

1 **Inorganic nitrate supplementation improves muscle**  
2 **oxygenation, O<sub>2</sub> uptake kinetics and exercise tolerance at**  
3 **high but not low pedal rates**

4  
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24

25 Running Head: Dietary nitrate and pedal cadence

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27

28 **Abstract**

29

30 The purpose of this study was to test the hypothesis that inorganic nitrate ( $\text{NO}_3^-$ )  
31 supplementation would improve muscle oxygenation, pulmonary  $\text{O}_2$  uptake ( $\dot{V}_{\text{O}_2}$ ) kinetics  
32 and exercise tolerance ( $T_{\text{lim}}$ ) to a greater extent when cycling at high compared low pedal  
33 rates. In a randomised, placebo-controlled, cross-over study, seven subjects (mean  $\pm$  SD, age  
34  $21 \pm 2$  yr, body mass  $86 \pm 10$  kg) completed severe-intensity step cycle tests at pedal  
35 cadences of 35 rpm and 115 rpm during separate 9 day supplementation periods with  $\text{NO}_3^-$ -  
36 rich beetroot juice (BR; providing  $8.4 \text{ mmol NO}_3^- \cdot \text{day}^{-1}$ ) and placebo (PLA). Compared to  
37 PLA, plasma nitrite concentration increased 178% with BR ( $P < 0.01$ ). There were no  
38 significant differences in muscle oxyhemoglobin concentration ( $[\text{O}_2\text{Hb}]$ ), phase II  $\dot{V}_{\text{O}_2}$   
39 kinetics or  $T_{\text{lim}}$  between BR and PLA when cycling at 35 rpm ( $P > 0.05$ ). However, when  
40 cycling at 115 rpm, muscle  $[\text{O}_2\text{Hb}]$  was higher at baseline and throughout exercise, phase II  
41  $\dot{V}_{\text{O}_2}$  kinetics was faster ( $47 \pm 16$  s vs.  $61 \pm 25$  s;  $P < 0.05$ ) and  $T_{\text{lim}}$  was greater ( $362 \pm 137$  s  
42 vs.  $297 \pm 79$  s;  $P < 0.05$ ) with BR compared to PLA. In conclusion, these results suggest that  
43 short-term BR supplementation can increase muscle oxygenation, expedite the adjustment of  
44 oxidative metabolism and enhance exercise tolerance when cycling at a high, but not a low,  
45 pedal cadence in healthy recreationally-active subjects. These findings support recent  
46 observations that  $\text{NO}_3^-$  supplementation may be particularly effective at improving  
47 physiological and functional responses in type II muscle fibers.

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

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## 62 **Introduction**

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64 Nitric oxide (NO) is a diffusible gas that positively impacts a plethora of physiological  
65 responses including skeletal muscle perfusion, metabolism, force production and fatigue  
66 resistance (53). It is well documented that NO is produced by the nitric oxide synthase  
67 enzymes, which catalyze the complex five-electron oxidation of the semi-essential amino  
68 acid, L-arginine (10). More recently, there has been a growing appreciation of the potential  
69 for NO synthesis from the simple one-electron reduction of nitrite ( $\text{NO}_2^-$ ), in a reaction  
70 catalyzed by numerous  $\text{NO}_2^-$  reductases (42, 54). Importantly, increasing the intake of  
71 dietary inorganic nitrate ( $\text{NO}_3^-$ ), which passes into the enterosalivary circulation for  
72 subsequent reduction to  $\text{NO}_2^-$  by oral anaerobes (20), has been shown to positively impact  
73 NO biomarkers, exercise efficiency and exercise tolerance in recreationally active subjects  
74 (3-4, 12, 40-41, 55, 58). Therefore, supplementation with  $\text{NO}_3^-$  appears to represent an  
75 effective dietary intervention to improve NO bioavailability, contractile efficiency, and  
76 fatigue resistance.

77

78 Results from *in vitro* studies suggest that the effect of NO donors on mammalian skeletal  
79 muscle contractility is influenced by contraction frequency (5, 21, 43, 46). Hernandez et al.  
80 (33) harvested whole EDL muscles and single FDB muscle fibers from  $\text{NO}_3^-$  supplemented  
81 mice and reported increased *in vitro* contractile force up to 50 Hz stimulation and a more  
82 rapid rate of force development in the single FDB muscle fibers at 100 Hz stimulation.  
83 Similarly, increased evoked contractile force has been observed in human skeletal muscle *in*  
84 *vivo* during low-frequency sub-maximal stimulation and over the initial stages of high-  
85 frequency maximal stimulation following  $\text{NO}_3^-$  supplementation (29). However,  $\text{NO}_3^-$   
86 ingestion has been shown to increase human peak knee extensor torque at  $360^\circ \cdot \text{s}^{-1}$ , but not  
87  $90^\circ \cdot \text{s}^{-1}$ ,  $180^\circ \cdot \text{s}^{-1}$  and  $270^\circ \cdot \text{s}^{-1}$ , *in vivo* (16). Collectively, these findings suggest that dietary  
88  $\text{NO}_3^-$  supplementation enhances skeletal muscle contractile function, and that it might be  
89 particularly effective at augmenting contractility at higher contraction velocities. However,  
90 heretofore, human *in vivo* studies reporting improvements in exercise tolerance following  
91  $\text{NO}_3^-$  supplementation have utilized cycle ergometer exercise with pedal cadences of 70-90  
92 rpm (i.e., at “mid-range” contraction frequency/velocity; e.g., see 4, 12, 55, 58).  
93 Consequently, it is unclear whether  $\text{NO}_3^-$  supplementation may be more effective at  
94 improving skeletal muscle fatigue resistance at a higher contraction frequency/velocity.

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

108

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

127

128 The purpose of this study was to evaluate the effects of short term dietary  $\text{NO}_3^-$   
129 supplementation on muscle oxygenation, pulmonary  $\dot{V}_{\text{O}_2}$  kinetics and exercise tolerance  
130 when cycling at a very high (115 rpm) and a very low (35 rpm) pedal cadence at the same  
131 relative exercise intensity. Given the effects of cadence on the physiological responses  
132 evoked during cycling and the physiological benefits afforded by  $\text{NO}_3^-$  supplementation (see  
133 above), we hypothesized that muscle oxygenation, pulmonary  $\dot{V}_{\text{O}_2}$  kinetics and exercise  
134 tolerance would be improved to a greater extent with  $\text{NO}_3^-$  when cycling at 115 rpm than at  
135 35 rpm.

136

## 137 **Methods**

138

### 139 *Subjects*

140 Seven healthy male subjects (mean  $\pm$  SD age,  $21 \pm 2$  yrs; body mass,  $86 \pm 10$  kg; height,  $1.82$   
141  $\pm 0.08$  m) volunteered to participate in this study. None of the subjects were tobacco  
142 smokers or users of dietary supplements. The subjects participated in exercise at a  
143 recreational level, but were not highly trained. All subjects were familiar with laboratory  
144 exercise testing procedures, having previously participated in studies employing cycle  
145 ergometry in our laboratory. The procedures employed in this study were approved by the  
146 Institutional Research Ethics Committee and all subjects were required to give their written  
147 informed consent prior to the commencement of the study after the experimental procedures,  
148 associated risks, and potential benefits of participation had been explained. Subjects were  
149 instructed to arrive at the laboratory in a rested and fully hydrated state, at least 3 h  
150 postprandial, and to avoid strenuous exercise in the 24 h preceding each testing session. All  
151 tests were performed at the same time of day ( $\pm 2$  h). Each subject was asked to refrain from  
152 caffeine and alcohol intake for 6 h and 24 h before each test, respectively. Subjects were also  
153 provided with a list of foods rich in  $\text{NO}_3^-$  and instructed to avoid the consumption of these  
154 foods and to abstain from the use of antibacterial mouthwash, which eliminates the oral  
155 bacteria that reduce  $\text{NO}_3^-$  to  $\text{NO}_2^-$  (26), for the duration of the study.

156

### 157 *Experimental Design*

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

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

168

### 169 *Incremental Tests*

170 Before the intervention period, subjects completed a ramp incremental test at 35 and 115 rpm  
171 on an electronically-braked cycle ergometer (Lode Excalibur Sport, Groningen, the  
172 Netherlands) in a randomized order. At least 48 hours separated the two ramp incremental  
173 tests. Initially, subjects performed 3 min of baseline cycling at 20 W, after which the work  
174 rate was increased by 30 W/min until the limit of tolerance. The saddle and handle bar height  
175 and configuration during the first incremental test was recorded and reproduced in subsequent  
176 tests. During the baseline period and the incremental test, subjects cycled at a pre-determined  
177 pedal rate (either 35 or 115 rpm) until volitional exhaustion or the cadence fell by > 10 rpm  
178 for 3 consecutive seconds. Breath-by-breath pulmonary gas-exchange data were collected  
179 continuously during the incremental tests and averaged over consecutive 10-s periods. The  
180  $\dot{V}_{O_{2max}}$  was taken as the highest 30-s mean value attained prior to exhaustion in the test. The  
181 GET was determined from a cluster of measurements including: 1) the first disproportionate  
182 increase in CO<sub>2</sub> production ( $\dot{V}_{CO_2}$ ) from visual inspection of individual plots of  $\dot{V}_{CO_2}$  vs.  $\dot{V}_{O_2}$ ;  
183 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  
184 increase in end-tidal O<sub>2</sub> tension with no fall in end-tidal CO<sub>2</sub> tension. The data collected  
185 during the incremental tests were used to calculate cadence-specific work rates which were  
186 employed during the subsequent severe-intensity step tests. Specifically, the work rates that  
187 would require 80% of the difference between the  $\dot{V}_{O_2}$  at the GET and  $\dot{V}_{O_{2max}}$  (80% $\Delta$ ) were  
188 estimated with account taken of the mean response time of the  $\dot{V}_{O_2}$  response to ramp exercise  
189 (57).

190

### 191 *Step Exercise Tests*

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

194 days 4 and 5 of the supplementation period with the other cadence applied on days 8 and 9 of  
195 the supplementation period. The order in which the 35 and 115 rpm tests were administered  
196 over the first supplementation period was randomised (3 subjects started on 35 rpm) and this  
197 order was replicated over the second supplementation period. The step tests comprised 4  
198 minutes of baseline cycling at 20 W, followed by a step increment to a severe-intensity  
199 (80%Δ) constant work rate that was continued until exhaustion (same criteria as described  
200 above for the incremental exercise test). For each condition, the mean time to exhaustion was  
201 used for subsequent analysis.

202

### 203 ***Supplementation Procedures***

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

222

### 223 ***Measurements***

224 After reporting to the laboratory on days 4 and 8 of each dietary intervention (the first day  
225 that each cadence-specific step test was performed), a venous blood sample was drawn into a  
226 lithium-heparin tube and centrifuged at 4000 rpm and 4°C for 10 min, within 3 min of

227 collection. Plasma was subsequently extracted and immediately frozen at  $-80^{\circ}\text{C}$  for later  
228 analysis of  $[\text{NO}_2^-]$  in duplicate via ozone-based chemiluminescence (58).

229

230 During all tests, pulmonary gas exchange and ventilation were measured breath-by-breath  
231 with subjects wearing a nose clip and breathing through a low-dead-space, low-resistance  
232 mouthpiece and impeller turbine assembly (Jaeger Triple V). The inspired and expired gas  
233 volume and gas concentration signals were continuously sampled at 100 Hz, the latter using  
234 paramagnetic ( $\text{O}_2$ ) and infrared ( $\text{CO}_2$ ) analyzers (Jaeger Oxycon Pro, Hoechberg, Germany)  
235 via a capillary line connected to the mouthpiece. The gas analyzers were calibrated before  
236 each test with gases of known concentration and the turbine volume transducer was calibrated  
237 with a 3-liter syringe (Hans Rudolph, Kansas City, MO). The volume and concentration  
238 signals were time-aligned by accounting for the delay in the capillary gas transit and the  
239 analyzer rise time relative to the volume signal. Pulmonary gas exchange and ventilation  
240 were calculated and displayed breath-by-breath.

241

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

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

264

265 The oxygenation status of the *m. vastus lateralis* of the right leg was monitored using a  
266 commercially available NIRS system (model NIRO 300, Hamamatsu Photonics KK,  
267 Hiugashi-ku, Japan) on days 4 and 8 of each dietary intervention. The system consisted of an  
268 emission probe that irradiates laser beams and a detection probe. Four different wavelength  
269 laser diodes provided the light source (776, 826, 845, and 905 nm) and the light returning  
270 from the tissue was detected by a photomultiplier tube in the spectrometer. The intensity of  
271 incident and transmitted light was recorded continuously at 2 Hz and used to estimate  
272 concentration changes from the resting baseline for oxygenated, deoxygenated, and total  
273 tissue hemoglobin/myoglobin. Therefore, the NIRS data represent a relative change based on  
274 the optical density measured in the first datum collected. The deoxygenated  
275 hemoglobin/myoglobin concentration ([HHb]) signal was assumed to provide an estimate of  
276 changes in fractional O<sub>2</sub> extraction in the field of interrogation (25, 27, 36). It should be  
277 noted here that the contribution of deoxygenated myoglobin to the NIRS signal is presently  
278 unclear, and, as such, the terms [O<sub>2</sub>Hb], and [HHb] used in this paper should be considered to  
279 refer to the combined concentrations of oxygenated and deoxygenated hemoglobin and  
280 myoglobin, respectively.

281

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

293

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

300

### 301 *Data Analysis Procedures*

302 The breath-by-breath  $\dot{V}_{O_2}$  data from each test were initially examined to exclude errant  
303 breaths caused by coughing, swallowing, sighing, etc., and those values lying more than four  
304 standard deviations from the local mean were removed. The breath-by-breath data were  
305 subsequently linearly interpolated to provide second-by-second values and, for each  
306 individual, the two identical repetitions for each experimental condition were time-aligned to  
307 the start of exercise and ensemble-averaged. The first 20 s of data after the onset of exercise  
308 (i.e., the phase I response) were deleted and a nonlinear least-square algorithm was used to fit  
309 the data thereafter until the point of exhaustion. A bi-exponential model was used to  
310 characterize the  $\dot{V}_{O_2}$  responses to severe exercise, as described in the following equation:

311

$$312 \dot{V}_{O_2}(t) = \dot{V}_{O_2 \text{ baseline}} + A_p(1 - e^{-(t-TD_p/\tau_p)}) + A_s(1 - e^{-(t-TD_s/\tau_s)}) \quad (\text{Eqn. 2})$$

313

314 where  $\dot{V}_{O_2}(t)$  represents the absolute  $\dot{V}_{O_2}$  at a given time  $t$ ;  $\dot{V}_{O_2 \text{ baseline}}$  represents the mean  $\dot{V}_{O_2}$   
315  $O_2$  in the baseline period;  $A_p$ ,  $TD_p$ , and  $\tau_p$  represent the amplitude, time delay, and time  
316 constant, respectively, describing the phase II increase in  $\dot{V}_{O_2}$  above baseline; and  $A_s$ ,  $TD_s$ ,  
317 and  $\tau_s$  represent the amplitude of, time delay before the onset of, and time constant describing  
318 the development of, the  $\dot{V}_{O_2}$  slow component, respectively.

319

320 An iterative process was used to minimize the sum of the squared errors between the fitted  
321 function and the observed values.  $\dot{V}_{O_2 \text{ baseline}}$  was defined as the mean  $\dot{V}_{O_2}$  measured over the  
322 final 90 s of the resting baseline period. The  $\dot{V}_{O_2}$  at the limit of tolerance ( $T_{lim}$ ) was defined  
323 as the mean  $\dot{V}_{O_2}$  measured over the final 30 s of the exhaustive exercise bout. Because the  
324 asymptotic value ( $A_s$ ) of the exponential term describing the  $\dot{V}_{O_2}$  slow component may  
325 represent a higher value than is actually reached at the end of the exercise, the actual  
326 amplitude of the  $\dot{V}_{O_2}$  slow component at exhaustion was defined as  $A_s'$ .

327

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

$$332 \quad Y(t) = Y_{\text{baseline}} + A(1 - e^{-(t/\text{MRT})}) \quad (\text{Eqn. 3})$$

334  
335 where  $Y(t)$  represents the absolute value of the parameter of interest at a given time  $t$ ;  $Y_{\text{baseline}}$   
336 represents the mean value of the parameter of interest in the baseline period;  $A$  and  
337 MRT represent the amplitude and mean response time, respectively, describing the increase  
338 in  $Y$  above baseline.

339  
340 To provide information on muscle oxygenation, we also modelled the [HHb] response to  
341 exercise. A mono-exponential model was applied to the data with the fitting window  
342 commencing at the time at which the [HHb] signal increased 1 SD above the baseline mean.  
343 The [HHb] kinetics were determined by constraining the fitting window to the point at which  
344 mono-exponentiality became distorted, consequent to a [HHb] slow component, as  
345 determined by visual inspection of the residual plots. The [HHb] TD and  $\tau$  values were  
346 summed to provide information on the overall [HHb] response dynamics in the fundamental  
347 phase of the response.

348  
349 The [O<sub>2</sub>Hb] response does not approximate an exponential and was, therefore, not modelled.  
350 Rather, we determined the [O<sub>2</sub>Hb] at baseline (90-s preceding step transition), 120 s (30 s  
351 mean surrounding 120 s) and exhaustion (mean response over the final 30 s of exercise). The  
352 muscle [HHb] and [O<sub>2</sub>Hb] responses were summed at these time points ([Hb<sub>tot</sub>]) to provide  
353 information on the total [Hb] in the NIRS area of interrogation.

354

### 355 **Statistics**

356 A two-way, treatment (PLA and BR)  $\times$  cadence (35 and 115 rpm), repeated-measures  
357 ANOVA was employed to assess differences in plasma [NO<sub>2</sub><sup>-</sup>],  $\dot{V}_{\text{O}_2}$  kinetics, HR, SV,  $\dot{Q}$ ,  
358 muscle [HHb], muscle [O<sub>2</sub>Hb], muscle [Hb<sub>tot</sub>] and exercise tolerance across the experimental  
359 conditions. Significant effects were further explored using post-hoc  $t$ -tests with the alpha  
360 level adjusted via a Fisher's LSD correction. Relationships between the outcome variables

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

363

## 364 **Results**

365

366 The PLA and BR supplements administered in this study were well tolerated by all subjects  
367 with no negative side effects reported. Subjects consumed all doses of the supplement for  
368 each experimental condition and their diet was consistent across all the dietary interventions.  
369 After all experimental testing for the study was completed, all subjects confirmed that they  
370 were unaware that the PLA condition was not NaNO<sub>3</sub>.

371

372 The  $\dot{V}_{O_{2peak}}$  and peak work rate attained in the ramp incremental tests at 35 rpm and 115 rpm  
373 were, respectively,  $3.84 \pm 0.57$  L·min<sup>-1</sup> and  $4.11 \pm 0.56$  L·min<sup>-1</sup>, and  $304 \pm 43$  W and  $319 \pm$   
374  $52$  W. The  $\dot{V}_{O_2}$  and work rate at the GET during the incremental tests were  $1.62 \pm 0.31$   
375 L·min<sup>-1</sup> and  $122 \pm 18$  W at 35 rpm, and  $2.53 \pm 0.33$  L·min<sup>-1</sup> and  $122 \pm 8$  W at 115 rpm,  
376 respectively. There were no significant differences in  $\dot{V}_{O_{2peak}}$  or peak work rate attained  
377 during the ramp tests at 35 rpm and 115 rpm. However, the  $\dot{V}_{O_2}$  at the GET was significantly  
378 greater during the 115 rpm incremental test than the 35 rpm incremental test ( $P < 0.001$ ). The  
379 work rates which corresponded to 80%  $\Delta$ , which were imposed during the experimental tests  
380 conducted at 35 rpm and 115 rpm, were  $244 \pm 35$  W and  $258 \pm 47$  W, respectively. These  
381 work rates were not significantly different from each other ( $P > 0.05$ ).

382

### 383 *Plasma [NO<sub>2</sub><sup>-</sup>] and Blood Pressure*

384 There was a significant main effect for supplement on plasma [NO<sub>2</sub><sup>-</sup>] ( $P < 0.01$ ), systolic blood  
385 pressure (SBP;  $P < 0.05$ ) and mean arterial pressure (MAP;  $P < 0.05$ ), but not diastolic blood  
386 pressure (DBP;  $P > 0.05$ ). Plasma [NO<sub>2</sub><sup>-</sup>] was significantly higher in both 35-BR and 115-BR  
387 compared to 35-PLA and 115-PLA ( $P < 0.01$ ), with plasma [NO<sub>2</sub><sup>-</sup>] increased by 179% above  
388 PLA conditions with BR when all data were pooled ( $P < 0.01$ ; Figure 1). There were no  
389 differences in plasma [NO<sub>2</sub><sup>-</sup>] between 35-PLA and 115-PLA, and 35-BR and 115-BR  
390 ( $P > 0.05$ ). Systolic blood pressure and mean arterial pressure were lowered by 8 mmHg and 4  
391 mmHg across the BR conditions when all data were pooled ( $P < 0.05$ ).

392

393

### 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  $\tau$  with a higher  $\dot{V}_{O_2}$  phase II  $\tau$  (slower  
406  $\dot{V}_{O_2}$  kinetics) in 115-PLA ( $61 \pm 25$  s) and 115-BR ( $47 \pm 16$  s) compared to both 35-PLA ( $32$   
407  $\pm 10$  s) and 35-BR ( $32 \pm 6$  s;  $P<0.05$ ; Table 1). In addition there was a significant treatment  
408  $\times$  cadence interaction effect on the  $\dot{V}_{O_2}$  phase II  $\tau$  ( $P<0.05$ ). Post hoc analyses demonstrated  
409 that there was no significant difference in the  $\dot{V}_{O_2}$  phase II  $\tau$  between 35-PLA and 35-BR  
410 ( $P>0.05$ ), but the  $\dot{V}_{O_2}$  phase II  $\tau$  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.

424

425

426

### 427 *Muscle Oxygenation Parameters*

428 NIRS-derived [HHb], [O<sub>2</sub>Hb] and [Hb<sub>tot</sub>] responses are presented in Table 3 with group mean  
429 [HHb] and [O<sub>2</sub>Hb] responses illustrated in Figures 3 and 4, respectively. There were no  
430 significant main effects or interaction effects on the [HHb] at baseline or throughout the step  
431 exercise test ( $P>0.05$ ; Table 3). The ANOVA revealed a significant main effect of  
432 supplement on the [HHb]  $\tau + TD$  ( $P<0.05$ ) and a significant main effect of cadence on the  
433 [HHb] amplitude ( $P<0.05$ ; Table 3; Figure 3). There were no significant differences in these  
434 variables across the individual experimental conditions ( $P>0.05$ ). There were significant  
435 main effects of cadence and a significant treatment  $\times$  cadence interaction effect on the [O<sub>2</sub>Hb]  
436 at baseline, 120 s and at exhaustion ( $P<0.05$ ). Further analyses revealed that the [O<sub>2</sub>Hb] was  
437 greater in 115-BR at baseline compared to 115-PLA, at 120 s compared to all other  
438 conditions and at exhaustion compared to 35-PLA and 35-BR ( $P<0.05$ ). There were no  
439 significant main or interaction effects on [Hb<sub>tot</sub>].

440

### 441 *Exercise Tolerance*

442 The effects of BR on T<sub>lim</sub> at 35 rpm and 115 rpm are illustrated in Figure 5.  
443 Supplementation with BR significantly increased T<sub>lim</sub> when cycling at 115 rpm (115-PLA:  
444  $297 \pm 79$  s vs. 115-BR:  $362 \pm 137$  s;  $P<0.05$ ), but did not significantly alter T<sub>lim</sub> at 35 rpm  
445 (35-PLA:  $341 \pm 99$  s vs. 35-BR:  $344 \pm 74$  s;  $P>0.05$ ; Figure 5). There was a significant  
446 correlation between the change in T<sub>lim</sub> and muscle [O<sub>2</sub>Hb] at 120 s in 115-BR compared to  
447 115-PLA ( $r = 0.76$ ,  $P<0.05$ ) and a trend for a correlation between the change in muscle  
448 [O<sub>2</sub>Hb] at T<sub>lim</sub> and the change in T<sub>lim</sub> in 115-BR compared to 115-PLA ( $r = 0.76$ ,  $P=0.07$ ).  
449 There were no other correlations between the physiological and performance variables  
450 assessed in this study ( $P>0.05$ ).

451

## 452 **Discussion**

453 The principal novel findings from this study are that short term dietary NO<sub>3</sub><sup>-</sup> supplementation,  
454 which enhanced the potential for O<sub>2</sub>-independent NO synthesis by elevating plasma [NO<sub>2</sub><sup>-</sup>],  
455 increased muscle oxygenation, speeded phase II  $\dot{V}_{O_2}$  kinetics and improved severe-intensity  
456 exercise tolerance (T<sub>lim</sub>) when cycling at 115 rpm. In contrast, there were no changes in  
457 muscle oxygenation,  $\dot{V}_{O_2}$  kinetics or exercise tolerance with BR at 35 rpm. There were no  
458 changes in  $\dot{Q}$  with BR at either cadence, but muscle [O<sub>2</sub>Hb] was higher in 115-BR compared  
459 to 115-PLA. These findings suggest that BR increased muscle O<sub>2</sub> delivery at 115 rpm

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

464

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

484

485 Pulmonary  $\dot{V}_{O_2}$  was significantly higher at baseline and throughout exercise when cycling at  
486 115 rpm compared to 35 rpm, as reported previously (19, 25, 60). While subjects were  
487 cycling at the same external work rate at baseline (20 W), and the same relative exercise  
488 intensity (80% $\Delta$ ) during the step tests, internal work (56) and muscle ATP turnover rate and  
489  $\dot{V}_{O_2}$  (22) are increased at higher contraction frequencies, which accounts for the higher  
490 pulmonary  $\dot{V}_{O_2}$  observed in the 115 rpm trials. The phase II  $\dot{V}_{O_2}$   $\tau$  was higher ( $\dot{V}_{O_2}$  kinetics  
491 was slower) when cycling at 115 rpm than 35 rpm, which corroborates findings of a slower  
492 adjustment of  $\dot{V}_{O_2}$  when cycling at a higher pedal cadence (11, 19).

493

494 In concert with slower phase II  $\dot{V}_{O_2}$  kinetics, there was a trend for slower  $\dot{Q}$  kinetics in the  
495 115 rpm trials, suggesting that the slower  $\dot{V}_{O_2}$  kinetics might have been related to a slower  
496 adjustment of bulk blood flow and  $O_2$  delivery to the contracting muscles in the higher  
497 cadence condition. However, there is strong evidence that bulk muscle blood flow is  
498 enhanced at higher contraction frequencies (22, 30, 47, 52). Despite slower phase II  $\dot{V}_{O_2}$   
499 kinetics at the higher pedal cadence, there were no differences in muscle microvascular  
500 [HHb] kinetics ( $\tau + TD$ ) between 115 rpm and 35 rpm. Since muscle [HHb] is considered a  
501 non-invasive proxy for muscle  $O_2$  extraction (25, 36), this suggests that the slower phase II  $\dot{V}_{O_2}$   
502  $O_2$  kinetics at 115 rpm might be a function of slower microvascular blood flow kinetics and,  
503 therefore, to a relative shortfall in muscle  $O_2$  delivery compared to muscle  $O_2$  demand over  
504 the initial stages of the severe-intensity step test. The slower phase II  $\dot{V}_{O_2}$  kinetics at the  
505 higher pedal cadence might also reflect increased fast-twitch fiber recruitment (7), because  
506 fast-twitch fibers are believed to exhibit slower  $\dot{V}_{O_2}$  kinetics (18, 39). Alternatively, the  
507 higher baseline metabolic rate that is elicited by cycling at a higher pedal cadence might have  
508 slowed phase II  $\dot{V}_{O_2}$  kinetics due to the altered energetic state (9, 12).

509

510 Supplementation with BR did not significantly impact HR, SV,  $\dot{Q}$ , muscle oxygenation or  $\dot{V}_{O_2}$   
511  $O_2$  kinetics when cycling at 35 rpm in this study. Conversely, phase II  $\dot{V}_{O_2}$  kinetics was 23%  
512 faster in 115-BR than 115-PLA. The faster  $\dot{V}_{O_2}$  kinetics in 115-BR was not a function of  
513 enhanced HR, SV or  $\dot{Q}$ , as these were similar in 115-BR and 115-PLA. However, muscle  
514 [ $O_2$ Hb] was increased at baseline and throughout exercise in 115-BR compared to 115-PLA  
515 suggesting that the faster phase II  $\dot{V}_{O_2}$  kinetics in 115-BR might have been linked to  
516 increased delivery of  $O_2$  to the muscle microvasculature. Since the slower  $\dot{V}_{O_2}$  kinetics in the  
517 115 rpm trials compared to the 35 rpm trials appears to be linked to slower muscle  $O_2$   
518 delivery (as described above), the increase in muscle  $O_2$  delivery at the higher cadence with  
519 BR might have partly corrected for the compromised muscle  $O_2$  delivery permitting faster  $\dot{V}_{O_2}$   
520  $O_2$  kinetics in 115-BR compared to 115-PLA. This interpretation is in keeping with the  $O_2$   
521 tipping point theory proposed by Poole and Jones (48), which stipulates that phase II  $\dot{V}_{O_2}$   
522 kinetics is only speeded by interventions which enhance muscle  $O_2$  delivery when muscle  $O_2$   
523 delivery is initially limited, at least in healthy adults.

524

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

527 accumulation of fatigue-related metabolites, thereby promoting enhanced exercise tolerance  
528 (13, 35). This is supported by our observation that exercise tolerance was enhanced by ~22%  
529 when phase II  $\dot{V}_{O_2}$  kinetics was speeded by BR (i.e., at 115 rpm where the phase II  $\tau$  was  
530 ~23% shorter after supplementation) and unchanged when no speeding was present (i.e., at  
531 35 rpm; see figure 5). Furthermore, the improvements in exercise tolerance and muscle  
532  $[O_2Hb]$  at 120 s with BR at 115 rpm were significantly correlated. This suggests that the  
533 increase in muscle  $O_2$  delivery with BR was an important contributor to the ergogenic effects  
534 of BR at 115 rpm. Plasma  $[NO_2^-]$  was increased with BR and since  $NO_2^-$  can positively  
535 impact on vascular function directly (2), or indirectly through its reduction to NO (28, 42,  
536 54), this is likely to account for the improved muscle  $O_2$  delivery with BR. Indeed, previous  
537 studies have shown that  $NO_3^-$  supplementation can increase muscle blood flow in the running  
538 rat (23) and that  $NO_2^-$  infusion can increase blood flow in humans completing forearm  
539 exercise (17). The potential for enhanced perfusion with BR is likely to be more pronounced  
540 in the microvasculature of fast-twitch muscle, where  $P_{O_2}$  during contractions is lower (8, 44),  
541 since the reduction of  $NO_2^-$  to NO is augmented as  $P_{O_2}$  declines (14). This might explain why  
542  $NO_3^-$  supplementation has been shown to preferentially distribute blood flow towards the  
543 more fatigue-susceptible fast-twitch muscle fibers in rats (23) and to speed phase II  $\dot{V}_{O_2}$   
544 kinetics when a greater proportion of fast-twitch muscle fibers are likely to be recruited (12).  
545 Therefore, assuming the recruitment of fast-twitch muscle was greater at the higher cadence  
546 (6-7, but see 1), this might account for the increased muscle  $[O_2Hb]$  in 115-BR, but not 35-  
547 BR, in this study. This increased muscle  $O_2$  delivery in 115-BR likely reduced the mismatch  
548 between muscle  $O_2$  delivery and muscle  $O_2$  utilization at the higher pedal cadence which, in  
549 turn, promoted a more rapid adjustment of phase II  $\dot{V}_{O_2}$  kinetics and improved exercise  
550 tolerance in 115-BR relative to 115-PLA. Alternatively, or in conjunction with enhanced  
551 fast-twitch muscle perfusion, inorganic  $NO_3^-$  supplementation has been shown to enhance  
552 calcium-handling proteins in fast-twitch muscle and to increase cytoplasmic [calcium] (33).  
553 This might provide a greater stimulus for oxidative phosphorylation in fast-twitch muscle  
554 fibers, potentially promoting faster  $\dot{V}_{O_2}$  kinetics in 115-BR (31). It is also possible that the  
555 higher baseline metabolic rate evoked by the higher pedal cadence was responsible for the  
556 effects of BR at 115 rpm, but not 35 rpm, in this study (12). Bowen et al. (9) suggested that  
557 elevating metabolic rate during cycling exercise negatively impacted the intramuscular  
558 energy state leading to slower phase II  $\dot{V}_{O_2}$  kinetics. Since  $NO_3^-$  supplementation has been  
559 shown to blunt intramuscular adenosine diphosphate and inorganic phosphate accumulation,  
560 and phosphocreatine utilization during exercise (3), the faster  $\dot{V}_{O_2}$  kinetics and improved

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

563

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

578

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

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592

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594 **References**

595

596 **1. Ahlquist LE, Bassett DR Jr, Sufit R, Nagle FJ, Thomas DP.** The effect of pedaling  
597 frequency on glycogen depletion rates in type I and type II quadriceps muscle fibers during  
598 submaximal cycling exercise. *Eur J Appl Physiol Occup Physiol.* 65: 360-364, 1992.

599

600 **2. Alzawahra WF, Talukder MA, Liu X, Samouilov A, Zweier JL.** Heme proteins mediate  
601 the conversion of nitrite to nitric oxide in the vascular wall. *Am J Physiol Heart Circ Physiol.*  
602 295: 499-508, 2008.

603

604 **3. Bailey SJ, Fulford J, Vanhatalo A, Winyard PG, Blackwell JR, DiMenna FJ,**  
605 **Wilkerson DP, Benjamin N, Jones AM.** Dietary nitrate supplementation enhances muscle  
606 contractile efficiency during knee-extensor exercise in humans. *J Appl Physiol.* 109: 135-148,  
607 2010.

608

609 **4. Bailey SJ, Winyard P, Vanhatalo A, Blackwell JR, Dimenna FJ, Wilkerson DP, Tarr**  
610 **J, Benjamin N, Jones AM.** Dietary nitrate supplementation reduces the O<sub>2</sub> cost of low-  
611 intensity exercise and enhances tolerance to high-intensity exercise in humans. *J Appl*  
612 *Physiol.* 107: 1144-1155, 2009.

613

614 **5. Barclay JK, Reading SA, Murrant CL, Woodley NE.** Inotropic effects on mammalian  
615 skeletal muscle change with contraction frequency. *Can J Physiol Pharmacol.* 81: 753-758,  
616 2003.

617

618 **6. Beelen A, Sargeant AJ.** Effect of prior exercise at different pedalling frequencies on  
619 maximal power in humans. *Eur J Appl Physiol Occup Physiol.* 66: 102-107, 1993.

620

621 **7. Beelen A, Sargeant AJ, Lind A, de Haan A, Kernell D, van Mechelen W.** Effect of  
622 contraction velocity on the pattern of glycogen depletion in human muscle fibre types. In A.  
623 J. Sargeant and D. Kernell (Eds) *Neuromuscular fatigue*. North Holland, Amsterdam, pp. 93-  
624 95, 1993.

625

- 626 **8. Behnke BJ, McDonough P, Padilla DJ, Musch TI, Poole DC.** Oxygen exchange profile  
627 in rat muscles of contrasting fibre types. *J Physiol.* 549: 597-605, 2003.  
628
- 629 **9. Bowen TS, Murgatroyd SR, Cannon DT, Cuff TJ, Lainey AF, Marjerrison AD,**  
630 **Spencer MD, Benson AP, Paterson DH, Kowalchuk JM, Rossiter HB.** A raised metabolic  
631 rate slows pulmonary O<sub>2</sub> uptake kinetics on transition to moderate-intensity exercise in  
632 humans independently of work rate. *Exp Physiol.* 96: 1049-61, 2011.  
633
- 634 **10. Bredt DS, Hwang PM, Glatt CE, Lowenstein C, Reed RR, Snyder SH.** Cloned and  
635 expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. *Nature.*  
636 351: 714-718, 1991.  
637
- 638 **11. Breese BC, Armstrong N, Barker AR, Williams CA.** The effect of pedal rate on  
639 pulmonary O<sub>2</sub> uptake kinetics during very heavy intensity exercise in trained and untrained  
640 teenage boys. *Respir Physiol Neurobiol.* 177: 149-154, 2011.  
641
- 642 **12. Breese BC, McNarry MA, Marwood S, Blackwell JR, Bailey SJ, Jones AM.** Beetroot  
643 juice supplementation speeds O<sub>2</sub> uptake kinetics and improves exercise tolerance during  
644 severe-intensity exercise initiated from an elevated metabolic rate. *Am J Physiol Regul Integr*  
645 *Comp Physiol.* 305: 1441-1450, 2013.  
646
- 647 **13. Burnley M, Jones AM.** Oxygen uptake kinetics as a determinant of sports performance.  
648 *Eur J Sports Sci* 7: 63-79, 2007.  
649
- 650 **14. Castello PR, David PS, McClure T, Crook Z, Poyton RO.** Mitochondrial cytochrome  
651 oxidase produces nitric oxide under hypoxic conditions: implications for oxygen sensing and  
652 hypoxic signaling in eukaryotes. *Cell Metab.* 3: 277-287, 2006.  
653
- 654 **15. Charloux A, Lonsdorfer-Wolf E, Richard R, Lampert E, Oswald-Mammoser M,**  
655 **Mettauer B, Geny B, Lonsdorfer J.** A new impedance cardiograph device for the non-  
656 invasive evaluation of cardiac output at rest and during exercise: comparison with the "direct"  
657 Fick method. *Eur J Appl Physiol.* 82: 313-320, 2000.  
658

- 659 **16. Coggan AR, Leibowitz JL, Kadkhodayan A, Thomas DT, Ramamurthy S, Spearie**  
660 **CA, Waller S, Farmer M, Peterson LR.** Effect of acute dietary nitrate intake on maximal  
661 knee extensor speed and power in healthy men and women. *Nitric Oxide*. In press  
662
- 663 **17. Cosby K, Partovi KS, Crawford JH, Patel RP, Reiter CD, Martyr S, Yang BK,**  
664 **Waclawiw MA, Zalos G, Xu X, Huang KT, Shields H, Kim-Shapiro DB, Schechter AN,**  
665 **Cannon RO 3rd, Gladwin MT.** Nitrite reduction to nitric oxide by deoxyhemoglobin  
666 vasodilates the human circulation. *Nat Med*. 9: 1498-1505, 2003.  
667
- 668 **18. Crow MT, Kushmerick MJ.** Chemical energetics of slow- and fast-twitch muscles of  
669 the mouse. *J Gen Physiol*. 79: 147-66, 1982.  
670
- 671 **19. DiMenna FJ, Wilkerson DP, Burnley M, Bailey SJ, Jones AM.** Influence of extreme  
672 pedal rates on pulmonary O<sub>2</sub> uptake kinetics during transitions to high-intensity exercise from  
673 an elevated baseline. *Respir Physiol Neurobiol*. 169: 16-23, 2009.  
674
- 675 **20. Duncan C, Dougall H, Johnston P, Green S, Brogan R, Leifert C, Smith L, Golden**  
676 **M, Benjamin N.** Chemical generation of nitric oxide in the mouth from the enterosalivary  
677 circulation of dietary nitrate. *Nat Med*. 1: 546-551, 1995.  
678
- 679 **21. Evangelista AM, Rao VS, Filo AR, Marozkina NV, Doctor A, Jones DR, Gaston B,**  
680 **Guilford WH.** Direct regulation of striated muscle myosins by nitric oxide and endogenous  
681 nitrosothiols. *PLoS One*. 5: e11209, 2010.  
682
- 683 **22. Ferguson RA, Ball D, Krustrup P, Aagaard P, Kjaer M, Sargeant AJ, Hellsten Y,**  
684 **Bangsbo J.** Muscle oxygen uptake and energy turnover during dynamic exercise at different  
685 contraction frequencies in humans. *J Physiol*. 536: 261-271, 2001.  
686
- 687 **23. Ferguson SK, Hirai DM, Copp SW, Holdsworth CT, Allen JD, Jones AM, Musch**  
688 **TI, Poole DC.** Impact of dietary nitrate supplementation via beetroot juice on exercising  
689 muscle vascular control in rats. *J Physiol*. 591: 547-557, 2013.  
690  
691

- 692 **24. Ferguson SK, Hirai DM, Copp SW, Holdsworth CT, Allen JD, Jones AM, Musch**  
693 **TI, Poole DC.** Effects of nitrate supplementation via beetroot juice on contracting rat skeletal  
694 muscle microvascular oxygen pressure dynamics. *Respir Physiol Neurobiol.* 187: 250-255,  
695 2013.
- 696
- 697 **25. Ferreira LF, Lutjemeier BJ, Townsend DK, Barstow TJ.** Effects of pedal frequency  
698 on estimated muscle microvascular O<sub>2</sub> extraction. *Eur J Appl Physiol.* 96: 558-563, 2006.
- 699
- 700 **26. Govoni M, Jansson EA, Weitzberg E, Lundberg JO.** The increase in plasma nitrite  
701 after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash. *Nitric*  
702 *Oxide.* 19: 333-337, 2008.
- 703
- 704 **27. Grassi B, Pogliaghi S, Rampichini S, Quaresima V, Ferrari M, Marconi C, Cerretelli**  
705 **P.** Muscle oxygenation and pulmonary gas exchange kinetics during cycling exercise on-  
706 transitions in humans. *J Appl Physiol.* 95: 149-158, 2003.
- 707
- 708 **28. Gruetter CA, Barry BK, McNamara DB, Gruetter DY, Kadowitz PJ, Ignarro L.**  
709 Relaxation of bovine coronary artery and activation of coronary arterial guanylate cyclase by  
710 nitric oxide, nitroprusside and a carcinogenic nitrosoamine. *J Cyclic Nucleotide Res.* 5: 211-  
711 224, 1979.
- 712
- 713 **29. Haider G, Folland JP.** Nitrate Supplementation Enhances the Contractile Properties of  
714 Human Skeletal Muscle. *Med Sci Sports Exerc.* 46: 2234-2243, 2014.
- 715
- 716 **30. Hamann JJ, Kluess HA, Buckwalter JB, Clifford PS.** Blood flow response to muscle  
717 contractions is more closely related to metabolic rate than contractile work. *J Appl Physiol.*  
718 98: 2096-2100, 2005.
- 719
- 720 **31. Hansford RG.** Role of calcium in respiratory control. *Med Sci Sports Exerc.* 26: 44-51,  
721 1994.
- 722
- 723 **32. Haycock GB, Schwartz GJ, Wisotsky DH.** Geometric method for measuring body  
724 surface area: a height-weight formula validated in infants, children, and adults. *J Pediatr.* 93:  
725 62-66, 1978.

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759

**33. Hernández A, Schiffer TA, Ivarsson N, Cheng AJ, Bruton JD, Lundberg JO, Weitzberg E, Westerblad H.** Dietary nitrate increases tetanic  $[Ca^{2+}]_i$  and contractile force in mouse fast-twitch muscle. *J Physiol.* 590: 3575-3583, 2012.

**34. Hogan MC, Ingham E, Kurdak SS.** Contraction duration affects metabolic energy cost and fatigue in skeletal muscle. *Am J Physiol.* 274: 397-402, 1998.

**35. Jones AM, Burnley M.** Oxygen uptake kinetics: an underappreciated determinant of exercise performance. *Int J Sports Physiol Perform.* 4: 524-532, 2009.

**36. Koga S, Poole DC, Fukuoka Y, Ferreira LF, Kondo N, Ohmae E, Barstow TJ.** Methodological validation of the dynamic heterogeneity of muscle deoxygenation within the quadriceps during cycle exercise. *Am J Physiol Regul Integr Comp Physiol.* 301: 534-541, 2011.

**37. Krstrup P, Söderlund K, Mohr M, Bangsbo J.** The slow component of oxygen uptake during intense, sub-maximal exercise in man is associated with additional fibre recruitment. *Pflugers Arch.* 447: 855-866, 2004.

**38. Krstrup P, Söderlund K, Relu MU, Ferguson RA, Bangsbo J.** Heterogeneous recruitment of quadriceps muscle portions and fibre types during moderate intensity knee- extensor exercise: effect of thigh occlusion. *Scand J Med Sci Sports.* 19: 576-84, 2009.

**39. Kushmerick MJ, Meyer RA, Brown TR.** Regulation of oxygen consumption in fast- and slow-twitch muscle. *Am J Physiol.* 263: 598-606, 1992.

**40. Larsen FJ, Schiffer TA, Borniquel S, Sahlin K, Ekblom B, Lundberg JO, Weitzberg E.** Dietary inorganic nitrate improves mitochondrial efficiency in humans. *Cell Metab.* 13: 149-159, 2011.

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

- 760 **42. Lundberg JO, Weitzberg E.** NO generation from inorganic nitrate and nitrite: Role in  
761 physiology, nutrition and therapeutics. *Arch Pharm Res.* 32: 1119-1126, 2009.  
762
- 763 **43. Maréchal G, Beckers-Bleukx G.** Effect of nitric oxide on the maximal velocity of  
764 shortening of a mouse skeletal muscle. *Pflugers Arch.* 436: 906-913, 1998.  
765
- 766 **44. McDonough P, Behnke BJ, Padilla DJ, Musch TI, Poole DC.** Control of microvascular  
767 oxygen pressures in rat muscles comprised of different fibre types. *J Physiol.* 563: 903-913,  
768 2005.  
769
- 770 **45. Modin A, Björne H, Herulf M, Alving K, Weitzberg E, Lundberg JO.** Nitrite-derived  
771 nitric oxide: a possible mediator of 'acidic-metabolic' vasodilation. *Acta Physiol Scand.* 171:  
772 9-16, 2001.  
773
- 774 **46. Murrant CL, Frisbee JC, Barclay JK.** The effect of nitric oxide and endothelin on  
775 skeletal muscle contractility changes when stimulation is altered. *Can J Physiol Pharmacol.*  
776 75: 414-422, 1997.  
777
- 778 **47. Osada T, Rådegran G.** Femoral artery inflow in relation to external and total work rate  
779 at different knee extensor contraction rates. *J Appl Physiol.* 92: 1325-1330, 2002.  
780
- 781 **48. Poole DC, Jones AM.** Oxygen uptake kinetics. *Compr Physiol.* 2: 933-996, 2012.  
782
- 783 **49. Pringle JS, Doust JH, Carter H, Tolfrey K, Jones AM.** Effect of pedal rate on primary  
784 and slow-component oxygen uptake responses during heavy-cycle exercise. *J Appl Physiol.*  
785 94:1501-1507, 2003.  
786
- 787 **50. Richard R, Lonsdorfer-Wolf E, Charloux A, Doutreleau S, Buchheit M, Oswald-**  
788 **Mammoser M, Lampert E, Mettauer B, Geny B, Lonsdorfer J.** Non-invasive cardiac  
789 output evaluation during a maximal progressive exercise test, using a new impedance  
790 cardiograph device. *Eur J Appl Physiol.* 85: 202-207, 2001.  
791

- 792 **51. Richardson RS, Leigh JS, Wagner PD, Noyszewski EA.** Cellular  $P_{O_2}$  as a determinant  
793 of maximal mitochondrial  $O_2$  consumption in trained human skeletal muscle. *J Appl Physiol.*  
794 87: 325-331, 1999.  
795
- 796 **52. Sjøgaard G, Hansen EA, Osada T.** Blood flow and oxygen uptake increase with total  
797 power during five different knee-extension contraction rates. *J Appl Physiol.* 93: 1676-1684,  
798 2002.  
799
- 800 **53. Stamler JS, Meissner G.** Physiology of nitric oxide in skeletal muscle. *Physiol Rev.* 81:  
801 209-237, 2001.  
802
- 803 **54. van Faassen EE, Bahrami S, Feelisch M, Hogg N, Kelm M, Kim-Shapiro DB, Kozlov**  
804 **AV, Li H, Lundberg JO, Mason R, Nohl H, Rassaf T, Samouilov A, Slama-Schwok A,**  
805 **Shiva S, Vanin AF, Weitzberg E, Zweier J, Gladwin MT.** Nitrite as regulator of hypoxic  
806 signaling in mammalian physiology. *Med Res Rev.* 29: 683-741, 2009.  
807
- 808 **55. Vanhatalo A, Bailey SJ, Blackwell JR, DiMenna FJ, Pavey TG, Wilkerson DP,**  
809 **Benjamin N, Winyard PG, Jones AM.** Acute and chronic effects of dietary nitrate  
810 supplementation on blood pressure and the physiological responses to moderate-intensity and  
811 incremental exercise. *Am J Physiol Regul Integr Comp Physiol.* 299: 1121-1131, 2010.  
812
- 813 **56. Wells R, Morrissey M, Hughson R.** Internal work and physiological responses during  
814 concentric and eccentric cycle ergometry. *Eur J Appl Physiol Occup Physiol.* 55: 295-301,  
815 1986.  
816
- 817 **57. Whipp BJ, Ward SA, Lamarra N, Davis JA, Wasserman K.** Parameters of ventilatory  
818 and gas exchange dynamics during exercise. *J Appl Physiol*, 52: 1506-1513, 1982.  
819
- 820 **58. Wylie LJ, Kelly J, Bailey SJ, Blackwell JR, Skiba PF, Winyard PG, Jeukendrup AE,**  
821 **Vanhatalo A, Jones AM.** Beetroot juice and exercise: pharmacodynamic and dose-response  
822 relationships. *J Appl Physiol.* 115: 325-336, 2013.  
823

824 **59. Wylie LJ, Mohr M, Krstrup P, Jackman SR, Ermudis G, Kelly J, Black MI, Bailey**  
825 **SJ, Vanhatalo A, Jones AM.** Dietary nitrate supplementation improves team sport-specific  
826 intense intermittent exercise performance. *Eur J Appl Physiol.* 113: 1673-1684, 2013.

827

828 **60. Zoladz JA, Duda K, Majerczak J.**  $\text{VO}_2$ /power output relationship and the slow  
829 component of oxygen uptake kinetics during cycling at different pedaling rates: relationship  
830 to venous lactate accumulation and blood acid-base balance. *Physiol Res.* 47: 427-438, 1998.

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858 **Figure Legends**

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860 **Figure 1:** Resting plasma nitrite concentration ( $[\text{NO}_2^-]$ ) following placebo (PLA) and nitrate-  
861 rich beetroot juice (BR) supplementation. The *open bar* represents the group mean  $\pm$  SEM  
862 plasma  $[\text{NO}_2^-]$  from the PLA trials, while the *filled bar* represents the group mean  $\pm$  SEM  
863 plasma  $[\text{NO}_2^-]$  from the BR trials. The solid grey lines represent the individual changes in  
864 plasma  $[\text{NO}_2^-]$  following BR supplementation. \* indicates significantly different from PLA  
865 ( $P < 0.01$ ). Note the increase in plasma  $[\text{NO}_2^-]$  with BR supplementation.

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867 **Figure 2:** Pulmonary oxygen uptake ( $\dot{V}_{\text{O}_2}$ ) 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  $\dot{V}_{\text{O}_2}$  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  $\dot{V}_{\text{O}_2}$   
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  $\dot{V}_{\text{O}_2}$  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  $\dot{V}_{\text{O}_2}$  with BR following the  
875 step increment in work rate at 115 rpm, but not 35 rpm. Data are truncated at 180 s.

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877 **Figure 3:** Muscle deoxyhemoglobin concentration ( $[\text{HHb}]$ ) group mean responses to a step  
878 increment from an unloaded baseline to a severe-intensity work rate. The *upper panel*  
879 illustrates the  $[\text{HHb}]$  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  $[\text{HHb}]$  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  $[\text{HHb}]$   
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  $[\text{HHb}]$   
885 following the step increment in work rate with BR for both conditions. Data are truncated at  
886 180 s.

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888 **Figure 4:** Muscle oxyhemoglobin concentration ( $[\text{O}_2\text{Hb}]$ ) group mean responses to a step  
889 increment from an unloaded baseline to a severe-intensity work rate. The *upper panel*  
890 illustrates the  $[\text{O}_2\text{Hb}]$  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

892 [O<sub>2</sub>Hb] responses whilst cycling at a cadence of 115 rpm following placebo (PLA) and  
893 nitrate-rich beetroot juice (BR) supplementation. Data are expressed as 5-s averages and the  
894 dashed vertical line indicates the point of the abrupt increase in work rate. Note the  
895 significantly higher [O<sub>2</sub>Hb] during baseline cycling and during the severe-intensity cycling  
896 bout with BR at 115 rpm, but not 35 rpm.

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

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

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

928 Values are presented as the mean ± SD. \* = significantly different from 35-PLA ( $P < 0.05$ );  $\phi$  =  
 929 significantly different from 35-BR ( $P < 0.05$ );  $\Psi$  = significantly different from 115-PLA  
 930 ( $P < 0.05$ ).

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

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

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

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965 **Table 3. Near-infrared spectroscopy determined muscle oxyhemoglobin concentration**  
 966 **([O<sub>2</sub>Hb]), deoxyhemoglobin concentration ([HHb]) and total hemoglobin concentration**  
 967 **([Hb<sub>tot</sub>]) measures during severe-intensity cycle exercise at 35 rpm and 115 rpm after**  
 968 **placebo (PLA) and nitrate-rich beetroot juice (BR) supplementation.**

	<b>35-PLA</b>	<b>35-BR</b>	<b>115-PLA</b>	<b>115-BR</b>
<b>Muscle [HHb]</b>				
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
<b>Muscle [O<sub>2</sub>Hb]</b>				
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
<b>Muscle [Hb<sub>tot</sub>]</b>				
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.

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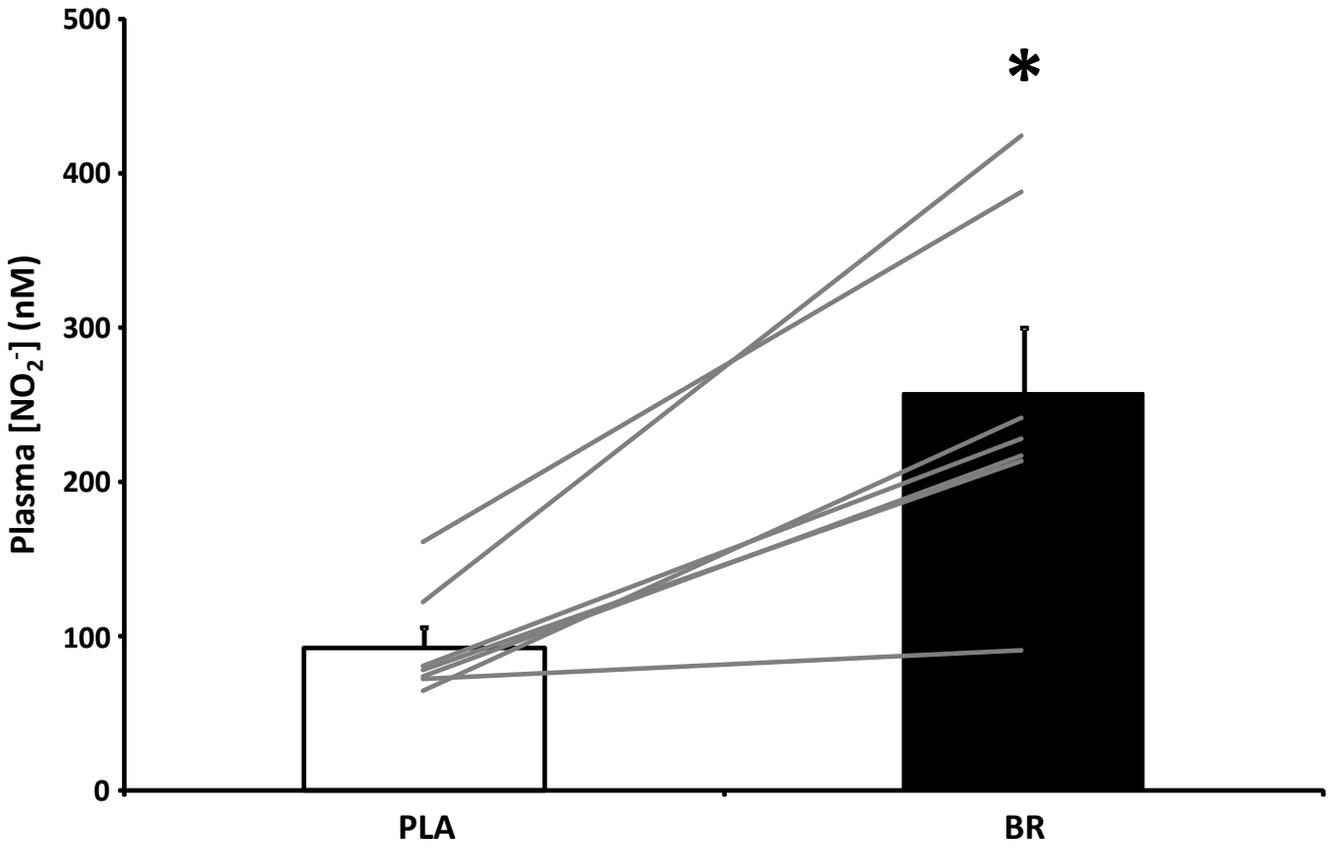


Fig 1

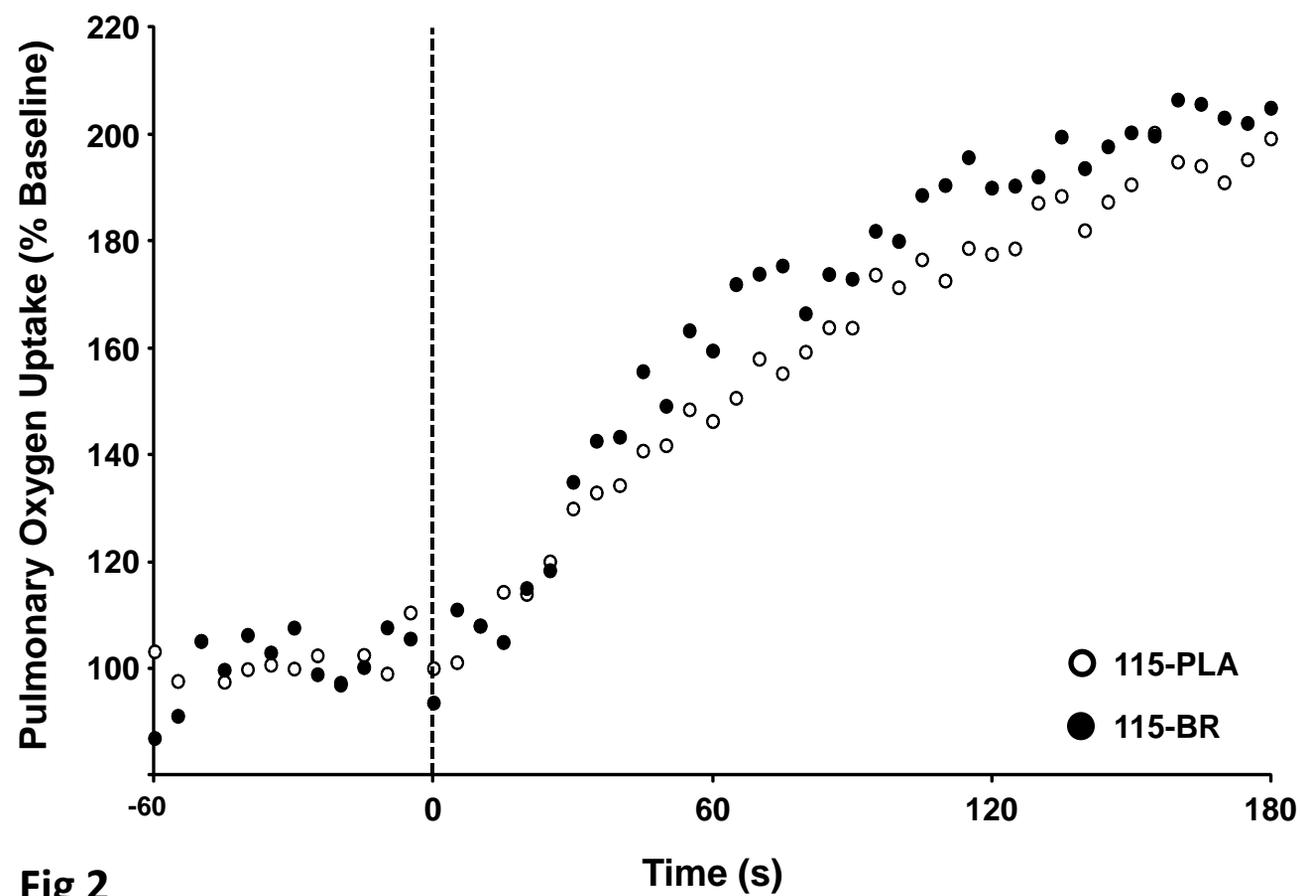
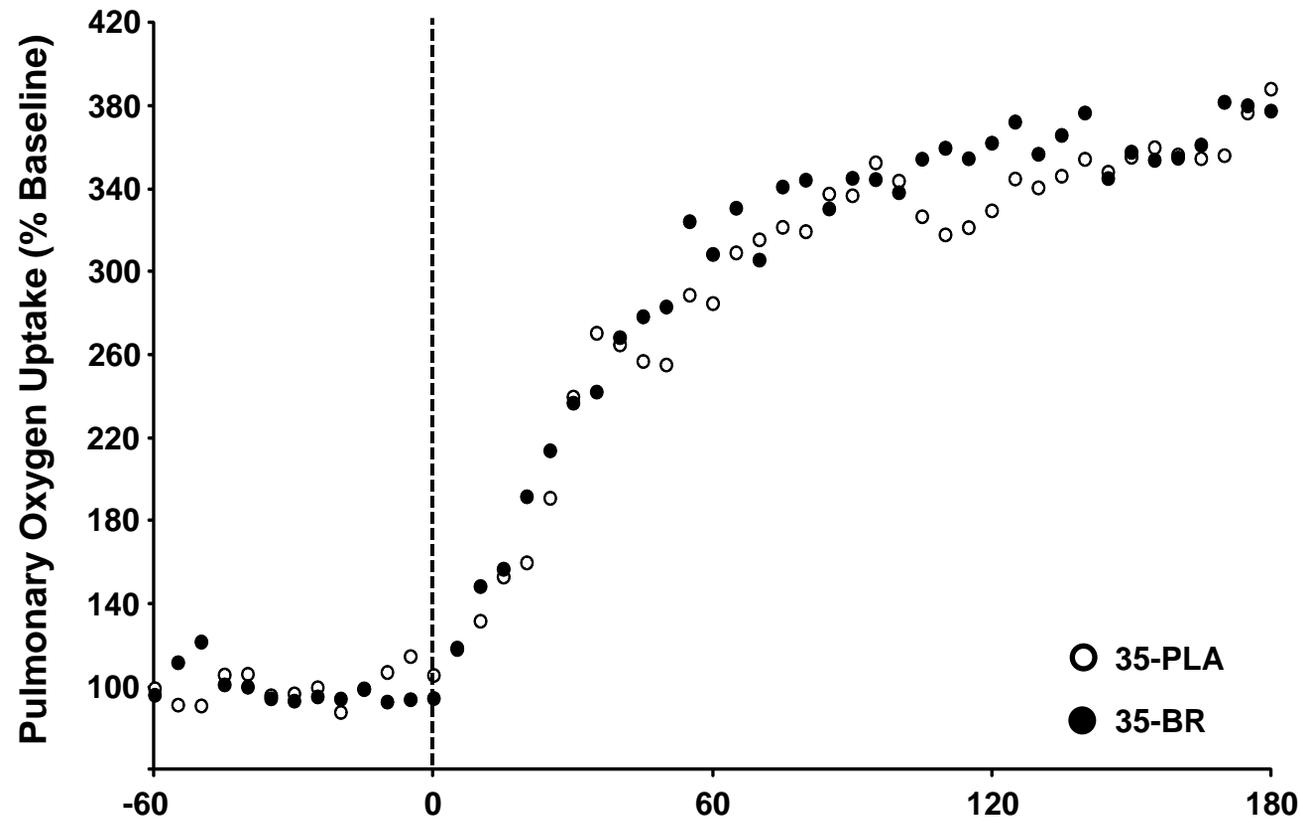


Fig 2

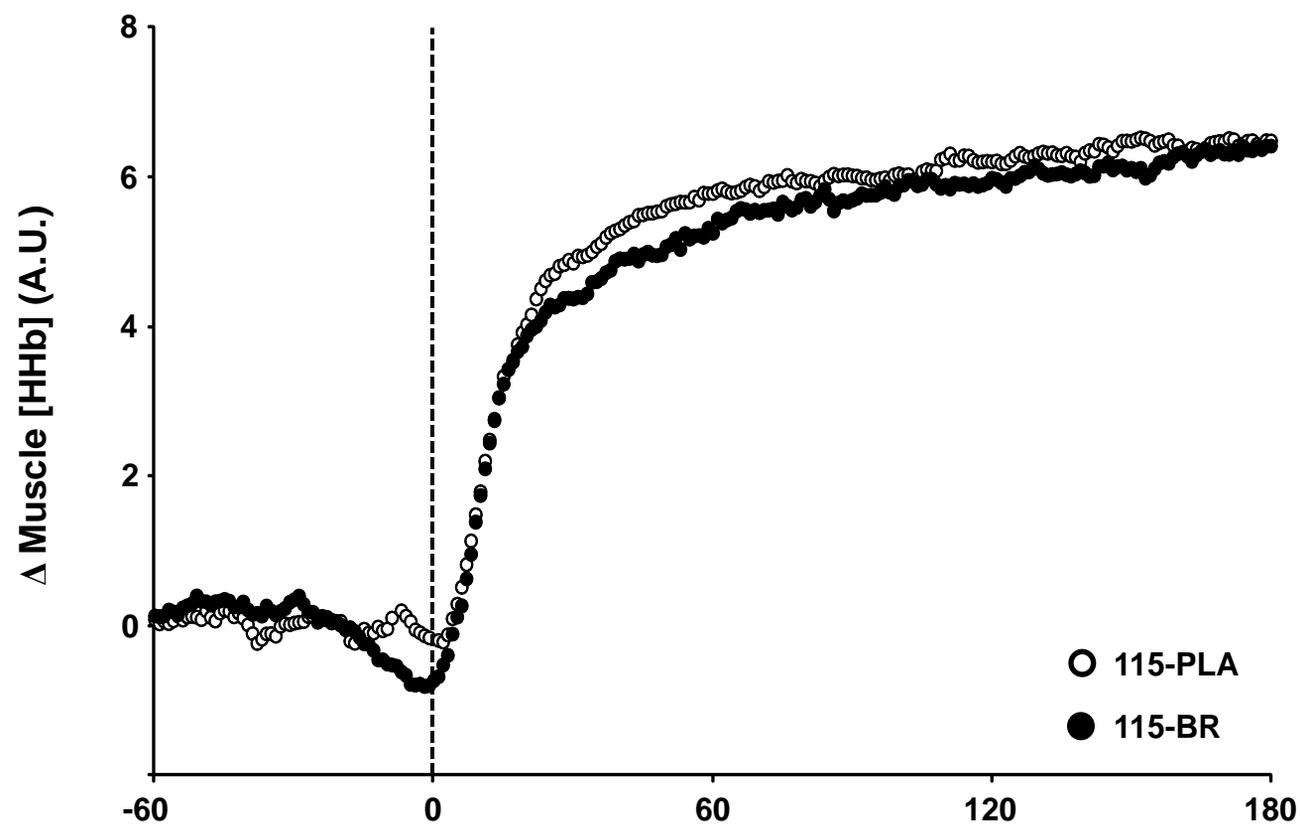
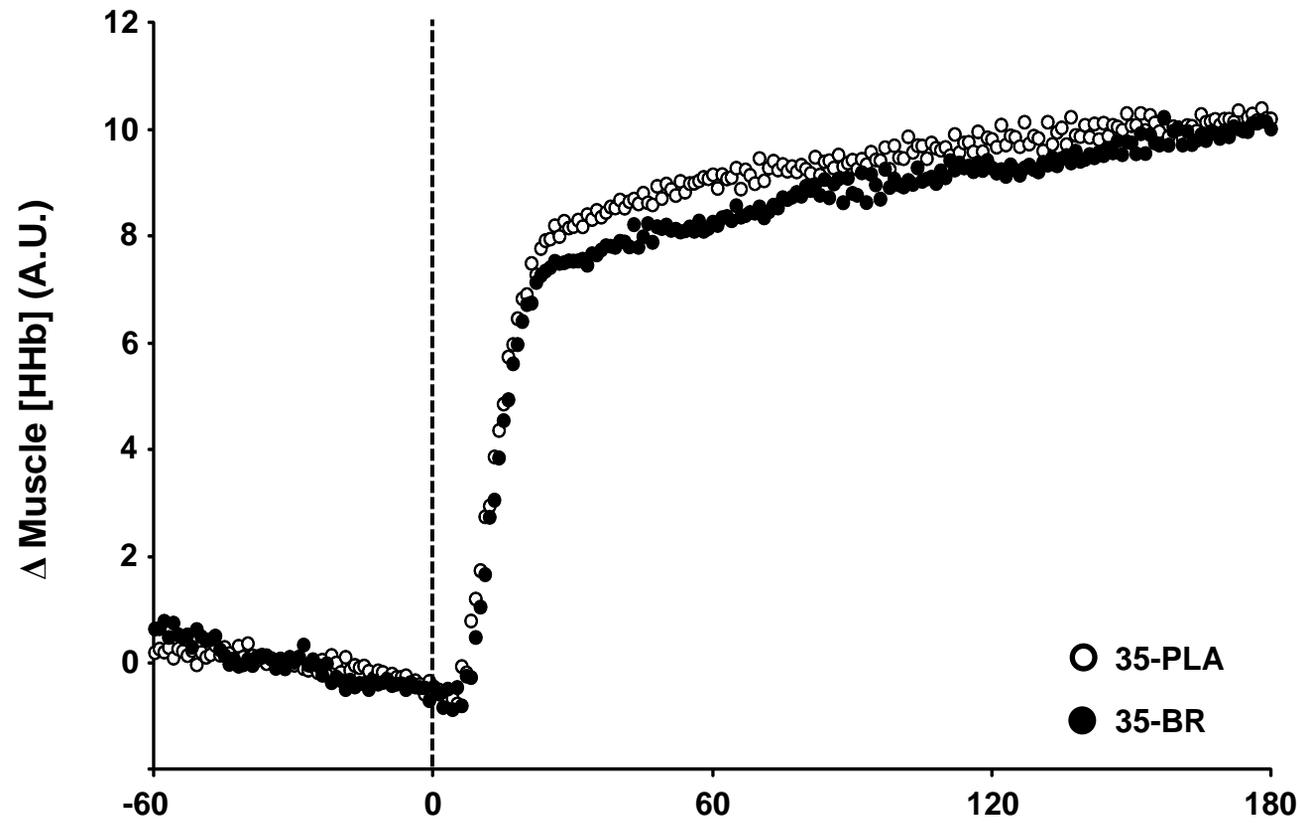


Fig 3

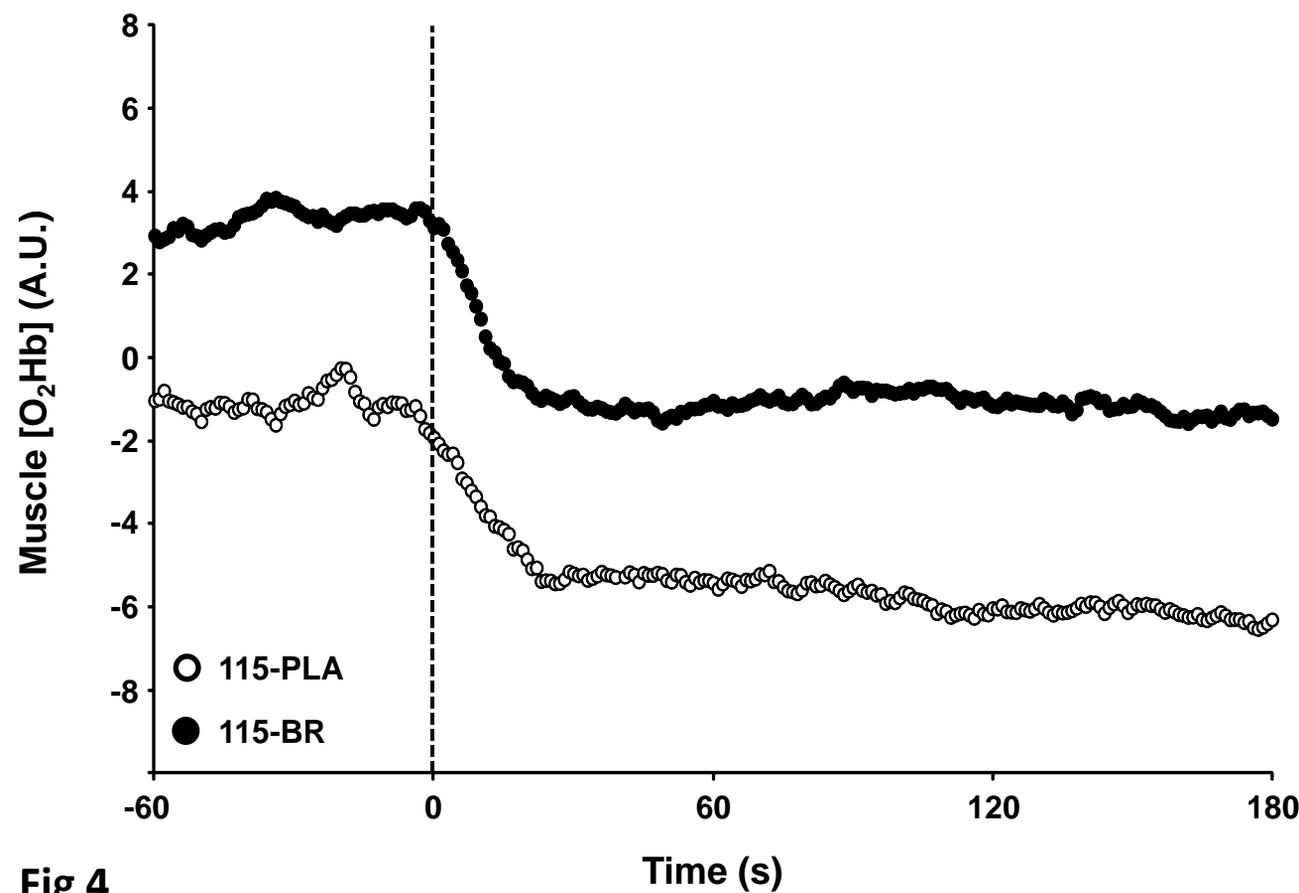
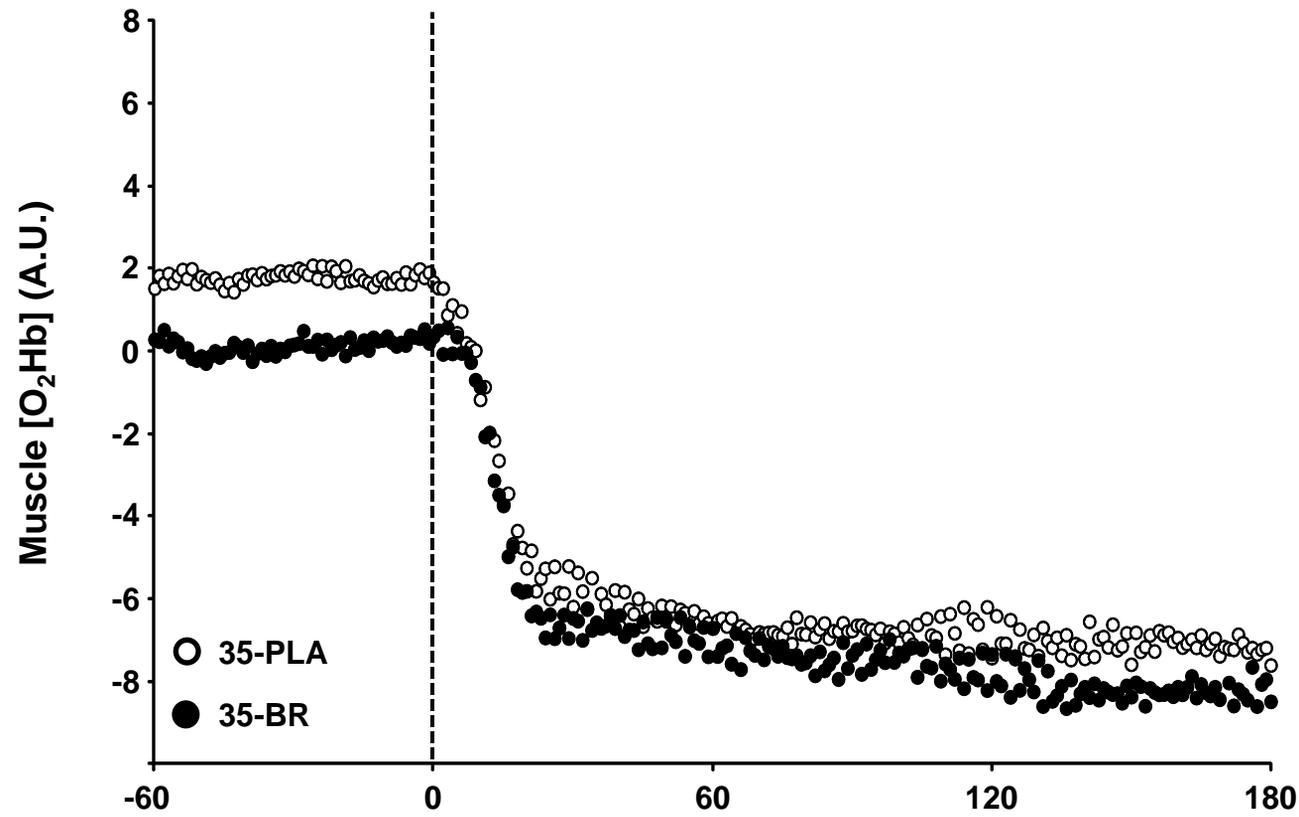


Fig 4

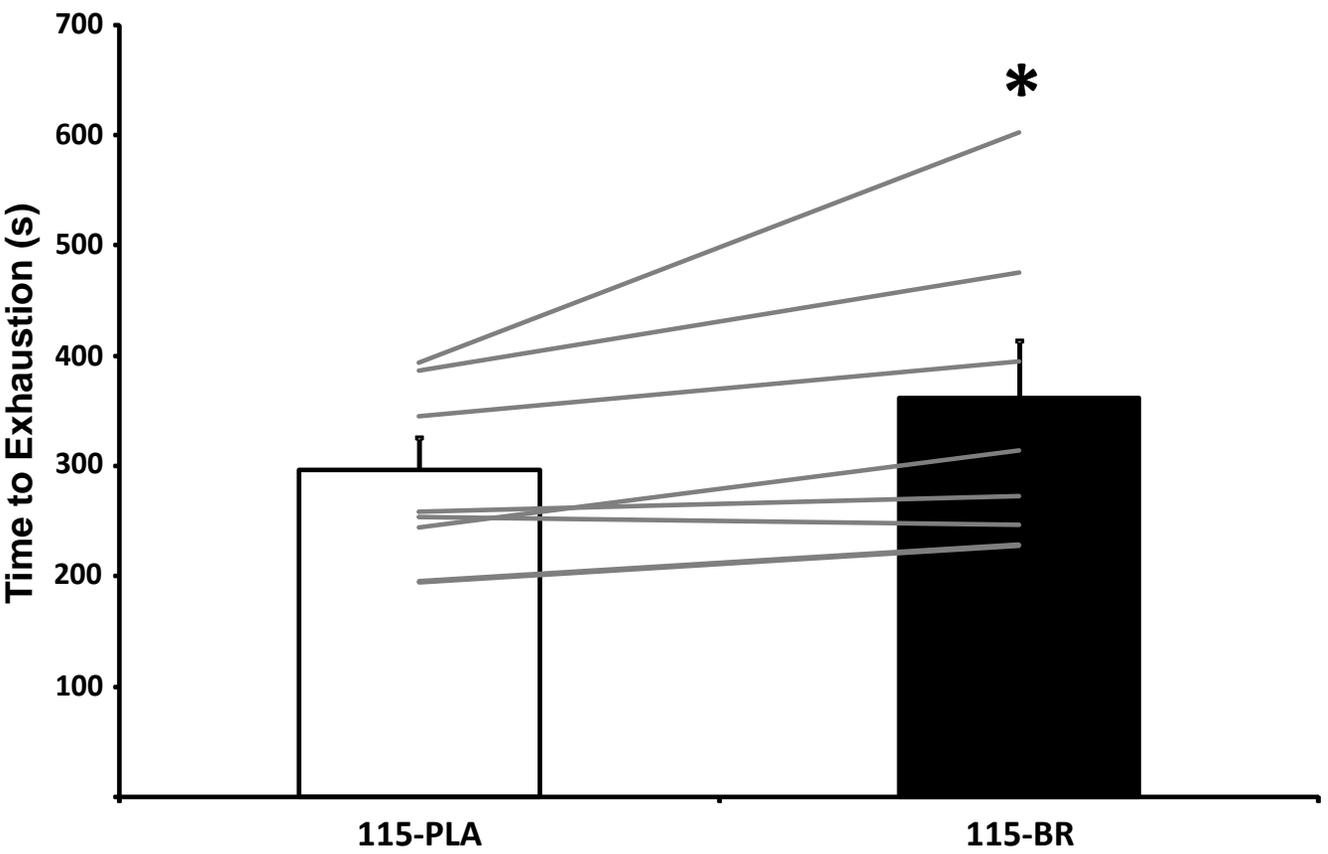
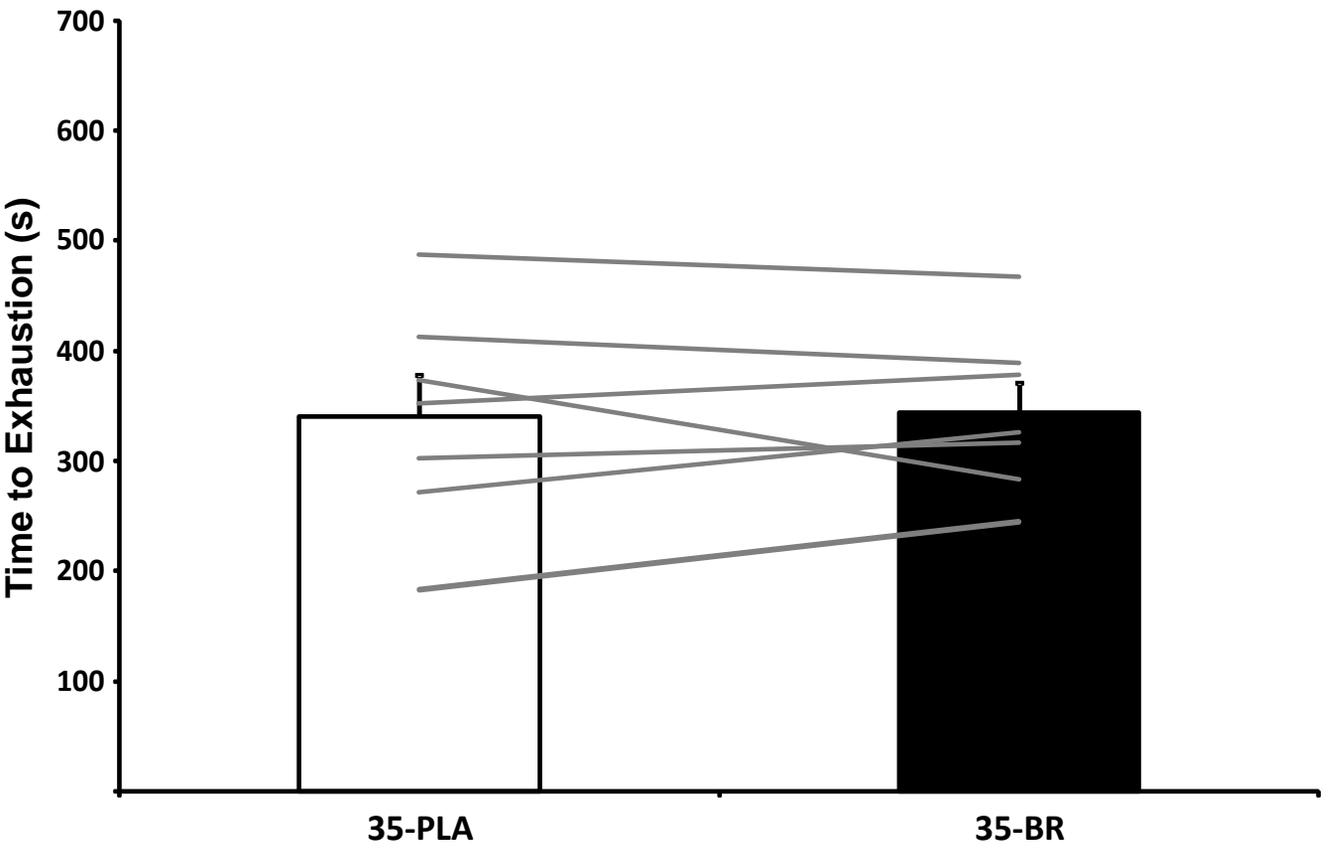


Fig 5