

Acute L-arginine supplementation reduces the O₂ cost of moderate-intensity exercise and enhances high-intensity exercise tolerance

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Bailey SJ, Winyard PG, Vanhatalo A, Blackwell JR, DiMenna FJ, Wilkerson DP, Jones AM. Acute L-arginine supplementation reduces the O₂ cost of moderate-intensity exercise and enhances high-intensity exercise tolerance. *J Appl Physiol* 109: 1394–1403, 2010. First published August 19, 2010; doi:10.1152/jappphysiol.00503.2010.—It has recently been reported that dietary nitrate (NO₃⁻) supplementation, which increases plasma nitrite (NO₂⁻) concentration, a biomarker of nitric oxide (NO) availability, improves exercise efficiency and exercise tolerance in healthy humans. We hypothesized that dietary supplementation with L-arginine, the substrate for NO synthase (NOS), would elicit similar responses. In a double-blind, crossover study, nine healthy men (aged 19–38 yr) consumed 500 ml of a beverage containing 6 g of L-arginine (Arg) or a placebo beverage (PL) and completed a series of “step” moderate- and severe-intensity exercise bouts 1 h after ingestion of the beverage. Plasma NO₂⁻ concentration was significantly greater in the Arg than the PL group (331 ± 198 vs. 159 ± 102 nM, *P* < 0.05) and systolic blood pressure was significantly reduced (123 ± 3 vs. 131 ± 5 mmHg, *P* < 0.01). The steady-state O₂ uptake ($\dot{V}O_2$) during moderate-intensity exercise was reduced by 7% in the Arg group (1.48 ± 0.12 vs. 1.59 ± 0.14 l/min, *P* < 0.05). During severe-intensity exercise, the $\dot{V}O_2$ slow component amplitude was reduced (0.58 ± 0.23 and 0.76 ± 0.29 l/min in Arg and PL, respectively, *P* < 0.05) and the time to exhaustion was extended (707 ± 232 and 562 ± 145 s in Arg and PL, respectively, *P* < 0.05) following consumption of Arg. In conclusion, similar to the effects of increased dietary NO₃⁻ intake, elevating NO bioavailability through dietary L-Arg supplementation reduced the O₂ cost of moderate-intensity exercise and blunted the $\dot{V}O_2$ slow component and extended the time to exhaustion during severe-intensity exercise.

exercise economy; muscle efficiency; oxygen uptake; nitric oxide; exercise performance; oxygen uptake kinetics

AT THE ONSET OF MODERATE-INTENSITY exercise [i.e., exercise performed at work rates below the gas exchange threshold (GET)], pulmonary O₂ uptake ($\dot{V}O_2$) rises in an exponential fashion to attain a “steady state” within ~2–3 min in healthy humans (74). In this exercise intensity domain, the $\dot{V}O_2$ steady state reflects the rate of ATP turnover within the recruited myocytes and is linearly related to the external work rate, with the functional “gain” (i.e., increase in $\dot{V}O_2$ per unit increment in external work rate) approximating 10 ml·min⁻¹·W⁻¹ during cycle ergometry (32). The steady-state $\dot{V}O_2$ for a given moderate-intensity work rate is impervious to a variety of acute exercise, nutritional, and pharmacological interventions and is only minimally impacted by age and training (3, 16, 42, 75). During supra-GET exercise, muscle contractile efficiency pro-

gressively declines and a $\dot{V}O_2$ “slow component,” which delays the attainment of steady state during heavy-intensity exercise (performed below the critical power) or sets the $\dot{V}O_2$ on a trajectory toward its maximum [peak $\dot{V}O_2$ ($\dot{V}O_{2peak}$)] during severe-intensity exercise (above critical power), is manifest (60, 74). Interventions that reduce the $\dot{V}O_2$ slow component amplitude have been reported to improve severe-intensity exercise tolerance (1–4, 15, 31).

The signaling molecule nitric oxide (NO) is produced by the NO synthase (NOS) family of enzymes, which catalyze the oxidation of L-arginine, yielding NO and L-citrulline (13, 54–56). A complementary, NOS-independent pathway for NO production, involving the reduction of inorganic nitrite (NO₂⁻) to NO, particularly in acidic/hypoxic conditions, has also been described (21, 26). It is well known that changes in NO production can affect vasodilatation and blood pressure (1, 4, 21, 25, 47, 48, 67), but there is increasing evidence that interventions that influence NO bioavailability can also alter the O₂ cost of exercise in humans (1, 4, 34, 47, 48) and other mammals (37, 53, 65). For example, it has recently been reported that 3–6 days of pharmacological (sodium nitrate) (47, 48) or dietary (beetroot juice) (1, 4) nitrate (NO₃⁻) administration can reduce the steady-state $\dot{V}O_2$ during submaximal cycle exercise. Moreover, during severe-intensity exercise, the amplitude of the $\dot{V}O_2$ slow component is reduced, and exercise tolerance is enhanced following dietary NO₃⁻ supplementation (1, 4). Conversely, the amplitude of the $\dot{V}O_2$ slow component is increased following infusion of the NOS inhibitor nitro-L-arginine methyl ester (L-NAME) (33). L-NAME administration has also been shown to increase tissue $\dot{V}O_2$ in dogs (37, 65).

Given the findings described above, it is possible that dietary supplementation with the NOS substrate L-arginine might reduce the O₂ cost of moderate-intensity exercise and the amplitude of the $\dot{V}O_2$ slow component and enhance exercise tolerance during severe-intensity exercise. Despite the presence of an abundant intracellular L-arginine concentration, which can be as high as 0.1–1.0 mM, significantly exceeding the *K_m* of endothelial NOS (eNOS) for L-arginine of 2.9 μmol/l (76), exogenous L-arginine causes NO-mediated biological effects, a phenomenon that has been termed the arginine paradox (12, 45, 76). For example, exogenous L-arginine administration has been reported to increase urinary NO₃⁻ concentration ([NO₃⁻]) (53), plasma NO₂⁻ concentration ([NO₂⁻] + [NO₃⁻] (NO_x)) (77), and L-citrulline concentration ([L-citrulline]) (64) and to reduce resting systolic blood pressure (67). Conversely, an arginine-free diet has been shown to reduce plasma arginine flux and NO synthesis (18). Despite this evidence that L-arginine can alter NO production, recent reports suggest that dietary L-arginine supplementation does not influence the

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steady-state O_2 cost of moderate-intensity cycle exercise (41) or submaximal treadmill running (8). However, markers of NO bioavailability were not demonstrably increased in these two earlier studies (8, 41), possibly because the supplementation regimens involved the ingestion of a relatively small amount of L-arginine on a number of occasions each day. In this respect, it is possible that the ingestion of an acute "bolus" of L-arginine might result in a rapid rise in markers of NO bioavailability, with corresponding physiological effects. It has been reported that the maximal concentration of plasma L-arginine is reached 90 min following the ingestion of 6 g of L-arginine (9). Also, whereas L-arginine supplementation has been reported to improve exercise tolerance in patient populations (7, 22, 58), the effects in healthy humans are less clear. Specifically, improvements in repeated sprint performance (14) or muscle power and fatigue resistance (17, 69) have been observed in some studies following L-arginine supplementation. However, other studies have reported no effect of L-arginine supplementation on intermittent anaerobic exercise (52) or marathon running performance (19). The L-arginine supplementation regimen differed considerably between these studies, and markers of NO bioavailability were either not measured (14, 17, 19, 69) or were found to be not different following L-arginine supplementation (52). Therefore, the possible influence of dietary L-arginine supplementation on the physiological responses to exercise and on exercise tolerance is controversial.

The purpose of the present study was to investigate the effects of acute L-arginine ingestion on indexes of NO synthesis and the physiological responses to low- and high-intensity exercise. The pharmacokinetic relationship between acute L-arginine ingestion and plasma L-arginine concentration (9) was used to inform the study design. We hypothesized that acute L-arginine supplementation would elevate plasma $[NO_2^-]$ and reduce systolic blood pressure, consistent with an enhanced NO bioavailability (38, 51). On the basis of the results of previous studies that used dietary NO_3^- to enhance NO bioavailability (1, 4), we also hypothesized that L-arginine supplementation would reduce the O_2 cost of moderate-intensity exercise and improve severe-intensity exercise tolerance by reducing the $\dot{V}O_2$ slow component amplitude.

METHODS

Subjects

Nine healthy, recreationally active men (mean \pm SD: 26 \pm 6 yr old, 1.81 \pm 0.04 m height, 84 \pm 5 kg body mass) volunteered to participate in the study after responding to poster advertisements placed around the University of Exeter campus. None of the subjects were tobacco smokers or users of dietary supplements. All subjects were fully familiar with laboratory exercise-testing procedures, having previously participated in studies employing cycle ergometry in our laboratory. The procedures were approved by the Institutional Research Ethics Committee. All subjects gave their written informed consent after the experimental procedures, associated risks, and potential benefits of participation had been explained. Subjects were instructed to arrive at the laboratory \geq 3 h postprandial and to refrain from caffeine and alcohol intake 6 and 24 h before each test, respectively. All tests were performed at the same time of day (\pm 2 h) to minimize the effects of diurnal biological variation on physiological responses and exercise performance.

Procedures

The subjects were required to report to the laboratory on seven occasions, over a 4- to 5-wk period. During the first visit to the laboratory, the subjects performed a ramp incremental exercise test for determination of $\dot{V}O_{2peak}$ and GET. All cycle tests were performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands). Initially, each subject completed 3 min of "unloaded" baseline cycling; then the work rate was increased by 30 W/min until the subject was unable to continue. The subjects cycled at a self-selected pedal rate (70–90 rpm), and this pedal rate, along with the saddle and handlebar height and configuration, was recorded and reproduced in subsequent tests. The breath-by-breath pulmonary gas exchange data were collected continuously during the incremental tests and averaged over consecutive 10-s periods. $\dot{V}O_{2peak}$ was taken as the highest 30-s mean value attained prior to the subject's volitional exhaustion. GET was determined as described previously (1–3). The work rates that would require 80% of the GET (moderate-intensity exercise) and 70% Δ (70% of the difference between the power output at the GET and $\dot{V}O_{2peak}$ plus the power output at GET; i.e., severe-intensity exercise) were subsequently calculated, with account taken of the mean response time (MRT) for $\dot{V}O_2$ during ramp exercise.

After completion of the ramp test, subjects were randomly assigned, using a double-blind cross-over design, to receive 3 consecutive days of dietary supplementation with a commercially available L-arginine product (Arg; ARK 1, Arkworld International; administered in 0.5 liter of water) or placebo (PL; 0.5 liter of blackcurrant-flavor cordial). Participants received a 20-g dose of the ARK 1 supplement, which contained 6 g of L-arginine, along with trace amounts of vitamins (E, C, B₆, and B₁₂), other amino acids (L-glutamine, L-leucine, L-valine, L-carnitine, L-citrulline, L-cysteine, and L-isoleucine), and fructose (11 g) at a dose that would not be expected to elicit performance gains (30). A 10-day washout period separated the supplementation periods. The order between the Arg and PL supplementation periods was randomized. The subjects were not aware of the experimental hypotheses to be tested but were informed that the purpose of the study was to compare the physiological responses to exercise following the consumption of two commercially available beverages. Throughout the study period, subjects were instructed to maintain their normal daily activities and diet. The subjects kept a food diary and consumed an identical diet during the two periods of exercise testing.

On the 3 days of supplementation, the subjects completed "step" exercise tests from a 20-W baseline to moderate- and severe-intensity work rates for the determination of pulmonary $\dot{V}O_2$ dynamics. After arrival at the laboratory, the subjects rested for 20 min and then were instructed to consume the Arg or PL beverage within a 5-min period; exercise tests were initiated 1 h after ingestion. On *day 1* of supplementation the subjects completed two 6-min bouts of moderate-intensity cycling, on *day 2* they completed one 6-min bout of moderate-intensity cycling followed by one 6-min bout of severe-intensity cycling, and on *day 3* they completed one 6-min bout of moderate-intensity cycling followed by one bout of severe-intensity cycling that was continued until task failure as a measure of exercise tolerance. The two bouts of exercise on each day were separated by 25 min of rest. The time to task failure was recorded when the pedal rate fell by >10 rpm below the required pedal rate. Before each exercise bout, blood pressure was measured and venous blood samples were collected for subsequent determination of plasma $[NO_2^-]$ (see *Measurements*). The supplementation protocol was based on the pharmacokinetics of L-arginine: it has been reported that the maximal concentration of plasma L-arginine is reached 90 min following the ingestion of 6 g of L-arginine (9).

Measurements

During all tests, pulmonary gas exchange and ventilation were measured continuously using a portable metabolic cart (MetaMax 3B,

Cortex Biophysik, Leipzig, Germany), as described previously (1–3). A digital volume transducer turbine measured inspired and expired airflow, while an electrochemical cell O_2 analyzer and a nondispersive infrared CO_2 analyzer measured expired gases. The inspired and expired gas volume and gas concentration signals were continuously sampled via a capillary line connected to the mouthpiece and displayed breath-by-breath. Heart rate (HR) was measured during all tests via short-range radiotelemetry (Polar S610, Polar Electro, Kempele, Finland). During one of the transitions to moderate- and severe-intensity exercise, for both supplementation periods, a blood sample was collected from a fingertip into a capillary tube over the 20 s preceding the step transition in work rate and within the last 20 s of exercise. A capillary blood sample was also collected at the limit of tolerance for the severe-intensity exercise bout performed on *day 3* of each supplementation period. These whole blood samples were subsequently analyzed to determine blood lactate concentration ([lactate]; YSI 1500, Yellow Springs Instruments, Yellow Springs, OH) within 30 s of collection. Blood lactate accumulation (Δ blood [lactate]) was calculated as the difference between blood [lactate] at end exercise and blood [lactate] at baseline.

The blood pressure in the brachial artery was measured with subjects in a rested, seated position prior to each exercise bout via an automated sphygmomanometer (Dinamap Pro, GE Medical Systems, Tampa, FL). Three measurements were taken, and the mean of the second and third blood pressure measurements was recorded. Venous blood samples were also drawn into lithium-heparin tubes prior to each exercise bout and centrifuged at 4,000 rpm and 4°C for 10 min, within 3 min of collection. Plasma was subsequently extracted and immediately placed in a freezer at –80°C for later analysis of $[NO_2^-]$ via chemiluminescence, as described previously (1).

Data Analysis Procedures

The breath-by-breath $\dot{V}O_2$ data from each test were initially examined to exclude errant breaths caused by coughing, swallowing, sighing, etc., and those values lying >4 SDs from the local mean were removed. The breath-by-breath data were subsequently linearly interpolated to provide second-by-second values, and, for each individual, identical repetitions were time-aligned to the start of exercise and ensemble-averaged. In this way, the $\dot{V}O_2$ responses to the four moderate-intensity and the two severe-intensity exercise bouts were averaged prior to analysis to reduce breath-to-breath noise and enhance confidence in the parameters derived from the modeling process (46). The first ~20–25 s of data after the onset of exercise (i.e., the phase I response) were deleted, and a nonlinear least-squares algorithm was used to fit the data thereafter. A single-exponential model was used to characterize the $\dot{V}O_2$ responses to moderate-intensity exercise, and a biexponential model was used for severe-intensity exercise, as described in *Eqs. 1* and *2* for moderate- and severe-intensity exercise, respectively

$$\dot{V}O_2(t) = \dot{V}O_{2\text{baseline}} + A_p[1 - e^{-(t-TD_p)/\tau_p}] \quad (1)$$

$$\dot{V}O_2(t) = \dot{V}O_{2\text{baseline}} + A_p[1 - e^{-(t-TD_p)/\tau_p}] + A_s[1 - e^{-(t-TD_s)/\tau_s}] \quad (2)$$

where $\dot{V}O_2(t)$ represents the absolute $\dot{V}O_2$ at a given time t ; $\dot{V}O_{2\text{baseline}}$ represents the mean $\dot{V}O_2$ in the baseline period; A_p , TD_p , and τ_p represent the amplitude, time delay (TD), and time constant (τ), respectively, describing the phase II (primary) increase in $\dot{V}O_2$ above baseline; and A_s , TD_s , and τ_s represent the amplitude, TD before the onset, and τ describing the development, of the $\dot{V}O_2$ slow component, respectively.

An iterative process was used to minimize the sum of the squared errors between the fitted function and the observed values. $\dot{V}O_{2\text{baseline}}$ was defined as the mean $\dot{V}O_2$ measured over the final 90 s of baseline pedaling. The “end-exercise” $\dot{V}O_2$ was defined as the mean $\dot{V}O_2$ measured over the final 30 s of the 6-min exercise bouts (i.e., between

330 and 360 s); the $\dot{V}O_2$ at exhaustion for the severe-intensity exercise bouts was calculated as the mean $\dot{V}O_2$ over the final 30 s before the subjects reached the limit of tolerance. Because the asymptotic value (A_s) of the exponential term describing the $\dot{V}O_2$ slow component may represent a higher value than is actually reached at the end of the exercise, the actual amplitude of the $\dot{V}O_2$ slow component at the end of exercise was defined as A'_s . The A'_s parameter was compared at isotime (360 s) under both supplementation periods. The amplitude of the $\dot{V}O_2$ slow component was also described relative to the entire $\dot{V}O_2$ response.

Because of possible concerns over the inclusion of nonessential parameters in *Eq. 2*, we also fitted the severe-intensity exercise data using the methods of Rossiter et al. (63). Briefly, with use of a purpose-designed program (LabView, version 6.1, National Instruments, Newbury, UK), *Eq. 1* was initially fit up to the first 60 s of exercise and then increased iteratively by 1 s to 360 s of exercise. The best-fit curve for the phase II portion of the response was established using 1) a plot of the $\dot{V}O_2$ time constant (τ) against time to identify the point at which the influence of the $\dot{V}O_2$ slow component lengthened the estimated τ following an initial plateau and 2) deviation from an optimal fitting of the model as judged by a systematic departure of the model’s residuals. The magnitude of the $\dot{V}O_2$ slow component was then calculated as the difference between the phase II asymptote and the mean $\dot{V}O_2$ value between 330 and 360 s of exercise.

For moderate-intensity exercise, the functional gain (i.e., the reciprocal of “delta” efficiency) of the $\dot{V}O_2$ response was computed by dividing A_p by the Δ work rate. In addition, to determine the overall kinetics of the $\dot{V}O_2$ response to moderate- and severe-intensity exercise, data were fit with a monoexponential model from 0 s to the end of exercise, without TD. For moderate-intensity exercise, the MRT so derived was used in the computation of the O_2 deficit ($A_p \cdot \text{MRT}/60$).

We also modeled the HR response to exercise in each condition. For this analysis, HR data were linearly interpolated to provide second-by-second values, and, for each individual, identical repetitions from like-transitions were time-aligned to the start of exercise and ensemble-averaged. Nonlinear least-squares mono- and biexponential models without TD were used to fit the data to moderate- and severe-intensity exercise, respectively, with the fitting window commencing at 0 s.

Statistics

Differences in the cardiorespiratory variables between conditions were analyzed with two-tailed, paired-samples *t*-tests. Alterations in blood pressure and plasma $[NO_2^-]$ were determined via a two-tailed, two-way (supplement \times time), repeated-measures ANOVA. Significant effects were further explored using Fisher’s least significant difference. Relationships between variables were assessed using Pearson’s product moment correlation coefficient. Data are presented as means \pm SD. Statistical significance was accepted when $P < 0.05$.

RESULTS

During the ramp incremental test, subjects attained a peak work rate of 367 ± 32 W and $\dot{V}O_{2\text{peak}}$ of 4.03 ± 0.37 l/min. The moderate- and severe-intensity work rates used in the main part of the study were 82 ± 14 and 274 ± 21 W, respectively.

Plasma $[NO_2^-]$ and Blood Pressure

The group mean plasma $[NO_2^-]$ values obtained before each of the two exercise bouts completed on each of *days 1, 2, and 3* of the Arg and PL supplementation periods are illustrated in *Fig. 1*. There was a significant main effect for supplement ($P < 0.05$), while the main effect for time and the interaction effect were not significant ($P > 0.05$). Plasma $[NO_2^-]$ was significantly higher during Arg supplementation than in the PL

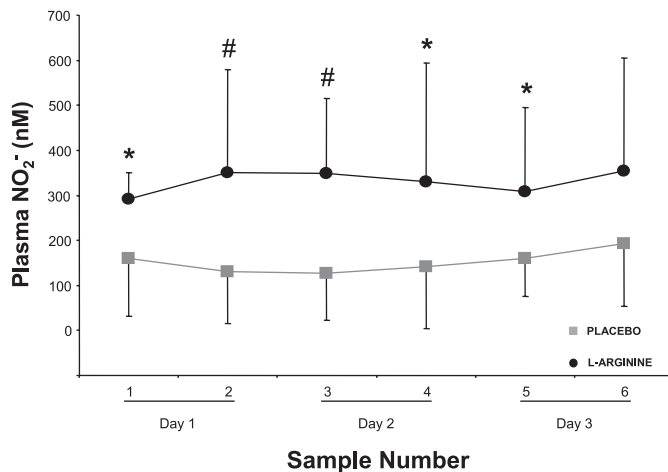


Fig. 1. Plasma nitrite (NO_2^-) concentration ($[\text{NO}_2^-]$) following acute L-arginine or placebo supplementation over 3 consecutive days. The 2 sample points within each day represent measurements obtained before the 1st and 2nd exercise bouts. Values are group means \pm SD. Note significantly greater plasma $[\text{NO}_2^-]$ following L-arginine supplementation. Significantly different from placebo: * $P < 0.05$; # $P < 0.01$.

condition for the first five sample points (Fig. 1). On average, across the six sample points, plasma $[\text{NO}_2^-]$ was 108% greater during Arg supplementation than in the PL condition ($P < 0.01$). The Arg-induced elevations in plasma $[\text{NO}_2^-]$ were not different between days 1, 2, and 3.

The mean systolic and diastolic blood pressure values measured before each of the six exercise bouts during Arg supplementation and the PL condition are shown in Fig. 2. Similar to the plasma $[\text{NO}_2^-]$ response to Arg supplementation, we observed a significant main effect for supplement ($P < 0.05$), while the main effect for time and the interaction effect were not significant ($P > 0.05$). The ingestion of Arg significantly reduced systolic blood pressure at three of the six sample points relative to PL (Fig. 2). Overall, systolic blood pressure was reduced by 8 mmHg when averaged over the six sample points ($P < 0.01$ compared with PL). The Arg-induced reductions in systolic blood pressure were not significantly different between days 1, 2, and 3. The ~ 3 -mmHg reduction in diastolic blood pressure following Arg ingestion was not significantly different from PL (Fig. 2).

$\dot{V}O_2$ Dynamics and Exercise Tolerance

Moderate-intensity exercise. The pulmonary $\dot{V}O_2$ response during moderate-intensity exercise is shown in Fig. 3, and the parameters derived from the model fit are presented in Table 1. Arg supplementation resulted in a 10% reduction in the amplitude of the pulmonary $\dot{V}O_2$ response, relative to PL, following a step increment to the same absolute moderate-intensity cycling work rate (0.67 ± 0.13 and 0.60 ± 0.13 l/min for PL and Arg, respectively, $P < 0.05$; Fig. 3), with no difference in $\dot{V}O_2$ during the baseline period of very low-intensity (20-W) cycling. Accordingly, the functional gain (i.e., the ratio of the increase in O_2 consumed per minute to the increase in external work rate) was reduced from $10.8 \text{ ml}\cdot\text{min}^{-1}\cdot\text{W}^{-1}$ in the PL condition to $9.7 \text{ ml}\cdot\text{min}^{-1}\cdot\text{W}^{-1}$ following Arg supplementation. The absolute $\dot{V}O_2$ over the final 30 s of moderate-intensity exercise was also significantly lower following Arg ingestion (1.59 ± 0.13 and 1.48 ± 0.12 l/min for PL and Arg, respec-

tively, $P < 0.05$; Fig. 3), as was the O_2 deficit (0.45 ± 0.15 and 0.39 ± 0.12 liter for PL and Arg, respectively, $P < 0.05$). The phase II τ was not significantly altered by Arg supplementation (27 ± 5 and 26 ± 8 s for PL and Arg, respectively, $P > 0.05$). The 95% confidence interval for the estimation of the phase II τ was 3 ± 1 s for both conditions. The baseline and end-exercise values of HR, CO_2 output, respiratory exchange ratio, minute ventilation, and blood [lactate] were not significantly different between the conditions (Tables 1 and 2).

Severe-intensity exercise. The pulmonary $\dot{V}O_2$ response during severe-intensity exercise is shown in Fig. 4, and the parameters derived from the biexponential fit (Eq. 2) are presented in Table 1. In contrast to the effects observed for moderate-intensity exercise, the primary $\dot{V}O_2$ amplitude during severe-intensity exercise was significantly elevated following Arg supplementation (2.27 ± 0.14 and 2.45 ± 0.12 l/min for PL and Arg, respectively, $P < 0.01$; Table 1, Fig. 4). The phase II τ was not significantly different following Arg supplementation relative to the PL condition (34 ± 10 and 39 ± 12 s for PL and Arg, respectively, $P > 0.05$; Table 1). The 95% confidence intervals for the estimation of the phase II τ were 5 ± 2 and 4 ± 2 s in the PL condition and following Arg

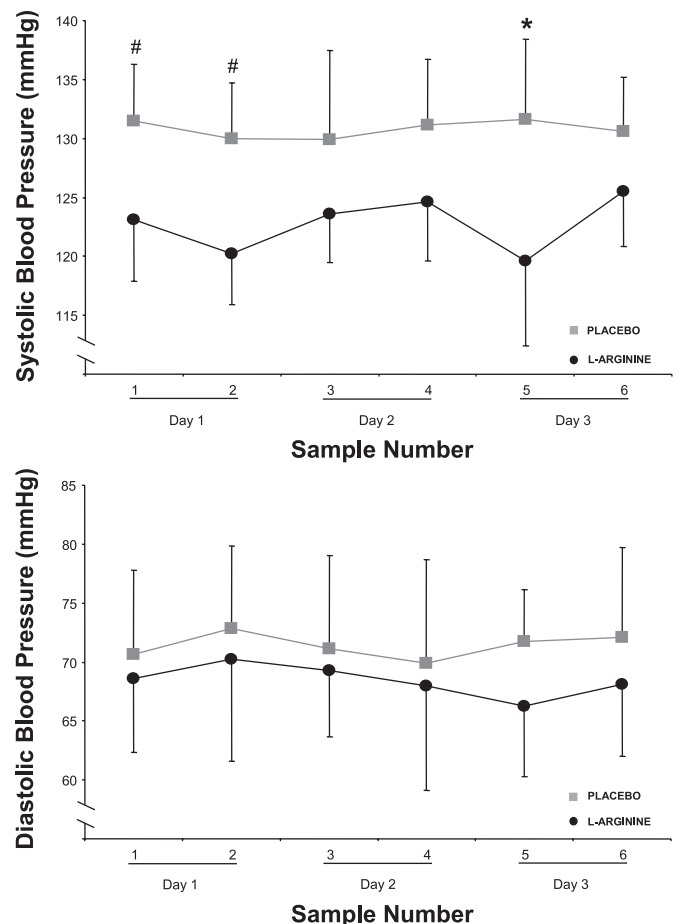


Fig. 2. Systolic (top) and diastolic (bottom) blood pressure following acute L-arginine or placebo supplementation over 3 consecutive days. The 2 sample points within each day represent measurements obtained before the 1st and 2nd exercise bouts. Values are group means \pm SD. Systolic blood pressure was significantly reduced following L-arginine supplementation. Significantly different from placebo: * $P < 0.05$; # $P < 0.01$.

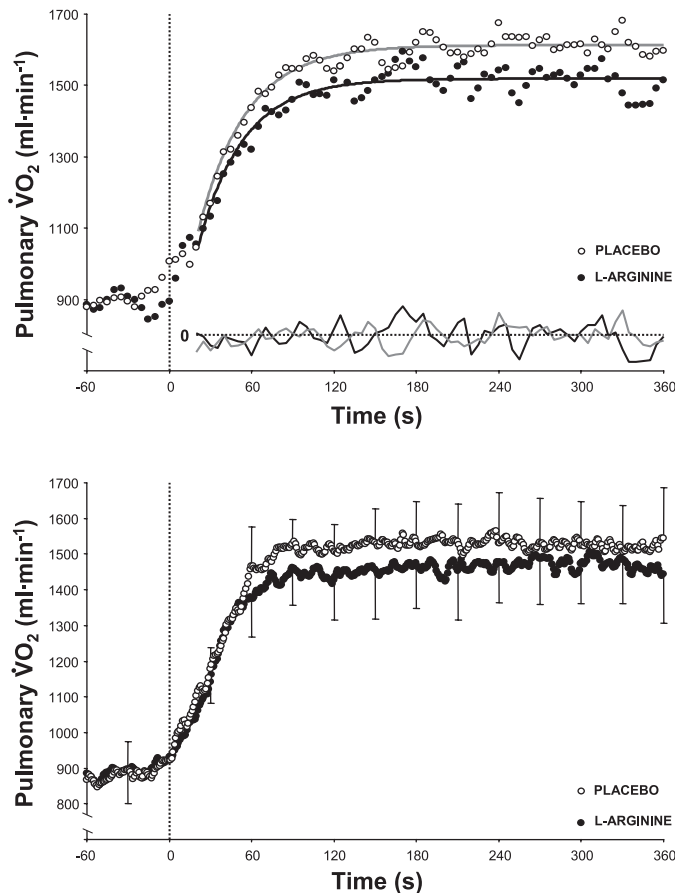


Fig. 3. Pulmonary $\dot{V}O_2$ following L-arginine and placebo supplementation after a step increment to a moderate-intensity work rate. Dotted vertical line represents abrupt imposition of the moderate-intensity work rate from a baseline of cycling at 20 W. *Top*: $\dot{V}O_2$ response of a representative individual (data are shown at 5-s intervals). Curve fits are shown as solid lines, and residuals are shown at the foot of the panel. *Bottom*: group mean $\dot{V}O_2$ response, with SD bars shown every 30 s for clarity. Steady-state $\dot{V}O_2$ during moderate-intensity exercise was reduced following L-arginine supplementation.

supplementation, respectively. The amplitude of the $\dot{V}O_2$ slow component was significantly smaller following Arg supplementation (0.76 ± 0.29 and 0.58 ± 0.23 l/min for PL and Arg, respectively, $P < 0.05$; Fig. 4) and, therefore, represented a smaller proportion of the overall $\dot{V}O_2$ response (20 ± 6 and $15 \pm 6\%$ for PL and Arg, respectively, $P < 0.05$). The essential results were the same when the alternative modeling procedure of Rossiter et al. (63) was employed. Specifically, the phase II τ was not significantly different (30 ± 11 and 33 ± 7 s for PL and Arg, respectively, $P > 0.05$), the primary $\dot{V}O_2$ amplitude was increased (2.12 ± 0.19 and 2.24 ± 0.27 l/min for PL and Arg, respectively, $P < 0.05$), and the $\dot{V}O_2$ slow component was reduced (0.87 ± 0.23 and 0.71 ± 0.25 l/min for PL and Arg, respectively, $P < 0.01$) following Arg supplementation. The $\dot{V}O_2$ at exhaustion in the constant work rate tests was not significantly different between supplements or from the $\dot{V}O_{2peak}$, as determined in the initial ramp incremental test. The baseline and end-exercise values of CO_2 output, respiratory exchange ratio, and minute ventilation were not significantly different between the conditions (Table 1). Blood [lactate] at 6 min of exercise and at exhaustion, as well as HR

dynamics, were not significantly different between the conditions (Table 2).

Exercise tolerance was enhanced following Arg supplementation, as demonstrated by the 20% increase in the time to task failure (562 ± 145 and 707 ± 232 s for PL and Arg, respectively, $P < 0.05$). After Arg supplementation, exercise tolerance was significantly related to the absolute plasma $[NO_2^-]$ prior to the exhaustive severe-intensity exercise bout ($r = 0.82$, $P < 0.05$). However, there were no significant correlations between the changes in plasma $[NO_2^-]$ following Arg and changes in $\dot{V}O_2$ kinetics or exercise tolerance.

DISCUSSION

The principal novel finding of this investigation was that acute L-arginine supplementation, which significantly increased plasma $[NO_2^-]$ and reduced systolic blood pressure, resulted in a reduced O_2 cost of moderate-intensity cycle exercise, along with a reduced $\dot{V}O_2$ slow component amplitude and improved time to task failure during severe-intensity exercise. These findings confirm our experimental hypotheses and are consistent with the improvement in the physiological and performance parameters we observed previously following

Table 1. Gas exchange and ventilation responses during moderate- and severe-intensity exercise following supplementation with L-arginine and placebo

	Placebo	L-Arginine
<i>Moderate-intensity exercise</i>		
O_2 uptake, l/min		
Baseline	0.92 ± 0.10	0.89 ± 0.07
End-exercise	1.59 ± 0.14	$1.48 \pm 0.12^*$
Phase II time constant, s	27 ± 5	26 ± 8
Mean response time, s	40 ± 6	39 ± 4
Primary amplitude, l/min	0.67 ± 0.13	$0.60 \pm 0.13^*$
O_2 deficit, liter	0.45 ± 0.15	$0.39 \pm 0.12^*$
CO_2 output, l/min		
Baseline	0.86 ± 0.08	0.83 ± 0.12
End-exercise	1.32 ± 0.18	1.31 ± 0.14
Minute ventilation, l/min		
Baseline	26 ± 2	25 ± 4
End-exercise	36 ± 4	36 ± 5
Respiratory exchange ratio		
Baseline	0.93 ± 0.10	0.93 ± 0.11
End-exercise	0.83 ± 0.06	0.88 ± 0.03
<i>Severe-intensity exercise</i>		
O_2 uptake, l/min		
Baseline	0.97 ± 0.08	0.99 ± 0.06
End-exercise	4.00 ± 0.26	4.02 ± 0.29
Phase II time constant, s	34 ± 10	39 ± 12
Primary amplitude, l/min	2.27 ± 0.14	$2.45 \pm 0.12^\dagger$
Slow component amplitude		
l/min	0.76 ± 0.29	$0.58 \pm 0.23^*$
%	20 ± 6	$15 \pm 6^*$
CO_2 output, l/min		
Baseline	0.88 ± 0.10	0.90 ± 0.10
End-exercise	4.07 ± 0.35	4.05 ± 0.32
Minute ventilation, l/min		
Baseline	25 ± 5	26 ± 4
End-exercise	142 ± 17	147 ± 22
Respiratory exchange ratio		
Baseline	0.91 ± 0.13	0.91 ± 0.09
End-exercise	1.02 ± 0.04	1.01 ± 0.04

Values are means \pm SD. Significantly different from placebo: $*P < 0.05$; $^\dagger P < 0.01$.

Table 2. Heart rate and blood [lactate] responses to moderate- and severe-intensity exercise following supplementation with L-arginine and placebo

	Placebo	L-Arginine
<i>Moderate-intensity exercise</i>		
Heart rate, beats/min		
Baseline	80 ± 9	81 ± 8
End-exercise	96 ± 10	98 ± 13
Time constant, s	29 ± 14	31 ± 16
Amplitude, beats/min	17 ± 6	16 ± 17
Blood [lactate], mM		
Baseline	0.8 ± 0.3	0.9 ± 0.1
End-exercise	1.0 ± 0.4	1.1 ± 0.3
Δ	0.2 ± 0.2	0.2 ± 0.4
<i>Severe-intensity exercise</i>		
Heart rate, beats/min		
Baseline	86 ± 7	88 ± 8
End-exercise	172 ± 7	170 ± 8
Time constant, s	15 ± 7	17 ± 6
Blood [lactate], mM		
Baseline	1.0 ± 0.2	1.0 ± 0.1
End-exercise	6.8 ± 1.2	6.5 ± 1.3
Δ	5.8 ± 1.3	5.5 ± 1.4
Exhaustion	10.3 ± 1.7	9.2 ± 1.6

Values are means ± SD. [lactate], Lactate concentration.

dietary NO_3^- supplementation (1). The similar physiological and performance effects following L-arginine and NO_3^- supplementation suggest that these effects are attributable, in large part, to the increased NO availability afforded by these dietary interventions.

Effects of L-Arginine Supplementation on Indexes of NO Production

NO is synthesized through the activity of the NOS family of enzymes, which includes three major isoforms: eNOS, neuronal NOS (nNOS), and inducible NOS (68). These enzymes produce NO and L-citrulline through catalysis of the five-electron oxidation of L-arginine in a reaction requiring O_2 (13). eNOS and nNOS are constitutively expressed in skeletal muscle (39). Skeletal muscles produce NO at low levels at rest and at higher levels during contraction, resulting in a significant increase in NO synthesis during exercise (5, 38). The in vivo quantification of NO is technically challenging; therefore, changes in the plasma concentration of the oxidation products of NO, namely, NO_2^- and NO_3^- , are often measured to provide an indication of NO production (38, 50, 51). NO_2^- is formed by the enzymatic oxidation of NO by ceruloplasmin (66) and through the binding of NO to the Cu^{2+} active site of cytochrome *c* oxidase (20), while NO_3^- is derived through the reaction of NO with oxyhemoglobin (23). Of these NO markers, NO_2^- is considered to provide the best indication of eNOS activity in humans (51, 61). In the present study, plasma $[NO_2^-]$ was essentially doubled following acute L-arginine supplementation, and this is indicative of an increased NOS activity and NO production. L-Arginine administration has also been reported to increase urinary $[NO_3^-]$ (53), plasma $[NO_2^-]$ and $[NO_3^-]$ (77), and plasma [L-citrulline] (64), consistent with an enhanced NO production.

While NO is involved in a multitude of physiological processes in metazoan species (68), a reduction in blood pressure

is one of the most recognized manifestations of an increased NO bioavailability (1, 4, 47, 48, 67). Indeed, NO is known to be an important endothelium-relaxing factor, through its activation of guanylate cyclase, which subsequently metabolizes GTP to cGMP, culminating in smooth muscle relaxation (28). In keeping with the significantly elevated plasma $[NO_2^-]$, we observed a ~8-mmHg reduction in systolic blood pressure following acute L-arginine administration, consistent with the observations of Siani et al. (67). Taken together, these findings confirm that L-arginine administration resulted in an enhanced NO production in healthy humans, as reported elsewhere in healthy mammals (53, 77). As well as serving as a substrate for NOS, L-arginine has a number of important antioxidant properties that can also serve to increase NO bioavailability through NOS-independent mechanisms. Although the physiological relevance is unclear, these mechanisms include the reaction between arginine and H_2O_2 to form NO (57) and the L-arginine-induced inhibition of superoxide (O_2^-) production and increased O_2^- scavenging (72). O_2^- reacts rapidly with NO to form the highly reactive peroxynitrite anion. A reduction in the concentration of O_2^- could potentially lower the amount of

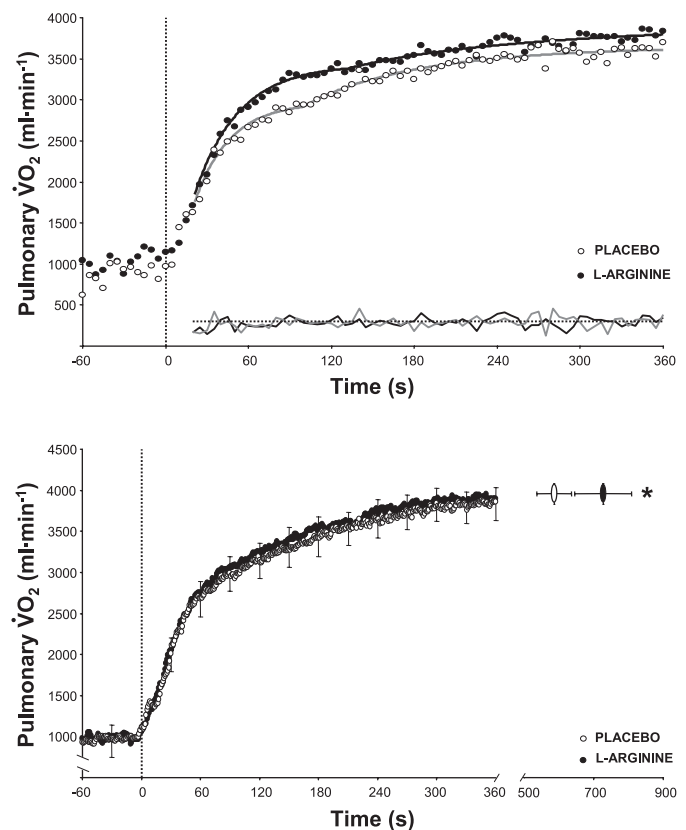


Fig. 4. Pulmonary $\dot{V}O_2$ following L-arginine and placebo supplementation after a step increment to a severe-intensity work rate. Dotted vertical line represents abrupt imposition of the severe-intensity work rate from a baseline of cycling at 20 W. *Top*: $\dot{V}O_2$ response of a representative individual (data are shown at 5-s intervals). Curve fits are shown as solid lines, and residuals are shown at the foot of the panel. *Bottom*: group mean $\dot{V}O_2$ response to 6 min of severe-intensity exercise, with SD bars shown every 30 s for clarity; group mean ± SD $\dot{V}O_2$ at task failure is also shown. Primary $\dot{V}O_2$ amplitude is elevated and $\dot{V}O_2$ slow component is reduced following L-arginine supplementation, effects that are akin to those of priming exercise (2, 15). *Time to exhaustion is significantly different from placebo ($P < 0.05$).

scavenging of NO by O_2^- , thereby increasing NO bioavailability.

Effects of L-Arginine on the Physiological Responses to Moderate-Intensity Exercise

After acute L-arginine supplementation, the amplitude of the $\dot{V}O_2$ response from baseline cycling to the steady state during moderate-intensity exercise was significantly reduced (by 10%), the absolute steady-state $\dot{V}O_2$ was significantly reduced (by 7%), and Δ efficiency was increased from $\sim 27\%$ to $\sim 30\%$. A reduction in the O_2 cost of low-intensity exercise has also been reported following pharmacological and dietary NO_3^- administration in association with increases in markers of NO synthesis (1, 4, 47, 48). Moreover, administration of the NOS inhibitor L-NAME has been associated with an increased O_2 cost of muscular contraction in dogs (65). While two recent studies have reported that the O_2 cost of low-intensity cycling (41) and running (8) was unaffected by L-arginine administration, it is noteworthy that markers of NO synthesis were not significantly altered in either study. In the study of Bescós et al. (8), plasma $[NO_3^-]$ was not significantly different between three diets in which dietary L-arginine was manipulated with a combination of foodstuffs and supplementation (5.5, 9.0, and 20.5 g/day for 3 days). In the study of Koppo et al. (41), 14 days of supplementation with L-arginine hydrochloride capsules (7.2 g/day in 3 equal doses) significantly increased serum arginine concentration but did not alter resting blood pressure or urinary $[NO_3^-]$. In contrast, in the present study, plasma $[NO_2^-]$ was significantly increased and systolic blood pressure was significantly reduced (consistent with increased NO bioavailability and production) ~ 60 – 90 min following the consumption of a beverage containing 6 g of L-arginine.

It is possible that the differences in the effects of L-arginine on $\dot{V}O_2$ dynamics and exercise tolerance between the present study and previous studies (8, 41) are related to the supplementation regimen (i.e., acute bolus vs. more regular, but smaller, doses of L-arginine given over 3–14 days) and the timing of the exercise test in relation to the administration of the supplement. Specifically, with chronic administration of L-arginine, it is unlikely that a sufficiently large dose of L-arginine is ingested at any time to result in a substantial increase in plasma L-arginine (9) or NO bioavailability (e.g., as estimated by plasma $[NO_2^-]$), such that no differences in resting blood pressure or the physiological responses to exercise would be expected. It is also possible that continued L-arginine supplementation in previous studies (8, 41) resulted in increased production of asymmetric dimethylarginine, a naturally occurring endogenous L-arginine metabolite that can inhibit all NOS isoforms (71). Consequently, the net effect of prolonged L-arginine supplementation may be no alteration in NO synthesis, as the potential for increased NOS activity with L-arginine supplementation may be offset by NOS inhibition by asymmetric dimethylarginine. The results of the present study, by contrast, indicate that ingestion of an acute bolus of L-arginine within 60–90 min of the start of exercise increases NO bioavailability (as indicated by increased plasma $[NO_2^-]$ and reduced systolic blood pressure) and positively influences the physiological responses to exercise and exercise tolerance.

Although a 10% reduction in the primary $\dot{V}O_2$ amplitude during moderate-intensity exercise following acute L-arginine

supplementation is impressive, we previously reported an even greater reduction (19%) following dietary NO_3^- supplementation (1). The NOS enzymes that catalyze L-arginine conversion to NO require O_2 as an essential cosubstrate. The eNOS and nNOS isoforms have been reported to have a K_m for O_2 of 2.3 and 202 Torr, respectively (70). In human skeletal muscle, P_{O_2} can fall to ~ 2 – 5 Torr during exercise (62). Therefore, while the activity of eNOS will be largely preserved during exercise, the activity of nNOS might be reduced, potentially limiting the total NO yield through this pathway. The NO_3^- - NO_2^- -NO pathway has fewer rate-limiting steps, such that it is possible that this pathway might be preferable for NO production during exercise (26). This might explain the greater effects of NO_3^- than L-arginine supplementation on exercise efficiency. However, the effects of dietary NO_3^- supplementation on exercise efficiency in our earlier studies (1, 4) were likely amplified, because habitual dietary NO_3^- intake was restricted throughout the study period.

Given that dietary NO_3^- and L-arginine supplementation reduce the O_2 cost of low-intensity exercise and that both of these dietary regimens increase indexes of NO production, it seems reasonable to conclude that it is the increased NO generation afforded by these dietary interventions that elicits the improvement in exercise efficiency. To have lowered the O_2 cost of low-intensity exercise, L-arginine administration would have been required to reduce the ATP cost of force production (i.e., to improve muscle contractile efficiency), the O_2 cost of ATP resynthesis (i.e., increased ratio of mitochondrial phosphorylated ADP to $\dot{V}O_2$), or both. To investigate the mechanistic bases of the reduced O_2 cost of moderate-intensity exercise following dietary NO_3^- supplementation, we recently determined skeletal muscle energetics (using ^{31}P -MRS) and pulmonary gas exchange dynamics during knee-extension exercise (4). This study confirmed that dietary NO_3^- supplementation reduced steady-state $\dot{V}O_2$ and demonstrated that this effect was proportionately similar to the blunted changes in intramuscular phosphocreatine, P_i , and ADP concentrations measured across the transition from rest to moderate-intensity exercise (4). Moreover, the estimated total ATP turnover rate was significantly reduced following dietary NO_3^- supplementation (4). These data suggest that the improved exercise efficiency afforded by a NO_3^- -rich diet might be consequent to a reduced ATP cost of force production. Given that both dietary NO_3^- and L-arginine yield NO, it is possible that the reduced O_2 cost of moderate-intensity exercise following L-arginine supplementation has a similar mechanistic basis, although this remains to be confirmed.

In addition to its effects on the steady-state O_2 cost of moderate-intensity exercise, NO can also influence the kinetics with which $\dot{V}O_2$ rises following the onset of exercise. Indeed, faster $\dot{V}O_2$ kinetics have been reported following L-NAME administration (33, 34, 36), an effect that was ascribed to the alleviation of the competitive inhibition of cytochrome *c* oxidase by NO (20). Consistent with this finding, dietary NO_3^- supplementation resulted in significantly slower phase II $\dot{V}O_2$ kinetics in our previous study (1). Surprisingly, Koppo et al. (41) recently reported a slight (< 2 s), but statistically significant, acceleration of phase II $\dot{V}O_2$ kinetics following L-arginine supplementation. However, this was countered by an increased TD_p of similar magnitude, such that the overall kinetics (i.e., MRT) were similar (~ 32 s) and the magnitude of the O_2 deficit

was unaltered (41). In the present study, we also found no significant difference in the MRT; however, the significant reduction in the primary $\dot{V}O_2$ amplitude resulted in a significantly reduced O_2 deficit following L-arginine supplementation. The explanation for the effects on $\dot{V}O_2$ kinetics in the present study compared with other studies that have attempted to alter NO bioavailability (1, 33, 34, 36, 41) is unclear but may be related to the potency of the intervention.

Effects of L-Arginine on the Physiological Responses to Severe-Intensity Exercise

In contrast to the reduction in the primary $\dot{V}O_2$ amplitude observed during moderate-intensity exercise, L-arginine supplementation resulted in an increased primary $\dot{V}O_2$ amplitude and a reduced $\dot{V}O_2$ slow component amplitude during severe-intensity exercise. These findings are consistent with, and of similar magnitude to, our recent findings with dietary NO_3^- supplementation (1). In our recent study (1), we also observed a reduced steady-state $\dot{V}O_2$ during moderate-intensity cycle exercise but an elevated primary $\dot{V}O_2$ amplitude and reduced $\dot{V}O_2$ slow component during severe-intensity cycle exercise. In contrast, during knee-extension exercise in which the work rates were not classified relative to the GET, $\dot{V}O_2$ was lower during “low-intensity” and “high-intensity” exercise following dietary NO_3^- supplementation compared with placebo (4). The influence of NO bioavailability on the O_2 cost of exercise, therefore, appears to be intensity domain and, perhaps, also exercise modality, specific. It is possible that these differential effects are related to differences in the limitations to $\dot{V}O_2$ kinetics for moderate-intensity compared with severe-intensity exercise (32). It is well documented that an increased NO production elevates blood flow (68) and that interventions that increase blood flow are associated with an increased $\dot{V}O_2$ primary component and a reduced $\dot{V}O_2$ slow component amplitude (1, 2, 16, 40). We previously observed an increased $\dot{V}O_2$ slow component amplitude following L-NAME infusion (34) and a reduced $\dot{V}O_2$ slow component amplitude following dietary NO_3^- supplementation (1, 4). These data indicate that manipulation of NO synthesis influences the magnitude of the $\dot{V}O_2$ slow component. Given the regulatory influence of NO on blood flow (68), it is conceivable that these effects on the $\dot{V}O_2$ slow component are related to changes in muscle perfusion. This, in turn, would be expected to influence the rate of muscle fatigue development and the pattern of motor unit recruitment; the latter has been suggested to be mechanistically linked to the $\dot{V}O_2$ slow component phenomenon (32).

Dietary supplementation with L-arginine has previously been reported to reduce blood lactate and ammonium accumulation (64) and to increase maximum $\dot{V}O_2$ in healthy mammals (53). These factors would be expected to predispose to improved exercise tolerance (15, 31). However, the influence of L-arginine supplementation on exercise performance in healthy humans is equivocal (14, 17, 19, 52, 69). In the present study, blood [lactate] and the $\dot{V}O_2$ measured at 360 s of severe-intensity exercise or at exhaustion (which was not significantly different from the maximum $\dot{V}O_2$ attained in the ramp incremental test) were not significantly altered by L-arginine ingestion. However, the time to task failure was extended by 20% following L-arginine administration; this improvement in exercise tolerance was accompanied by an increase in plasma

$[NO_2^-]$ prior to exercise and improved $\dot{V}O_2$ dynamics. There is a growing appreciation that plasma $[NO_2^-]$, as an indicator of NOS activity (38, 50, 51, 61) and through its role as a reservoir for NO production (26), provides an important indication of the capacity to tolerate high-intensity exercise (1, 4, 47, 48, 61). Our recent studies indicate that interventions that increase plasma $[NO_2^-]$ can improve $\dot{V}O_2$ dynamics (1, 4; present study). Indeed, the reduced $\dot{V}O_2$ slow component amplitude observed in the present study following L-arginine supplementation and, in previous studies following NO_3^- supplementation (1, 4), would be expected to spare the utilization of the anaerobic reserves (4, 43, 63) and the accumulation of metabolites related to the fatigue process (4), leading to improved exercise tolerance (1–4, 15, 31).

Although the enhanced exercise tolerance following L-arginine supplementation is striking, relatively small changes in oxidative function can result in substantial changes in exercise tolerance due to the hyperbolic nature of the power-duration relationship for severe-intensity exercise (73). Nevertheless, the effect is equivalent to a 1–2% reduction in the time taken to complete a set distance (where the duration in the control condition is ~ 10 min) and is therefore likely to be meaningful for athletic performance (29). Ageing and several pathologies result in impaired endothelial function, which limits the capacity for NO generation and may explain, at least in part, the reduced exercise capacity in these populations (24, 27, 49). There is evidence that L-arginine administration increases maximal walking distance in patients with peripheral arterial disease (10, 11) and that the increase in exercise capacity following exercise training in congestive heart failure patients is associated with an increased L-arginine transport (59). The possibility that L-arginine administration might enhance exercise efficiency and performance in senescent and patient populations requires further study.

A limitation of the present study was that the commercial L-arginine supplement contained small amounts of other compounds that might also be considered “active” or might have acted synergistically with L-arginine. While we cannot exclude this possibility, we consider it to be unlikely, given the similarity of our results to other studies in which NO availability was enhanced to a similar degree with use of pharmacological (47, 48) or dietary (1, 4) NO_3^- interventions.

Conclusions

Acute dietary supplementation with 6 g of L-arginine, which increased indexes of NO synthesis, reduced the steady-state $\dot{V}O_2$ during moderate-intensity exercise and also reduced the $\dot{V}O_2$ slow component and increased the time to task failure during severe-intensity exercise in healthy adults. These findings are similar to recent observations in which NO availability was increased via dietary NO_3^- supplementation (1, 4, 47, 48). Therefore, a diet rich in the amino acid L-arginine and/or NO_3^- , which increases plasma $[NO_2^-]$, a key marker of NO bioavailability, appears to reduce systolic blood pressure and to improve exercise efficiency and exercise tolerance in healthy humans. While the precise mechanisms responsible for the latter effects remain to be elucidated, they likely involve an increased muscle O_2 supply and direct effects of NO on muscle contractile efficiency and/or mitochondrial function.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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