

Nitric Oxide Synthase Inhibition Reduces Glucose Uptake During Exercise in Individuals With Type 2 Diabetes More Than in Control Subjects

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Nitric oxide (NO) synthase inhibition reduces leg glucose uptake during cycling without reducing leg blood flow (LBF) in young, healthy individuals. This study sought to determine the role of NO in glucose uptake during exercise in individuals with type 2 diabetes. Nine men with type 2 diabetes and nine control subjects matched for age, sex, peak pulmonary oxygen uptake ($\dot{V}O_2$ peak), and weight completed two 25-min bouts of cycling exercise at $60 \pm 2\%$ $\dot{V}O_2$ peak, separated by 90 min. N^G -monomethyl-L-arginine (L-NMMA) (total dose 6 mg/kg) or placebo was administered into the femoral artery for the final 15 min of exercise in a counterbalanced, blinded, crossover design. LBF was measured by thermodilution in the femoral vein, and leg glucose uptake was calculated as the product of LBF and femoral arteriovenous glucose difference. During exercise with placebo, glucose uptake was not different between control subjects and individuals with diabetes; however, LBF was lower and arterial plasma glucose and insulin levels were higher in individuals with diabetes. L-NMMA had no effect on LBF or arterial plasma glucose and insulin concentrations during exercise in both groups. L-NMMA significantly reduced leg glucose uptake in both groups, with a significantly greater reduction ($P = 0.04$) in the diabetic group ($75 \pm 13\%$, 5 min after L-NMMA) compared with the control group ($34 \pm 14\%$, 5 min after L-NMMA). These data suggest a greater reliance on NO for glucose uptake during exercise in individuals with type 2 diabetes compared with control subjects. *Diabetes* 51:2572–2580, 2002

Type 2 diabetes is the most prevalent form of diabetes, accounting for >85% of patients with diabetes. The incidence of this disease is steadily increasing in Western countries, affecting 5–7% of the population and posing a significant health problem

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AMPK, AMP-activated protein kinase; a-v, arteriovenous; eNOS, endothelial nitric oxide synthase; LBF, leg blood flow; L-NMMA, N^G -monomethyl-L-arginine; NOS, nitric oxide synthase; NO_x , nitrate/nitrite; RER, respiratory exchange ratio; So_2 , oxygen saturation; STPD, standard temperature and pressure, dry; $\dot{V}O_2$ peak, peak pulmonary oxygen uptake.

in terms of both individual suffering and economic burden (1). Because chronic exposure to hyperglycemia as a result of insulin resistance is the primary causative factor in the development of complications in patients with diabetes, there is substantial interest in the mechanisms that control glucose uptake. Skeletal muscle is the major site of glucose disposal in the body, and the two most physiologically relevant stimuli for glucose uptake into skeletal muscle are insulin and muscle contraction. Although it is well established that both stimuli increase translocation of the GLUT-4 glucose transporter from intracellular pools to the plasma membrane, the mechanisms differ (2). In particular, whereas insulin acts through insulin receptor substrate-1 and phosphatidylinositol 3-kinase (3–6), contraction does not (4,5,7). Calcium, protein kinase C, AMP-activated protein kinase (AMPK), and nitric oxide (NO) all have been implicated in contraction-mediated GLUT-4 translocation (2,8).

Of the putative mechanisms proposed for contraction-mediated glucose uptake, only NO has been directly implicated in humans (9,10). Specifically, NO synthase (NOS) inhibition with N^G -monomethyl-L-arginine (L-NMMA) during cycle exercise reduced leg glucose uptake by 48% in humans in the absence of any effect on leg blood flow (LBF) or plasma insulin (9). This inhibitory effect was reversed by the NOS substrate L-arginine and implicates NO in control of glucose extraction during exercise. Although controversial (11,12), this role has been further substantiated by rat studies in which translocation of the GLUT-4 glucose transporter from intracellular pools to the cell surface is enhanced in the presence of NO donors (12) and reduced after exercise by NOS inhibitors (13). This NO mechanism seems to be distinct from and/or additive to insulin-stimulated skeletal muscle glucose uptake (11,14), which is impaired in individuals with diabetes (15,16).

During exercise, glucose uptake can occur in the absence of insulin (17). Under physiological conditions, however, both insulin and contraction are important synergistic mediators of glucose uptake during exercise such that for any given level of contractile activity, insulin further increases glucose uptake (18,19). Individuals with type 2 diabetes (15) and insulin-resistant obese Zucker rats (20) have impaired insulin-stimulated GLUT-4 translocation; however, exercise-stimulated GLUT-4 translocation is normal (21,22). Furthermore, despite deficits in insulin-mediated GLUT-4 translocation, skeletal muscle glucose utilization during exercise is normal (23,24) or supranor-

TABLE 1
Subject characteristics

	Control subjects	Individuals with type 2 diabetes	P
Age (years)	46 ± 2	48 ± 1	0.23
Weight (kg)	86.8 ± 4.1	88.2 ± 4.8	0.82
BMI (kg/m ²)	25.9 ± 1.0	28.1 ± 1.4	0.25
Systolic blood pressure (mmHg)	120 ± 2	128 ± 6	0.27
Diastolic blood pressure (mmHg)	74 ± 2	80 ± 3	0.05*
Fasting blood glucose (mmol/l)	5.21 ± 0.15	9.07 ± 0.87	<0.001*
Fasting plasma insulin (pmol/l)	61 ± 4	87 ± 18	0.20
HbA _{1c} (%)	4.97 ± 0.08	6.23 ± 0.38	0.005*
Total cholesterol (mmol/l)	5.08 ± 0.22	4.75 ± 0.38	0.46
HDL cholesterol (mmol/l)	1.39 ± 0.09	1.02 ± 0.08	0.01*
LDL cholesterol (mmol/l)	3.25 ± 0.20	2.89 ± 0.33	0.37
Triglycerides (mmol/l)	0.98 ± 0.14	1.82 ± 0.35	0.04*
VO ₂ peak (l/min)	2.37 ± 0.20	2.52 ± 0.15	0.52
VO ₂ peak (ml · kg ⁻¹ · min ⁻¹)	27.3 ± 2.0	28.8 ± 1.4	0.54
VO ₂ during exercise (% VO ₂ peak)	60.6 ± 1.9	60.0 ± 2.0	0.84
Workload at 60% VO ₂ peak (W)	91 ± 8	97 ± 7	0.55

Data are means ± SE: Blood pressures were from brachial sphygmomanometry measured at the initial screening visit. *P < 0.05 vs. control subjects.

mal in individuals with type 2 diabetes (25–27). This may relate in part to increased facilitated diffusion brought about by high blood glucose levels. Alternatively, mechanisms independent of glucose delivery may compensate for impaired insulin action through increased glucose uptake via the contraction pathway. The current study aimed to investigate the potential role of NO in mediation of glucose uptake during exercise in individuals with type 2 diabetes and normal control subjects matched for age, sex, peak pulmonary oxygen uptake (VO₂ peak), and weight.

RESEARCH DESIGN AND METHODS

Subjects. After providing written informed consent, nine men with type 2 diabetes (aged 48 ± 4 years; mean ± SD) and nine control subjects (aged 46 ± 5 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 nonsmokers, were free of overt coronary disease (stress electrocardiogram), and had a BMI <35 kg/m². Control subjects did not take any medication and had fasting and plasma glucose levels <6.1 mmol/l after a 2-h 75-g oral glucose load, whereas individuals with type 2 diabetes had fasting plasma glucose >7 mmol/l and/or plasma glucose levels of >11.1 mmol/l after a 75-g oral glucose load (Table 1) (28,29). Of the individuals with type 2 diabetes, seven were controlled by diet and two were medicated with metformin (half-life 3.5 h). Of those medicated, one was also taking gliclazide (half-life 12 h). All were normally active but were not specifically exercise trained.

Subject preparation. The two medicated individuals with type 2 diabetes did not take their medication the night before or the morning of the screening or experimental days and thus had a 24-h drug-free period before all measurements. VO₂ peak was determined during continuous incremental upright cycling to volitional exhaustion on an electronically braked ergometer (Ergoline 900 ergometer, Bitz, West Germany). Expired air was analyzed for volume, O₂, and CO₂ using calibrated analyzers (Cosmed Quark b², Rome, Italy). During a subsequent visit, subjects were familiarized with supine cycling during a single 30-min bout on an electronically braked ergometer (Siemens-Elema, 380B ergometry system, Stockholm, Sweden) at a workload eliciting 60% of the upright VO₂ peak. Previous studies by our group (9) and others (30) have demonstrated that supine VO₂ peak is equivalent to upright VO₂ peak.

Experimental design. On the experimental day, subjects performed two submaximal 25-min bouts of supine cycling at 60 ± 2% VO₂ peak, separated by 90 min of rest. After the first 10 min of each exercise session, subjects were administered either L-NMMA or placebo into the femoral artery in a counter-balanced, blinded, crossover design. These infusions were continued for the remainder of the exercise bout. LBF was measured and arterial and venous

femoral blood was obtained at rest and during exercise at 10, 15, 20, and 25 min.

Experimental procedures. Subjects were requested to refrain from exercise, alcohol, and caffeine for 24 h before the experimental trial. After an overnight fast, subjects attended the Alfred Hospital at 08:00 h. Teflon catheters were placed in the right femoral artery (3.0F; Cook Australia, Brisbane, Australia) and right femoral vein (4.0F; Cook Australia) under local anesthetic (1% lignocaine) using strict aseptic conditions. The artery was cannulated ~2 cm and the vein ~4 cm below the inguinal ligament, and the catheters were advanced ~10 cm and ~4 cm centrally, 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 (31). The catheters were used for simultaneous arterial and venous blood sampling, arterial blood pressure measurement, arterial drug infusions, and venous blood flow measurement. Chest electrodes were positioned for monitoring of heart rate by electrocardiogram. 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 analog-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, LBF was measured, heart rate and blood pressure were recorded, and blood samples were obtained simultaneously from the two catheters. Subjects then cycled at the predetermined workload (Table 1) eliciting 60 ± 2% VO₂ peak for 25 min. After 10 min of cycling, an intra-arterial infusion of either L-NMMA solution (2 ml/min; L-NMMA: 0.4 mg · kg body mass⁻¹ · min⁻¹) or placebo (saline, 2 ml/min) was commenced via the right femoral artery. This infusion continued for the remainder of the exercise bout (from 10 to 25 min of exercise). The total dose of L-NMMA administered was 6 mg/kg and was selected on the basis of previous findings and designed to induce peripheral but not systemic effects (9). After 90 min of rest, a second 25-min exercise bout with the other infusion protocol was performed.

At 10 (before L-NMMA or placebo infusion), 15, 20, and 25 min of exercise, LBF, pulmonary oxygen uptake, and respiratory exchange ratio (RER) were measured and heart rate was recorded. At the later three time points, after the LBF measurement, the intra-arterial infusion (L-NMMA or placebo) 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. L-NMMA was diluted in 30–35 ml of 0.9% NaCl to a concentration of 0.2 mg · kg⁻¹ · ml⁻¹. An equal volume of saline was prepared to act as a placebo.

LBF. Right femoral venous blood flow was measured by constant-rate infusion of cold saline according to the thermolimitation principle (32). Cold saline was drawn from a reservoir and then immediately infused through the femoral venous catheter (5–8°C) using an Angiomat 3000 Injector (Leibel-Flarsheim, Sybron, Cincinnati, OH). At rest, LBF was measured at an infusion

rate of 0.7 ml/s for 20 s. During exercise, LBF was measured using a constant infusion rate of between 1.5 and 2.4 ml/s for 15 s, titrated to produce ~ 0.9 – 1.2°C decrease in blood temperature (32). The coefficient of variation in blood flow measurement during exercise was 6.7%.

Blood sampling and analysis. Simultaneous blood samples were drawn from the femoral artery and vein at 0, 10 (before L-NMMA or placebo infusion), 15, 20, and 25 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, fluoride/oxalate tubes (4 ml) for glucose and lactate, and EDTA tubes for plasma nitrate/nitrite (NO_x) determination. The heparinized blood was immediately placed on ice and analyzed within 15 min for temperature-corrected PO_2 , PCO_2 , and pH (ABL 500; Radiometer, Copenhagen, Denmark) and for percentage saturation of Hb (SO_2) and Hb concentration in g/100 ml (OSM2 hemoximeter; Radiometer). Oxygen content (in ml/100 ml standard temperature and pressure, dry [STPD]) was calculated as the sum of bound and dissolved oxygen by the equation $(\text{SO}_2/100) \times [\text{Hb}] \times 1.34 + \alpha\text{PO}_2$, where 1.34 is the amount of O_2 (in ml) carried by 1 g of Hg when fully saturated, α is the solubility coefficient for O_2 in whole blood (0.0031 ml [STPD] of $\text{O}_2/0.100$ ml of blood per mmHg of O_2 at 37°C), and PO_2 is the partial pressure of O_2 in mmHg. Carbon dioxide content was calculated as described by Douglas et al. (33) and was used to determine leg respiratory quotient.

Blood for biochemical analysis was immediately placed on ice, then centrifuged at 1,500g at the end of each trial with the plasma frozen at -80°C for later analysis. Plasma total, LDL, and HDL cholesterol and triglycerides, glucose (triplicate), and lactate (triplicate) concentrations were measured using enzymatic, spectrophotometric techniques with a Cobas-BIO centrifugal analyzer (Roche Diagnostic Systems, Basel, Switzerland). Arterial plasma insulin concentration was measured in duplicate by radioimmunoassay (Linco Research, St. Charles, MO). Insulin samples for one control subject were not analyzed because of technical difficulties. Plasma concentrations of NO_x were determined in duplicate using the Griess reaction (34) with the Cayman chemical kit 7800. The product of arteriovenous (a-v) difference and LBF was used to calculate whole leg oxygen consumption, leg glucose uptake, leg net NO_x uptake, and leg net lactate release (v-a).

Statistics. All results are expressed as means \pm SE. Group characteristics were compared using unpaired *t* tests. Resting and 10-min parameters were compared using ANOVA to examine the effects of treatment and order. For examining the effect of L-NMMA (15, 20, 25 min) relative to both pre-L-NMMA infusion (10 min) and placebo, the percentage change in glucose uptake and leg a-v glucose difference from 10 min was calculated at 15, 20 and 25 min for all trials. Measurements made during the two exercise protocols were compared using ANOVA for repeated measures to determine the effects of patient group (control or diabetic), treatment, time, and order of intervention. When significance was achieved, the Fisher's least significant difference test was used to compare individual means. The null hypothesis was rejected at $P < 0.05$.

RESULTS

Screening data. Control subjects and individuals with type 2 diabetes were not different in age, weight, or BMI (Table 1). Resting brachial systolic blood pressure was not different between groups, but diastolic pressure was higher in individuals with type 2 diabetes. Fasting plasma glucose and triglycerides were significantly higher and HDL cholesterol was significantly lower in individuals with type 2 diabetes (Table 1). There was no difference between groups in either total or LDL cholesterol (Table 1). HbA_{1c} was elevated in individuals with type 2 diabetes, attesting to their diabetic status, but the elevation was only mild, indicating that they were well controlled. Furthermore, although fasting insulin tended to be higher in individuals with type 2 diabetes, it was not significantly higher (Table 1). VO_2 peak (relative and absolute) and both trial workload and VO_2 as a percentage of VO_2 peak were not different between groups (Table 1), and there were no significant order effects for any parameter between trials. The two medicated subjects were randomized to receive L-NMMA in opposite orders, and their responses did not differ from the other subjects, indicating that there were no systematic effects related to medication washout.

Blood pressure and heart rate. There was no difference

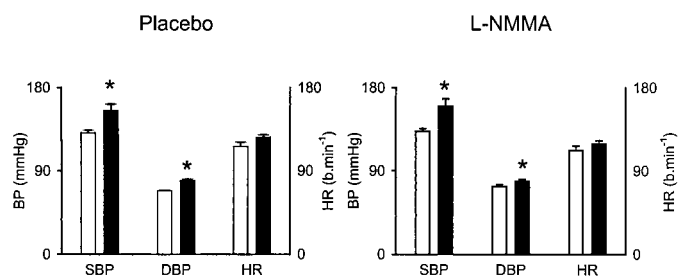


FIG. 1. Average systolic blood pressure (SBP) and diastolic blood pressure (DBP) and heart rate (HR) during 25 min of exercise at $60 \pm 2\%$ VO_2 peak in control subjects (□) and individuals with type 2 diabetes (■) for the placebo (left) and L-NMMA (right) trials. Data are means \pm SE. *Significant difference between control subjects and individuals with type 2 diabetes; $P < 0.05$.

in resting heart rate or intra-arterial blood pressure at rest before the two trials (placebo and L-NMMA) or between the diabetic and control groups. During exercise, heart rate tended to be higher in individuals with type 2 diabetes (control subjects 115 ± 5 ; individuals with type 2 diabetes 126 ± 3 beats/min during placebo; $P = 0.07$) (Fig. 1). Blood pressure was higher in individuals with type 2 diabetes throughout exercise (control subjects $131/68 \pm 3/3$; individuals with type 2 diabetes $155/79 \pm 7/2$ mmHg during placebo; $P < 0.01$) (Fig. 1). There was no effect of L-NMMA infusion on either blood pressure or heart rate during exercise (Fig. 1).

LBF. LBF was not different between control subjects and individuals with type 2 diabetes before exercise but was significantly lower in individuals with type 2 diabetes during exercise (mean 10–25 min; control subjects 3.73 ± 0.38 ; individuals with type 2 diabetes 2.78 ± 0.19 l/min during placebo; $P = 0.04$) (Fig. 2, top left). For both groups, there was no difference in LBF between the two protocols, and L-NMMA infusion did not result in a change in LBF compared with 10 min (Fig. 2, top right). There was no effect of order of intervention ($P = 0.98$).

Leg glucose uptake. At rest, arterial glucose concentration (Fig. 3, top) was higher in individuals with type 2 diabetes compared with control subjects ($P < 0.01$), but leg glucose uptake was similar despite trends ($P = 0.07$ during placebo) for greater leg a-v glucose difference in individuals with type 2 diabetes (Fig. 2, middle and bottom). Arterial glucose concentration decreased during exercise in individuals with type 2 diabetes ($P = 0.001$) but not in control subjects ($P = 0.13$) and remained higher in the individuals with type 2 diabetes compared with the control subjects during both trials ($P < 0.05$) (Fig. 3, top). As a result of the lower LBF in individuals with type 2 diabetes, mean glucose delivery during exercise was not different between control subjects and individuals with type 2 diabetes (control subjects 19.9 ± 1.9 ; individuals with type 2 diabetes 21.0 ± 2.3 mmol/min during placebo; $P = 0.74$). Glucose uptake increased with exercise to a similar degree in both groups in the placebo trial (Fig. 2). A-v glucose difference in the individuals with type 2 diabetes tended to be greater and compensated for lower exercise LBF such that average glucose uptake during exercise in the placebo trial was not different between individuals with type 2 diabetes and control subjects (control subjects 0.87 ± 0.10 ; individuals with type 2 diabetes 0.79 ± 0.13 mmol/min during placebo; $P = 0.63$).

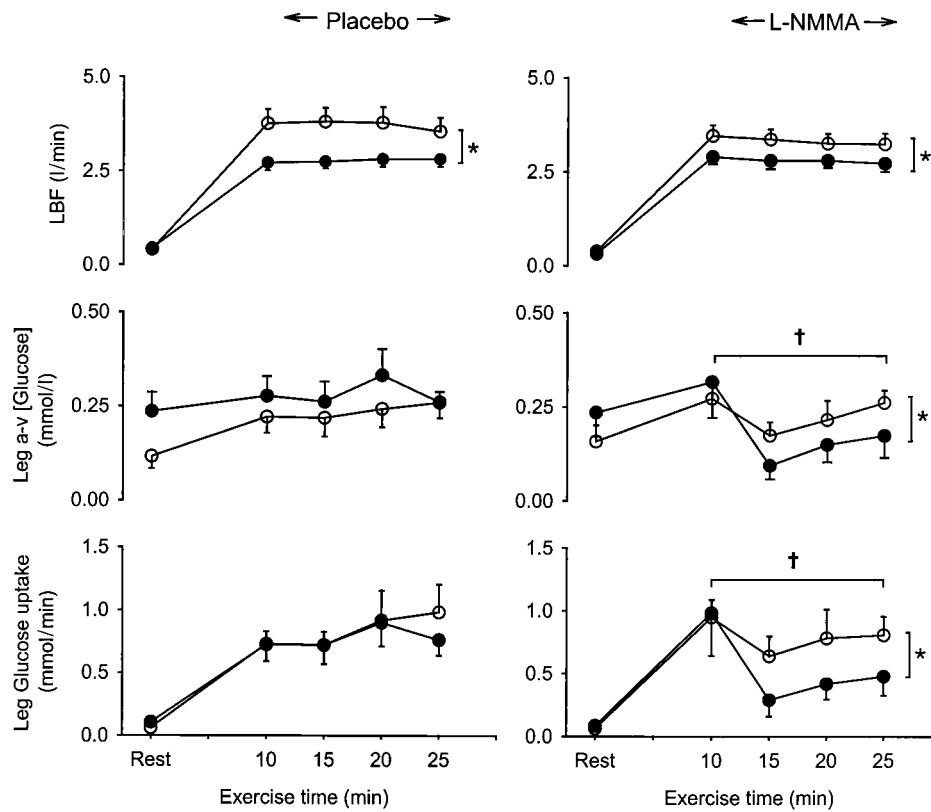


FIG. 2. Top panels show leg blood flow (LBF), middle panels show leg a-v glucose concentration, and bottom panels show leg glucose uptake for control subjects (○) and individuals with type 2 diabetes (●) for the placebo (left) and L-NMMA (right) trials during 25 min of exercise at $60 \pm 2\%$ $\dot{V}O_2$ peak. Data are means \pm SE. †Significant reduction from 10 min (% change at 15, 20, and 25 min) compared with placebo for both control subjects and individuals with type 2 diabetes (repeated measures ANOVA); *significant difference between control subjects and individuals with type 2 diabetes at 15, 20, and 25 min (repeated measures ANOVA).

Leg a-v glucose difference and leg glucose uptake were similar at 10 min of exercise in both groups and did not differ significantly between the placebo and L-NMMA trials (Fig. 2, middle and bottom). There was, however, a trend for the 10-min value to be higher in the L-NMMA trial, but this was not significant. The placebo trial acted as a time

control, whereas the 10-min pre-drug infusion value was an internal exercise bout control. Therefore, to minimize the variation unrelated to L-NMMA infusion, the percentage change in glucose uptake and leg a-v glucose difference from 10 min was calculated at 15, 20, and 25 min for all trials. In comparison with placebo, there was a significant

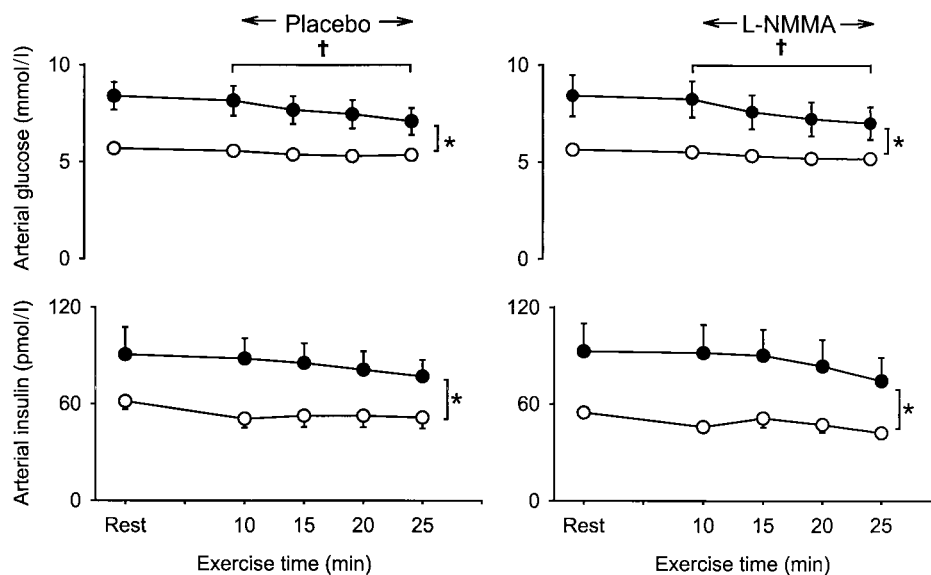


FIG. 3. Arterial plasma glucose (top, $n = 9$) and insulin (bottom, $n = 8$) for control subjects (○) and individuals with type 2 diabetes ($n = 9$, ●) for the placebo (left) and L-NMMA (right) trials during 25 min of exercise at $60 \pm 2\%$ $\dot{V}O_2$ peak. Data are means \pm SE. *Significant difference between control subjects and individuals with type 2 diabetes; †significant decline over time during exercise in individuals with type 2 diabetes; $P < 0.05$.

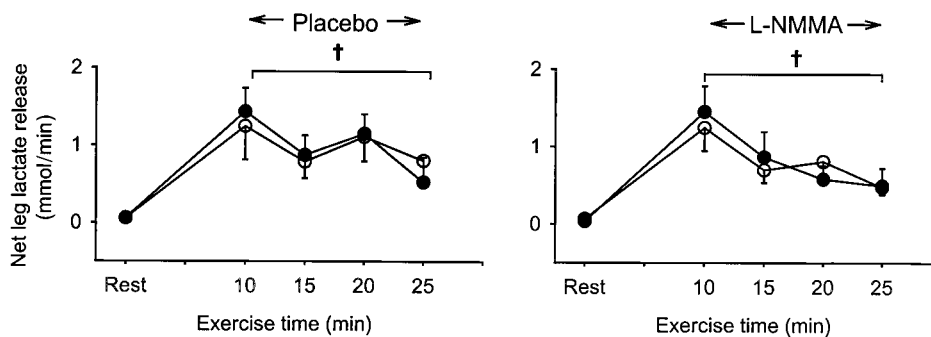


FIG. 4. Net leg lactate release for control subjects (○) and individuals with type 2 diabetes (●) for the placebo (left) and L-NMMA (right) trials during 25 min of exercise at 60 ± 2% V_{O_2} peak. Data are means ± SE. †Significant decline over time during exercise ($P < 0.05$).

reduction in glucose uptake and leg a-v glucose difference in both groups during L-NMMA infusion ($P < 0.05$) (Fig. 2, right middle and bottom). The reduction was greatest at 15 min, 5 min after commencement of L-NMMA infusion. For the control subjects, the reduction at 15 min compared with 10 min was $34 \pm 14\%$ (from 0.95 ± 0.14 to 0.64 ± 0.16 mmol/min; $P = 0.04$); for individuals with type 2 diabetes, the reduction was $75 \pm 13\%$ (0.98 ± 0.34 to 0.29 ± 0.13 mmol/min; $P = 0.02$). This reduction in individuals with type 2 diabetes was significantly greater than for the control subjects ($P = 0.04$) (Fig. 2, right bottom). The effects of L-NMMA on glucose uptake were entirely due to a reduction in leg a-v glucose difference (at 15 min: individuals with type 2 diabetes $74 \pm 14\%$; control subjects $31 \pm 15\%$; $P = 0.04$), as LBF was unchanged. There was no significant effect of order of intervention on leg glucose delivery ($P = 0.97$) or leg glucose uptake ($P = 0.50$).

Plasma insulin. Arterial plasma insulin concentrations were higher in individuals with type 2 diabetes compared with control subjects throughout exercise (Fig. 3, bottom). As was the case with glucose delivery, average insulin delivery during exercise calculated by arterial insulin concentration multiplied by LBF was not different between groups (control subjects 204 ± 30 [$n = 8$]; individuals with type 2 diabetes 225 ± 31 pmol/min [$n = 9$]; $P = 0.63$ during placebo). Neither exercise nor L-NMMA infusion had any effect on the arterial concentration of insulin.

Plasma lactate. Leg v-a lactate difference and net leg lactate release were not different between groups before either trial. In all trials, net lactate release increased from

resting values similarly after 10 min of exercise and was lower at the 15-, 20-, and 25-min time points compared with 10 min (Fig. 4). There was no difference between groups and no effect of L-NMMA infusion on net leg lactate release (Fig. 4) or v-a lactate difference during exercise (data not shown). **Gas exchange.** There was no difference in whole-body oxygen consumption or RER during exercise between groups or between the placebo and L-NMMA trials (Table 2). Femoral arteriovenous oxygen difference increased significantly from rest to 10 min of exercise during all trials (data not shown). Throughout exercise, there was no difference in leg oxygen consumption or leg respiratory quotient between groups, and, importantly, L-NMMA did not cause a change in oxygen consumption, RER, or leg respiratory quotient (Table 2).

NO_x. During exercise, there was net uptake of NO_x across the leg in both control subjects and individuals with type 2 diabetes. The magnitude of NO_x uptake was greater ($P < 0.001$) in the control compared with the diabetic group (Fig. 5). This was due primarily to a trend for higher arterial levels in the control compared with the diabetic group, whereas venous levels were similar in the two groups (data not shown). There was no significant effect of L-NMMA on net NO_x uptake during exercise in either group (Fig. 5).

DISCUSSION

The major new finding of this study was that NOS inhibition during exercise decreased glucose uptake more in

TABLE 2
Gas exchange during exercise

	Control subjects	Individuals with type 2 diabetes	<i>P</i>
Whole-body oxygen consumption (l/min)			
Placebo	1.44 ± 0.20	1.57 ± 0.13	0.48
L-NMMA	1.41 ± 0.11	1.51 ± 0.14	0.57
Leg oxygen consumption (l/min)			
Placebo	0.477 ± 0.044	0.408 ± 0.034	0.23
L-NMMA	0.429 ± 0.032	0.423 ± 0.041	0.74
RER			
Placebo	0.88 ± 0.01	0.88 ± 0.1	0.96
L-NMMA	0.89 ± 0.01	0.88 ± 0.01	0.86
Leg respiratory quotient			
Placebo	0.93 ± 0.03	0.93 ± 0.02	0.77
L-NMMA	0.95 ± 0.03	0.94 ± 0.01	0.90

Data are means ± SE. Whole-body and single-leg oxygen consumption, RER, and leg respiratory quotient during the placebo and L-NMMA protocols for control subjects and individuals with type 2 diabetes during 25 min of exercise at 60 ± 2% V_{O_2} peak.

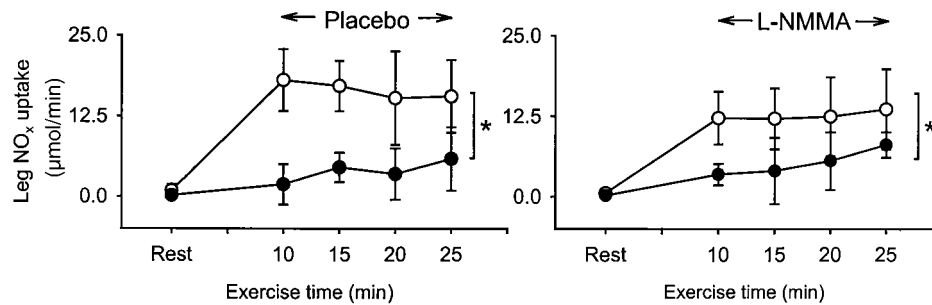


FIG. 5. Net leg NO_x uptake for control subjects (○) and individuals with type 2 diabetes (●) for the placebo (left) and L-NMMA (right) trials during 25 min of exercise at 60 ± 2% V_{O₂} peak. Data are means ± SE. *Significant difference between control subjects and individuals with type 2 diabetes; *P* < 0.05.

individuals with type 2 diabetes than in control subjects. These data implicate NO as a key mediator of the majority of glucose extraction by skeletal muscle during exercise in individuals with type 2 diabetes. Although we have previously shown this mechanism to be operative in young, healthy individuals (9), the extent to which individuals with type 2 diabetes rely on NO for glucose uptake during exercise was not known. The magnitude of the reduction in glucose uptake with NOS inhibition was approximately double in individuals with type 2 diabetes compared with matched control subjects, suggesting that NO may compensate for deficiencies in insulin-mediated GLUT-4 translocation (15,20) and glucose uptake during exercise. This finding may explain, in part, why glucose uptake during exercise is normal in individuals with type 2 diabetes despite impaired insulin action. Furthermore, NO signaling pathways may represent potential therapeutic targets for patients with type 2 diabetes.

Glucose uptake. It is evident from the whole-body and leg oxygen consumption, RER, and leg respiratory quotient data that both the diabetic and control groups were exercising at the same absolute and relative intensities (Table 2). Despite lower blood flow during exercise, glucose uptake was normal in individuals with type 2 diabetes (Fig. 2), which concurs with previous studies reporting either normal (23,24) or supranormal (25–27) glucose uptake during exercise in this patient group. It is interesting that glucose and insulin delivery to skeletal muscle during exercise were also similar in both groups (Fig. 3), because although concentrations of both were higher in individuals with type 2 diabetes, LBF was lower (Fig. 2). Consistent with previous studies, arterial glucose concentration declined more during exercise in individuals with type 2 diabetes than in control subjects (24,26,27). The greater decline in the individuals with type 2 diabetes likely results from attenuated liver glucose production because glucose uptake was normal.

The reduction in glucose uptake by L-NMMA was independent of effects on LBF in both control subjects and individuals with type 2 diabetes (Fig. 2). The greater magnitude of this effect in individuals with type 2 diabetes is consistent with greater reliance on contraction-mediated glucose uptake stimulated via an NO pathway in individuals with type 2 diabetes. Animal studies (13) and, importantly, a recent human study (9) provided evidence that NO is important for glucose uptake in healthy individuals. L-NAME administered in the drinking water of Sprague-Dawley rats before exercise completely pre-

vented the increase in glucose transport and GLUT-4 translocation after ~40 min of exhaustive treadmill running (13). The involvement of NO in contraction-mediated glucose uptake, however, is not without controversy. In two rat studies, NOS inhibitors failed to reduce contraction-stimulated glucose uptake (11,12). The main difference between these studies and those showing reduced glucose uptake with NOS inhibitors (9,13) is the timing of glucose uptake/transport measurements relative to exercise. Whereas the current study and that of Bradley et al. (9) examined the effects of NOS inhibition on glucose uptake during exercise, studies showing negative effects applied NOS inhibition before and during contraction or exercise but examined glucose uptake after exercise in isolated, incubated muscle preparations (11,12). When glucose uptake was examined during exercise, we previously demonstrated that up to 48% of exercise glucose uptake was via an NO-dependent mechanism in young, healthy humans (9). In the current study, NOS inhibition reduced glucose uptake by 34 ± 14% in control subjects and 75 ± 13% in individuals with type 2 diabetes (5 min after L-NMMA administration) (Fig. 2) in the absence of any changes in plasma insulin. Given that insulin and contraction act synergistically to increase glucose uptake during exercise (18,19) and that glucose uptake during exercise is normal (23–26) in individuals with type 2 diabetes despite impairment of the insulin mechanism (16), these data suggest that a contraction-mediated signaling pathway involving NO may represent a compensatory mechanism activating GLUT-4 translocation during exercise in individuals with type 2 diabetes. The time taken for the maximum L-NMMA response (5 min) indicates that our hypothesized actions of NO with respect to GLUT-4 translocation are very rapid but not inconsistent with studies in L6 myoblasts in which the half-life for disappearance of GLUT-4 from the cell surface was 1.5 min after removal of insulin (35).

Because leg respiratory quotient was unaffected, it seems that the reduction in glucose uptake mediated by NOS inhibition was compensated for by increased glycogenolysis. For individuals with type 2 diabetes, the average increased contribution from glycogen during the L-NMMA trial would have been 0.07 g/min. This amounts to the contribution of glycogen to carbohydrate oxidation increasing from 70 to 85%. Although this represents a very small proportion of muscle glycogen available, during more prolonged exercise, which is associated with muscle glycogen depletion, blood glucose becomes an increasingly

important contributor to carbohydrate oxidation. Inhibition of glucose uptake by L-NMMA therefore could have a substantial impact on endurance exercise performance.

Origin of NO. NO sources relevant to skeletal muscle include enzymatic production via NOS from L-arginine in both the vascular endothelium (mainly endothelial NOS [eNOS] or NOS III) (36) and skeletal muscle (mainly neuronal [nNOS] or NOS I) (36,37). Acute exercise is known to increase skeletal muscle NOS activity (38), although the mechanisms of activation remain speculative. Vascular shear stress, which is substantially elevated by exercise, stimulates endothelial NO release, which may then diffuse into the adjacent skeletal muscle. In addition, electrical stimulation has been shown to increase NO release from rat extensor digitorum longus preparations *in vitro* (39). Given that calcium is a cofactor for activation of both eNOS and nNOS, it is possible that calcium release from the sarcoplasmic reticulum associated with excitation-contraction coupling causes release of NO from skeletal muscle. In addition, skeletal muscle AMPK has been shown to phosphorylate skeletal muscle nNOS in response to acute exercise in humans (40), although it is not clear whether such phosphorylation increases nNOS activity. Furthermore, skeletal muscle glucose uptake stimulated by 5-aminoimidazole-4-carboxamide ribonucleoside, a potent stimulator of AMPK, can be blocked by a NOS inhibitor (41). NO thus may exert its actions via both paracrine (i.e., from vascular endothelium) and autocrine (i.e., produced within the muscle fiber) regulation of exercise-stimulated glucose uptake.

Plasma nitrates and nitrites have previously been used as markers of NO production at rest; however, they may also serve as substrates for NO production (42–45). NO production from NO_x may be particularly relevant during exercise, and indeed Gladwin et al. (46) reported forearm nitrite extraction from the blood during exercise in the presence of NOS inhibition. The current data are the first to report net uptake of NO_x during exercise in the absence of NOS inhibition. In the current study, net NO_x uptake was significantly lower in individuals with type 2 diabetes compared with control subjects (Fig. 5). These data reflect net flux and thus are difficult to interpret without separate knowledge of NO_x production and uptake. Thus, the lower NO_x uptake in individuals with type 2 diabetes may have resulted from greater NO_x production and/or reduced NO_x uptake. The former conclusion certainly would be consistent with the greater effects of NOS inhibition on glucose uptake in the individuals with type 2 diabetes. However, L-NMMA had no effect on NO_x uptake, which perhaps is not surprising given that the data will be confounded by the fact that NOS inhibitors may break down to nitrates and nitrites (47).

Although NO seems to be important for glucose uptake in individuals with type 2 diabetes, there is evidence in humans to suggest that individuals with type 2 diabetes have impaired resting NO-dependent vascular responses (48) and in animals that resting skeletal muscle NOS expression and activity are lower than in relevant control subjects (49,50). These results suggest that NOS protein and activity at rest may be reduced in humans with type 2 diabetes. That NOS inhibition reduced glucose uptake to a greater extent during exercise in the individuals with type

2 diabetes suggests that skeletal muscle and/or eNOS activity may actually be higher during exercise in individuals with type 2 diabetes than in control subjects. It is also possible that NO production is higher in type 2 diabetes through elevation of inducible NOS expression in skeletal muscle in response to cytokines (51).

Clinical implications. It is likely that NO mediates its effects via stimulation of soluble guanylate cyclase and production of cGMP. Inhibition of cGMP breakdown with Zaprinast, a specific inhibitor of cGMP type 5 phosphodiesterase in incubated rat soleus muscle, raised skeletal muscle cGMP levels by ~90% and substantially increased glucose uptake (49). This finding, together with the known stimulatory effect of NO donors such as sodium nitroprusside on skeletal muscle glucose uptake (12,14), highlights the NO-glucose uptake mechanism as a potential therapeutic target in patients with type 2 diabetes.

Hemodynamics. Compared with control subjects, throughout exercise, individuals with type 2 diabetes had significantly lower LBF (Fig. 2) despite higher blood pressure (Fig. 1), signifying higher leg vascular resistance. The current study is the first that we are aware of to show lower LBF during dynamic leg exercise in type 2 diabetes. A previous study in relatively fit individuals with type 2 diabetes (maximum $\dot{V}O_2$ 3.63 ± 0.28 l/min) reported normal blood flow during exercise using dye dilution (24). The other main difference between these two trials was exercise posture, which was upright in the previous study and supine in this study. Although speculative, it is possible that unloading the baroreceptors in the supine posture unmasks differences in peripheral vascular control, which are normally obscured by reflex increases in vascular tone during upright exercise. Our LBF data are consistent with an impaired vasodilatory response to exercise in individuals with type 2 diabetes and may relate to both vascular endothelium-dependent (52) and -independent (53) mechanisms. Lipid abnormalities, defects in the vascular action of insulin (52), and elevated blood glucose (54) all are likely contributors to impaired vascular control during exercise.

Consistent with most previous studies in humans, NOS inhibition did not change LBF during exercise (9,55,56). Substantial controversy currently surrounds the role of NO in exercise hyperemia (10). Conflicting results arise from differences in flow methodology, protocols, and exercise modalities (10). Our study again demonstrates that in humans, NO is not obligatory for vasodilation during exercise. This does not mean, however, that NO is not important under normal exercise circumstances. Indeed, animal studies have indicated substantial redundancies in vasodilator mechanisms. In the coronary vasculature, for example, the important role of NO in coronary vasodilation during exercise was unmasked only after blockade of K⁺ ATP channels and adenosine receptors (57). 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.

Although the effects of L-NMMA on glucose uptake were independent of changes in total LBF, the possibility remains that blood flow redistribution within the muscle contributed to our findings (58). If L-NMMA preferentially

reduced flow through metabolically active (nutritive) areas while flow through metabolically inactive (nonnutritive) areas was increased, then glucose uptake might be reduced in the absence of any net change in flow (58). If operative, then this mechanism suggests greater NO-dependent dilation of nutritive vascular networks in individuals with diabetes compared with control subjects, which seems unlikely given the known impairment in NO-mediated endothelium-dependent dilation in these patients (59). This mechanism would also predict reduction in net oxygen uptake and elevation in net lactate release in concert with the reduced glucose uptake. There were, however, no changes in leg oxygen uptake or lactate production in association with NOS inhibition (Fig. 4), which argues against the possibility of changes in nutritive and nonnutritive flow. Studies examining the effects of NOS inhibition on both GLUT-4 translocation and nutritive/nonnutritive flow, however, will be required to substantiate our hypothesis that NO affects glucose uptake through effects on GLUT-4 translocation.

CONCLUSION

These data are consistent with the hypothesis that NO increases glucose uptake during exercise and that this mechanism is more important in individuals with type 2 diabetes than in age-matched healthy control subjects. Additional studies are required to show definitively whether this is a direct effect on GLUT-4 translocation. NO-mediated glucose uptake may compensate for impaired insulin action and account for the normal glucose uptake in individuals with type 2 diabetes during exercise. The NO pathway therefore may represent a potential therapeutic target in patients with type 2 diabetes.

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