Nitric Oxide Synthase Inhibition Reduces Leg Glucose Uptake but not Blood Flow During Dynamic Exercise in Humans

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Nitric oxide (NO) appears to play a role in contraction-stimulated glucose uptake in isolated rodent skeletal muscle; however, no studies have examined this question in humans. Seven healthy men completed two 30-min bouts of supine cycling exercise at 60 ± 2% peak pulmonary oxygen uptake (Vo_2 peak), separated by 90 min of rest. The NO synthase inhibitor N^G-monomethyl-L-arginine ([L-NMMA]; total dose 5 mg/kg body weight) or saline (control) were administered via the femoral artery for the final 20 min of exercise in a randomized blinded crossover design. L-Arginine (5 mg/kg body weight) was co-infused during the final 5 min of each exercise bout. Leg blood flow (LBF) was measured by thermodilution in the femoral vein, and leg glucose uptake was calculated as the product of LBF and femoral arteriovenous (AV) glucose difference. L-NMMA infusion significantly ($P < 0.05$) reduced leg glucose uptake compared with control (48 ± 12% lower at 15 min, mean ± SE). The reduction in glucose uptake was due solely to a decrease in AV glucose difference, as there was no effect of L-NMMA infusion on LBF during exercise. Co-infusion of L-arginine restored glucose uptake during L-NMMA infusion to levels similar to control. These results indicate that NO production contributes substantially to exercise-mediated skeletal muscle glucose uptake in humans independent of skeletal muscle blood flow. *Diabetes* 48:1815–1821, 1999

Since the discovery of nitric oxide (NO) as an important mediator of vasoregulation, NO has been found to be a diverse biological messenger (1,2). More recently, the role of NO in skeletal muscle metabolism and contractile function has been increasingly recognized (3).

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ANOVA, analysis of variance; AV, arteriovenous; cGMP, cyclic guanosine monophosphate; ECG, electrocardiogram; eNOS, endothelial isoform of nitric oxide synthase; LBF, leg blood flow; L-NAME, N^ω-nitro-L-arginine methyl ester; L-NMMA, N^G-monomethyl-L-arginine; nNOS, neuronal isoform of nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; PDE-5, phosphodiesterase type 5; RQ, respiratory quotient; SNP, sodium nitroprusside; STPD, standard temperature (0°C) and pressure (760 mmHg) for dry gas; 2-DG, 2-deoxyglucose; Vo_2 peak, peak pulmonary oxygen uptake.

During exercise, the large increase in blood glucose utilization by skeletal muscle is mediated by the translocation of the glucose transporter GLUT4 from an intracellular pool to the sarcolemma of skeletal muscle myocytes. The exact mechanisms underlying contraction-induced GLUT4 translocation are unclear; however, it has recently been found that NO mediates glucose uptake into skeletal muscle during exercise by affecting this GLUT4 translocation (4). NO donors, such as sodium nitroprusside (SNP), cause a dose-dependent increase in skeletal muscle glucose transport (5–7) and result in an increase in cell-surface GLUT4 in isolated rat skeletal muscle preparations (6). Nitric oxide synthase (NOS) inhibition with the competitive NOS inhibitor N^G-monomethyl-L-arginine (L-NMMA) reduces both basal and contraction-stimulated 2-deoxyglucose (2-DG) transport in isolated rat extensor digitorum longus (5). Furthermore, NOS inhibition with N^ω-nitro-L-arginine methyl ester (L-NAME) administered before exercise prevents an increase in GLUT4 in sarcolemmal membranes from rat skeletal muscle during exercise (4). No studies have been undertaken in humans to investigate the potential role of NO in skeletal muscle glucose uptake during exercise.

Glucose uptake into skeletal muscle is dependent on both glucose extraction (i.e., arteriovenous [AV] glucose difference) and muscle blood flow. Not only may NO mediate skeletal muscle glucose uptake directly by contributing to glucose extraction, but it may also affect metabolism indirectly by regulating skeletal muscle blood flow. Although the continual release of NO is important for resting vascular tone (8,9), the role of NO in exercise hyperemia is less certain. Most (10–15), but not all (16–18), studies in animals suggest that NO has an important role in exercise hyperemia. Investigations of the role of NO in exercise hyperemia in humans have provided conflicting results. Whereas some studies in the forearm have reported a decrease in forearm blood flow during exercise following NOS inhibition (19,20), others have found no role for NO in exercise hyperemia (21–23). The interpretation of these results is confounded by the necessity to interrupt exercise for a brief period while forearm blood flow is measured by venous occlusion plethysmography. The only two studies examining the role of NO in blood-flow regulation during large muscle group leg exercise again provide conflicting results. Rådegran and Saltin (24) reported that femoral artery blood flow measured by Doppler ultrasound was not influenced by infusion of L-NMMA during prolonged single-leg extension exercise. By contrast, Hickner et al. (25) reported that regional vastus lateralis muscle blood flow during cycling exercise was substantially reduced around microdialysis probes infused with

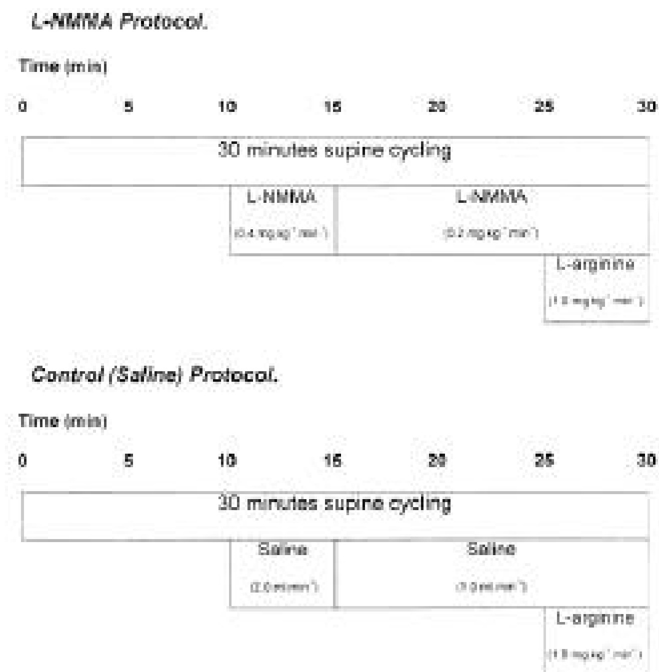

FIG. 1. Experimental protocol. Subjects performed both the L-NMMA protocol and the control protocol in a counterbalanced double-blind crossover design, separated by ~90 min of rest. L-NMMA concentration was such that infusion of L-NMMA occurred at the same rate as the saline infusion (i.e., 2.0 and 1.0 ml/min, respectively).

L-NMMA. Clearly, the role of NO in exercise hyperemia during dynamic exercise remains to be resolved.

Therefore, the aims of the current study were twofold: first, to determine the role of NO in skeletal muscle glucose uptake in humans during exercise; and second, to determine whether NO plays a role in exercise hyperemia during dynamic lower-limb exercise.

RESEARCH DESIGN AND METHODS

Subjects. After providing written informed consent, seven men aged 23–35 years participated in the study, which was approved by the Alfred Hospital Ethics Committee and conducted in accordance with the Declaration of Helsinki of the World Medical Association. All subjects were unmedicated nonsmokers with BMI < 25 kg/m^2, blood pressure <140/90 mmHg, fasting glucose <6.0 mmol/l[1], total cholesterol <5.5 mmol/l[1], and triglycerides <2.0 mmol/l[1]. All were normally active but were not specifically exercise trained. Before participation, all subjects received a medical examination, including a 12-lead electrocardiogram (ECG).

Subject preparation. During the initial visit, subjects performed continuous incremental upright cycling to volitional exhaustion on an electronically-braked ergometer (Ergo-metrics 900 ergometer; Ergoline, Bitz, Germany) for determination of peak pulmonary oxygen uptake (V_{O_2} peak). Expired air was analyzed for volume, O_2, and CO_2 using calibrated analyzers (Medical Graphics CAD/Net System 2001, St. Paul, MN). During a subsequent visit, subjects were familiarized with supine cycling during a single 30-min bout on an electronically-braked ergometer (380B ergometry system; Siemens-Elema, Stockholm). Supine exercise was performed at a workload eliciting 60% of the upright V_{O_2} peak determined during the initial visit. This session was completed at least 3 days, but no more than 7 days, before the experimental trial. Preliminary studies in five subjects demonstrated that supine V_{O_2} peak was equivalent to upright V_{O_2} peak, confirming the work of Perrault et al. (26). Therefore, in all subsequent studies, upright V_{O_2} peak was used to determine the required supine exercise workload.

Experimental design. Subjects performed two submaximal 30-min bouts of supine cycling, separated by 90 min of rest. After the first 10 min of each exercise session, subjects were administered either L-NMMA or saline (control) into the femoral artery in a counterbalanced double-blinded crossover design. These infusions were continued for the remainder of the exercise bout. During the final 5 min of each exercise session, subjects received a co-infusion of L-arginine (Fig. 1). Arterial and venous femoral blood was obtained, and leg blood flow (LBF) was measured at rest and during exercise.

Experimental procedures. Subjects were requested to refrain from exercise, alcohol, and caffeine for at least 24 h before the experimental trial. After an overnight fast, subjects attended the Alfred Hospital at 8:00 A.M. Teflon catheters were placed in the right femoral artery (4.0F; Cook Australia, Brisbane, Australia), and right femoral vein (5.0F; Cook Australia) under local anaesthetic (1% lignocaine; Astra, Sydney, Australia) using strict aseptic conditions, and the tips advanced centrally to ~2–3 cm above, and just below, the inguinal ligament, respectively. A thermistor probe (Edslab 94-030-2.5F; Baxter Healthcare, Irvine, CA) was inserted through the venous catheter and advanced ~8 cm beyond the catheter tip (27). The catheters were used for simultaneous arterial/venous blood sampling, arterial blood pressure measurement, arterial drug infusions, and for venous blood flow measurement. Chest electrodes were positioned for monitoring of heart rate by ECG. Blood pressure, blood temperature, and infusate temperature were digitized at 500 Hz using a 486/50 IBM-compatible PC and a data-acquisition system incorporating a 12-bit analogue-to-digital converter (McPherson Scientific, Melbourne, Australia). Systolic, diastolic, and mean blood pressure and heart rate were derived on a beat-to-beat basis from the blood pressure signal using a variable threshold peak-detection technique. Electronic calipers were used to average these signals over appropriate time intervals.

After the subjects rested for 30 min, blood samples were simultaneously obtained from the two catheters, LBF was measured, and heart rate and blood pressure were recorded. Subjects then cycled at the predetermined workload eliciting 60 ± 2% V_{O_2} peak (mean ± SE) for ~30 min (Fig. 1). After 10 min of cycling, an intra-arterial infusion of either L-NMMA solution at 2 ml/min (L-NMMA: 0.4 mg · kg^{-1} body mass · min^{-1}) or saline (2 ml/min) was commenced via the right femoral artery. This loading-dose infusion of L-NMMA continued for 5 min (i.e., from 10–15 min of exercise), after which the infusion rate was decreased to 1 ml/min L-NMMA solution (L-NMMA 0.2 mg · kg^{-1} · min^{-1}) or 1 ml/min saline for the remainder of the exercise bout (i.e., from 15–30 min exercise). The total dose of L-NMMA administered was 5 mg/kg. L-NMMA infusion rate was based on pilot studies in six subjects and designed to induce peripheral but not systemic effects. During the final 5 min of each exercise session, a co-infusion of L-arginine solution at 2 ml/min (L-arginine 1 mg · kg^{-1} · min^{-1}, total dose 5 mg/kg) was administered (i.e., from 25–30 min of exercise). After 90 min of rest, a second 30-min exercise bout with the other infusion protocol was performed. The infusion protocols were administered in a counterbalanced design.

At 10, 15, 20, 25, and 30 min of exercise, LBF and pulmonary oxygen uptake were measured and heart rate recorded. At these times, the intra-arterial infusion (L-NMMA ± L-arginine or saline ± L-arginine) was discontinued for a short time (–1 min) to allow simultaneous arterial and venous blood sampling and recording of femoral arterial blood pressure.

Preparation of drug infusions. A total of 550 mg of L-NMMA (Calbiochem-Novabiochem, Läufalfingen, Switzerland) was diluted in 0.9% NaCl to a concentration of 0.2 mg L-NMMA · kg^{-1} body mass · ml^{-1} saline. An equal volume of saline was prepared to act as control. A total of 1,000 mg of L-arginine (Clinalfa; Calbiochem-Novabiochem) was diluted in 0.9% NaCl to a concentration of 0.5 mg · kg^{-1} body mass · ml^{-1}. At the commencement of each infusion and after arterial blood samples were taken, the infusion line was primed with the appropriate solution before continuation of the infusion at the appropriate rate.

LBF. Right femoral venous blood flow was measured by constant-rate infusion of cold saline according to the thermodilution principle (28). Cold saline was drawn from a reservoir and then immediately infused (–5°C) through the femoral venous catheter using an Angiomat 3000 Injector (Leibel-Flarsheim, Sybron, Cincinnati, OH). At rest, LBF was measured in triplicate using an infusion rate of 0.7 ml/s (42 ml/min) for 20 s each. During exercise, venous blood flow was measured in duplicate using an infusion rate of 1.5–2.4 ml/s (90–144 ml/min) for 15 s, titrated to produce ~0.9–1.2°C decrease in blood temperature (28). The coefficient of variation in blood flow measurement during exercise was 4.3%.

Blood sampling and analysis. Simultaneous blood samples were drawn from the femoral artery (10 ml) and vein (8 ml) at 0, 10, 15, 20, 25, and 30 min of each exercise bout and separated as follows: heparinized syringe (2 ml) for measurement of blood gases, lithium heparin tube (2 ml) for insulin, and sequestrene tubes (4 ml) for glucose and lactate. The heparinized blood was immediately placed on ice and analyzed within 15 min for temperature-corrected P_{O_2}, P_{CO_2}, and pH (ABL500 blood gas analyzer; Radiometer, Copenhagen, Denmark) and for percent saturation of hemoglobin (SO_2) and hemoglobin [Hb] concentration in g/100 ml (OSM2 hemoximeter; Radiometer, Copenhagen, Denmark). Oxygen content (in ml/100 ml at standard temperature [0°C] and pressure [760 mmHg] for dry gas [STPD]) was calculated as the sum of bound and dissolved oxygen by the equation $(SO_2/100) \times [Hb] \times 1.34 + \alpha P_{O_2}$, in which 1.34 is the amount of O_2 (in ml) carried by 1 g of Hb when fully saturated (29), α is the solubility coefficient for O_2 in whole blood (0.0031 ml [STPD] of O_2/100 ml of blood per mmHg of O_2 at 37°C), and P_{O_2} is the partial pressure of O_2 in mmHg. Carbon dioxide content was calculated as de-scribed by Douglas et al. (29a), and used to determine leg respiratory quotient (RQ).

Blood for biochemical analysis was immediately placed on ice, then centrifuged with the plasma frozen at –20°C for later analysis. Plasma glucose con-

TABLE 1
Subject characteristics

Age (years)	27 ± 2
Height (m)	1.78 ± 0.03
Weight (kg)	74.3 ± 2.4
BMI (kg/m^2)	23.4 ± 0.5
Systolic blood pressure (mmHg)	115 ± 3
Diastolic blood pressure (mmHg)	69 ± 4
Fasting blood glucose (mmol/l)	5.4 ± 0.1
Cholesterol (mmol/l)	4.2 ± 0.3
Triglycerides (mmol/l)	0.9 ± 0.1
V_{O_2} peak (l/min)	3.51 ± 0.19
V_{O_2} peak ($ml \cdot kg^{-1} \cdot min^{-1}$)	47.7 ± 3.5

Data are means ± SE.

centration (Sigma Diagnostics, St. Louis, MO) and plasma lactate concentration (Roche [Boehringer Mannheim], Sydney, Australia) were measured in triplicate using enzymatic spectrophotometric techniques. Arterial plasma insulin concentration was measured in duplicate by radioimmunoassay (Linco Research, St. Louis, MO). The product of AV difference and LBF was used to calculate whole-leg oxygen consumption, leg glucose uptake, and leg net lactate release.

Statistics. All results are expressed as means ± SE. Baseline parameters were compared using paired *t* test to determine the effects of treatment and order. Measurements made during the two exercise protocols were compared using analysis of variance (ANOVA) for repeated measures to determine the effects of treatment, time, and order of intervention. The Fisher's least significant differences test was used to compare individual means. The null hypothesis was rejected when $P < 0.05$.

RESULTS

Subject characteristics are presented in Table 1. In the trials, subjects exercised at 142 ± 9 W, representing 60 ± 2% of upright V_{O_2} peak. There were no order effects between trials, although heart rate tended to be higher during the second exercise bout than the first ($P = 0.06$).

Hemodynamics. There was no difference in resting heart rate or blood pressure before the two protocols (Table 2). Heart rate and blood pressure increased from rest in both trials; however, there was no difference between the two protocols. Importantly, there were no effects on blood pressure or heart rate of any of the drug infusions (Table 2).

LBF. Due to technical difficulties, a value for LBF could not be obtained in one subject. There was no difference in resting LBF before exercise, although LBF tended to be higher before the control trial than before the L-NMMA trial (control 351 ± 38 ml/min and L-NMMA 278 ± 15 ml/min, $P = 0.18$) (Fig. 2). After 10 min of exercise, before commencement of infusions, LBF again tended to be higher during the control trial ($P = 0.09$, Fig. 2). There was no difference in LBF between the two protocols, although the nonsignificant difference in LBF observed at 10 min was maintained throughout exercise: LBF averaged 2,910 ± 260 ml/min during the L-NMMA protocol and 3,150 ± 370 ml/min during the control protocol. Most importantly, the L-NMMA infusion did not result in a change in LBF compared with flow at 10 min (Fig. 2). That is to say, although LBF was slightly lower in the L-NMMA trial compared with the control trial at 10 min, the L-NMMA infusion was associated with no further change in LBF. There was no effect of order of intervention ($P = 0.21$).

Leg glucose uptake. Before the commencement of exercise, arterial glucose concentration (Table 3) and leg glucose uptake (Fig. 2) were similar in the two trials. During exercise, leg glucose uptake markedly increased compared with that during rest in both trials, due to increases in both AV glucose difference and LBF (Fig. 2). Arterial glucose concentration was not different between the two trials throughout exercise (Table 3), and AV glucose difference and leg glucose uptake were similar at 10 min of exercise in both protocols. The L-NMMA protocol was associated with a decrease in leg glucose uptake compared with the control protocol ($P < 0.05$). Leg glucose uptake at 15 min during the L-NMMA protocol was reduced to 58 ± 13% of leg glucose uptake at 10 min and was 52 ± 12% of leg glucose uptake at 15 min in the control protocol. This decrease in leg glucose uptake in L-NMMA was entirely due to a decrease in AV glucose difference, as LBF was unchanged. After co-infusion of L-arginine, there was no difference in leg glucose uptake between the L-NMMA protocol and the control protocol (i.e., at 30 min exercise) (Fig. 2). There was no significant effect of order of intervention on leg glucose delivery ($P = 0.22$) or leg glucose uptake ($P = 0.97$).

Leg oxygen consumption. Femoral AV oxygen difference increased significantly from rest to 10 min of exercise during both protocols (Table 3). Throughout exercise, there was no difference in whole-leg oxygen extraction between the two

TABLE 2
Blood pressure and heart rate throughout the L-NMMA and control trials

		Exercise (min)				
	Rest	10	15	20	25	30
Systolic blood pressure (mmHg)						
L-NMMA	119 ± 6	142 ± 5	141 ± 5	143 ± 4	143 ± 5	140 ± 4
Control	126 ± 4	143 ± 4	144 ± 5	145 ± 3	142 ± 3	141 ± 3
Diastolic blood pressure (mmHg)						
L-NMMA	69 ± 4	78 ± 2	80 ± 3	77 ± 3	78 ± 4	77 ± 4
Control	70 ± 2	78 ± 3	78 ± 3	80 ± 4	76 ± 4	77 ± 4
Mean blood pressure (mmHg)						
L-NMMA	88 ± 4	107 ± 4	107 ± 5	106 ± 4	106 ± 4	105 ± 4
Control	91 ± 3	107 ± 4	106 ± 4	108 ± 4	104 ± 4	105 ± 4
Heart rate (min^{-1})						
L-NMMA	63 ± 2	139 ± 4	139 ± 4	139 ± 3	142 ± 3	141 ± 3
Control	61 ± 3	139 ± 2	141 ± 2	145 ± 3	145 ± 3	146 ± 3

Data are means ± SE. L-NMMA infusion (L-NMMA protocol) or saline infusion (control protocol) commenced after 10 min of exercise. Subjects received co-infusion of L-arginine during the final 5 min of each protocol.

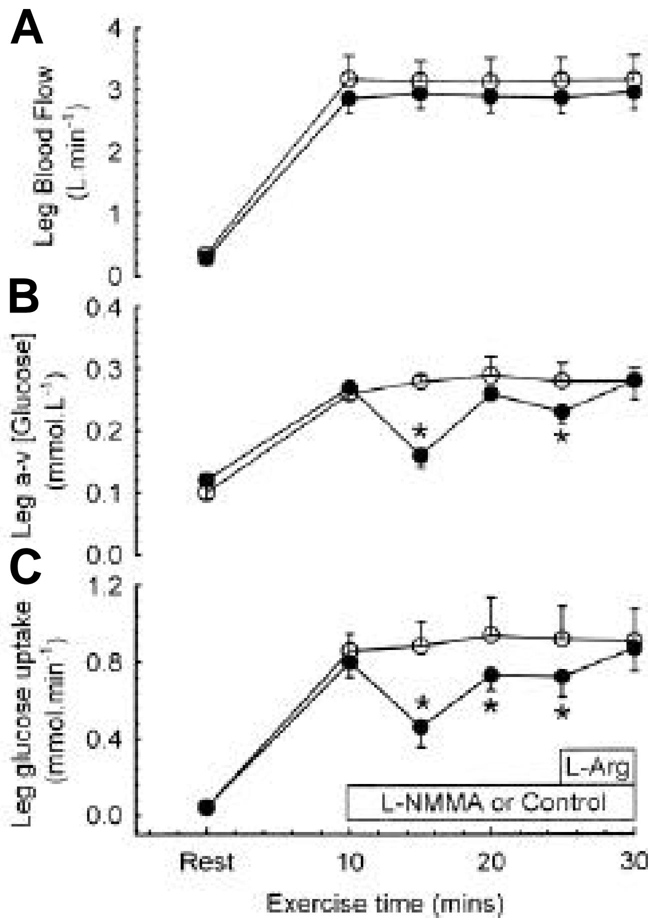

FIG. 2. LBF (*A*), leg AV glucose difference (*B*), and leg glucose uptake (*C*) in six healthy male subjects (*n* = 7 for AV glucose difference) during 30 min of supine cycling. Values are means ± SE. ●, L-NMMA protocol; ○, control protocol. * *P* < 0.05 between trials.

protocols, and importantly, L-NMMA did not cause a change in AV oxygen difference during exercise (Table 3). This was reflected in total leg oxygen consumption (product of LBF and AV oxygen difference), which was not different between the two protocols (Table 3). Furthermore, there was no difference in leg RQ between the two protocols (Table 3).

Plasma lactate. Resting arterial lactate was similar in both protocols (Table 3). During exercise, arterial lactate increased from resting values; however, there was no difference between the L-NMMA and control trials. There was no difference in AV lactate difference between the two trials (results not shown).

Plasma insulin. Arterial plasma insulin concentrations were similar at rest and throughout exercise during both protocols (Table 3). Insulin tended to decrease from rest to exercise in the two protocols. L-NMMA infusion had no effect on arterial insulin levels (P = 0.09), although insulin tended to increase in both trials by co-infusion of L-arginine (Table 3).

DISCUSSION

During exercise, skeletal muscle glucose uptake and blood flow substantially increase. Although much information has accumulated to describe these processes, the regulatory mechanisms associated with them are still to be fully elucidated. Recently, the role of NO in skeletal muscle metabolism has received much attention; however, this is the first study in humans to comprehensively investigate the role of NO in skeletal muscle blood flow and glucose uptake during exercise. Our results suggest that NO production is not essential for exercise hyperemia, but contributes substantially to muscle glucose uptake during exercise.

Glucose uptake. The decrease in glucose uptake with L-NMMA administration was due solely to a decrease in leg glucose extraction (i.e., decrease in AV glucose difference), because LBF was not affected by the L-NMMA infusion (Fig. 2).

TABLE 3
Gas exchange and metabolic parameters

	Rest	Exercise (min)				
		10	15	20	25	30
Leg A-V O_2 difference (ml/100 ml)						
L-NMMA	5.0 ± 0.5	14.5 ± 0.4	14.8 ± 0.3	14.7 ± 0.3	14.4 ± 0.3	14.4 ± 0.2
Control	4.8 ± 0.3	14.3 ± 0.4	14.3 ± 0.4	14.3 ± 0.4	14.3 ± 0.5	14.4 ± 0.5
Leg O_2 uptake (ml/min)						
L-NMMA	13 ± 2	425 ± 44	446 ± 46	434 ± 42	422 ± 43	433 ± 38
Control	16 ± 1	465 ± 61	459 ± 56	458 ± 64	464 ± 60	467 ± 67
Leg RQ						
L-NMMA	0.82 ± 0.02	0.97 ± 0.01	0.95 ± 0.04	0.97 ± 0.02	0.99 ± 0.02	0.99 ± 0.02
Control	0.87 ± 0.02	0.94 ± 0.02	0.96 ± 0.03	0.96 ± 0.03	0.97 ± 0.01	0.99 ± 0.02
Arterial glucose (mmol/l)						
L-NMMA	5.40 ± 0.13	4.99 ± 0.14	4.89 ± 0.20	4.92 ± 0.19	4.86 ± 0.21	4.87 ± 0.21
Control	5.41 ± 0.03	4.98 ± 0.11	4.96 ± 0.11	4.89 ± 0.14	4.90 ± 0.15	4.89 ± 0.14
Arterial insulin (µU/ml)						
L-NMMA	7.0 ± 0.8	4.9 ± 0.8	5.9 ± 1.1	—	4.6 ± 0.9	7.4 ± 1.8
Control	7.6 ± 0.6	5.2 ± 0.7	5.2 ± 0.2	—	5.3 ± 0.3	6.7 ± 0.5
Arterial lactate (mmol/l)						
L-NMMA	0.92 ± 0.12	3.90 ± 0.39	3.79 ± 0.46	3.40 ± 0.39	3.17 ± 0.35	3.11 ± 0.38
Control	0.72 ± 0.04	3.38 ± 0.71	3.09 ± 0.71	3.21 ± 0.81	3.14 ± 0.71	3.16 ± 0.78

Data are means ± SE for single-leg oxygen uptake, RQ, arterial glucose, insulin, and lactate at rest and throughout exercise during the L-NMMA and control protocols. Leg A-V O_2 difference, femoral arteriovenous oxygen difference.

It has previously been suggested that NO mediates exercise-stimulated glucose uptake, perhaps by playing an integral role in the contraction-induced translocation of GLUT4 from an intracellular pool to the sarcolemma of skeletal muscle (4). L-NAME administered in the drinking water of Sprague-Dawley rats before exercise completely prevented the increase in glucose transport after ~40 min of exhaustive treadmill running (4). In addition, NOS inhibition prevented the normal exercise-induced sarcolemmal GLUT4 translocation (4). Our present study demonstrated that inhibition of NO production with L-NMMA in vivo resulted in a 42 ± 13% reduction in leg (and presumably skeletal muscle) glucose uptake during exercise in humans (Fig. 2). Although GLUT4 translocation was not determined in the present study, it is probable that a decrease in skeletal muscle GLUT4 translocation was associated with the reduction in glucose uptake seen with infusion of L-NMMA (4). There were no changes in arterial plasma glucose or insulin during the L-NMMA trial that could explain the reduction in glucose uptake (Table 3). Furthermore, after co-infusion of L-arginine, there was no difference in glucose uptake between L-NMMA and control (Fig. 2). We feel that this indicates that the reduction in glucose uptake during L-NMMA infusion was due to a blocking of NOS rather than nonspecific effects of L-NMMA on glucose uptake.

There is evidence that NO is produced in both the skeletal muscle blood vessels and the muscle fibers themselves. NO production occurs not only in larger resistance vessels, but also in skeletal muscle microvascular endothelium (31), and indeed the endothelial isoform of NOS (eNOS) is present in human skeletal muscle microvasculature (32). It is reasonable to suggest that the NO produced in these vessels during exercise may diffuse to the adjacent skeletal muscle fibers in the same way that it diffuses into vascular smooth muscle in resistance vessels. Furthermore, in recent years, the neuronal isoform of NOS (nNOS) has been identified within human skeletal muscle fibers (32,33). NO is released from resting rat extensor digitorum longus preparations in vitro (34), and prior electrical stimulation causes an increase in NO release (34). It is therefore possible that NO in skeletal muscle provides both paracrine (i.e., from vascular endothelium) and autocrine (i.e., produced within the muscle fiber) regulation of exercise-stimulated glucose uptake.

NO stimulates soluble guanylate cyclase in most tissues (35), and NO donors such as SNP have been shown to increase cyclic guanosine monophosphate (cGMP), presumably as a result of activation of guanylate cyclase (7). LY-83583, an inhibitor of soluble guanylate cyclase, decreased basal 2-DG transport in isolated rat muscle to a similar extent as L-NMMA (30%) (36) and completely abolished SNP-augmented 2-DG transport (7,36), suggesting that NO may mediate its metabolic effects via a cGMP-related mechanism. Indeed, Young and Leighton (36) have recently shown in incubated rat soleus muscle that addition of zaprinast, a selective inhibitor of cGMP-specific phosphodiesterase type 5 (PDE-5), reduced the breakdown of cGMP, raised skeletal muscle cGMP levels by ~90%, and substantially increased glucose uptake. Based on these findings, and the fact that both eNOS and nNOS typically require calcium as a co-factor for activation (38), it is logical to hypothesize that during excitation-contraction coupling, calcium released from the sarcoplasmic reticulum not only induces contraction but also stimulates the production of NO, in turn activating soluble guanylate cyclase and increasing cGMP. Then, cGMP may increase skeletal muscle glucose transport through as-yet-to-be-determined mechanisms.

It has been suggested that skeletal muscle contains both nutritive and nonnutritive vascular beds. Blood flow through nutritive vascular beds promotes metabolism in surrounding muscle, whereas blood flow through nonnutritive beds inhibits skeletal muscle metabolism. Furthermore, it has been proposed that vasoconstrictors affect oxygen consumption and glucose uptake by altering the relative proportion of nutritive and nonnutritive flow (39). In the current study, there were no changes in whole-leg oxygen extraction in association with NOS inhibition (Table 3), making the possibility of changes in nutritive and nonnutritive flow unlikely. It is therefore suggested that NO plays a direct role in regulating exercise-induced skeletal muscle glucose uptake.

Exercise blood flow. During exercise, a number of vasoactive metabolic by-products are produced within skeletal muscle. Although these substances may explain, at least in part, the vasodilation of blood vessels within the muscle, the large increases in skeletal muscle blood flow during exercise also require dilation of the "feed" arteries into the muscles. Theoretically, NO is an attractive mediator of exercise hyperemia. In addition to local agonist action on the vascular endothelium to increase NO production, shear stress may also stimulate NO in both "feed" and intramuscular blood vessels.

Despite the importance of the continual basal release of NO in maintaining resting vascular tone (8), the contribution of NO production to exercise hyperemia is uncertain. In humans, a number of studies have reported an increase in expired NO (40–43), plasma nitrates (44,45), or urinary nitrates (46) with exercise, suggesting that NO production is increased during exercise and that NO may play a role in exercise hemodynamic control.

Typically, the magnitude of vasoconstriction, and, therefore, the decrease in blood flow after NOS inhibition, serves as the basis for the design of most experiments investigating the contribution of NO to vasodilatation. Most (10–15), but not all (16–18), studies in animals suggest that NO has an important role in exercise hyperemia. Several studies in humans have investigated exercise hyperemia in the forearm using intrabrachial artery infusions of L-NMMA. These studies have reported either that forearm blood flow is decreased during exercise in the presence of L-NMMA (19,20) or that L-NMMA produces a decrease in resting forearm blood flow but has no further effect on blood flow during exercise (21–23). Similarly, studies in the leg have provided conflicting results. Rådegran and Saltin (24) reported no difference in femoral artery blood flow during prolonged single-leg kicking exercise with or without L-NMMA infusion, whereas Hickner et al. (25) reported that blood flow around a microdialysis probe perfused with L-NMMA was 50% of flow around a control probe during cycling exercise. Finally, it has been reported that the infusion of L-NMMA at doses that cause increases in peripheral vascular resistance and blood pressure at rest (9,47,48) does not do so during exercise (24,47).

In the current study, we found that NO inhibition with L-NMMA did not alter LBF during dynamic cycling exercise in humans. There are several possible explanations for the conflicting findings of our study compared with studies previously performed.

Some studies have infused NOS inhibitors at rest before exercise, and others have infused the inhibitors during exercise. The distribution of blood flow in contracting muscle is likely to differ from that in resting muscles. When NO synthase inhibitors are given at rest (e.g., 21), the agent may not reach a high proportion of the vessels involved in exercise hyperemia due to closure of precapillary sphincters. By contrast, when NOS inhibitors are infused during exercise, all of the vessels involved in exercise hyperemia are likely to be exposed (e.g., 19). During our study, infusion of the NOS inhibitor L-NMMA started after the commencement of exercise, ensuring that the vessels responsible for hyperemia would definitely be exposed to the L-NMMA.

Differences in the intensity and modality of exercise may also explain the conflicting results. The forearm studies used a variety of handgrip and wrist flexion-extension exercises. Static handgrip exercise as used by Endo et al. (21) may be expected to occlude skeletal muscle blood flow more so than dynamic exercise and, therefore, not provide a true indication of normal exercise hyperemic regulation. In general, forearm studies involve only a small muscle mass at relatively low exercise intensities, not typical of exercise used for locomotion or advocated for cardiovascular health. Two exercise models have been used in the lower limb. The study by Rådegran and Saltin (24) that reported no effect of L-NMMA on LBF during exercise used the knee-extensor model (49). This model results in very high perfusion-to-muscle mass ratio and also results in an increased pressor response. Although the knee-extensor model is attractive in that it provides a model that accurately identifies the mass of skeletal muscle involved in exercise, for the above reasons it may not be an appropriate model for investigating hemodynamic control. The study by Hickner et al. (25) that reported a significant decrease in regional vastus lateralis blood flow with L-NMMA infusion used cycling exercise, an exercise modality producing more typical perfusion-to-muscle mass ratio. We chose to use supine cycling, an exercise model similar to that used by Hickner et al. and typical of dynamic exercise recommended for cardiovascular health.

Perhaps the major explanation for the conflicting results from previous studies is the variation in the method of blood flow measurement. All forearm studies have used venous occlusion plethysmography (19–23). These blood flow measurements can be made only during a brief cessation of exercise, and therefore the blood flow measurements made may reflect early recovery blood flow as opposed to true exercise blood flow. Indeed, in the study by Rådegran and Saltin (24) mentioned above, it was shown that although exercise LBF was not reduced in the presence of L-NMMA, LBF was reduced during recovery from exercise. Finally, some controversy remains about the accuracy of blood flow measurements obtained using the ethanol outflow-inflow ratio used in the microdialysis blood flow measurement technique (50). Therefore, the results from the study by Hickner et al. (25) examining the effect of NOS inhibition on LBF are contentious. Constant-rate thermodilution, a widely accepted method for measuring exercise blood flow, which does not require a cessation of exercise for the measurements to be made, was used to measure LBF during this study.

Our results showed that L-NMMA infusion was not associated with a change in femoral venous blood flow during exercise (Fig. 2). This finding would suggest that NO is not essential for the regulation of exercise hyperemia and supports the finding of Rådegran and Saltin (24). Further, L-NMMA infusion during exercise was not associated with a change in blood pressure or heart rate. Significant vasoconstriction of leg vascular beds, or alternatively systemic vascular beds, would be expected to increase blood pressure and reduce heart rate (via baroreceptor reflexes). Our results support the finding of Brett et al. (46), who found that administration of L-NMMA (3 or 6 mg/kg) had a diminished effect on hemodynamics with increasing exercise intensity compared with rest.

Although our study demonstrates that NO production is not essential for normal exercise hyperemia, our results do not exclude the possibility that, under normal conditions, vascular NO does play a role in blood flow regulation during exercise. There is a large number of vasoactive metabolites released into the interstitial space during exercise. Under exercise conditions when NO production is inhibited, it is feasible that these other agents provide an increased contribution to exercise hyperemia and thus maintain normal exercise blood flow.

Clinical significance. The possible role of NO in regulation of skeletal muscle glucose uptake may have important clinical significance. It has been reported that obese Zucker rats (insulin-resistant) have reduced skeletal muscle NOS activity compared with insulin-sensitive lean Zucker rats (37). Furthermore, obese insulin-resistant human subjects have decreased skeletal muscle NOS activity (51). The finding that nNOS protein expression is increased fourfold in skeletal muscle from endurance-trained rats (5) suggests that physical activity may regulate skeletal muscle NOS activity and therefore contribute to normal muscle metabolism.

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