Nitric Oxide Does Not Significantly Contribute to Changes in Pulse Pressure Amplification During Light Aerobic Exercise

James E. Sharman, Carmel M. McEniery, Ross Campbell, Pawan Pusalkar, Ian B. Wilkinson, Jeff S. Coombes, John R. Cockcroft

Abstract—NO modulates resting blood pressure and wave reflection. The effect of NO on exercise central hemodynamics is unknown but has important implications relating to cardiovascular risk. The aim of this study was to determine the contribution of NO to pulse pressure (PP) amplification and wave reflection during exercise. Twelve healthy men aged 29±1 years (mean±SEM) undertook cycle exercise at 60% of their maximal heart rate. Noninvasive measures of central blood pressure, estimated aortic pulse wave velocity, and wave reflection (augmentation index) were obtained by pulse wave analysis during intravenous infusion of saline (control), Nω-monomethyl-L-arginine (a NO-synthase inhibitor), or noradrenaline (control vasoconstrictor). PP amplification was defined as the ratio of peripheral to central PP. Cardiac output and stroke volume were determined by electric bioimpedance. Both Nω-monomethyl-L-arginine and noradrenaline caused a significant increase in mean arterial pressure (P<0.01) and augmentation index (P<0.01), as well as reduced ratio of peripheral to central PP (P<0.05) at baseline. Exercise caused a significant increase in the ratio of peripheral to central PP (P<0.001), whereas augmentation index and estimated aortic pulse wave velocity declined (for both P<0.05) during all 3 of the infusion protocols. However, no significant differences were observed in augmentation index, ratio of peripheral to central PP, or estimated aortic pulse wave velocity between infusion procedures (P>0.50) during exercise. Also, heart rate, peripheral vascular resistance, and cardiac output did not differ during exercise between saline, Nω-monomethyl-L-arginine, or noradrenaline. Although we cannot rule out other vasodilator mechanisms having adjusted for NO blockade, our results indicate that NO does not solely contribute to systemic arterial stiffness or altered blood pressure amplification during light exercise. (Hypertension. 2008;51:856-861.)

Key Words: NO ■ exercise ■ radial artery ■ aorta ■ hemodynamics

Bradial blood pressure (BP) predicts cardiovascular risk at rest1 and during exercise.2-3 Because of pressure wave travel and reflection through vessels of varied morphology, pulse pressure (PP) is amplified from the large central arteries to the peripheral vessels (ie, brachial artery), where BP is traditionally measured. Thus central BP is not representative of peripheral BP. This has clinical implications, because pathological remodelling of the myocardium4,5 and large central arteries6 is more dependent on central PP and markers of wave reflection (eg, augmentation index [AIx]) rather than on brachial pressure. Indeed, central PP and AIx predict cardiovascular morbidity and mortality independent of brachial BP.7-10

Pulse pressure amplification is decreased with age and hypercholesterolemia11,12 but increased during exercise.13 Interestingly, the normal exercise-induced increase in PP amplification is significantly blunted in men with high blood cholesterol.14 Hypercholesterolemia is associated with increased systemic arterial stiffness12 and forearm endothelial dysfunction,15 probably because of reduced bioavailability of NO,16 a molecule known to modulate large artery distensibility.17 Inhibition of NO synthase, by infusion of Nω-monomethyl-L-arginine (l-NMMA), increases vascular resistance in the human forearm,18 as well as mean arterial pressure19 and central, but not peripheral, PP.20 Thus, basal release of NO has a major influence on regulating the tone of both small peripheral and large central arteries, thereby affecting BP amplification at rest. To date, no study has assessed the role of NO on central hemodynamics during light aerobic exercise.

Increased vasodilation and large artery distensibility occur after dynamic aerobic exercise,21,22 probably attributed in part to augmented NO release.23,24 Although the contribution of NO to large artery stiffness, wave reflection (AIx), and pressure amplification during exercise has never been tested, it is an important consideration relevant to cardiac structure.
and cardiovascular risk. If reduced NO bioavailability underlies abnormal exercise hemodynamics in a fashion similar to that found in men with hypercholesterolemia, we may expect that induction of endothelial dysfunction in healthy men (by blockade of NO synthase) should lead to increased AIx and reduced PP amplification. We tested this hypothesis in young men who performed submaximal cycle exercise after intravenous infusion of L-NMMA compared with an NO-independent control vasoconstrictor (noradrenaline [NE]) and placebo (saline).

**Methods**

**Subjects**

Twelve healthy young men aged 29 ± 1 years (mean ± SEM) were recruited from the University of Wales College of Medicine and Wales Heart Research Institute (Cardiff, Wales). The Bro Taf Local Research Ethics Committee approved all of the procedures. Subjects gave their written informed consent, and the study was performed according to the Declaration of Helsinki. Cigarette smokers and subjects with a clinical history of cardiovascular disease or diabetes, hypercholesterolemia (total cholesterol > 6.0 mmol L⁻¹ and low-density lipoprotein cholesterol > 3.5 mmol L⁻¹), hypertension (≥ 140/90 mm Hg), or those taking medication were excluded.

**Study Protocol**

Subjects were studied in a quiet, temperature-controlled room (22 ± 2°C) on 4 occasions, each separated by 4 to 10 days. Before each visit, subjects were asked to avoid caffeinated drinks and heavy exercise for 3 hours and 24 hours, respectively. At the first visit, a fasted blood sample was obtained for measurement of lipids and glucose. Subjects then performed a maximal exercise test in which their maximal oxygen consumption and maximal heart rate were recorded. For each of the next 3 visits, participants were randomly assigned to receive saline, L-NMMA, or NE in a single-blind, placebo-controlled, crossover study design. At each of these visits, after placement of the study apparatus, subjects were seated quietly for 15 minutes during infusion of saline, at the end of which all of the hemodynamic measurements were taken in triplicate (saline baseline). Drug or saline infusions were then commenced, and all of the measurements were repeated in triplicate after 15 minutes (drug baseline). Subjects then exercised on a stationary bicycle ergometer (Monark Exercise AB) and performed a maximal exercise test in which their maximal oxygen consumption and maximal heart rate were recorded. Subjects commenced cycling at 60% of maximal heart rate has shown the mean ± SD for PPP:CPP to be 1.86 ± 0.07. Using these data we determined that 12 subjects would enable 87% power to detect a 5% change in exercise PPP:CPP, with α = 0.05.

**Cardiorespiratory Fitness**

An incremental exercise protocol on a cycle ergometer (874E, Monark Exercise AB) was used to determine maximal oxygen consumption and maximal heart rate. Subjects commenced cycling at an initial workload of 60 W, which was increased thereafter by 30 W every 3 minutes until 180 W, then by 20 W/min until volitional fatigue. An ECG was recorded at baseline and at the end of each cycle workload. Expired air was analyzed every 30 seconds during exercise using mass spectrometry gas analysis (Pulmolab Ex 670, Morgan Medical Ltd).

**Data Analysis**

Data are presented as means ± SEMs and were analyzed using SPSS software (version 10, SPSS Inc). Changes from saline baseline to drug baseline for each of the 3 visits were analyzed by paired t tests. Differences between interventions were analyzed by 1-way ANOVA. Exercise and postexercise data were analyzed separately by repeated-measures ANOVA. All of the ANOVA tests were corrected with Bonferroni’s posthoc tests. Significance was considered P < 0.05.

**Results**

**Resting Hemodynamics**

Baseline characteristics of subjects are presented in Table 1. Tables 2 and 3 show the central and peripheral hemodynamics at baseline, during cycling at 60% of maximal heart rate, and postexercise after infusion of saline, L-NMMA, and NE. There were no significant differences for any measured variables between the saline baselines at each of the 3 visits or after continued infusion of saline at the drug baseline (P > 0.05). Infusion of L-NMMA and NE caused similar significant increases in mean arterial pressure (9 ± 2% and 10 ± 1%, respectively; for each P < 0.001). At baseline, infusion of L-NMMA and NE caused a significant increase in AIx (for each P < 0.01).

Central BP was obtained by applanation tonometry and pulse wave analysis using a SphygmoCor apparatus (version 7.01, AtCor Medical). Radial artery pressure waveforms were obtained by use of a servo-controlled tonometer (Colin CBM-7000, Colin Corp). Movement artifact was eliminated by lightly taping the subject’s forearm to a platform that was mounted on the bicycle handlebars, fully supporting the arm. A central (ascending aortic) pressure waveform was synthesized from the radial pressure waveform using a transfer function validated, and shown to have excellent reproducibility, during exercise.

AIx, a composite measure of wave reflection and systemic arterial stiffness was calculated on the radial and central pressure waveforms by the difference between the second and first systolic peaks, expressed as a percentage of the PP. The estimated aortic pulse wave velocity (Tₐ) was calculated as the time between the foot of the pressure wave and the first inflection point and has been shown to correlate with aortic pulse wave velocity. PP amplification was calculated as the ratio of the peripheral to central PP (PPP:CPP). An increase in arterial stiffness would be expected to reduce Tₐ and PPP:CPP but increase central and radial AIx.

Mean arterial pressure was calculated from integration of the central pressure waveform. Cardiac output and stroke volume at each time point were calculated from the average of 2 minutes of continuous monitoring using a validated electric bioimpedance device (Task Force monitor model 3040, CNSystems). Peripheral vascular resistance was calculated by mean arterial pressure (mm Hg)/cardiac output (cm³ min⁻¹)×1000 expressed as peripheral resistance units. Our previous work in 20 young men exercising at 60% of maximal heart rate has shown the mean ± SD for PPP:CPP to be 1.86 ± 0.07. Using these data we determined that 12 subjects would enable 87% power to detect a 5% change in exercise PPP:CPP, with α = 0.05.

and cardiovascular risk. If reduced NO bioavailability underlies abnormal exercise hemodynamics in a fashion similar to that found in men with hypercholesterolemia, we may expect that induction of endothelial dysfunction in healthy men (by blockade of NO synthase) should lead to increased AIx and reduced PP amplification. We tested this hypothesis in young men who performed submaximal cycle exercise after intravenous infusion of L-NMMA compared with an NO-independent control vasoconstrictor (noradrenaline [NE]) and placebo (saline).
and there was a trend for decreased $T_R$ with NE infusion ($P=0.07$) but not for L-NMMA ($P=0.35$). There was a significant increase in radial AIx after infusion of NE (from $54\pm3\%$ to $64\pm4\%$; $P<0.001$) and L-NMMA (from $56\pm4\%$ to $64\pm4\%$; $P<0.01$) but not saline (from $55\pm4\%$ to $55\pm2\%$; $P>0.05$).

### Exercise Hemodynamics

There was no significant difference between infusions for data recorded at any time period during exercise. Therefore, data in the tables are only presented for minutes 4 and 8 of exercise. The Figure shows the PPP:CPP changes at baseline, during exercise, and during recovery. The PPP:CPP ratio was significantly reduced after infusion of L-NMMA and NE ($P<0.05$) at baseline. Compared with the baseline, there was a significant increase in PPP:CPP ($P<0.001$) and a significant decrease in $T_R$ and central and radial AIx ($P<0.05$ for all) during exercise for each of the infusion procedures. However, there was no significant difference in PPP:CPP ($P=0.63$), $T_R$ ($P=0.59$), or central or radial AIx ($P=0.50$ for each) between infusion of saline, L-NMMA, or NE.

For each infusion protocol, compared with the baseline there was a significant decrease in peripheral vascular resistance ($P<0.001$) together with an increase in heart rate ($P<0.001$) and cardiac output ($P<0.001$) during exercise. Mean arterial pressure was significantly higher during exercise after NE infusion compared with saline (Table 3). However, during exercise, there was no significant difference in peripheral vascular resistance ($P=0.64$), heart rate ($P=0.98$), or cardiac output ($P=0.86$) between infusion of saline or drugs. During exercise, both mean arterial pressure and cardiac output were $\approx 10\%$ higher during NE infusion compared with saline. Thus, overall, there was no significant difference between NE and saline infusion for peripheral vascular resistance during exercise. There was no significant difference in the rating of perceived exertion during exercise.

### Table 1. Baseline Characteristics of Study Participants (n=12)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>29±1</td>
</tr>
<tr>
<td>BMI, kg . m$^{-2}$</td>
<td>23.8±0.9</td>
</tr>
<tr>
<td>VO$_{2}\text{max}$, mL . kg$^{-1}$ . min$^{-1}$</td>
<td>49.5±1.4</td>
</tr>
<tr>
<td>Total cholesterol, mmol . L$^{-1}$</td>
<td>4.18±0.21</td>
</tr>
<tr>
<td>HDL cholesterol, mmol . L$^{-1}$</td>
<td>1.33±0.08</td>
</tr>
<tr>
<td>LDL cholesterol, mmol . L$^{-1}$</td>
<td>2.24±0.25</td>
</tr>
<tr>
<td>VLDL cholesterol, mmol . L$^{-1}$</td>
<td>0.66±0.12</td>
</tr>
<tr>
<td>Triglycerides, mmol . L$^{-1}$</td>
<td>1.34±0.24</td>
</tr>
<tr>
<td>Glucose, mmol . L$^{-1}$</td>
<td>5.33±0.19</td>
</tr>
</tbody>
</table>

BMI indicates body mass index; VO$_{2}\text{max}$, maximal oxygen consumption; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

### Table 2. Central and Peripheral BPs in Healthy Male Subjects (n=12) at Baseline and During and Postexercise After Infusion of Saline, L-NMMA (6 mg . kg$^{-1}$ . h$^{-1}$), and NE (50 ng . kg$^{-1}$ . h$^{-1}$)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Seated Baseline</th>
<th>Cycling at 60% of Maximum Heart Rate</th>
<th>Seated Postexercise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4 min</td>
<td>8 min</td>
</tr>
<tr>
<td>Brachial SBP, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>118±3</td>
<td>117±3</td>
<td>153±6</td>
</tr>
<tr>
<td>L-NMMA</td>
<td>117±3</td>
<td>119±4</td>
<td>158±5</td>
</tr>
<tr>
<td>NE</td>
<td>121±3</td>
<td>130±3*†</td>
<td>170±3</td>
</tr>
<tr>
<td>Brachial DBP, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>66±3</td>
<td>69±2</td>
<td>73±3</td>
</tr>
<tr>
<td>L-NMMA</td>
<td>66±3</td>
<td>75±2*</td>
<td>76±2</td>
</tr>
<tr>
<td>NE</td>
<td>69±3</td>
<td>75±2*</td>
<td>76±2</td>
</tr>
<tr>
<td>Brachial PP, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>49±3</td>
<td>48±4</td>
<td>80±7</td>
</tr>
<tr>
<td>L-NMMA</td>
<td>50±3</td>
<td>44±2</td>
<td>82±5</td>
</tr>
<tr>
<td>NE</td>
<td>53±3</td>
<td>55±4</td>
<td>94±3</td>
</tr>
<tr>
<td>Aortic SBP, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>101±2</td>
<td>100±3</td>
<td>119±3</td>
</tr>
<tr>
<td>L-NMMA</td>
<td>99±2</td>
<td>105±3*</td>
<td>124±3</td>
</tr>
<tr>
<td>NE</td>
<td>102±2</td>
<td>114±3*†</td>
<td>132±2‡</td>
</tr>
<tr>
<td>Aortic PP, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>31±2</td>
<td>30±3</td>
<td>43±4</td>
</tr>
<tr>
<td>L-NMMA</td>
<td>32±7</td>
<td>30±3</td>
<td>44±3</td>
</tr>
<tr>
<td>NE</td>
<td>33±2</td>
<td>38±3*</td>
<td>51±2</td>
</tr>
</tbody>
</table>

Data are means±SEMs. SBP indicates systolic BP; DBP, diastolic BP.

* $P<0.01$ indicates significant difference from seated baseline saline infusion.

† $P<0.05$ indicates significant difference from the saline visit after infusion of L-NMMA or NE.

‡ $P<0.05$ indicates a significant difference from saline during exercise.
exercise (saline 11.0±0.2, L-NMMA 11.0±0.3, and NE 11.0±0.3; P=0.17) or the intensity of cycling (saline 126.0±5.0 W, L-NMMA 118.0±7.0 W, and NE 129.0±9.0 W; P=0.53) between visits. In the postexercise recovery period, there were no significant differences in any measured variable between infusion protocols (data not shown; P>0.05 for all).

### Differential Response of NE Compared With L-NMMA

Infusion of NE caused a significantly higher increase in AIX, mean arterial pressure, and brachial and aortic systolic BP compared with L-NMMA infusion (Tables 2 and 3). On the other hand, NE infusion did not significantly change resting cardiac output, whereas this was significantly decreased with L-NMMA infusion. A greater increase in peripheral vascular resistance was also elicited with L-NMMA compared with NE infusion at rest. Furthermore, resting aortic PP was significantly increased from saline baseline with NE but not L-NMMA. The response of NE and L-NMMA on exercise BP and waveform indices was similar, except for higher aortic systolic BP and mean arterial pressure with NE (as discussed previously). The Figure shows that both NE and L-NMMA obtained similar responses for PPP:CPP at rest and during and postexercise. In the recovery period 10 minutes after exercise, brachial systolic BP was significantly higher after NE infusion.

### Discussion

The novel findings of this study were that inhibition of NO, by intravenous infusion of L-NMMA, had no significant effect on PP amplification or systemic arterial stiffness (AIX) during dynamic aerobic exercise. These findings may suggest that discrete mechanisms differentially affect pressure amplification and myocardial afterload under resting conditions compared with low-level exercise similar to that of daily living. It may also be that NO still plays some role in regulating exercise hemodynamics, but other vasodilatory compounds (eg, adenosine, bradykinin, and/or prostaglandins) released during exercise, or other stimuli associated
with exercise (eg, temperature or acidity), have a stronger influence than NO on large artery function during aerobic exercise.34–36 Alternatively, other vasodilatory mechanisms may have compensated for the blockade of NO.

**NO and Exercise Hemodynamics**

NO acts to lessen myocardial afterload by attenuating reflected pressure waveforms and is a key determinant of resting BP and ventricular-vascular coupling.19,20 Evidence is conflicting on the role of NO in the hyperemic response to exercise, with some studies finding a significant contributory role,23,24,37,38 whereas others have not.36,39–42 When all of these data are considered, blood flow in the recovery period after exercise is lower when NO synthase is blocked. It also seems clear that, when the change in blood flow from baseline is evaluated, NO synthase inhibition does not have a significant effect on the net dilation response to exercise.39,43,44 Thus, the influence of peripheral vasodilation on central BP indices, such as AIX, may be largely unaffected with L-NMMA infusion during exercise, but a response may be observed in the recovery period. This notion is supported by a recent study showing that L-NMMA attenuates wave reflection during recovery from exercise.45 Our own data in the immediate postexercise period also show a trend toward similar findings, with a nonsignificant increase in AIX together with a decrease in PPP:CPP when L-NMMA was infused.

**Limitations**

We used the noninvasive technique of radial tonometry to derive the central BP waveform instead of direct central BP measurement. Therefore, some differences in the true central pressures would be expected, particularly because the radial waveform was calibrated by brachial cuff BP. However, we have shown the tonometric central BP technique to be highly reproducible46 and to correlate closely with invasive central pressures during exercise.29 The study was also performed in a small group of healthy men, and the findings may not be applicable to larger populations or patient groups. We also used $T_r$ as an indirect measure of aortic stiffness. This surrogate was chosen because sequential planimetry of the carotid and femoral arteries (which is required for more direct measurement of aortic pulse wave velocity) is not possible during upright exercise. Furthermore, the control constrictor (NE) had a differential hemodynamic response compared with L-NMMA and may have also altered metabolism and affected cardiovascular mechanics in a nonphysiological manner. Finally, functional sympatholysis, to some degree, may have also negated the effect of NE with exercise.

**Perspectives**

To date, the mechanisms relating to pressure amplification during exercise have not been elucidated but have potentially important implications for cardiovascular risk25,46,47 and mortality.2,3,48 NO is known to play a modulatory role in large artery stiffness, pressure amplification, and myocardial loading during resting conditions, and this present work supports this concept. However, this is the first study to report that NO does not affect large artery hemodynamics and wave reflection such that ventricular-vascular interaction is substantially altered during low-intensity aerobic exercise.

**Sources of Funding**

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**Disclosures**

None.

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NO and Exercise Central Hemodynamics

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