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L-citrulline supplementation improves O_2 uptake kinetics and high-intensity exercise performance in humans

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Running Head: L-citrulline and exercise performance

Abstract

The purpose of this study was to compare the effects of L-citrulline (CIT) and L-arginine (ARG) supplementation on nitric oxide (NO) biomarkers, pulmonary O_2 uptake ($\dot{V}O_2$) kinetics and exercise performance. In a randomised, placebo-controlled, cross-over study, ten healthy adult males completed moderate- and severe-intensity cycling exercise on days 6 and 7 of a 7 day supplementation period with placebo (PLA), 6 $g \cdot day^{-1}$ of ARG and 6 $g \cdot day^{-1}$ of CIT. Compared to PLA, plasma [ARG] was increased by a similar magnitude with ARG and CIT supplementation, but plasma [CIT] was only increased ($P<0.001$) with CIT supplementation. Plasma nitrite concentration ($[NO_2^-]$) was increased with ARG ($P<0.05$), and tended to increase with CIT ($P=0.08$), compared to PLA (PLA: 83 ± 25, ARG: 106 ± 41, CIT: 100 ± 38 nM); however, mean arterial blood pressure was only lower ($P<0.05$) after CIT supplementation. The steady state $\dot{V}O_2$ amplitude during moderate-intensity cycle exercise was not significantly different between supplements, but CIT speeded overall $\dot{V}O_2$ kinetics (PLA: 59 ± 8, CIT: 53 ± 5 s; $P<0.05$) during severe-intensity exercise, improved tolerance to severe-intensity exercise (PLA: 589 ± 101, CIT: 661 ± 107 s) and increased the total amount of work completed in the exercise performance test (PLA: 123 ± 18, CIT: 125 ± 19 kJ; $P<0.05$). These variables were not altered by ARG supplementation ($P>0.05$). In conclusion, these results suggest that short-term CIT, but not ARG, supplementation can improve blood pressure, $\dot{V}O_2$ kinetics and exercise performance in healthy adults.

Key Words: nitric oxide; blood pressure; near-infrared spectroscopy; metabolism; fatigue

Introduction

The multifaceted physiological signalling molecule, nitric oxide (NO), can be synthesised endogenously through the nitrate-nitrite-NO pathway (37) and through the five-electron oxidation of L-arginine (ARG) in a reaction catalyzed by the nitric oxide synthase (NOS) enzymes (55). While studies have shown that dietary nitrate supplementation can increase NO biomarkers, reduce blood pressure and improve exercise economy/efficiency and exercise tolerance in healthy adults (see 4 for review), the extent to which these variables are impacted by dietary ARG supplementation is less clear (see 1 for review). However, when ARG treatment increases NO biomarkers, exercise economy and exercise performance are improved (5, 52), whereas exercise economy and exercise performance are not improved when ARG treatment does not influence NO synthesis (9, 31, 35). Therefore, while there is some evidence to suggest that ARG treatment might improve physiological responses in conjunction with elevated NO synthesis, an optimal ARG administration procedure to enhance NO synthesis and associated physiological responses has yet to be established.

A significant obstacle to increasing ARG delivery to NOS via ARG supplementation is that orally ingested ARG is subjected to a number of presystemic and systemic elimination processes. Approximately 40% of ingested oral ARG is catabolised by intestinal bacteria and arginases on the first pass (13, 67), with a further 10-15% of systemic ARG extracted and metabolized by the liver (13, 44, 48, 59, 71). While both acute (57) and short term (34) ARG ingestion have been shown to increase plasma [ARG], the intracellular utilisation of this additional substrate by NOS might be restricted by the competition between ARG, asymmetric dimethylarginine (ADMA) and other ARG analogues for the transporter, y^+ carrier hCAT-2B (14). This regulation of ARG transport and metabolism could account for the finding that only ~ 1% of an oral ARG dose is utilised as substrate by NOS (11). Based on these restrictions, it appears that oral ARG supplementation might not be the optimal method to stimulate NO production through the NOS pathway.

L-citrulline (CIT) is co-produced with NO as an end product of NOS activity. It is well documented that NOS-derived CIT is efficiently recycled into ARG for

subsequent NO production through the CIT-NO cycle (24). Therefore, exogenous CIT administration might represent an attractive alternative to increase ARG provision to NOS. An advantage of oral CIT treatment is that, unlike ARG, catabolism of this amino acid in the intestines is limited since CIT is not metabolized by arginases and bacteria, and the activity of arginoinosuccinate synthase, the enzyme that initiates CIT metabolism, is low in enterocytes (68). Moreover, and also in contrast to ARG, CIT is not extracted from the systemic circulation for clearance by the liver (59, 65). Consequently, the majority of an oral CIT bolus passes into the systemic circulation (43). CIT is then extracted by the kidneys to be converted, in sequence, to argininosuccinate and ARG by the enzymes arginoinosuccinate synthase and arginoinosuccinate lyase, respectively (15, 24, 59, 65, 70). Synthesis of ARG from CIT is prevalent in other tissues (15, 23, 70) and this process is facilitated since CIT does not compete with ADMA for cell transport (50, 69). Importantly, it has been shown that oral CIT supplementation is more effective at increasing the circulating (32, 57, 53, 62) and tissue (63) [ARG] compared to an equivalent dose of ARG, and that CIT supplementation can increase NOS activation (63) and NO biomarkers (46, 53). Therefore, these findings suggest that CIT might serve as an important precursor for NO production.

Chronic supplementation with L-citrulline malate has been shown to enhance skeletal muscle power output in concert with a greater oxidative energy turnover and a lower power/pH ratio (7), and a lower ATP cost of muscle force production (19). These data suggest that short-term L-citrulline malate supplementation might improve skeletal muscle metabolism and/or contractile efficiency, which would be expected to predispose to greater fatigue resistance. However, since these experiments administered CIT as L-citrulline malate, and since malate is an important tricarboxylic acid cycle intermediate that might itself influence muscle function (61), it is unclear whether these beneficial effects can be attributed to CIT, *per se*. Hickner et al. (26) reported compromised endurance performance in concert with a lower plasma concentration of [nitrate] + [nitrite] ([NOx]) in humans following the acute ingestion of pure CIT. In contrast, seven days of supplementation with pure CIT has been shown to improve endurance exercise performance in mice (55). While these data suggest that chronic CIT supplementation has greater potential to improve endurance exercise performance than acute CIT ingestion, this has yet to be investigated in

humans. Moreover, since NO biomarkers have not been assessed in studies reporting positive effects of CIT on muscle function and metabolism (7, 19, 56), it is unclear whether these improvements are linked to an increase in NOS-derived NO. It is also unclear whether the improvements in muscle metabolism and performance with chronic CIT (7, 19, 56) are linked to improved oxygen uptake ($\dot{V}_{O_2}$) kinetics. Therefore, further research is required to assess whether short-term oral CIT can influence NO synthesis and exercise performance, and the underlying mechanisms for any performance gains with CIT.

The purpose of this study was to investigate the effects of short-term ARG and CIT supplementation on plasma [ARG], [CIT] and [NO_2^-], a sensitive marker of NOS activity (34), as well as blood pressure, $\dot{V}_{O_2}$ kinetics and exercise performance compared to a taste- and energy-matched placebo. We hypothesised that, when compared to placebo: 1) plasma [ARG] would be increased to a greater extent with ARG than CIT; and 2) that CIT but not ARG would elevate plasma [NO_2^-], reduce blood pressure and improve $\dot{V}_{O_2}$ kinetics, cycling efficiency and exercise performance.

Methods

Subjects

Ten healthy, recreationally-active males (mean ± SD, age 19 ± 1 yr, height 1.80 ± 0.08 m, body mass 79 ± 11 kg) volunteered to participate in this study. None of the subjects were tobacco smokers or users of dietary supplements. The procedures employed in this study were approved by the Institutional Research Ethics Committee. All subjects gave their written informed consent prior to the commencement of the study, after the experimental procedures, associated risks, and potential benefits of participation had been explained. Subjects were instructed to arrive at the laboratory in a rested and fully hydrated state, at least 3 h postprandial, and to avoid strenuous exercise in the 24 h preceding each testing session. Each subject was also asked to refrain from caffeine and alcohol 6 and 24 h before each test, respectively. All tests were performed at the same time of day (± 2 hours).

Experimental Design

Subjects were required to report to the laboratory on eight occasions over 6-7 weeks to complete the experimental testing. On the first visit to the laboratory subjects completed a ramp incremental exercise test for determination of the gas exchange threshold (GET) and the peak oxygen uptake ($\dot{V}_{O_{2peak}}$). Subjects were familiarized with the two exercise performance tests employed in this study during the second laboratory testing session. After these preliminary exercise tests, subjects returned to the laboratory on days six and seven of 7-day supplementation periods with placebo (PLA), L-arginine (ARG) and L-citrulline (CIT) to complete the experimental testing. During these tests, resting blood pressure, pulmonary $\dot{V}_{O_2}$ kinetics, muscle oxygenation and exercise performance were assessed and a resting venous blood sample was obtained. The supplements were administered orally in a randomized order as part of a double blind, cross-over experimental design. Each supplementation period was separated by 7-10 days of washout. Subjects were provided with a food diary for the first supplementation intervention and were instructed to replicate their diet over subsequent supplementation periods.

Incremental Test

During the first laboratory visit subjects completed a ramp incremental cycle test on an electronically-braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands). Initially, subjects performed 3 min of baseline cycling at 0 W, after which the work rate was increased by 30 W/min until the limit of tolerance. The subjects cycled at a self-selected pedal rate (70-90 rpm) and this pedal rate along with saddle and handle bar height and configuration was recorded and reproduced in subsequent tests. Breath-by-breath pulmonary gas-exchange data were collected continuously during the incremental tests and averaged over consecutive 10-s periods. The $\dot{V}_{O_{2max}}$ was taken as the highest 30-s mean value attained prior to the subject's volitional exhaustion in the test. 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 $\dot{V}_E$ / $\dot{V}_{CO_2}$, and 3) an increase in end-tidal O_2 tension with no fall in end-tidal CO_2 tension. The work rates that would require 90% of the GET (moderate-intensity exercise) and 70% Δ (GET plus 70% of the difference between the work rate at the GET and $\dot{V}_{O_{2max}}$; severe-intensity

exercise) were subsequently calculated with account taken of the mean response time for $\dot{V}_{O_2}$ during ramp exercise (i.e., two thirds of the ramp rate was deducted from the work rate at GET and peak).

Familiarization Tests

To avoid any order effect on the performance results as a consequence of a potential 'learning effect', subjects were familiarized with all performance tests prior to the experimental testing. Subjects completed a severe-intensity step exercise test terminating with an all-out sprint (exercise performance test) followed, after a 45 min passive recovery period, by a severe-intensity constant-work-rate step exercise test that was continued until the limit of tolerance (exercise tolerance test).

Supplementation Procedures

Experimental testing was conducted during a 7-day supplementation period with PLA, ARG and CIT. The PLA supplement consisted of 10.7 g of maltodextrin; the ARG supplement consisted of 6 g L-arginine + 4.3 g of maltodextrin; and the CIT supplement consisted of 6 g L-citrulline + 4.3 g of maltodextrin. All supplements were energy-matched containing 40 kcal per serving. Pure maltodextrin, L-arginine and L-citrulline powders were provided by NOW Sports Nutrition (NOW Foods, Bloomingdale, IL, USA) and were mixed with 500 mL water and 75 ml blackcurrant cordial in the proportions described above to produce the PLA, ARG and CIT supplements. On days 1-5 of supplementation, subjects were instructed to drink the beverage slowly over the course of the day. On days 6 and 7 of supplementation, subjects were instructed to consume the beverage over a 10 minute window such that the entire beverage had been consumed 60 min before the subject was required to report to the laboratory.

Experimental Tests

After reporting to the laboratory on days 6 and 7 of the supplementation interventions, subjects were required to rest in a seated position for 10 min in an isolated room. Thereafter, blood pressure of the brachial artery was measured whilst the subject was seated using an automated sphygmomanometer (Dinamap Pro, GE Medical Systems, Tampa, USA). Four measurements were taken and the mean of the measurements was calculated. A venous blood sample was then drawn into a lithium-heparin tube

and centrifuged at 4000 rpm and 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^-] in duplicate via chemiluminescence (6) and [ARG], [CIT] and [L-ornithine] ([ORN]) using high-performance liquid chromatography (HPLC; see below for details).

Thirty minutes after arriving at the laboratory (90 minutes after the ingestion of the supplement), subjects completed a series of cycle exercise tests. We elected to commence exercise testing 90 minutes after supplement consumption since published pharmacokinetic data has shown that this time frame should coincide with peak plasma [ARG] after orally ingesting 6 g CIT (53) or 6 g ARG (10). The exercise protocol consisted of three 'step' exercise tests including two moderate-intensity step tests followed by one severe-intensity exercise bout. Moderate-intensity step tests were completed to assess $\dot{V}_{O_2}$ kinetics and cycling economy in the absence of a $\dot{V}_{O_2}$ slow component, while severe-intensity step tests were completed to assess $\dot{V}_{O_2}$ kinetics in the presence of a $\dot{V}_{O_2}$ slow component where $\dot{V}_{O_{2max}}$ is attained and the tolerable duration of exercise is <20 min (49, 66). We conducted repeated step tests on the same laboratory visit since a prior moderate-intensity step exercise bout does not impact on $\dot{V}_{O_2}$ kinetics during subsequent moderate- or severe-intensity cycle exercise (12, 16). Therefore, all subjects performed a total of four bouts of moderate-intensity exercise and two bouts of severe-intensity exercise for each experimental condition.

Each transition began with 3 min of baseline cycling at 20 W before an abrupt transition to the target work rate. A passive recovery of 5 min separated the transitions. The moderate-intensity steps were each of 6 min duration. On day 6 of each supplementation condition, subjects cycled for 6 min at a severe-intensity constant-work-rate (70% Δ) followed immediately by a 60 s all-out sprint. The resistance on the pedals during the 60 s all-out effort was set using the linear mode of the Lode ergometer so that the subject would attain the power output calculated to be 50% Δ if they attained their preferred cadence (linear factor = power/preferred cadence^2). Subjects were provided with a 5 s countdown prior to the sprint and were instructed to attain the peak power as quickly as possible and to continue exercising maximally for the duration of the sprint. No time feedback was given to the subjects

at any point during the sprint. On day 7 of the supplementation period, the severe-intensity constant-work-rate bout was continued to the limit of tolerance. The time to task failure was used as a measure of exercise tolerance and was recorded when the pedal rate fell by > 10 rpm below the required pedal rate.

Measurements

During all 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). 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 (Jaeger Oxycon Pro, Hoechberg, Germany) via a capillary line connected to the mouthpiece. The gas analyzers were calibrated before each test with gases of known concentration and the turbine volume transducer was calibrated with a 3-liter 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 analyzer rise time relative to the volume signal. Pulmonary gas exchange and ventilation were calculated and displayed breath-by-breath.

During the exercise trials, a blood sample was collected from a fingertip into a capillary tube over the 20 s preceding the step transition in work rate, the 20 s preceding the completion of 360 s of moderate and severe cycling exercise, and also immediately following the all-out sprint and immediately after exhaustion during the severe-intensity constant-work-rate trial. These whole blood samples were subsequently analyzed to determine blood [lactate] (YSI 1500, Yellow Springs Instruments, Yellow Springs, OH, United States) within 30 s of collection.

The oxygenation status of the *m. vastus lateralis* of the right leg was monitored using a commercially available near-infrared spectroscopy (NIRS) system (model NIRO 300, Hamamatsu Photonics KK, Hiugashi-ku, Japan). The system consisted of an emission probe that irradiates laser beams and a detection probe. Four different wavelength laser diodes provided the light source (776, 826, 845, and 905 nm) and the light returning from the tissue was detected by a photomultiplier tube in the spectrometer. The intensity of incident and transmitted light was recorded

continuously at 2 Hz and used to estimate concentration changes from the resting baseline for oxygenated, deoxygenated, and total tissue hemoglobin/myoglobin. Therefore, the NIRS data represent a relative change based on the optical density measured in the first datum collected. The deoxygenated hemoglobin/myoglobin concentration ([HHb]) signal was assumed to provide an estimate of changes in fractional O_2 extraction in the field of interrogation (e.g., 21). It should be noted here that the contribution of deoxygenated myoglobin to the NIRS signal is presently unclear, and, as such, the terms [HbO_2], and [HHb] used in this paper should be considered to refer to the combined concentrations of oxygenated and deoxygenated hemoglobin and myoglobin, respectively. The tissue oxygenation index (TOI) was calculated using the following equation:

$$\mathrm{TOI} = \frac{[\mathrm{HbO_2}]}{[HbO_2] + [HHb]} \times 100 \qquad \text{(Eqn 1)}$$

The leg was initially cleaned and shaved around the belly of the muscle, and the optodes were placed in the holder, which was secured to the skin with adhesive at 20 cm above the fibular head. To secure the holder and wires in place, an elastic bandage was wrapped around the subject's leg. The wrap helped to minimize the possibility that extraneous light could influence the signal and also ensured that the optodes did not move during exercise. Indelible pen marks were made around the holder to enable precise reproduction of the placement in subsequent tests. The probe gain was set with the subject at rest in a seated position with the leg extended at down stroke on the cycle ergometer before the first exercise bout, and NIRS data were collected continuously throughout the exercise protocols. The data were subsequently downloaded onto a personal computer, and the resulting text files were stored on disk for later analysis.

Plasma [ARG], [CIT] and [ORN] were determined by *o*-phthaldialdehyde (OPA) derivatised, fluorescence-detection HPLC, using methods adapted from Jones and Gilligan (27). The HPLC apparatus was a Perkin Elmer Flexar LC system with Chromera software (Perkin Elmer, MASS, USA). In brief, plasma was de-proteinised in 1.5N perchloric acid, neutralised in 2N potassium hydrogen carbonate, and centrifuged. 100µL of supernatant, 100µL of 1.2% benzoic acid, and 1.4mL H_2O

were added to HPLC vials. 50µL of unknowns/standards were mixed with 50µL of an OPA solution containing 2-mercaptoethanol (Fluoraldehyde OPA reagent solution, Thermo Scientific, IL, USA), enabling the pre-column derivatization of amino acids with a highly fluorescent OPA adduct. 25µL of derivatised sample was mixed in mobile phase and eluted at 0.8 ml.min^{-1} through a 4.6 x 150mm, 2.7 µm Brownlee SPP C18 reverse-phase analytical column with 5mm guard column with matching specification. A gradient protocol of aqueous mobile phase A (0.05M potassium phosphate buffer, pH 7.2) with organic mobile phase B (acetonitrile/methanol/water, 40/40/20) was performed: 0 – 1.5 min, 80% Mobile A; 1.5 – 18.5, 80 – 65%; 23.5, 50%; 32.5, 40%; 36.5, 30%; 43.5, 0%; 51.5, 80%. Fluorescence was monitored at excitation and emission wavelengths of 340 and 455 nm, respectively. Amino acid concentrations were determined against standards calibration curves between 0 and 500 µM (nmol.mL^{-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 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 characterize 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:

$$\dot{V}_{O_2}(t) = \dot{V}_{O_2\,baseline} + A_p(1-e^{-(t-TDp/\tau p)}) \quad \text{(moderate)} \quad \text{(Eqn. 2)}$$

$$\dot{V}_{O_2}(t) = \dot{V}_{O_2\,baseline} + A_p(1-e^{-(t-TDp/\tau p)}) + A_s(1-e^{-(t-TDs/\tau s)}) \quad \text{(severe)} \quad \text{(Eqn. 3)}$$

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; and A_s, TD_s, and τ_s represent the amplitude of, time delay before the onset of, and time constant 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. $\dot{V}O_{2baseline}$ was defined as the mean $\dot{V}O_2$ measured over the final 90 s of the resting baseline period. The $\dot{V}O_2$ at 360 s was taken as the mean $\dot{V}O_2$ between 330 and 360 s, while the $\dot{V}O_2$ at the limit of tolerance (T_{lim}) was defined as the mean $\dot{V}O_2$ measured over the final 30 s of the exhaustive exercise bout. 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 the same iso-time (360-s) for all dietary interventions. The amplitude of the slow component was also described relative to the entire $\dot{V}O_2$ response. In addition, the functional 'gain' (G) of the fundamental $\dot{V}O_2$ response was computed by dividing A_p by the Δ work rate. To determine the overall kinetics of the $\dot{V}O_2$ response to both moderate- and severe-intensity exercise, the data were also fit with a mono-exponential model from 0-s to end-exercise without time delay. This mean response time (MRT) was used to calculate the O_2 deficit using the following equation:

$$O_2 \text{ Deficit (L)} = \text{MRT(min)} \times \Delta \dot{V}o_2 \text{ (L)} \qquad \text{(Eqn 4)}$$

where $\Delta \dot{V}O_2$ was the difference in $\dot{V}O_2$ at 360 s and baseline.

To provide information on muscle oxygenation, we also modelled the [HHb] response to exercise. Mono- and bi-exponential models, similar to those described above, were applied to the ensemble averaged data with the exception that the fitting window commenced at the time at which the [HHb] signal increased 1 SD above the baseline mean. The [HHb] kinetics for moderate exercise were determined by constraining the fitting window to the point at which mono-exponentiality became distorted, consequent to a gradual fall in [HHb], as determined by visual inspection of the residual plots. The [HHb] kinetics for severe exercise were determined by fitting a bi-exponential model from the first data point, which was 1 SD above the baseline mean through the entire response. The [HHb] TD and τ values were summed to provide information on the overall [HHb] response dynamics in the fundamental phase of the response. The [HbO_2] response does not approximate an exponential and was, therefore, not modelled. Rather, we assessed this by determining the [HbO_2] at

baseline (90-s preceding step transition), 120 s (30 s mean surrounding 120 s) and end exercise (mean response over the final 30 s of exercise). The TOI responses were assessed using the same data analysis procedures.

Statistics

A one-way repeated-measures ANOVA was employed to assess between-supplement differences in blood pressure; plasma [ARG], [CIT], [ORN] and [NO_2^-]; $\dot{V}_{O_2}$; NIRS-derived [HHb], [HbO_2] and TOI; and exercise performance. Significant effects were further explored using simple contrasts with the alpha level adjusted via a Fisher's LSD correction. Data are presented as mean ± SD, unless otherwise stated. Statistical significance was accepted when $P<0.05$.

Results

The PLA, ARG and CIT supplements administered in this study were well tolerated by all subjects with no negative side effects reported. Subjects consumed all doses of the supplement for each experimental condition and their diet was consistent across all the dietary interventions. The $\dot{V}_{O_{2peak}}$ attained in the ramp incremental test was 3.94 ± 0.51 $L \cdot min^{-1}$ which equated to a relative $\dot{V}_{O_{2peak}}$ of 50 ± 9 $ml \cdot kg^{-1} \cdot min^{-1}$. The work rates which corresponded to 90% GET and 70% Δ were 120 ± 23 W and 284 ± 40 W, respectively.

Plasma [NO_2^-], [ARG], [CIT] and [ORN]

The plasma [NO_2^-], [ARG], [CIT] and [ORN] data for the PLA, ARG and CIT conditions are reported in Table 1. The ANOVA revealed a significant main effect for supplement on plasma [ARG], [CIT] and [ORN] (all $P<0.001$). Plasma [ARG] was increased above PLA (57 ± 14 μM) in ARG (151 ± 31 μM) and CIT (135 ± 22 μM; both $P<0.001$), with no differences between ARG and CIT ($P>0.05$; Table 1). Plasma [CIT] was not significantly different between PLA (23 ± 5 μM) and ARG (26 ± 6 μM; $P>0.05$), but was significantly greater that both these conditions with CIT (665 ± 205 μM; $P<0.001$; Table 1). Plasma [ORN] was significantly greater in CIT (50 ± 6 μM) than PLA (26 ± 8 μM; $P<0.001$) and significantly greater in ARG (62 ± 14) than both PLA and CIT ($P>0.05$; Table 1). Plasma [NO_2^-] was significantly

increased in ARG (106 ± 41 nM; $P<0.05$), but not following CIT supplementation (100 ± 38 nM; $P=0.08$), compared to PLA (83 ± 25 nM; Table 1).

Blood Pressure

The blood pressure data for the PLA, ARG and CIT conditions are reported in Table 2. There was a significant main effect for supplement on systolic blood pressure (SBP; $P<0.05$), with follow up analyses showing that SBP was lower after CIT (118 ± 6 mmHg; $P<0.05$), but not after ARG (120 ± 7 mmHg; $P>0.05$), compared to PLA (122 ± 7 mmHg; Table 2). While there was no main effect for supplement on diastolic blood pressure (DBP; $P>0.05$), there was a significant main effect for supplement on mean arterial pressure (MAP; $P<0.05$). Relative to PLA (87 ± 3 mmHg), MAP was lower after CIT (85 ± 2 mmHg; $P<0.05$), but not ARG (86 ± 2 mmHg; $P>0.05$; Table 2).

Pulmonary $\dot{V}_{O_2}$ Kinetics

The pulmonary gas exchange data from the moderate- and severe-intensity cycle tests are reported in Table 3. There were no significant between-supplement differences for the baseline and end-exercise $\dot{V}_{O_2}$ during the moderate-intensity step exercise tests ($P>0.05$). Accordingly, the fundamental $\dot{V}_{O_2}$ amplitude was not significantly different between the conditions (PLA: 0.87 ± 0.21, ARG: 0.87 ± 0.22, CIT: 0.86 ± 0.23 $L \cdot min^{-1}$; $P>0.05$; Table 3). The phase II τ was also not significantly different between conditions (PLA: 24 ± 7, ARG: 22 ± 7, CIT: 21 ± 6 s; $P>0.05$; Table 3).

The baseline $\dot{V}_{O_2}$ and phase II $\dot{V}_{O_2}$ kinetics during severe-intensity exercise were not significantly impacted by the dietary interventions employed in this investigation ($P>0.05$ for all comparisons). The $\dot{V}_{O_2}$ at exhaustion was not significantly different between experimental conditions and was also not significantly different from the $\dot{V}_{O_{2peak}}$ attained in the ramp incremental test ($P>0.05$ for all comparisons). No significant differences in the fundamental $\dot{V}_{O_2}$ amplitude (PLA: 2.23 ± 0.34, ARG: 2.26 ± 0.42, CIT: 2.29 ± 0.45 $L \cdot min^{-1}$) or $\dot{V}_{O_2}$ slow component (PLA: 0.66 ± 0.09, ARG: 0.60 ± 0.12, CIT: 0.58 ± 0.13 $L \cdot min^{-1}$; $P>0.05$; Table 3) were observed across the experimental conditions. However, there was a significant main effect for supplement on the MRT ($P<0.05$), with faster overall $\dot{V}_{O_2}$ kinetics observed after CIT compared to PLA supplementation (PLA: 60 ± 8, CIT: 54 ± 5 s; $P<0.05$; Figure 1).

There were no significant differences in $\dot{V}CO_2$ and RER between the PLA, ARG and CIT conditions during moderate- or severe-intensity cycle exercise (P>0.05 for all comparisons, data not shown). There were also no between condition differences in blood [lactate] at any time comparison in this study (P>0.05, data not shown).

NIRS Variables

The NIRS-derived muscle [HHb], [HbO_2] and TOI data during moderate- and severe-intensity cycle exercise with PLA, ARG and CIT supplementation are reported in Table 4. There were no significant differences between the experimental conditions for the [HbO_2] and TOI responses during moderate-intensity exercise (P>0.05 for all comparisons). However, the [HHb] amplitude during moderate-intensity cycling exercise was significantly lower after CIT supplementation (PLA: 8 ± 4, CIT: 6 ± 4 A.U.; P>0.05; Figure 2). While there were no significant between-supplement differences in [HHb] dynamics or in muscle [HbO_2] during severe-intensity cycle exercise in this study, the muscle TOI was significantly elevated over the first 360 s of severe-intensity exercise with CIT supplementation (P<0.05; Table 4; Figure 3).

Exercise Performance

The power profiles for the three experimental conditions during the 60 s all-out sprint that followed the 6 min bout of severe-intensity exercise (the exercise performance test) are shown in Figure 4, while the times to exhaustion during the severe-intensity constant-work-rate cycle trials (the exercise tolerance test) are shown in Figure 5. A significant main effect for supplement was observed for the peak power attained and total work completed during the 60 s all-out sprint that concluded the exercise performance test (P<0.05). Follow up analyses showed that, compared to PLA, CIT supplementation increased the test peak power by 9% (PLA: 480 ± 98, CIT: 524 ± 94 W; P<0.05; Figure 4) and the total work completed during the 60 s sprint by 7% (PLA: 21 ± 4, CIT: 23 ± 4 kJ; P<0.05; Figure 4). Neither peak power output (482 ± 102 W) nor total sprint work completed (21 ± 5 kJ) were significantly impacted by ARG supplementation (P>0.05). The total work completed over the entire exercise performance test was greater with CIT (125 ± 19 kJ; P<0.05), but not ARG (124 ± 19 kJ; P>0.05), compared to PLA (123 ± 18 kJ; P<0.05). There was a strong trend for a main effect of supplement on the time-to-exhaustion during the exercise tolerance test (P=0.07). When between-condition analyses were conducted, there was a significant

12% increase in exercise tolerance time after CIT supplementation relative to PLA (PLA: 589 ± 101, CIT: 661 ± 107 s; $P<0.05$; Figure 5). Exercise tolerance was not significantly improved with ARG (612 ± 150 s) compared to PLA ($P>0.05$). The changes in exercise tolerance after CIT supplementation were not related to changes in plasma [NO_2^-], $\dot{V}O_2$ kinetics or muscle oxygenation ($P>0.05$ for all comparisons).

Discussion

The principal novel findings from this study are that short-term supplementation with pure CIT enhanced endurance exercise performance in concert with faster overall $\dot{V}O_2$ kinetics and a 21% increase in the sensitive NO biomarker, plasma [NO_2^-]. This is in contrast to previous research demonstrating that acute CIT supplementation lowers plasma [NOx] and compromises exercise tolerance (26), but consistent with studies showing that short term supplementation with L-citrulline malate can positively impact on skeletal muscle power output and metabolic responses (7, 19). These findings are important since they suggest that CIT might be responsible for the positive effects previously reported following L-citrulline malate supplementation, and offer new insights into the mechanisms by which CIT supplementation might be ergogenic. Conversely, no significant differences in $\dot{V}O_2$ kinetics and exercise performance were observed following short-term ARG supplementation. These findings suggest that short-term CIT supplementation, but not short term ARG supplementation, might be an effective dietary intervention to improve oxidative metabolism and exercise performance in healthy adults.

Influence of ARG and CIT supplementation on plasma [ARG], [CIT] and [NO_2^-]

In an attempt to overcome the well developed inter-organ system for ARG clearance, recent studies have investigated the efficacy of oral CIT supplementation as an alternative method to enhance NO production via NOS. Oral CIT supplementation is appealing in this regard since CIT is not significantly metabolized in the gut (68) and liver (59, 65), and less than 1% of orally ingested CIT is excreted in the urine (50). As such the majority of an oral CIT load passes into the systemic circulation, as reflected by a significant increase in plasma [CIT] after CIT ingestion in the current study and numerous previous reports (8, 16, 42, 51, 53). Thereafter the bulk of

plasma CIT is converted into ARG, mostly in the kidneys (15, 24, 59, 65, 70), but also in several other tissues (15, 23, 70). This is compatible with the significant increase in plasma [ARG] in this study, and several previous studies (32, 47, 53, 62), following CIT supplementation. It is important to note that, not only does CIT increase systemic [ARG] by avoiding catabolism along the intestinal-renal axis, but CIT might also be expected to enhance ARG bioavailability given that CIT can function as an allosteric inhibitor of arginase (54). This is supported by our finding of a lower plasma [ORN], the product of ARG metabolism by arginase (70), after CIT compared to ARG supplementation in this study. However, in spite of this potential for greater systemic ARG bioavailability following oral CIT compared to oral ARG supplementation, and in contrast to previous studies reporting a greater increase in plasma [ARG] after CIT ingestion relative to ARG ingestion (32, 47, 53, 62), plasma [ARG] was increased by a similar magnitude when the same dose of CIT and ARG was orally administered in this study. These conflicting findings might be a function of between-study differences in the experimental subjects and supplementation regimes. Therefore, our results do not support the notion of a greater systemic ARG availability after CIT compared to ARG supplementation, at least in healthy adult males undergoing the ARG and CIT supplementation procedures employed in this study.

As well as increasing NOS substrate provision, there is some evidence to suggest that NO production is enhanced after CIT treatment (46, 53, 63) and that CIT can restore NO production in conditions where NO production is compromised (16, 36). However, there is also a suggestion that CIT ingestion tends to lower NO production, as inferred from plasma [NOx] (26). Plasma [NO_2^-] better reflects human NOS activity than plasma [NOx] (34) and is likely to provide a more accurate assessment of NOS-derived NO. In this study CIT supplementation increased plasma [NO_2^-] by 21%, but this increase did not attain statistical significance (P=0.08). On the other hand, ARG supplementation resulted in a statistically significant (28%) increase in plasma [NO_2^-]. Taken together, these data suggest that short-term ARG supplementation might be more effective than CIT supplementation at increasing the sensitive biomarker of NOS activity, plasma [NO_2^-] (28, 34). However, the extent to which plasma [NO_2^-] reflects skeletal muscle NOS activity is unclear. In this study plasma [ORN] was lower following CIT than ARG. It is known that ORN competes

567 with ARG for cellular uptake via the y^+ carrier system (70). As such, this might have
568 facilitated greater skeletal muscle ARG uptake after CIT compared to ARG
569 supplementation in this study. The finding of a greater increase in tissue [ARG] (63)
570 after CIT ingestion relative to ARG ingestion supports this postulate. Moreover, the
571 lower plasma [ORN] following CIT than ARG, despite a similar increase in plasma
572 [ARG], implies a lower arginase activity following CIT (70). Providing muscle
573 [ORN] was also lower following CIT compared to ARG supplementation in this
574 study, muscle arginase activity may have been downregulated. While there appears to
575 be some controversy regarding the levels of arginase in human skeletal muscle (20,
576 45), the potential for CIT to inhibit arginase might be important since arginase-II
577 content in human skeletal muscle can be similar to that observed in the kidney, i.e.,
578 relatively high (45). Therefore, the functional effects of CIT (described below) may
579 be muscle-specific and not detected as changes in plasma [NO_2^-], which may be more
580 indicative of gross changes in NOS activity throughout the body. Likewise, the ARG-
581 induced increase in plasma [NO_2^-], observed in the present study, may be due to non-
582 endothelial NOS-mediated NO production or NOS-mediated NO production at sites
583 other than skeletal muscle.
584
585 *Influence of ARG and CIT supplementation on blood pressure*
586 A hallmark of enhanced NO synthesis is a reduction in blood pressure owing to NO-
587 induced smooth muscle relaxation (22). It has also recently been demonstrated that
588 circulating NO_2^- itself can act as a *source* for NO synthesis via endogenous human
589 nitrite reductase activities, associated with proteins such as xanthine oxidase and
590 deoxyhemoglobin (see 37 for review). However, in spite of a significant increase in
591 plasma [NO_2^-] after ARG supplementation, resting blood pressure was not
592 significantly lowered. This suggests that the increase in plasma [NO_2^-] after short-
593 term ARG supplementation might not have been sufficient to lower resting blood
594 pressure in normotensive adults. Conversely, CIT supplementation, which did not
595 significantly increase plasma [NO_2^-], significantly reduced resting blood pressure.
596 Although previous studies have shown a reduction in arterial stiffness (46), enhanced
597 endothelium-dependent vasorelaxation in response to acetylcholine (25) and an
598 association between the change in the ARG/ADMA ratio and flow mediated dilation
599 (53) with CIT, we have shown for the first time that pure CIT supplementation can
600 reduce blood pressure in healthy normotensive adults. An increase in cyclic

guanosine monophosphate (cGMP) has been reported after short-term CIT consumption (49) which suggests that the reduction in blood pressure with CIT might result from NO-cGMP-related smooth muscle relaxation. Alternatively, CIT might alter vascular tone through another endothelium-derived relaxing factor such as prostacyclin or endothelium-derived hyperpolarizing factors independent of, or alongside, an increase in NO. Further research is required to investigate the mechanisms by which CIT might positively impact on vascular and other physiological responses.

Influence of ARG and CIT supplementation on $\dot{V}O_2$ kinetics

Giannesini et al. (19) reported that short-term L-citrulline malate supplementation lowered both the oxidative and phosphocreatine cost of skeletal muscle force production in the rat gastrocnemius muscle *in situ*. We have previously shown that short-term dietary nitrate supplementation can also lower skeletal muscle ATP turnover by attenuating ATP flux through oxidative phosphorylation and phosphocreatine (PCr) hydrolysis in association with lower $\dot{V}O_2$ in humans completing knee-extensor exercise (2). Therefore, we hypothesized that short term CIT supplementation might lower $\dot{V}O_2$ in humans completing cycle exercise. However, in contrast to our previous findings with dietary nitrate supplementation (6) and our experimental hypothesis, short term CIT supplementation did not significantly lower $\dot{V}O_2$ during moderate-intensity cycle ergometry exercise. Our findings in this study might differ with those reported by Giannesinin et al. (19) as a consequence of differences in the experimental model (human skeletal muscle contraction *in vivo vs*. isolated rat skeletal muscle *in situ*) or differences in the L-citrulline supplementation procedures (pure L-citrulline supplementation *vs*. L-citrulline malate supplementation).

In the present study short term CIT supplementation did not significantly increase plasma [NO_2^-] and moderate exercise $\dot{V}O_2$ was not significantly altered. We have recently shown that acute ARG ingestion did not increase plasma [NO_2^-] or lower moderate exercise $\dot{V}O_2$ (60). However, plasma [NO_2^-] was increased by 28% following short term ARG in the present study without impacting on moderate exercise $\dot{V}O_2$. Importantly neither ARG nor CIT increased plasma [NO_2^-] to the extent observed when moderate exercise $\dot{V}O_2$ is lowered by dietary nitrate supplementation

(2, 5-6, 33) and this likely accounts for the similar moderate exercise $\dot{V}O_2$ across PLA, ARG and CIT in the present study. While neither the steady-state $\dot{V}O_2$ nor the rate at which $\dot{V}O_2$ increased following the onset of moderate-intensity exercise were significantly impacted by ARG or CIT supplementation, the NIRS-derived muscle [HHb] amplitude was lower with CIT. Since the NIRS-derived muscle [HHb] signal is considered a non-invasive proxy for muscle fractional O_2 extraction (e.g., 21), the lower muscle [HHb] amplitude with CIT in the absence of a change in $\dot{V}O_2$ suggests that CIT may have improved O_2 availability/distribution within the muscle microvasculature.

During severe-intensity exercise, the overall $\dot{V}O_2$ kinetics was faster following CIT supplementation compared to PLA. These data support the findings of Bendahan *et al*. (7) who reported that L-citrulline malate supplementation elevated muscle oxidative ATP production determined *in vivo* using ^{31}P-MRS. The faster overall $\dot{V}O_2$ kinetics after CIT supplementation was accompanied by an increased NIRS-derived TOI throughout the exercise bout. This suggests that CIT supplementation improved the distribution of O_2 within the muscle microvasculature which, in turn, permitted a greater $\dot{V}O_2$ over the initial stages of severe-intensity cycle exercise. Previous studies have also reported that overall $\dot{V}O_2$ kinetics during severe-intensity exercise is speeded by interventions that enhance muscle O_2 availability (e.g., 3, 64). However, it is known that muscle NIRS measures manifest significant special heterogeneities across the contracting quadriceps (29) so it is unclear whether a lower [HHb] amplitude during moderate-intensity exercise, and the increased TOI during severe-intensity exercise, at a discrete site of the *vastus lateralis* is reflective of an improved matching between muscle O_2 delivery and muscle O_2 consumption across the contracting quadriceps. Indeed, some studies report good agreement between NIRS markers of muscle oxygenation and mixed venous P_{O_2} or oxygenation (e.g., 39), while other studies do not (e.g., 38). It has also been suggested that the interpretation of NIRS data may be complicated by increased skin blood flow (30, 40), as develops during exercise. However, the NIRS-derived [HHb] (30) and TOI (40) that were used to draw inferences on muscle oxygenation in this study are not altered by increased skin blood flow. Alternatively, since short-term L-citrulline malate supplementation has been shown to speed the rate of muscle phosphocreatine resynthesis following exercise (7, 18), a process coupled to the maximal rate of ATP derived from

669 mitochondrial oxidative phosphorylation (41), it is also possible that CIT speeded
670 overall $\dot{V}O_2$ kinetics through enhancing muscle oxidative metabolism independent of
671 enhanced muscle O_2 delivery. There was no improvement in NIRS-derived TOI or $\dot{V}$
672 O_2 dynamics during severe exercise in this study after ARG supplementation.
673
674 *Influence of ARG and CIT supplementation on exercise performance*
675 Severe exercise tolerance was increased by 12% and subjects completed 7% more
676 sprint work during an exercise performance test with CIT supplementation. While L-
677 citrulline malate supplementation has been shown to increase muscle force production
678 (7, 19), to improve muscle contractile efficiency (19) and to prevent the decline in
679 muscle force production with endotoxemia (18), studies investigating the effects of
680 CIT on exercise performance or muscle fatigue resistance are limited and equivocal.
681 Of the two studies investigating the influence of CIT supplementation on exercise
682 performance to date, one has reported improved exercise tolerance in mice performing
683 swimming exercise (56), while the other showed that CIT compromised incremental
684 exercise performance in humans (26). It is unclear why our findings contrast with
685 those of Hickner et al. (26). While Hickner et al. (26) designed an experiment to
686 assess the influence of acute ingestion of either 3 or 9 g of CIT on incremental
687 treadmill exercise performance, we investigated the effects of 7 days of 6 $g \cdot day^{-1}$
688 supplementation with CIT on cycling exercise tolerance and performance. These
689 conflicting findings might therefore be a consequence of different exercise
690 performance tests and dosing procedures, with 6-7 days of supplementation being
691 more effective than acute CIT ingestion. Moreover, an important difference between
692 these studies is the influence of CIT supplementation on NO biomarkers.
693 Specifically, we have shown that short term CIT tended (P=0.08) to increase plasma
694 [NO_2^-] and improved exercise performance, whereas Hickner et al. (26) reported a
695 surprising tendency for acute CIT to lower plasma [NOx] (plasma [nitrite] + [nitrate])
696 and to compromise exercise performance. Therefore, the extent to which CIT
697 influences exercise performance appears to be linked to the duration of the
698 supplementation period and its impact on NO bioavailability.
699
700 The improved exercise performance after CIT supplementation was accompanied by
701 faster overall $\dot{V}O_2$ kinetics although there was no significant correlation between the
702 two. An increase in muscle oxidative ATP turnover in concert with a lower pH/power

ratio after L-citrulline malate supplementation has also been reported previously (7). While we observed no change in blood [lactate] during and after severe-intensity exercise with CIT supplementation in this study, previous studies have reported lower end-exercise blood [lactate] and [ammonia] (56), as well as a lower rate of muscle phosphocreatine degradation (19) with CIT. Taken together, these findings suggest that CIT supplementation might increase the proportional energy contribution from oxidative metabolism thereby limiting the utilization of the finite anaerobic energy reserves and reducing the accumulation of metabolites linked to the process of muscle fatigue.

The findings presented in this study suggest that short-term supplementation with L-citrulline powder might lower blood pressure, speed $\dot{V}O_2$ kinetics and improve exercise tolerance/performance. An alternative, natural, method to increase dietary L-citrulline intake is via the consumption of watermelon (*Citrullus lanatus*), which contains ~2.33 g of L-citrulline/L of unpasteurized watermelon juice (58). Accordingly, subjects would be required to consume a daily watermelon dose of ~2.5 L in order to ingest the same dose of L-citrulline administered in this study. Further research is required to determine whether the effects reported following short term CIT supplementation in this study can be reproduced using watermelon juice supplementation.

In conclusion, this study has shown that short-term CIT supplementation can reduce blood pressure, speed $\dot{V}O_2$ kinetics and enhance endurance exercise performance. Supplementation with ARG, on the other hand, did not significantly impact on these parameters. Therefore, the results of this study suggest that chronic supplementation with CIT might represent a practical, dietary intervention to reduce blood pressure, and enhance oxidative metabolism and exercise performance in young, healthy adults.

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Figure Legends

Figure 1: The pulmonary $\dot{V}_{O_2}$ mean response time (MRT) during severe-intensity constant-work-rate cycle exercise after placebo (PLA), L-arginine (ARG) and L-citrulline (CIT) supplementation. The *upper panel* compares the $\dot{V}_{O_2}$ MRT following placebo (PLA) and L-arginine (ARG) supplementation. The *lower panel* compares the $\dot{V}_{O_2}$ MRT following following PLA and L-citrulline (CIT) supplementation. The *filled bars* represent the group mean ± SEM responses after ARG and CIT supplementation, while the *open bars* represent the group mean ± SEM responses after PLA supplementation. The solid grey lines represent the individual responses to the supplements. * indicates significantly different from PLA (P<0.05). Note the significant decrease in the $\dot{V}_{O_2}$ MRT after CIT but not ARG supplementation.

Figure 2: Group mean NIRS-derived muscle [deoxyhemoglobin] ([HHb]) during a moderate-intensity step cycle test following placebo (PLA), L-arginine (ARG) and L-citrulline (CIT) supplementation. Note the significant reduction in the [HHb] amplitude during moderate-intensity cycling exercise after CIT, but not ARG supplementation, compared to PLA.

Figure 3: Group mean NIRS-derived muscle tissue oxygenation index (TOI) during a severe-intensity step cycle test following placebo (PLA), L-arginine (ARG) and L-citrulline (CIT) supplementation. Note the significant increase in muscle oxygenation during severe-intensity cycling exercise after CIT, but not ARG supplementation, compared to PLA.

Figure 4: Group mean power profiles during a 60 s all-out cycle sprint commenced immediately after 6 min of severe-intensity cycle exercise following placebo (PLA), L-arginine (ARG) and L-citrulline (CIT) supplementation. Note the significant increase in peak and mean power output during the 60 s all-out sprint after CIT, but not ARG supplementation, compared to PLA.

Figure 5: Time-to-exhaustion during severe-intensity constant-work-rate cycle exercise after placebo (PLA), L-arginine (ARG) and L-citrulline (CIT) supplementation. The *upper panel* compares time-to-exhaustion following placebo

(PLA) and L-arginine (ARG) supplementation. The *lower panel* compares time-to-exhaustion following PLA and L-citrulline (CIT) supplementation. The *filled bars* represent the group mean ± SEM responses after ARG and CIT supplementation, while the *open bars* represent the group mean ± SEM responses after PLA supplementation. The solid grey lines represent the individual responses to the supplements. * indicates significantly different from PLA ($P<0.05$). Note the significant increase in exercise tolerance after CIT but not ARG supplementation.

Table 1. Resting plasma [nitrite] and blood pressure measures following placebo (PLA), L-arginine (ARG) and L-citrulline (CIT) supplementation.

	PLA	ARG	CIT
Plasma [nitrite] (nM)	83 ± 25	106 ± 41*	100 ± 38
Plasma [L-arginine] (μM)	57 ± 14	151 ± 31*	135 ± 22*
Plasma [L-citrulline] (μM)	23 ± 5	26 ± 6	665 ± 205*#
Plasma [L-ornithine] (μM)	26 ± 8	62 ± 14*	50 ± 6*#

Values are presented as the mean ± SD. * = significantly different from placebo ($P<0.05$); # = significantly different from ARG ($P<0.05$).

Table 2. Resting blood pressure measures following placebo (PLA), L-arginine (ARG) and L-citrulline (CIT) supplementation.

	PLA	ARG	CIT
Systolic blood pressure (mmHg)	122 ± 7	121 ± 5	118 ± 6*
Diastolic blood pressure (mmHg)	65 ± 6	63 ± 4	64 ± 5
Mean arterial pressure (mmHg)	87 ± 3	86 ± 2	85 ± 2*

Values are presented as the mean ± SD. * = significantly different from placebo ($P<0.05$).

Table 3. Pulmonary gas exchange measures during moderate- and severe-intensity cycle exercise after placebo (PLA), L-arginine (ARG) and L-citrulline (CIT) supplementation.

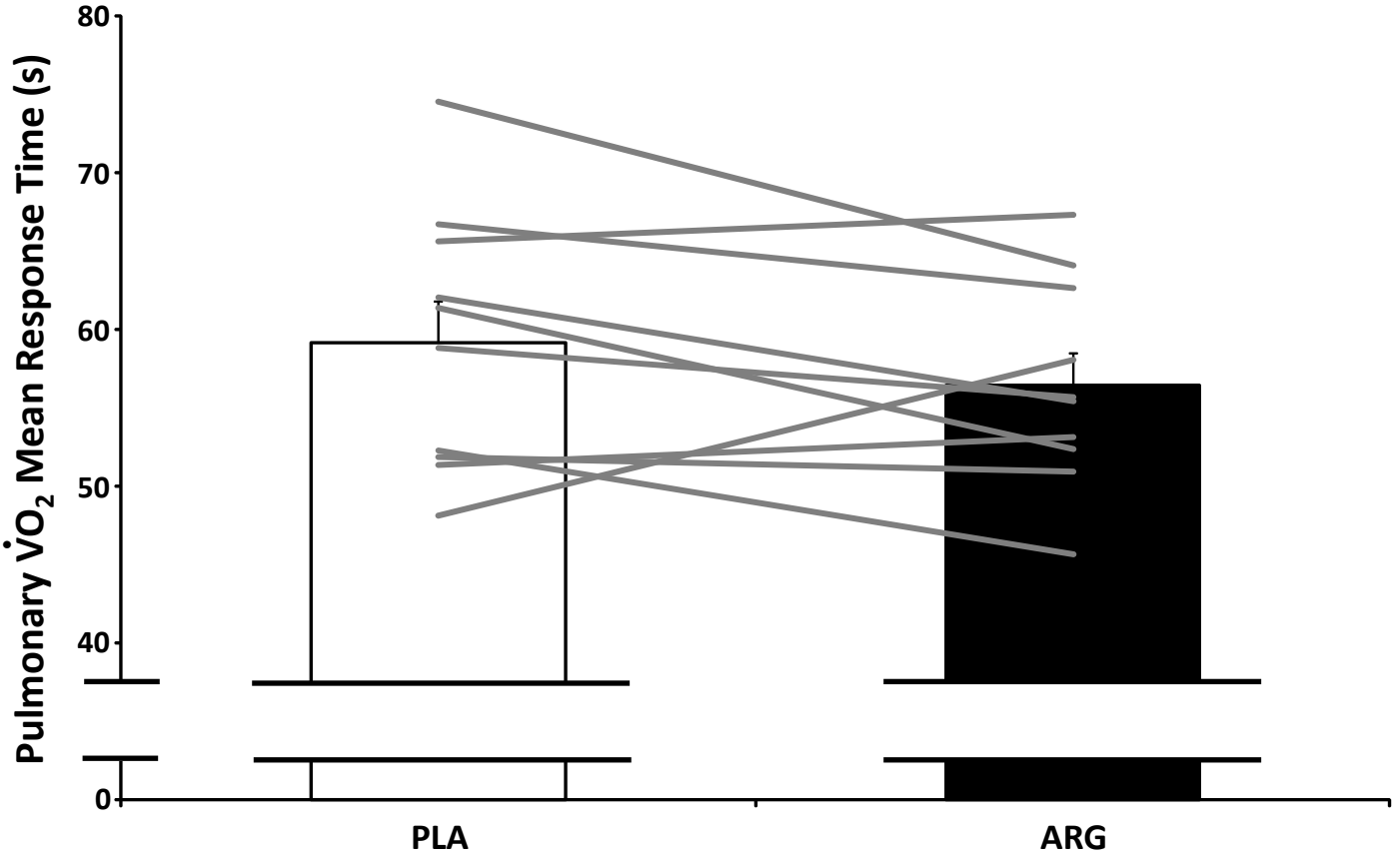
	PLA	ARG	CIT
Moderate-intensity exercise			
Oxygen Uptake ($\dot{V}_{O_2}$)			
Baseline ($L \cdot min^{-1}$)	1.07 ± 0.83	1.09 ± 0.11	1.09 ± 0.13
End-exercise ($L \cdot min^{-1}$)	1.94 ± 0.29	1.96 ± 0.28	1.93 ± 0.30
Phase II time constant (s)	24 ± 7	22 ± 7	21 ± 6
Mean response time (s)	37 ± 7	38 ± 7	36 ± 6
Fundamental amplitude ($L \cdot min^{-1}$)	0.87 ± 0.21	0.87 ± 0.22	0.86 ± 0.23
Fundamental gain ($ml \cdot min^{-1} \cdot W^{-1}$)	8.7 ± 0.5	8.7 ± 0.6	8.7 ± 0.7
Oxygen deficit (L)	0.54 ± 0.16	0.55 ± 0.16	0.51 ± 0.15
Severe-intensity exercise			
Oxygen Uptake ($\dot{V}_{O_2}$)			
Baseline ($L \cdot min^{-1}$)	1.12 ± 0.10	1.13 ± 0.13	1.13 ± 0.11
360 s ($L \cdot min^{-1}$)	3.94 ± 0.49	3.95 ± 0.49	3.94 ± 0.51
Exhaustion ($L \cdot min^{-1}$)	4.12 ± 0.50	4.09 ± 0.49	4.13 ± 0.56
Phase II time Constant (s)	26 ± 7	26 ± 6	25 ± 6
Fundamental amplitude ($L \cdot min^{-1}$)	2.23 ± 0.34	2.26 ± 0.42	2.29 ± 0.45
Slow component amplitude ($L \cdot min^{-1}$)	0.66 ± 0.09	0.60 ± 0.12	0.58 ± 0.13
Slow component amplitude (%)	23 ± 2	21 ± 4	20 ± 5
Overall mean response time (s)	60 ± 8	56 ± 7	54 ± 5*

Values are presented as the mean ± SD. * = significantly different from placebo ($P<0.05$).

Table 4. Near-infrared spectroscopy measures during moderate- and severe-intensity cycle exercise after placebo (PLA), L-arginine (ARG) and L-citrulline (CIT) supplementation.

	PLA	ARG	CIT
Moderate-intensity exercise			
Muscle [HHb]			
Baseline [HHb] (A.U)	-3 ± 5	-2 ± 4	-5 ± 4
120 s (A.U)	4 ± 7	5 ± 7	1 ± 5
End-exercise [HHb] (A.U)	4 ± 8	7 ± 6	1 ± 6
[HHb] τ (s)	11 ± 2	10 ± 3	9 ± 5
[HHb] τ + TD (s)	19 ± 3	18 ± 3	19 ± 4
[HHb] amplitude (A.U)	8 ± 4	9 ± 7	6 ± 4*
Muscle [HbO_2]			
Baseline [HbO_2] (A.U)	5 ± 2	4 ± 3	4 ± 4
120 s (A.U)	3 ± 2	0 ± 3	2 ± 3
End-exercise [HbO_2] (A.U)	5 ± 2	3 ± 4	4 ± 4
Tissue oxygenation index			
Baseline TOI (%)	67 ± 2	67 ± 2	69 ± 7
120 s (%)	60 ± 5	59 ± 7	63 ± 10
End-exercise TOI (%)	61 ± 6	59 ± 7	64 ± 11
Severe-intensity exercise			
Muscle [HHb]			
Baseline [HHb] (A.U)	-6 ± 5	-3 ± 4	-7 ± 4
120 s (A.U)	15 ± 13	16 ± 11	10 ± 8
End-exercise [HHb] (A.U)	16 ± 12	18 ± 11	12 ± 9
[HHb] primary τ (s)	8 ± 2	9 ± 2	9 ± 2
[HHb] primary τ + TD (s)	10 ± 2	11 ± 2	11 ± 2
[HHb] primary amplitude (A.U)	19 ± 10	19 ± 12	16 ± 9
[HHb] slow-phase amplitude (A.U)	3 ± 1	3 ± 2	3 ± 2
Muscle [HbO_2]			
Baseline [HbO_2] (A.U)	12 ± 3	10 ± 5	10 ± 5
120 s (A.U)	-6 ± 8	-8 ± 8	-5 ± 4
End-exercise [HbO_2] (A.U)	-10 ± 9	-11 ± 9	-8 ± 5
Tissue oxygenation index			
Baseline TOI (%)	72 ± 3	71 ± 3	75 ± 8
120 s (%)	47 ± 12	46 ± 12	52 ± 13*
End-exercise TOI (%)	43 ± 12	43 ± 12	49 ± 13*

Values are presented as the mean ± SD. * = significantly different from placebo (P<0.05).


80
70
60
50
40
0
Pulmonary $\dot{V}O_2$ Mean Response Time (s)
PLA
ARG

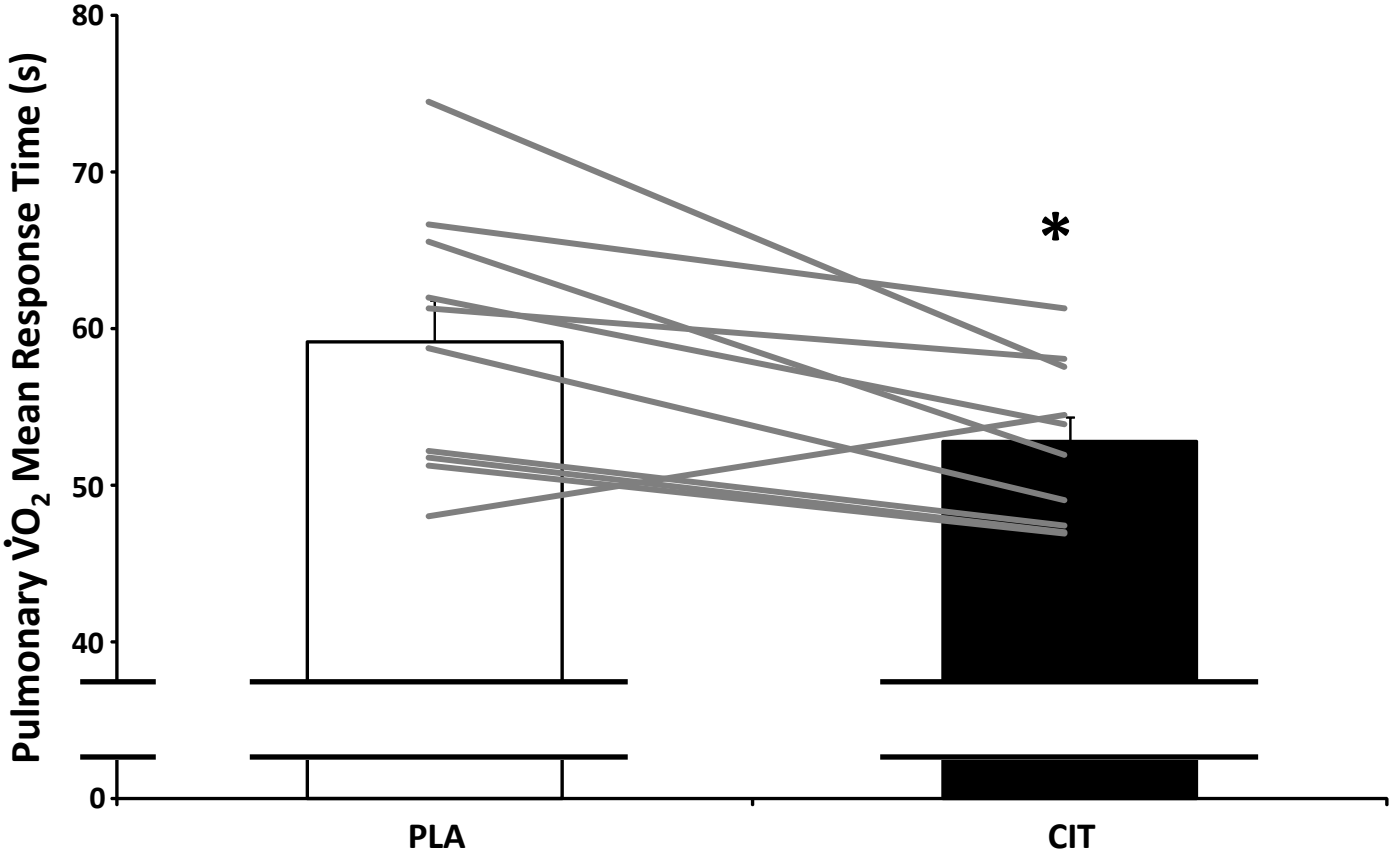

80
70
60
50
40
0
Pulmonary $\dot{V}O_2$ Mean Response Time (s)
*
PLA
CIT

Fig 1.

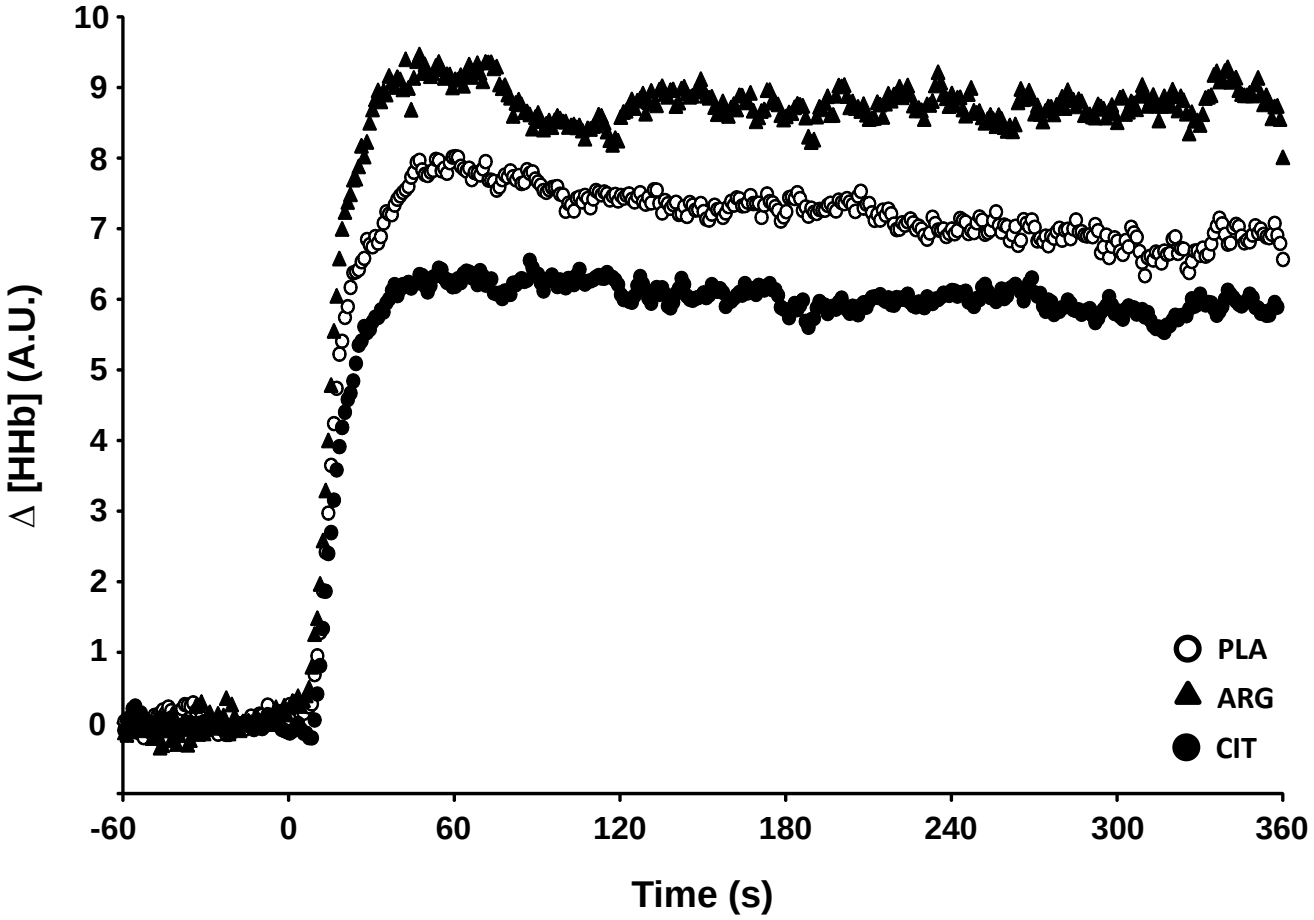

10
9
8
7
6
5
4
3
2
1
0
Δ [HHb] (A.U.)
-60
0
60
120
180
240
300
360
Time (s)
PLA
ARG
CIT

Fig 2.

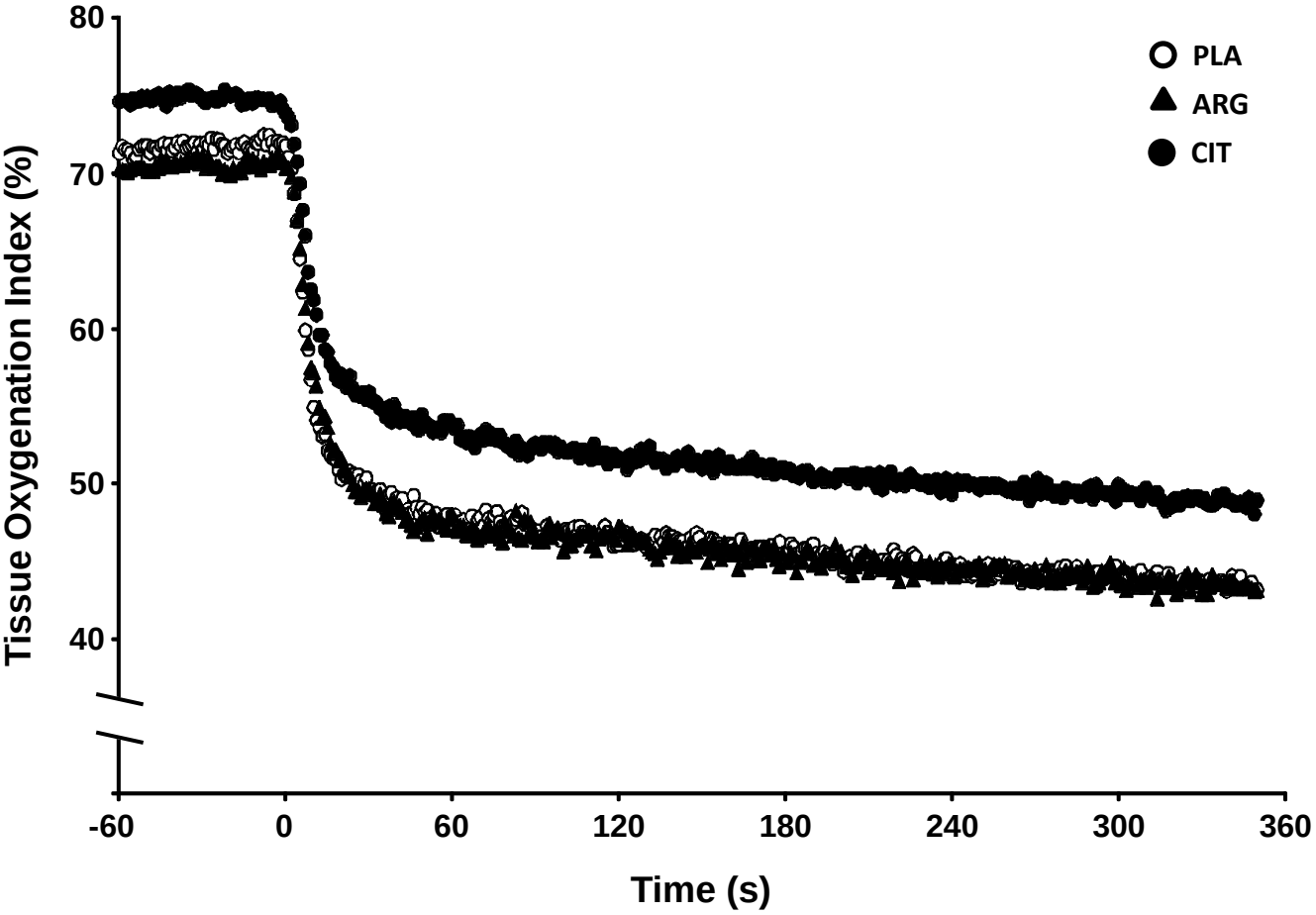

80
70
60
50
40
Tissue Oxygenation Index (%)
PLA
ARG
CIT
-60
0
60
120
180
240
300
360
Time (s)

Fig 3.

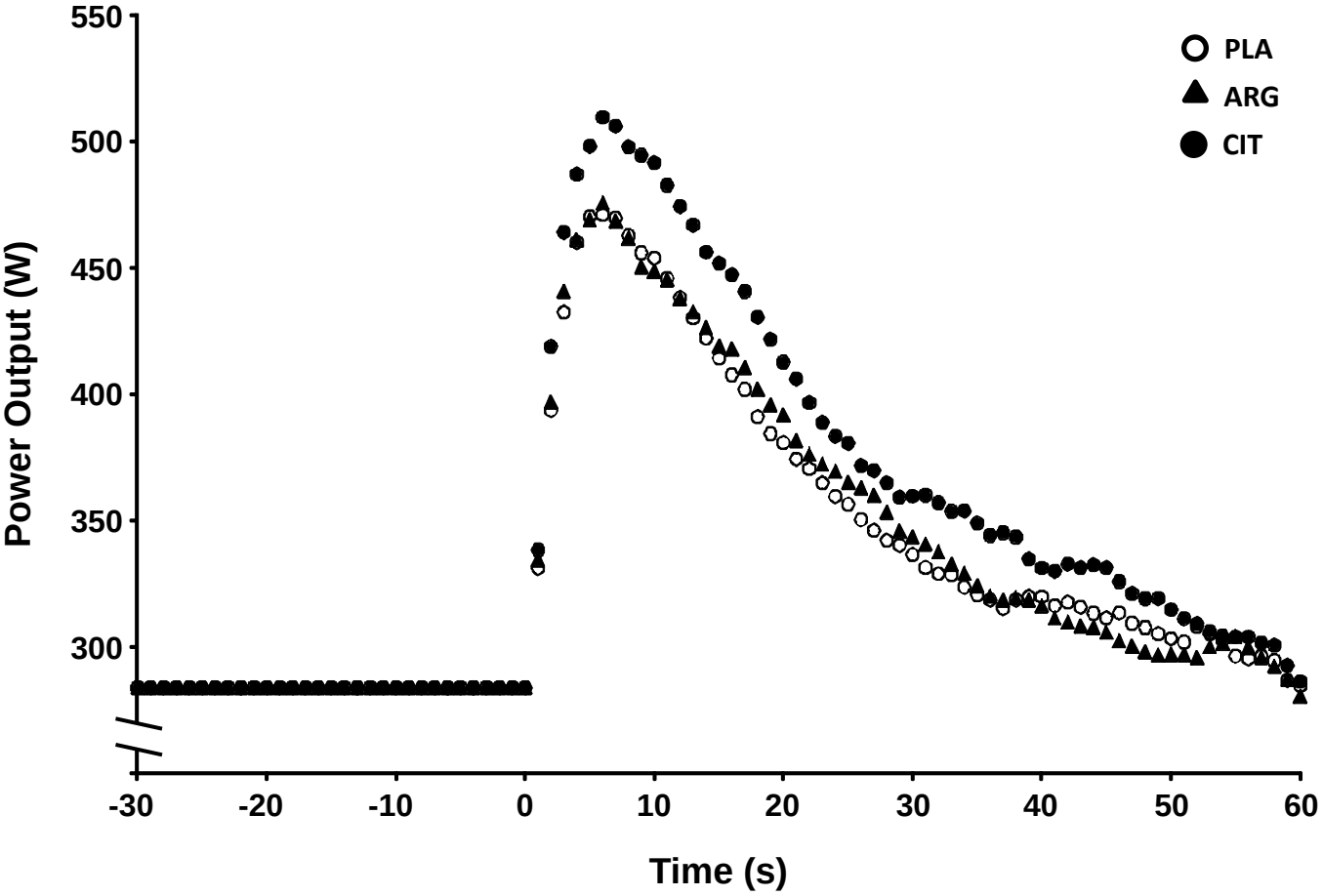
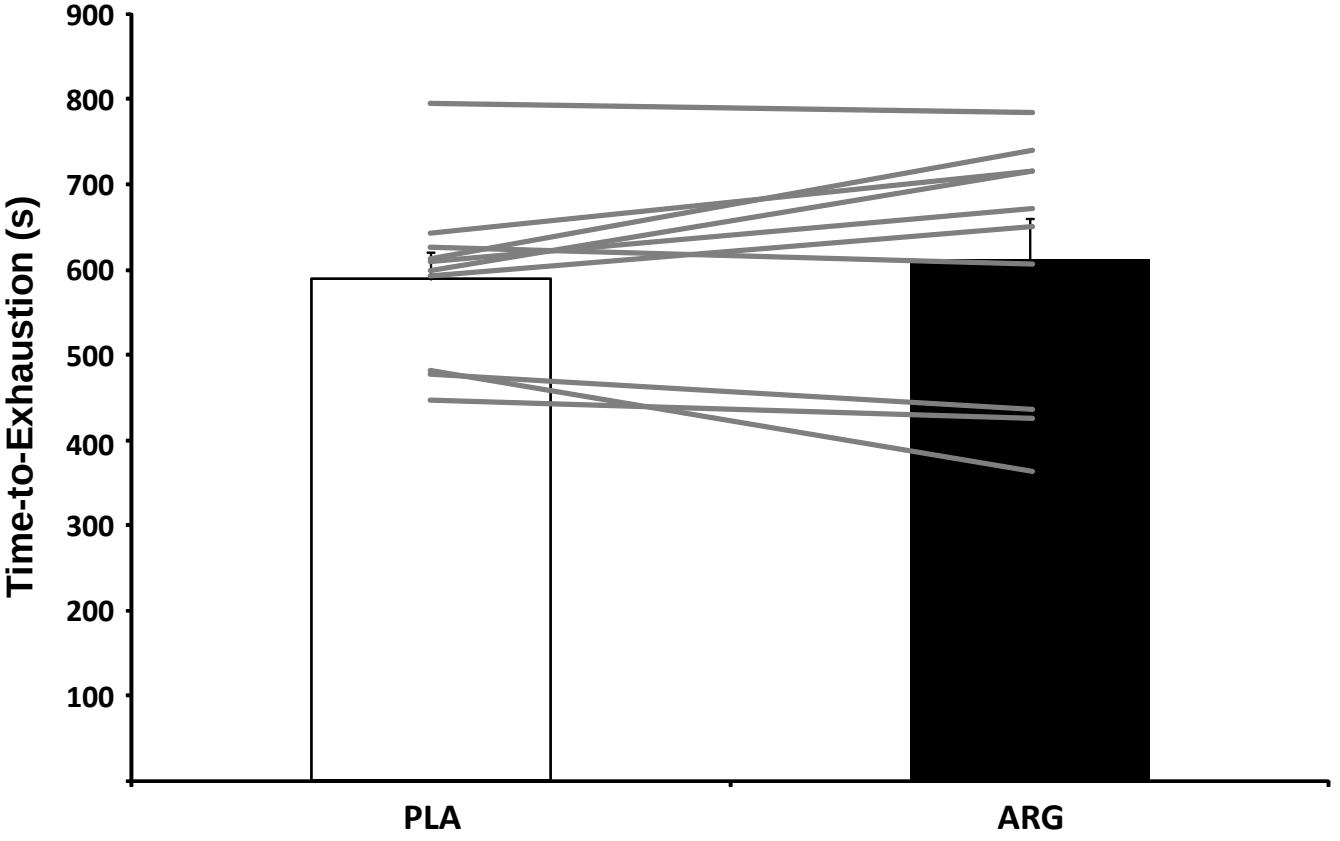

550
500
450
400
350
300
Power Output (W)
PLA
ARG
CIT
-30
-20
-10
0
10
20
30
40
50
60
Time (s)

Fig 4.


900
800
700
600
500
400
300
200
100
Time-to-Exhaustion (s)
PLA
ARG

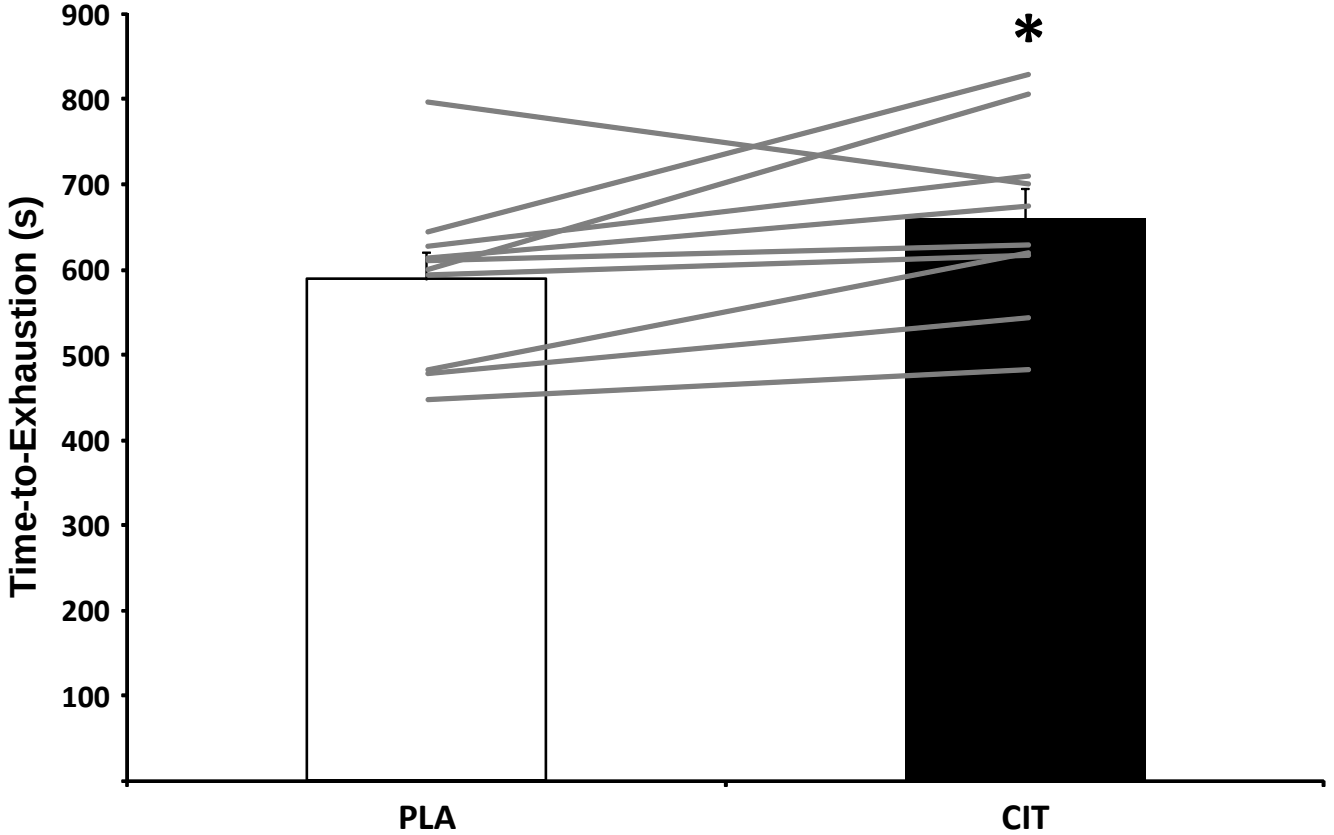

900
800
700
600
500
400
300
200
100
Time-to-Exhaustion (s)
*
PLA
CIT

Fig 5.