L-Arginine infusion increases glucose clearance during prolonged exercise in humans

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McConell, G. K., N. N. Huynh, R. S. Lee-Young, B. J. Canny, and G. D. Wadley. L-Arginine infusion increases glucose clearance during prolonged exercise in humans. Am J Physiol Endocrinol Metab 290: E60–E66, 2006. First published August 16, 2005; doi:10.1152/ajpendo.00263.2005.—Nitric oxide synthase (NOS) inhibition has been shown in humans to attenuate exercise-induced increases in muscle glucose uptake. We examined the effect of infusing the NO precursor L-arginine (L-Arg) on glucose kinetics during exercise in humans. Nine endurance-trained males cycled for 120 min at 72 ± 1% V˙O2peak followed immediately by a 15-min “all-out” cycling performance bout. A [6,6-3H]glucose tracer was infused throughout exercise, and either saline alone (Control, CON) or saline containing L-Arg HCl (L-Arg, 30 g at 0.5 g/min) was coinfused in a double-blind, randomized order during the last 60 min of exercise. L-Arg augmented the increases in glucose rate of appearance, glucose rate of disappearance, and glucose clearance rate (L-Arg: 16.1 ± 1.8 ml·min⁻¹·kg⁻¹; CON: 11.9 ± 0.7 ml·min⁻¹·kg⁻¹ at 120 min, P < 0.05) during exercise, with a net effect of reducing plasma glucose concentration during exercise. L-Arg infusion had no significant effect on plasma insulin concentration but attenuated the increase in nonesterified fatty acid and glycerol concentrations during exercise. L-Arg infusion had no effect on cycling exercise performance. In conclusion, L-Arg infusion during exercise significantly increases skeletal muscle glucose clearance in humans. Because plasma insulin concentration was unaffected by L-Arg infusion, greater NO production may have been responsible for this effect.

SKELETAL MUSCLE GLUCOSE UPTAKE is increased by translocation of the GLUT4 glucose transporter from intracellular vesicles to the plasma membrane (23). Both insulin and muscle contraction increase skeletal muscle GLUT4 translocation and glucose uptake, but the mechanisms involved differ (23, 36, 37, 49). The factors regulating glucose uptake into skeletal muscle during exercise are unclear with calcium, calcium/calmodulin-dependent protein kinase, protein kinase C, AMP-activated protein kinase (AMPK), and nitric oxide (NO) all implicated (23, 49).

We have evidence in humans that NO may be playing an essential role in the regulation of skeletal muscle glucose uptake during exercise (9, 34). We found during cycling exercise in young, healthy individuals that femoral artery infusion of an NO synthase (NOS) inhibitor reduced leg glucose uptake during exercise by 40–50% without influencing leg blood flow (LBF), blood pressure, or arterial plasma insulin concentration (9). In a follow-up study, we found that NOS inhibition reduced leg glucose uptake during exercise to a greater extent in people with type 2 diabetes than in matched controls (34). Although our results in humans are clear, studies in rats examining the effect of NOS inhibition on contraction-stimulated glucose uptake have yielded conflicting results (3, 17, 26, 51, 53, 57).

Aside from NOS inhibition, another way to examine the potential importance of NO in skeletal muscle glucose disposal during exercise in humans is to examine the effect of increasing the availability of L-arginine, which is the substrate of NOS. In skeletal muscle, the major form of NOS expressed is the alternatively spliced neuronal form (nNOSµ) (Ref. 54 and Bradley SJ, unpublished observations), which is associated with the sarcolemma. There is a small amount of endothelial NOS expressed in skeletal muscle which, in humans, appears to be confined to the endothelium of blood vessels (19, 20). We hypothesize that L-arginine enters skeletal muscle, where it is converted to NO and L-citrulline by NOSµ. Skeletal muscle NOS activity is increased during treadmill exercise in rats (50), and electrical stimulation increases NO production in primary rat skeletal muscle cell culture (55) and in isolated rat skeletal muscle (4). Exercise appears to increase NO production in humans on the basis of increases in urinary nitrate/nitrite and cGMP levels in humans (7). L-Arginine infusion also increases plasma and urinary nitrate/nitrite content and cGMP concentrations in humans (5, 6, 27), suggesting increased NO production.

We postulate that any effects of L-arginine infusion on glucose kinetics during exercise, if they exist, will most likely occur independently of changes in hemodynamics, in particular muscle blood flow. Intravenous infusion of L-arginine during exercise in healthy individuals (11) and patients with coronary heart disease (45) has no effect on heart rate or blood pressure during exercise. In addition, infusing L-arginine through a microdialysis probe into skeletal muscle has no effect on human skeletal muscle blood flow during exercise (25) and 5 min of L-arginine infusion into the femoral artery has no effect on LBF during leg exercise in humans (9). Furthermore, NOS inhibition has no effect on LBF during leg exercise in humans (9, 18, 34, 46). Therefore, it appears that, although NO clearly affects blood flow at rest (46), neither increases (via L-arginine) nor decreases (via NOS inhibition) in NO production have any effect on blood flow during exercise in humans.

In humans, L-arginine infusion at rest results in an approximately twofold increase in plasma insulin concentration (6, 45). It is not known whether L-arginine infusion increases plasma insulin levels during prolonged exercise in humans (i.e.,
under conditions when increases in plasma catecholamines inhibit insulin secretion). It is important to determine this, because insulin is additive on skeletal muscle glucose uptake during exercise (16). Two weeks of oral L-arginine-L-aspartate supplementation had no effect on plasma insulin concentration during ~3 h of running in endurance-trained athletes (15).

No study has examined the effect of L-arginine infusion on prolonged exercise performance in humans. It is possible that if L-arginine infusion increases glucose uptake into muscle during prolonged exercise it may also improve exercise capacity. All previous studies examining the effects of L-arginine on exercise capacity have examined only short, intense, incremental exercise (~8–9% increase in VO2max) in hypercholesterolemic and normal mice (38). In humans, results have been contradictory (60), but on balance it would appear that chronic oral L-arginine supplementation improves VO2max exercise capacity in patients with cardiovascular diseases (12, 13, 48). Chronic oral L-arginine supplementation appears to have no effect on VO2max test exercise time in healthy individuals (1). In addition, acute L-arginine infusion appears to have no effect on VO2max test exercise time in all human studies, including in patients with chronic heart failure (32) and patients with hypercholesterolemia (61).

Therefore, the first aim of this study was to determine whether L-arginine infusion during exercise increases glucose disposal in humans. The second aim was to determine whether L-arginine infusion influences prolonged exercise performance in healthy humans. We hypothesized that L-arginine infusion during exercise would increase the rate of glucose disappearance but not significantly influence exercise performance during exercise in healthy humans. We chose to use endurance-trained participants in this study to minimize the possibility of adaptive changes between the two experimental trials (CON and L-Arg). In addition, to detect an exercise performance effect of a particular treatment it is known to be most appropriate to employ exercise-trained participants, who are more reproducible in their exercise performance (29).

METHODS

Participants

Nine endurance-trained males provided informed, written consent to participate in this study, which was approved by the Monash University Standing Committee for Research on Humans and the Human Research Ethics Committee of The University of Melbourne and conducted in accordance with the Declaration of Helsinki. The participants’ age, weight, and height were 28 ± 2 yr, 73.7 ± 2.8 kg, and 180 ± 1 cm, respectively (means ± SE). The participants cycled on average 283 ± 35 km/wk.

Experimental Procedures

Preliminary testing and diet control. Peak pulmonary oxygen consumption during cycling (VO2peak) was determined using a graded exercise test to volitional exhaustion on an ergometer (Lode, Groningen, The Netherlands) and averaged 4.65 ± 0.23 l/min (63.0 ± 1.9 ml·kg⁻¹·min⁻¹). On a separate day, participants completed a familiarization trial where they cycled for 120 min at 236 ± 12 W (72 ± 1% VO2peak), immediately followed by completion of as much work as possible in 15 min with the ergometer placed in the “linear mode.” In this linear mode, participants are able increase the power output by increasing their cadence [rpm; power output (W) = “linear factor” × (rpm)²]. On average, the participants completed 249 ± 14 KJ during the 15 min. This amount of work (249 ± 14 KJ) was then completed as quickly as possible following the 120-min of exercise at 72 ± 1% VO2peak in the experimental trials and comprised the performance measurement (time trial). Approximately 1 wk later, the first of the two experimental trials was undertaken.

The day before each experimental trial, exercise and diet were standardized. Approximately 24 h before each experimental trial, participants were required to come to the laboratory to exercise for 1 h at 70% VO2peak (standard ride), after which participants were asked to abstain from exercise for the remainder of the day. After the standard ride participants were provided with a diet (food and drinks) for the day (i.e., breakfast, lunch, dinner, and snacks) and dietary L-arginine supplementation increases aerobic capacity during prolonged exercise in healthy humans. We chose to use endurance-trained participants in this study to minimize the possibility of adaptive changes between the two experimental trials (CON and L-Arg). Infusion of 30 g of L-arginine into the participants (overnight fasted) reported to the laboratory in the morning having abstained from exercise, alcohol, and caffeine for 24 h. One catheter was inserted into an antecubital forearm vein for infusion of a stable isotope glucose tracer ([6,2H]glucose; Cambridge Isotope Laboratories, MA) and another into the contralateral forearm for blood sampling. A blood sample was obtained, and then a bolus of 44.4 ± 0.6 μmol/kg tracer was administered before a 2-h preexercise constant infusion (0.63 ± 0.03 μmol/kg⁻¹·min⁻¹), which was continued throughout exercise (i.e., until the end of the time trial). Participants then cycled for 120 min at 72 ± 1% VO2peak (236 ± 12 W; 59 ± 2% of peak power output during VO2peak test). Blood was sampled at ~120, ~30, ~10, and 0 min and then every 15 min of exercise and at the end of the performance ride. Plasma insulin, glyceral, and nonesterified fatty acid (NEFA) concentrations were not measured between the onset and 60 min of exercise, as the treatment (L-argin infusion vs. saline) did not start until 75 min, and therefore there was no reason to expect any differences between the two trials during the first 60 min of exercise. At the commencement of exercise 8 ml/kg body wt of water were ingested followed by 2 ml/kg every 15 min until 120 min to ensure that the participant’s hydration levels were similar between each trial. Expired air was collected into Douglas bags during the last 3 min of each 15 min of exercise (until 120 min of exercise). Heart rate was monitored throughout exercise (Polar Favor, Oulu, Finland). Rating of perceived exertion was assessed using the Borg scale (ranging from 6: easiest, to 19: hardest) (8). In a double-blind, randomized, cross-over design, participants were confused intravenously after 75 min of exercise with either 30 g of L-arginine hydrochloride (Ophthalmic Laboratories for Pharmalab, Brookvale, NSW, Australia) mixed with saline (L-Arg: 0.5 g/min iv) or a placebo (CON) treatment (0.9% saline; Baxter Healthcare, Toongabbie, NSW, Australia). Infusion of 30 g of L-arginine into humans over 30–60 min results in plasma L-arginine concentration increasing from ~0.1 to ~6.2–7.2 mM (5, 27). Immediately following the initial 120 min of exercise, the ergometer was placed into the “linear” mode function, and the participants completed 249 ± 14 KJ as quickly as possible (performance ride). To standardize motivational factors, no encouragement was given to the participants during the performance rides of the experimental trials, and the participants were permitted to see only the accumulating kilojoules and not the clock or the instantaneous power output or rpm. The last Douglas bag collection period was completed by 120 min of exercise just before commencement of the performance ride so that participants could concentrate on their performance. Because it has been shown that infusion of 30 g of L-arginine at rest can result in decreases in blood pressure,
Table 1. Mean physiological responses during the last 30 min (90, 105, and 120 min) of 120 min of steady-state exercise at 72 ± 1% \( V\dot{O}_2 \) peak and time to complete the performance ride (249 ± 14 kJ) in CON and L-Arg conditions

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>L-Arg</th>
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<tr>
<td>( V\dot{O}_2 ), l/min</td>
<td>3.37±0.17</td>
<td>3.38±0.17</td>
</tr>
<tr>
<td>RER</td>
<td>0.90±0.01</td>
<td>0.89±0.01</td>
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<tr>
<td>Heart rate, beats/min</td>
<td>149±4</td>
<td>147±4</td>
</tr>
<tr>
<td>Rating of perceived exertion</td>
<td>12.8±0.4</td>
<td>13.0±0.5</td>
</tr>
<tr>
<td>Performance time, min</td>
<td>14.5±0.9</td>
<td>14.1±0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n = 9 \). \( V\dot{O}_2 \), oxygen uptake; CON, control saline infusion; L-Arg, l-arginine infusion; RER, respiratory exchange ratio.

the participants were instructed to lie down for 30 min after each experimental trial. No participant complained of feeling light headed during this recovery period.

Analytical Techniques

Blood analysis. Plasma glucose and lactate were determined using an automated glucose oxidase and l-lactate oxidase method, respectively (YSI 2300 Stat, Yellow Springs, OH), plasma NEFA by an enzymatic colorimetric procedure (NEFA-C test; Wako, Osaka, Japan), plasma glycerol by an enzymatic fluorometric method (14), and plasma insulin using a human insulin-specific radioimmunoassay kit (Linco Research, St. Charles, MO). Glucose kinetics at rest and during exercise were estimated using a modified one-pool, non-steady-state model, as proposed by Steele et al. (56), which has been validated by Radziuk et al. (47). We assumed 0.65 as the rapidly mixing portion of the glucose pool and estimated the apparent glucose space as 25% of body weight. Rates of plasma glucose appearance (Ra) and glucose disappearance (Rd) were determined from the changes in percent enrichment of [6,6-2H]glucose and the plasma glucose concentration. The glucose clearance rate (glucose CR) was calculated by dividing the glucose Ra by the plasma glucose concentration (indicates the glucose disposal per unit of plasma glucose). During cycling exercise at ~60% of \( V\dot{O}_2 \) max workload, over 95% of tracer-determined glucose Ra is oxidized (30).

Statistical analysis. Results were analyzed using two-factor repeated-measures analysis of variance (ANOVA). Because the treatment was not initiated until 75 min of exercise, the ANOVA was partitioned and analyzed to include data up to and including 75 min and data from 75 min onwards (including 75 min). As was expected, no significant differences were found between the two trials during the first 75 min of exercise. If the ANOVA revealed a significant trial by time interaction, specific differences between mean values were located using the Fisher’s least significance difference test. Performance times were compared using Student’s paired t-test. All data are presented as means ± SE. The level of significance was set at \( P < 0.05 \).

RESULTS

Respiratory Measures, Rating of Perceived Exertion, Heart Rate, and Exercise Performance

During the first 75 min of steady-state exercise (before commencement of saline/l-Arg infusion) there were no significant differences (\( P > 0.05 \)) in oxygen consumption, carbon dioxide production, respiratory exchange ratio (RER), heart rate (HR), or rating of perceived exertion (RPE) between the two treatments (data not shown). In addition, there were no significant differences (\( P > 0.05 \)) in oxygen consumption, carbon dioxide production (data not shown), RER, HR, or RPE between the two treatments during the last 30 min of steady-state exercise after the saline/l-Arg infusion commenced (Table 1).

Immediately following the 120 min of steady-state exercise, the participants completed 249 ± 14 kJ as quickly as possible (the amount of work completed in 15 min in the familiarization ride). This time trial was used to determine the exercise capacity (exercise performance). The time taken to complete the work did not differ significantly between the two trials (\( P = 0.62 \); Table 1). Accordingly, there was no significant difference in the mean power output between the two time trials (CON: 292 ± 19 watts, L-Arg: 298 ± 19 watts, \( P = 0.62 \)). In addition, there were no significant differences between trials in the HR (CON: 176 ± 4 beats/min, L-Arg: 171 ± 4 beats/min, \( P = 0.19 \)) or RPE (CON: 18.1 ± 0.4 Borg scale units, L-Arg: 18.3 ± 0.3 Borg scale units, \( P = 0.44 \)) at the end of the performance ride.

Glucose Kinetics

Plasma glucose concentration decreased during the second hour of the steady-state exercise in both trials and was significantly lower in the L-Arg trial at 105 min and 120 min (Fig. 1A). Plasma glucose increased significantly (\( P < 0.05 \)) during the performance ride in both trials and did not differ significantly (\( P > 0.05 \)) between trials at the end of exercise.

Plasma Ra, glucose Rd, and glucose CR increased during exercise in both trials (Fig. 2). Each of glucose Ra, glucose Rd, and glucose CR were analyzed using two-factor repeated-measures analysis of variance (ANOVA) and there were no significant differences (\( P > 0.05 \)) between trials at any time point at the end of exercise.

Fig. 1. Plasma glucose concentration (A) and plasma insulin concentration (B) at rest, during 120 min of steady-state exercise at 72 ± 1% \( V\dot{O}_2 \) peak, and at the end of the performance ride (249 ± 14 kJ) time trial (TT) in saline control (CON) and l-arginine infusion (L-Arg) conditions. Values are means ± SE; \( n = 9 \). * \( P < 0.05 \) vs. CON.

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and glucose CR were significantly higher in L-Arg during the last 30 min of the steady-state exercise. During the performance ride glucose $R_a$, glucose $R_d$, and glucose CR increased in both trials, and at the end of exercise there were no significant differences between the trials in any of these parameters ($P > 0.05$; Fig. 2).

**Plasma Insulin, NEFA, Glycerol, and Lactate**

Plasma insulin decreased during exercise in both trials with no significant difference between the two trials at any time point (Fig. 1B). Plasma glycerol concentration increased during exercise in both trials but was significantly lower during the latter stages of exercise in L-Arg (Fig. 3A). Plasma NEFA increased during the second hour of exercise in both trials (Fig. 3B). Plasma NEFA was significantly lower at 105 and 120 min of exercise in L-Arg compared with CON. There was a small, significant increase in plasma lactate concentration during the first 120 min of exercise in both trials with no difference between trials (data not shown). Plasma lactate then increased greatly during the performance ride in both trials, with the concentration at the end of exercise being not significantly different between the two trials (CON: 7.8 ± 1.0 mmol/l, L-Arg: 6.8 ± 0.7 mmol/l).

**DISCUSSION**

The main finding of this study was that L-arginine infusion during exercise augmented the normal exercise-induced increases in glucose disposal in humans. One interpretation of this finding is that L-arginine infusion increased NO production, which increased the level of glucose utilization by the contracting muscles, probably via increased GLUT4 translocation. Taken with our earlier findings where NOS inhibition reduced leg glucose uptake during cycling exercise (9, 34), our results reinforce the hypothesis that NO is a central regulator of skeletal muscle glucose uptake during exercise in humans.

Our studies are novel, as no other groups have examined the role of NO in glucose uptake during exercise in humans. In rats...
it has been universally found that NO donors increase basal skeletal muscle glucose uptake (3, 17, 26, 63), but the effects of NOS inhibition on contraction-stimulated glucose uptake are variable (3, 17, 26, 51, 53, 57). The rat studies are difficult to compare with our human studies because, in general, both in vivo exercise and in vitro contraction rat studies involving NOS inhibition assessed glucose transport/uptake 30 min or more after the series of contractions or treadmill exercise. One exception is a study by Rottman et al. (53), which reported that glucose uptake measured during mouse treadmill exercise was not reduced by 3 days of ingestion of a NOS inhibitor.

Because local muscle infusion of L-arginine (25), femoral artery infusion of L-arginine (9), and femoral artery infusion of a NOS inhibitor (9, 34, 46) have no effect on blood flow during exercise in humans, we think that it is likely that L-arginine infusion increased glucose uptake during exercise by increasing GLUT4 translocation to the plasma membrane rather than the mechanism(s) behind this response is unclear but may relate, in part, to the relative hypoglycemia caused by L-arginine infusion. Liver glucose output is exquisitely sensitive to small changes in plasma glucose levels during exercise in humans, so the decrease in plasma glucose would be expected to increase liver glucose output (28). Therefore, it is possible that the greater glucose uptake in L-Arg caused a decrease in the plasma glucose concentration, which then stimulated glucose uptake. It is also possible that L-Arg infusion increased the plasma gluconeogenic concentration, which then increased liver glucose output (28). Therefore, it is possible that the greater glucose uptake in L-Arg contributed to the greater glucose uptake during exercise.

L-Arginine infusion actually significantly increased glucose uptake during exercise compared with CON (Fig. 2A). The mechanism(s) behind this response is unclear but may relate, in part, to the relative hypoglycemia caused by L-arginine infusion. Liver glucose output is exquisitely sensitive to small changes in plasma glucose levels during exercise in humans, so the decrease in plasma glucose would be expected to increase liver glucose output (28). Therefore, it is possible that the greater glucose uptake in L-Arg caused a decrease in the plasma glucose concentration, which then stimulated glucose uptake. It is also possible that L-Arg infusion increased the plasma glucose concentration, which then increased liver glucose output (28). Therefore, it is possible that the greater glucose uptake in L-Arg contributed to the greater glucose uptake in that trial, since plasma glucose levels were lower, therefore decreasing the glucose gradient.

Although infusion of L-arginine increases glucose uptake during exercise, it does not appear to affect endurance exercise performance. In both trials, the participants were able to increase the exercise intensity during the performance ride above that of the first 120 min of exercise at 72% of \( \dot{V}O_2 \) peak, but there was no difference between the trials in the time to complete the set amount of work or the average power output during the performance ride. Accordingly there were similar increases in plasma glucose concentration, in glucose uptake, and in plasma lactate and decreases in plasma NEFA during the performance ride in the two trials. The reason for including a performance ride in the present study was that we postulated that if L-arginine increased glucose uptake during exercise, it might improve exercise performance. We have shown, in studies utilizing similar methodologies as those used in the present study (39, 40), that carbohydrate ingestion increases glucose uptake and improves exercise performance. However, the increases in glucose uptake with carbohydrate ingestion during prolonged exercise (39) were substantially greater than the increases observed in the present study with L-arginine infusion (Fig. 2B). Indeed, carbohydrate ingestion increases the rate of carbohydrate oxidation during prolonged exercise (39–41), but in the present study the respiratory exchange ratio was identical in the CON and L-Arg trials at all time points. It has been shown that prior L-arginine infusion has no effect on \( \dot{V}O_2 \) max test exercise time in patients with chronic heart failure (32), and several weeks of
Insulin concentration, we suggest that L-arginine infusion lowers plasma NEFA concentration during L-arginine infusion. Given that we and others have previously shown that studies show no effect of alterations in NEFA on muscle played a role in the higher glucose disposal, although most attempts can be made to develop pharmacological agents that mimic exercise for people with type 2 diabetes who cannot exercise regularly.

In conclusion, L-arginine infusion during prolonged exercise in humans decreased plasma glucose concentration. Despite this lower concentration gradient for facilitated glucose translocation and glucose uptake, but their GLUT4 translocation (33) and glucose uptake during exercise are normal (23, 34). If we can better understand the mechanisms regulating skeletal muscle glucose uptake during exercise, attempts can be made to develop pharmacological agents that mimic exercise for people with type 2 diabetes who cannot exercise regularly.

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GRANTS

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REFERENCES


