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

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Abstract

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 nitric oxide (NO) in vascular endothelium with exercise. Nine healthy men (age: 22~28 yr) performed a 5-min single-leg cycling exercise (30 watt) in the supine position under an intra-venous infusion of $N^3$-monomethyl-L-arginine (L-NMMA: 3 mg·kg$^{-1}$ during the initial 5 min and subsequent continuous infusion of 50µg·kg$^{-1}$·min$^{-1}$ in saline) or vehicle (saline) at 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 the exercise. Under the control condition, the exercised leg PWV significantly decreased after the exercise (P<0.05), whereas the non-exercised leg PWV did not show a significant change throughout the experiment. Under the L-NMMA administration condition, the exercised leg PWV significantly increased by the infusion (P<0.05) but significantly decreased after the exercise (P<0.05). The non-exercised leg PWV increased with the L-NMMA administration and maintained significantly higher level over the administration compared with the baseline (before the
infusion, all $P<0.05$). The NOS blockade × time interaction on the exercised leg PWV was not significant ($P=0.706$). These results suggest that the increased production of NO is not a major factor in the decrease of the regional arterial stiffness with low-intensity, short-duration aerobic exercise.

**Key words:** femoral artery, single leg exercise, pulse wave velocity
**Introduction**

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 with a higher buffering capacity can efficiently absorb the energy during the systolic component of pulsatile blood flow and reduce the energy loss by making the blood flow smooth. During exercise, the 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 (15) aerobic exercise induced an acute decrease in conduit arterial stiffness. Kingwell et al. (11) showed that a 30-min bout of moderate cycling by both legs induced decreases in central (aorta) and peripheral (femoral to dorsalis pedis arteries) arterial stiffness (which was assessed by pulse wave velocity [PWV]) at 30 min after the exercise. Naka et al. (15) 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 the 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., endothelial-derived vasoactive substances, exercised muscle-derived metabolites) factors can alter the smooth muscle tone (11, 15), but conclusive evidence about whether these are major factors affecting arterial stiffness in this case has not been reported. We previously demonstrated that low-intensity and short-duration single-leg exercise (20~30 watts, 5 min) in healthy subjects induced a significant decrease in the PWV of the exercised leg, but not in that of the non-exercised leg (29). 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 the vascular endothelial cells and advances the production of various vasodilatory substances, e.g. nitric oxide (NO) (2, 9, 13, 23, 24), prostacyclin (1, 8), endothelial-derived hyperpolarizing factor (EDHF) (16). Especially, NO is a
potent endothelial-dependent vasodilator that moreover reduces the vasoconstrictor response to $\alpha$-adrenergic receptor stimulation (21). Recent studies have demonstrated that NO modulates the 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 tested this hypothesis by examining the effects of systemic NO synthase (NOS) inhibition on the changes of the 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 23.1 ± 0.5 (21.1~26.0) in body mass index, 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 hypercholesterolaemia and insulin resistance have been shown to correlate with abnormal blood pressure responses to exercise (3) 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 using a counter-balanced design in a blind manner on separate days, i.e., systemic NOS inhibition and control conditions. All the experiments were done after an overnight fast. The subjects abstained from alcohol, caffeine, and the intense exercise for at least 24 h before the experiments. All the experiments were carried out in a temperature-controlled room (25°C).

All the subjects rested for at least 30 min in the supine position to
establish a stable baseline. Each subject received an initial bolus intra-venous (left brachial vein) infusion of $N^G$-monomethyl-L-arginine (L-NMMA: 3 mg·kg$^{-1}$) or vehicle (saline) over 5 min, and a subsequent continuous infusion of L-NMMA (50µg·kg$^{-1}$·min$^{-1}$) or vehicle during the experiments. The procedure and dose of L-NMMA infusion accorded with the method of Mayer et al. (14).

Heart rate and blood pressure were continuously monitored during the experiments at the finger with a Portapres 2.0 (TNO-Biomedical Instrumentation, Amsterdam, The Netherlands). Before and more than 5 min after the start of the constant infusion, when the heart rate and blood pressure were in the steady state, the PWV in both legs was measured by using an automatic PWV measurement system (form-PWV/ABI, Colin Co., Komaki, Japan). After these measurements, each subject performed a 5-min single-leg (left) cycling at 30 watt workload using a cycle ergometer (232C-EX, Combi Co., Tokyo, Japan). The measurements of the 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 right inguinal region to record pressure wave forms of the right
common femoral artery. Cuffs were wrapped over the both ankles to record pressure wave forms of posterior tibial arteries. These pressure waveforms were simultaneously recorded at 1200 Hz (common femoral artery) or 240 Hz (posterior tibial arteries). The delay times between the sharp systolic upstroke starts of the right femoral and the 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. Based on the phase velocity theory, the sharp systolic upstroke starts was determined. As mean phase velocity >2.5 Hz is constant and coincides with the wave front velocity (12), 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 about 30 Hz. Accordingly, to extract the high frequency components a band-pass filter with a lower cut-off frequency of 5 Hz and higher cut-off frequency at around 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 with a tape measure in duplicate, and the mean value was calculated. The PWV was determined from the distance between the two recording sites of 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 during corresponding periods of PWV measurements (1 min) were calculated from the beat-to-beat Portapres data.

**Statistical analysis**

All values are expressed as means ± SE. Results were analyzed by repeated measures ANOVA (leg × NOS inhibition status × time course). In regard to significant F values, the Fisher’s LSD 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 the L-NMMA administration (P<0.05) and returned to the baseline (before the infusion) after the exercise. Under the control condition, heart rate was not affected by the saline administration and then significantly increased after the exercise (P<0.05). Under the NOS inhibition condition, systolic blood pressure progressively and significantly increased with the L-NMMA administration (P<0.05) and with the exercise (P<0.05). Under the control condition, systolic blood pressure was not affected by the saline administration and then showed significantly increased with the exercise (P<0.05). Diastolic blood pressure significantly increased with the L-NMMA and saline administrations (both P<0.05). After the exercise, diastolic blood pressure returned and had no significant differences with the baseline (before the infusion) under the both conditions. Mean arterial pressure significantly increased with the L-NMMA administration (P<0.05) and maintained significant higher level after the 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 the 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), the exercised leg PWV decreased 8.3% after the exercise (P<0.05) from the pre-exercise level, whereas the non-exercised leg PWV did not show a significant change throughout the experiment (+0.1% from the pre-exercise level). Under the NOS inhibition condition (i.e. L-NMMA infusion), the exercised leg PWV increased 7.9% with the L-NMMA administration (P<0.05) but decreased 7.5% after the exercise (P<0.05) from the pre-exercise level. The non-exercised leg PWV increased 7.4% with the L-NMMA administration (P<0.05) but did not change significantly after the exercise from the pre-exercise level (+0.4% from the pre-exercise level). The NOS blockade × time interaction on the exercised leg PWV was not significant (P=0.706).

Discussion

The primary findings of the present study were as follows. Irrespective of whether or not systemic NOS inhibition by the intra-venous 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 non-exercised leg. Thus, systemic NOS inhibition appeared to have no effect on the decrease in the 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 the systemic NOS inhibition by intra-venous 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. (14). Mayer et al. (14) reported that the bolus infusion of 3 mg·kg\(^{-1}\) L-NMMA resulted in the maximal plasma concentration of about 13 \(\mu g\cdot ml^{-1}\) with an approximately 1-h elimination half-time, and caused a small hypertensive response, decreased cardiac output, and increased systemic vascular resistance. Mayer et al. (14) also described that the continuous infusion of 50 \(\mu g\cdot kg^{-1}\cdot min^{-1}\) 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 intra-venous bolus infusion of 3 mg·kg\(^{-1}\) 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 the heart rate and the blood pressures by the 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 out after the exercise. Several previous studies indicated that the basal production of endothelium-derived NO affects the basal arterial stiffness via the regulation of smooth muscle tone of arterial wall and/or via the 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, Nω-nitro-L-arginine methyl ester (L-NAME), was infused into the jugular vein (5). In human study, however, the aortic PWV (carotid artery-femoral artery) was suggested to be increased mainly via the increase in mean arterial pressure when the basal NO release was systemically inhibited by the L-NMMA (28). The PWV measured in the common iliac artery of sheep was increased by the intra-arterial (iliac artery) L-NMMA infusion (30). In humans, the compliance of the brachial artery was decreased and the PWV was increased by intra-arterial (brachial artery) infusion of L-NMMA (10). Taken together, changes in peripheral arterial stiffness could be produced by NOS inhibitor due to the
depression of NO production per se. In the present study, the L-NMMA administration induced the significant increase in the PWV of both the exercised and non-exercised legs. The PWV after the exercise was also higher than that before the L-NMMA administration, in the non-exercised 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 the 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 the 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, L-NMMA administration would interfere with the exercise-induced decrease of PWV. It was suggested that the production of NO might be increased in non-exercised limbs during exercise at the moderate-high intensity (e.g., 60~160 W) but not at the lower intensity (e.g., 40 W) (7). Under the
condition without L-NMMA administration, the PWV was decreased with low-intensity, short-duration exercise in the exercised leg, but not in the non-exercised 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 may 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 (4). 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.

The NOS inhibition does not affect hemodynamics during exercise (4, 22) but reduces post-exercise hyperemic flow (6, 22, 26). The reduced hyperemic flow is presumable through the impaired vasodilation and the 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, the systemic arterial pressure was increased with administration of L-NMMA. Nevertheless, under the NOS inhibition, the 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. Then, it is considered that the decrease in the exercised-leg PWV is explained by the effect of the decreased vascular smooth muscular tone, which might surmount the effect of the increased arterial pressure. Additionally, it has been indicated that an arterial pressure is not an independent determinant of PWV in young healthy males and that PWV does not correlate with systemic vascular resistance (17).

Prostacyclin is a potential factor that may induce the post-exercise 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 (16). Interstitial metabolites (e.g., lactate, adenosine, phosphate, H⁺) which are released by exercising muscle also attenuate the arterial smooth muscular tone in the proximal arteries by an upstream transmission of vasodilatory stimuli (25). Our data, however, cannot specify which factor induced the post-exercise decrease of the arterial stiffness in the exercised leg.

In summary, the PWV of the exercised leg was decreased by low-intensity single-leg cycling exercise, but that of the non-exercised leg was not under both the systemic inhibition of NOS by the intra-venous administration of L-NMMA and under 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 the middle-sized muscular arterial stiffness with exercise.
Acknowledgement

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References


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Figure legend

Figure 1. Simultaneous recordings of arterial pressure waves at the right common femoral artery and right and left posterior tibial arteries. Arrows show the sharp systolic upstroke starts of arterial pressure waves.
Table 1. Changes in heart rate and blood pressure.

<table>
<thead>
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<th>Variable</th>
<th>Before infusion</th>
<th>Before exercise</th>
<th>2 min after exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beat·min⁻¹)</td>
<td></td>
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<td></td>
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<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>
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<td>Systolic blood pressure (mmHg)</td>
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<td></td>
<td></td>
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<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>
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<td></td>
<td></td>
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<tr>
<td>Saline</td>
<td>71 ± 2</td>
<td>75 ± 2 *</td>
<td>74 ± 1</td>
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<tr>
<td>L-NMMA</td>
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<td>77 ± 3 *</td>
<td>77 ± 2</td>
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<tr>
<td>Mean arterial pressure (mmHg)</td>
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<td></td>
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<tr>
<td>Saline</td>
<td>88 ± 2</td>
<td>91 ± 3</td>
<td>92 ± 2</td>
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<tr>
<td>L-NMMA</td>
<td>88 ± 2</td>
<td>95 ± 3 *</td>
<td>94 ± 2 *</td>
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Data are means ± SE. L-NMMA = NG-monomethyl-L-arginine. *: P<0.05 vs. before infusion; †: P<0.05 vs. before exercise.
**Table 2.** Changes in pulse wave velocity (PWV)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Non-exercised leg PWV</th>
<th>Exercised leg PWV</th>
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<tr>
<td></td>
<td>Before infusion</td>
<td>Before exercise</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>L-NMMA</td>
</tr>
<tr>
<td>Non-exercised leg PWV</td>
<td>895 ± 17</td>
<td>889 ± 33</td>
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<td>(cm·sec⁻¹)</td>
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<td>948 ± 23 *</td>
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<tr>
<td>Exercised leg PWV</td>
<td>915 ± 22</td>
<td>908 ± 35</td>
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<tr>
<td>(cm·sec⁻¹)</td>
<td></td>
<td>972 ± 24 *</td>
</tr>
<tr>
<td>L-NMMA</td>
<td></td>
<td></td>
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<td></td>
<td>Data are means ± SE.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L-NMMA = N(^G)-monomethyl-L-arginine. *: P&lt;0.05 vs. before infusion; †: P&lt;0.05 vs. before exercise.</td>
<td></td>
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</tbody>
</table>
Right femoral arterial wave
Right posterior tibial arterial wave
Left posterior tibial arterial wave