

1 **Discrete physiological effects of beetroot juice and potassium**  
2 **nitrate supplementation following 4 weeks sprint interval training**

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11 **Running head:** Dietary nitrate and sprint interval training

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19

20 **Abstract**

21 The physiological and exercise performance adaptations to sprint interval training (SIT) may  
22 be modified by dietary nitrate ( $\text{NO}_3^-$ ) supplementation. However, it is possible that different  
23 types of  $\text{NO}_3^-$  supplementation evoke divergent physiological and performance adaptations to  
24 SIT. The purpose of this study was to compare the effects of 4 weeks SIT with and without  
25 concurrent dietary  $\text{NO}_3^-$  supplementation administered as either  $\text{NO}_3^-$ -rich beetroot juice (BR)  
26 or potassium  $\text{NO}_3^-$  ( $\text{KNO}_3$ ). Thirty recreationally-active subjects completed a battery of  
27 exercise tests before and after a 4 week intervention in which they were allocated to one of  
28 three groups: 1) SIT undertaken without dietary  $\text{NO}_3^-$  supplementation (SIT); 2) SIT  
29 accompanied by concurrent BR supplementation (SIT+BR); or 3) SIT accompanied by  
30 concurrent  $\text{KNO}_3$  supplementation (SIT+ $\text{KNO}_3$ ). During severe-intensity exercise,  $\dot{V}\text{O}_{2\text{peak}}$  and  
31 time to task failure were improved to a greater extent with SIT+BR than SIT and SIT+ $\text{KNO}_3$   
32 ( $P<0.05$ ). There was also a greater reduction in the accumulation of muscle lactate at 3-min of  
33 severe-intensity exercise in SIT+BR compared to SIT+ $\text{KNO}_3$  ( $P<0.05$ ). Plasma  $[\text{NO}_2^-]$  fell to  
34 a greater extent during severe-intensity exercise in SIT+BR compared to SIT and SIT+ $\text{KNO}_3$   
35 ( $P<0.05$ ). There were no differences between groups in the reduction in the muscle  
36 phosphocreatine recovery time constant from pre- to post-intervention ( $P>0.05$ ). These  
37 findings indicate that 4 weeks SIT with concurrent BR supplementation results in greater  
38 exercise capacity adaptations compared to SIT alone and SIT with concurrent  $\text{KNO}_3$   
39 supplementation. This may be the result of greater NO-mediated signalling in SIT+BR  
40 compared to SIT+ $\text{KNO}_3$ .

41 **Keywords:** nitric oxide; fitness; diet; endurance; ergogenic aid; nutrition.

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43 New and Noteworthy: We compared the influence of different forms of dietary nitrate  
44 supplementation on the physiological and performance adaptations to sprint interval training  
45 (SIT). Compared to SIT alone, supplementation with nitrate-rich beetroot juice, but not KNO<sub>3</sub>,  
46 enhanced some physiological adaptations to training.

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## 50 **Introduction**

51 Over the last decade, dietary supplementation with inorganic nitrate ( $\text{NO}_3^-$ ) has emerged as a  
52 popular pre-competition nutrition strategy to enhance athletic performance. This practice  
53 originates from evidence that dietary  $\text{NO}_3^-$  supplementation can enhance a range of  
54 physiological processes, including skeletal muscle metabolic (2, 35, 62), contractile (11, 28,  
55 29, 66) and vascular function (16, 17) with attendant implications for improved performance  
56 in a range of exercise settings (5, 33, 58, 60, 69).

57 Favourable effects on resting blood pressure (BP) have been observed following  $\text{NO}_3^-$  ingested  
58 in the form of both  $\text{NO}_3^-$ -rich beetroot juice (BR; 31, 64) and  $\text{NO}_3^-$  salts ( $\text{KNO}_3$  or  $\text{NaNO}_3$ ; 1,  
59 32, 34). However, some recent studies suggest that BR/ $\text{NO}_3^-$ -rich vegetables and  $\text{NO}_3^-$  salts can  
60 evoke disparate physiological effects (18, 30). The improvement in some physiological  
61 responses in the contracting skeletal muscle following  $\text{NO}_3^-$  supplementation has been  
62 attributed to enhanced nitroso signalling facilitated by elevated nitrite ( $\text{NO}_2^-$ ), s-nitrosothiol  
63 and/or NO bioavailability (40, 57, 64). Importantly, the co-ingestion of the betacyanins and  
64 polyphenols found in BR may increase the capacity for NO synthesis from  $\text{NO}_2^-$  (21, 50).  
65 Moreover, chlorogenic acid, a constituent of BR, has been shown to promote NO release by  
66 human saliva at the acidic pH of the stomach (50). Therefore, ingesting  $\text{NO}_3^-$  as BR, which is  
67 accompanied by the co-ingestion of compounds that might facilitate the  $\text{NO}_3^-$ -  $\text{NO}_2^-$  -NO  
68 pathway, has the potential to enhance NO-mediated physiological signalling compared to a  
69 similar dose of  $\text{NO}_3^-$  administered as  $\text{NO}_3^-$  salts.

70 In addition to enhanced physiological and performance responses following  $\text{NO}_3^-$   
71 supplementation in an acute exercise setting, recent evidence suggest that  $\text{NO}_3^-$   
72 supplementation in the form of BR (59), a high  $\text{NO}_3^-$  gel (44) or  $\text{NaNO}_3$  (14) can modulate  
73 some of the physiological and performance adaptations to sprint interval training (SIT).

74 Increased exposure to  $\text{NO}_3^-$  or  $\text{NO}_2^-$  has been reported to activate PGC1- $\alpha$  (51) and AMPK (42)  
75 which initiate signalling cascades that promote adaptive skeletal muscle remodelling to  
76 exercise (23). However, while consumption of BR or  $\text{NO}_3^-$ -rich vegetables can lower the  $\text{O}_2$   
77 cost of submaximal exercise (18) and BP (30) compared to an equivalent dose of  $\text{NaNO}_3$ , it  
78 has yet to be determined whether equimolar doses of inorganic  $\text{NO}_3^-$  administered through  
79 different supplementation vehicles elicit comparable or divergent physiological and  
80 performance adaptations to an exercise training program.

81 As well as serving as an NO precursor, BR contains a number of compounds with antioxidant  
82 properties (55, 67). The most abundant betacyanin present in BR, betanin, has been shown to  
83 possess high antioxidant activity (9). There is some evidence to suggest that supplementation  
84 with antioxidant compounds during an exercise training program can blunt some of the skeletal  
85 muscle adaptive responses to exercise training (26, 43, 48). It has been reported that BR  
86 contains a high total antioxidant capacity relative to other vegetable juices (67), although this  
87 would be substantially lower than in training studies reporting compromised training  
88 adaptations in which high doses of vitamin C (1 g) and vitamin E (up to 294 mg) were  
89 administered (43, 48). Therefore, while BR has the potential to augment NO-mediated  
90 signalling, and by extension the activation of transcription pathways integral to muscle  
91 remodelling to exercise, it remains possible that this benefit might be offset by the antioxidant  
92 effects of BR supplementation and the potential suppression of exercise-evoked adaptive  
93 signalling cascades. Therefore, further research is required to address the influence of the  $\text{NO}_3^-$   
94 administration vehicle on the adaptations to an exercise training program.

95 The purpose of this study was to compare the physiological and exercise performance  
96 adaptations to 4 weeks SIT accompanied by concurrent supplementation with BR (SIT+BR)  
97 or potassium  $\text{NO}_3^-$  ( $\text{KNO}_3$ ) (SIT+ $\text{KNO}_3$ ) or SIT undertaken without dietary  $\text{NO}_3^-$   
98 supplementation (SIT). It was hypothesised that exercise performance, muscle oxidative

99 capacity measured *in vivo* using <sup>31</sup>phosphorus magnetic resonance spectroscopy (<sup>31</sup>P-MRS),  
100 and muscle metabolic adaptations measured from muscle biopsy samples would be improved  
101 following all SIT interventions, but that these variables would be improved more in the  
102 SIT+BR group compared to the SIT+KNO<sub>3</sub> and SIT groups due to enhanced NO-mediated  
103 physiological signalling in SIT+BR.

## 104 **Methods**

### 105 *Subjects*

106 Eighteen male (mean ± SD: age 25 ± 6 years, height 1.82 ± 0.07 m, body mass 85 ± 12 kg,  
107  $\dot{V}O_{2peak}$  46.6 ± 7.5 mL·kg<sup>-1</sup>·min<sup>-1</sup>) and 12 female (mean ± SD: age 22 ± 3 years, height 1.71 ±  
108 0.08 m, body mass 66 ± 10 kg,  $\dot{V}O_{2peak}$  39.9 ± 3.9 mL·kg<sup>-1</sup>·min<sup>-1</sup>) volunteers were recruited.  
109 The subjects were involved in team and/or endurance sports but were not highly trained.  
110 Following an explanation of the experimental procedures, associated risks, potential benefits  
111 and likely value of the possible findings, subjects gave their written informed consent to  
112 participate. The study was approved by the Institutional Research Ethics Committee and  
113 conformed to the code of ethics of the Declaration of Helsinki.

### 114 *Experimental design*

115 Subjects initially visited the laboratory on 3 separate occasions over a 5 day period. On visit 1,  
116 subjects completed an incremental exercise test on a cycle ergometer for the determination of  
117  $\dot{V}O_{2peak}$  and gas exchange threshold (GET). The work rates requiring 80% of the GET (moderate  
118 exercise) and 85%Δ (GET plus 85% of the difference between the work rate at GET and  
119  $\dot{V}O_{2peak}$ ; severe exercise) were calculated and adjusted for mean response time for  $\dot{V}O_2$  during  
120 incremental exercise (65). Following this, subjects were familiarized to the exercise testing  
121 procedures, including completion of a severe-intensity bout of cycle ergometry until  
122 exhaustion. On visit 2, subjects completed the <sup>31</sup>P-MRS protocol (see below) before a 5-min

123 bout of moderate-intensity cycling and an incremental exercise test were performed. On visit  
124 3, subjects completed 2 bouts of severe-intensity cycling, the first for 3-min and the second  
125 until task failure.

126 A third party (not associated with data collection and analysis) received the baseline  
127 characteristics of each participant before assigning participants, in a group characteristic  
128 matched manner, to one of three SIT groups. Thereafter, in a double-blind, independent-groups  
129 design, subjects were enrolled onto a 4-week supervised SIT program and assigned to receive  
130 either  $\text{NO}_3^-$  rich BR (SIT+BR; age  $25 \pm 7$  years, height  $1.76 \pm 0.11$  m, body mass  $80 \pm 19$  kg),  
131  $\text{KNO}_3^-$  (SIT+ $\text{KNO}_3$ ; age  $25 \pm 3$  years, height  $1.76 \pm 0.09$  m, body mass  $75 \pm 13$  kg) or water  
132 (SIT; age  $22 \pm 3$  years, height  $1.79 \pm 0.08$  m, body mass  $78 \pm 12$  kg) for 28 days. The SIT+BR  
133 and SIT+ $\text{KNO}_3$  groups were deliberately misinformed that they might be consuming  
134 supplements that were either active ( $\text{NO}_3^-$ -rich) or placebo alternatives. All three groups  
135 consisted of 6 male and 4 female subjects. Each group consumed 1 x 70 mL of their allocated  
136 supplements (SIT+BR; ~6.4 mmol of  $\text{NO}_3^-$  per 70 mL; Beet it, James White Drinks Ltd.,  
137 Ipswich, UK; SIT+ $\text{KNO}_3$ : ~6.4 mmol of  $\text{NO}_3^-$  per 70 mL; Minerals-Water.ltd, Purfleet, UK)  
138 in the morning and 1 x 70 mL in the evening of each day for the duration of the training period.  
139 This approach is expected to result in elevated plasma [ $\text{NO}_3^-$ ] and [ $\text{NO}_2^-$ ] for each 24 h period  
140 (68). On experimental visits following the intervention period, subjects consumed 2 x 70 mL of  
141 their allocated supplement 2.5 h prior to the exercise tests. Compliance to the supplementation  
142 procedures was confirmed by the return of empty bottles each week and via the completion of  
143 questionnaires during and following the intervention period.

144 All groups completed the same exercise tests (at the same absolute work rates) and  
145 physiological assessments both before and after the 28-day intervention period. Laboratory  
146 visits were scheduled at the same time of day ( $\pm 2$  h). Subjects were asked to maintain their

147 normal dietary and exercise behavior throughout the study. However, subjects were instructed  
148 to record their diet during the 24 h preceding the first laboratory visit and to repeat this for all  
149 subsequent laboratory visits. On training days, subjects were asked to arrive at the training  
150 venue  $\geq 1$  h post-prandial and to complete a 5 min self-paced warm up before training  
151 commenced. On experimental days, subjects were instructed to arrive at the laboratory  $\geq 3$  h  
152 post-prandial having avoided strenuous exercise and the consumption of alcohol and caffeine  
153 in the 24 h preceding each exercise test. For the duration of the study, subjects were asked to  
154 refrain from taking other dietary supplements and to avoid the use of antibacterial mouthwash  
155 as this inhibits the reduction of  $\text{NO}_3^-$  to  $\text{NO}_2^-$  in the oral cavity by eliminating commensal  
156 bacteria (27).

#### 157 *Training intervention*

158 During the training sessions, all subjects completed a series of 30-s “all-out” sprints (i.e.  
159 Wingate test) against a resistance equivalent to 7.5% body mass on a mechanically-braked  
160 cycle ergometer (model 814E, Monark, Stockholm, Sweden). Each sprint was separated by a  
161 4-min period of rest in which subjects cycled at a low cadence against a light resistance to  
162 reduce venous pooling and sensations of nausea. During weeks 1 and 2 of training, subjects  
163 performed 4 x 30-s sprints three times per week, while during weeks 3 and 4, subjects  
164 performed 5 x 30-s sprints four times per week. Following a 5-min warm up of cycling against  
165 a light resistance, subjects were given a 10-s count down and instructed to pedal maximally for  
166 2 s before the appropriate load was applied. Subjects were verbally encouraged to maintain  
167 maximal cadence throughout each 30-s sprint. All groups completed a total of 14 supervised  
168 training sessions over a 4-week period, with at least 24 h separating each training session. The  
169 post-intervention laboratory tests were performed at least 48 h following, but within 4 days of  
170 the completion of the final training session. All subjects completed 100% of the training  
171 sessions and 100% of the sprints during the intervention.

172 *Incremental exercise tests*

173 Subjects completed all ramp incremental exercise tests on an electronically braked cycle  
174 ergometer (Lode Excalibur Sport, Groningen, Netherlands). The self-selected cadence (75-90  
175 rpm), saddle and handle bar height and configuration for each subject were recorded on the  
176 first visit and reproduced in subsequent visits. Initially, subjects performed 3-min of baseline  
177 cycling at 20 W, after which the work rate was increased by 30 W/min until task failure. Breath-  
178 by-breath pulmonary gas exchange data (Oxycon Pro, Jaeger, Hoechberg, Germany) were  
179 collected continuously throughout all incremental tests and were averaged over 10-s periods.  
180  $\dot{V}O_{2\text{peak}}$  and GET were determined as previously described (61).

181 *Step exercise tests*

182 A 5-min moderate-intensity “step” test was performed on the first laboratory visit before and  
183 following the intervention. This was completed 10 min before the ramp incremental test  
184 protocol was initiated. On the second laboratory visit before and following the intervention,  
185 two severe-intensity step tests were performed, separated by a 20 min period of rest; the first  
186 until 3-min, and the second until task failure. The time to task failure was recorded once the  
187 pedal rate fell by >10 rpm below the target cadence. All step tests began with 3-min of pedaling  
188 at 20 W before a sudden transition to the target work rate. Muscle biopsies were obtained before  
189 and following the 3-min severe-intensity exercise bout and again at task failure in the second  
190 bout. Breath-by-breath pulmonary gas exchange data were collected continuously throughout  
191 all step tests.

192 *<sup>31</sup>P-MRS protocol*

193 Before the initial experimental visit, participants were familiarised to the <sup>31</sup>P-MRS protocol  
194 using a custom-built, non-ferrous ergometer in the bore of a purpose built ‘mock’ magnetic  
195 resonance scanner. Before and following the intervention, subjects completed four bouts of

196 single-leg knee extension exercise (over a distance of ~0.22 m) in a prone position within the  
197 bore of a 1.5 T superconducting magnet (Gyrosan Clinical Intera, Philips, The Netherlands)  
198 using a custom-built, non-ferrous ergometer at the University of Exeter Magnetic Resonance  
199 Research Centre (Exeter, UK) as previously described (20, 62). Each 24-s bout of high-  
200 intensity exercise ( $20 \pm 4$  W) was separated by 4 min.  $^{31}\text{P}$ -MRS was used for the assessment  
201 of muscle PCr recovery kinetics with data acquired every 1.5 s leading to a spectrum every 6 s  
202 via a four phase cycle protocol. Due to technical issues with the scanner at the start of data  
203 collection the subject number included in the analyses was restricted within each group to  $n =$   
204 9.

#### 205 *Measurements*

##### 206 *Blood pressure*

207 Before and following the intervention the BP at the brachial artery was measured using an  
208 automated sphygmomanometer (Dinamap Pro: GE Medical Systems, Tampa, FL). Following  
209 10 min seated rest in an isolated room, three measurements were recorded. The means of the  
210 systolic and diastolic measurements were used for data analysis.

##### 211 *Blood analysis*

212 Venous blood was sampled at rest (baseline) before each experimental test. Blood samples  
213 were also obtained at 1-min, at 3-min and at exhaustion during the severe-intensity exercise  
214 bout. The blood samples collected during the severe-intensity exercise bout were drawn from  
215 a cannula (Insyte-WTM, Becton Dickinson, Madrid, Spain) inserted into the subject's  
216 antecubital vein and were collected into lithium-heparin vacutainers (Becton Dickinson, New  
217 Jersey, USA). Blood [lactate] and [glucose], as well as plasma [ $\text{NO}_2^-$ ] were analyzed in all  
218 samples (square brackets denote concentration). 200  $\mu\text{L}$  of blood was immediately extracted  
219 from the lithium-heparin vacutainers and hemolysed in 200  $\mu\text{L}$  of Triton X-100 solution (Triton

220 X-100, Amresco, Salon, OH) before blood [lactate] and [glucose] were measured (YSI 2300,  
221 Yellow Springs Instruments, Yellow Springs, OH). The remaining whole blood from each  
222 sample was centrifuged at 4000 rpm for 8 min at 4 °C within 2 min of collection. Plasma was  
223 immediately extracted, frozen at -80 °C and subsequently analyzed for [NO<sub>2</sub><sup>-</sup>] using  
224 chemiluminescence, as previously described (68).

#### 225 *Muscle biopsy*

226 Muscle samples were obtained from two incisions from the medial region of the *m. vastus*  
227 *lateralis* under local anesthesia (1% lidocaine) using the percutaneous Bergström needle biopsy  
228 technique with suction (4). Muscle samples were taken at three different time points before and  
229 following the intervention: at rest; following 3-min of severe-intensity exercise; and at task  
230 failure during severe-intensity exercise. The post-exercise biopsies were taken while subjects  
231 remained on the cycle ergometer and were typically collected within 10 s of the completion of  
232 the exercise bout. Biopsy samples were immediately frozen in liquid nitrogen and stored at -  
233 80 °C for subsequent analysis.

#### 234 *Muscle metabolites*

235 Following a freeze-drying process, samples were dissected to remove visible blood, fat, and  
236 connective tissue. Approximately 2 mg aliquots of isolated muscle fibers were weighed on fine  
237 balance scales (Mettler Toledo XS105, Leicester, UK) and stored in 500 µL microcentrifuge  
238 tubes at -80 °C. Prior to metabolite analysis, 200 µL of 3 M perchloric acid was added to ~2  
239 mg dry weight (d.w.) muscle tissue. Following 3 min centrifugation and 30 min incubation on  
240 ice, 170 µL of supernatant was transferred to a fresh microcentrifuge tube and 255 µL of cooled  
241 2 M potassium bicarbonate (KHCO<sub>3</sub>) was added. This was centrifuged, and the supernatant  
242 analyzed for [PCr], [ATP] and [lactate] by fluorometric assays as previously described (39).

#### 243 *Muscle glycogen and pH*

244 Glycogen was extracted from ~2 mg d.w. muscle in 500  $\mu$ L of 1 M hydrochloric acid (HCl)  
245 and hydrolyzed at 100 °C for 3 h to glycosyl units, which were measured using an automated  
246 glucose analyser (YSI 2300, Yellow Springs Instruments, Yellow Springs, OH) to determine  
247 muscle [glycogen]. Muscle pH was measured using a micro-pH meter (Sentron SI600, Roden,  
248 The Netherlands) following homogenization of ~1 mg d.w. muscle in 500  $\mu$ L of a non-  
249 buffering solution (145 mM KCl, 10 mM NaCl and 5 mM NaF).

#### 250 *Muscle fiber type*

251 Approximately 20 mg of tissue obtained from each resting muscle biopsy sample was  
252 embedded in Tissue-Tek® O.T.C.™ compound (Sakura Finetek Europe BV Zoeterwoude,  
253 The Netherlands), rapidly frozen in liquid nitrogen-cooled isopentane, and stored at -80 °C for  
254 subsequent histochemical analysis of myocellular characteristics. Serial cross sections (~10  $\mu$ M  
255 thick) were cut in a cryostat (Cryostar NX50, Thermo Scientific, USA) maintained at -16 °C.  
256 Sections were mounted on 3 separate slides and pre-incubated at pH values of 4.3, 4.6 and 10.3.  
257 According to the lability to the acid and alkaline pre-incubation, the fibers were stained for  
258 myofibrillar ATPase, identified as type I, IIa, or IIx and counted under an Olympus CKX41  
259 microscope with cellSens Dimension software (Olympus Corporation, Tokyo, Japan).

#### 260 *Data analysis procedures*

261 *Oxygen uptake.* The breath-by-breath  $\dot{V}O_2$  data from each step exercise test were initially  
262 examined to exclude values lying more than four SDs from the local mean. The filtered data  
263 were subsequently linearly interpolated to provide second-by-second values and time-aligned  
264 to the start of exercise for each individual. The baseline  $\dot{V}O_2$  was defined as the mean  $\dot{V}O_2$   
265 measured over the final 60 s of the 3-min baseline period. The end-exercise  $\dot{V}O_2$  was defined  
266 as the mean  $\dot{V}O_2$  measured over the final 30 s of exercise.

267 *PCr recovery kinetics.* The acquired spectra were quantified using the jMRUI (version 3)  
268 software package employing the AMARES peak fitting algorithm as previously described (20,  
269 62). Using Prism 6 software (GraphPad Software Inc., La Jolla, CA, USA), the PCr recovery  
270 for each 24 s recovery period was fitted individually by a single exponential of the form

$$271 \text{PCr}(t) = \text{PCr}_{\text{end}} + \text{PCr}_{(0)} (1 - e^{-(t/\tau)})$$

272 where  $\text{PCr}_{\text{end}}$  is the end exercise PCr value,  $\text{PCr}_{(0)}$  is the difference between  $\text{PCr}_{\text{end}}$  and full  
273 recovery,  $t$  is the time from exercise cessation and  $\tau$  is the time constant for the exponential  
274 recovery of PCr. A value of  $\tau$  was calculated for each trial and then a mean determined from  
275 the four individual 24 s recovery periods.

### 276 *Statistical analyses*

277 Physiological and performance differences consequent to the interventions were assessed using  
278 analysis of covariance (ANCOVA) with baseline values used as a covariate. This approach was  
279 used to adjust for any small but potentially physiologically important chance imbalances at  
280 baseline (54). Significant effects were followed up by Fisher's LSD post hoc tests. Data that  
281 were not normally distributed were log transformed before applying the ANCOVA. All values  
282 are reported as mean  $\pm$  SD. Statistical significance was accepted at  $P < 0.05$ .

## 283 **Results**

### 284 *Plasma [NO<sub>2</sub><sup>-</sup>]*

285 Resting plasma [NO<sub>2</sub><sup>-</sup>] was increased in SIT+BR (Pre: 39  $\pm$  18 vs. Post: 257  $\pm$  112 nM) and  
286 SIT+KNO<sub>3</sub> (Pre: 75  $\pm$  20 vs. Post: 310  $\pm$  123 nM) compared to SIT (Pre: 45  $\pm$  52 vs. Post: 55  
287  $\pm$  83 nM) ( $P < 0.05$ ). There was no difference in the change in resting plasma [NO<sub>2</sub><sup>-</sup>] between  
288 SIT+BR and SIT+KNO<sub>3</sub> ( $P > 0.05$ ). However, the change in plasma [NO<sub>2</sub><sup>-</sup>] during severe-

289 intensity cycling was greater at 3-min in SIT+BR ( $104 \pm 92$  nM reduction) compared to  
290 SIT+KNO<sub>3</sub> ( $59 \pm 108$  nM reduction) ( $P < 0.05$ ; Fig. 1).

### 291 *Blood Pressure*

292 Systolic BP was reduced in SIT+BR compared to SIT ( $P < 0.05$ ) but not SIT+KNO<sub>3</sub> ( $P > 0.05$ ;  
293 Table 1). The change in systolic BP in SIT was not different compared to SIT+KNO<sub>3</sub> ( $P > 0.05$ ).  
294 Diastolic BP was reduced in SIT+BR compared to both SIT and SIT+KNO<sub>3</sub> ( $P < 0.05$ ; Table  
295 1). There was no difference in diastolic BP between SIT and SIT+KNO<sub>3</sub> ( $P > 0.05$ ).

### 296 *Incremental exercise test*

297 None of the interventions resulted in a significant change in body mass (SIT+BR: Pre:  $80 \pm 19$   
298 kg, Post:  $80 \pm 19$  kg; SIT+KNO<sub>3</sub>: Pre:  $75 \pm 13$  kg, Post:  $74 \pm 13$  kg; SIT: Pre:  $78 \pm 12$  kg, Post:  
299  $77 \pm 11$  kg;  $P > 0.05$ ). The  $\dot{V}O_{2peak}$  was increased to a greater extent in SIT+BR (11% increase)  
300 compared to both SIT (6% increase) and SIT+KNO<sub>3</sub> (4% increase) ( $P < 0.05$ ; Table 1). There  
301 was no difference in the change in  $\dot{V}O_{2peak}$  between SIT and SIT+KNO<sub>3</sub> ( $P > 0.05$ ). The increase  
302 in peak WR was not different between groups ( $P > 0.05$ ; Table 1).

### 303 *Step exercise tests*

304 The steady-state  $\dot{V}O_2$  during moderate-intensity exercise was reduced in SIT+BR and  
305 SIT+KNO<sub>3</sub> compared to SIT ( $P < 0.05$ ; Table 1). There was no difference in the extent of the  
306 reduction in steady-state  $\dot{V}O_2$  between SIT+BR and SIT+KNO<sub>3</sub> ( $P > 0.05$ ). There were no  
307 differences in baseline cycling  $\dot{V}O_2$  between groups ( $P > 0.05$ ).

308 The time to task failure during severe-intensity cycling was increased to a greater extent in  
309 SIT+BR (71% increase) compared to both SIT (47% increase) and SIT+KNO<sub>3</sub> (42% increase)  
310 ( $P < 0.05$ ; Fig. 2). There was no difference in the change in time to task failure between SIT and

311 SIT+KNO<sub>3</sub> ( $P>0.05$ ).  $\dot{V}O_{2peak}$  attained during severe-intensity cycling was not different to that  
312 attained during incremental cycling. The change from pre- to post-intervention in blood  
313 [lactate] sampled at 1-min of severe-intensity cycling was significantly different in SIT+BR  
314 (Pre:  $5.1 \pm 1.4$  vs. Post:  $3.5 \pm 0.8$  mM) compared to SIT (Pre:  $3.0 \pm 1.4$  vs. Post:  $1.9 \pm 0.9$  mM)  
315 ( $P<0.05$ ) but not SIT+KNO<sub>3</sub> (Pre:  $3.9 \pm 1.7$  vs. Post:  $2.6 \pm 0.7$  mM) ( $P>0.05$ ). There was no  
316 difference in blood [lactate] between SIT and SIT+KNO<sub>3</sub> ( $P>0.05$ ). Blood [glucose] measured  
317 during severe-intensity exercise was not different between groups ( $P>0.05$ ).

### 318 *PCr recovery kinetics*

319 The reduction in muscle PCr recovery time constant following the cessation of 24 s high  
320 intensity knee-extension exercise was not different between groups (SIT: Pre:  $31.6 \pm 10.3$  vs.  
321 Post:  $24.7 \pm 6.2$  s; SIT+BR: Pre:  $28.4 \pm 4.9$  vs. Post:  $25.9 \pm 3.5$  s; SIT+KNO<sub>3</sub>: Pre:  $31.7 \pm 8.0$   
322 vs. Post:  $26.7 \pm 7.3$  s;  $P>0.05$ ; Fig. 3).

### 323 *Muscle substrates and metabolites*

324 The concentrations of muscle ATP, PCr and glycogen, and muscle pH measured during severe-  
325 intensity cycling were not different between groups ( $P>0.05$ ; Table 2). However, muscle  
326 [lactate] was different between groups at 3-min of severe-intensity cycling ( $P<0.05$ ; Table 2).  
327 Specifically, the accumulation of muscle lactate from rest to 3-min of severe-intensity cycling  
328 was reduced in SIT+BR compared to SIT+KNO<sub>3</sub> ( $P<0.05$ ; Table 2) but not SIT.

### 329 *Muscle fiber type*

330 There was no difference in the change in proportion of type I or type IIx muscle fibres between  
331 groups ( $P>0.05$ ). However, the increase in the proportion of type IIa muscle fibers was greater  
332 in SIT and SIT+BR compared to SIT+KNO<sub>3</sub> ( $P<0.05$ ; Table 3).

333 **Discussion**

334 The principal original finding of this study is that SIT with concurrent  $\text{NO}_3^-$  supplementation  
335 in the form of a natural dietary source (BR) results in superior physiological and exercise  
336 performance adaptations compared to both SIT alone and SIT with concurrent  
337 supplementation of a  $\text{NO}_3^-$  salt ( $\text{KNO}_3$ ). Specifically, SIT+BR enhanced time to task failure  
338 during severe-intensity cycling to a greater extent than SIT+ $\text{KNO}_3$  and SIT. Moreover, despite  
339 the administration of equimolar  $\text{NO}_3^-$  doses, SIT+BR resulted in a greater reduction in resting  
340 BP and a greater fall in plasma  $[\text{NO}_2^-]$  during exercise compared to SIT+ $\text{KNO}_3$ . These findings  
341 suggest that BR, but not  $\text{KNO}_3$ , supplementation may augment the improvements in exercise  
342 capacity and some physiological responses to SIT.

343 *Influence of SIT+BR and SIT+KNO<sub>3</sub> on exercise capacity*

344 In the present study, peak work rate,  $\dot{V}\text{O}_{2\text{peak}}$  and the time to task failure during severe-intensity  
345 exercise were increased following the intervention period in all SIT groups. This confirms the  
346 efficacy of low volume, high-intensity interval training to increase exercise capacity (7, 24, 38,  
347 52). Interestingly, we found that  $\dot{V}\text{O}_{2\text{peak}}$  and time to task failure were increased to a greater  
348 extent when SIT was combined with BR compared to SIT alone. This is consistent with our  
349 previous study showing that 4 weeks SIT combined with BR improved  $\dot{V}\text{O}_{2\text{peak}}$  whereas SIT  
350 combined with  $\text{NO}_3^-$ -depleted beetroot juice did not (59). Surprisingly, and in contrast to our  
351 previous study (59), the greater increase in  $\dot{V}\text{O}_{2\text{peak}}$  in SIT+BR was not accompanied by a  
352 significantly greater increase in peak WR during incremental exercise in the present study.  
353 However, although not significantly different, it is interesting to note that 7 out of 10  
354 participants in SIT+BR improved peak WR to a greater extent than the pooled group mean  
355 change (equivalent to an ~8% improvement), whereas fewer participants exhibited this trend  
356 in SIT (3/10) and SIT+ $\text{KNO}_3$  (2/10).

357 The greater improvement in  $\dot{V}O_{2\text{peak}}$  and time to task failure in SIT+BR might be explained by  
358 differences in muscle fiber type transformation and/or changes in muscle metabolic responses  
359 to exercise between groups. PGC-1 $\alpha$  regulates the exercise-stimulated skeletal muscle  
360 remodelling from glycolytic type IIx to more oxidative type IIa and type I muscle fibers (36,  
361 47). The transcription of PGC-1 $\alpha$  may be stimulated by both low volume SIT (37) and elevated  
362 NO bioavailability (42, 45, 46). It is therefore possible that, by activating common signalling  
363 pathways, dietary NO $_3^-$  may augment some of the oxidative metabolic adaptations typical of  
364 exercise training. Consistent with this, Roberts et al. (51) have recently demonstrated that both  
365 exercise and NO $_3^-$  increased the expression of PGC-1 $\alpha$  in human and rat muscle. The change  
366 from type IIx towards type IIa and type I fibers typically associated with exercise training was  
367 observed in both the soleus (comprising  $\leq 20\%$  type IIa + IIx fibers (13)) and gastrocnemius  
368 (comprising  $\sim 100\%$  type IIa + IIx fibers (13)) muscle of NO $_3^-$  supplemented rats (51). In the  
369 present study, we found a significant increase in type IIa muscle fibers following the  
370 intervention period in SIT and SIT+BR but not SIT+KNO $_3$ . However, in contrast to recent  
371 findings in hypoxia (14) and normoxia (59), we did not find that concurrent dietary NO $_3^-$   
372 supplementation modulated muscle fiber type transformation compared to SIT alone.

373 SIT+BR was associated with more favorable changes in blood and muscle markers of skeletal  
374 muscle metabolism during exercise following the intervention period. Specifically, blood  
375 lactate accumulation was reduced to a greater extent at 1-min severe-intensity exercise in  
376 SIT+BR compared to SIT and muscle lactate accumulation was reduced to a greater extent at  
377 3-min severe-intensity exercise in SIT+BR compared to SIT+KNO $_3$ . Dietary NO $_3^-$   
378 supplementation may reduce physiological strain during severe-intensity cycling by: 1)  
379 lowering the O $_2$  cost of a given work rate within the severe-intensity domain (see below; 18);  
380 2) reducing the ATP and PCr cost of muscle force production (2, 20); 3) improving Ca $^{2+}$

381 handling in type II muscle fibers (29); and 4) improving blood flow distribution towards type  
382 II muscle and elevating the driving pressure for capillary-myocyte O<sub>2</sub> flux (16, 17).

383 *Influence of SIT+BR and SIT+KNO<sub>3</sub> on the O<sub>2</sub> cost of exercise*

384 SIT alone had no effect on the O<sub>2</sub> cost of exercise but submaximal  $\dot{V}O_2$  was lowered post-  
385 training in both SIT+BR and SIT+KNO<sub>3</sub>. This is consistent with several studies that have  
386 assessed steady-state  $\dot{V}O_2$  after acute and short-term BR supplementation (3, 61, 70) and with  
387 two studies following 4 weeks NO<sub>3</sub><sup>-</sup> supplementation (59, 70). The one other study that has  
388 assessed the effect of KNO<sub>3</sub> supplementation on  $\dot{V}O_2$  during exercise found that acute KNO<sub>3</sub>  
389 supplementation was ineffective at lowering the O<sub>2</sub> cost of exercise in elite athletes (49). Fleuck  
390 et al. (18) previously reported that acute BR ingestion is more effective at reducing the O<sub>2</sub> cost  
391 of exercise than an equimolar concentration of NO<sub>3</sub><sup>-</sup> administered as NaNO<sub>3</sub>, perhaps due to  
392 differential effects on NO<sub>3</sub><sup>-</sup>/NO<sub>2</sub><sup>-</sup> metabolism and NO bioavailability evoked by the different  
393 vehicles of NO<sub>3</sub><sup>-</sup> supplementation (See *Effect of SIT+BR and SIT+KNO<sub>3</sub> on markers of NO*  
394 *bioavailability* below). However, in the present study, both SIT+BR and SIT+KNO<sub>3</sub> reduced  
395 submaximal  $\dot{V}O_2$  compared to SIT, with no difference in the magnitude of change between the  
396 two different types of NO<sub>3</sub><sup>-</sup> supplementation.

397 *Influence of SIT+BR and SIT+KNO<sub>3</sub> on markers of NO bioavailability*

398 Systolic and diastolic BP, and plasma [NO<sub>2</sub><sup>-</sup>] were unchanged following 4 weeks of SIT.  
399 However, despite the administration of equimolar concentrations of NO<sub>3</sub><sup>-</sup>, there were disparate  
400 effects of SIT+BR and SIT+KNO<sub>3</sub> on BP. Resting plasma [NO<sub>2</sub><sup>-</sup>] did not differ between the  
401 interventions in which dietary NO<sub>3</sub><sup>-</sup> was administered as BR or as NO<sub>3</sub><sup>-</sup> salt. However, greater  
402 reductions in BP were observed in SIT+BR compared to SIT+KNO<sub>3</sub>. Acute supplementation  
403 with KNO<sub>3</sub> has been shown to lower systolic and diastolic BP (1, 32) but no previous study has  
404 compared the effects of chronic supplementation with KNO<sub>3</sub> to BR. Our results are consistent

405 with Jonvik et al. (30) who reported that systolic and diastolic BP were reduced following BR  
406 but were unchanged following the same dose (~800 mg) of  $\text{NO}_3^-$  administered as a  $\text{NaNO}_3$ -  
407 containing beverage. Collectively, these results indicate that dietary  $\text{NO}_3^-$  consumed as BR is  
408 more effective at lowering BP than dietary  $\text{NO}_3^-$  consumed as a  $\text{NO}_3^-$  salt. This effect may be  
409 linked to the presence of other compounds within the BR beverage which may facilitate the  
410 conversion of  $\text{NO}_2^-$  into bioactive NO and other reactive nitrogen intermediates (21, 50). In  
411 this regard, it is pertinent that the relatively high total antioxidant content of BR (67) did not  
412 appear to blunt the adaptations to training in the present study. This is in contrast to previous  
413 studies in which high doses of vitamins C and E have been administered (43, 48).

414 A possible greater effect of BR compared to  $\text{KNO}_3$  on NO synthesis may be particularly  
415 important during exercise to support NO-mediated physiological responses in contracting  
416 muscle. In this regard, it is interesting that, plasma  $[\text{NO}_2^-]$  declined to a greater extent during  
417 the severe-intensity exercise test in SIT+BR compared to SIT+ $\text{KNO}_3$  (Fig. 1), which is perhaps  
418 indicative of enhanced NO synthesis from  $\text{NO}_2^-$  during exercise in SIT+BR. Given the  
419 importance of  $\text{NO}_2^-$  availability to exercise performance (15), and evidence that the decline in  
420 plasma  $[\text{NO}_2^-]$  during exercise is correlated to improvements in performance (60, 69),  
421 differences in the magnitude of  $\text{NO}_2^-$  reduction may explain the superior improvements in  
422 exercise capacity observed in SIT+BR compared to SIT+ $\text{KNO}_3$  and SIT in the present study.

423 We have previously reported that, in the absence of exercise training, exercise capacity  
424 improved following 15 days (61) and 28 days (59) of  $\text{NO}_3^-$  supplementation in the form of BR.  
425 However, it remains unclear whether similarly protracted periods of dietary  $\text{NO}_3^-$   
426 supplementation in the form of a  $\text{NO}_3^-$  salt, could elicit comparable improvements in exercise  
427 performance. It is possible that different levels of exposure to NO bioavailability incurred by  
428 the different  $\text{NO}_3^-$ -based supplements evoke discrete skeletal muscle adaptive responses which  
429 may have contributed to the differences in exercise capacity reported herein.

430 *Influence of SIT+BR and SIT+KNO<sub>3</sub> on oxidative capacity measured in vivo using <sup>31</sup>P-MRS*

431 Elevating NO bioavailability via long term dietary NO<sub>3</sub><sup>-</sup> administration may stimulate  
432 angiogenesis (22), mitochondrial biogenesis (45, 46) and the transformation towards a more  
433 oxidative skeletal muscle fiber type (47, 56), effects which may enhance muscle oxidative  
434 capacity. Owing to the equilibrium of the creatine kinase reaction, post-exercise muscle PCr  
435 resynthesis is a function of mitochondrial ATP production (53). Therefore, we measured the  
436 resynthesis of PCr following brief, high-intensity exercise, where the time constant for the  
437 exponential recovery of PCr is proportional to muscle mitochondrial oxidative capacity (10,  
438 41). In contrast to our hypothesis, 4 weeks of SIT combined with dietary NO<sub>3</sub><sup>-</sup> supplementation  
439 in the form of either BR or KNO<sub>3</sub> did not enhance muscle oxidative capacity to a greater extent  
440 than SIT alone.

441 Mitochondrial oxidative capacity can also be estimated by measuring the activity of enzymes  
442 such as citrate synthase (12); markers that have been shown to be elevated following SIT (6, 8,  
443 25, 63). However, to our knowledge, this is the first study to demonstrate that 4 weeks SIT  
444 improved oxidative capacity *in vivo* using <sup>31</sup>P-MRS. The reduction in the PCr recovery time  
445 constant in our study (~16%) was similar to that reported by Forbes et al. (19) in the recovery  
446 from moderate-intensity exercise following 2 weeks SIT. Mitochondrial biogenesis might be  
447 anticipated following exercise training (37, 38). Furthermore, interventions that elevate NO  
448 bioavailability such as dietary NO<sub>3</sub><sup>-</sup> supplementation may also increase mitochondrial mass via  
449 comparable signaling pathways (42, 45, 46). However, in the present study, the effects of  
450 exercise training appear responsible for the speeding of PCr recovery observed in all SIT  
451 groups, with no additional benefit afforded by dietary NO<sub>3</sub><sup>-</sup> supplementation.

452 *Experimental considerations*

453 A key strength of the present investigation is that, in addition to comparing changes in  
454 exercise capacity between conditions, we assessed changes in markers of NO bioavailability,  
455 muscle metabolism (with biopsy) and oxidative capacity (using  $^{31}\text{P}$ -MRS techniques) to  
456 explore the mechanistic bases to any functional improvements observed. Furthermore, the  
457 inclusion of a SIT only control group allowed us to isolate the effects of SIT from the effects  
458 of dietary  $\text{NO}_3^-$  supplementation alongside SIT in the SIT+BR and SIT+KNO<sub>3</sub> groups. In our  
459 study design, we elected to simulate the approach that athletes might adopt during training  
460 and in preparation for competition: that is, daily  $\text{NO}_3^-$  supplementation during training and  
461 then an acute  $\text{NO}_3^-$  dose prior to the criterion exercise trial. However, one disadvantage to  
462 this approach is that it complicates differentiation of the effects of training with  $\text{NO}_3^-$   
463 supplementation, *per se*, from potential effects of acute  $\text{NO}_3^-$  supplementation on some of the  
464 physiological responses to exercise. For this reason, we cannot exclude the possibility that  
465 some of the physiological and exercise performance effects observed in the SIT+BR and  
466 SIT+KNO<sub>3</sub> groups may have been influenced by the acute  $\text{NO}_3^-$  ingestion. Our results do,  
467 however, indicate that the combination of SIT+BR and acute BR ingestion results in superior  
468 physiological and performance outcomes compared to SIT alone or SIT+KNO<sub>3</sub> and acute  
469 KNO<sub>3</sub> ingestion. Further research is necessary to partition out the possible differences in the  
470 physiological adaptations to SIT alongside chronic  $\text{NO}_3^-$  supplementation with and without  
471 the addition of acute  $\text{NO}_3^-$  supplementation prior to post-training performance tests.

472 The BR and KNO<sub>3</sub> supplements were consumed by participants in this study as they might be  
473 used by athletes in training, i.e., no attempt was made to match the macronutrient, micronutrient  
474 or energy content of the supplements. Therefore, it is possible, though we believe unlikely, that  
475 the additional carbohydrate and/or energy intake provided by the BR supplement (~31g/day or  
476 ~120 kcal/day) might have influenced, either positively or negatively, the adaptations to SIT  
477 compared to the other training groups. There was no change in body mass from pre-to post-

478 training in any of the groups indicating that energy balance was maintained. It should also be  
479 acknowledged that, since the total work completed in each of the training programs was not  
480 quantified, it is unclear whether the enhanced adaptations in the SIT+BR group relative to the  
481 SIT and SIT+KNO<sub>3</sub> groups were a result of a greater overall training load, due to an  
482 aggregation of acute ergogenic effects of BR, or to a greater physiological remodeling to the  
483 same training load.

#### 484 *Summary*

485 The present study demonstrated that SIT combined with dietary NO<sub>3</sub><sup>-</sup> supplementation in the  
486 form of BR improved exercise capacity to a greater extent than SIT alone and SIT combined  
487 with dietary NO<sub>3</sub><sup>-</sup> supplementation in the form of KNO<sub>3</sub>. These findings may be linked to  
488 greater NO synthesis from NO<sub>2</sub><sup>-</sup> or a greater increase in other bioactive nitroso compounds in  
489 SIT+BR compared to the other SIT groups. Increased dietary NO<sub>3</sub><sup>-</sup> intake, including via  
490 supplementation with a natural NO<sub>3</sub><sup>-</sup>-rich product such as BR, may promote greater exercise  
491 capacity adaptations and NO-mediated physiological responses to exercise training. These  
492 findings might have important implications for augmenting some of the physiological and  
493 performance adaptations to a short-term SIT program.

494

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498

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724 **Figure Legends**

725 Figure 1. Plasma [NO<sub>2</sub><sup>-</sup>] declined to a greater extent from baseline to 3min during severe-  
726 intensity exercise in SIT+BR (panel A) compared to SIT+KNO<sub>3</sub> (panel B) (\* *P*<0.05). Open  
727 circles represent plasma [NO<sub>2</sub><sup>-</sup>] pre-intervention, closed circles represent plasma [NO<sub>2</sub><sup>-</sup>] post-  
728 intervention. Values presented as mean ± SE.

729 Figure 2. Percentage increase in time to task failure and  $\dot{V}O_{2peak}$  during severe-intensity exercise  
730 in SIT (solid grey line), SIT+BR (solid black line) and SIT+KNO<sub>3</sub> (dotted black line). Values  
731 presented as mean ± SE. The ANCOVA indicated the changes in time to task failure and  
732  $\dot{V}O_{2peak}$  during severe-intensity exercise were greater in SIT+BR compared to SIT (# *P*<0.05)  
733 and compared to SIT+KNO<sub>3</sub> (\* *P*<0.05).

734 Figure 3. Muscle phosphocreatine (PCr) time constant in the recovery from brief high-intensity  
735 exercise. The ANCOVA indicated that change in PCr recovery time constant with training was  
736 not different between groups (*P*>0.05). The dashed lines represent individual responses. The  
737 bars represent the mean response for each group (±SE).

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**Table 1. Physiological and performance variables pre- and post-intervention.**

	SIT		SIT+BR		SIT+KNO <sub>3</sub>	
	Pre	Post	Pre	Post	Pre	Post
<b>Blood pressure</b>						
SBP (mmHg)	114 ± 10	118 ± 8	119 ± 10	113 ± 10#	114 ± 11	112 ± 19
DBP (mmHg)	63 ± 8	65 ± 6	67 ± 10	63 ± 8#*	61 ± 8	64 ± 7
<b>Incremental test</b>						
Peak WR (W)	313 ± 62	335 ± 65	310 ± 97	335 ± 92	304 ± 62	324 ± 673
$\dot{V}O_{2\text{ peak}}$ (L·min <sup>-1</sup> )	3.56 ± 0.81	3.76 ± 0.81	3.27 ± 1.11	3.62 ± 1.04#*	3.31 ± 0.81	3.45 ± 0.81
<b>Moderate-intensity exercise</b>						
End-exercise $\dot{V}O_2$ (L·min <sup>-1</sup> )	1.72 ± 0.41	1.77 ± 0.45	1.70 ± 0.41	1.64 ± 0.40#	1.56 ± 0.41	1.46 ± 0.33#
<b>Severe-intensity exercise</b>						
Time to task failure (s)	317 ± 88	467 ± 164	254 ± 55	434 ± 138#*	269 ± 67	383 ± 80

Values are means ± SD. SBP, systolic blood pressure; DBP, diastolic blood pressure;  $\dot{V}O_{2\text{ peak}}$ , peak oxygen uptake; WR, work rate. #  $P < 0.05$  compared to change in SIT; \*  $P < 0.05$  compared to change in SIT+KNO<sub>3</sub>.

**Table 2. Muscle metabolites and substrates during severe-intensity cycling exercise. Baseline values were used as a covariate in ANCOVA. Values presented as mean  $\pm$  SD. \*  $P < 0.05$  compared to change in SIT+KNO<sub>3</sub>.**

	SIT						SIT+BR						SIT+KNO <sub>3</sub>					
	Pre			Post			Pre			Post			Pre			Post		
	Rest	3 min	Ex	Rest	3 min	Ex	Rest	3 min	Ex									
[ATP] mmol·kg d.w. <sup>-1</sup>	28 $\pm$ 11	22 $\pm$ 6	22 $\pm$ 4	22 $\pm$ 5	21 $\pm$ 6	22 $\pm$ 7	22 $\pm$ 8	22 $\pm$ 7	21 $\pm$ 13	21 $\pm$ 5	20 $\pm$ 3	22 $\pm$ 8	26 $\pm$ 14	20 $\pm$ 14	21 $\pm$ 11	23 $\pm$ 11	16 $\pm$ 7	23 $\pm$ 9
[PCr] mmol·kg d.w. <sup>-1</sup>	77 $\pm$ 16	40 $\pm$ 24	32 $\pm$ 10	76 $\pm$ 17	44 $\pm$ 17	28 $\pm$ 9	69 $\pm$ 13	32 $\pm$ 15	30 $\pm$ 13	77 $\pm$ 10	41 $\pm$ 17	28 $\pm$ 9	57 $\pm$ 14	20 $\pm$ 10	17 $\pm$ 9	60 $\pm$ 12	18 $\pm$ 3	21 $\pm$ 7
[Glycogen] mmol·kg d.w. <sup>-1</sup>	354 $\pm$ 83	301 $\pm$ 95	239 $\pm$ 92	509 $\pm$ 160	407 $\pm$ 117	353 $\pm$ 122	416 $\pm$ 109	342 $\pm$ 123	214 $\pm$ 88	544 $\pm$ 141	458 $\pm$ 140	346 $\pm$ 118	379 $\pm$ 131	338 $\pm$ 100	270 $\pm$ 33	511 $\pm$ 111	405 $\pm$ 120	282 $\pm$ 54
[Lactate] mmol·kg d.w. <sup>-1</sup>	4 $\pm$ 3	43 $\pm$ 22	60 $\pm$ 25	5 $\pm$ 2	35 $\pm$ 23	63 $\pm$ 35	4 $\pm$ 3	53 $\pm$ 35	74 $\pm$ 37	5 $\pm$ 3	29 $\pm$ 23 *	66 $\pm$ 29	4 $\pm$ 2	77 $\pm$ 56	71 $\pm$ 33	4 $\pm$ 2	70 $\pm$ 30	68 $\pm$ 37
pH	7.4 $\pm$ 0.2	7.0 $\pm$ 0.2	6.9 $\pm$ 0.2	7.3 $\pm$ 0.1	7.1 $\pm$ 0.2	6.9 $\pm$ 0.1	7.3 $\pm$ 0.3	6.9 $\pm$ 0.3	6.8 $\pm$ 0.2	7.4 $\pm$ 0.1	7.1 $\pm$ 0.2	6.9 $\pm$ 0.1	7.3 $\pm$ 0.2	7.0 $\pm$ 0.2	6.8 $\pm$ 0.1	7.2 $\pm$ 0.2	7.1 $\pm$ 0.1	6.9 $\pm$ 0.1

**Table 3. The effect of SIT with and without dietary NO<sub>3</sub><sup>-</sup> in the form of BR or KNO<sub>3</sub> on muscle fibre type proportions. Values presented as mean ± SD. \***

***P*<0.05**

**SIT**

**SIT+BR**

**SIT+KNO<sub>3</sub>**

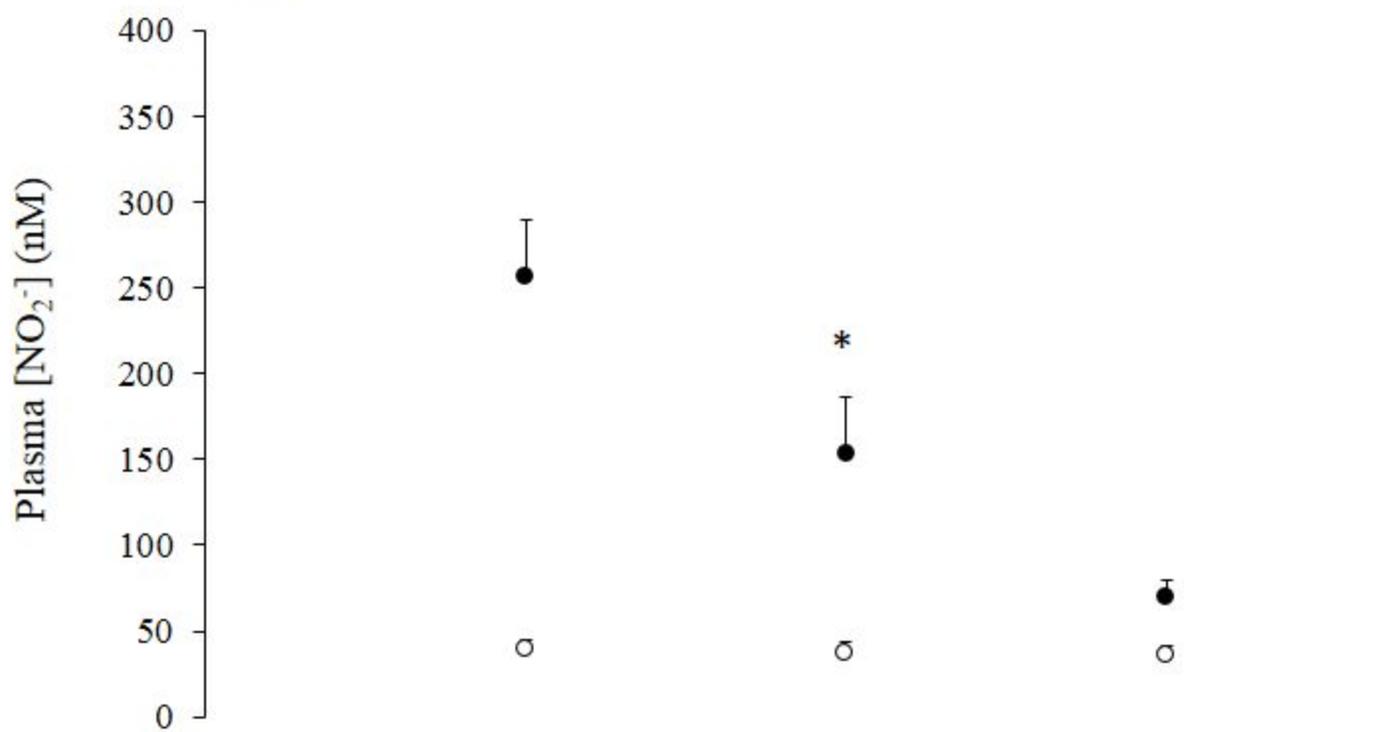
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**compared to change in SIT+KNO<sub>3</sub>.**

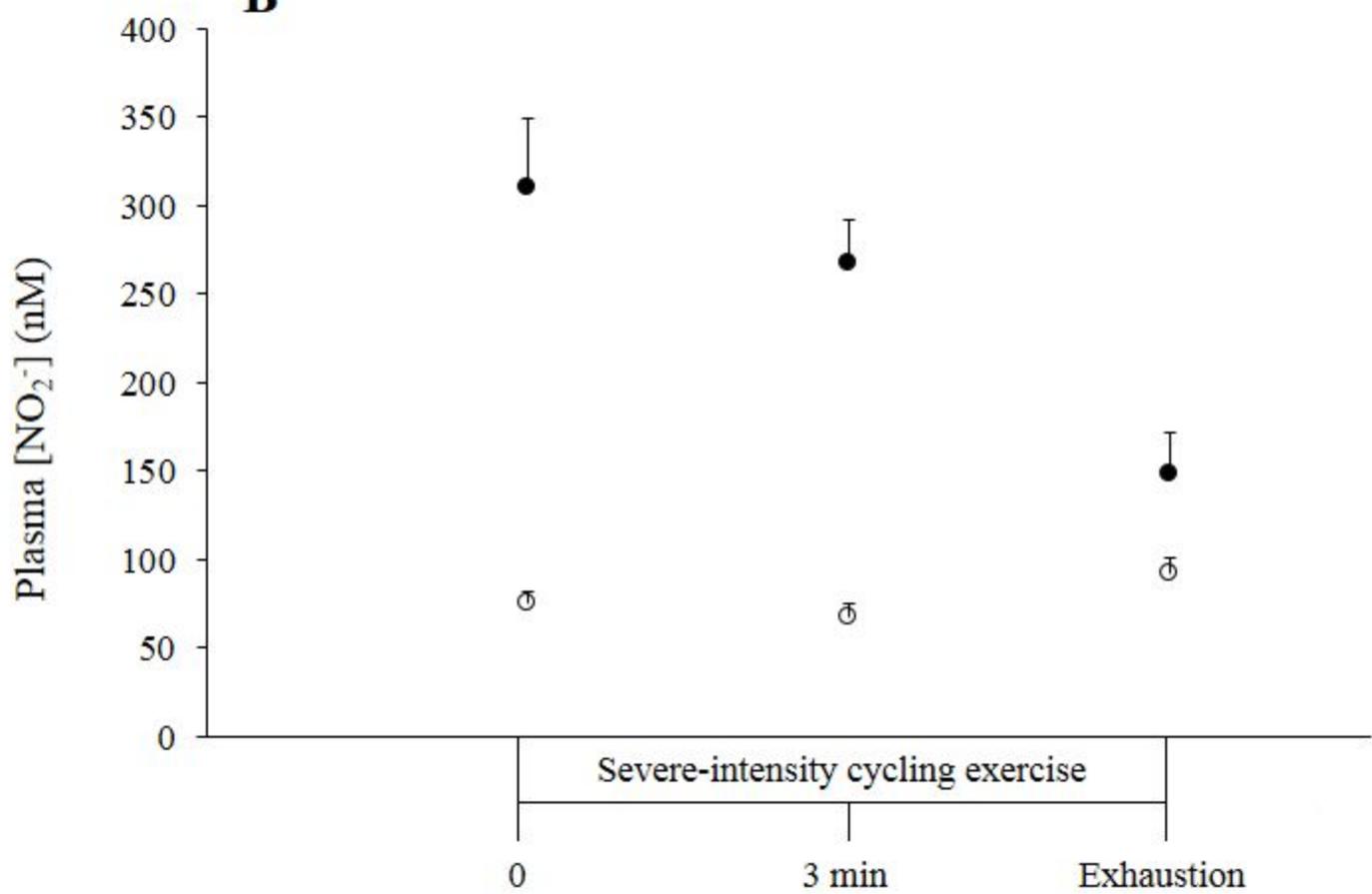
	<b>Pre</b>	<b>Post</b>	<b>Pre</b>	<b>Post</b>	<b>Pre</b>	<b>Post</b>
Type I (%)	53 ± 16	46 ± 15	57 ± 17	55 ± 13	55 ± 12	64 ± 14
Type IIa (%)	40 ± 15	48 ± 19 *	36 ± 13	41 ± 9 *	42 ± 13	34 ± 14
Type IIx (%)	7 ± 15	6 ± 11	7 ± 9	4 ± 6	3 ± 2	2 ± 2

**Figure 1**

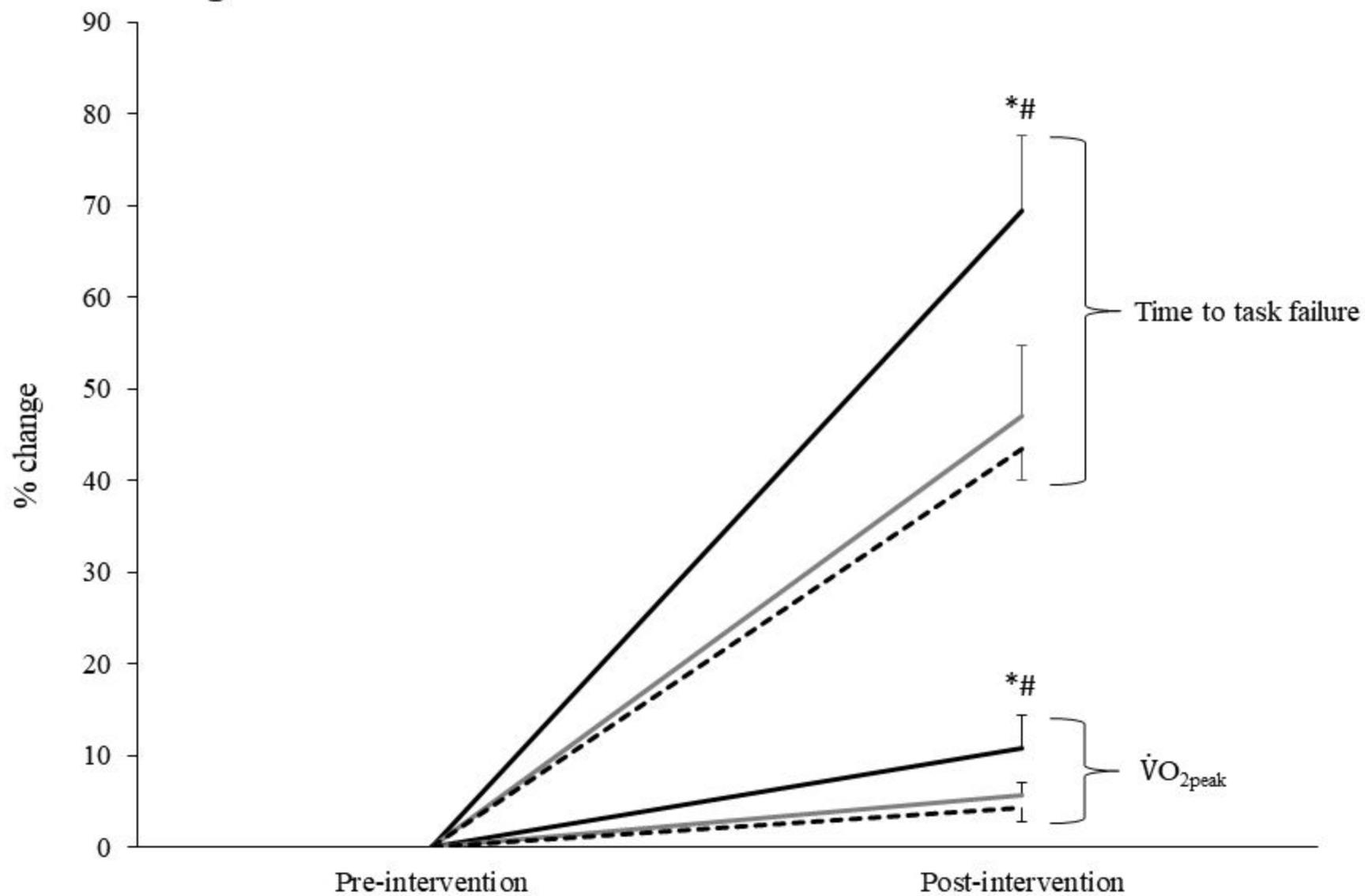
**A**



**B**



**Figure 2**



**Figure 3**

