

1 **Influence of dietary nitrate supplementation on physiological and**
2 **muscle metabolic adaptations to sprint interval training**

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

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20 **Abstract**

21 We hypothesized that 4 weeks of dietary nitrate supplementation would enhance exercise
22 performance and muscle metabolic adaptations to sprint interval training (SIT). Thirty six
23 recreationally-active subjects, matched on key variables at baseline, completed a series of
24 exercise tests before and following a 4 week period in which they were allocated to one of the
25 following groups: 1) SIT and NO₃⁻-depleted beetroot juice as a placebo (SIT+PL); 2) SIT and
26 NO₃⁻-rich beetroot juice (~13mmol NO₃⁻/day; SIT+BR); or 3) no training and NO₃⁻-rich
27 beetroot juice (NT+BR). During moderate-intensity exercise, pulmonary $\dot{V}O_2$ was reduced by
28 4% following 4 weeks of SIT+BR and NT+BR ($P<0.05$) but not SIT+PL. The peak work rate
29 attained during incremental exercise increased more in SIT+BR than in SIT+PL ($P<0.05$) or
30 NT+BR ($P<0.001$). The reduction in muscle and blood [lactate] and the increase in muscle
31 pH from pre- to post-intervention was greater at 3 min of severe-intensity exercise in
32 SIT+BR compared to SIT+PL and NT+BR ($P<0.05$). However, the change in severe-
33 intensity exercise performance was not different between SIT+BR and SIT+PL ($P>0.05$). The
34 relative proportion of type IIX muscle fibers in the m. vastus lateralis was reduced in SIT+BR
35 only ($P<0.05$). These findings suggest that BR supplementation may enhance some aspects of
36 the physiological adaptations to SIT.

37 **New and Noteworthy:** We investigated the influence of nitrate-rich and nitrate-depleted
38 beetroot juice on the muscle metabolic and physiological adaptations to 4 weeks of sprint
39 interval training. Compared to placebo, dietary nitrate supplementation reduced the O₂ cost of
40 submaximal exercise, resulted in greater improvement in incremental (but not severe-
41 intensity) exercise performance, and augmented some muscle metabolic adaptations to
42 training. Nitrate supplementation may facilitate some of the physiological responses to sprint
43 interval training.

44 **Key words:** beetroot juice supplementation, exercise training, training adaptation, muscle
45 metabolism.

46

47 **Introduction**

48 The gaseous biological signaling molecule, nitric oxide (NO), is known to modulate several
49 physiological responses to exercise including skeletal muscle perfusion, energy metabolism
50 and contractile function (41, 69). Nitric oxide synthase (NOS) enzymes catalyze the oxygen
51 (O_2)-dependent production of NO from L-arginine and it is now known that the products of
52 NO oxidation, nitrate (NO_3^-) and nitrite (NO_2^-), can be reduced *in vivo* to form NO (52, 72).
53 Interestingly, hypoxia and acidosis, physiological environments typical of muscular exercise,
54 facilitate the reduction of NO_2^- to NO (72). Increasing the dietary intake of inorganic NO_3^- to
55 augment circulating NO_3^- and NO_2^- pools may therefore represent a natural means to increase
56 NO bioavailability during exercise.

57 The physiological effects of NO_3^- ingestion in humans are well documented and may include
58 a reduction in blood pressure (BP) at rest and reduced oxygen uptake ($\dot{V}O_2$) during sub-
59 maximal exercise (5, 16, 45, 73). Moreover, several studies suggest that NO_3^-
60 supplementation can improve performance in a variety of exercise settings, at least in sub-
61 elite athletes (2, 14, 71, 73, 78, cf. 40). It has recently been reported that short-term (3-7 days)
62 NO_3^- supplementation may favorably impact the metabolic and contractile properties of
63 skeletal muscle (30, 46, 76). Specifically, the improvements in exercise efficiency and
64 performance that have been observed following dietary NO_3^- supplementation may be related
65 to altered mitochondrial function (46, cf. 76) and to enhanced muscle force or power
66 production (19, 30) which, in turn, might be related to increased perfusion and contractile
67 function (22, 32). It is unclear whether more protracted periods (several weeks) of NO_3^-
68 supplementation may more favorably impact the physiological response to exercise and
69 improve exercise performance. However, given that dietary NO_3^- may specifically enhance
70 the physiological responses of type II muscle fibers to exercise (22, 23, 32, 35), and improve

71 performance during repeated sprint exercise (2, 71, 78), it is possible that NO_3^-
72 supplementation may be of particular value to athletes engaging in high-intensity training.

73 Sprint interval training (SIT) is known to provide a potent and relatively time-efficient
74 stimulus for enhancing aerobic capacity and endurance exercise performance (11, 12, 13, 26).

75 However, the effects of a high- NO_3^- dietary supplement, such as beetroot juice, consumed
76 daily as part of an exercise training program, on the physiological and muscle metabolic
77 adaptations to training has received limited attention (21, 57). It is possible that the NO-
78 mediated inhibition of O_2 consumption at cytochrome c oxidase (10, 17) and resultant local
79 hypoxia may initiate signaling cascades that may be synergistic (or antagonistic) to those
80 generated by SIT (27). Also, similar to the effects of training, elevated NO bioavailability
81 may stimulate angiogenesis (25), mitochondrial biogenesis (58) and the transformation of
82 muscle fiber phenotype (59, 68) through cGMP-dependent gene expression and the activation
83 of regulatory factors, in particular peroxisome proliferator-activated receptor-gamma
84 coactivator-1 alpha (PGC-1 α ; 37, 43). It could also be anticipated that the lower $\dot{\text{V}}\text{O}_2$ and
85 reduced adenosine triphosphate (ATP) and phosphocreatine (PCr) cost of muscle force
86 production during high-intensity exercise following NO_3^- supplementation (6, 24) might
87 enable a higher training intensity for the same effort which, over time, may lead to greater
88 training adaptation (34). An increase in cytosolic calcium concentration ($[\text{Ca}^{2+}]$) and force
89 production during muscle contraction following NO_3^- supplementation (32) may also permit a
90 higher training intensity to be maintained. Given the potentially complementary effects of
91 exercise training and NO bioavailability on metabolic regulation, it is possible that NO_3^-
92 supplementation could augment the physiological adaptations to SIT.

93 Two recent studies have used different approaches to address this question and have
94 produced somewhat disparate results (21, 57). Muggeridge et al. (57) reported that, compared
95 to placebo, NO_3^- supplementation with gels during 3 weeks of SIT (4-6 x 15-s sprints, 3 times

96 per week) tended to increase peak work rate during incremental exercise (8.7 vs. 4.7 %;
97 $P=0.07$) and reduce the fatigue index during repeated sprint exercise (0.5 vs.7.3 %; $P=0.06$).
98 De Smet et al. (21) reported that, compared to placebo, NaNO_3 supplementation during 5
99 weeks of SIT (4-6 x 30-s sprints, 3 times per week), performed in hypoxia, did not improve
100 either incremental exercise or 30-min time trial performance but did result in a significant
101 increase in the proportion of type IIa fibers in the m. vastus lateralis. Neither study measured
102 potential training-related differences in muscle metabolic responses to exercise with NO_3^-
103 compared to placebo supplementation (for example, [PCr], pH, [lactate] and [glycogen] as
104 determined from muscle biopsy) or compared the effects of training with NO_3^- or placebo to
105 the physiological effects of NO_3^- supplementation alone. It would be of interest to determine
106 whether the intriguing change in muscle fiber type proportions when SIT in hypoxia was
107 performed with NO_3^- supplementation (21) is also evident following SIT in normoxia.
108 Additional studies are clearly required to explore the influence of NO_3^- supplementation on
109 the muscle metabolic adaptations and submaximal and maximal exercise responses to
110 training.

111 The purpose of this study was therefore to evaluate the independent and combined
112 performance and physiological effects of SIT and NO_3^- supplementation during a 4 week
113 intervention involving: SIT with concurrent NO_3^- -depleted beetroot juice supplementation as
114 a placebo (PL); SIT with concurrent NO_3^- -rich beetroot juice supplementation (BR); and
115 NO_3^- -rich beetroot juice supplementation with no training. We tested the hypothesis that 4
116 weeks SIT and 4 weeks BR supplementation would independently improve physiological
117 responses and exercise performance, but that these effects would be greater when BR
118 supplementation and SIT were combined.

119

120 **Methods**

121 *Subjects*

122 Eighteen male (mean \pm SD: age 27 ± 8 years, height 1.79 ± 0.08 m, body mass 80 ± 13 kg,
123 $\dot{V}O_{2\text{peak}}$ 50.4 ± 11.4 mL \cdot kg $^{-1}\cdot$ min $^{-1}$) and 18 female (mean \pm SD: age 23 ± 4 years, height 166
124 ± 5 cm, body mass 65 ± 9 kg, $\dot{V}O_{2\text{peak}}$ 39.8 ± 5.8 mL \cdot kg $^{-1}\cdot$ min $^{-1}$) participants were recruited.
125 The subjects were recreationally-active sportspeople involved in team and/or endurance
126 sports but they were not highly trained. Following an explanation of the experimental
127 procedures, associated risks, potential benefits and likely value of the possible findings,
128 subjects gave their written informed consent to participate. The study was approved by the
129 Institutional Research Ethics Committee and conformed to the code of ethics of the
130 Declaration of Helsinki.

131

132 *Experimental Design*

133 Subjects initially visited the laboratory on 3 separate occasions over a 5 day period. On visit
134 1, subjects performed an incremental exercise test on a cycle ergometer for the determination
135 of $\dot{V}O_{2\text{peak}}$ and gas exchange threshold (GET). The work rates requiring 80% of the GET
136 (moderate exercise) and 85% Δ (GET plus 85% of the difference between the work rate at
137 GET and $\dot{V}O_{2\text{peak}}$; severe exercise) were calculated and adjusted for mean response time for
138 $\dot{V}O_2$ during incremental exercise (75). Following this, subjects were familiarized to the
139 exercise testing procedures, including completion of a severe-intensity bout of cycle
140 ergometry until exhaustion. On visit 2, subjects completed a 5-min bout of moderate-intensity
141 cycling and an incremental exercise test. On visit 3, subjects completed 2 bouts of severe-
142 intensity cycling, the first for 3 min and the second until task failure.

143

144 In a double-blind, independent-groups design, subjects were then assigned to receive NO_3^-
145 rich beetroot juice (BR) or NO_3^- depleted beetroot juice (PL) for 28 days. Three independent
146 groups ($n = 12$, comprising 6 males and 6 females) were matched at baseline for physical
147 characteristics (i.e. mass, height and age) as well as physiological and performance variables
148 of interest, principally BP and peak WR during incremental exercise and secondarily $\dot{V}\text{O}_{2\text{ peak}}$
149 and GET. Subjects were then either enrolled onto a 4-week supervised SIT program with PL
150 (SIT+PL) or BR (SIT+BR) supplementation, or received the NO_3^- -rich beetroot juice for 28
151 days without undergoing a training intervention (NT+BR).

152

153 All groups completed the same exercise tests (at the same absolute work rates) and
154 physiological assessments both before and after the 28-day intervention period. Also, after 14
155 days, subjects visited the laboratory for an incremental exercise test to assess the short-term
156 changes in aerobic capacity that may be expected following the interventions (11, 65, 73).

157

158 Laboratory visits were scheduled at the same time of day (± 2 h). Subjects were asked to
159 maintain their normal dietary and exercise behavior throughout the study. However, subjects
160 were instructed to record their diet during the 24 h preceding the first laboratory visit and to
161 repeat this for all subsequent laboratory visits. On days of training, subjects were asked to
162 arrive at the training venue ≥ 1 h post-prandial and to complete a 5 min self-paced warm up
163 before training commenced. On experimental days, subjects were instructed to arrive at the
164 laboratory ≥ 3 h post-prandial having avoided strenuous exercise and the consumption of
165 alcohol and caffeine in the 12 h preceding each exercise test. For the duration of the study,
166 subjects were asked to refrain from taking other dietary supplements, and also to avoid using
167 antibacterial mouthwash as this inhibits the reduction of NO_3^- to NO_2^- in the oral cavity by
168 eliminating commensal bacteria (29).

169

170

171 *Supplementation*

172 Following the pre-intervention laboratory visits, subjects were allocated to receive
173 concentrated NO_3^- -rich beetroot juice (BR; beetroot juice; ~ 6.4 mmol of NO_3^- per 70 mL;
174 Beet it, James White Drinks Ltd., Ipswich, UK) or NO_3^- -depleted beetroot juice (PL; placebo
175 beetroot juice; ~ 0.04 mmol NO_3^- per 70 mL; Beet it, James White Drinks Ltd., Ipswich, UK).
176 Subjects consumed 1 x 70 mL of their allocated supplement each morning and evening for
177 the duration of the training or non-training intervention and recorded their intake in a diary.
178 This approach would be expected to result in elevated plasma [NO_3^-] and [NO_2^-] for each 24
179 h period (79). Compliance was checked by the return of empty bottles each week and via
180 questionnaire at 2 and 4 weeks. BR and PL doses were administered using a double blind
181 design. On experimental visits at the mid-intervention point and following the intervention
182 period, subjects consumed 2 x 70 mL of their allocated supplement 2.5 hours prior to the
183 exercise tests.

184

185 *Incremental exercise tests*

186 On the first laboratory visit before and following the intervention period as well as at the mid-
187 intervention point, subjects completed a ramp incremental exercise test on an electronically
188 braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). The self-selected
189 cadence (75-90 rpm), saddle and handle bar height and configuration for each subject were
190 recorded on the first visit and reproduced in subsequent visits. Initially, subjects performed 3
191 min of baseline cycling at 20 W, after which the work rate was increased by 30 W/min until
192 the limit of tolerance. Breath-by-breath pulmonary gas exchange data (Oxycon Pro, Jaeger,
193 Hoechberg, Germany) were collected continuously throughout all incremental tests and were
194 averaged over 10-s periods. $\dot{V}\text{O}_{2\text{peak}}$ and GET were determined as previously described (73).

195

197 *Step exercise tests*

198 A 5-min moderate-intensity “step” test was performed on the first laboratory visit before and
199 following the intervention. This was completed 10 min before the ramp incremental test
200 protocol was initiated. On the second laboratory visit before and following the intervention,
201 two severe-intensity step tests were performed, separated by a 20 min period of rest; the first
202 until 3 min, and the second, after 20 min of passive recovery, until task failure. The time to
203 task failure was recorded once the pedal rate fell by >10 rpm below the target cadence. All
204 step tests began with 3 min of pedaling at 20 W before a sudden transition to the target work
205 rate. Muscle biopsies were obtained before and following the 3-min severe intensity exercise
206 bout and again at task failure in the second bout. Breath-by-breath pulmonary gas exchange
207 data were collected continuously throughout all step tests.

208

209 *Training intervention*

210 Following the initial laboratory visits, subjects were allocated to one of the two SIT groups:
211 SIT with PL supplementation (SIT+PL; age 25 ± 7 years, height 174 ± 10 cm, body mass 73
212 ± 10 kg); SIT with BR supplementation (SIT+BR; mean \pm SD, age 24 ± 7 years, height $174 \pm$
213 11 cm, body mass 78 ± 18 kg); or the non-training group with BR supplementation (NT+BR;
214 age 25 ± 7 years, height 170 ± 6 cm, body mass 68 ± 9 kg). All three groups consisted of 6
215 male and 6 female subjects. Both SIT groups completed a total of 14 supervised training
216 sessions over a 4-week period, with at least 24-h separating each training session, while the
217 NT group maintained their habitual exercise patterns. The post intervention laboratory tests
218 were performed at least 48h following, but within 4 days of, completing the final training
219 session.

220

221 During the training sessions, the SIT groups completed a series of 30-s “all-out” sprints (i.e.
222 Wingate test) against a resistance equivalent to 7.5% body mass on a mechanically-braked
223 ergometer (model 814E bicycle ergometer, Monark, Stockholm, Sweden; 11, 12, 13). Each
224 sprint was separated by a 4-min period of rest in which subjects cycled at a low cadence
225 against a light resistance to reduce venous pooling and sensations of nausea. During weeks 1
226 and 2 of training, subjects performed 4 x 30-s sprints three times per week, while during
227 weeks 3 and 4, subjects performed 5 x 30-s sprints four times per week. Following a 5-min
228 warm up of cycling against a light resistance, subjects were given a 10-s count down and
229 instructed to pedal maximally for 2 s before the appropriate load was applied. Subjects were
230 verbally encouraged to maintain maximal cadence throughout each 30-s sprint.

231

232 *Measurements*

233 *Blood pressure and heart rate*

234 Before and following the intervention, as well as at the mid-intervention point, the BP at the
235 brachial artery was measured using an automated sphygmomanometer (Dinamap Pro: GE
236 Medical Systems, Tampa, FL). Following 10 min seated rest in an isolated room, three
237 measurements were recorded. MAP was calculated as $1/3$ systolic pressure + $2/3$ diastolic
238 pressure. The mean of the systolic, diastolic and MAP measurements were used for data
239 analysis.

240

241 *Blood analysis*

242 Venous blood was sampled at rest (baseline) before each experimental test. Blood samples
243 were also obtained at 1-min, at 3 min and at exhaustion during the severe-intensity exercise
244 bout. The blood samples collected during the severe-intensity exercise bout were drawn from
245 a cannula (Insyte-WTM, Becton Dickinson, Madrid, Spain) inserted into the subject’s

246 antecubital vein and were collected into lithium-heparin vacutainers (Becton Dickinson, New
247 Jersey, USA). Blood [lactate] and [glucose], as well as plasma [NO₂⁻] and [NO₃⁻] were
248 analyzed in all samples (square brackets denote concentration). 200 µL of blood was
249 immediately extracted from the lithium-heparin vacutainers and hemolysed in 200 µL of
250 Triton X-100 solution (Triton X-100, Amresco, Salon, OH) before blood [lactate] and
251 [glucose] were measured (YSI 2300, Yellow Springs Instruments, Yellow Springs, OH). The
252 remaining whole blood from each sample was centrifuged at 4000 rpm for 8 min at 4 °C
253 within 2 min of collection. Plasma was immediately extracted, frozen at -80 °C and
254 subsequently analyzed for [NO₂⁻] and [NO₃⁻] using chemiluminescence, as described by
255 Wylie et al. (79).

256

257 *Muscle biopsy*

258 Muscle samples were obtained from two incisions from the medial region of the *m. vastus*
259 *lateralis* under local anesthesia (1% lidocaine) using the percutaneous Bergström needle
260 biopsy technique (7) with suction. Muscle samples were taken at three different time points
261 before and following the intervention: at rest; following 3 min of severe-intensity exercise;
262 and at task failure from severe-intensity exercise. The post-exercise biopsies were taken
263 while subjects remained on the cycle ergometer and were typically collected within 5-10 s of
264 the completion of the exercise bout. Biopsy samples were immediately frozen in liquid
265 nitrogen and stored at -80 °C for subsequent analysis.

266

267 *Muscle metabolites*

268 Following a freeze-drying process, samples were dissected to remove visible blood, fat, and
269 connective tissue. Approximately 2 mg aliquots of isolated muscle fibers were weighed on
270 fine balance scales (Mettler Toledo XS105, Leicester, UK) and stored in 500 µL

271 microcentrifuge tubes at -80 °C. Prior to metabolite analysis, 200 µL of 3 M perchloric acid
272 was added to ~2 mg dry weight muscle tissue. Following 3 min centrifugation and 30 min
273 incubation on ice, 170 µL of supernatant was transferred to a fresh microcentrifuge tube and
274 255 µL of cooled 2 M potassium bicarbonate (KHCO₃) was added. This was centrifuged, and
275 the supernatant analyzed for [PCr], [ATP] and [lactate] by fluorometric assays as previously
276 described (51).

277

278 *Muscle glycogen and pH*

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

285

286 *Muscle fiber type*

287 Approximately 20 mg of tissue obtained from each resting muscle biopsy sample was
288 embedded in Tissue-Tek® O.T.C.™ compound (Sakura Finetek Europe BV Zoeterwoude,
289 The Netherlands), rapidly frozen in liquid nitrogen-cooled isopentane, and stored at -80 °C
290 for subsequent histochemical analysis of myocellular characteristics. Serial cross sections
291 (~10 µM thick) were cut in a cryostat (Cryostar NX50, Thermo Scientific, USA) maintained
292 at -16 °C. Sections were mounted on 3 separate slides and pre-incubated at pH values of 4.3,
293 4.6 and 10.3. According to the lability to the acid and alkaline pre-incubation, the fibers were
294 stained for myofibrillar ATPase, identified as type I, IIa, or IIx (9) and counted under an
295 Olympus CKX41 microscope with cellSens Dimension software (Olympus Corporation,

296 Tokyo, Japan). For each subject, 214 (\pm 104) fibers were analyzed, and each fiber type was
297 expressed as a percentage of the total number counted.

298

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

305

306 **Statistical analyses**

307 Differences between groups in pre-intervention physiological and performance values were
308 tested using a one way ANOVA. Time by group ANOVAs with repeated measures for time
309 were employed to determine the physiological and performance effects consequent to the
310 interventions. In addition, one-way ANOVAs were used to assess differences between groups
311 in the change values for physiological and performance variables pre- to post-intervention.
312 All significant main and interaction effects were followed up by Fisher's LSD post hoc tests.
313 Data that were not normally distributed were log transformed before applying the ANOVA.
314 All values are reported as mean \pm SD. Statistical significance was accepted at $P < 0.05$

315 **Results**

316 *Compliance*

317 All subjects within the training groups completed 100% of the training sessions and 100% of
318 the sprints within each training session. All subjects reported that they fully adhered to the

319 supplementation regimen and did not alter dietary and exercise behavior outside of their
320 assigned group-specific intervention.

321

322 *Plasma [NO₃⁻] and [NO₂⁻]*

323 Pre-intervention resting plasma [NO₃⁻] values were not different between groups ($P>0.05$). A
324 significant main effect for time ($P<0.001$) and an interaction effect ($P<0.001$) was observed
325 for the plasma [NO₃⁻] measured at rest. Compared to pre-intervention, SIT+BR increased
326 resting plasma [NO₃⁻] by ~590% at 2 weeks and ~960% at 4 weeks (both $P<0.001$; Fig. 1A)
327 and NT+BR increased resting plasma [NO₃⁻] by ~505% at 2 weeks and ~1050% at 4 weeks
328 (both $P<0.001$; Fig. 1A) but there was no change in resting plasma [NO₃⁻] with SIT+PL
329 ($P>0.05$; Fig. 1A). Resting plasma [NO₃⁻] was also greater at 4 weeks compared to 2 weeks
330 in both SIT+BR and NT+BR ($P<0.05$; Fig. 1A).

331 Pre-intervention resting plasma [NO₂⁻] values were higher in SIT+PL (74 ± 62 nM) compared
332 to SIT+BR (29 ± 19 nM; $P<0.05$) and NT+BR (26 ± 13 nM; $P<0.05$) but were similar
333 between SIT+BR and NT+BR ($P>0.05$). There was a significant main effect for time
334 ($P<0.001$) and an interaction effect ($P<0.001$) for the plasma [NO₂⁻] measured at rest.
335 Compared to pre-intervention, SIT+BR increased resting plasma [NO₂⁻] by ~485% at 2
336 weeks and ~715% at 4 weeks (both $P<0.001$; Fig. 1B) and NT+BR increased resting plasma
337 [NO₂⁻] by ~600% at 2 weeks and ~690% at 4 weeks (both $P<0.001$; Fig. 1B) but there was no
338 change in resting plasma [NO₂⁻] with SIT+PL ($P>0.05$; Fig. 1B). There were no differences
339 in the plasma [NO₂⁻] measured at rest between 2 weeks and 4 weeks in any of the groups
340 ($P>0.05$).

341 *Blood Pressure*

342 Systolic BP was not different between the groups before the interventions ($P>0.05$; Table 1)
343 but there was a significant main effect for time ($P<0.05$) and an interaction effect ($P<0.05$).
344 Post hoc tests revealed that, compared to pre-intervention, systolic BP was reduced at 2
345 weeks and at 4 weeks ($P<0.05$) by 5 ± 6 mmHg and 6 ± 4 mmHg in SIT+BR, respectively

346 ($P<0.05$), and by 4 ± 5 mmHg and 10 ± 6 mmHg in NT+BR, respectively ($P<0.05$), whereas
347 systolic BP remained unaltered in SIT+PL ($P>0.05$; Table 1). Diastolic BP was not different
348 between groups at pre-intervention ($P>0.05$) and remained unaltered at 2 weeks and at 4
349 weeks (both $P>0.05$) in all interventions (Table 1). The MAP was not different between
350 groups at pre-intervention but there was a significant main effect for time ($P<0.05$) such that
351 MAP was reduced by 3 ± 5 mmHg at 4 weeks in both SIT+BR and NT+BR ($P<0.05$) but was
352 unchanged with SIT+PL (Table 1). Relative to post-intervention resting baseline, plasma
353 $[\text{NO}_2^-]$ declined by $\sim 65\%$ at task failure during severe-intensity exercise ($P<0.001$) in
354 SIT+BR and NT+BR. The reduction in plasma $[\text{NO}_2^-]$ following 3 min of severe-intensity
355 exercise was greater in NT+BR compared to SIT+BR ($P<0.05$).

356 *Incremental exercise test*

357 Peak WR was not different between the groups at pre-intervention ($P>0.05$; Table 1). There
358 was a significant main effect by time ($P<0.001$) and an interaction effect ($P<0.05$). Post hoc
359 tests revealed that peak WR was improved at 4 weeks compared to pre-intervention in all
360 groups ($P<0.05$; Table 1). However, peak WR increased more from pre- to post-intervention
361 in SIT+BR than in SIT+PL ($P<0.001$; Fig. 2). Additionally, peak WR was improved at 2
362 weeks compared to pre-intervention in SIT+BR only ($P<0.05$; Fig. 2).

363 $\dot{V}\text{O}_{2\text{peak}}$ was not different between the groups at pre-intervention ($P>0.05$; Table 1). There was
364 a significant main effect by time on $\dot{V}\text{O}_{2\text{peak}}$ ($P<0.05$). Post hoc analysis revealed that,
365 compared to pre-intervention, $\dot{V}\text{O}_{2\text{peak}}$ was increased after 2 weeks and 4 weeks with SIT+BR
366 ($P<0.05$; Table 1) but remained unchanged in SIT+PL and NT+BR ($P>0.05$; Table 1).
367 However, there were no differences between the three groups in the change in $\dot{V}\text{O}_{2\text{peak}}$ from
368 pre- to post-intervention ($P>0.05$). There were no significant changes in body mass from pre-
369 to post-intervention in any of the groups.

370 The $\dot{V}O_2$ at the GET was not different between the groups at pre-intervention ($P>0.05$; Table
371 1) and was not altered by any intervention ($P>0.05$; Table 1). The WR associated with the
372 GET was not different between groups at pre-intervention ($P>0.05$; Table 1). There was a
373 significant main effect for time such that the WR at the GET was increased pre- to post-
374 intervention in SIT+BR only ($P<0.05$; Table 1). However, there were no differences between
375 the three groups in the change in the WR at the GET from pre- to post-intervention ($P>0.05$).

376 *Step exercise tests: Moderate-intensity exercise*

377 The $\dot{V}O_2$ measured during baseline cycling at 20 W preceding the transition to moderate-
378 intensity exercise was not different between groups at pre-intervention ($P>0.05$; Table 1) and
379 was not affected by any intervention ($P>0.05$; Table 1). The end-exercise $\dot{V}O_2$ during
380 moderate-intensity exercise was not different between groups at pre-intervention ($P>0.05$;
381 Table 1). There was a significant main effect by time ($P<0.05$) and an interaction effect
382 ($P<0.05$) on end-exercise $\dot{V}O_2$. Post hoc analyses revealed that, compared to pre-intervention,
383 end-exercise $\dot{V}O_2$ was significantly reduced in SIT+BR ($P<0.05$) and NT+BR ($P<0.05$) but
384 was unaltered in SIT+PL ($P>0.05$; Table 1). There was no difference in the change in end-
385 exercise $\dot{V}O_2$ from pre- to post-intervention between the SIT+BR and NT+BR groups
386 ($P>0.05$).

387 *Step exercise tests: Severe-intensity exercise*

388 The time to task failure during severe-intensity exercise was not different between groups at
389 pre-intervention ($P>0.05$; Table 1). There was a significant main effect by time ($P<0.05$) and
390 an interaction effect ($P<0.05$) such that time to task failure was improved by 163 ± 144 s pre-
391 to post- intervention in SIT+PL ($P<0.05$; Table 1) and by 170 ± 90 s pre- to post-intervention
392 in SIT+BR ($P<0.05$; Table 1) but was unaltered by NT+BR ($P>0.05$; Table 1). There was no

393 difference in the change in the time to task failure from pre- to post-intervention between the
394 SIT+BR and SIT+PL groups ($P>0.05$).

395 Blood [lactate] was not different between groups during severe-intensity exercise at pre-
396 intervention ($P>0.05$). There was a main effect by time on blood [lactate] ($P<0.05$). Post-hoc
397 analysis revealed that blood [lactate] was lower at 1 min (1.2 ± 1.1 mM decrease from same
398 time point pre-intervention; $P<0.05$; Fig. 3) and at 3 min (1.6 ± 1.5 mM decrease from same
399 time point pre-intervention; $P<0.05$, Fig. 3) during severe-intensity exercise in SIT+BR but
400 not SIT+PL or NT+BR ($P>0.05$). Further analyses revealed that the increase in blood
401 [lactate] from rest to 3 min was attenuated post-intervention compared to pre-intervention in
402 SIT+BR (2.7 ± 0.9 vs. 3.9 ± 0.8 mM) ($P<0.05$). This attenuation was significantly greater
403 than the equivalent change in blood [lactate] from rest to 3 min in SIT+PL (post-intervention:
404 4.1 ± 1.9 vs. pre-intervention: 3.7 ± 1.2 mM; $P<0.05$).

405 *Muscle substrates and metabolites*

406 Pre-intervention values for muscle substrates and metabolites during severe-intensity exercise
407 were not different between groups ($P>0.05$) and muscle [ATP] and [PCr] were unchanged by
408 the interventions in all groups ($P>0.05$).

409 There were main effects by time on the muscle [lactate] and pH measured at 3 min of severe-
410 intensity exercise ($P<0.05$). Post hoc tests revealed that, compared to pre-intervention,
411 muscle [lactate] was lower and pH was higher at 3 min of severe-intensity exercise post-
412 intervention in SIT+BR ($P<0.05$; Fig. 4). Further analyses revealed that, compared to pre-
413 intervention, the increase in muscle [lactate] and the decrease in muscle pH from rest to 3
414 min of exercise tended to be attenuated post-intervention in SIT+ BR (both $P=0.09$).

415 There was a main effect by time on muscle [glycogen] measured at rest, at 3 min and at
416 exhaustion (all $P<0.05$). Post hoc tests revealed that, compared to pre-intervention, muscle
417 [glycogen] was higher at all three time points post-intervention compared to pre-intervention
418 in SIT+BR ($P<0.05$; Fig 5). Muscle [glycogen] was also higher at all three time points post-
419 intervention in SIT+BR and SIT+PL compared to NT+BR ($P<0.05$; Fig 4). There were no
420 differences between SIT+BR and SIT+PL in the change in muscle [glycogen] from pre- to
421 post- intervention at rest, 3 min of exercise or at exhaustion ($P>0.05$).

422 *Muscle fiber type*

423 The relative proportion of type I (SIT+BR: $57 \pm 16\%$; SIT+PL: $59 \pm 10\%$; NT+BR: $48 \pm$
424 16%), type IIa (SIT+BR: $36 \pm 12\%$; SIT+PL: $36 \pm 16\%$; NT+BR: $44 \pm 16\%$) and type IIx
425 (SIT+BR: $7 \pm 8\%$; SIT+PL: $5 \pm 7\%$; NT+BR: $8 \pm 12\%$) muscle fibers at pre-intervention
426 were not different between groups ($P>0.05$). There was a significant effect of time and an
427 interaction effect on the proportion of type IIx fibers. Post hoc tests revealed that the
428 proportion of type IIx fibers identified in SIT+BR was lower post-intervention ($4 \pm 5\%$)
429 compared to pre-intervention ($7 \pm 8\%$; $P<0.05$). In contrast, the proportion of type IIx fibers
430 identified in SIT+PL tended to be higher post-intervention ($10 \pm 9\%$) compared to pre-
431 intervention ($5 \pm 7\%$; $P=0.07$). The change in type IIx fibers was significantly different in
432 SIT+BR compared to SIT+PL ($P<0.05$) but not NT+BR ($P>0.05$). There were no differences
433 in the proportion of type I (SIT+BR: $55 \pm 12\%$; SIT+PL: $58 \pm 10\%$; NT+BR: $50 \pm 17\%$) or
434 type IIa (SIT+BR: $41 \pm 9\%$; SIT+PL: $32 \pm 16\%$; NT+BR: $43 \pm 14\%$) muscle fibers following
435 any intervention ($P>0.05$). However, there was a significant interaction effect on the
436 proportion of type I and type IIa fibers combined (type I+IIa; $P<0.05$). Post hoc tests revealed
437 that the proportion of type I+IIa fibers identified in SIT+BR was higher post-intervention (96
438 $\pm 6\%$) compared to pre-intervention ($93 \pm 8\%$; $P<0.05$). In contrast, the proportion of type
439 I+IIa fibers identified in SIT+PL tended to be lower post-intervention ($90 \pm 9\%$) compared to

440 pre-intervention ($95 \pm 7\%$; $P=0.07$). The change in type I+IIa fibers was significantly
441 different in SIT+BR and NT+BR compared to SIT+PL ($P<0.05$).

442

443 **Discussion**

444 This is the first study to investigate the combined effect of SIT and NO_3^- supplementation,
445 administered in the form of beetroot juice, on muscle metabolic adaptations and the
446 physiological responses to ramp incremental, moderate-intensity and severe-intensity
447 exercise performance in normoxia. We compared the effects of chronic NO_3^- supplementation
448 alone (NT+BR) with the effects of concurrent NO_3^- -rich (SIT+BR) and NO_3^- -depleted
449 (SIT+PL) beetroot juice supplementation during a SIT intervention. Consistent with our
450 hypotheses, the separate 4 week interventions of SIT and chronic BR supplementation
451 independently induced several beneficial physiological and/or performance effects. However,
452 the main finding of the present study was that the combination of SIT and BR
453 supplementation provided greater improvements in incremental exercise performance
454 compared to either intervention alone and led to greater improvements in some indices of
455 muscle metabolic adaptation.

456 Plasma $[\text{NO}_3^-]$ and $[\text{NO}_2^-]$ were elevated, and systolic BP was lowered following 2 weeks and
457 4 weeks of BR supplementation, changes which are consistent with elevated systemic NO
458 bioavailability. Interestingly, however, resting plasma $[\text{NO}_3^-]$ and $[\text{NO}_2^-]$ were not altered
459 following 4 weeks of SIT+PL. Previous studies have reported that subjects with higher
460 aerobic fitness and/or training status have higher resting plasma $[\text{NO}_3^-]$ and $[\text{NO}_2^-]$ compared
461 to less fit and/or sedentary subjects (53, 63). The results of the present study may therefore
462 indicate that short-term SIT, at least when combined with PL supplementation, does not
463 substantially modify NOS activity or protein expression. The reduction in plasma $[\text{NO}_2^-]$
464 from resting baseline to task failure during severe-intensity exercise was similar between
465 NT+BR and SIT+BR (~65% decline). However, the reduction in plasma $[\text{NO}_2^-]$ from resting
466 baseline to 3 min of severe-intensity exercise was attenuated in SIT+BR (~25% decline)
467 compared to NT+BR (~45% decline). It is possible that this may be related to differences in

468 training status induced by the separate interventions; for example, less reduction of NO_2^- to
469 NO may have been required following SIT+BR due to training-related improvements in
470 muscle capillarity and oxygenation (18).

471 The reductions in systolic BP (SIT+ BR: -4% and -5%, NT+BR: -6% and -9%, at 2 weeks
472 and 4 weeks, respectively) reported in the present study are similar to those previously
473 reported in healthy volunteers following shorter supplementation periods (5, 44, 73, 74).
474 Diastolic BP was unaltered in NT+BR and SIT+PL but was reduced by 7% in SIT+BR. MAP
475 was lowered by ~4% in both NO_3^- supplemented groups (SIT+BR and NT+BR), but was
476 unaltered in SIT+PL. Collectively, these data indicate that 4 weeks of NO_3^- supplementation
477 may result in a greater reduction in BP than 4 weeks of SIT alone.

478 *The effect of SIT and BR on sub-maximal $\dot{V}\text{O}_2$ and $\dot{V}\text{O}_{2\text{peak}}$*

479 A high exercise economy, i.e. a low $\dot{V}\text{O}_2$ for a given power output, is an important
480 determinant of exercise performance (33). It has been postulated that exercise training can
481 lower the O_2 cost of submaximal cycling (55). However, in the present study, the O_2 cost of
482 moderate-intensity exercise was only reduced following training in SIT+BR and the
483 magnitude of the reduction in the O_2 cost of exercise was not different to that observed with
484 NT+BR, suggesting that 4 weeks of SIT *per se* has no influence on the O_2 cost of submaximal
485 cycling. This finding is consistent with previous work indicating that the O_2 cost of exercise
486 may be reduced by dietary NO_3^- supplementation (5, 45, 46, 73). The physiological bases for
487 the improved efficiency following NO_3^- ingestion are likely related to a reduced ATP cost of
488 muscle force production (6) and/or a reduced O_2 cost of mitochondrial ATP resynthesis (46,
489 cf. 76).

490 Despite the low training volume, SIT has emerged as a potent strategy to increase aerobic
491 capacity and endurance exercise performance in as little as two weeks (11, 65, 70). We found

492 that $\dot{V}O_{2\text{peak}}$ was not significantly altered by 4 weeks of either NT+BR or SIT+PL. The
493 former result is consistent with the majority of studies that have assessed $\dot{V}O_{2\text{peak}}$ following
494 acute or short-term NO_3^- supplementation (5, 6, 39, 45, 78). The lack of effect of SIT on
495 $\dot{V}O_{2\text{peak}}$ is also consistent with some (11, 12, 13, 26), but not all (65, 70), previous
496 investigations. The physiological and muscle metabolic adaptations to SIT are likely
497 dependent upon the initial training status of the subjects along with the exact nature of the
498 training stimulus, including the frequency and duration of both the sprint and recovery
499 periods (66). In this respect, it is important to highlight that our exercise training protocol
500 was shorter in duration to some studies (13) and the progression in training volume was more
501 gradual than in other studies (4, 65) in which $\dot{V}O_{2\text{peak}}$ was increased.

502 Although the change in $\dot{V}O_{2\text{peak}}$ from pre- to post-intervention was not different between the
503 three groups, the increase in $\dot{V}O_{2\text{peak}}$ was only greater from pre- to post intervention in
504 SIT+BR, suggesting that NO_3^- supplementation may enhance the adaptation of $\dot{V}O_{2\text{peak}}$ to
505 SIT. Further work is required to confirm this observation and to elucidate the potential
506 cardiovascular and/or metabolic mechanisms which may be responsible.

507 *The effect of SIT and BR on exercise performance*

508 The peak WR during incremental exercise at 4 weeks was improved in both training groups.
509 Interestingly, peak WR was also significantly improved following NT+BR. Although this
510 effect was small, it is consistent with an earlier study which reported a significant increase in
511 peak WR during incremental exercise following 15 days BR supplementation (73).
512 Interestingly, a greater peak WR at 2 weeks of training was only observed with SIT+BR.
513 Moreover, the improvement in peak WR at 4 weeks was greater in SIT+BR than in SIT+PL
514 and NT+BR. The greater, and more rapidly attained, improvements in incremental exercise
515 test performance with SIT+BR is presumably a function of the improved exercise economy

516 and/or favorable muscle metabolic profile which would be expected to result in an extended
517 time to reach $\dot{V}O_{2\text{peak}}$. Our results are consistent with a recent study by Muggeridge et al. (57)
518 which reported that 3 weeks of SIT (4-6 repeated 15-s sprints) increased peak WR during
519 incremental exercise to a greater extent when subjects were supplemented with NO_3^-
520 compared to placebo.

521 The time to task failure during severe-intensity exercise was significantly increased after 4
522 weeks of both SIT+BR (group mean change: +69%) and SIT+PL (+55%), but not NT+BR
523 (+3%). Despite evidence for an enhanced muscle metabolic response to severe-intensity
524 exercise in SIT+BR compared to SIT+PL (see below), this did not translate into a greater
525 improvement in severe-intensity exercise performance. It is not clear why this was the case
526 nor why ramp incremental exercise test performance was improved with SIT+BR when time
527 to task failure during severe-intensity exercise was not, although greater variability in time-
528 to-exhaustion tests may have contributed to the difference (20). Indeed, it is interesting to
529 note that the improvement in time to task failure ranged from 37-116% in SIT+BR (with 9/12
530 subjects improving by more than 50%) and from 4-122% in SIT+PL (with 4/12 subjects
531 improving by more than 50%). Our results are similar to those of Puype et al. (64) who found
532 that 6 weeks of endurance training in normobaric hypoxia with BR supplementation did not
533 improve 30-min time trial performance relative to the placebo condition. However, it remains
534 unclear whether BR supplementation during training could improve performance in other
535 types of exercise. Recent studies indicate that BR may be ergogenic during high-intensity
536 intermittent exercise (2, 71, 78) and that, compared to placebo, NO_3^- supplementation during
537 SIT improves fatigue resistance during repeated sprint exercise (57) and may enhance mean
538 power output in a 30 s sprint (21). Further studies are required to investigate whether the
539 subtle enhancements of skeletal muscle adaptation to training with BR might translate into
540 improved performance during these other forms of exercise.

542 *The effect of SIT and BR on the muscle metabolic response to exercise*

543 Although conflicting data exist, SIT has been implicated in rapid skeletal muscle remodeling
544 (11, 12, 13, 26). The extreme perturbations in substrate availability and metabolite
545 accumulation caused by repeated sprint efforts require substantial oxidative energy turnover
546 to restore homeostasis (8). The fluctuations in ATP availability and local O₂ tension are
547 potent stimulators of signaling pathways and may induce mitochondrial biogenesis and
548 oxidative enzyme adaptation via the transcription of PGC-1 α (28, 31, 50). Recent findings
549 indicate that dietary NO₃⁻ may favorably affect the contractility (30, 32) and perfusion (22,
550 23) of type II muscle fibers, and reduce the energetic cost of muscle force production during
551 high-intensity exercise (6, 24). Similar to SIT (3, 13, 47, 48, 49, 61, 62), elevating NO₂⁻ and
552 NO bioavailability with chronic NO₃⁻-rich BR supplementation may also stimulate the
553 transcription of PGC-1 α (43, 54, 58), a key regulator of mitochondrial biogenesis (77) and
554 angiogenesis (1, 15). We therefore determined the effects of 4 weeks SIT and 4 weeks BR
555 supplementation on the muscle metabolic responses during exercise and tested the hypothesis
556 that these adaptations may be amplified when the interventions were combined.

557 There were no differences in muscle [ATP], [PCr], [lactate] or pH at rest or at task failure
558 during severe-intensity exercise, post-intervention compared to pre-intervention, in any
559 group. However, at 3 min into severe-intensity exercise, there was evidence of reduced
560 metabolic perturbation, post-intervention compared to pre-intervention, in the SIT+BR group
561 only. Specifically, muscle [lactate] as well as blood [lactate] was lower, and muscle pH was
562 higher, at 3 min of severe-intensity exercise following SIT+BR but not SIT+PL or NT+BR
563 (Figs. 3 and 4), suggesting an enhanced muscle metabolic adaptation to SIT when combined
564 with BR supplementation.

565 The reason for the small difference in muscle acidosis at the 3 min exercise iso-time with
566 SIT+BR compared to SIT+PL is unclear. However, this may be the result of differences in
567 exercise efficiency between the training groups. The lower O₂ cost of exercise measured at
568 the same submaximal work rate in SIT+BR would be expected to lower the physiological
569 strain and potentially reduce substrate-level phosphorylation and lactate production during
570 exercise (34). Furthermore, BR supplementation has been shown to elevate microvascular
571 PO₂ in type II muscles of exercising rats thus promoting O₂ exchange between the capillary
572 and the myocyte and enabling a better preservation of intramuscular homeostasis (22, 23). By
573 better maintaining oxidative function, this mechanism may be important in delaying lactate
574 accumulation during severe-intensity exercise which is known to mandate an increased
575 recruitment of type II fibers to sustain power output (42). While NO₃⁻ intake alone would be
576 expected to promote some of these effects (for example, a lower O₂ cost of sub-maximal
577 exercise in the NT+BR group in the present study), NO₃⁻ intake combined with training may
578 synergistically improve the muscle metabolic response to severe-intensity exercise. In
579 particular, the SIT+BR group evidenced improved exercise efficiency (which was observed
580 with NT+BR but not SIT+PL) and improved performance and physiological
581 responses/adaptations to maximal exercise (which were observed with SIT+PL but to a much
582 lesser extent with NT+BR).

583 None of the interventions influenced the proportion of type I muscle fibers identified
584 following training. Interestingly, there was a disparity in the muscle phenotypic response to
585 training between SIT+BR and SIT+PL. Specifically, SIT+BR resulted in a significant
586 reduction in the proportion of type IIx muscle fibers. In contrast, SIT+PL resulted in a trend
587 towards a greater proportion of type IIx fibers following the intervention period. These results
588 suggest that a remodeling of skeletal muscle towards a more oxidative phenotype following
589 SIT (26, 27) may be facilitated by BR supplementation and perhaps hampered by PL

590 supplementation. Our findings are consistent with a recent study which also reported changes
591 in muscle fiber type composition following 5 weeks of SIT with ~5 mmol daily NO_3^-
592 supplementation (21). These authors reported that SIT performed in hypoxia resulted in a
593 significant increase in the relative number of type IIa fibers in the m. vastus lateralis (from
594 ~45 to 56%) when subjects ingested NO_3^- compared to placebo. It is possible that the
595 differences in the muscle metabolic or performance response to exercise following SIT when
596 combined with NO_3^- compared to placebo supplementation (present study; 57) are related to
597 changes in muscle fiber type composition – i.e., a greater reduction in type IIX fibers and/or a
598 greater increase in type IIa fibers (present study; 21).

599 It is important to highlight that both the BR and PL supplements contain high concentrations
600 of antioxidants including betacyanins and polyphenols (38, 67) which may potentially
601 interfere with skeletal muscle adaptations to training (56, 60). It is possible, therefore, that the
602 adaptations to training in the SIT+PL group were attenuated in the present study due to the
603 simultaneous intake of antioxidants. However, it is also possible that the potential for chronic
604 NO_3^- administration to enhance muscular adaptations and exercise performance with SIT was
605 underestimated in the SIT+BR group for the same reason. On the other hand, it has recently
606 been reported that BR supplementation increases hydrogen peroxide emission from the
607 mitochondria, an effect that could promote redox signaling (76) and enhance training
608 adaptations. Moreover, the combination of NO_3^- with antioxidants might promote the
609 reduction of NO_2^- to NO and facilitate physiological effects (36). Further research should
610 investigate the influence of NO_3^- alone (as NaNO_3 or KNO_3) and BR on the skeletal muscle
611 adaptations to training. Our study design involved 4 weeks of daily BR supplementation with
612 the final dose being consumed on the morning of the post-intervention laboratory tests. Our
613 measurements therefore reflect the combined effects of chronic and acute BR (or PL)
614 supplementation superimposed on exercise training. It has been reported recently that 4

615 weeks of BR supplementation continues to exert physiological effects for at least 48 hours
616 following the cessation of supplementation (80). Future studies might therefore be designed
617 to partition out the influence of chronic NO_3^- or BR supplementation (without additional
618 acute supplementation) on the adaptations to training.

619 *Conclusions*

620 In the absence of training, chronic BR ingestion resulted in a significant reduction in the O_2
621 cost of moderate-intensity exercise and a small but significant increase in peak WR. SIT+PL
622 resulted in improvements in peak WR during incremental exercise and time to task failure
623 during severe-intensity exercise. Greater changes in peak WR during incremental exercise
624 were found with SIT+BR compared to SIT+PL and NT+BR. In addition, type IIX muscle
625 fiber proportion was reduced and, at the 3-min iso-time during severe-intensity exercise,
626 muscle pH was higher and muscle (and blood) [lactate] was lower in SIT+BR only. These
627 findings suggest that the independent physiological and performance effects of SIT and BR
628 supplementation may be enhanced when these interventions are combined. Dietary NO_3^-
629 supplementation in the form of BR may potentiate some exercise performance and muscle
630 metabolic adaptations to SIT.

631

632

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637

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642

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889

890 **Figure Legends**

891 Figure 1. Mean \pm SD resting plasma $[\text{NO}_3^-]$ (panel A) and plasma $[\text{NO}_2^-]$ (panel B) responses
892 in SIT+BR (solid black line), SIT+PL (solid grey line) and NT+BR (dotted black line). * =
893 different from pre-intervention ($P<0.05$); † = different from mid-intervention ($P<0.05$); ‡ =
894 different from SIT+PL ($P<0.05$).

895 Figure 2. Mean \pm SD changes (Δ) in peak WR at mid- and post-intervention in the three
896 groups expressed relative to pre-intervention baseline. The change in peak WR from pre- to
897 post-intervention was greater in SIT+BR (solid black line) than SIT+PL (solid grey line) and
898 NT+BR (dotted black line). * = different from pre-intervention ($P<0.05$), † = different from
899 mid-intervention ($P<0.05$), # = different from NT+BR ($P<0.05$), ‡ = different from SIT+PL
900 ($P<0.05$).

901 Figure 3. Mean \pm SD blood [lactate] at rest (black bars), 1 min (patterned bars), 3 min (grey
902 bars) and at task failure (open bars) during severe-intensity exercise. * = different to pre-
903 intervention ($P<0.05$).

904 Figure 4. Mean \pm SD muscle [lactate] (panel A), muscle pH (panel B) and muscle [glycogen]
905 (panel C) at rest (black bars), 3 min (grey bars) and at task failure (open bars) during severe-
906 intensity exercise. * = different to pre-intervention ($P<0.05$); # = different to post-
907 intervention NT+BR ($P<0.05$); ‡ = different to post-intervention SIT+PL ($P<0.05$).

908

Table 1. Physiological and performance variables pre-, mid- and post-intervention

	SIT+PL			SIT+BR			NT+BR		
	Pre	Mid	Post	Pre	Mid	Post	Pre	Mid	Post
Blood pressure									
SBP (mmHg)	116 ± 13	115 ± 14	116 ± 10	118 ± 11	113 ± 11*	112 ± 10*‡	117 ± 13	113 ± 10*	107 ± 17*‡
DBP (mmHg)	67 ± 8	64 ± 7	66 ± 4	67 ± 9	64 ± 9	62 ± 7	63 ± 5	63 ± 7	62 ± 8
MAP (mmHg)	83 ± 8	81 ± 8	83 ± 6	84 ± 8	80 ± 9	79 ± 7*	82 ± 7	80 ± 7	77 ± 7*
Incremental test									
Peak WR (W)	303 ± 78	306 ± 72	318 ± 73*†	298 ± 93	305 ± 90*	321 ± 91*†	296 ± 66	295 ± 67	300 ± 67*
Δ Peak WR (W)	-	4 ± 13	16 ± 15*†#	-	7 ± 10*#	24 ± 8*†#‡	-	0 ± 9	4 ± 4*
$\dot{V}O_{2\text{ peak}}$ (L·min ⁻¹)	3.43 ± 0.99	3.49 ± 0.97	3.50 ± 0.86	3.19 ± 1.03	3.39 ± 1.06*	3.47 ± 1.02*	3.28 ± 1.03	3.42 ± 0.99	3.42 ± 1.08
$\dot{V}O_{2\text{ at GET}}$ (L·min ⁻¹)	1.55 ± 0.49	1.49 ± 0.41	1.62 ± 0.44	1.60 ± 0.37	1.58 ± 0.37	1.64 ± 0.43	1.61 ± 0.46	1.62 ± 0.52	1.61 ± 0.4
WR at GET (W)	110 ± 32	103 ± 34	112 ± 27	102 ± 30	105 ± 32	110 ± 27*	105 ± 34	102 ± 29	112 ± 27

Moderate-intensity exercise									
End-exercise $\dot{V}O_2$ (L·min ⁻¹)	1.57 ± 0.41	-	1.67 ± 0.44	1.64 ± 0.41	-	1.58 ± 0.42*‡	1.73 ± 0.32	-	1.65 ± 0.34*‡
Severe-intensity exercise									
Time to task failure (s)	297 ± 69	-	460 ± 186*#	248 ± 53	-	418 ± 132*#	266 ± 82	-	275 ± 84

Values are means ± SD. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; $\dot{V}O_2$, oxygen uptake; $\dot{V}O_{2\text{ peak}}$, peak $\dot{V}O_2$; WR, work rate; GET, gas exchange threshold; SIT+BR, high-intensity interval training plus NO₃⁻-rich beetroot juice; SIT+PL, high-intensity interval training plus NO₃⁻-depleted juice; NT+BR, no-training plus NO₃⁻-rich beetroot juice. * = different from Pre-intervention ($P < 0.05$), † = different from Mid-intervention ($P < 0.05$), # = different from NT+BR ($P < 0.05$), ‡ = different from SIT+PL ($P < 0.05$).

Figure 1

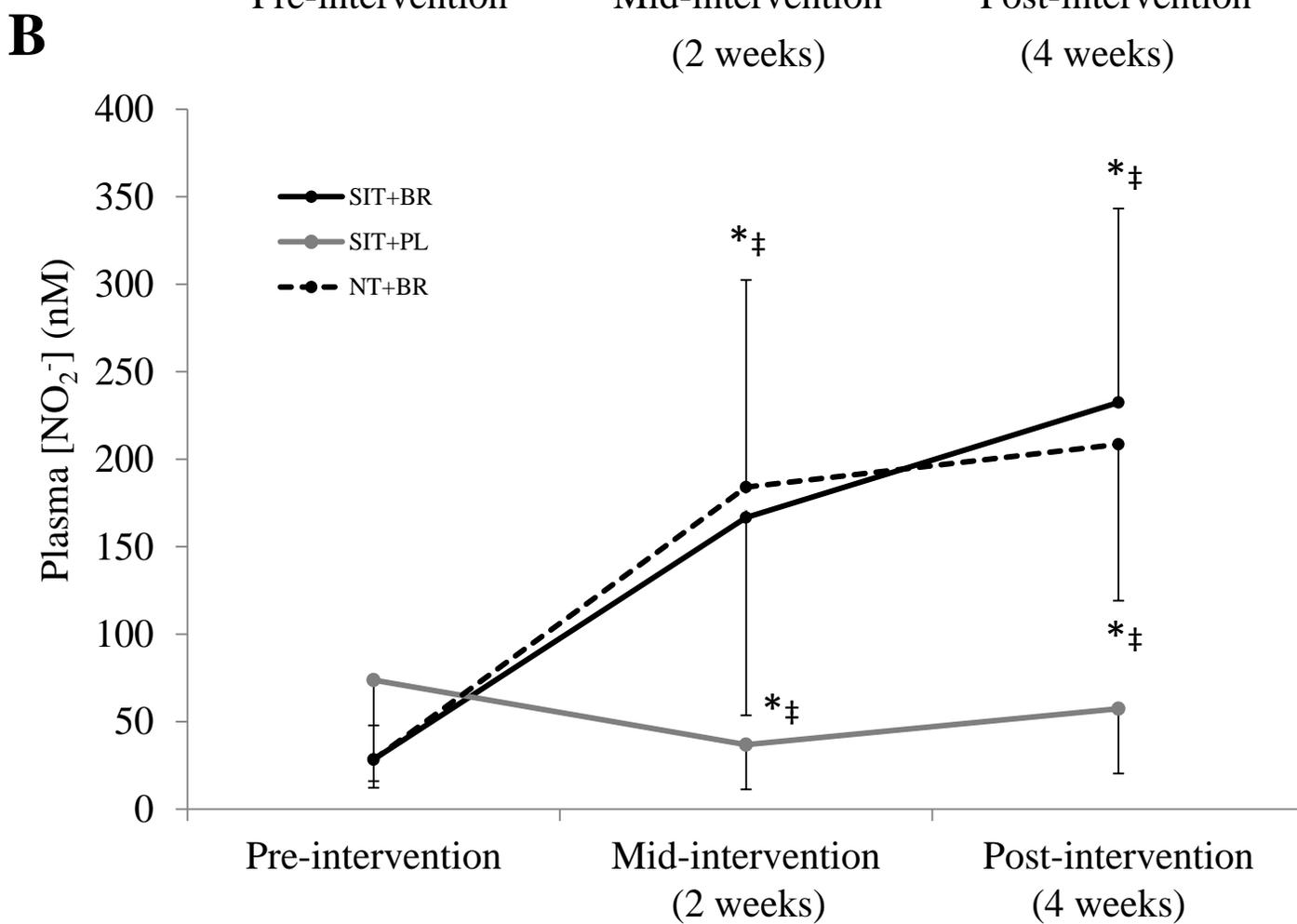
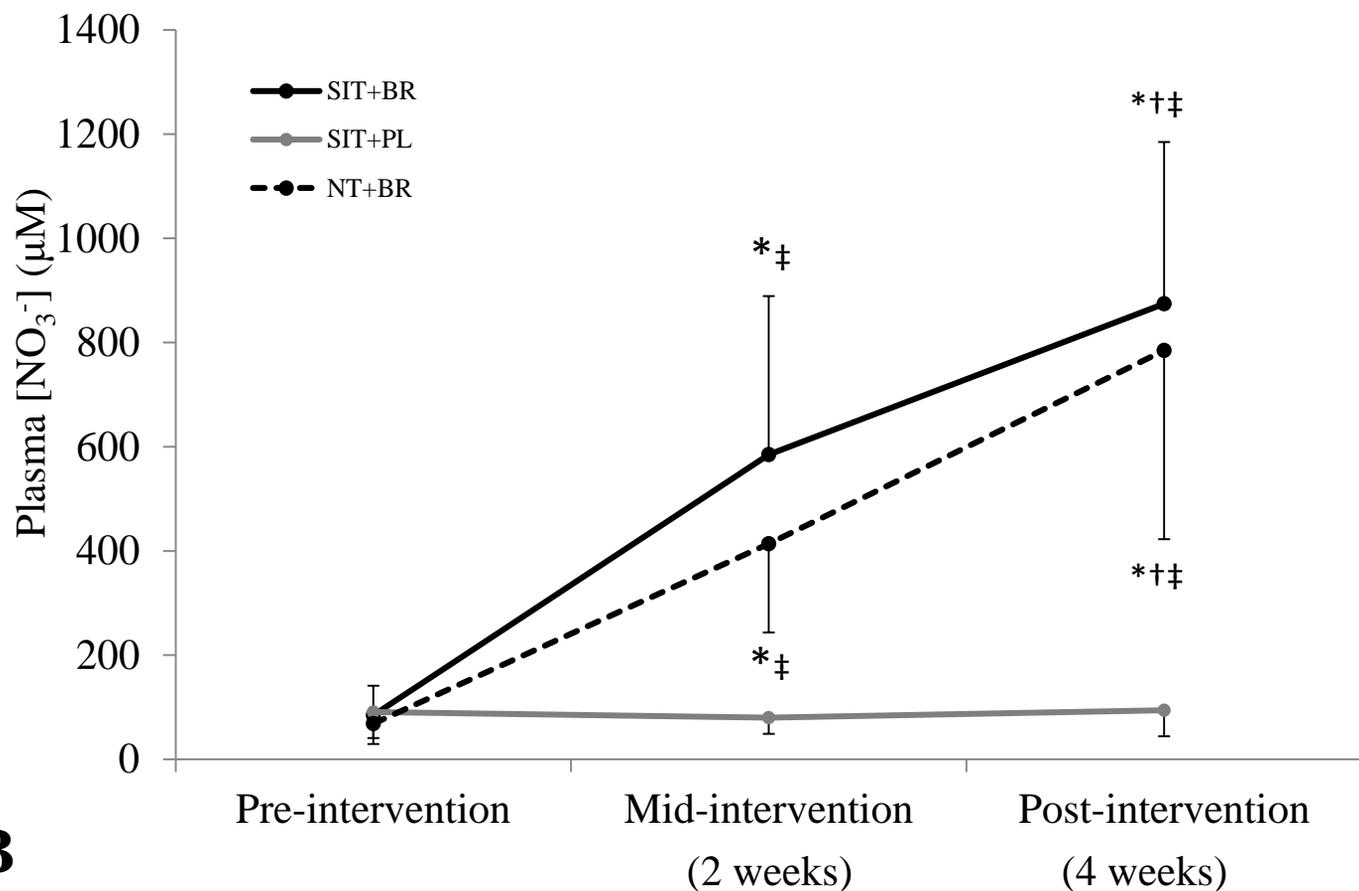


Figure 2

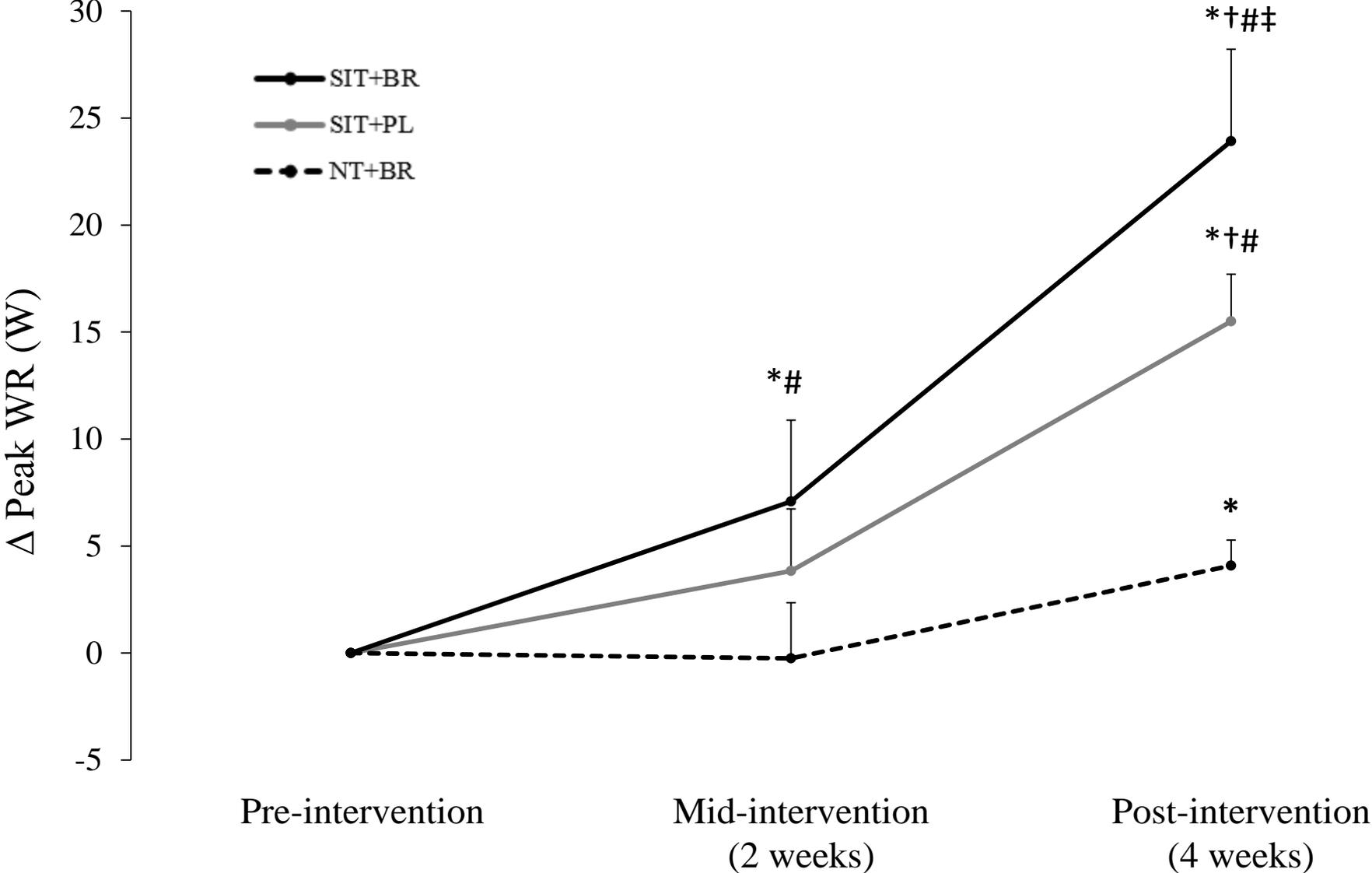


Figure 3

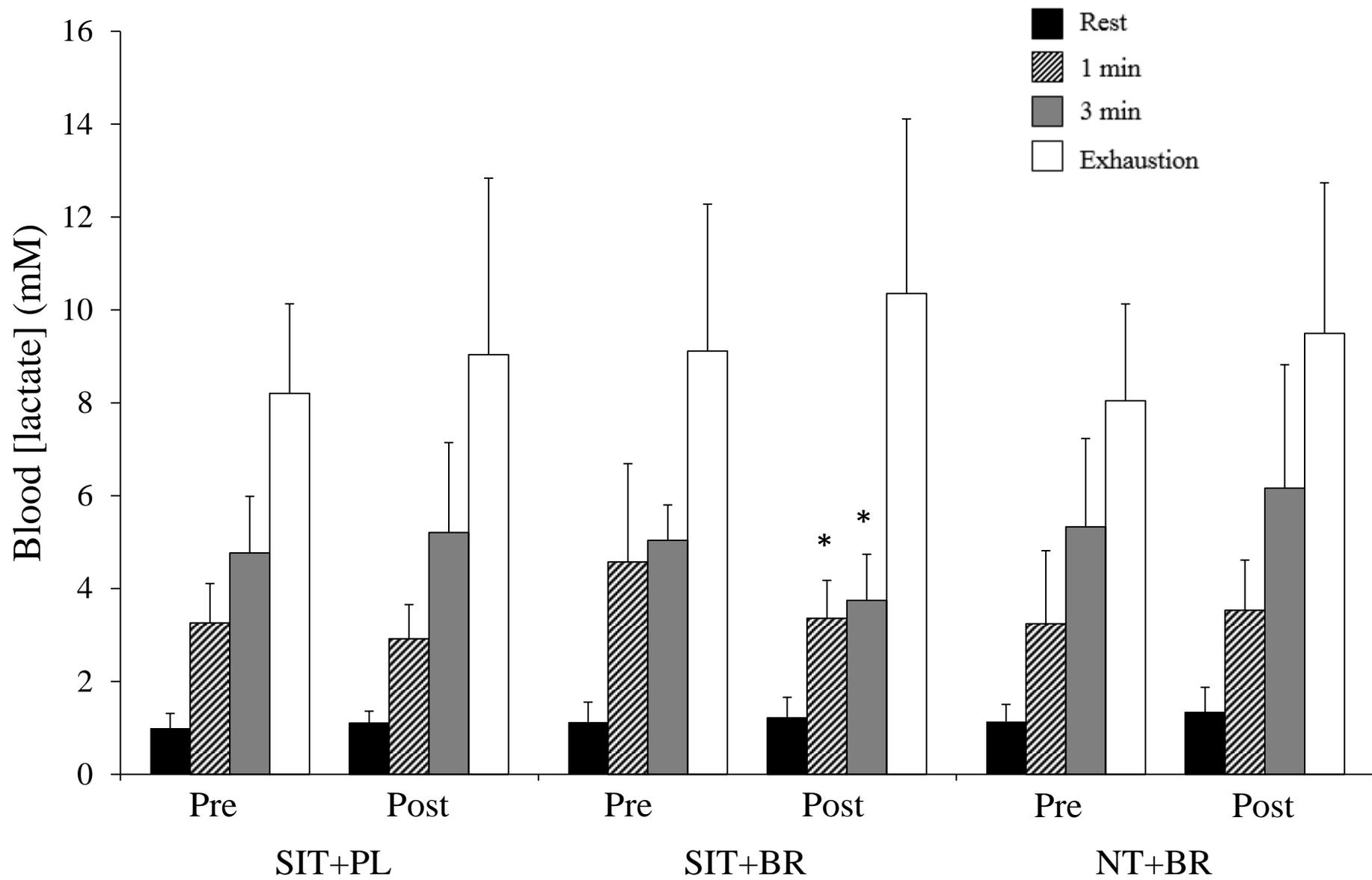
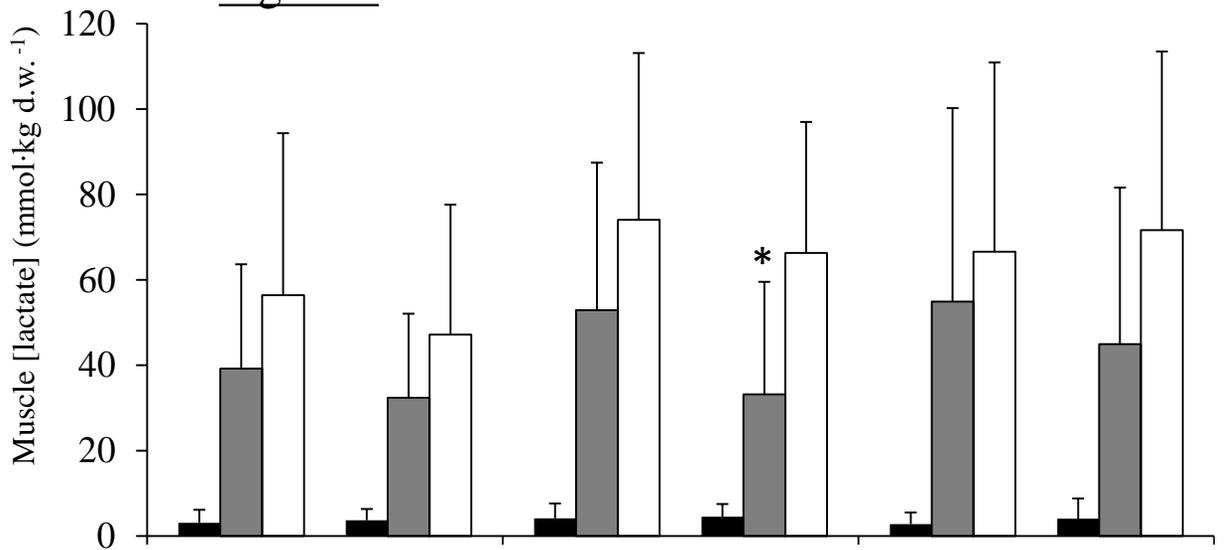
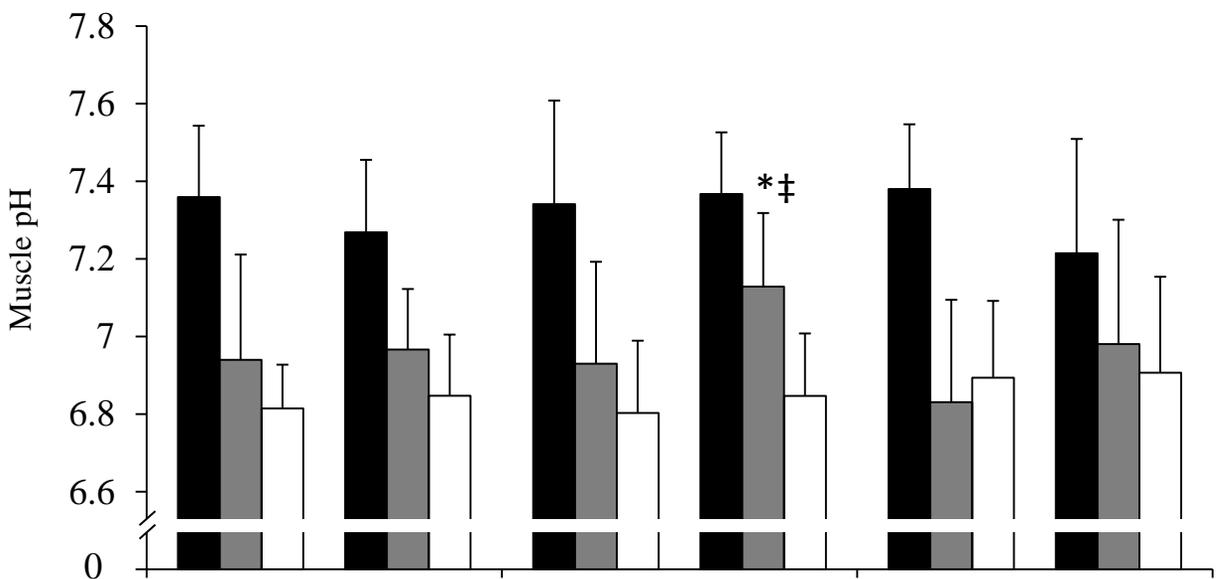


Figure 4

A



B



C

