Inorganic nitrate supplementation improves muscle oxygenation, O_2 uptake kinetics and exercise tolerance at high but not low pedal rates

Stephen J. Bailey[1], Richard L. Varnham[2], Fred J. DiMenna[3], Brynmor C. Breese[4], Lee J. Wylie[1], and Andrew M. Jones[1]

[1] Sport and Health Sciences, College of Life and Environmental Sciences, St. Luke's Campus, University of Exeter, Heavitree Road, Exeter, UK; [2] Sport and Health Sciences, Faculty of Health and Life Science, Oxford Brookes University, Gipsy Lane, Headington, Oxford, UK; [3] Teachers College, Department of Biobehavioral Sciences, Columbia University, New York, USA; [4] School of Biological and Biomedical Sciences, Portland Square Building, Plymouth University, Drake Circus, Plymouth, UK.

Address for Correspondence:
Stephen J Bailey PhD
College of Life and Environmental Sciences
University of Exeter, St. Luke's Campus
Exeter, Devon, EX1 2LU, UK.
Tel: 01392 722882
Fax: 01392 264726
E-mail: S.J.Bailey@exeter.ac.uk

Running Head: Dietary nitrate and pedal cadence

Abstract

The purpose of this study was to test the hypothesis that inorganic nitrate (NO_3^-) supplementation would improve muscle oxygenation, pulmonary O_2 uptake ($\dot{V}O_2$) kinetics and exercise tolerance (Tlim) to a greater extent when cycling at high compared low pedal rates. In a randomised, placebo-controlled, cross-over study, seven subjects (mean ± SD, age 21 ± 2 yr, body mass 86 ± 10 kg) completed severe-intensity step cycle tests at pedal cadences of 35 rpm and 115 rpm during separate 9 day supplementation periods with NO_3^--rich beetroot juice (BR; providing 8.4 mmol NO_3^-·day^{-1}) and placebo (PLA). Compared to PLA, plasma nitrite concentration increased 178% with BR (P<0.01). There were no significant differences in muscle oxyhemoglobin concentration ([O_2Hb]), phase II $\dot{V}O_2$ kinetics or Tlim between BR and PLA when cycling at 35 rpm (P>0.05). However, when cycling at 115 rpm, muscle [O_2Hb] was higher at baseline and throughout exercise, phase II $\dot{V}O_2$ kinetics was faster (47 ± 16 s vs. 61 ± 25 s; P<0.05) and Tlim was greater (362 ± 137 s vs. 297 ± 79 s; P<0.05) with BR compared to PLA. In conclusion, these results suggest that short-term BR supplementation can increase muscle oxygenation, expedite the adjustment of oxidative metabolism and enhance exercise tolerance when cycling at a high, but not a low, pedal cadence in healthy recreationally-active subjects. These findings support recent observations that NO_3^- supplementation may be particularly effective at improving physiological and functional responses in type II muscle fibers.

Key Words: nitric oxide; vascular function; oxidative metabolism; exercise performance; fatigue

Introduction

Nitric oxide (NO) is a diffusible gas that positively impacts a plethora of physiological responses including skeletal muscle perfusion, metabolism, force production and fatigue resistance (53). It is well documented that NO is produced by the nitric oxide synthase enzymes, which catalyze the complex five-electron oxidation of the semi-essential amino acid, L-arginine (10). More recently, there has been a growing appreciation of the potential for NO synthesis from the simple one-electron reduction of nitrite (NO_2^-), in a reaction catalyzed by numerous NO_2^- reductases (42, 54). Importantly, increasing the intake of dietary inorganic nitrate (NO_3^-), which passes into the enterosalivary circulation for subsequent reduction to NO_2^- by oral anaerobes (20), has been shown to positively impact NO biomarkers, exercise efficiency and exercise tolerance in recreationally active subjects (3-4, 12, 40-41, 55, 58). Therefore, supplementation with NO_3^- appears to represent an effective dietary intervention to improve NO bioavailability, contractile efficiency, and fatigue resistance.

Results from *in vitro* studies suggest that the effect of NO donors on mammalian skeletal muscle contractility is influenced by contraction frequency (5, 21, 43, 46). Hernandez et al. (33) harvested whole EDL muscles and single FDB muscle fibers from NO_3^- supplemented mice and reported increased *in vitro* contractile force up to 50 Hz stimulation and a more rapid rate of force development in the single FDB muscle fibers at 100 Hz stimulation. Similarly, increased evoked contractile force has been observed in human skeletal muscle *in vivo* during low-frequency sub-maximal stimulation and over the initial stages of high-frequency maximal stimulation following NO_3^- supplementation (29). However, NO_3^- ingestion has been shown to increase human peak knee extensor torque at $360°\cdot s^{-1}$, but not $90°\cdot s^{-1}$, $180°\cdot s^{-1}$ and $270°\cdot s^{-1}$, *in vivo* (16). Collectively, these findings suggest that dietary NO_3^- supplementation enhances skeletal muscle contractile function, and that it might be particularly effective at augmenting contractility at higher contraction velocities. However, heretofore, human *in vivo* studies reporting improvements in exercise tolerance following NO_3^- supplementation have utilized cycle ergometer exercise with pedal cadences of 70-90 rpm (i.e., at "mid-range" contraction frequency/velocity; e.g., see 4, 12, 55, 58). Consequently, it is unclear whether NO_3^- supplementation may be more effective at improving skeletal muscle fatigue resistance at a higher contraction frequency/velocity.

Relative to a lower contraction frequency (60 rpm), a higher contraction frequency (100 rpm) has been shown to increase $\dot{V}_{O_2}$ and to reduce contractile efficiency in human skeletal muscle *in vivo* (22). Similarly, when contraction duration is shortened, $\dot{V}_{O_2}$ (30, 34) and the ATP cost of force production (34) are increased in mammalian skeletal muscle *in situ* relative to contractions of longer duration when the same work-rest ratio is applied. There is also evidence to suggest that pulmonary $\dot{V}_{O_2}$ kinetics is slower (11, 19) and the $\dot{V}_{O_2}$ slow component is increased (11, 49) when cycling at very high compared to very low pedal cadences. Dietary NO_3^- supplementation has been shown to increase muscle blood flow (23), lower the O_2 (3-4, 40-41, 55, 58) and ATP (3) requirements of muscle contraction and improve the matching between muscle O_2 supply and muscle O_2 utilization (4, 24). Therefore, this potential for enhanced contractile efficiency and perfusion distribution with NO_3^- supplementation might improve muscle oxygenation, $\dot{V}_{O_2}$ kinetics and exercise tolerance, particularly when more rapid muscle contractions are completed.

In addition to compromising muscle contractile efficiency, it has been suggested that the proportional contribution of fast-twitch muscle fibers to force production is greater at higher pedal cadences (6-7, but see 1). Importantly, dietary NO_3^- supplementation has been shown, at least in rodent models, to: 1) increase bulk muscle blood flow and preferentially distribute this towards fast-twitch muscle fibers (23); and 2) increase calcium handling proteins and twitch and tetanic force production in fast-twitch muscle, but not in slow-twitch muscle (33). In support of a targeted effect of NO_3^- supplementation on fast-twitch muscle, Breese et al. (12) have recently reported that NO_3^- supplementation speeds oxygen uptake ($\dot{V}_{O_2}$) and muscle deoxyhemoglobin (HHb; reflective of the balance between muscle O_2 utilization and muscle O_2 delivery [25, 36]) kinetics during a severe-intensity step exercise test initiated from a moderate-intensity baseline. Conversely, there was no effect when a moderate-intensity step test was initiated from a low-intensity baseline. This is important because, compared to the latter, the former would be expected to involve a greater recruitment of fast-twitch fibers (37-38). Taken together, these findings suggest that dietary NO_3^- supplementation may preferentially augment physiological responses within, and in the miscrovasculature surrounding, fast-twitch muscle fibers. Based on these observations, the effects of NO_3^- might be more pronounced when humans cycle at a very high compared to a very low pedal cadence; however, this has yet to be investigated.

The purpose of this study was to evaluate the effects of short term dietary NO_3^- supplementation on muscle oxygenation, pulmonary $\dot{V}_{O_2}$ kinetics and exercise tolerance when cycling at a very high (115 rpm) and a very low (35 rpm) pedal cadence at the same relative exercise intensity. Given the effects of cadence on the physiological responses evoked during cycling and the physiological benefits afforded by NO_3^- supplementation (see above), we hypothesized that muscle oxygenation, pulmonary $\dot{V}_{O_2}$ kinetics and exercise tolerance would be improved to a greater extent with NO_3^- when cycling at 115 rpm than at 35 rpm.

Methods

Subjects

Seven healthy male subjects (mean ± SD age, 21 ± 2 yrs; body mass, 86 ± 10 kg; height, 1.82 ± 0.08 m) volunteered to participate in this study. None of the subjects were tobacco smokers or users of dietary supplements. The subjects participated in exercise at a recreational level, but were not highly trained. All subjects were familiar with laboratory exercise testing procedures, having previously participated in studies employing cycle ergometry in our laboratory. The procedures employed in this study were approved by the Institutional Research Ethics Committee and all subjects were required to give 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. All tests were performed at the same time of day (± 2 h). Each subject was asked to refrain from caffeine and alcohol intake for 6 h and 24 h before each test, respectively. Subjects were also provided with a list of foods rich in NO_3^- and instructed to avoid the consumption of these foods and to abstain from the use of antibacterial mouthwash, which eliminates the oral bacteria that reduce NO_3^- to NO_2^- (26), for the duration of the study.

Experimental Design

Subjects were required to report to the laboratory on 10 occasions over a 5-7 week timeframe. Prior to the experimental testing, subjects completed cadence-specific ramp incremental tests at 35 and 115 rpm in a randomised order so that the same relative exercise intensity could be

prescribed at both pedal cadences during the experimental tests. Over the remaining eight laboratory visits, subjects completed severe-intensity step exercise tests at 35 and 115 rpm during separate 9-day supplementation periods with BR (35-BR and 115-BR, respectively) and PLA (35-PLA and 115-PLA, respectively) for determination of heart rate (HR), stroke volume (SV), cardiac output ($\dot{Q}$), muscle oxygenation, $\dot{V}_{O_2}$ kinetics and exercise tolerance. The two supplementation periods were administered as part of a randomised (3 subjects started on BR), cross-over, double-blinded experimental design.

Incremental Tests

Before the intervention period, subjects completed a ramp incremental test at 35 and 115 rpm on an electronically-braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands) in a randomized order. At least 48 hours separated the two ramp incremental tests. Initially, subjects performed 3 min of baseline cycling at 20 W, after which the work rate was increased by 30 W/min until the limit of tolerance. The saddle and handle bar height and configuration during the first incremental test was recorded and reproduced in subsequent tests. During the baseline period and the incremental test, subjects cycled at a pre-determined pedal rate (either 35 or 115 rpm) until volitional exhaustion or the cadence fell by > 10 rpm for 3 consecutive seconds. 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 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 data collected during the incremental tests were used to calculate cadence-specific work rates which were employed during the subsequent severe-intensity step tests. Specifically, the work rates that would require 80% of the difference between the $\dot{V}_{O_2}$ at the GET and $\dot{V}_{O_{2max}}$ (80%Δ) were estimated with account taken of the mean response time of the $\dot{V}_{O_2}$ response to ramp exercise (57).

Step Exercise Tests

Subjects completed one severe-intensity step test on days 4, 5, 8 and 9 of each dietary condition (PLA and BR). The same pedal cadence (either 35 or 115 rpm) was applied on

days 4 and 5 of the supplementation period with the other cadence applied on days 8 and 9 of the supplementation period. The order in which the 35 and 115 rpm tests were administered over the first supplementation period was randomised (3 subjects started on 35 rpm) and this order was replicated over the second supplementation period. The step tests comprised 4 minutes of baseline cycling at 20 W, followed by a step increment to a severe-intensity (80%Δ) constant work rate that was continued until exhaustion (same criteria as described above for the incremental exercise test). For each condition, the mean time to exhaustion was used for subsequent analysis.

Supplementation Procedures

Following the initial ramp tests, subjects underwent two 9-day supplementation periods with BR and PLA, with each period separated by at least ten-days of washout. Subjects recorded an 11-day food diary, which commenced two days prior to the first 9-day supplementation period, and were asked to use the diary to replicate and record their diet in the second supplementation period. The BR (Beet It Sport, James White Drinks, Ipswich, UK) was administered in 70 ml doses providing 6.2 mmol NO_3^- per serving. Sodium chloride (NaCl) was administered as the PLA in doses of 0.1 $mmol \cdot kg^{-1}$ body mass (41). Subjects were informed that the PLA was $NaNO_3$, and that the purpose of the study was to compare the effects of 'NaNO3' relative to NO_3^--rich BR. All labels and packaging were removed from the BR supplements and the PLA was provided in transparent plastic capsules which were placed in a small transparent plastic bag labelled sodium nitrate. Subjects were instructed to open the PLA capsules and mix the content with 200-300 ml of water for consumption. On days 1-3 and 6-7 of the supplementation periods (when no exercise tests were conducted), one dose of supplement was consumed in the morning and evening. On the days of the experimental testing (days 4-5 and 8-9), subjects were instructed to consume both supplement doses 2.5 hours before arriving at the laboratory (58). An additional dose of supplement was consumed 2 hours following each experimental exercise test to counteract the marked depletion in plasma [NO_2^-] that occurs during intense exhaustive exercise (59).

Measurements

After reporting to the laboratory on days 4 and 8 of each dietary intervention (the first day that each cadence-specific step test was performed), a venous blood sample was 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 ozone-based chemiluminescence (58).

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.

HR, SV and $\dot{Q}$ data were recorded beat-by-beat using thoracic impedance cardiography (PhysioFlow PF-05; Manatec Biomedical, Paris, France) on days 4 and 8 of each dietary intervention. The PhysioFlow measures impedance to a high-frequency (75 kHz), low-magnitude (3.8 mA) alternating electrical current applied across the thorax and subsequently estimates SV based on changes in impedance over the cardiac cycle. The current was transmitted through two electrodes placed superior to the supraclavicular fossa on the left lateral triangle of the neck and detected through two electrodes placed on the xiphoid process. To acquire an electrocardiogram (ECG) signal, two additional electrodes were placed at the V1 and V6 positions. Body surface area was estimated using the Haycock formula (32) which, along with estimated SV and ECG-derived HR, was used to calculate $\dot{Q}$. The PhysioFlow method and calculations have been described previously (15) and, when compared to the direct Fick method, the PhysioFlow has been shown to provide a valid estimate of $\dot{Q}$ during exhaustive exercise (50). All of the relevant skin sites were treated with an abrasive skin gel and alcohol prior to the application of the electrodes (Blue Sensor R; Ambu, Ballerup, Denmark), and leads were secured to the subjects using an adhesive tape. Following the application of the electrodes, subject rested for 10 min on the cycle ergometer. Four resting blood pressure measurements were taken using an automated sphygmomanometer (Dinamap Pro, GE Medical Systems, Tampa, FL) with the arm

supported at the level of the heart. The mean of the final 3 measurements was recorded. The PhysioFlow device was auto-calibrated prior to exercise using the manufacture's software that gathered impedance waveforms throughout 30 cardiac cycles. Following the test, data were downloaded onto a personal computer for subsequent analysis.

The oxygenation status of the *m. vastus lateralis* of the right leg was monitored using a commercially available NIRS system (model NIRO 300, Hamamatsu Photonics KK, Hiugashi-ku, Japan) on days 4 and 8 of each dietary intervention. 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 (25, 27, 36). It should be noted here that the contribution of deoxygenated myoglobin to the NIRS signal is presently unclear, and, as such, the terms [O_2Hb], and [HHb] used in this paper should be considered to refer to the combined concentrations of oxygenated and deoxygenated hemoglobin and myoglobin, respectively.

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 for later analysis.

Fingertip capillary blood samples (~20μl) were collected during the final 30 seconds of baseline cycling and at exhaustion. Blood [lactate] was determined using an automated analyser (YSI 2300, Yellow Springs Instruments, Yellow Springs, OH, USA). The mean of the two baseline and exhaustion blood [lactate] values were calculated for each experimental condition prior to analysis. Blood [lactate] accumulation during exercise was taken as the change in the values from baseline to exhaustion.

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, the two identical repetitions for each experimental condition 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 until the point of exhaustion. A bi-exponential model was used to characterize the $\dot{V}_{O_2}$ responses to severe exercise, as described in the following equation:

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

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; 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 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 exhaustion was defined as A_s'.

The beat-by-beat HR, SV and $\dot{Q}$ data from each test were averaged into 5-s bins prior to analysis. To determine the overall kinetics (the mean response time [MRT]) of the central cardiovascular responses, the data were fit with a mono-exponential model from 0-s to exhaustion without time delay using the following equation:

$$Y(t) = Y_{baseline} + A(1-e^{-(t-/MRT)}) \quad \text{(Eqn. 3)}$$

where Y (*t*) represents the absolute value of the parameter of interest at a given time *t*; $Y_{baseline}$ represents the mean value of the parameter of interest in the baseline period; A and MRT represent the amplitude and mean response time, respectively, describing the increase in Y above baseline.

To provide information on muscle oxygenation, we also modelled the [HHb] response to exercise. A mono-exponential model was applied to the data with the fitting window commencing at the time at which the [HHb] signal increased 1 SD above the baseline mean. The [HHb] kinetics were determined by constraining the fitting window to the point at which mono-exponentiality became distorted, consequent to a [HHb] slow component, as determined by visual inspection of the residual plots. 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 [O_2Hb] response does not approximate an exponential and was, therefore, not modelled. Rather, we determined the [O_2Hb] at baseline (90-s preceding step transition), 120 s (30 s mean surrounding 120 s) and exhaustion (mean response over the final 30 s of exercise). The muscle [HHb] and [O_2Hb] responses were summed at these time points ([Hb_{tot}]) to provide information on the total [Hb] in the NIRS area of interrogation.

Statistics

A two-way, treatment (PLA and BR) × cadence (35 and 115 rpm), repeated-measures ANOVA was employed to assess differences in plasma [NO_2^-], $\dot{V}_{O_2}$ kinetics, HR, SV, $\dot{Q}$, muscle [HHb], muscle [O_2Hb], muscle [Hb_{tot}] and exercise tolerance across the experimental conditions. Significant effects were further explored using post-hoc *t*-tests with the alpha level adjusted via a Fisher's LSD correction. Relationships between the outcome variables

were assessed using Pearson's correlation coefficient (*r*). Data are presented as mean ± SD, unless otherwise stated. Statistical significance was accepted when $P<0.05$.

Results

The PLA and BR 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. After all experimental testing for the study was completed, all subjects confirmed that they were unaware that the PLA condition was not $NaNO_3$.

The $\dot{V}_{O2peak}$ and peak work rate attained in the ramp incremental tests at 35 rpm and 115 rpm were, respectively, 3.84 ± 0.57 L·min^{-1} and 4.11 ± 0.56 L·min^{-1}, and 304 ± 43 W and 319 ± 52 W. The $\dot{V}_{O2}$ and work rate at the GET during the incremental tests were 1.62 ± 0.31 L·min^{-1} and 122 ± 18 W at 35 rpm, and 2.53 ± 0.33 L·min^{-1} and 122 ± 8 W at 115 rpm, respectively. There were no significant differences in $\dot{V}_{O2peak}$ or peak work rate attained during the ramp tests at 35 rpm and 115 rpm. However, the $\dot{V}_{O2}$ at the GET was significantly greater during the 115 rpm incremental test than the 35 rpm incremental test ($P<0.001$). The work rates which corresponded to 80% Δ, which were imposed during the experimental tests conducted at 35 rpm and 115 rpm, were 244 ± 35 W and 258 ± 47 W, respectively. These work rates were not significantly different from each other ($P>0.05$).

Plasma [NO_2^-] and Blood Pressure

There was a significant main effect for supplement on plasma [NO_2^-] ($P<0.01$), systolic blood pressure (SBP; $P<0.05$) and mean arterial pressure (MAP; $P<0.05$), but not diastolic blood pressure (DBP; $P>0.05$). Plasma [NO_2^-] was significantly higher in both 35-BR and 115-BR compared to 35-PLA and 115-PLA ($P<0.01$), with plasma [NO_2^-] increased by 179% above PLA conditions with BR when all data were pooled ($P<0.01$; Figure 1). There were no differences in plasma [NO_2^-] between 35-PLA and 115-PLA, and 35-BR and 115-BR ($P>0.05$). Systolic blood pressure and mean arterial pressure were lowered by 8 mmHg and 4 mmHg across the BR conditions when all data were pooled ($P<0.05$).

394 $\dot{V}_{O_2}$ *Kinetics*

395 Pulmonary $\dot{V}_{O_2}$ at designated time points and the key parameters derived from the bi-
396 exponential modelling are presented in Table 1 and illustrated for a representative individual
397 in Figure 2. There was a significant main effect for cadence on $\dot{V}_{O_2}$ baseline and the $\dot{V}_{O_2}$ at
398 120 s and exhaustion (P<0.01) with $\dot{V}_{O_2}$ at these time points being higher in both 115-PLA
399 and 115-BR compared to both 35-PLA and 35-BR (P<0.05; Table 1). The $\dot{V}_{O_2}$ at exhaustion
400 was not significantly between 35-PLA and 35-BR and these values were not different to the
401 $\dot{V}_{O_{2peak}}$ attained in the incremental test at 35 rpm (P>0.05). Likewise, the $\dot{V}_{O_2}$ at exhaustion
402 in 115-PLA and 115-BR and the $\dot{V}_{O_{2peak}}$ attained in the incremental test at 115 rpm were not
403 significantly different from each other (P>0.05). However, the $\dot{V}_{O_2}$ at exhaustion was higher
404 in 115-PLA relative to 35-PLA and 115-BR relative to 35-BR (both P<0.01). There was a
405 significant main effect for cadence on the $\dot{V}_{O_2}$ phase II τ with a higher $\dot{V}_{O_2}$ phase II τ (slower
406 $\dot{V}_{O_2}$ kinetics) in 115-PLA (61 ± 25 s) and 115-BR (47 ± 16 s) compared to both 35-PLA (32
407 ± 10 s) and 35-BR (32 ± 6 s; P<0.05; Table 1). In addition there was a significant treatment
408 × cadence interaction effect on the $\dot{V}_{O_2}$ phase II τ (P<0.05). Post hoc analyses demonstrated
409 that there was no significant difference in the $\dot{V}_{O_2}$ phase II τ between 35-PLA and 35-BR
410 (P>0.05), but the $\dot{V}_{O_2}$ phase II τ was lower in 115-BR compared to 115-PLA (P<0.05; Table
411 1; Figure 2). There were no significant between-supplement differences in the $\dot{V}_{O_2}$
412 fundamental amplitude (P>0.05), but there were significant between-cadence differences in
413 the $\dot{V}_{O_2}$ fundamental amplitude (P<0.05; Table 1). The $\dot{V}_{O_2}$ slow component amplitude was
414 not significantly different between any of the experimental conditions (P<0.05; Table 1).
415
416 *Central Cardiovascular Parameters*

417 There were no significant between-supplement differences in HR, SV or $\dot{Q}$ at baseline, 120 s
418 or exhaustion (P>0.05 Table 2); however, there was a trend towards a main effect for cadence
419 on the HR (P=0.071) and $\dot{Q}$ (P=0.079) MRT. Indeed, the HR MRT tended to be lower in 35-
420 PLA and 35-BR compared to both 115-PLA and 115-BR (P=0.07-0.08), but the HR MRT
421 was not significantly different within cadences (P>0.05). The $\dot{Q}$ MRT was lower in 35-PLA
422 and 35-BR compared to 115-PLA (P<0.05) with no differences between the other
423 experimental conditions.
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Muscle Oxygenation Parameters

NIRS-derived [HHb], [O_2Hb] and [Hb_{tot}] responses are presented in Table 3 with group mean [HHb] and [O_2Hb] responses illustrated in Figures 3 and 4, respectively. There were no significant main effects or interaction effects on the [HHb] at baseline or throughout the step exercise test ($P>0.05$; Table 3). The ANOVA revealed a significant main effect of supplement on the [HHb] τ + TD ($P<0.05$) and a significant main effect of cadence on the [HHb] amplitude ($P<0.05$; Table 3; Figure 3). There were no significant differences in these variables across the individual experimental conditions ($P>0.05$). There were significant main effects of cadence and a significant treatment × cadence interaction effect on the [O_2Hb] at baseline, 120 s and at exhaustion ($P<0.05$). Further analyses revealed that the [O_2Hb] was greater in 115-BR at baseline compared to 115-PLA, at 120 s compared to all other conditions and at exhaustion compared to 35-PLA and 35-BR ($P<0.05$). There were no significant main or interaction effects on [Hb_{tot}].

Exercise Tolerance

The effects of BR on Tlim at 35 rpm and 115 rpm are illustrated in Figure 5. Supplementation with BR significantly increased Tlim when cycling at 115 rpm (115-PLA: 297 ± 79 s vs. 115-BR: 362 ± 137 s; $P<0.05$), but did not significantly alter Tlim at 35 rpm (35-PLA: 341 ± 99 s vs. 35-BR: 344 ± 74 s; $P>0.05$; Figure 5). There was a significant correlation between the change in Tlim and muscle [O_2Hb] at 120 s in 115-BR compared to 115-PLA ($r = 0.76$, $P<0.05$) and a trend for a correlation between the change in muscle [O_2Hb] at Tlim and the change in Tlim in 115-BR compared to 115-PLA ($r = 0.76$, $P=0.07$). There were no other correlations between the physiological and performance variables assessed in this study ($P>0.05$).

Discussion

The principal novel findings from this study are that short term dietary NO_3^- supplementation, which enhanced the potential for O_2-independent NO synthesis by elevating plasma [NO_2^-], increased muscle oxygenation, speeded phase II $\dot{V}_{O_2}$ kinetics and improved severe-intensity exercise tolerance (Tlim) when cycling at 115 rpm. In contrast, there were no changes in muscle oxygenation, $\dot{V}_{O_2}$ kinetics or exercise tolerance with BR at 35 rpm. There were no changes in $\dot{Q}$ with BR at either cadence, but muscle [O_2Hb] was higher in 115-BR compared to 115-PLA. These findings suggest that BR increased muscle O_2 delivery at 115 rpm

facilitating faster phase II $\dot{V}_{O_2}$ kinetics and improved severe-intensity exercise tolerance. This is consistent with our experimental hypothesis and suggests that BR supplementation is more effective at improving muscle O_2 delivery, phase II $\dot{V}_{O_2}$ kinetics and fatigue resistance at higher pedal cadences.

In line with other studies administering NO_3^--rich BR (3-4, 12, 40-41, 55, 58), plasma [NO_2^-] was increased in this study. Importantly, plasma [NO_2^-] was not significantly different between the 2 BR trials and between the 2 PLA trials. This observation is consistent with our previous finding that plasma [NO_2^-] remains consistently elevated above baseline with BR, at least up to 15 days of supplementation (55). An increase in the circulating plasma [NO_2^-] reflects an increase in the reserve for NOS-independent NO production given that NO_2^- undergoes a simple one-electron reduction to NO through numerous NO_2^- reductatses (42, 54). The reduction of NO_2^- to NO is upregulated as O_2 tension (14) and pH (45) decline and, since muscle P_{O_2} (51) and pH (3) are lowered during intense muscle contractions, NO_2^- reduction is likely to be an important source of NO during exercise. Aside from its reduction to NO, it should be acknowledged that NO_2^- itself can participate in post-translational protein modifications that positively impact on physiological processes (2). Moreover, a few days of NO_3^- supplementation can increase mitochondrial (40) and calcium-handling (33) proteins promoting enhanced metabolic and contractile function, respectively. Therefore, the short term exposure to inorganic NO_3^- and the associated increase in plasma [NO_2^-] in this study may have been sufficient to increase NO signalling and elicit favorable physiological and functional responses. Indeed, resting arterial blood pressure was lowered by BR supplementation in this study, in accord with established effects of elevated NO on vascular tone (28).

Pulmonary $\dot{V}_{O_2}$ was significantly higher at baseline and throughout exercise when cycling at 115 rpm compared to 35 rpm, as reported previously (19, 25, 60). While subjects were cycling at the same external work rate at baseline (20 W), and the same relative exercise intensity (80%Δ) during the step tests, internal work (56) and muscle ATP turnover rate and $\dot{V}_{O_2}$ (22) are increased at higher contraction frequencies, which accounts for the higher pulmonary $\dot{V}_{O_2}$ observed in the 115 rpm trials. The phase II $\dot{V}_{O_2}$ τ was higher ($\dot{V}_{O_2}$ kinetics was slower) when cycling at 115 rpm than 35 rpm, which corroborates findings of a slower adjustment of $\dot{V}_{O_2}$ when cycling at a higher pedal cadence (11, 19).

In concert with slower phase II $\dot{V}_{O_2}$ kinetics, there was a trend for slower $\dot{Q}$ kinetics in the 115 rpm trials, suggesting that the slower $\dot{V}_{O_2}$ kinetics might have been related to a slower adjustment of bulk blood flow and O_2 delivery to the contracting muscles in the higher cadence condition. However, there is strong evidence that bulk muscle blood flow is enhanced at higher contraction frequencies (22, 30, 47, 52). Despite slower phase II $\dot{V}_{O_2}$ kinetics at the higher pedal cadence, there were no differences in muscle microvascular [HHb] kinetics (τ + TD) between 115 rpm and 35 rpm. Since muscle [HHb] is considered a non-invasive proxy for muscle O_2 extraction (25, 36), this suggests that the slower phase II $\dot{V}_{O_2}$ kinetics at 115 rpm might be a function of slower microvascular blood flow kinetics and, therefore, to a relative shortfall in muscle O_2 delivery compared to muscle O_2 demand over the initial stages of the severe-intensity step test. The slower phase II $\dot{V}_{O_2}$ kinetics at the higher pedal cadence might also reflect increased fast-twitch fiber recruitment (7), because fast-twitch fibers are believed to exhibit slower $\dot{V}_{O_2}$ kinetics (18, 39). Alternatively, the higher baseline metabolic rate that is elicited by cycling at a higher pedal cadence might have slowed phase II $\dot{V}_{O_2}$ kinetics due to the altered energetic state (9, 12).

Supplementation with BR did not significantly impact HR, SV, $\dot{Q}$, muscle oxygenation or $\dot{V}_{O_2}$ kinetics when cycling at 35 rpm in this study. Conversely, phase II $\dot{V}_{O_2}$ kinetics was 23% faster in 115-BR than 115-PLA. The faster $\dot{V}_{O_2}$ kinetics in 115-BR was not a function of enhanced HR, SV or $\dot{Q}$, as these were similar in 115-BR and 115-PLA. However, muscle [O_2Hb] was increased at baseline and throughout exercise in 115-BR compared to 115-PLA suggesting that the faster phase II $\dot{V}_{O_2}$ kinetics in 115-BR might have been linked to increased delivery of O_2 to the muscle microvasculature. Since the slower $\dot{V}_{O_2}$ kinetics in the 115 rpm trials compared to the 35 rpm trials appears to be linked to slower muscle O_2 delivery (as described above), the increase in muscle O_2 delivery at the higher cadence with BR might have partly corrected for the compromised muscle O_2 delivery permitting faster $\dot{V}_{O_2}$ kinetics in 115-BR compared to 115-PLA. This interpretation is in keeping with the O_2 tipping point theory proposed by Poole and Jones (48), which stipulates that phase II $\dot{V}_{O_2}$ kinetics is only speeded by interventions which enhance muscle O_2 delivery when muscle O_2 delivery is initially limited, at least in healthy adults.

A more rapid adjustment in oxidative metabolism following the onset of exercise would be expected to spare the utilization of the finite anaerobic energy reserves and attenuate the

accumulation of fatigue-related metabolites, thereby promoting enhanced exercise tolerance (13, 35). This is supported by our observation that exercise tolerance was enhanced by ~22% when phase II $\dot{V}_{O_2}$ kinetics was speeded by BR (i.e., at 115 rpm where the phase II τ was ~23% shorter after supplementation) and unchanged when no speeding was present (i.e., at 35 rpm; see figure 5). Furthermore, the improvements in exercise tolerance and muscle [O_2Hb] at 120 s with BR at 115 rpm were significantly correlated. This suggests that the increase in muscle O_2 delivery with BR was an important contributor to the ergogenic effects of BR at 115 rpm. Plasma [NO_2^-] was increased with BR and since NO_2^- can positively impact on vascular function directly (2), or indirectly through its reduction to NO (28, 42, 54), this is likely to account for the improved muscle O_2 delivery with BR. Indeed, previous studies have shown that NO_3^- supplementation can increase muscle blood flow in the running rat (23) and that NO_2^- infusion can increase blood flow in humans completing forearm exercise (17). The potential for enhanced perfusion with BR is likely to be more pronounced in the microvasculature of fast-twitch muscle, where P_{O_2} during contractions is lower (8, 44), since the reduction of NO_2^- to NO is augmented as P_{O_2} declines (14). This might explain why NO_3^- supplementation has been shown to preferentially distribute blood flow towards the more fatigue-susceptible fast-twitch muscle fibers in rats (23) and to speed phase II $\dot{V}_{O_2}$ kinetics when a greater proportion of fast-twitch muscle fibers are likely to be recruited (12). Therefore, assuming the recruitment of fast-twitch muscle was greater at the higher cadence (6-7, but see 1), this might account for the increased muscle [O_2Hb] in 115-BR, but not 35-BR, in this study. This increased muscle O_2 delivery in 115-BR likely reduced the mismatch between muscle O_2 delivery and muscle O_2 utilization at the higher pedal cadence which, in turn, promoted a more rapid adjustment of phase II $\dot{V}_{O_2}$ kinetics and improved exercise tolerance in 115-BR relative to 115-PLA. Alternatively, or in conjunction with enhanced fast-twitch muscle perfusion, inorganic NO_3^- supplementation has been shown to enhance calcium-handling proteins in fast-twitch muscle and to increase cytoplasmic [calcium] (33). This might provide a greater stimulus for oxidative phosphorylation in fast-twitch muscle fibers, potentially promoting faster $\dot{V}_{O_2}$ kinetics in 115-BR (31). It is also possible that the higher baseline metabolic rate evoked by the higher pedal cadence was responsible for the effects of BR at 115 rpm, but not 35 rpm, in this study (12). Bowen et al. (9) suggested that elevating metabolic rate during cycling exercise negatively impacted the intramuscular energy state leading to slower phase II $\dot{V}_{O_2}$ kinetics. Since NO_3^- supplementation has been shown to blunt intramuscular adenosine diphosphate and inorganic phosphate accumulation, and phosphocreatine utilization during exercise (3), the faster $\dot{V}_{O_2}$ kinetics and improved

exercise tolerance in 115-BR might be linked to an improved intramuscular energy state, regardless of muscle fiber recruitment patterns, in the higher cadence condition.

In addition to the faster phase II $\dot{V}_{O_2}$ kinetics in 115-BR, there is also evidence to demonstrate that NO_3^- supplementation can increase force during the initial stages of high-frequency maximal stimulation (29) and at high but not low contraction velocities (16) in human skeletal muscle *in vivo*. Moreover, NO_3^- supplementation can increase the rate of force development during high-frequency stimulation in mouse skeletal muscle *in vitro* (33). Taken together with the enhanced muscle oxygenation and faster phase II $\dot{V}_{O_2}$ kinetics observed in this study, these findings suggest that the effects of NO_3^- supplementation on skeletal muscle vascular function, metabolism and force production are enhanced at higher contraction velocities and frequencies and that these positive physiological responses are likely to account for the improved fatigue resistance observed with BR at 115 rpm, but not 35 rpm, in the current study. The results of this study might have important implications for improving skeletal muscle fatigue resistance at a high muscle contractile frequency or velocity, when greater recruitment of type II muscle fibers and a lower contractile efficacy might be expected.

In conclusion, short-term dietary supplementation with inorganic NO_3^- increased muscle $[O_2Hb]$ during cycling at 115 rpm, without changing muscle $[Hb_{tot}]$, suggesting improved muscle oxygenation. This increase in muscle oxygenation was accompanied by faster phase II $\dot{V}_{O_2}$ kinetics and was correlated with improved exercise tolerance in 115-BR. There were no changes in muscle oxygenation, phase II $\dot{V}_{O_2}$ kinetics and exercise tolerance with BR when cycling at very slow pedal cadence (35 rpm). Considered together, our findings suggest that NO_3^- supplementation is more effective at improving muscle microvascular oxygenation, pulmonary $\dot{V}_{O_2}$ kinetics and exercise tolerance during cycle ergometry exercise when a higher pedal cadence is employed. These findings might have important implications for speeding the adjustment of pulmonary $\dot{V}_{O_2}$ and improving fatigue resistance in exercise settings where more type II muscle fibers are recruited and/or muscle O_2 delivery is compromised.

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858 **Figure Legends**

859

860 **Figure 1:** Resting plasma nitrite concentration ($[NO_2^-]$) following placebo (PLA) and nitrate-
861 rich beetroot juice (BR) supplementation. The *open bar* represents the group mean ± SEM
862 plasma $[NO_2^-]$ from the PLA trials, while the *filled bar* represents the group mean ± SEM
863 plasma $[NO_2^-]$ from the BR trials. The solid grey lines represent the individual changes in
864 plasma $[NO_2^-]$ following BR supplementation. * indicates significantly different from PLA
865 ($P<0.01$). Note the increase in plasma $[NO_2^-]$ with BR supplementation.

866

867 **Figure 2:** Pulmonary oxygen uptake ($\dot{V}_{O_2}$) responses to a step increment from an unloaded
868 baseline to a severe-intensity work rate in a representative subject. The *upper panel*
869 illustrates the $\dot{V}_{O_2}$ responses whilst cycling at a cadence of 35 rpm following placebo (PLA)
870 and nitrate-rich beetroot juice (BR) supplementation. The *lower panel* shows the $\dot{V}_{O_2}$
871 responses whilst cycling at a cadence of 115 rpm following placebo (PLA) and nitrate-rich
872 beetroot juice (BR) supplementation. Data are expressed as a percentage of the $\dot{V}_{O_2}$ during
873 baseline cycling and displayed as 5-s averages. The dashed vertical line indicates the point of
874 the abrupt increase in work rate. Note the more rapid increase in $\dot{V}_{O_2}$ with BR following the
875 step increment in work rate at 115 rpm, but not 35 rpm. Data are truncated at 180 s.

876

877 **Figure 3:** Muscle deoxyhemoglobin concentration ([HHb]) group mean responses to a step
878 increment from an unloaded baseline to a severe-intensity work rate. The *upper panel*
879 illustrates the [HHb] responses whilst cycling at a cadence of 35 rpm following placebo
880 (PLA) and nitrate-rich beetroot juice (BR) supplementation. The *lower panel* shows the
881 [HHb] responses whilst cycling at a cadence of 115 rpm following placebo (PLA) and nitrate-
882 rich beetroot juice (BR) supplementation. Data are expressed as the change in muscle [HHb]
883 above baseline values and displayed as 5-s averages. The dashed vertical line indicates the
884 point of the abrupt increase in work rate. Note the slower adjustment of muscle [HHb]
885 following the step increment in work rate with BR for both conditions. Data are truncated at
886 180 s.

887

888 **Figure 4:** Muscle oxyhemoglobin concentration ($[O_2Hb]$) group mean responses to a step
889 increment from an unloaded baseline to a severe-intensity work rate. The *upper panel*
890 illustrates the $[O_2Hb]$ responses whilst cycling at a cadence of 35 rpm following placebo
891 (PLA) and nitrate-rich beetroot juice (BR) supplementation. The *lower panel* shows the

[O_2Hb] responses whilst cycling at a cadence of 115 rpm following placebo (PLA) and nitrate-rich beetroot juice (BR) supplementation. Data are expressed as 5-s averages and the dashed vertical line indicates the point of the abrupt increase in work rate. Note the significantly higher [O_2Hb] during baseline cycling and during the severe-intensity cycling bout with BR at 115 rpm, but not 35 rpm.

Figure 5: Time-to-exhaustion responses during a step increment from an unloaded baseline to a severe-intensity constant work rate. The *upper panel* compares time-to-exhaustion following placebo (PLA) and nitrate-rich beetroot juice (BR) supplementation whilst cycling at 35 rpm. The *lower panel* compares time-to-exhaustion following placebo (PLA) and nitrate-rich beetroot juice (BR) supplementation whilst cycling at 115 rpm. The *open bars* represent the group mean ± SEM responses after PLA supplementation, while the *filled bars* represent the group mean ± SEM responses after BR supplementation. The solid grey lines represent the individual changes in time-to-exhaustion following BR supplementation. * indicates significantly different from PLA (P<0.05). Note the significant increase in exercise tolerance after BR at 115 rpm, but not 35 rpm.

Table 1. Pulmonary oxygen uptake ($\dot{V}_{O_2}$) measures during severe-intensity cycle exercise at 35 rpm and 115 rpm after placebo (PLA) and nitrate-rich beetroot juice (BR) supplementation.

	35-PLA	35-BR	115-PLA	115-BR
Oxygen Uptake ($\dot{V}_{O_2}$)				
Baseline ($L \cdot min^{-1}$)	0.92 ± 0.14	0.89 ± 0.07	1.93 ± 0.34*ϕ	1.89 ± 0.25*ϕ
120 s ($L \cdot min^{-1}$)	3.41 ± 0.55	3.45 ± 0.51	3.65 ± 0.49* ϕ	3.68 ± 0.47*ϕ
Exhaustion ($L \cdot min^{-1}$)	3.83 ± 0.58	3.86 ± 0.52	4.24 ± 0.61*ϕ	4.32 ± 0.75*ϕ
Phase II τ (s)	32 ± 10	32 ± 6	61 ± 25*ϕ	47 ± 16*ϕΨ
Fundamental amplitude ($L \cdot min^{-1}$)	2.58 ± 0.40	2.61 ± 0.43	2.01 ± 0.24*ϕ	1.92 ± 0.30*ϕ
Slow component amplitude ($L \cdot min^{-1}$)	0.44 ± 0.15	0.35 ± 0.18	0.38 ± 0.32	0.53 ± 0.42

Values are presented as the mean ± SD. * = significantly different from 35-PLA ($P<0.05$); ϕ = significantly different from 35-BR ($P<0.05$); Ψ = significantly different from 115-PLA ($P<0.05$).

Table 2. Heart rate (HR), stroke volume (SV) and cardiac output ($\dot{Q}$) measures during severe-intensity cycle exercise at 35 rpm and 115 rpm after placebo (PLA) and nitrate-rich beetroot juice (BR) supplementation.

	35-PLA	**35-BR**	**115-PLA**	**115-BR**
Heart Rate (HR)				
Baseline (beats·min^{-1})	89 ± 10	88 ± 11	124 ± 19*ϕ	131 ± 21*ϕ
120 s (beats·min^{-1})	153 ± 15	153 ± 13	162 ± 17*ϕ	165 ± 13*ϕ
Exhaustion (beats·min^{-1})	168 ± 16	166 ± 15	178 ± 17*ϕ	178 ± 12*ϕ
Stroke Volume (SV)				
Baseline (mL·beat^{-1})	93 ± 6	87 ± 15	102 ± 14ϕ	98 ± 17
120 s (mL·beat^{-1})	108 ± 12	100 ± 19	111 ± 14	105 ± 11
Exhaustion (mL·beat^{-1})	102 ± 13	99 ± 16	111 ± 17	104 ± 13
Cardiac Output ($\dot{Q}$)				
Baseline (L·min^{-1})	8 ± 1	8 ± 2	12 ± 2*ϕ	13 ± 4*ϕ
120 s (L·min^{-1})	16 ± 2	15 ± 4	18 ± 2	17 ± 3
Exhaustion (L·min^{-1})	17 ± 2	16 ± 4	20 ± 3ϕ	18 ± 2

Values are presented as the mean ± SD. * = significantly different from 35-PLA (P<0.05); ϕ = significantly different from 35-BR (P<0.05);

965 **Table 3. Near-infrared spectroscopy determined muscle oxyhemoglobin concentration**
966 **([O_2Hb]), deoxyhemoglobin concentration ([HHb]) and total hemoglobin concentration**
967 **([Hb_{tot}]) measures during severe-intensity cycle exercise at 35 rpm and 115 rpm after**
968 **placebo (PLA) and nitrate-rich beetroot juice (BR) supplementation.**

	35-PLA	**35-BR**	**115-PLA**	**115-BR**
Muscle [HHb]				
Baseline (A.U.)	-4.0 ± 3.8	-3.9 ± 2.5	-2.0 ± 4.2	-3.0 ± 3.9
120 s (A.U.)	5.8 ± 6.1	5.4 ± 5.8	4.3 ± 4.2	2.9 ± 5.9
Exhaustion (A.U.)	6.4 ± 6.7	7.0 ± 7.0	4.9 ± 4.3	4.1 ± 6.5
[HHb] τ + TD (s)	19 ± 6	23 ± 13	24 ± 14	36 ± 24
[HHb] amplitude (A.U.)	10 ± 5	9 ± 6	6 ± 4	6 ± 3
Muscle [O_2Hb]				
Baseline (A.U.)	1.8 ± 3.1	0.1 ± 4.5	-1.1 ± 3.5*	3.4 ± 4.9Ψ
120 s (A.U.)	-6.9 ± 2.9	-7.7 ± 7.1	-6.1 ± 3.7	-1.0 ± 6.3*ϕΨ
Exhaustion (A.U.)	-6.9 ± 3.1	-7.0 ± 6.7	-6.0 ± 5.4	-0.6 ± 7.8*ϕ
Muscle [Hb_{tot}]				
Baseline (A.U.)	-2.2 ± 3.2	-3.8 ± 6.4	-3.1 ± 3.1	0.3 ± 5.5
120 s (A.U.)	-1.1 ± 5.9	-2.3 ± 8.1	-1.8 ± 3.2	1.9 ± 5.5
Exhaustion (A.U.)	-0.5 ± 5.7	-0.1 ± 8.9	-1.2 ± 4.3	3.5 ± 7.0

969 Values are presented as the mean ± SD. * = significantly different from 35-PLA (P<0.05); ϕ =
970 significantly different from 35-BR (P<0.05); Ψ = significantly different from 115-PLA
971 (P<0.05). A.U., arbitrary units.
972

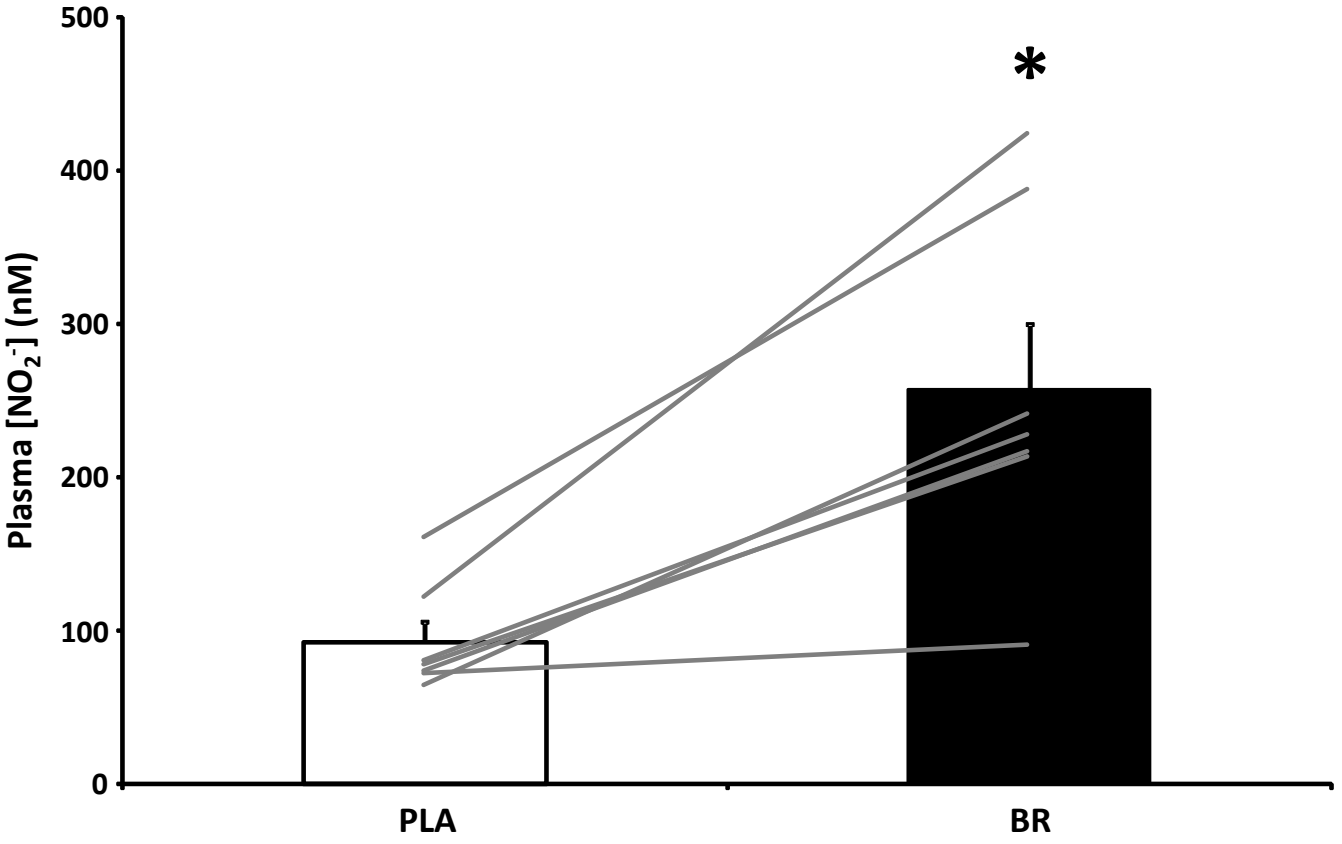


Fig 1

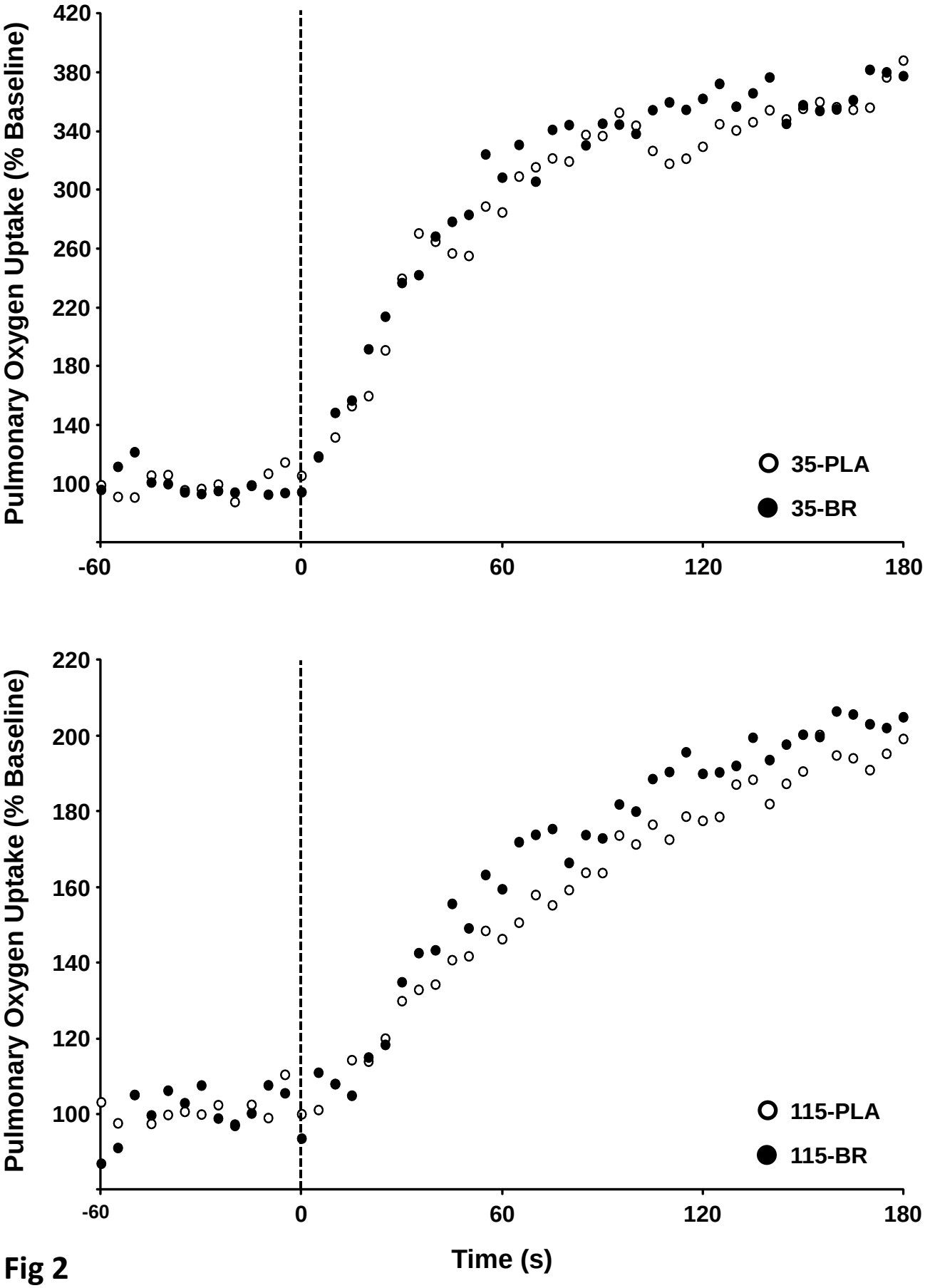
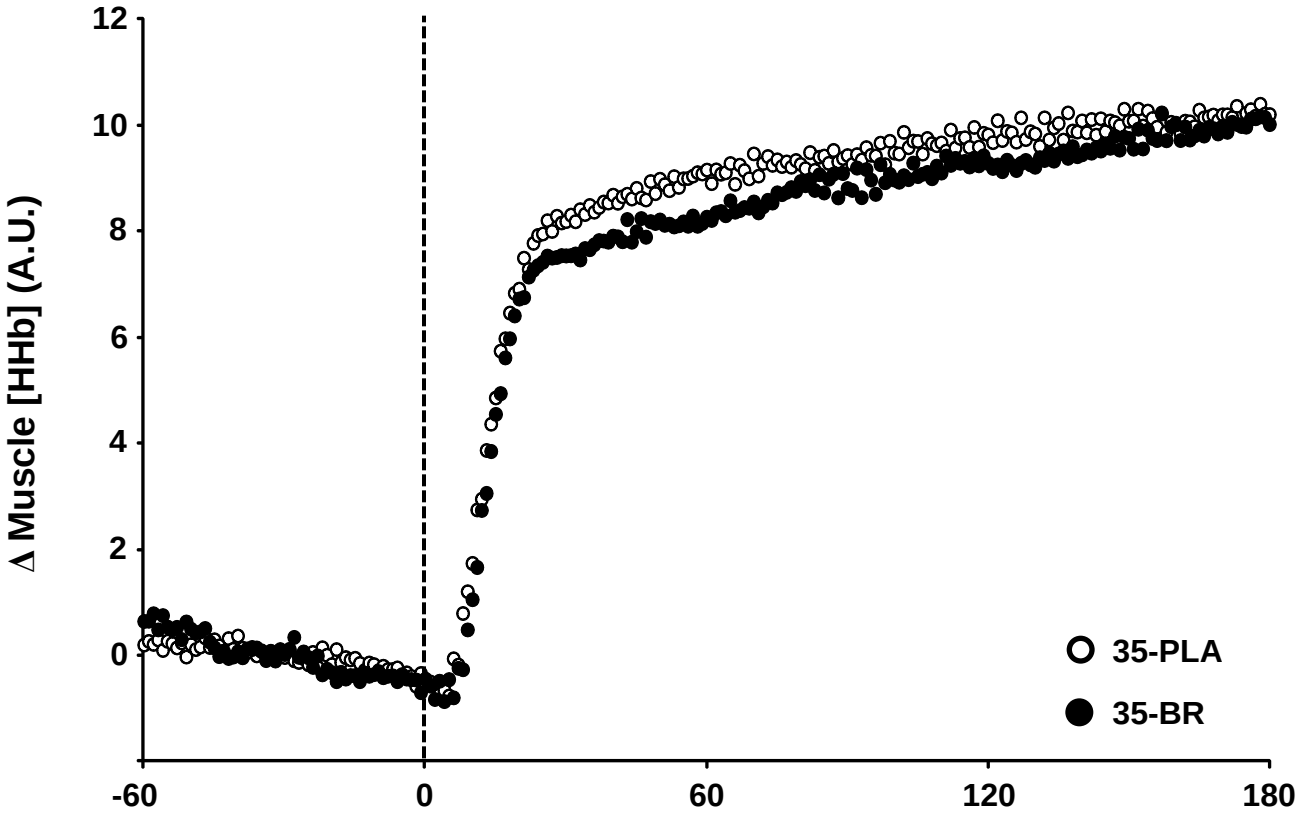

Pulmonary Oxygen Uptake (% Baseline)
420
380
340
300
260
220
180
140
100
-60
0
60
120
180
35-PLA
35-BR
Pulmonary Oxygen Uptake (% Baseline)
220
200
180
160
140
120
100
-60
0
60
120
180
115-PLA
115-BR
Time (s)

Fig 2


12
10
8
6
4
2
0
Δ Muscle [HHb] (A.U.)
-60
0
60
120
180
35-PLA
35-BR

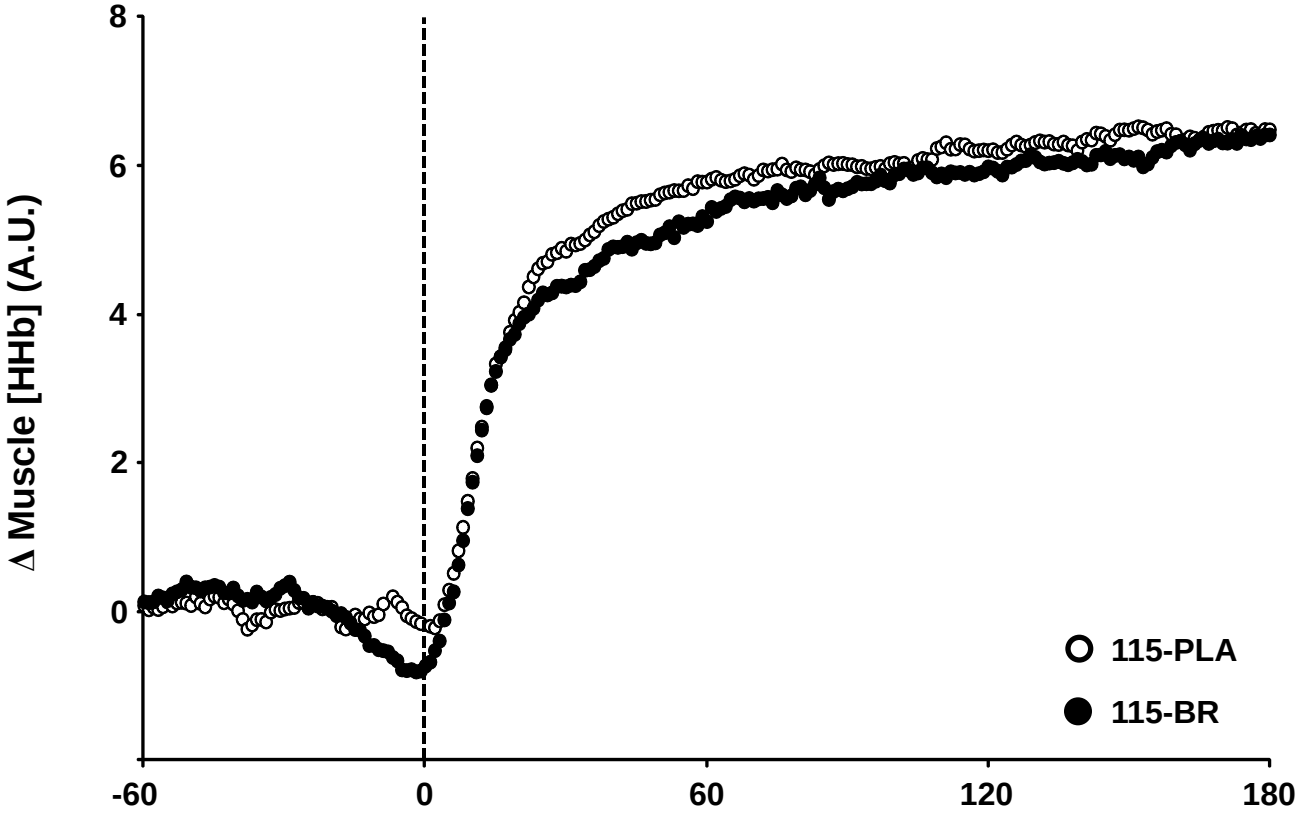

8
6
4
2
0
Δ Muscle [HHb] (A.U.)
-60
0
60
120
180
115-PLA
115-BR

Fig 3

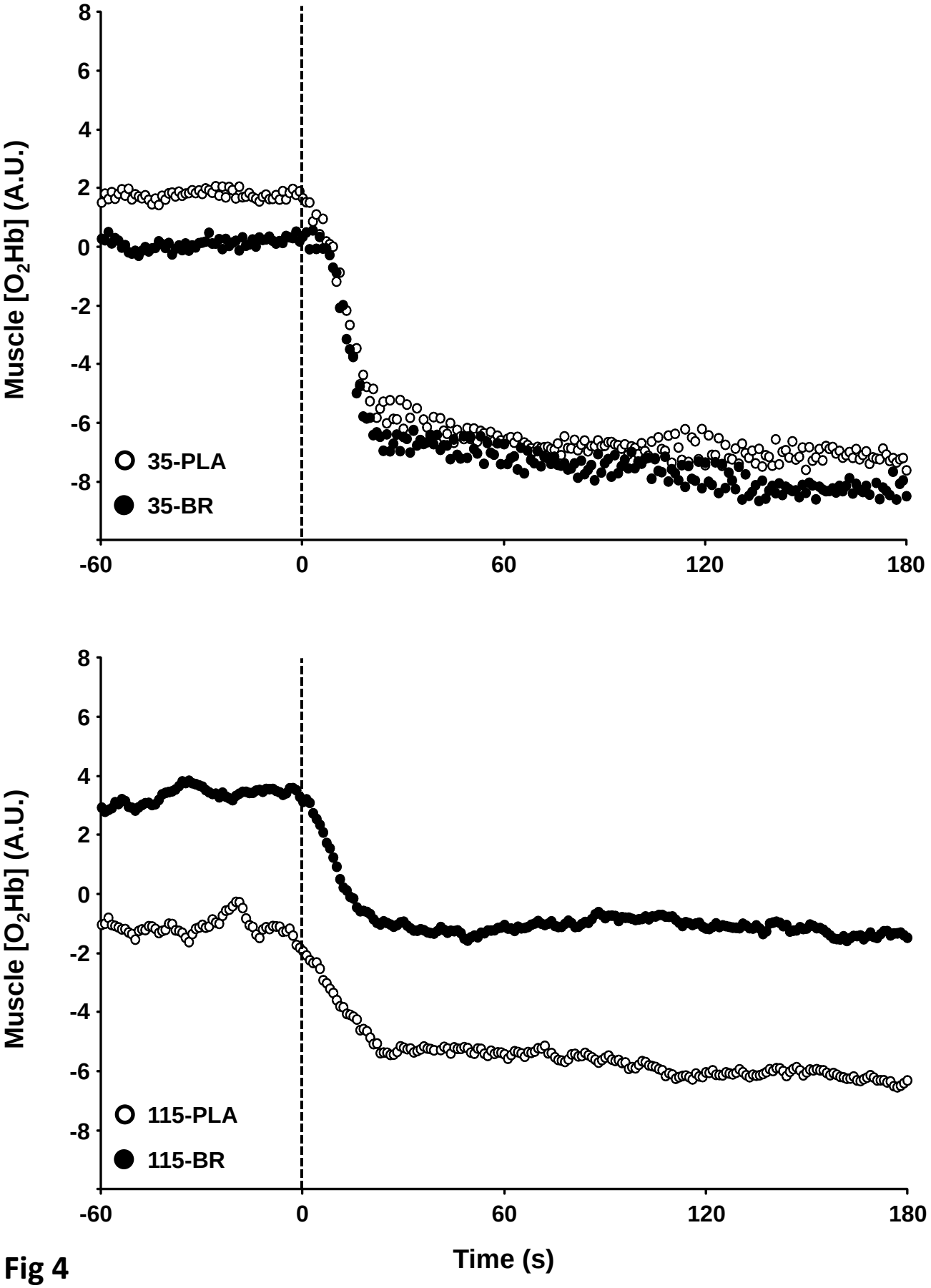
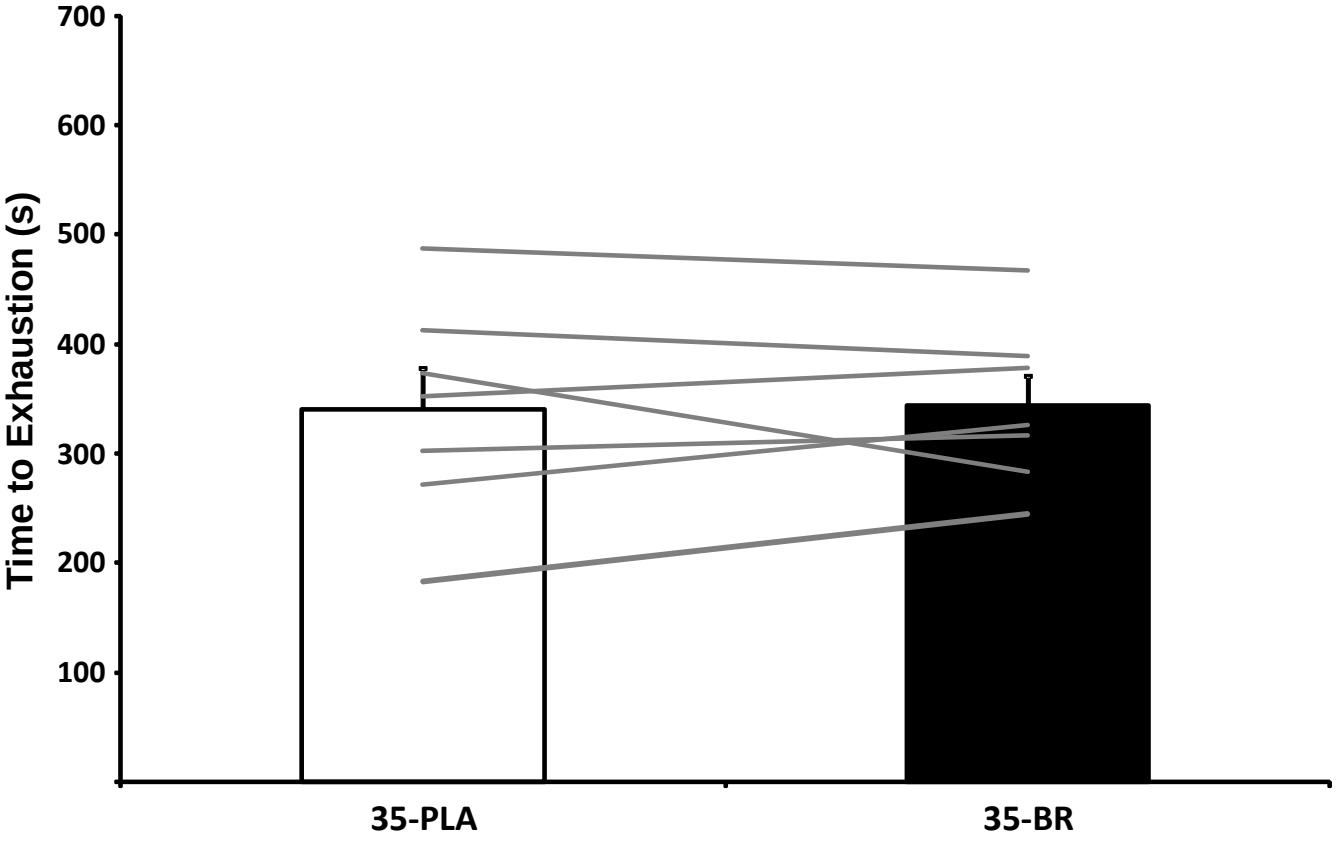

Muscle [O2Hb] (A.U.)
8
6
4
2
0
-2
-4
-6
-8
35-PLA
35-BR
-60
0
60
120
180
Muscle [O2Hb] (A.U.)
115-PLA
115-BR
Time (s)

Fig 4


700
600
500
400
300
200
100
Time to Exhaustion (s)
35-PLA
35-BR

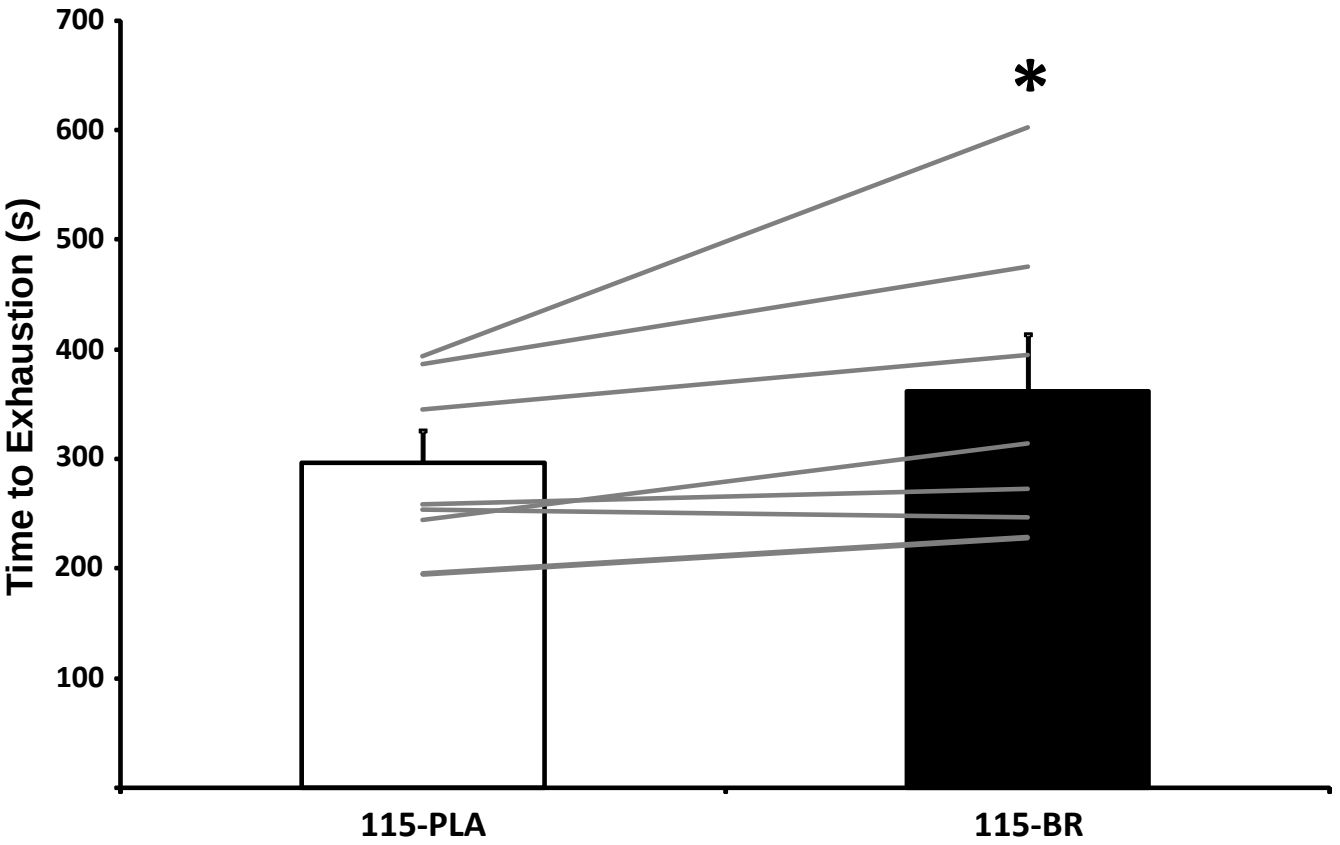

700
600
500
400
300
200
100
Time to Exhaustion (s)
*
115-PLA
115-BR

Fig 5