Effect of lower limb exercise on forearm vascular function: contribution of nitric oxide

DANIEL GREEN,1,2,3 CRAIG CHEETHAM,1,3 LOUISE MAVADDAT,1 KATIE WATTS,1 MATTHEW BEST,3 ROGER TAYLOR,3 AND GERARD O’DRISCOLL2,3

1Department of Human Movement and Exercise Science, The University of Western Australia, Nedlands 6907; and 2Cardiac Transplant Unit, 3Department of Cardiology and Medicine, Royal Perth Hospital, Perth 6000, Western Australia

Received 22 January 2002; accepted in final form 3 May 2002

Green, Daniel, Craig Cheetham, Louise Mavaddat, Katie Watts, Matthew Best, Roger Taylor, and Gerard O’Driscoll. Effect of lower limb exercise on forearm vascular function: contribution of nitric oxide. Am J Physiol Heart Circ Physiol 283: H899–H907, 2002. First published May 23 2002; 10.1152/ajpheart.00049.2002.—We examined vascular function in an inactive muscle bed, the forearm, during lower limb exercise and determined the contribution of endothelium-derived nitric oxide (NO) to the hyperemic response. Eight young males were randomized to participate in two studies, each consisting of two bouts of lower limb exercise, separated by a 30-min recovery. Peak forearm blood flow (PFBF) and mean blood flow (MBBF) were continuously recorded at baseline and during exercise using continuous high-resolution vascular ultrasound and Doppler flow velocity measurement. During one session, the brachial artery was cannulated to allow continuous infusion of saline or Nω-monomethyl-L-arginine (L-NMMA), an inhibitor of NO synthase. The alternate session was performed to control for possible effects of repeated exercise. At 60, 100, and 160 W, L-NMMA significantly decreased both PFBF and MBBF compared with the saline infusion. These results suggest that systemic production of NO occurs during exercise in resting vessel beds, which do not feed metabolically active tissue. This finding provides a plausible explanation for the antiatherogenic benefits of exercise.

blood flow; high-resolution ultrasound; Doppler

NITRIC OXIDE (NO) is a vasodilator that possesses antiproliferative, antiadhesive, and antithrombotic properties and is released from the endothelium in humans both basally and in response to pharmacological and physiological stimuli (31). The biological activity of NO is impaired in subjects with cardiovascular disease or with vascular risk factors (3), and numerous interventions that improve endothelial NO-mediated vasodilator function are also cardioprotective (28, 29, 34–36). This evidence, along with recent studies indicating that endothelial dysfunction predicts cardiovascular events (1, 14, 33, 37, 42, 43), supports the notion that NO-related endothelial dysfunction is an early and integral manifestation of atherosclerotic disease (4, 5, 40, 54).

A number of studies performed in animals provide evidence that supports the contention that NO contributes to exercise hyperemia (15, 16, 21) and that it may coordinate the vascular response to exercise (44). However, studies performed in humans have been equivocal, some supporting (6, 7, 11, 30, 39, 46) and others repudiating (8, 52) significant involvement of NO in the acute hyperemic response to exercise. The disparity in these findings may relate to differences in subjects studied, the type of exercise performed, or to the limitations of blood flow assessment techniques such as strain-gauge plethysmography, which is subject to motion artifact and limits measurements to brief periods between or following muscle contractions or exercise (22). It is well established that pulsatile flow, through shear stress, provides a physiological stimulus to NO production and that, in turn, NO may act to regulate shear stress on the vessel wall (32). Indeed, recent studies in which lower limb exercise training was performed and forearm exercise specifically avoided found that the capacity for forearm NO vasodilator activity increases after exercise training, indicating that exercise training is associated with a sustained and systemic increase in the capacity for NO production (27–29). It seems likely that this sustained exercise increase in NO bioactivity could be dependent on repeated augmentation of NO bioactivity during acute bouts of exercise, suggesting that there may be a general augmentation of vascular NO production during the performance of exercise in addition to effects in the vascular bed of the exercising muscle.

The purpose of the present study was to describe the changes in vascular caliber, blood velocity, and flow that occur in the resting forearm during lower limb cycle ergometer exercise and to examine the hypothesis that NO function increases during exercise in vascular beds not directly involved in the exercise stimulus.
METHODS

Subjects and Screening Measures

The eight subjects were young, healthy males with no evidence or history of vascular disease. Those enrolled had the following characteristics: age 22.4 ± 4.4 (SE) yr, height 178.8 ± 4.8 cm, weight 78.7 ± 10.2 kg, resting heart rate 71 ± 7 beats/min, systolic blood pressure 125 ± 9 mmHg, and diastolic blood pressure 83 ± 7 mmHg. The study procedures were approved by the Ethics Committee of Royal Perth Hospital, and all subjects gave prior written consent.

Experimental Design

To determine the magnitude of exercise hyperemia in the forearm during lower limb exercise and the contribution of NO to this response, vascular function was assessed during two separate visits randomized in their order. Before attendance, subjects received an information sheet instructing them to abstain from food within 4 h and alcohol and/or caffeine within 12 h of testing. Each study was conducted for any given subject at the same time of the morning, controlling for possible circadian variation. Each session contained two identical exercise protocols separated by a 30-min rest period. One of the sessions involved brachial artery cannulation and infusion of physiological saline during the initial exercise protocol and infusion of \( \text{N}^\circ \)-monomethyl-L-arginine (\( L\)-NMMA; Clinalfa) during the subsequent one. This session was completed by all subjects, whereas the alternate session, which was undertaken to examine the possibility of a difference in repeated bouts of exercise without drug infusion, was not completed by two of eight subjects due to limitations associated with travel.

Experimental Procedures

\( L\)-NMMA infusion session. Investigations were conducted in a quiet, temperature-controlled laboratory. A 20-gauge arterial cannula (Arrow; Reading, PA) was introduced into the brachial artery of the nondominant arm, under local anesthesia with <2 ml of 1% lidocaine (Astra Pharmaceuticals), to transduce pressure and for the infusion of \( L\)-NMMA or physiological saline. Intra-arterial pressure was measured continuously (Transpac; Abbot Laboratories) throughout the study. \( L\)-NMMA and saline infusions were administered using an infusion pump (IVAC 770) at a constant rate of 60 ml/h.

After cannulation, saline was infused to maintain patency throughout a 30-min stabilization period during which subjects were seated quietly on an electronically braked bicycle ergometer (Orival 400, Lode). After this, a 2-min baseline recording of brachial artery diameter and velocity was undertaken, followed by a 15-min lower limb cycling exercise protocol consisting of five 3-min incremental epochs (40, 60, 80, 100, and 160 W). Brachial artery diameter and velocity were continuously recorded throughout the exercise protocol and for the first 5 min of a subsequent 30-min recovery period during which subjects again rested quietly on the ergometer. The infusion of saline was then replaced with \( L\)-NMMA at a constant dose of 8 \( \mu \)mol/min, and a second resting baseline was recorded. This was followed by a repeat of the bicycle ergometer protocol detailed above in the presence of continuous \( L\)-NMMA infusion to inhibit NO production.

Control session. During the \( L\)-NMMA infusion session outlined above, the order of saline and \( L\)-NMMA administration was not randomized because of the prolonged vasoconstriction that follows brachial artery \( L\)-NMMA infusion (50). The possibility of an order effect was therefore investigated by duplicating the above-stated exercise protocols in the absence of \( L\)-NMMA. This enabled an assessment of the effect of repeated bicycle ergometer protocols, separated by a 30-min rest period, on forearm blood flow responses.

Experimental Measurements

Forearm blood flow assessment. Blood flow was calculated by using high-resolution vascular ultrasonography with synchronized Doppler velocity assessment. A 12- to 15-MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (Aspen; Acuson) was used to visualize the artery in the distal third of the upper arm. Ultrasonic parameters were then set to optimize longitudinal B-mode images of the lumen/arterial wall interface. Once set, these parameters remained constant throughout the session. The probe was held in a constant position during the study, and its precise location was recorded and standardized for the repeat session by measurement of the proximal and distal distance of the probe from the radiale. Continuous Doppler velocity assessment was also recorded using the Aspen with an insonation angle of 60°.

Posttest analysis of brachial artery diameter was performed using custom-designed edge-detection and tracking software, which is independent of investigator bias, as described elsewhere (53). Briefly, B-mode images were either recorded on a S-VHS tape inside the ultrasound machine and then played back on a separate S-VHS video recorder for analysis or, alternatively, the video signal was taken directly from the ultrasound machine and, using the IMAQ-PCI-1407 card, was directly encoded and stored as a digital dicom file on the personal computer. Subsequent software analysis of these data, at ~20–30 frames/s, was performed using an icon-based graphical programming language (LabView 6.02, National Instruments; Austin, TX) and toolkit (IMAQ, National Instruments) in which developers build software programs called virtual instruments. To perform the analysis, the operator selects four regions of interest on the B-mode images, which are accessed from the video or stored DICOM files and displayed on the personal computer. Data collected from all regions of interest are used in subsequent acquisition of S-VHS or DICOM image files from which synchronized diameter and velocity measures are stored for each analyzed frame at 20–30 Hz. Details of this analysis software are available in a recent publication (12).

Display and analysis of results. Once the study has been acquired, a data “display” virtual instrument plots a graph of the arterial diameter (\( D \)) and velocity (\( v \)) against time. In addition, these synchronized velocity and diameter measurements are used to calculate and display the volume rate of blood flow as a continuous plot across the cardiac cycle. The volume rate of blood flow was calculated as the product of cross-sectional area (CSA) and \( v \), where CSA was calculated from the software-derived arterial diameter measures using the equation: \( \text{CSA} = \pi \text{radius}^2 \). Operator-controlled cursors can be used to select and zoom in on sections of the data set that are of interest (e.g., exercise epochs), and clearly erroneous data points may be manually removed by the observer or a smoothing algorithm applied if required. In the present study, the cursors were placed to calculate blood flow data from the final 20 s of each exercise epoch. Finally, data displayed between the cursors are analyzed and presented in a number of formats. “Mean” forearm blood flow rate (MFBF), \( v \), and \( D \) are calculated as the algebraic mean of all data points between the cursors, which may be placed on either side of a discreet cardiac cycle or at the beginning and
Fig. 1. Still frame of B-mode ultrasound data display acquisition software. Continuous traces of brachial artery diameter (top), velocity (middle), and flow (bottom) against time are shown. Vertical “begin” and “end” cursors are placed to zoom in on selected data, delete selected data, and, ultimately, calculate mean (MFBF) and average peak blood flows (PFBF). Typical diameter, velocity, and flow data at baseline (A), 60 W (B), and 100 W (C) are displayed for one subject. Note that the peak flows within each cardiac cycle have been automatically detected, and the appearance of substantial negative (retrograde) velocity and flow during exercise (dashed horizontal line represents zero flow).
end of an array of such cycles. Note that these values incorporate those during both systole and diastole so that MFBF is influenced by the magnitude of retrograde, diastolic flow (as described later). “Area under the curve” data for blood flow, D, and v is calculated as the time integral of each trace. “Peak” (systolic) forearm blood flow (PFBF), D, and v detection virtual instruments are used to identify and display the maximum data point within each cardiac cycle and to subsequently calculate the averages of these peaks. Finally, the area under the curve of all positive and all negative blood flow data points that lie between the cursors is presented to provide a global index of the volume of antegrade and retrograde blood flow per minute. All data are plotted across the cardiac cycle for the baseline and each of the exercise intensities. PFBF and MPBF measures for the baseline and exercise workloads were calculated across the final 20 s of each period.

**Treatment and Analysis of Data**

Results are expressed as means ± SE. The responses to exercise workloads were compared with the preceding baseline period using one-way ANOVA with post hoc t-tests. The contribution of NO to forearm vascular responses was determined by comparing saline and L-NMMA responses at each workload using two-way ANOVA with post hoc t-tests. Additionally, the differences in blood flow responses between L-NMMA and saline infusion conditions at each workload were compared with baseline differences using Student’s paired t-test. P < 0.05 was considered significant. Forearm vascular conductance (ml·min⁻¹·mmHg⁻¹) was calculated by dividing blood flow (ml/min) by blood pressure (mmHg).

**RESULTS**

An example of typical blood flows, v, and arterial diameter traces at baseline and during exercise stages are presented in Fig. 1. Satisfactory ultrasound and Doppler images were obtained for all subjects and at all workloads, including 160 W. There were no significant differences in systolic blood pressure, diastolic blood pressure, or mean arterial pressure between saline and L-NMMA infusions as analyzed by two-way ANOVA. Mean arterial pressures (mmHg) during saline and L-NMMA infusions, respectively, at each workload were as follows: 40 W, 102 ± 2 vs. 100 ± 3; 60 W, 105 ± 4 vs. 106 ± 5; 80 W, 108 ± 3 vs. 109 ± 4; 100 W, 110 ± 4 vs. 112 ± 4; and 160 W, 116 ± 6 vs. 117 ± 6. No differences were evident between conditions at any workload.

**Effect of L-NMMA on PFBF Responses to Incremental Cycle Exercise**

During lower limb exercise, PFBF significantly increased from the resting level (P < 0.001, one-way ANOVA). Post hoc t-tests revealed differences at all workloads: at 40 (P < 0.01), 100 (P < 0.05), 80 (P < 0.001), 100 (P < 0.001), and 160 W (P < 0.001) under the saline infusion condition (Fig. 2). Similarly, during L-NMMA infusion, PFBF significantly increased (P < 0.001, one-way ANOVA) at all workloads relative to the preceding baseline flow (all P < 0.01). When saline and L-NMMA conditions were compared, two-way ANOVA revealed a significant difference (P < 0.01). Post hoc t-tests revealed that L-NMMA reduced PFBF during lower limb exercise at 60 W (P < 0.05), 100 W (P < 0.05), and 160 W (P < 0.01). The average magnitude of decrease in PFBF induced by L-NMMA compared with saline infusion at baseline was 23.8 ± 37.7 ml/min, and the decreases induced by L-NMMA at both 100 W (104.4 ± 43.7 ml/min) and 160 W (229.4 ± 60.5 ml/min) were significantly greater than this difference at baseline (P < 0.05 and P < 0.01, respectively).

**Fig. 3. MFBF to the resting forearm across the cardiac cycle at baseline and during incremental cycle ergometer exercise in the presence of saline (C) and NO synthase inhibition with L-NMMA (■). Values are means ± SE. L-NMMA reduced MPBF responses compared with those during saline infusion during lower limb exercise at 60, 100, and 160 W (all P < 0.05). See text for details regarding the significance of changes relative to preceding baselines under both conditions.**
Peak forearm vascular conductance data also significantly increased with exercise intensity under both the saline and L-NMMA conditions ($P < 0.001$, one-way ANOVA). Post hoc $t$-tests revealed differences compared with baseline ($5.2 \pm 0.4 \text{ ml} \text{ min}^{-1} \text{ mmHg}^{-1}$) at the following workloads: 60 W ($7.0 \pm 0.6 \text{ ml} \text{ min}^{-1} \text{ mmHg}^{-1}$, $P < 0.01$), 80 W ($7.1 \pm 0.2 \text{ ml} \text{ min}^{-1} \text{ mmHg}^{-1}$, $P < 0.005$), 100 W ($8.2 \pm 0.4 \text{ ml} \text{ min}^{-1} \text{ mmHg}^{-1}$, $P < 0.001$), and 160 W ($9.7 \pm 0.9 \text{ ml} \text{ min}^{-1} \text{ mmHg}^{-1}$, $P < 0.001$) under the saline infusion condition. Under the L-NMMA condition, differences from baseline ($4.9 \pm 0.5 \text{ ml} \text{ min}^{-1} \text{ mmHg}^{-1}$) were evident at all workloads: 40 W ($6.1 \pm 0.6 \text{ ml} \text{ min}^{-1} \text{ mmHg}^{-1}$, $P < 0.001$), 60 W ($6.1 \pm 0.4 \text{ ml} \text{ min}^{-1} \text{ mmHg}^{-1}$, $P < 0.05$), 80 W ($7.0 \pm 0.4 \text{ ml} \text{ min}^{-1} \text{ mmHg}^{-1}$, $P < 0.001$), 100 W ($7.3 \pm 0.2 \text{ ml} \text{ min}^{-1} \text{ mmHg}^{-1}$, $P < 0.001$), and 160 W ($7.7 \pm 0.5 \text{ ml} \text{ min}^{-1} \text{ mmHg}^{-1}$, $P < 0.001$). When saline and L-NMMA conditions were compared, L-NMMA reduced peak forearm vascular conductance at 60 and 160 W (both $P < 0.05$).

**Effect of L-NMMA on MFBF Responses to Incremental Cycle Exercise**

During saline infusion, MFBF (Fig. 3) was significantly influenced by exercise workload ($P < 0.001$, one-way ANOVA). Post hoc $t$-tests revealed that MFBF was lower than baseline at 40 W ($P < 0.01$), similar at 60 W ($P = 0.07$) and 80 W ($P = 0.7$), and significantly higher than baseline at 100 W ($P < 0.01$) and 160 W ($P < 0.001$). A similar biphasic response was evident during L-NMMA infusion ($P < 0.001$, one-way ANOVA), with MFBF lower than baseline at 40 W ($P < 0.05$) and 60 W ($P < 0.05$), similar at 80 W ($P = 0.9$) and 100 W ($P = 0.08$), and significantly higher than baseline at 160 W ($P < 0.01$). The differences in the pattern of PFBBF and MFBF responses to lower limb exercise were due to the relative magnitude of changes in retrograde and antegrade flows as workload increased. These are fully explained in our recent methodological paper (12), and the implications for the time integrals of retrograde and antegrade flow during the cardiac cycle are described below. When saline and L-NMMA conditions were compared, two-way ANOVA revealed a significant difference ($P < 0.01$). Post hoc analysis indicated that, at baseline, MFBF was, on average, lower during L-NMMA but not significantly so ($90 \pm 19$ vs. $65 \pm 8 \text{ ml/min}$, $P = 0.2$), consistent with only a slight NO-induced vasodilation. In contrast, L-NMMA reduced MFBF during lower limb exercise at 60, 100, and 160 W (all $P < 0.05$) quite markedly at the higher levels of exercise; for example, at 160 W, MFBF was $203 \pm 24 \text{ ml/min}$ during saline infusion and $118 \pm 17 \text{ ml/min}$ during L-NMMA administration (see Fig. 3).

The average decrease in MFBF induced by L-NMMA compared with saline infusion at baseline was $25.1 \pm 18.6 \text{ ml/min}$. The decreases induced by L-NMMA at both 100 W ($75.0 \pm 29.5 \text{ ml/min}$) and 160 W ($85.0 \pm 27.0 \text{ ml/min}$) were significantly greater than this difference at baseline (each $P < 0.05$).

Mean forearm vascular conductance data also significantly increased with exercise intensity under both the saline and L-NMMA conditions ($P < 0.001$, one-way ANOVA). Post hoc $t$-tests revealed differences compared with baseline ($0.9 \pm 0.2 \text{ ml} \text{ min}^{-1} \text{ mmHg}^{-1}$) at the following workloads: 40 W ($0.5 \pm 0.2 \text{ ml} \text{ min}^{-1} \text{ mmHg}^{-1}$, $P < 0.01$), 60 W ($0.6 \pm 0.2 \text{ ml} \text{ min}^{-1} \text{ mmHg}^{-1}$, $P < 0.05$), 100 W ($1.4 \pm 0.3 \text{ ml} \text{ min}^{-1} \text{ mmHg}^{-1}$, $P < 0.05$), and 160 W ($1.8 \pm 0.2 \text{ ml} \text{ min}^{-1} \text{ mmHg}^{-1}$, $P < 0.01$) under the saline infusion condition. Under the L-NMMA condition, differences from baseline ($0.7 \pm 0.1 \text{ ml} \text{ min}^{-1} \text{ mmHg}^{-1}$) were evident at the following workloads: 40 W ($0.3 \pm 0.1 \text{ ml} \text{ min}^{-1} \text{ mmHg}^{-1}$, $P < 0.01$), 60 W ($0.3 \pm 0.1 \text{ ml} \text{ min}^{-1} \text{ mmHg}^{-1}$, $P < 0.01$), and 160 W ($1.0 \pm 0.1 \text{ ml} \text{ min}^{-1} \text{ mmHg}^{-1}$, $P < 0.05$). When saline and L-NMMA conditions were compared, L-NMMA reduced peak forearm vascular conductance at 60, 100, and 160 W (each $P < 0.05$).

**Effect of L-NMMA on Antegrade and Retrograde Brachial Artery Flows**

Figure 4 presents the area under the curve time integral of flow rate data for retrograde and antegrade Flows.
flows at baseline and during each exercise intensity under the saline and l-NMMA conditions. Relative to baseline, the magnitude of antegrade flow increased significantly ($P < 0.001$, one-way ANOVA), and post hoc tests revealed increases at all workloads: at 60 W ($P < 0.05$) and at 80, 100, and 160 W ($P < 0.001$) under the saline condition. Also during l-NMMA infusion, antegrade flow increased significantly ($P < 0.001$, one-way ANOVA) at all workloads: 40 W ($P < 0.001$), 60 W ($P < 0.05$), and at 80, 100, and 160 W ($P < 0.001$). The magnitude of retrograde flow increased at each workload compared with baseline under both saline and l-NMMA conditions (all $P < 0.01$). l-NMMA tended to decrease antegrade flow and to increase retrograde flow; it significantly attenuated antegrade flow at 160 W ($P < 0.05$), whereas it increased retrograde flows at 80 ($P < 0.01$) and 100 W ($P < 0.05$).

**Effect of Repeated Exercise Protocols on Forearm Blood Flow Responses**

Repeated bicycle ergometer protocols, separated by a 30-min rest period, were undertaken to examine the possibility of an order effect on forearm blood flow responses. The effect of repeated exercise protocols on PFBF and MFBF are presented in Fig. 5. No significant differences were evident between the initial and repeat bouts of exercise for either variable, suggesting that an order effect was unlikely to have compromised the l-NMMA responses.

**DISCUSSION**

We recently demonstrated in the same subjects as those investigated in the present study that during lower limb cycle ergometer exercise during which the upper limbs are at rest, peak forearm blood flow increases incrementally with exercise intensity (12). Conversely, the mean blood flow rate calculated across the cardiac cycle exhibits a biphasic response; increased flow relative to baseline at higher workloads is preceded by a decrease at lower intensities. The reason for this disparity in the pattern of peak and mean responses relates to the influence of retrograde diastolic flow, which has a greater relative impact on mean blood flow responses at lower workloads (12). In the present study, our principle aim was to investigate the possible contribution of NO to forearm blood flow responses during cycle ergometry. Our principle findings are that l-NMMA inhibited the magnitude of both PFBF and MFBF rate responses during lower limb exercise. Whereas there was only a small resting effect of l-NMMA to decrease MFBB, which was not actually statistically significant, the effect of l-NMMA on the forearm vascular bed was considerably greater during the higher exercise workloads, indicating inhibition of a larger NO vasodilatory effect. Importantly, because NO has a very short half-life and acts locally, the data indicate that NO is released from vascular beds other than those that feed metabolically active skeletal muscle during exercise.

l-NMMA exerted its effect across the cardiac cycle by attenuating the magnitude of antegrade flow during systole and exaggerating retrograde flow during diastole. These responses are consistent with vasoconstriction in the forearm vascular bed (2) resulting from the local inhibition of NO synthase by l-NMMA. That is, the relative increase in vascular resistance induced by l-NMMA in the forearm bed, coupled with the decrease in resistance in the actively working muscle, tended to reduce antegrade flow to the forearm and to increase retrograde flow. The latter may be due to an increase in a diastolic “steal” effect (12) or exaggerated pressure wave reflection (2).

This is the first study, to our knowledge, to investigate the contribution of NO to vasmotor control in a nonactive vascular bed during exercise in humans. Our rationale for undertaking this investigation derives from recent findings by ourselves (28, 29) and others (27), which indicate that sustained improvements in
upper limb vascular function occur as a result of lower limb exercise training programs, which excluded physical conditioning of the forearm musculature. These findings raised the possibility that the performance of exercise, either via some circulating metabolically derived stimulus or via a hemodynamic-mediated shear stress phenomenon, increases NO bioactivity in vessel beds distant from the exercising musculature. We have confirmed this hypothesis, finding that there is local increase in NO activity in the resting forearm during lower limb exercise, although the precise mechanisms responsible for NO release were not specifically determined.

It is well established that the likely physiological stimuli for NO release involve changes in flow and shear stress on the vessel wall (38, 41). Acute changes in flow and shear stress stimulate the release of NO during exercise in animal models (15, 16, 32) with peak release of NO from donor arteries dependent on both pulse frequency and pulse pressure amplitude (19). It has also been demonstrated that acute increases in blood flow through conduit arteries are associated with flow-mediated stress on the vessel wall, which, in turn, liberates NO from the endothelium (9, 24). In humans, this flow-mediated dilation is attenuated by coinfusion of L-NMMA, suggesting that conduit vessel dilation during exercise may, at least in part, be NO dependent (18, 20). Indeed, several (6, 7, 11, 39), but not all (8, 52), studies performed in humans suggest that exercise-induced hyperemia in the active vessel bed is partly NO dependent, and it has also been suggested that NO may coordinate the hyperemic response to exercise by amplifying metabolic vasodilator stimuli in the microvessels and transmitting these signals to upstream feed arteries (44). Although we speculate that our observation of increased NO activity in a resting vascular bed during exercise may be due to exercise-mediated hemodynamic modulation, we cannot rule out the possibility that some circulating metabolite may be responsible for stimulation of NO production.

The present study utilized a novel software analysis system to assess conduit artery diameter and blood velocity by using a combination of high resolution B-mode ultrasonography and Doppler velocity assessment to calculate blood flow across the cardiac cycle from these synchronized data (12). Although this technology allows noninvasive and continuously blood flow assessment in vivo with high temporal resolution, it does not assess relative changes in blood flow to the muscle and skin vascular beds. This limitation is important to acknowledge in the present study because increases in exercise intensity are likely associated with differential neural regulation of skin and muscle vascular beds (48, 49). Increases in skin blood flow, subserving thermoregulatory demands, likely contribute to increased forearm blood flows with increasing exercise intensity. Furthermore, we did not occlude blood flow to the hand during measurements in the present study, a practice that is common in plethysmography studies, which have reported transient forearm dilation followed by progressive constriction in the forearm during prolonged exercise (48, 49). Future studies employing laser Doppler flowmetry and the forearm blood flow methodology utilized in the present study should better characterize the relative changes that occur in skin and muscle blood flow during incremental exercise. Importantly, whereas changes in skin blood flow may have impacted the pattern of forearm blood flow response with increasing exercise, this does not compromise our principle finding; namely, that NO contributes to forearm blood flow responses during lower limb exercise.

The present findings have several important implications. In addition to being a vasodilator, NO is recognized to possess antiproliferative, antiadhesive, and antithrombotic properties, and numerous interventions that improve NO activity are cardioprotective (5, 28, 29, 34–36). The present data indicate that the performance of exercise is associated with a systemic increase in NO bioactivity, and we suggest that the repeated induction of generalized vascular NO activity leads to a sustained enhancement of the capacity for NO production. This would provide a plausible explanation for the long-term effects of exercise on NO-related vasodilation evident generally in the vasculature (27–29, 45, 47) and for the antiatherogenic benefits of exercise (51). In addition, animal studies suggest that chronic increases in flow enlarge (23) and chronic decreases in flow reduce (26) vessel caliber in vivo via endothelium-dependent mechanisms and that NO influences the synthesis, mitogenesis, and proliferation of vascular smooth muscle cells (10, 13, 17, 25). These and the present study imply that exercise may modulate systemic vascular architecture and promote vessel patency at least partly as a result of NO modulation.

In summary, this study is the first to use a novel blood flow assessment system, which calculates blood flow across the cardiac cycle at high frequency, to investigate the impact of exercise on NO activity in a vascular bed other than those specifically involved in the exercise stimulus. We demonstrate that exercise exerts a generalized effect on the vasculature by increasing NO activity in vessel beds other than those that perfuse the actively working muscle. Our data also indicate for the first time that L-NMMA impacts on both antegrade and retrograde components of flow during the cardiac cycle. Whereas it is likely that these observations are due to mechanotransduction of NO release in response to exercise-mediated increases in shear stress or pulsatile flow, we cannot rule out the possibility that some circulating exercise metabolite may be responsible for stimulation of NO bioactivity, for example, catecholamine-mediated stimulation of β-receptors. Regardless of the mechanism responsible, these data provide a plausible explanation for the antiatherogenic benefits of exercise, because NO is recognized as possessing antiproliferative, antiadhesive, and antithrombotic properties.

This study was supported by National Heart Foundation of Australia and the Medical Research Fund of Western Australia.
REFERENCES


