Nitric oxide in the regulation of vasomotor tone in human skeletal muscle

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Rådegran, G., and B. Saltin. Nitric oxide in the regulation of vasomotor tone in human skeletal muscle. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1951–H1960, 1999.—The role of nitric oxide (NO) as a regulator of vasomotor tone has been investigated in resting and exercising human skeletal muscle. At rest, NO synthase (NOS) inhibition by intra-arterial infusion of Nω-monomethyl-L-arginine decreased femoral artery blood flow (FABF, ultrasound Doppler) from 0.39 ± 0.08 to 0.18 ± 0.03 l/min (P < 0.01), i.e., by ∼52%, and increased leg O2 extraction from 62.1 ± 9.8 to 100.9 ± 4.5 ml/min (P < 0.004); thus leg O2 uptake (Vo2, 22 ± 4 ml/min, ∼0.75 ml·min−1·100 g−1) was unaltered [not significant (P = NS)]. Mean arterial pressure (MAP) increased by 8 ± 2 mmHg (P < 0.01). Heart rate (HR, 53 ± 3 beats/min) was unaltered (P = NS). The NOS inhibition had, however, no effect on the initial rate of rise or the magnitude of FABF (4.8 ± 0.4 l/min, ∼163 ml·min−1·100 g−1), MAP (117 ± 3 mmHg), HR (98 ± 5 beats/min), or leg Vo2 (704 ± 55 ml/min, ∼24 ml·min−1·100 g−1) during submaximal, one-legged dynamic knee-extensor exercise. Similarly, FABF (7.6 ± 1.0 l/min, ∼258 ml·min−1·100 g−1), MAP (140 ± 8 mmHg), and leg Vo2 (1,173 ± 139 ml/min, ∼40 ml·min−1·100 g−1) were unaffected at termination of peak effort (P = NS). Peak HR (137 ± 3 beats/min) was, however, lowered by 10% (P < 0.01). During recovery, NOS inhibition reduced FABF by ∼34% (P < 0.04), which was compensated for by an increase in the leg O2 extraction by ∼41% (P < 0.04); thus leg Vo2 was unaltered (P = NS). In conclusion, these findings indicate that NO is not essential for the initiation or maintenance of active hyperemia in human skeletal muscle but support a role for NO during rest, including recovery from exercise. Moreover, changes in blood flow during rest and recovery caused by NOS inhibition are accompanied by reciprocal changes in O2 extraction, and thus Vo2 is maintained.

Blood flow; circulation; exercise; metabolism; vasodilatation

Whereas muscle mechanical factors induce the initial rapid increase in muscle blood flow at onset of exercise, a concomitant vasodilatation accelerates the rate of rise within the first few contractions (18, 22, 23, 26). This vasodilatation has, in addition to metabolites released from the active muscle fibers (21, 25), also been suggested to be triggered by nitrinic oxide (NO) and/or ACh (4, 8, 29). The potential role of ACh is further supported by the fact that it is the main transmitter released from motor nerves in the end plate region. Besides the possibility that ACh may directly trigger an ascending vasodilatation, it is also the prime stimulator of endothelial release of NO. Moreover, inasmuch as blood flow momentarily increases at onset of exercise, the associated shear stress may stimulate a further endothelial release of NO and ACh (14), potentiating the vasodilatation, locally and/or upstream.

Previous findings support (4, 8) as well as reject (6, 24, 30) a role for NO in regulation of exercise hyperemia. However, these studies have limitations with regard to the methods used to measure blood flow in transition from rest to exercise, as well as during exercise. Recent improvements of the ultrasound-Doppler methodology offer not only the required time resolution but also the precision needed to continuously measure arterial inflow to a contracting muscle group (17).

Thus, by taking advantage of the ultrasound-Doppler methodology, we aimed to test the hypothesis that NO may play an important role in humans in the regulation of vasomotor tone and muscle blood flow before, during, and after muscular exercise. The one-legged dynamic knee-extensor model was used, inasmuch as it allows the study of local blood flow and O2 uptake (Vo2) of a single active muscle group in vivo. Femoral artery blood flow (FABF) was measured during each condition, before and after NOS inhibition, by continuous intra-arterial infusion of Nω-monomethyl-L-arginine (L-NMMA). The potency and reversibility of the NOS inhibition were assayed by intra-arterial infusion of ACh and L-arginine, respectively. In addition, whereas the present study focused on the role of endogenous NO, control experiments were also performed to address the peak vasodilatatory potency of intra-arterially infused sodium nitroprusside (SNP) and ACh compared with the exercise response.

Methods

Subjects

Thirteen healthy male volunteers, 25.4 ± 0.8 (21–31) yr of age, 180.9 ± 1.9 (168.5–192.7) cm height, and 76.0 ± 2.0 (67.7–88.3) kg body wt (means ± SE), participated. Their mean quadriceps muscle and thigh volume were 2.95 ± 0.09 (2.42–3.68) and 10.90 ± 0.20 (9.90–11.81) liters, respectively, as estimated from anthropometric measurements, with muscle insertion points measured from patella to os pubis (1). The subjects’ engagement in exercise training ranged from daily activities to regular endurance training. Before participation, the subjects were informed about the experimental procedures, the potential risks and discomfort, and that they could withdraw from the study at any time. They participated after they signed informed consent forms. The experiments were carried out with the approval of the Ethical Committees of Copenhagen and Frederiksberg (KF-01-013/96).
Experimental Design and Equipment

The subjects were familiarized with the one-legged, dynamic knee-extensor exercise model (1) by training at 60 rpm until they could fully relax the hamstring muscles, so that the work was done solely by the knee extensors. The mean peak power output they could sustain for 3 min at 60 rpm was $72.8 \pm 3.8 (65-90)$ W.

All subjects were required to abstain from caffeine, tea, and nicotine for $\approx 48$ h before the experiments. After they reported to the laboratory at $\approx 0800$, the femoral artery of both legs and the femoral vein of one leg were cannulated under local anesthesia (lidocaine, 20 mg/ml). The Seldinger technique was used to insert catheters (20 gauge; Ohmeda, Wiltshire, UK) $\approx 2$–5 cm below the inguinal ligament. The tip of the arterial catheter for drug infusion was positioned just above the femoral bifurcation.

A syringe pump (model 44, Harvard Apparatus) was used for drug infusion. The arterial and venous blood samples were analyzed for Hb, O$_2$ saturation (912 CO-OxyLite, AVL Medical Instruments, Schaffhausen, Switzerland), hematocrit, and blood PO$_2$, PCO$_2$, and pH (Compact 2 Blood Gas Analyzer, AVL Medical Instruments) as well as blood lactate (2300 Stat Plus, Yellow Springs Instrument, Yellow Springs, OH). Extraction and fluxes of O$_2$ and lactate from the leg were calculated from the femoral arterial and venous blood sample differences as well as the femoral artery blood flow (Fick’s principle) measured by ultrasound Doppler (17). Heart rate (HR, electrocardiogram [ECG]) and arterial blood pressure were continuously monitored (Dialogue 2000, Danica Elektronik, Copenhagen, Denmark). The knee-extensor force was monitored with a strain gauge attached to the ergometer lever arm. The equipment was connected, via a switch box, to an eight-channel analog-to-digital converter in an IBM-compatible Pentium-based personal computer with use of a data acquisition system obtained from the Institute of Physiology (Oslo, Norway). This allowed signal transfer with a frequency of 100 Hz as well as averaging for each cardiac cycle.

Femoral Artery Blood Flow

The procedure of blood flow measurements has previously been validated and shown to produce accurate absolute values at rest and during exercise (17). An ultrasound Doppler (model CFM 800, Viringed Sound, Horten, Norway) equipped with an annular phased array transducer (Viringed Sound) probe (11.5 mm diameter) operating at an imaging frequency of 7.5 MHz and variable Doppler frequencies of 4.0–6.0 MHz (high-pulsed repetition frequency mode, 4–36 kHz) was used.

The site for vessel diameter determination and blood velocity measurements in the common femoral artery was distal to the inguinal ligament but above the bifurcation into the superficial and profound femoral branch. The femoral artery was insonated at a fixed perpendicular angle. The diameter was determined along the central path of the ultrasound beam where the best spatial resolution is achieved. A diameter based on the relative time periods of the systolic (one-third) and diastolic (two-thirds) blood pressure phases was used to determine the cross-sectional area (17). The blood velocity was measured with the Doppler probe stabilized in a fixed position at as low an insonation angle as possible (17). The blood velocity and flow during exercise were specifically analyzed in relation to the muscle contraction force (strain gauge profile) (17). A cuff below the knee around the calf muscles was temporarily inflated before the flow measurements to a suprasystolic ($=240$ mmHg) blood pressure to eliminate blood flow contributions to the lower leg.

FABF $\times 10^4 \cdot V \cdot A (l/min)$, where $V$ is the measured mean blood velocity (m/s) and $A$ is the cross-sectional area was calculated over the parabolic velocity profile by multiplying the cross-sectional area of the femoral artery by the angle-corrected, time- and space-averaged, and amplitude (signal intensity)-weighted mean blood velocity. Vascular conductance ($VC$) was calculated from the following formula: $VC = FABF/(BPa - BPv)$, where $BPa$ and $BPv$ represent the arterial and venous blood pressure and $BPv$ is assumed to be zero.

Skin Blood Flow

Changes in skin blood flow (SKBF, red blood cell perfusion units) relative to rest control were monitored during the vasodilator infusion protocol with laser Doppler (Periflux 4001 Master, Perimed, Järfälla, Sweden) (10) to estimate the contribution of SKBF to FABF. The laser diode probes operated with divergent (noncollimated), continuous-wave (nonpulsed) light, at 780 nm, and with a maximum emission of 0.8 mW. The two probes (408 standard probe) of the fiber-optic cables were secured on the surface of the skin over the quadriceps muscle along a straight line between the pubic bone and the patella. The mean values from the two probes are given in the text.

Drugs

Sterile filtered SNP (Nipride) was obtained from Roche (Basel, Switzerland), ACH from Alexis (Läufelfingen, Switzerland), L-NMMA from Chemicon (Malmö, Sweden) and Alexis, and L-arginine from Alexis.

Experimental Design

Before exercise, seven subjects warmed up for $\approx 15$ min with one-legged, dynamic knee-extensor exercise at 30–50% of peak power output. They rested for $\approx 30$ min, i.e., until FABF, SKBF, HR, and mean arterial pressure (MAP), as well as the extraction and fluxes of O$_2$ and lactate, were normalized at baseline rest level (P = NS). To adopt the same specific work rate at onset of exercise with and without NOS inhibition, each subject’s leg that was attached to the ergometer lever arm (1) was moved by a technician with five to seven distinct movements until a rate of 60 rpm was reached (i.e., passive leg movements with regard to the subject). The voluntary exercise was then initiated at a workload of $\approx 47.1 \pm 1.8$ W ($\approx 65.4 \pm 3.6$% of peak power output), at which the subjects exercised for 5–8 min. Thereafter the intensity was increased 5–10 W every 30 s up to the peak load of $\approx 72.8 \pm 3.8$ (60–90) W.

The NOS inhibition trial was performed after $\approx 1$ h of rest, i.e., when blood flow had normalized to baseline rest level. The competitive inhibitor L-NMMA was infused for 5 min into the femoral artery at a rate of $5 \text{mg} \cdot \text{min}^{-1} \cdot l$, thigh volume$^{-1}$ (loading dose) and thereafter throughout the experiment at a rate of $1 \text{mg} \cdot \text{min}^{-1} \cdot l$, thigh volume$^{-1}$ (maintenance dose). The L-NMMA dose had previously been titrated in five subjects, where increasing doses within 10 min induced a stable peak leveling off in the inhibitory response (i.e., maximum and no further effect on FABF, MAP, and leg O$_2$ extraction) at the dose chosen for this experiment. The dose given was similar to or higher than that previously used in other studies (4, 6, 8, 30). L-NMMA was chosen for stereospecific NOS inhibition of the substrate L-arginine, since N$^\text{ON}$-nitro-L-arginine methyl ester (L-NAME) in addition may block muscarinic receptors or possibly donate its nitro group (2). The inhibitor was continuously administered during rest, exercise, and recovery to reach vascular beds that possibly were not perfused at rest. The inhibitory effect was specifi-
cally monitored at rest, by the response in FABF, MAP, and leg O2 extraction, after 1, 3, 6, and 10 min of infusion, as well as before the exercise was started. Femoral arterial and venous blood samples were taken during both trials at rest, as well as after 1, 3, and 5–8 min of submaximal exercise, at termination of peak effort, and after 1, 3, 6, and 10 min of recovery.

The extent of NOS inhibition was investigated in six subjects by following the attenuation of the blood flow response to ACh. The NOS inhibitor was specifically infused, as explained above, before and between each ACh dose. The reversibility was verified by infusion for 5 min of L-arginine, at a rate of ~26.4 ± 0.8 mg·min⁻¹·l thigh volume⁻¹ (total dose of ~1,160 ± 58.4 mg), corresponding to twice the amount of L-NMMA.

In control experiments the exercise response was compared with the vasodilator potency of SNP and ACh by continuous vasodilator infusions in the femoral artery of six subjects in the resting supine position. The continuous infusions were given for 3 min each at incremental rates with a factor of 3. SNP was infused via light-protected syringes (Original-Perfusor-Spritze, B. Braun Medical, Melsungen, Germany) and infusion tubes (Original-Perfusor-Leitung, B. Braun Medical). Femoral arterial and venous blood samples were drawn at rest and at the end of infusion. The infusions were given at intervals of ≥15 min; i.e., the next infusion was not given until FABF, SkBF, HR, and MAP, as well as the extraction and fluxes of O2 and lactate, were normalized at baseline rest level [not significant (P > NS)]. Similarly, the infusions of SNP and ACh were separated by ≥30 min, corresponding to when normalization to baseline rest level had occurred.

Statistics and Data Analysis

Parametric statistics were used for data analysis. Multiple ANOVA for repeated measures and Tukey's honestly significant difference post hoc tests were used for analyzing the effect of NOS inhibition at rest and during passive and voluntary exercise compared with control conditions, as well as for the different infusion concentrations of SNP and ACh.

RESULTS

NOS Inhibition With L-NMMA

Rest. The NOS inhibition was evident from the 3rd min of L-NMMA infusion, resulting in an increase in the leg O2 extraction from 62.1 ± 9.8 to 91.8 ± 7.3 ml/l (P < 0.004), after which it stabilized at 100.9 ± 4.5 ml/l (P > NS). The arterial saturation was unaffected and maintained constant at 96.9 ± 0.1% (P > NS). FABF decreased by ~52.0 ± 4.7%, i.e., from 0.39 ± 0.08 to 0.18 ± 0.03 l/min (~6.1 ml·min⁻¹·100 g⁻¹, P < 0.02; Fig. 1), keeping leg VO2 unaltered at 22.0 ± 4.0 ml/min (~0.75 ml·min⁻¹·100 g⁻¹, P > NS). The net release of lactate was unaltered at 0.024 ± 0.015 mmol/min (P > NS); MAP increased by ~8.0 ± 2.0 mmHg (P < 0.0001), whereas HR was unaltered (P > NS).

Passive and voluntary exercise. With passive leg movements, FABF increased to 1.15 ± 0.16 l/min (~39 ml·min⁻¹·100 g⁻¹, P < 0.04), but FABF was unaffected...
by the NOS inhibition (P = NS; Fig. 1). Also, at onset of submaximal, voluntary exercise, the NOS inhibition did not affect (P = NS) the initial rise in FABF, as measured for consecutive and corresponding knee-extensor duty cycles (Fig. 2A). The NOS inhibition did not affect FABF or leg VO₂ during steady-state submaximal exercise and 9.88 ± 1.92 mmol/min at peak intensity. However, HR (136.7 ± 3.2 beats/min) at peak intensity was lowered by 14 beats/min, i.e., by 10% (P < 0.005).

Recovery. The integrated FABF response during the 10 min of recovery with NOS inhibition was reduced to 66 ± 5% of control recovery (P < 0.0001; Fig. 2B). The reduction in FABF with NOS inhibition was immediately evident at initiation of recovery, but at 1 min a slight opposite response was found in one subject. The integrated leg VO₂ extraction with NOS inhibition was increased by 41 ± 9% (P < 0.04); thus leg VO₂ was unaffected (P = NS; Fig. 3). The net release of lactate was unaffected with NOS inhibition (P = NS): 7.58 ± 1.76, 4.6 ± 1.55, 1.49 ± 0.70, and 0.95 ± 0.17 mmol/min after 1, 3, 6, and 10 min of recovery, respectively. The integrated HR response with NOS inhibition was reduced to 88 ± 6% (P < 0.04; Fig. 3).

Interactions of L-NMMA, ACh, and L-Arginine at Rest

The potency and specific reversibility of the NOS inhibition were tested by intra-arterial femoral infusion of ACh and L-arginine, respectively. The arterial saturation remained constant at 96.7 ± 0.2% throughout the infusions (P = NS). ACh. At rest, FABF was 0.22 ± 0.02 l/min. FABF increased by 0.53 ± 0.23, 1.75 ± 0.68, and 3.56 ± 0.28 l/min (~18, 59, and 121 ml·min⁻¹·100 g⁻¹) during incremental rates of ACh infusion at 16, 48, and 144 µg·min⁻¹·l thigh volume⁻¹, respectively. The corresponding leg VO₂ extraction decreased from 64.5 ± 5.0 ml/l at rest control to 13.1 ± 4.4 ml/l at the highest infusion rate (P < 0.0005); thus leg VO₂ was unaffected at 10.4 ± 2.4 ml/min (~0.35 ml·min⁻¹·100 g⁻¹, P = NS). The net release of lactate was unaffected at 0.005 ± 0.005 mmol/min (P = NS). The FABF increase, for each ACh infusion rate, was markedly attenuated with NOS inhibition (P < 0.001) by 90.4, 68.6, and 36.5%, respectively. As the FABF response was attenuated, there was a corresponding increase in leg VO₂ extraction; thus leg VO₂ was unaffected (P = NS).

L-NMMA. NOS inhibition per se decreased FABF by 60.1 ± 8.4% (P < 0.05) and SkBF by 23.3 ± 6.7% (Fig. 4; P < 0.03). The leg VO₂ extraction increased correspondingly from 48.3 ± 1.9 to 70.6 ± 5.3 ml/l (P < 0.002),...
keeping leg \( \dot{V}O_2 \) unaltered at 14.0 \( \pm \) 3.1 ml/min (\( \approx \frac{0.47}{100} \) ml\( \cdot \)min\(^{-1}\)\( \cdot \)100 g\(^{-1}\), \( P = \text{NS} \)). The net release of lactate was unaltered at 0.005 \( \pm \) 0.005 mmol/min (\( P = \text{NS} \)). MAP increased by \( \approx 7.1 \pm 1.8 \) mmHg (\( P < 0.05 \)), whereas HR was unaltered (\( P = \text{NS} \)).

L-Arginine. Infusion of L-arginine into the femoral artery reversed the NOS inhibition and slightly increased FABF as well as SkBF (\( P < 0.02 \); Fig. 4). The corresponding leg \( \dot{V}O_2 \) extraction decreased from 70.6 \( \pm \) 5.3 to 35.4 \( \pm \) 2.8 ml/l (\( P < 0.0005 \)) but slightly increased leg \( \dot{V}O_2 \) from 12.4 \( \pm \) 3.4 to 19.7 \( \pm \) 3.0 ml/min (i.e., from \( \approx 0.42 \) to 0.67 ml\( \cdot \)min\(^{-1}\)\( \cdot \)100 g\(^{-1}\), \( P < 0.01 \)). The net release of lactate showed an \( \approx 1.3 \)-fold increase (\( P < 0.03 \)). MAP and HR remained unaltered (\( P = \text{NS} \); Fig. 4). All variables returned to rest control level within 5 min after termination of the L-arginine infusion.

SNP and ACh

Continuous infusion. SNP and ACh infusion (Fig. 5) increased FABF dose dependently (\( P < 0.04 \)) to a transient peak level of 4.08 \( \pm \) 0.71 and 5.41 \( \pm \) 0.61 l/min (\( \approx 138 \) and \( \approx 183 \) ml\( \cdot \)min\(^{-1}\)\( \cdot \)100 g\(^{-1}\)), respectively. Leg \( \dot{V}O_2 \) extraction decreased (\( P < 0.006 \)) dose dependently during the SNP and ACh infusions from 46.0 \( \pm \) 2.9 and 64.5 \( \pm \) 5.0 ml/l, respectively, to level off (\( P = \text{NS} \)) at 6.6 \( \pm \) 1.2 and 7.0 \( \pm \) 3.7 ml/l, respectively. Leg \( \dot{V}O_2 \) was unaltered (\( P = \text{NS} \)) at 11.7 \( \pm \) 2.7 and 10.4 \( \pm \) 2.4 ml/min (\( \approx 0.40 \) and \( \approx 0.35 \) ml\( \cdot \)min\(^{-1}\)\( \cdot \)100 g\(^{-1}\)), respectively. The net release of lactate was unaltered at 0.012 \( \pm \) 0.007 mmol/min (\( P = \text{NS} \)). After termination of the SNP and ACh infusions the half-life of the FABF response was 27 \( \pm \) 2 and 33 \( \pm \) 3 s, respectively.
DISCUSSION

The major finding of the present study was that inhibition of NOS by L-NMMA caused a potent and persistent reduction in FABF during rest, including postexercise recovery. However, NOS inhibition had no effect on FABF during dynamic exercise. This lack of effect was apparent during passive leg movement as well as in the initial and steady-state phases of submaximal voluntary exercise and at the end of peak intensity. Thus normal exercise hyperemia can occur, even though NOS is inhibited. Moreover, the NOS inhibition did not alter the leg VO₂.

Of note is also the effect of the passive leg movements, which momentarily elevated FABF almost five-fold. This is a function of mechanical factors squeezing blood out of the knee-extensor muscles and increasing the flow as passive filling of the vascular beds. However, the enlarged shear stress during the passive leg movements did not cause any further elevation in FABF that was associated with a release of NO, inasmuch as no differences in FABF were found between control and NOS inhibition bouts. In contrast, the first voluntary contractions caused a marked further elevation in FABF beyond the impact of the muscle mechanical component, indicating release of vasoactive agents. Thus the experiments with passive leg movements indicate that a shear stress-induced release of NO is of minor importance in comparison to muscle mechanical factors and not essential for the FABF increase at onset of exercise.

Fig. 5. Steady-state FABF, VC, SkBF (relative rest), HR, and MAP during infusion of sodium nitroprusside (SNP, A) and ACh (B) at incremental rates for 3 min each into femoral artery at rest. SNP and ACh infusions were separated by ~30 min. FABF increased dose dependently (P < 0.04) after an onset latency of 21.4 ± 3.8 and 37.3 ± 7.5 s, respectively. FABF stabilized during SNP infusion at level 38 ± 3% lower (P < 0.001) than its dose-dependent maximum, whereas FABF remained unaltered from its peak level (not significant) throughout ACh infusion. Note small SkBF response. SkBF dose dependently and gradually increased during SNP and ACh infusion (P < 0.001) to reach a maximum 2.6 ± 0.5- and 4.6 ± 1.2-fold rise above rest control, respectively, which at most constituted ~2.9 and ~1.9% of FABF, respectively. *Significantly different (P < 0.05) from rest. # Leveling off (not significant) in response.
Blood Flow Measurements in Previous Studies on the Role of NO

Recently, Shoemaker et al. (24) used ultrasound Doppler to measure forearm blood flow during rhythmic hand grip. Light exercise was performed before and after infusion of atropine or atropine + L-NMMA. Neither ACh nor NO was found to modulate the time course or the magnitude of blood flow response to exercise. The study was, however, not conclusive, since the NOS inhibition was not studied per se. Furthermore, atropine made it impossible to verify the potency of the NOS inhibition. Also, Shoemaker et al. did not address the role of NO during dynamic exercise at higher intensities.

Moreover, in the study of Shoemaker et al. (24) the blood velocity was averaged on a beat-by-beat basis in relation to an ECG. Such ECG-averaged velocities vary markedly because of variations in intramuscular pressure as well as the temporal dissociation between the cardiac and exercise cycles (18, 28). In addition, any failure in the insonation of the artery may be conveyed in the general variability of this measurement procedure (17). Thus the ability of this procedure to follow the velocity profiles and transitional changes in blood flow is compromised (17). To reduce the variability, Shoemaker et al. designed their study and analyzed their velocities over 3-s time intervals to include a contraction (1 s) and a relaxation (2 s) phase in each value. This, however, impairs the optimal temporal resolution of the ultrasound-Doppler method. In the present study, these limitations were overcome by sampling the blood velocity continuously...
and analyzing it in relation to each exercise duty cycle (17).

Our ultrasound-Doppler measurements have further advantages compared with plethysmography, inasmuch as the ultrasound Doppler allows continuous measurements during the contraction and the relaxation phase. Moreover, the differential effects of NO at rest, including recovery, compared with exercise, as found in the present study as well as by Shoemaker et al. (24), emphasize that previous studies on the role of NO with use of plethysmographic extrapolations during recovery to represent exercise must be interpreted with caution (4, 6, 8, 30).

Comparison to Previous Studies on the Role of NO

The previous diverse findings on the role of NO in exercise hyperemia (4–6, 8, 9, 11, 13, 15, 16, 19, 30) have been suggested to be due to differences in experimental design, type and intensity of exercise, or differences in the species and muscle types. In the human studies the dose of L-NMMA, as well as the timing and duration of administration, has varied. L-NMMA was initially infused into the brachial artery at rest at 0.1–0.2 mg·min⁻¹·100 ml forearm volume⁻¹ (30) and at −0.75–3.0 mg/min (6, 8). To ensure that the NOS inhibitor reached the resistance vasculature, which possibly was not open at rest, L-NMMA was subsequently infused also during exercise at 1–4 mg/min (4, 24). Therefore, in the present study, L-NMMA was also continuously infused during rest, exercise, and recovery at a dose similar to or greater than that used in previous studies (4, 6, 8, 24, 30).

Collectively, the present data indicate that our NOS inhibition was sufficient. In agreement with Vallance et al. (27), we found a peak plateau in the inhibitory response during the loading dose. The inhibition was also sustained during the maintenance dose as well as in recovery after exercise. Our decrease in blood flow by 50–60% at rest was, furthermore, larger than the 25% reported by Gilligan et al. (8), ~30% found by Shoemaker et al. (24), and ~40% observed by Wilson and Kapoor (30) but slightly lower than the ~70% reported by Endo et al. (6). Dyke et al. (4) did not study the effect at rest.

The effectiveness of our NOS inhibition was also strengthened by the ~90, 69, and 36% attenuation of the blood flow response to ACh infused at 16, 48, and 144 µg·min⁻¹·1 thigh volume⁻¹, respectively. Thus our blood flow attenuation was 1) greater than in the plethysmographic studies that supported a role for NO during exercise (~25–30% attenuation to ACh at 16 µg/min (4) and ~31 ± 21% attenuation to ACh at 7.5, 15, and 30 µg/min (8)) and 2) similar to or greater than the attenuation in the plethysmographic studies that rejected a role for NO during exercise (~80% attenuation to ACh at 5 µg·min⁻¹·100 ml forearm volume⁻¹ (6) and ~56% attenuation to ACh at 120 nmol/min, i.e., ~21.8 µg/min (30)). Part of the response to ACh may not be blocked because of an additional ascending vasodilatation or release of other endothelium-derived vasodilators (3, 7). Our theory of NOS inhibition was further supported by the finding that it persisted until it was reversed by intra-arterially infused L-arginine.

Even though a lack of effect of the NOS inhibition on exercise hyperemia does not totally exclude a role for NO, it demonstrates that the role of NO is not essential for the exercise response. It has been suggested, however, that a redundancy of other vasodilators could mask the effect of the NOS inhibition and that this would be most apparent during intensive exercise. Therefore, some argue that the effect of the NOS inhibition would be most obvious during mild exercise and especially in fatigue-resistant muscles with a high percentage of slow-twitch oxidative fibers, where the flow is “luxurious” and there is no need for release of other metabolic vasodilators (4, 9, 15). However, during very mild exercise a large portion of the blood flow increase may actually be governed by muscle mechanical factors (18, 22, 23, 26). Moreover, the NOS inhibitor has also been suggested to be more diluted at the large blood flows found during exercise and, therefore, to exert a smaller effect. However, this seems invalid, since the NOS inhibition potently decreases the blood flow at similar high flows during recovery from exercise as well as during intra-arterial infusion of ACh. It is, furthermore, important to note that we aimed to determine the role of NO in skeletal muscle of humans, where the fiber type distribution is ~50% slow- and ~50% fast-twitch fibers (20). We also reasoned that if NO was essential, its role would be larger at higher than at lower intensities. In addition, if NO was crucial for the exercise response, redundant mechanisms would not likely be able to fully compensate for NOS inhibition as potent as that achieved in our study.

Control Experiments With SNP and ACh

To further determine the vasodilator potency of NO in our subjects, intra-arterial infusion of the NO donor SNP as well as the NOS stimulator and ascending dilator ACh was performed. Both proved to be powerful vasodilators. The maximum ~15- to 25-fold increase in FABF during intra-arterial infusion of SNP and ACh did, however, not reach the maximum levels of blood flow, as found during exercise at peak effort in humans. Thus this may exclude them as potential sole determinants of peak exercise hyperemia in humans, which is in agreement with an additive effect of the muscle pump and metabolic vasodilators (18, 21–23, 25, 26).

Limb \( \dot{V}O_2 \)

Although it has been suggested that NO may impose a tonic inhibitory influence on cellular and mitochondrial respiration (12), NOS inhibition with L-NMMA did not alter the limb \( \dot{V}O_2 \). A decrease in blood flow induced at rest and during recovery was directly compensated for by an increase in the \( \dot{O}_2 \) extraction. Further support for an unaffected energy metabolism stems from the finding that the lactate fluxes were not affected by the NOS inhibition. The larger \( \dot{O}_2 \) extrac-
tion and unchanged anaerobic metabolism may then point to a reduction in the flow velocity in all the open capillaries of the microcirculation, rather than closing of sections of the capillary bed. An increased mean transit time could then allow for the enlarged O₂ extraction. An alternative explanation is that sections of capillaries are closed and that those mitochondria in which blood continues to flow in their vicinity actually are respiring at a higher rate than when NO was present under control conditions.

Summary
This study provides unique information concerning the differential roles of NO as a mediator of vasomotor tone in skeletal muscle of humans. The present ultrasound-Doppler blood flow measurements surmount previous methodological limitations for evaluating the role of NO during exercise in humans, inasmuch as it allows continuous measurements during rest, exercise, and recovery. By use of NOS inhibition with L-NMMA, it was demonstrated that NO is not essential for skeletal muscle hyperemia during the initiation of exercise and during sustained submaximal exercise as well as at termination at peak effort. Moreover, the lack of effect of the NOS inhibition on blood flow during passive leg movement and at onset of exercise indicates that the influence of muscle mechanical factors is of greater importance than a shear stress-induced release of NO. The study, however, confirms the role of NO in regulating basal vascular tone and ~50–60% of FABF at rest. NO also contributes to ~35% of the blood flow in recovery after exhaustive exercise. In addition, changes in blood flow caused by NOS inhibition in vivo in humans during rest including recovery are accompanied by reciprocal changes in leg O₂ extraction, with no net effect on leg VO₂.

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