resistance would have resulted in increased peripheral conduit arterial stiffness via increased arterial pressure. Although we did not estimate blood flow and, consequently, vascular resistance, systemic arterial pressure was increased with administration of L-NMMA. Nevertheless, under NOS inhibition, PWV in the exercised leg decreased regardless of the increased arterial pressure. In peripheral muscular arteries, stiffness is influenced by arterial pressure and/or by the tone of arterial smooth muscle. Thus it is considered that the decrease in the exercised leg PWV is explained by the effect of decreased vascular smooth muscle tone, which might surmount the effect of increased arterial pressure. Additionally, it has been indicated that an arterial pressure is not an independent determinant of PWV in young healthy men and that PWV does not correlate with systemic vascular resistance (19).

Prostacyclin is a potential factor that may induce the postexercise decrease in arterial stiffness. The production of prostacyclin is enhanced by an increase of shear stress in the regional vessels, and prostacyclin attenuates neurogenic and myogenic vasoconstriction (8). Furthermore, a recent study reported that flow-induced prostacyclin production might be enhanced by the inhibition of NOS (20). EDHF causes the relaxation of vascular smooth muscle cells (15). Interstitial metabolites (e.g., lactate, adenosine, phosphate, H⁺) that are released by exercising muscle also attenuate the arterial smooth muscle tone in the proximal arteries by an upstream transmission of vasodilatory stimuli (25). Our data, however, cannot specify which factor induced the postexercise decrease of arterial stiffness in the exercised leg.

In summary, PWV of the exercised leg was decreased by low-intensity single-leg cycling exercise, but that of the nonexercised leg was not, under both the systemic inhibition of NOS by intravenous administration of L-NMMA and the control condition. Thus, at least under the conditions of the present protocol, systemic NOS inhibition appears to have no effect on the decrease in middle-sized muscular arterial stiffness with exercise.

GRANTS
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REFERENCES