Eur J Appl Physiol (2013) 113:1805–1819
DOI 10.1007/s00421-013-2593-z

ORIGINAL ARTICLE

No effect of acute L-arginine supplementation on O_2 cost or exercise tolerance

Anni Vanhatalo · Stephen J. Bailey · Fred J. DiMenna · Jamie R. Blackwell · Gareth A. Wallis · Andrew M. Jones

Received: 5 December 2012 / Accepted: 16 January 2013 / Published online: 20 February 2013

Abstract The extent to which dietary supplementation with the nitric oxide synthase (NOS) substrate, L-arginine (ARG), impacts on NO production and NO-mediated physiological responses is controversial. This randomised, double blinded, cross-over study investigated the effects of acute ARG supplementation on NO biomarkers, O_2 cost of exercise and exercise tolerance in humans. In one experiment, 15 subjects completed moderate- and severe-intensity running bouts after acute supplementation with 6 g ARG or placebo (PLA). In another experiment, eight subjects completed moderate- and severe-intensity cycling bouts after acute supplementation with 6 g ARG plus 25 g of carbohydrate (ARG + CHO) or an energy-matched dose of carbohydrate alone (CHO). The plasma nitrite concentration was not different after ARG (Pre: 204 ± 79; Post: 241 ± 114 nM; $P > 0.05$) or ARG + CHO consumption (Pre: 304 ± 57; Post: 335 ± 116 nM; $P > 0.05$). During moderate-intensity exercise, the steady-state pulmonary $\dot{V}O_2$ was not different, relative to the respective placebo conditions, after ARG (PLA: 2,407 ± 318, ARG: 2,422 ± 333 mL min^{-1}) or ARG + CHO (CHO: 1,695 ± 304, ARG + CHO: 1,712 ± 312 mL min^{-1}) ingestion ($P > 0.05$). The tolerable duration of severe exercise was also not significantly different ($P > 0.05$) after ingesting ARG (PLA: 551 ± 140, ARG: 552 ± 150 s) or ARG + CHO (CHO: 457 ± 182, ARG + CHO: 441 ± 221 s). In conclusion, acute dietary supplementation with ARG or ARG + CHO did not alter biomarkers of NO synthesis, O_2 cost of exercise or exercise tolerance in healthy subjects.

Keywords Nitric oxide · Dietary supplementation · Exercise efficiency · Blood pressure

Communicated by Carsten Lundby.

A. Vanhatalo · S. J. Bailey · J. R. Blackwell · A. M. Jones (✉)
Sport and Health Sciences, College of Life and Environmental Sciences, University of Exeter, St. Luke's Campus, Exeter, Devon EX1 2LU, UK
e-mail: a.m.jones@exeter.ac.uk

F. J. DiMenna
Human Performance Laboratory, Adelphi University, Garden City, NY, USA

G. A. Wallis
School of Sport and Exercise Sciences, College of Life and Environmental Sciences, University of Birmingham, Birmingham, UK

Introduction

Nitric oxide (NO), a gaseous, diffusible signalling molecule, impacts on a number of physiological functions including vascular tone, cellular calcium handling, skeletal muscle glucose uptake, neurotransmission, mitochondrial respiration and skeletal muscle force production (Stamler and Meissner 2001). NO can be generated by the NO synthase (NOS) enzymes, which are expressed in the endothelium (endothelial NOS; eNOS) and in skeletal muscle (eNOS and neuronal NOS; nNOS) (Stamler and Meissner 2001), in an O_2-dependent reaction that requires L-arginine as substrate and nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), tetrahydrobiopterin (BH_4), haem and calmodulin as essential co-factors (Boucher et al. 1999). Alternatively, NO can be generated from the one electron reduction of nitrite (NO_2^-) in a reaction that is potentiated in acidic and hypoxic environments (Lundberg and Weitzberg 2010). The co-existence of these two complementary NO-generating pathways facilitates

NO synthesis across a diverse range of physiological conditions.

There is a growing body of evidence showing that dietary supplementation with inorganic nitrate (NO_3^-), which undergoes a stepwise reduction to nitrite (NO_2^-) and then NO (Lundberg and Weitzberg 2010), can reduce the O_2 cost of exercise and enhance exercise performance in moderately trained (Bailey et al. 2010; Cermak et al. 2012a; Larsen et al. 2007, 2010) but perhaps not highly trained (Bescós et al. 2012; Christensen et al. 2012; Wilkerson et al. 2012) subjects. Some studies have also reported enhanced indices of exercise performance and fatigue resistance (Bailey et al. 2010; Buford and Koch 2004; Camic et al. 2010a, b; Stevens et al. 2000) and a reduced O_2 cost of sub-maximal exercise (Bailey et al. 2010; Burtscher et al. 2005) following dietary supplementation with the NOS substrate, L-arginine. However, other studies have not detected significant effects of L-arginine on steady-state pulmonary O_2 uptake ($\dot{V}O_2$) (Bescós et al. 2009; Koppo et al. 2009) or performance (Abel et al. 2005; Beis et al. 2011; Liu et al. 2009; McConell et al. 2006). The above studies, with the exception of Bailey et al. (2010), did not measure the plasma NO_2^- or NO_3^- concentrations ([NO_2^-] and [NO_3^-], respectively), which are the key biomarkers of NOS-mediated NO production (Kleinbongard et al. 2003; Lauer et al. 2001). It is therefore difficult to ascertain whether the above dietary interventions were successful in enhancing endogenous NO synthesis. The discrepancies between findings may also be related to differences in dose, timing and duration of supplementation (i.e. acute single bolus vs. chronic intake for up to 8 weeks) (Álvares et al. 2011). L-arginine has been variously administered as free crystalline L-arginine (Camic et al. 2010a, b), arginine complexed with other compounds (e.g. arginine aspartate; Abel et al. 2005; Colombani et al. 1999), L-arginine hydrochloride (Koppo et al. 2009; Schaefer et al. 2002), arginine-α-ketoglutarate (AAKG; Campbell et al. 2006; Willoughby et al. 2011), glycerine-arginine-α-ketoisocaproic acid (GAKIC; Beis et al. 2011; Buford and Koch 2004; Stevens et al. 2000) or as part of a multiple-micronutrient supplement (Bailey et al. 2010). Unless free crystalline L-arginine is ingested or directly infused into the systemic circulation, it may not be possible to ascribe any physiological or performance enhancements to L-arginine per se. Therefore, it is unclear whether L-arginine is exclusively responsible for the improvements in exercise performance reported following AAKG (Campbell et al. 2006), GAKIC (Buford and Koch 2004; Stevens et al. 2000) and as part of a multiple-micronutrient supplement (Bailey et al. 2010).

We have previously reported that a multiple-micronutrient supplement which provided 6 g of L-arginine increased resting plasma [NO_2^-], reduced the O_2 cost of moderate-intensity cycling and improved the time-to-exhaustion during severe-intensity cycling by ~20 % (Bailey et al. 2010). However, it is important to note that, in addition to L-arginine, the supplement (Arkworld ARK-1, Arkworld International Inc., USA) contained a number of other potential performance-enhancing nutrients including: AAKG, citrus bioflavanoids, α-lipoic acid, L-glutamine, L-citrulline, acetyl L-carnitine, L-carnitine citrate, L-cysteine and betaine, and the powder was coloured using beetroot juice, which contains inorganic nitrate. Thus, it is possible that the positive effects we observed were consequent to the independent effects of one, or the cumulative effect of several, of the nutrients provided or to a synergistic interaction between L-arginine and one or more of the other ingredients. Therefore, as highlighted above and elsewhere (Álvares et al. 2011), further research is required to determine whether pure L-arginine can be recommended as an ergogenic aid to enhance endurance exercise performance.

Accordingly, the purpose of this study was to determine whether oral ingestion of L-arginine would enhance biomarkers of endogenous NO production, reduce the O_2 cost of exercise and improve exercise tolerance. In one experiment, we investigated the physiological and performance effects of ingesting 6 g L-arginine (ARG) compared to a taste-matched placebo (PLA). Given that cellular L-arginine uptake may be insulin dependent (Simmons et al. 1996) and that L-arginine alone may not induce a sufficient insulin response to facilitate cellular uptake (Broglio et al. 2003; Thams and Capito 1999), in a second experiment, we investigated the effects of combined L-arginine and carbohydrate (ARG + CHO) ingestion compared to carbohydrate (CHO) alone. We hypothesised that, relative to PLA and CHO, respectively, ARG and ARG + CHO would increase plasma [NO_3^-] and [NO_2^-], reduce the O_2 cost of sub-maximal exercise and increase high-intensity exercise tolerance.

Methods

Subjects

In total, 18 healthy, recreationally active male students (mean ± SD, age 22 ± 3 year, height 1.76 ± 0.06 m, body mass 75 ± 9 kg) volunteered to participate in the two studies that are reported herein. The studies 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 asked to continue their habitual dietary and physical activity patterns throughout the study period. None of the subjects

smoked tobacco or used dietary supplements. Subjects were instructed to arrive at the laboratory at least 3 h post-prandial 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 (±2 h) to minimise the effects of diurnal biological variation on physiological responses and exercise performance.

Experimental design

In the first experiment, 15 subjects (mean ± SD, age 22 ± 3 year, height 1.78 ± 0.07 m, body mass 73 ± 9 kg) reported to the laboratory on seven occasions over a 5- to 6-week period. This experiment investigated the influence of acute ARG vs. PLA administration on NO biomarkers, running economy and exercise tolerance. In the second experiment, eight subjects (mean ± SD, age 21 ± 1 year, height 1.76 ± 0.06 m, body mass 75 ± 10 kg), five of whom had participated in the first experiment, reported to the laboratory on four occasions over a 2- to 3-week period. This experiment investigated the influence of acute ARG + CHO vs. CHO ingestion on NO biomarkers, cycling efficiency and exercise tolerance. Subjects were familiarised for the first experiment by performing the step exercise test protocol in full on the treadmill on two separate visits, and for the second experiment, subjects were familiarised by one step exercise test protocol in full on the cycle ergometer. The tests in both experiments were conducted following familiarisation in a randomised-order using a double-blind, placebo-controlled design. The subjects ingested 6 g of ARG either 90 min (experiment 1) or 60 min (experiment 2) prior to exercise. This approach was based on the work of Bode-Böger et al. (1998) who reported that 6 g of oral L-arginine significantly elevated plasma [L-arginine] with the peak values being maintained between 60 and 150 min post-ingestion. This approach was also similar to that used in our previous study wherein 6 g L-arginine was ingested as part of a multi-compound supplement 60 min before exercise (Bailey et al. 2010). We measured plasma [NO_2^-] and [NO_3^-] as biomarkers of NO production via the L-arginine-NOS pathway. We studied two different exercise modalities (running in experiment 1 and cycling in experiment 2) to provide comprehensive evaluation of the effects of L-arginine on exercise performance.

Incremental tests

Prior to the supplementation interventions, subjects completed a ramp incremental test for the determination of the $\dot{V}O_{2peak}$ and the gas-exchange threshold (GET). For the first experiment investigating the effects of acute ARG administration, subjects completed a ramp incremental running test on a motorised treadmill (Woodway GmbH, Weil am Rhein, Germany) at a gradient of 1 % (Jones and Doust 1996). Initially, subjects completed 3 min of walking at 4 km h^{-1}, after which the treadmill speed was increased by 1 km h^{-1} every minute until the subject was unable to continue. For the second experiment investigating the effects of acute ARG + CHO administration, subjects completed a ramp incremental test on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands). Initially, subjects completed 3 min of 'unloaded' baseline cycling, after which the work rate was increased by 30 W min^{-1} 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 were recorded and reproduced in subsequent tests.

The breath-by-breath pulmonary gas-exchange data were collected continuously during all incremental tests and averaged over consecutive 10-s periods. The $\dot{V}O_{2peak}$ was taken as the highest 30-s mean value attained prior to volitional exhaustion. The GET was determined from a cluster of measurements including: (1) the first disproportionate increase in CO_2 production ($\dot{V}CO_2$) from visual inspection of individual plots of $\dot{V}CO_2$ vs. $\dot{V}O_2$; (2) an increase in expired ventilation ($\dot{V}_E$)/$\dot{V}O_2$ with no increase in $\dot{V}_E/\dot{V}CO_2$; (3) an increase in end-tidal O_2 tension with no fall in end-tidal CO_2 tension. The treadmill speed and cycling work rate that would require 80 % of the GET (moderate-intensity exercise) and 70 % Δ running speed and 80 % Δ cycling work rate (70 and 80 %, respectively, of the difference between the GET and $\dot{V}O_{2peak}$, severe-intensity exercise) were subsequently calculated with account taken of the mean response time for $\dot{V}O_2$ during ramp exercise.

Supplementation protocols

Upon arriving at the laboratory, subjects rested for 10 min in a seated position. After the rest period, the blood pressure of the brachial artery was determined using an automated sphygmomanometer (Dinamap Pro, GE Medical Systems, Tampa, USA). Four measurements were taken with the mean of the final three measurements being recorded. Thereafter, a venous blood sample (5 mL) was drawn from the antecubital vein into a lithium-heparin tube (Vacutainer, Becton–Dickinson, New Jersey, USA). Samples were centrifuged at 4,000 rpm at 4 °C for 10 min, within 3 min of collection. Plasma was subsequently extracted and immediately frozen at −80 °C, for later analysis of [NO_2^-] using a modification of the chemiluminescence technique (Bailey et al. 2009). Plasma [NO_3^-] was also determined from the reduction of NO_3^- to NO in the presence of VCl at 90 °C using a chemiluminescence

nitric oxide analyser (Sievers NOA 280i, Analytix Ltd, Durham, UK).

Following the blood pressure assessment and blood sampling, subjects were provided with a 500 mL beverage in an opaque plastic drinks bottle. For the acute ARG supplementation investigation, subjects ingested a 0.5-L beverage that contained 6.0 g of L-arginine (lemon flavoured powder, containing 0 g of CHO; GSK, IFN Ltd., Reading, UK) or a placebo beverage that contained no L-arginine (PLA; lemon flavoured powder, containing 0 g of CHO; GSK, IFN Ltd., Reading, UK). Blood pressure measurements were replicated and a venous blood sample was obtained 90 min after beverage consumption to coincide with the peak plasma [L-arginine] after an oral bolus of 6 g L-arginine (Bode-Böger et al. 1998). In addition, a blood sample was collected from a fingertip into a capillary tube for the determination of blood glucose concentration ([glucose]) within 30 s of collection (YSI 1500, Yellow Springs Instruments, Yellow Springs, OH, USA). For the acute ARG + CHO supplementation investigation, subjects ingested a 0.5-L beverage that contained 6 g of L-arginine and 25 g of CHO (lemon-flavoured powder; GSK, IFN Ltd., Reading, UK) or a placebo beverage that contained no L-arginine, but 37 g of CHO to ensure that these beverages were approximately energy matched (CHO; lemon-flavoured powder; GSK, IFN Ltd., Reading, UK). In this experiment, blood pressure was assessed and venous (for determination of plasma $[NO_3^-]$ and $[NO_2^-]$) and fingertip (for determination of blood [glucose]) blood samples were obtained 60 min after beverage consumption (Bailey et al. 2010). All beverages were ingested over 10 min and were taste-matched using similar acidity regulators, sweeteners and flavouring. Subjects rested in a seated position until the commencement of the exercise tests after beverage consumption.

Step exercise tests

All step exercise tests were initiated 5 min after the post-supplementation venous blood collection (i.e. 95 min after beverage ingestion in ARG vs. PLA study, and 65 min after beverage ingestion in ARG + CHO vs. CHO study). In the first experiment, subjects completed a series of three-step running tests on two occasions for both the ARG and PLA conditions. The protocol comprised two moderate-intensity running bouts of 6 min and one severe-intensity running bout which was continued until exhaustion. All running bouts were initiated from a walking baseline of 4 km h^{-1}, with 10 min of continuous walking at 4 km h^{-1} separating the step exercise tests. The time to the limit of tolerance was noted when the subject dismounted the treadmill at the point where they were unable to maintain the required running speed. All subjects completed a total of four bouts of moderate-intensity running and two bouts of severe-intensity running for both the ARG and PLA conditions. Exercise tolerance was reported as the mean time-to-exhaustion from the two severe exercise bouts completed in each of the two experimental conditions. In the second experiment, subjects completed a series of three-step cycling bouts on one occasion for both the ARG + CHO and CHO conditions. Two moderate-intensity cycle bouts of 5 min and one severe-intensity cycling bout which was continued to exhaustion were completed for each supplementation condition. Each step-cycle test began with 3 min of baseline pedalling at 20 W before an abrupt transition to the target work rate. Five minutes of passive recovery separated each step test. The time to exhaustion was noted when the pedal rate fell by >10 rpm below the required pedal rate.

Blood samples were collected from a fingertip into a capillary tube over the 20 s before the step transition in work rate and within the last 20 s of moderate exercise and immediately following exhaustion for severe exercise. These whole blood samples were analysed to determine the blood lactate concentration ([lactate]) within 30 s of collection (YSI 1500, Yellow springs Instruments, Yellow Springs, OH, USA). A venous blood sample was obtained at 2–3 min following the termination of the exhaustive severe-intensity tests for the determination of the plasma $[NO_2^-]$ and $[NO_3^-]$.

During all exercise tests, pulmonary gas exchange and ventilation were measured breath-by-breath with subjects wearing a nose clip and breathing through a low-dead-space, low-resistance mouthpiece and impeller turbine assembly (Jaeger Triple V, Jaeger GmbH, Hoechberg, Germany). The inspired and expired gas volume and gas concentration signals were continuously sampled at 100 Hz, the latter using paramagnetic (O_2) and infrared (CO_2) analyzers via a capillary line connected to the mouthpiece (Jaeger Oxycon Pro, Jaeger GmbH, Hoechberg, Germany). The gas analyzers were calibrated before each test with gases of known concentration, and the turbine volume transducer was calibrated with a 3-L syringe (Hans Rudolph, Kansas City, MO). The volume and concentration signals were time aligned by accounting for the delay in the capillary gas transit and the analyser rise time relative to the volume signal. Pulmonary gas exchange and ventilation were calculated and displayed breath-by-breath.

Data analysis

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 more than four standard deviations 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. The first 20 s of data after the onset of exercise (i.e. the phase I response) were deleted and a nonlinear least-square algorithm was used to fit the data thereafter. A single-exponential model was used to characterise the $\dot{V}O_2$ responses to moderate exercise, and a bi-exponential model was used for severe exercise, as described in the following equations:

$$\dot{V}O_2(t) = \dot{V}O_{2\,\text{baseline}} + A_p\left(1 - e^{-(t-TD_p/\tau_p)}\right) \quad \text{(moderate)} \tag{1}$$

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

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

An iterative process was used to minimise the sum of the squared errors between the fitted function and the observed values. $\dot{V}O_{2baseline}$ was defined as the mean $\dot{V}O_2$ measured over the final 90 s of the baseline period. The end-exercise $\dot{V}O_2$ was defined as the mean $\dot{V}O_2$ measured over the final 30 s of exercise. 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 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 the same iso-time for both supplementation periods. This was taken as the shortest exercise tolerance time in any severe exercise trial in the PLA and ARG experiment, and also in the CHO and CHO + ARG experiment. To determine the overall kinetics of the $\dot{V}O_2$ response to both moderate- and severe-intensity exercises, the data were also fit with a mono-exponential model from 0 s to end-exercise without time delay. For moderate-intensity cycle exercise in experiment two, we calculated the gross efficiency (%) as the quotient of work done and energy expenditure.

Statistical analysis

A two-way repeated-measures ANOVA was used to determine the effects of the supplementation interventions on the plasma [NO_2^-] and [NO_3^-] and blood pressure responses. Significant effects were further analysed using simple contrasts with Fisher's LSD. Two-tailed, paired-sample t tests were used to determine the effect of the nutritional supplement interventions on the exercise tolerance, $\dot{V}O_2$ and blood [lactate] responses. Correlations were assessed by Pearson's product moment correlation coefficient. Data are presented as mean ± SD. Statistical significance was accepted when $P < 0.05$.

Results

The ARG and ARG + CHO supplements administered in this study were well tolerated by subjects with no adverse side effects reported. The $\dot{V}O_{2peak}$ attained in the ramp incremental tests was 59 ± 6 and 55 ± 7 mL kg^{-1} min^{-1} for running and cycling, respectively. The running speeds which corresponded to 80 % GET and 70 % Δ were 7.8 ± 0.4 and 15.2 ± 1.2 km h^{-1}, respectively, while the work-rates at 80 % GET and 80 % Δ during cycle ergometry were 98 ± 34 W and 308 ± 65 W, respectively. There were no significant differences in the blood [glucose]

Table 1 Plasma [NO_3^-], plasma [NO_2^-] and blood pressure responses before and after acute PLA and ARG ingestion

	PLA	ARG
Plasma [NO_3^-]		
Pre-supplementation (μM)	45 ± 16	48 ± 19
Post-supplementation (μM)	48 ± 20	50 ± 23
End-exercise (μM)	52 ± 21#	54 ± 19#
Plasma [NO_2^-]		
Pre-supplementation (nM)	223 ± 107	204 ± 79
Post-supplementation (nM)	222 ± 105	241 ± 114
End-exercise (nM)	201 ± 96*	210 ± 93
Systolic blood pressure (mmHg)		
Pre-supplementation (mmHg)	127 ± 7	127 ± 8
Post-supplementation (mmHg)	128 ± 7	124 ± 7*
Diastolic blood pressure (mmHg)		
Pre-supplementation (mmHg)	70 ± 5	70 ± 6
Post-supplementation (mmHg)	69 ± 5	67 ± 5*
Mean arterial pressure (mmHg)		
Pre-supplementation (mmHg)	89 ± 5	89 ± 6
Post-supplementation (mmHg)	89 ± 5	86 ± 5*

Values are mean ± SD

PLA placebo condition, *ARG* L-arginine condition, *[NO_3^-]* nitrate concentration, *[NO_2^-]* nitrite concentration

* Significantly different from the pre-supplementation values ($P < 0.01$)

Significantly different from both the pre- and post-supplementation values ($P < 0.05$)

after PLA, ARG, CHO and ARG + CHO ingestion ($P > 0.05$; data not reported).

Plasma [NO_3^-] and [NO_2^-]

The plasma [NO_3^-] and [NO_2^-] results for the first experiment (ARG vs. PLA) are reported in Table 1 with individual responses shown in Figs. 1 and 2. The coefficient of variation for duplicate samples was 1.9 % for [NO_3^-] and 8.5 % for [NO_2^-]. The two-way ANOVA revealed that there was a significant main effect for time on the plasma [NO_3^-] ($P < 0.05$). Follow-up analyses revealed that the plasma [NO_3^-] was significantly greater after exhaustive exercise in both the PLA (52 ± 21 μM) and ARG (54 ± 19 μM) conditions compared to pre-supplementation values (PLA: 45 ± 16 μM; ARG 48 ± 19 μM; $P < 0.05$) and post-supplementation values (PLA: 48 ± 20 μM; ARG: 50 ± 23 μM; $P < 0.05$). However, there was no significant difference in the plasma [NO_3^-] between PLA and ARG at any sampling point ($P > 0.05$; Table 1). Similar to plasma [NO_3^-], there was a significant main effect for time on the plasma [NO_2^-] ($P < 0.05$); the plasma [NO_2^-] was 10 % lower after exhaustive exercise compared to the pre-supplementation values in PLA ($P < 0.05$; Table 1), and 13 % lower (nonsignificant) after exhaustive exercise with ARG supplementation compared to the post-supplementation values ($P = 0.16$; Table 1). There were no significant differences in the plasma [NO_2^-] between PLA and ARG at any sampling point ($P > 0.05$).

The plasma [NO_3^-] and [NO_2^-] results for the second experiment (ARG + CHO vs. CHO) are reported in Table 2 with individual responses shown in Figs. 1 and 2. There were no significant between-condition differences in the plasma [NO_3^-] at any time point ($P > 0.05$). There was a significant main effect for time on the plasma [NO_3^-] ($P < 0.01$) such that the plasma [NO_3^-] was significantly elevated at exhaustion (33 ± 5 μM) compared with the pre-supplementation value in the CHO condition (27 ± 6 μM; $P < 0.05$; Table 2). Similarly, the plasma [NO_3^-] at exhaustion (34 ± 5 μM) was greater than the pre (29 ± 8 μM) and post (31 ± 6 μM) supplementation plasma [NO_3^-] in the ARG + CHO condition ($P < 0.05$ for all comparisons). There were no differences in plasma [NO_2^-] within or between CHO and ARG + CHO at any time point ($P > 0.05$; Table 2). The plasma [NO_2^-] after severe-intensity exhaustive cycle exercise was 13 % lower compared to the post-supplementation values after CHO supplementation and 15 % lower after ARG + CHO supplementation,

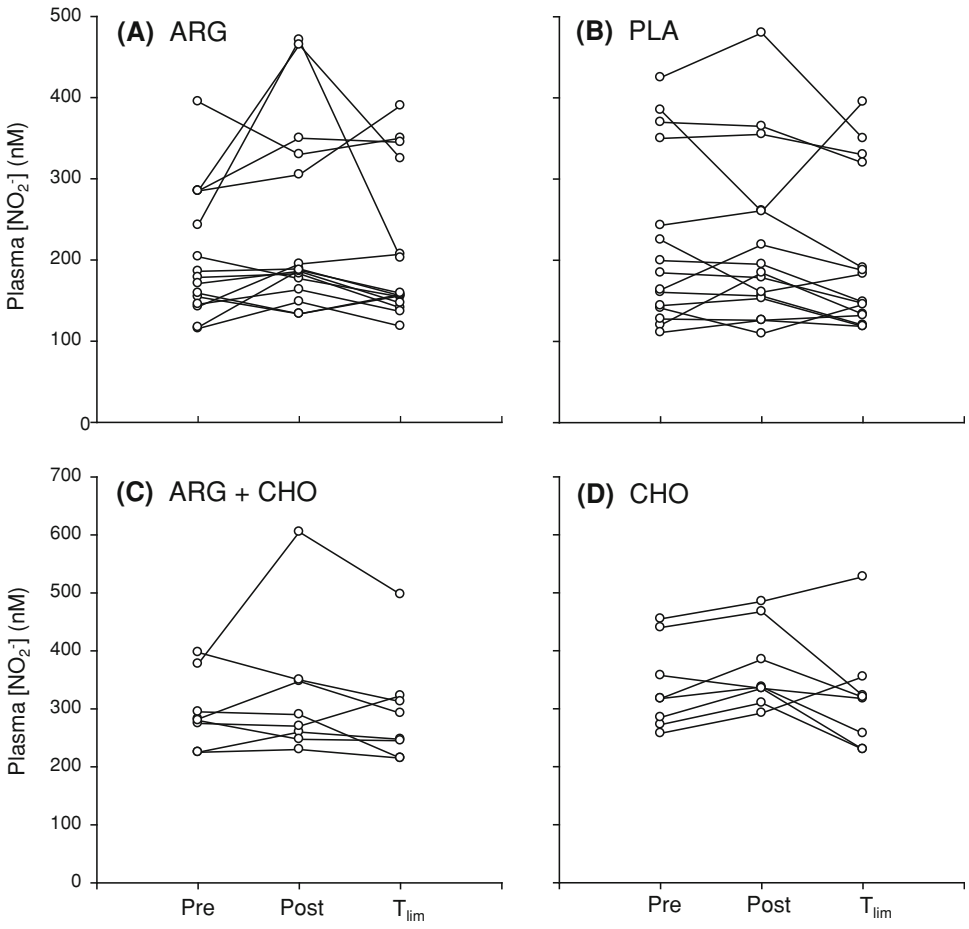

Fig. 1 Individual changes in plasma [NO_2^-] in experiment one (**a**, ARG; **b**, placebo) and experiment two (**c**, ARG + CHO; **d**, CHO). Pre, pre-supplementation; Post, post-supplementation but before exercise; T_{lim}, at the limit of tolerance during severe-intensity exercise

however, these decreases did not reach statistical significance ($P = 0.12$ and $P = 0.13$, respectively).

Blood pressure

In the first experiment, there was a significant supplement × time interaction effect on systolic blood pressure (SBP; $P < 0.01$) and mean arterial pressure (MAP; $P < 0.01$), and there was a significant main effect for time on diastolic blood pressure (DBP; $P < 0.01$). Post-hoc analyses showed that, relative to the pre-supplementation values, SBP (Pre: 127 ± 4; Post: 124 ± 7 mmHg; $P < 0.01$), DBP (Pre: 70 ± 6; Post: 67 ± 5 mmHg; $P < 0.01$) and MAP (Pre: 89 ± 6; Post: 86 ± 5 mmHg; $P < 0.01$) were reduced following ARG, but not PLA supplementation ($P > 0.05$; Table 1). The changes in blood pressure following ARG supplementation were not significantly correlated with changes in plasma [NO_2^-] (SBP, $r = 0.45$; DBP, $r = 0.05$; MAP, $r = 0.25$) or plasma [NO_3^-] (SBP, $r = 0.32$; DBP, $r = 0.01$; MAP, $r = 0.17$).

In the second experiment, there was a tendency for a significant supplement × time interaction effect on SBP ($P = 0.08$), and there was a significant main effect for time on MAP ($P < 0.01$). Follow-up analyses revealed that there was a tendency for both SBP (Pre: 125 ± 8; Post: 121 ± 8 mmHg; $P = 0.07$) and MAP (Pre: 89 ± 6; Post: 86 ± 7 mmHg; $P = 0.06$) to be lower after ARG + CHO supplementation relative to CHO supplementation ($P > 0.05$; Table 2). The changes in blood pressure following ARG + CHO supplementation were not significantly correlated with changes in plasma [NO_2^-] (SBP, $r = 0.35$; DBP, $r = 0.49$; MAP, $r = 0.21$) or plasma [NO_3^-] (SBP, $r = 0.25$; DBP, $r = 0.23$; MAP, $r = 0.32$).

Pulmonary $\dot{V}O_2$ kinetics

The pulmonary $\dot{V}O_2$ responses during moderate and severe exercise are shown in Fig. 3 for experiment one (ARG vs. PLA) and in Fig. 4 for experiment two (ARG + CHO vs. CHO). Figure 5 shows the individual end-exercise $\dot{V}O_2$ values during moderate-intensity exercise for experiment one (Panel A) and experiment two (Panel B). There were no significant differences in the $\dot{V}O_2$ primary phase τ (PLA: 22 ± 5, ARG: 21 ± 5 s), the $\dot{V}O_2$ amplitude (PLA: 1,411 ± 240, ARG: 1,418 ± 245 mL min^{-1}) or the end-exercise $\dot{V}O_2$ (PLA: 2,407 ± 318, ARG: 2,422 ± 333 mL min^{-1}) during moderate-intensity running between the PLA and ARG conditions ($P > 0.05$; Figs. 3 and 5). There was no significant difference in end-exercise RER between conditions (PLA: 0.92 ± 0.04, ARG: 0.92 ± 0.03). Likewise, there were no significant differences in the $\dot{V}O_2$ primary phase τ (CHO: 24 ± 8, ARG + CHO: 21 ± 6 s), the $\dot{V}O_2$ amplitude (CHO: 675 ± 340, ARG + CHO: 692 ± 317 mL min^{-1}) and the end-exercise $\dot{V}O_2$ (CHO: 1,695 ± 304, ARG + CHO: 1,712 ± 312 mL min^{-1}) during moderate-intensity cycling after CHO compared to ARG + CHO ingestion ($P > 0.05$; Figs. 4, 5). There was no significant difference in end-exercise RER between conditions (CHO: 0.97 ± 0.03, ARG + CHO: 0.97 ± 0.03). The gross efficiency was not different between conditions (CHO: 16.7 ± 2.6, ARG + CHO: 16.5 ± 2.5 %).

The pulmonary $\dot{V}O_2$ primary phase τ (PLA: 19 ± 3, ARG: 19 ± 4 s), the $\dot{V}O_2$ primary amplitude (PLA: 2,783 ± 370, ARG: 2,771 ± 385 mL min^{-1}), the $\dot{V}O_2$ slow component (PLA: 531 ± 132, ARG: 588 ± 187 mL min^{-1}) or the end-exercise $\dot{V}O_2$ (PLA: 4,336 ± 530, ARG: 4,401 ± 528 mL min^{-1}) were not significantly different during severe-intensity running between the PLA and ARG conditions ($P > 0.05$; Fig. 3). There was no significant difference in RER between conditions (end-exercise, PLA: 1.08 ± 0.06, ARG: 1.07 ± 0.05). Similarly, the $\dot{V}O_2$ primary phase τ (CHO: 30 ± 9, ARG + CHO: 28 ± 7 s), the $\dot{V}O_2$ primary amplitude (CHO: 2,524 ± 660, ARG + CHO: 2,478 ± 692 mL min^{-1}), the $\dot{V}O_2$ slow component (CHO: 750 ± 275, ARG + CHO: 719 ± 286 mL min^{-1}) and the end-exercise $\dot{V}O_2$ (CHO: 4,313 ± 784, ARG + CHO: 4,216 ± 745 mL min^{-1}) were also not significantly different during severe-intensity cycling after CHO compared to ARG + CHO ingestion ($P > 0.05$; Fig. 4). There was no significant difference in RER between conditions (end-exercise, CHO: 1.07 ± 0.07, ARG + CHO: 1.09 ± 0.07). There were no between-conditions differences in the blood [lactate] at any time point in the PLA and ARG experiments or in the CHO and ARG + CHO experiments ($P > 0.05$; data not reported).

Exercise tolerance

The time-to-exhaustion during severe-intensity treadmill running after PLA and ARG ingestion, and severe-intensity cycle ergometry after CHO and ARG + CHO ingestion are shown in Fig. 6. Exercise tolerance was not significantly altered by ARG (552 ± 150 s) compared to PLA (551 ± 140 s; $P > 0.05$) supplementation, or by ARG + CHO (441 ± 221 s) compared to CHO supplementation (457 ± 182 s; $P > 0.05$). The severe-intensity exercise tolerance tended to be positively associated with the pre-supplementation plasma [NO_2^-] in PLA ($r = 0.37$, $P = 0.17$), CHO ($r = 0.64$, $P = 0.09$) and CHO + ARG ($r = 0.55$, $P = 0.16$); however, these relationships did not attain statistical significance. There were no associations between severe-intensity exercise tolerance and the plasma [NO_3^-] for any of the supplements administered in this study ($P > 0.05$ for all comparisons).

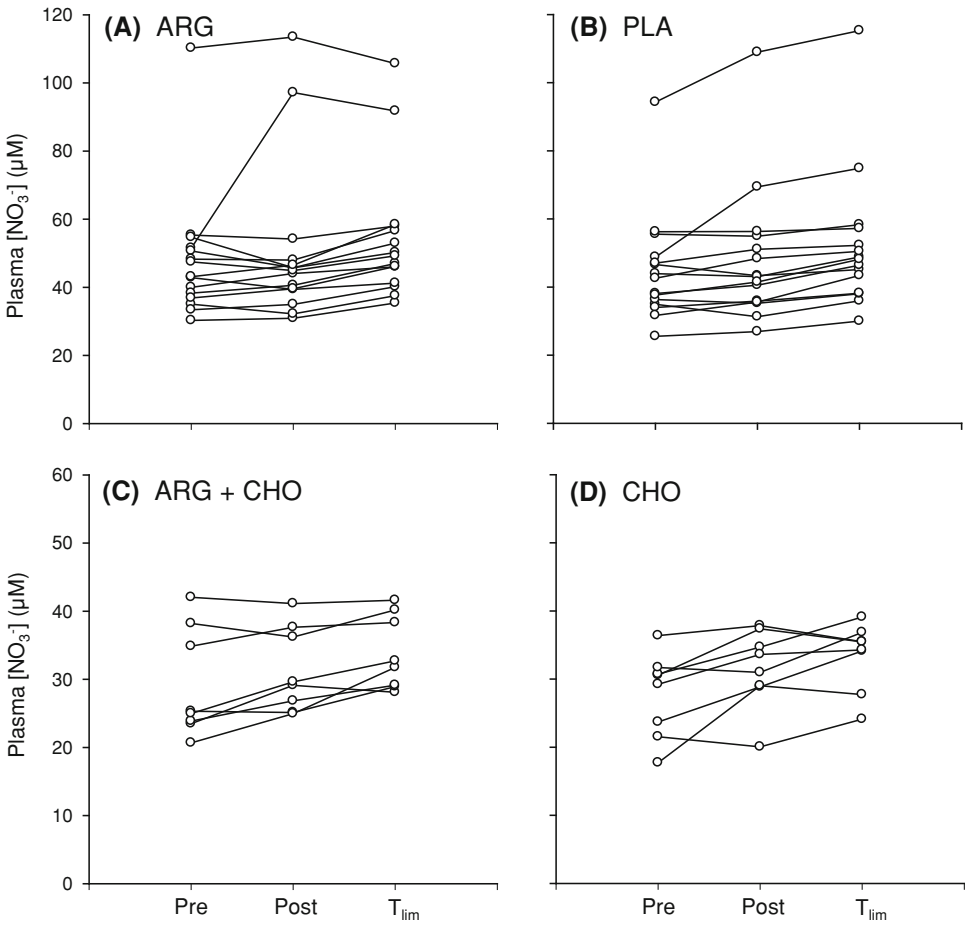

Fig. 2 Individual changes in plasma [NO_3^-] in experiment one (**a**, ARG; **b**, placebo) and experiment two (**c**, ARG + CHO; **d**, CHO). Pre, pre-supplementation; Post, post-supplementation but before exercise; T_{lim}, at the limit of tolerance during severe-intensity exercise

Discussion

The influence of L-arginine supplementation on NO production and exercise performance in healthy adults is controversial. To address this issue, we investigated the effects of dietary supplementation with ARG relative to PLA, and also with ARG + CHO relative to CHO to avoid concerns that insulin-mediated cellular L-arginine uptake may be compromised in the absence of CHO. While resting blood pressure was, in general, lower following ARG and ARG + CHO ingestion, neither ARG nor ARG + CHO supplementation significantly increased blood markers of NO synthesis (plasma [NO_3^-] or [NO_2^-]) or altered the O_2 cost of exercise or exercise tolerance relative to their respective placebo conditions. Following exhaustive severe-intensity exercise, venous plasma [NO_3^-] was increased; however, there were no between-condition differences in the [NO_3^-] response. Collectively, these data suggest that the oral administration of the NOS substrate L-arginine does not augment NO synthesis, reduce the O_2 cost of exercise or improve exercise tolerance.

It is well documented that the intracellular [L-arginine] by far exceeds the K_m of eNOS for L-arginine (Pollock et al. 1991; Wu and Morris 1998). Nevertheless, exogenous L-arginine administration has been shown to provoke NO-mediated or NO-like physiological responses, a phenomenon termed the L-arginine paradox (Wu and Morris 1998). There is some evidence that the oral administration of L-arginine reduces blood pressure in normotensive subjects, indicative of an NO-induced vasodilatory response (see Dong et al. 2011 for meta-analysis; cf. Greer and Jones 2011). In addition to serving as a direct substrate for NOS, L-arginine might increase NOS-derived NO bioavailability indirectly by scavenging superoxide anions (O_2^-), thereby reducing superoxide-induced breakdown of NO (Wascher et al. 1997). L-arginine might also increase NO production by stimulating the expression of GTP cyclohydrolase-I which synthesises the essential NOS co-factor BH_4 (Wu and Meininger 2009). However the NO biomarkers, plasma [NO_3^-] and [NO_2^-], were not altered after either ARG or ARG + CHO supplementation in the present study. These findings are consistent with earlier reports that L-arginine ingestion does not alter circulating NO_x ([NO_3^- + NO_2^-]; Liu et al. 2009; Willoughby et al. 2011), and do not support L-arginine supplementation as an effective intervention to enhance NO synthesis in healthy volunteers.

Table 2 Plasma [NO_3^-], plasma [NO_2^-] and blood pressure responses before and after acute CHO and ARG + CHO ingestion

	CHO	ARG + CHO
Plasma [NO_3^-]		
Pre-supplementation (μM)	27 ± 6	29 ± 8
Post-supplementation (μM)	32 ± 6	31 ± 6
End-exercise (μM)	33 ± 5*	34 ± 5[#]
Plasma [NO_2^-]		
Pre-supplementation (nM)	342 ± 72	304 ± 57
Post-supplementation (nM)	365 ± 74	335 ± 116
End-exercise (nM)	319 ± 96	286 ± 50
Systolic blood pressure (mmHg)		
Pre-supplementation (mmHg)	128 ± 8	125 ± 8
Post-supplementation (mmHg)	128 ± 7	121 ± 8
Diastolic blood pressure (mmHg)		
Pre-supplementation (mmHg)	69 ± 6	70 ± 7
Post-supplementation (mmHg)	68 ± 6	69 ± 8
Mean arterial pressure (mmHg)		
Pre-supplementation (mmHg)	89 ± 5	89 ± 6
Post-supplementation (mmHg)	88 ± 5	86 ± 7

Values are mean ± SD

CHO carbohydrate condition; ARG + CHO, L-arginine and carbohydrate condition, *[NO_3^-]* nitrate concentration, *[NO_2^-]* nitrite concentration

* Significantly different from the pre-supplementation values ($P < 0.05$)

[#] Significantly different from the pre- and post-supplementation values ($P < 0.05$)

Orally ingested L-arginine is subjected to a number of presystemic and systemic elimination processes that might limit L-arginine availability irrespective of the supplementation regime (dose, duration, and type of supplement). Approximately 40 % of ingested oral L-arginine is metabolised by bacteria and arginases located within the intestinal tract (Castillo et al. 1993; Wu 1998). Thereafter, approximately 10–15 % of the circulating L-arginine is extracted by the liver (van de Poll et al. 2007; Yu et al. 1996) and catabolised by intra-hepatocyte arginases (Castillo et al. 1993). Although both acute oral L-arginine ingestion (Tang et al. 2011) and short-term supplementation (Liu et al. 2009; Willoughby et al. 2011) have been shown to increase circulating [L-arginine] in humans, the utilisation of this additional substrate by NOS may be limited considering indirect evidence that: (1) in vitro, elevated [L-arginine] inhibits dimethylarginine dimethylaminohydrolase (DDAH), the enzyme which inactivates the endogenous NOS-inhibitor asymmetric dimethylarginine (ADMA) (MacAllister et al. 1996); and (2) L-arginine competes with ADMA for the cellular L-arginine transporter, y^+ carrier hCAT-2B (Closs et al. 1997). It is perhaps reflective of this tight regulation of L-arginine utilisation that in a rodent model, only ~1 % of isotope-labelled L-arginine administered via gastric cannulation was utilised as a substrate by NOS over 24 h (Böger et al. 2004), and in humans, intravenous infusion of L-arginine hydrochloride did not significantly increase muscle NOS activity (Linden et al. 2011). In accordance with these findings, the present study is the first to report that the plasma [NO_2^-] and [NO_3^-], sensitive biomarkers of NOS activity (Kleinbongard et al. 2003; Lauer et al. 2001), were not altered in healthy subjects following acute supplementation with pure L-arginine. L-arginine delivery to, and utilisation by, NOS after L-arginine ingestion is a tightly controlled process in vivo and, therefore, alternative dietary intervention strategies should be considered for enhancing NO synthesis in healthy humans.

Intravenous L-arginine administration has been reported to reduce plasma lactate and ammonia accumulation during exercise (Schaefer et al. 2002), and in some studies, oral ingestion of L-arginine, or multi-ingredient supplements containing L-arginine, has reduced the O_2 cost of exercise (Bailey et al. 2010; Burtscher et al. 2005) and indices of exercise performance (Bailey et al. 2010; Buford and Koch 2004; Camic et al. 2010a, b; Stevens et al. 2000). These findings are in contrast to the present results and to other studies which have reported that L-arginine has no effect on blood [lactate] (Koppo et al. 2009; Liu et al. 2009), O_2 cost of exercise (Bescós et al. 2009; Koppo et al. 2009) and exercise tolerance or performance (Abel et al. 2005; Beis et al. 2011; Liu et al. 2009; McConell et al. 2006). However, despite the lack of effects of acute L-arginine ingestion on these variables in the healthy subjects investigated herein, we cannot exclude the possibility that L-arginine may enhance NO synthesis and exercise tolerance in other populations, such as older people or those with poor aerobic fitness or cardiovascular health (e.g. Bednarz et al. 2004).

A major strength of the present study is that we investigated the physiological effects of a pure L-arginine supplement. In an earlier study, we reported that acute supplementation with a multiple-micronutrient supplement containing 6 g of L-arginine increased plasma [NO_2^-], reduced the O_2 cost of exercise and enhanced exercise tolerance (Bailey et al. 2010). However, due to the diverse nutritional composition of the supplement used in our previous study (Bailey et al. 2010), we pointed out in that study that the physiological and performance benefits might not be exclusively apportioned to L-arginine. It may be speculated that the L-glutamine and L-citrulline that were present in the supplement we investigated previously might have enhanced L-arginine appearance in the circulation (Morris 2002; Wu 1998). Moreover, the antioxidants (citrus bioflavonoids, α-lipoic acid, betaine, vitamins C and E) might have relieved NOS inhibition by ADMA by preserving the DDAH activity in the face of oxidative stress (Lin et al.

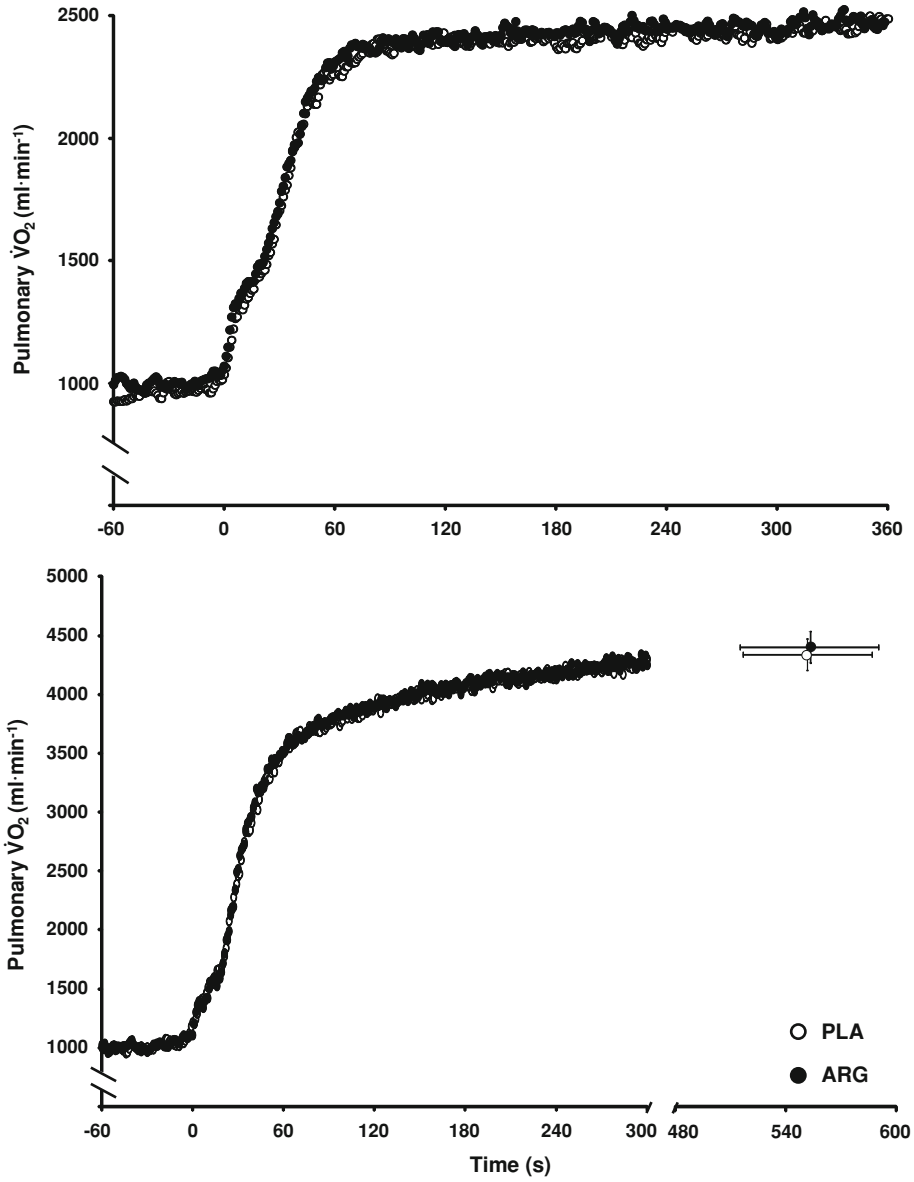

Fig. 3 Pulmonary $\dot{V}O_2$ responses during a step increment from a walking baseline to moderate-intensity (*upper panel*) and severe-intensity (*lower panel*) treadmill running. The placebo (PLA) is shown as *open circles* and L-arginine (ARG) as *filled circles*. The moderate $\dot{V}O_2$ responses are presented as the group mean of four averaged $\dot{V}O_2$ responses for each individual in each condition. The severe $\dot{V}O_2$ responses are presented as the group mean of two averaged $\dot{V}O_2$ responses in each experimental condition. The $\dot{V}O_2$ responses during moderate- and severe-intensity running were not different between the PLA and ARG conditions

2002; Stühlinger et al. 2001). There is also evidence that antioxidants stimulate eNOS activity (Rizza et al. 2011) and inactivate NO-scavenging superoxide (Powers and Jackson 2008), thereby enhancing NO bioavailability. Therefore, the multiple-micronutrient supplement we investigated previously (Bailey et al. 2010) may have enhanced the cellular L-arginine availability, limited ADMA activity and increased NOS function and NO bioavailability to a greater extent than the pure L-arginine we used in the present study.

It is also possible, however, that the supplement used in our previous study (Bailey et al. 2010) enhanced exercise performance though mechanisms which were unrelated to NO synthesis. For example, the supplement also contained betaine, which has been suggested to be ergogenic (Hoffman et al. 2009), without enhancing NO biomarkers in humans (Bloomer et al. 2011). Importantly, although this supplement contained beetroot juice as a colouring agent, the NO_3^- content at the dose administered (Bailey et al. 2010) was ~5 μmol (unpublished observations), which would not be expected to have physiological effects (Lansley et al. 2011). Similar to the uncertainty surrounding the mechanisms by which the supplement we tested previously (Bailey et al. 2010) was ergogenic, it is unclear whether the positive effects reported following AAKG (Campbell et al. 2006) and GAKIC (Buford and Koch 2004; Stevens et al. 2000) supplementations occur as a result of their L-arginine content or the α-ketoglutarate or glycerine-arginine-α-ketoisocaproic acid content, respectively. Therefore, further research is required to ascertain the mechanistic bases for the performance enhancement

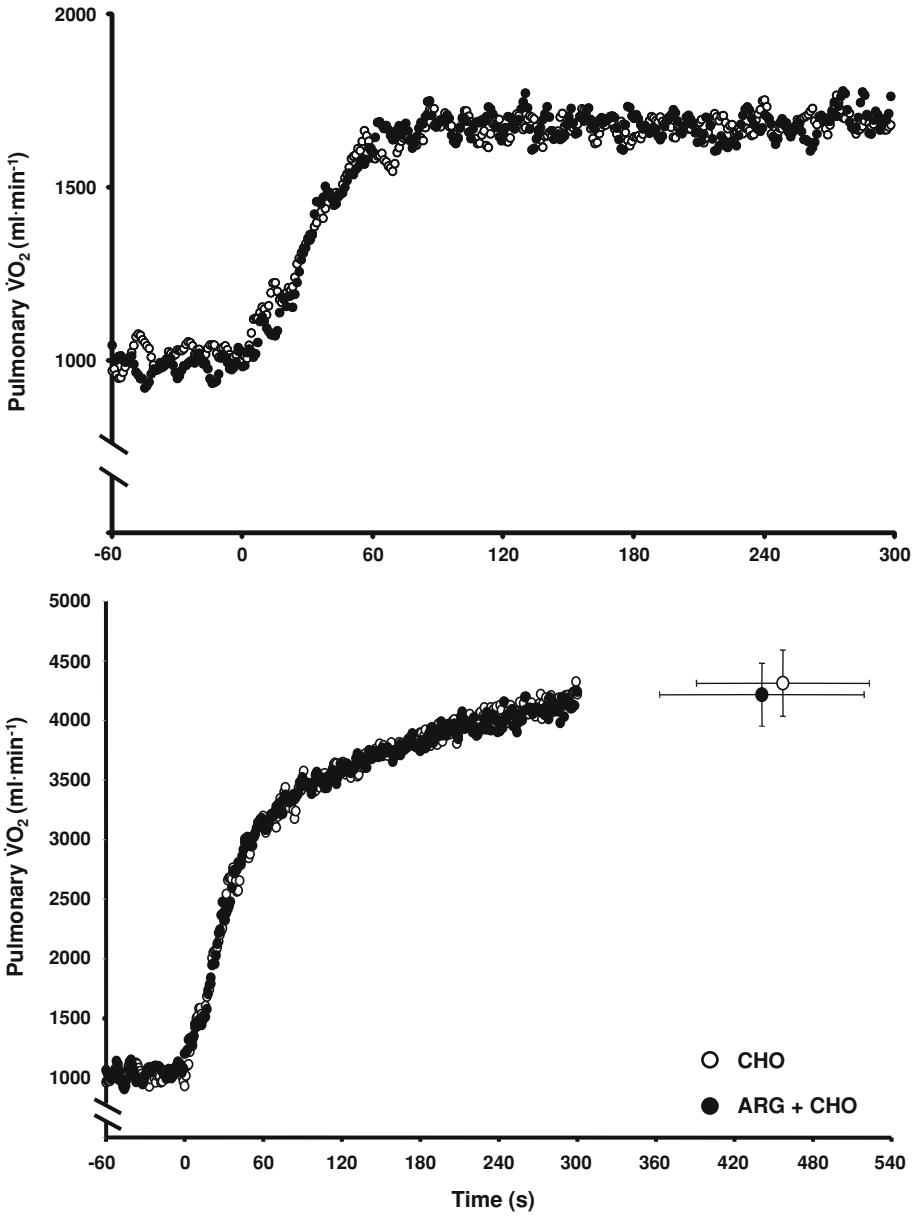

Fig. 4 Pulmonary $\dot{V}O_2$ responses during a step increment from a 20-W baseline to moderate-intensity (*upper panel*) and severe-intensity (*lower panel*) cycle exercise. The carbohydrate (CHO) is shown as *open circles* and L-arginine plus CHO (ARG + CHO) as *filled circles*. The moderate $\dot{V}O_2$ responses are presented as the group mean of two averaged $\dot{V}O_2$ responses for each individual in each experimental condition. The severe $\dot{V}O_2$ responses to a single exercise transition are presented for the group mean in each condition. The $\dot{V}O_2$ responses during moderate- and severe-intensity cycling were not different between the CHO and ARG + CHO conditions

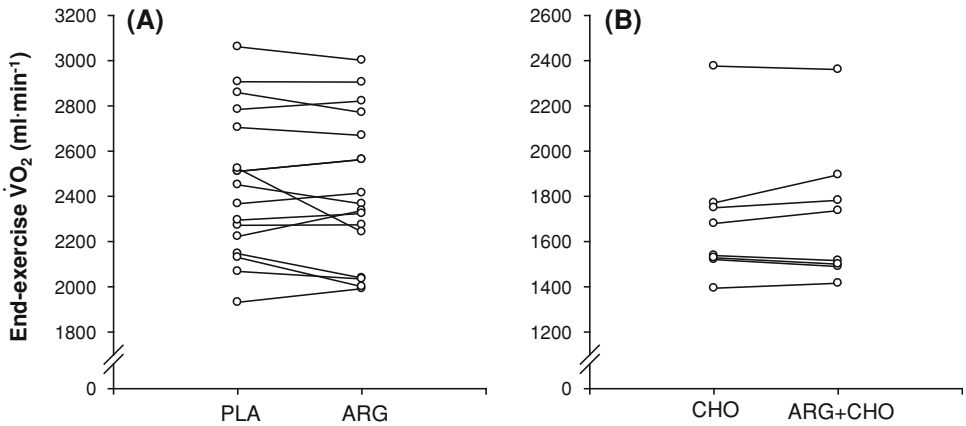

Fig. 5 Individual changes in end-exercise (steady-state) $\dot{V}O_2$ during moderate-intensity exercise in experiment one (**a**; PLA vs. ARG) and experiment two (**b**; CHO vs. ARG + CHO)

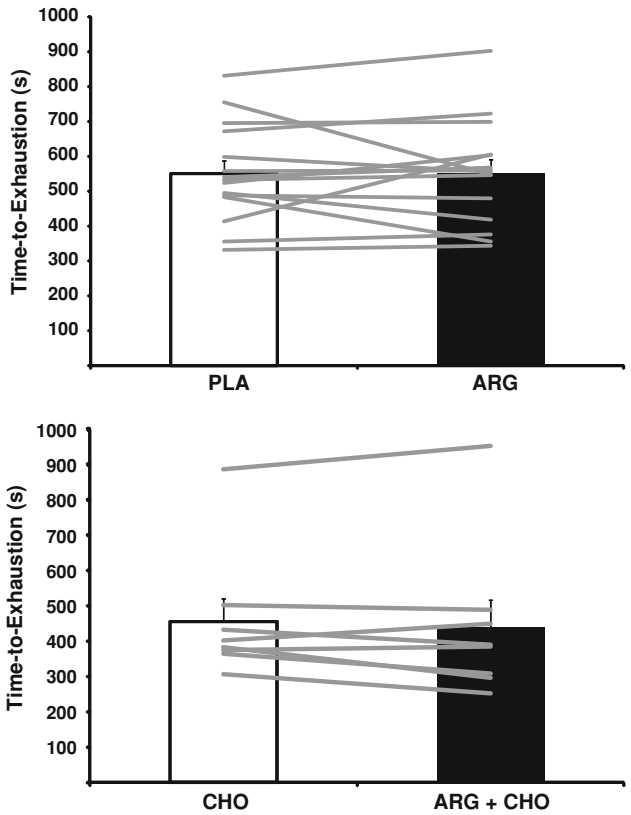

Fig. 6 Group mean ± SEM severe exercise tolerance during treadmill running after placebo (PLA; *open bars*) and L-arginine ingestion (ARG; *filled bars*) (*upper panel*) and during cycle ergometry after acute carbohydrate (CHO; *open bars*) and ARG + CHO ingestion (ARG + CHO; *filled bars*) (*lower panel*). The *grey lines* indicate the individual changes in exercise tolerance. Relative to PLA and CHO, respectively, ARG and ARG + CHO did not alter exercise tolerance

reported in previous studies using L-arginine-containing supplements (AAKG, GAKIC, or ARK-1).

To provide an insight into NO synthesis during exhaustive severe-intensity exercise, we measured the plasma $[NO_3^-]$ and $[NO_2^-]$ before and after exercise using the highly sensitive ozone-based chemiluminescence method. The venous plasma $[NO_3^-]$ was increased from pre-supplementation values by ~13–22 % across the experimental conditions after exhaustive exercise, consistent with an enhanced NO production. The plasma $[NO_2^-]$ decreased by ~10–15 % from pre- to post-exercise. The reported influence of exercise on venous plasma $[NO_2^-]$ has been variable with some studies reporting an increase (Allen et al. 2010; Rassaf et al. 2007) and others reporting a decrease (Bescós et al. 2011; Dreissigacker et al. 2010; Gladwin et al. 2000; Larsen et al. 2010). Since plasma $[NO_2^-]$ is an important biomarker of NOS-derived NO (Kleinbongard et al. 2003; Lauer et al. 2001), and also serves as a reservoir for NOS-independent NO production (Gladwin et al. 2000; Lundberg and Weitzberg 2010), the plasma $[NO_2^-]$ after exercise likely reflects the net balance between NOS-derived NO and the reduction of NO_2^- to NO during exercise. The arterio-venous differences in plasma $[NO_2^-]$ at rest and during exercise (Gladwin et al. 2000) and the decrease in venous plasma $[NO_2^-]$ following intense exercise (Dreissigacker et al. 2010; Gladwin et al. 2000; Larsen et al. 2010) suggest that the reduction of NO_2^- to NO might outweigh the synthesis of NO by NOS in the contracting myocytes, and/or in the muscle microvasculature, during exhaustive exercise. The decline in plasma $[NO_2^-]$ across an intense exercise bout supports the notion that the NO_2^- in plasma (and perhaps tissue) is depleted to support an increased demand for NO production during exercise (Bescós et al. 2011; Larsen et al. 2010). Although the relationship between pre-exercise plasma $[NO_2^-]$ and the time-to-exhaustion did not reach statistical significance in the present study, a positive relationship has been noted in previous literature (Dreissigacker et al. 2010; Totzeck et al. 2012) suggesting that the potential for O_2-independent NO production may be linked to exercise tolerance.

The present finding that resting blood pressure was significantly lower in the pure L-arginine condition compared to placebo (Table 1), is consistent with previous findings in normotensive subjects (Dong et al. 2011). The underlying mechanism for this effect, however, is not obvious, given that the plasma $[NO_3^-]$ and $[NO_2^-]$ were not altered by L-arginine supplementation. Importantly, there were no significant correlations between changes in plasma $[NO_2^-]$ or $[NO_3^-]$ and changes in SBP, DBP or MAP following L-arginine supplementation. In the present study, the $[NO_2^-]$ was measured in the venous circulation and the blood pressure was measured at the brachial artery. Considering that arterial $[NO_2^-]$ has been reported to be ~15 % greater than venous $[NO_2^-]$ at rest (Cannon et al. 2001; Gladwin et al. 2000; cf. Lauer et al. 2001), it may be postulated that arterial NO production may have been elevated, resulting in arterial vasodilatation despite no change in venous $[NO_2^-]$. However, this seems unlikely given that the changes in blood pressure correlate with changes in venous plasma $[NO_2^-]$ (Kapil et al. 2010). Further research is warranted to clarify the mechanism by which blood pressure may be lowered in the absence of significant change in biomarkers of NO production following L-arginine supplementation.

There were several aspects of the experimental design in our study that are worthy of consideration. We did not measure plasma [L-arginine] but rather focused on plasma $[NO_2^-]$ and $[NO_3^-]$ as biomarkers of NO production. We therefore do not know whether the interventions were successful in significantly elevating plasma [L-arginine] although we show here that the plasma $[NO_2^-]$ and $[NO_3^-]$ were not significantly altered. However, doses of

approximately 6 g of oral L-arginine have been previously shown to substantially elevate the plasma [L-arginine] (Bode-Böger et al. 1998; Forbes and Bell 2011). In addition, we do not know whether a higher oral dose of L-arginine might have resulted in physiological benefits, although research suggests that this is unlikely. Indeed, 6 g is a standard oral dose used in L-arginine supplementation studies (Álvares et al. 2011) and has been shown previously to be beneficial (Bailey et al. 2010; Buford and Koch 2004; Stevens et al. 2000). In the present study, 6 g oral L-arginine amounted to approximately 0.08 g kg^{-1} body mass. Forbes and Bell (2011) have reported that increasing the oral L-arginine dose from 0.075 to 0.15 g kg^{-1} body mass did not further increase plasma [L-arginine] and that neither dose altered [NOx]. Another consideration is that it is possible that subject training status might influence the extent to which NO precursors are efficacious. Acute dietary nitrate supplementation is apparently less effective in aerobically well-trained compared to moderately trained subjects perhaps due to training-related enhancements of NO bioavailability in the vasculature and skeletal muscle (Bescós et al. 2012; Cermak et al. 2012b; Wilkerson et al. 2012). Similarly, it is possible that the difference in the results between the present study and our earlier study (Bailey et al. 2010) is related in part to the differences in subject training status ($\dot{V}O_{2peak}$ of ~55–59 mL kg^{-1} min^{-1} in the present study and ~48 mL kg^{-1} min^{-1} in Bailey et al. 2010). Finally, we elected to use a time-to-exhaustion protocol at a fixed high-intensity power output rather than a time trial to assess exercise performance. This approach is necessary to evaluate differences in $\dot{V}O_2$ kinetics between experimental conditions, and time-to-exhaustion is a sensitive index of changes in exercise capacity resulting from nutritional and other interventions. We acknowledge, however, that such tests have less ecological validity than time trials.

In conclusion, we have shown that acute dietary supplementation with the NOS substrate, L-arginine, does not increase venous plasma [NO_3^-] and [NO_2^-], reduce the O_2 cost of exercise or improve exercise tolerance, although it may reduce resting blood pressure. The results were similar when ARG + CHO was compared to CHO suggesting that the L-arginine supplementation was not ineffective due to an insufficient insulin-mediated cellular L-arginine uptake. In contrast to our experimental hypotheses, dietary supplementation with 6 g pure L-arginine or in combination with CHO does not enhance markers of NO synthesis or indices of endurance exercise performance in healthy subjects.

Acknowledgments This study was supported by a Grant from GlaxoSmithKline Nutrition, UK.

Conflict of interest Authors report no conflict of interest.

References

Abel T, Knechtle B, Perret C, Eser P, von Arx P, Knecht H (2005) Influence of chronic supplementation of arginine aspartate in endurance athletes on performance and substrate metabolism—a randomized, double-blind, placebo-controlled study. Int J Sports Med 26:344–349

Allen JD, Stabler T, Kenjale A, Ham KL, Robbins JL, Duscha BD, Dobrosielski DA, Annex BH (2010) Plasma nitrite flux predicts exercise performance in peripheral arterial disease after 3 months of exercise training. Free Radic Biol Med 49:1138–1144

Álvares TS, Meirelles CM, Bhambhani YN, Paschoalin VM, Gomes PS (2011) L-arginine as a potential ergogenic aid in healthy subjects. Sports Med 41:233–248

Bailey SJ, Winyard P, Vanhatalo A, Blackwell JR, DiMenna FJ, Wilkerson DP, Tarr J, Benjamin N, Jones AM (2009) Dietary nitrate supplementation reduces the O_2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. J Appl Physiol 107:1144–1155

Bailey SJ, Winyard PG, Vanhatalo A, Blackwell JR, DiMenna FJ, Wilkerson DP, Jones AM (2010) Acute L-arginine supplementation reduces the O_2 cost of moderate-intensity exercise and enhances high-intensity exercise tolerance. J Appl Physiol 109:1394–1403

Bednarz B, Jaxa-Chamiec T, Gebalska J, Herbaczyńska-Cedro K, Ceremuzyński L (2004) L-arginine supplementation prolongs exercise capacity in congestive heart failure. Kardiol Pol 60:348–353

Beis L, Mohammad Y, Easton C, Pitsiladis YP (2011) Failure of glycine-arginine-α-ketoisocaproic acid to improve high-intensity exercise performance in trained cyclists. Int J Sport Nutr Exerc Metab 21:33–39

Bescós R, Gonzalez-Haro C, Pujol P, Drobnic F, Alonso E, Santolaria ML, Ruiz O, Esteve M, Galilea P (2009) Effects of dietary L-arginine intake on cardiorespiratory and metabolic adaptation in athletes. Int J Sport Nutr Exerc Metab 19:355–365

Bescós R, Rodríguez FA, Iglesias X, Ferrer MD, Iborra E, Pons A (2011) Acute administration of inorganic nitrate reduces VO_{2peak} in endurance athletes. Med Sci Sports Exerc 43:1979–1986

Bescós R, Ferrer-Roca V, Galilea PA, Roig A, Drobnic F, Sureda A, Martorell M, Cordova A, Tur JA, Pons A (2012) Sodium nitrate supplementation does not enhance performance of endurance athletes. Med Sci Sports Exerc 44:2400–2409

Bloomer RJ, Farney TM, Trepanowski JF, McCarthy CG, Canale RE (2011) Effect of betaine supplementation on plasma nitrate/nitrite in exercise-trained men. J Int Soc Sports Nutr 8:5

Bode-Böger SM, Böger RH, Galland A, Tsikas D, Frölich JC (1998) L-arginine-induced vasodilation in healthy humans: pharmacokinetic-pharmacodynamic relationship. Br J Clin Pharmacol 46:489–497

Böger RH, Tsikas D, Bode-Böger SM, Phivthong-Ngam L, Schwedhelm E, Frölich JC (2004) Hypercholesterolemia impairs basal nitric oxide synthase turnover rate: a study investigating the conversion of L-[guanidino-(15)N(2)]-arginine to (15)N-labeled nitrate by gas chromatography–mass spectrometry. Nitric Oxide 11:1–8

Boucher JL, Moali C, Tenu JP (1999) Nitric oxide biosynthesis, nitric oxide synthase inhibitors and arginase competition for L-arginine utilization. Cell Mol Life Sci 55(8–9):1015–1028

Broglio F, Gottero C, Benso A, Prodam F, Destefanis S, Gauna C, Maccario M, Deghenghi R, van der Lely AJ, Ghigo E (2003) Effects of ghrelin on the insulin and glycemic responses to glucose, arginine, or free fatty acids load in humans. J Clin Endocrinol Metab 88:4268–4272

Buford BN, Koch AJ (2004) Glycine-arginine-alpha-ketoisocaproic acid improves performance of repeated cycling sprints. Med Sci Sports Exerc 36:583–587

Burtscher M, Brunner F, Faulhaber M, Hotter B, Likar R (2005) The prolonged intake of L-arginine-L-aspartate reduces blood lactate accumulation and oxygen consumption during submaximal exercise. J Sports Sci Med 4:314–322

Camic CL, Housh TJ, Mielke M, Zuniga JM, Hendrix CR, Johnson GO, Schmidt RJ, Housh DJ (2010a) The effects of 4 weeks of an arginine-based supplement on the gas exchange threshold and peak oxygen uptake. Appl Physiol Nutr Metab 35:286–293

Camic CL, Housh TJ, Zuniga JM, Hendrix RC, Mielke M, Johnson GO, Schmidt RJ (2010b) Effects of arginine-based supplements on the physical working capacity at the fatigue threshold. J Strength Cond Res 24:1306–1312

Campbell B, Roberts M, Kerksick C, Wilborn C, Marcello B, Taylor L, Nassar E, Leutholtz B, Bowden R, Rasmussen C, Greenwood M, Kreider R (2006) Pharmacokinetics, safety, and effects on exercise performance of L-arginine alpha-ketoglutarate in trained adult men. Nutrition 22:872–881

Cannon RO 3rd, Schechter AN, Panza JA, Ognibene FP, Pease-Fye ME, Waclawiw MA, Shelhamer JH, Gladwin MT (2001) Effects of inhaled nitric oxide on regional blood flow are consistent with intravascular nitric oxide delivery. J Clin Invest 108:279–287

Castillo L, deRojas TC, Chapman TE, Vogt J, Burke JF, Tannenbaum SR, Young VR (1993) Splanchnic metabolism of dietary arginine in relation to nitric oxide synthesis in normal adult man. Proc Natl Acad Sci USA 90:193–197

Cermak NM, Gibala MJ, van Loon LJC (2012a) Nitrate supplementation's improvement of 10-km time-trial performance in trained cyclists. Int J Sport Nutr Exerc Metab 22:64–71

Cermak NM, Res P, Stinkens R, Lundberg JO, Gibala MJ, van Loon LJC (2012b) No improvement in endurance performance after a single dose of beetroot juice. Int J Sport Nutr Exerc Metab 22:470–478

Christensen PM, Nyberg M, Bangsbo J (2012) Influence of nitrate supplementation on VO_2 kinetics and endurance of elite cyclists. Scand J Med Sci Sports. doi:10.1111/sms.12005

Closs EI, Basha FZ, Habermeier A, Förstermann U (1997) Interference of L-arginine analogues with L-arginine transport mediated by the y+ carrier hCAT-2B. Nitric Oxide 1:65–73

Colombani PC, Bitzi R, Frey-Rindova P, Frey W, Arnold M, Langhans W, Wenk C (1999) Chronic arginine aspartate supplementation in runners reduces total plasma amino acid level at rest and during a marathon run. Eur J Nutr 38:263–270

Dong JY, Qin LQ, Zhang Z, Zhao Y, Wang J, Arigoni F, Zhang W (2011) Effect of oral L-arginine supplementation on blood pressure: a meta-analysis of randomized, double-blind, placebo-controlled trials. Am Heart J 162:959–965

Dreissigacker U, Wendt M, Wittke T, Tsikas D, Maassen N (2010) Positive correlation between plasma nitrite and performance during high-intensive exercise but not oxidative stress in healthy men. Nitric Oxide 23:128–135

Forbes SC, Bell GJ (2011) The acute effects of a low and high dose of oral L-arginine supplementation in young active males at rest. Appl Physiol Nutr Metab 36:405–411

Gladwin MT, Shelhamer JH, Schechter AN, Pease-Fye ME, Waclawiw MA, Panza JA, Ognibene FP, Cannon RO 3rd (2000) Role of circulating nitrite and S-nitrosohemoglobin in the regulation of regional blood flow in humans. Proc Natl Acad Sci USA 97:11482–11487

Greer BK, Jones BT (2011) Acute arginine supplementation fails to improve muscle endurance or affect blood pressure responses to resistance training. J Strength Cond Res 25:1789–1794

Hoffman JR, Ratamess NA, Kang J, Rashti SL, Faigenbaum AD (2009) Effect of betaine supplementation on power performance and fatigue. J Int Soc Sports Nutr 6:7

Jones AM, Doust JH (1996) A 1 % treadmill grade most accurately reflects the energetic cost of outdoor running. J Sports Sci 14:321–327

Kapil V, Milsom AB, Okorie M, Maleki-Toyserkani S, Akram F, Rehman F, Arghandawi S, Pearl V, Benjamin N, Loukogeorgakis S, Macallister R, Hobbs AJ, Webb AJ, Ahluwalia A (2010) Inorganic nitrate supplementation lowers blood pressure in humans: role for nitrite-derived NO. Hypertension 56:274–281

Kleinbongard P, Dejam A, Lauer T, Rassaf T, Schindler A, Picker O, Scheeren T, Gödecke A, Schrader J, Schulz R, Heusch G, Schaub GA, Bryan NS, Feelisch M, Kelm M (2003) Plasma nitrite reflects constitutive nitric oxide synthase activity in mammals. Free Radic Biol Med 35:790–796

Koppo K, Taes YE, Pottier A, Boone J, Bouckaert J, Derave W (2009) Dietary arginine supplementation speeds pulmonary VO_2 kinetics during cycle exercise. Med Sci Sports Exerc 41:1626–1632

Lansley KE, Winyard PG, Fulford J, Vanhatalo A, Bailey SJ, Blackwell JR, DiMenna FJ, Gilchrist M, Benjamin N, Jones AM (2011) Dietary nitrate supplementation reduces the O_2 cost of walking and running: a placebo-controlled study. J Appl Physiol 110:591–600

Larsen FJ, Weitzberg E, Lundberg JO, Ekblom B (2007) Effects of dietary nitrate on oxygen cost during exercise. Acta Physiol 191:59–66

Larsen FJ, Weitzberg E, Lundberg JO, Ekblom B (2010) Dietary nitrate reduces maximal oxygen consumption while maintaining work performance in maximal exercise. Free Radic Biol Med 48:342–347

Lauer T, Preik M, Rassaf T, Strauer BE, Deussen A, Feelisch M, Kelm M (2001) Plasma nitrite rather than nitrate reflects regional endothelial nitric oxide synthase activity but lacks intrinsic vasodilator action. Proc Natl Acad Sci USA 2001(98):12814–12819

Lin KY, Ito A, Asagami T, Tsao PS, Adimoolam S, Kimoto M, Tsuji H, Reaven GM, Cooke JP (2002) Impaired nitric oxide synthase pathway in diabetes mellitus: role of asymmetric dimethylarginine and dimethylarginine dimethylaminohydrolase. Circulation 106:987–992

Linden KC, Wadley GD, Garnham AP, McConell GK (2011) Effects of L-arginine infusion on glucose disposal during exercise in humans. Med Sci Sports Exerc 43:1626–1634

Liu TH, Wu CL, Chiang CW, Lo YW, Tseng HF, Chang CK (2009) No effect of short-term arginine supplementation on nitric oxide production, metabolism and performance in intermittent exercise in athletes. J Nutr Biochem 20:462–468

Lundberg JO, Weitzberg E (2010) NO-synthase independent NO generation in mammals. Biochem Biophys Res Commu 396: 39–45

MacAllister RJ, Parry H, Kimoto MT, Russell RJ, Hodson H, Whitley GS, Vallance P (1996) Regulation of nitric oxide synthesis by dimethylarginine dimethylaminohydrolase. Br J Pharmacol 119:1533–1540

McConell GK, Huynh NN, Lee-Young RS, Canny BJ, Wadley GD (2006) L-Arginine infusion increases glucose clearance during prolonged exercise in humans. Am J Physiol Endocrinol Metab 290:E60–E66

Morris SM Jr (2002) Regulation of enzymes of the urea cycle and arginine metabolism. Annu Rev Nutr 22:87–105

Pollock JS, Förstermann U, Mitchell JA, Warner TD, Schmidt HH, Nakane M, Murad F (1991) Purification and characterization of

particulate endothelium-derived relaxing factor synthase from cultured and native bovine aortic endothelial cells. Proc Natl Acad Sci USA 88:10480–10484

Powers SK, Jackson MJ (2008) Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. Physiol Rev 88:1243–1276

Rassaf T, Lauer T, Heiss C, Balzer J, Mangold S, Leyendecker T, Rottler J, Drexhage C, Meyer C, Kelm M (2007) Nitric oxide synthase-derived plasma nitrite predicts exercise capacity. Br J Sports Med 41:669–673

Rizza S, Muniyappa R, Iantorno M, Kim JA, Chen H, Pullikotil P, Senese N, Tesauro M, Lauro D, Cardillo C, Quon MJ (2011) Citrus polyphenol hesperidin stimulates production of nitric oxide in endothelial cells while improving endothelial function and reducing inflammatory markers in patients with metabolic syndrome. J Clin Endocrinol Metab 96:E782–E792

Schaefer A, Piquard F, Geny B, Doutreleau S, Lampert E, Mettauer B, Lonsdorfer J (2002) L-arginine reduces exercise-induced increase in plasma lactate and ammonia. Int J Sports Med 23:403–407

Simmons WW, Closs EI, Cunningham JM, Smith TW, Kelly RA (1996) Cytokines and insulin induce cationic amino acid transporter (CAT) expression in cardiac myocytes. Regulation of L-arginine transport and no production by CAT-1, CAT-2A, and CAT-2B. J Biol Chem 271:11694–11702

Stamler JS, Meissner G (2001) Physiology of nitric oxide in skeletal muscle. Physiol Rev 81:209–237

Stevens BR, Godfrey MD, Kaminski TW, Braith RW (2000) High-intensity dynamic human muscle performance enhanced by a metabolic intervention. Med Sci Sports Exerc 32:2102–2108

Stühlinger MC, Tsao PS, Her JH, Kimoto M, Balint RF, Cooke JP (2001) Homocysteine impairs the nitric oxide synthase pathway: role of asymmetric dimethylarginine. Circulation 104:2569–2575

Tang JE, Lysecki PJ, Manolakos JJ, MacDonald MJ, Tarnopolsky MA, Phillips SM (2011) Bolus arginine supplementation affects neither muscle blood flow nor muscle protein synthesis in young men at rest or after resistance exercise. J Nutr 141:195–200

Thams P, Capito K (1999) L-arginine stimulation of glucose-induced insulin secretion through membrane depolarization and independent of nitric oxide. Eur J Endocrinol 140:87–93

Totzeck M, Hendgen-Cotta UB, Rammos C, Frommke LM, Knackstedt C, Predel HG, Kelm M, Rassaf T (2012) Higher endogenous nitrite levels are associated with superior exercise capacity in highly trained athletes. Nitric Oxide 2012(27):75–81

van de Poll MC, Siroen MP, van Leeuwen PA, Soeters PB, Melis GC, Boelens PG, Deutz NE, Dejong CH (2007) Interorgan amino acid exchange in humans: consequences for arginine and citrulline metabolism. Am J Clin Nutr 85:167–172

Wascher TC, Posch K, Wallner S, Hermetter A, Kostner GM, Graier WF (1997) Vascular effects of L-arginine: anything beyond a substrate for the NO-synthase? Biochem Biophys Res Commun 234:35–38

Wilkerson DP, Hayward GM, Bailey SJ, Vanhatalo A, Blackwell JR, Jones AM (2012) Influence of acute dietary nitrate supplementation on 50 mile time trial performance in well-trained cyclists. Eur J Appl Physiol 112:4127–4134

Willoughby DS, Boucher T, Reid J, Skelton G, Clark M (2011) Effects of 7 days of arginine-alpha-ketoglutarate supplementation on blood flow, plasma L-arginine, nitric oxide metabolites, and asymmetric dimethyl arginine after resistance exercise. Int J Sport Nutr Exerc Metab 21:291–299

Wu G (1998) Intestinal mucosal amino acid catabolism. J Nutr 128:1249–1252

Wu G, Meininger CJ (2009) Nitric oxide and vascular insulin resistance. BioFactors 35:21–27

Wu G, Morris SM Jr (1998) Arginine metabolism: nitric oxide and beyond. Biochem J 336:1–17

Yu YM, Burke JF, Tompkins RG, Martin R, Young VR (1996) Quantitative aspects of interorgan relationships among arginine and citrulline metabolism. Am J Physiol 271:E1098–E1109