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

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Abstract

We hypothesized that 4 weeks of dietary nitrate supplementation would enhance exercise performance and muscle metabolic adaptations to sprint interval training (SIT). Thirty six recreationally-active subjects, matched on key variables at baseline, completed a series of exercise tests before and following a 4 week period in which they were allocated to one of the following groups: 1) SIT and NO_3^--depleted beetroot juice as a placebo (SIT+PL); 2) SIT and NO_3^--rich beetroot juice (~13mmol NO_3^-/day; SIT+BR); or 3) no training and NO_3^--rich beetroot juice (NT+BR). During moderate-intensity exercise, pulmonary $\dot{V}O_2$ was reduced by 4% following 4 weeks of SIT+BR and NT+BR (P<0.05) but not SIT+PL. The peak work rate attained during incremental exercise increased more in SIT+BR than in SIT+PL (P<0.05) or NT+BR (P<0.001). The reduction in muscle and blood [lactate] and the increase in muscle pH from pre- to post-intervention was greater at 3 min of severe-intensity exercise in SIT+BR compared to SIT+PL and NT+BR (P<0.05). However, the change in severe-intensity exercise performance was not different between SIT+BR and SIT+PL (P>0.05). The relative proportion of type IIx muscle fibers in the m. vastus lateralis was reduced in SIT+BR only (P<0.05). These findings suggest that BR supplementation may enhance some aspects of the physiological adaptations to SIT.

New and Noteworthy: We investigated the influence of nitrate-rich and nitrate-depleted beetroot juice on the muscle metabolic and physiological adaptations to 4 weeks of sprint interval training. Compared to placebo, dietary nitrate supplementation reduced the O_2 cost of submaximal exercise, resulted in greater improvement in incremental (but not severe-intensity) exercise performance, and augmented some muscle metabolic adaptations to training. Nitrate supplementation may facilitate some of the physiological responses to sprint interval training.

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

Introduction

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

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

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

Sprint interval training (SIT) is known to provide a potent and relatively time-efficient stimulus for enhancing aerobic capacity and endurance exercise performance (11, 12, 13, 26). However, the effects of a high-NO_3^- dietary supplement, such as beetroot juice, consumed daily as part of an exercise training program, on the physiological and muscle metabolic adaptations to training has received limited attention (21, 57). It is possible that the NO-mediated inhibition of O_2 consumption at cytochrome c oxidase (10, 17) and resultant local hypoxia may initiate signaling cascades that may be synergistic (or antagonistic) to those generated by SIT (27). Also, similar to the effects of training, elevated NO bioavailability may stimulate angiogenesis (25), mitochondrial biogenesis (58) and the transformation of muscle fiber phenotype (59, 68) through cGMP-dependent gene expression and the activation of regulatory factors, in particular peroxisome proliferator-activated receptor-gamma coactivator-1 alpha (PGC-1α; 37, 43). It could also be anticipated that the lower $\dot{V}O_2$ and reduced adenosine triphosphate (ATP) and phosphocreatine (PCr) cost of muscle force production during high-intensity exercise following NO_3^- supplementation (6, 24) might enable a higher training intensity for the same effort which, over time, may lead to greater training adaptation (34). An increase in cytosolic calcium concentration ($[Ca^{2+}]$) and force production during muscle contraction following NO_3^- supplementation (32) may also permit a higher training intensity to be maintained. Given the potentially complementary effects of exercise training and NO bioavailability on metabolic regulation, it is possible that NO_3^- supplementation could augment the physiological adaptations to SIT.

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

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

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

Methods

Subjects

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

Experimental Design

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

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 $\dot{V}O_{2\ peak}$
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

Supplementation

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

Incremental exercise tests

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

196

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 ± SD, age 24 ± 7 years, height 174 ±
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

antecubital vein and were collected into lithium-heparin vacutainers (Becton Dickinson, New Jersey, USA). Blood [lactate] and [glucose], as well as plasma [NO_2^-] and [NO_3^-] were analyzed in all samples (square brackets denote concentration). 200 µL of blood was immediately extracted from the lithium-heparin vacutainers and hemolysed in 200 µL of Triton X-100 solution (Triton X-100, Amresco, Salon, OH) before blood [lactate] and [glucose] were measured (YSI 2300, Yellow Springs Instruments, Yellow Springs, OH). The remaining whole blood from each sample was centrifuged at 4000 rpm for 8 min at 4 ºC within 2 min of collection. Plasma was immediately extracted, frozen at -80 ºC and subsequently analyzed for [NO_2^-] and [NO_3^-] using chemiluminescence, as described by Wylie et al. (79).

Muscle biopsy

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

Muscle metabolites

Following a freeze-drying process, samples were dissected to remove visible blood, fat, and connective tissue. Approximately 2 mg aliquots of isolated muscle fibers were weighed on 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 ($KHCO_3$) 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,

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

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

Statistical analyses

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

Results

Compliance

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

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

Plasma [NO_3^-] and [NO_2^-]

Pre-intervention resting plasma [NO_3^-] values were not different between groups (P>0.05). A significant main effect for time (P<0.001) and an interaction effect (P<0.001) was observed for the plasma [NO_3^-] measured at rest. Compared to pre-intervention, SIT+BR increased resting plasma [NO_3^-] by ~590% at 2 weeks and ~960% at 4 weeks (both P<0.001; Fig. 1A) and NT+BR increased resting plasma [NO_3^-] by ~505% at 2 weeks and ~1050% at 4 weeks (both P<0.001; Fig. 1A) but there was no change in resting plasma [NO_3^-] with SIT+PL (P>0.05; Fig. 1A). Resting plasma [NO_3^-] was also greater at 4 weeks compared to 2 weeks in both SIT+BR and NT+BR (P<0.05; Fig. 1A).

Pre-intervention resting plasma [NO_2^-] values were higher in SIT+PL (74 ± 62 nM) compared to SIT+BR (29 ± 19 nM; P<0.05) and NT+BR (26 ± 13 nM; P<0.05) but were similar between SIT+BR and NT+BR (P>0.05). There was a significant main effect for time (P<0.001) and an interaction effect (P<0.001) for the plasma [NO_2^-] measured at rest. Compared to pre-intervention, SIT+BR increased resting plasma [NO_2^-] by ~485% at 2 weeks and ~715% at 4 weeks (both P<0.001; Fig. 1B) and NT+BR increased resting plasma [NO_2^-] by ~600% at 2 weeks and ~690% at 4 weeks (both P<0.001; Fig. 1B) but there was no change in resting plasma [NO_2^-] with SIT+PL (P>0.05; Fig. 1B). There were no differences in the plasma [NO_2^-] measured at rest between 2 weeks and 4 weeks in any of the groups (P>0.05).

Blood Pressure

Systolic BP was not different between the groups before the interventions (P>0.05; Table 1) but there was a significant main effect for time (P<0.05) and an interaction effect (P<0.05). Post hoc tests revealed that, compared to pre-intervention, systolic BP was reduced at 2 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 [NO_2^-] declined by ~65% at task failure during severe-intensity exercise (P<0.001) in
354 SIT+BR and NT+BR. The reduction in plasma [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 $\dot{V}O_{2peak}$ was not different between the groups at pre-intervention (P>0.05; Table 1). There was
364 a significant main effect by time on $\dot{V}O_{2peak}$ (P<0.05). Post hoc analysis revealed that,
365 compared to pre-intervention, $\dot{V}O_{2peak}$ 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 $\dot{V}O_{2peak}$ 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.

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

Step exercise tests: Moderate-intensity exercise

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

Step exercise tests: Severe-intensity exercise

The time to task failure during severe-intensity exercise was not different between groups at pre-intervention (P>0.05; Table 1). There was a significant main effect by time (P<0.05) and an interaction effect (P<0.05) such that time to task failure was improved by 163 ± 144 s pre- to post- intervention in SIT+PL (P<0.05; Table 1) and by 170 ± 90 s pre- to post-intervention 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).

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

Muscle fiber type

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

440 pre-intervention (95 ± 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

Discussion

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

Plasma [NO_3^-] and [NO_2^-] were elevated, and systolic BP was lowered following 2 weeks and 4 weeks of BR supplementation, changes which are consistent with elevated systemic NO bioavailability. Interestingly, however, resting plasma [NO_3^-] and [NO_2^-] were not altered following 4 weeks of SIT+PL. Previous studies have reported that subjects with higher aerobic fitness and/or training status have higher resting plasma [NO_3^-] and [NO_2^-] compared to less fit and/or sedentary subjects (53, 63). The results of the present study may therefore indicate that short-term SIT, at least when combined with PL supplementation, does not substantially modify NOS activity or protein expression. The reduction in plasma [NO_2^-] from resting baseline to task failure during severe-intensity exercise was similar between NT+BR and SIT+BR (~65% decline). However, the reduction in plasma [NO_2^-] from resting baseline to 3 min of severe-intensity exercise was attenuated in SIT+BR (~25% decline) 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* $\dot{V}O_2$ *and* $\dot{V}O_{2peak}$

479 A high exercise economy, i.e. a low $\dot{V}O_2$ 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 $\dot{V}O_{2peak}$ 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 $\dot{V}O_{2peak}$ following
494 acute or short-term NO_3^- supplementation (5, 6, 39, 45, 78). The lack of effect of SIT on
495 $\dot{V}O_{2peak}$ 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 $\dot{V}O_{2peak}$ was increased.

502 Although the change in $\dot{V}O_{2peak}$ from pre- to post-intervention was not different between the
503 three groups, the increase in $\dot{V}O_{2peak}$ was only greater from pre- to post intervention in
504 SIT+BR, suggesting that NO_3^- supplementation may enhance the adaptation of $\dot{V}O_{2peak}$ 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

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

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

541

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

Although conflicting data exist, SIT has been implicated in rapid skeletal muscle remodeling (11, 12, 13, 26). The extreme perturbations in substrate availability and metabolite accumulation caused by repeated sprint efforts require substantial oxidative energy turnover to restore homeostasis (8). The fluctuations in ATP availability and local O_2 tension are potent stimulators of signaling pathways and may induce mitochondrial biogenesis and oxidative enzyme adaptation via the transcription of PGC-1α (28, 31, 50). Recent findings indicate that dietary NO_3^- may favorably affect the contractility (30, 32) and perfusion (22, 23) of type II muscle fibers, and reduce the energetic cost of muscle force production during high-intensity exercise (6, 24). Similar to SIT (3, 13, 47, 48, 49, 61, 62), elevating NO_2^- and NO bioavailability with chronic NO_3^--rich BR supplementation may also stimulate the transcription of PGC-1α (43, 54, 58), a key regulator of mitochondrial biogenesis (77) and angiogenesis (1, 15). We therefore determined the effects of 4 weeks SIT and 4 weeks BR supplementation on the muscle metabolic responses during exercise and tested the hypothesis that these adaptations may be amplified when the interventions were combined.

There were no differences in muscle [ATP], [PCr], [lactate] or pH at rest or at task failure during severe-intensity exercise, post-intervention compared to pre-intervention, in any group. However, at 3 min into severe-intensity exercise, there was evidence of reduced metabolic perturbation, post-intervention compared to pre-intervention, in the SIT+BR group only. Specifically, muscle [lactate] as well as blood [lactate] was lower, and muscle pH was higher, at 3 min of severe-intensity exercise following SIT+BR but not SIT+PL or NT+BR (Figs. 3 and 4), suggesting an enhanced muscle metabolic adaptation to SIT when combined 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 O_2 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 PO_2 in type II muscles of exercising rats thus promoting O_2 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 NO_3^- intake alone would be
576 expected to promote some of these effects (for example, a lower O_2 cost of sub-maximal
577 exercise in the NT+BR group in the present study), NO_3^- 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 betacyacins 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

633 **Acknowledgements**

634 The authors thank Chiara Gattoni, Tom Male, Charlie Dean, Scott Hobbs, Louis Bowers and
635 Taro Isidore for assisting during exercise testing and training. We also thank Professor R
636 Hugh Morton for statistical advice.

637

638 **Additional Information**

639 Competing Interests: None of the authors has any conflicts of interests.

640 Funding: This study was supported by grant 2012-1891470 from PepsiCo, Il, USA. Jonathan
641 Fulford's salary was supported via an NIHR grant.

642

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888

889

890 **Figure Legends**

891 Figure 1. Mean ± SD resting plasma [NO_3^-] (panel A) and plasma [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 ± 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 ± 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 ± 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

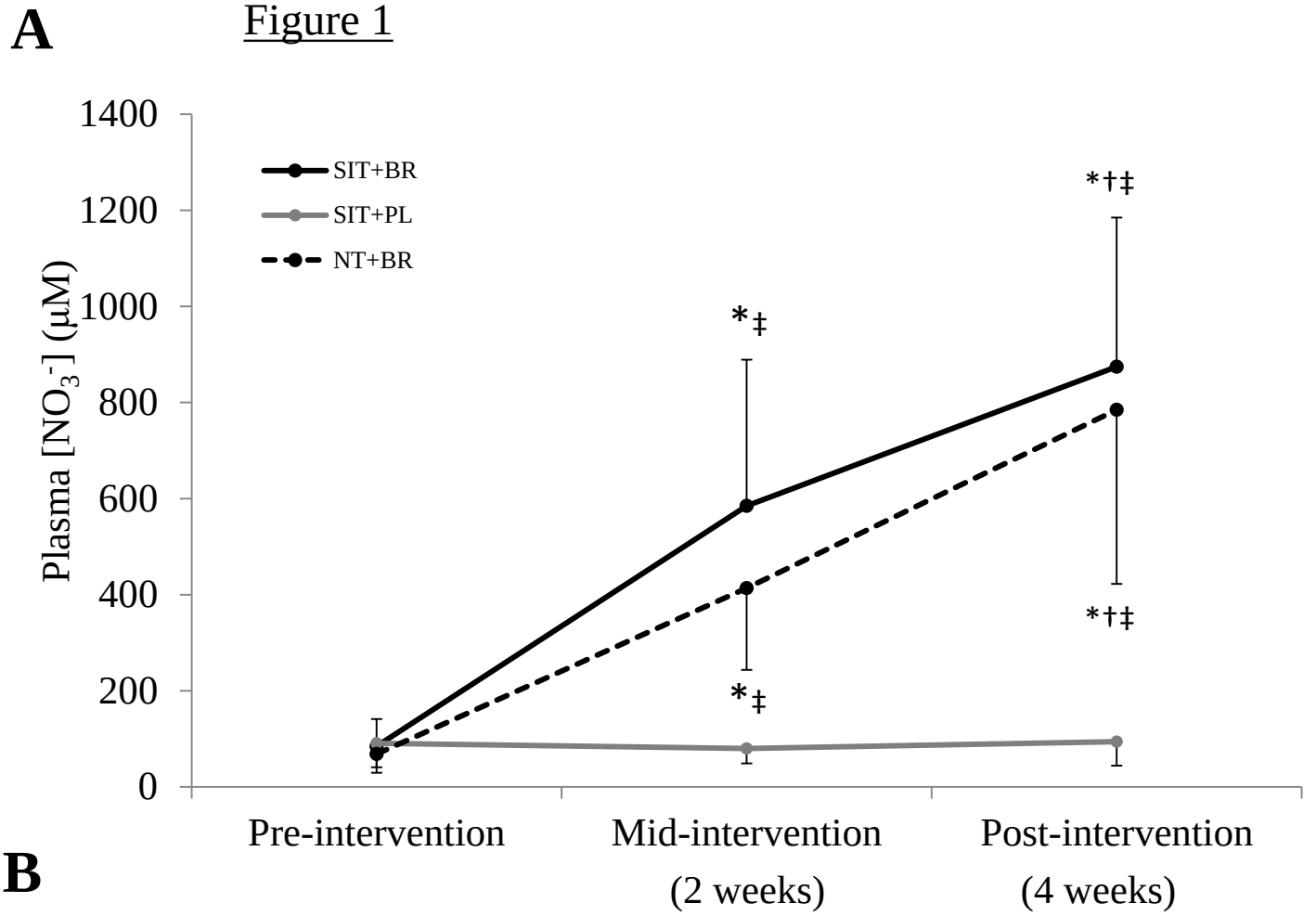
Table 1. Physiological and performance variables pre-, mid- and post-intervention

	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*
$\dot{V}O_{2\ peak}$ ($L \cdot min^{-1}$)	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
$\dot{V}O_2$ at GET ($L \cdot min^{-1}$)	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 $\dot{V}O_2$ ($L \cdot min^{-1}$)	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; $\dot{V}O_{2\ peak}$, peak $\dot{V}O_2$; WR, work rate; GET, gas exchange threshold; SIT+BR, high-intensity interval training plus NO_3^--rich beetroot juice; SIT+PL, high-intensity interval training plus NO_3^--depleted juice; NT+BR, no-training plus NO_3^--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


A
1400
1200
1000
800
600
400
200
0
Plasma [NO3-] (μM)
SIT+BR
SIT+PL
NT+BR
*‡
*†‡
*‡
*†‡
Pre-intervention
Mid-intervention
(2 weeks)
Post-intervention
(4 weeks)

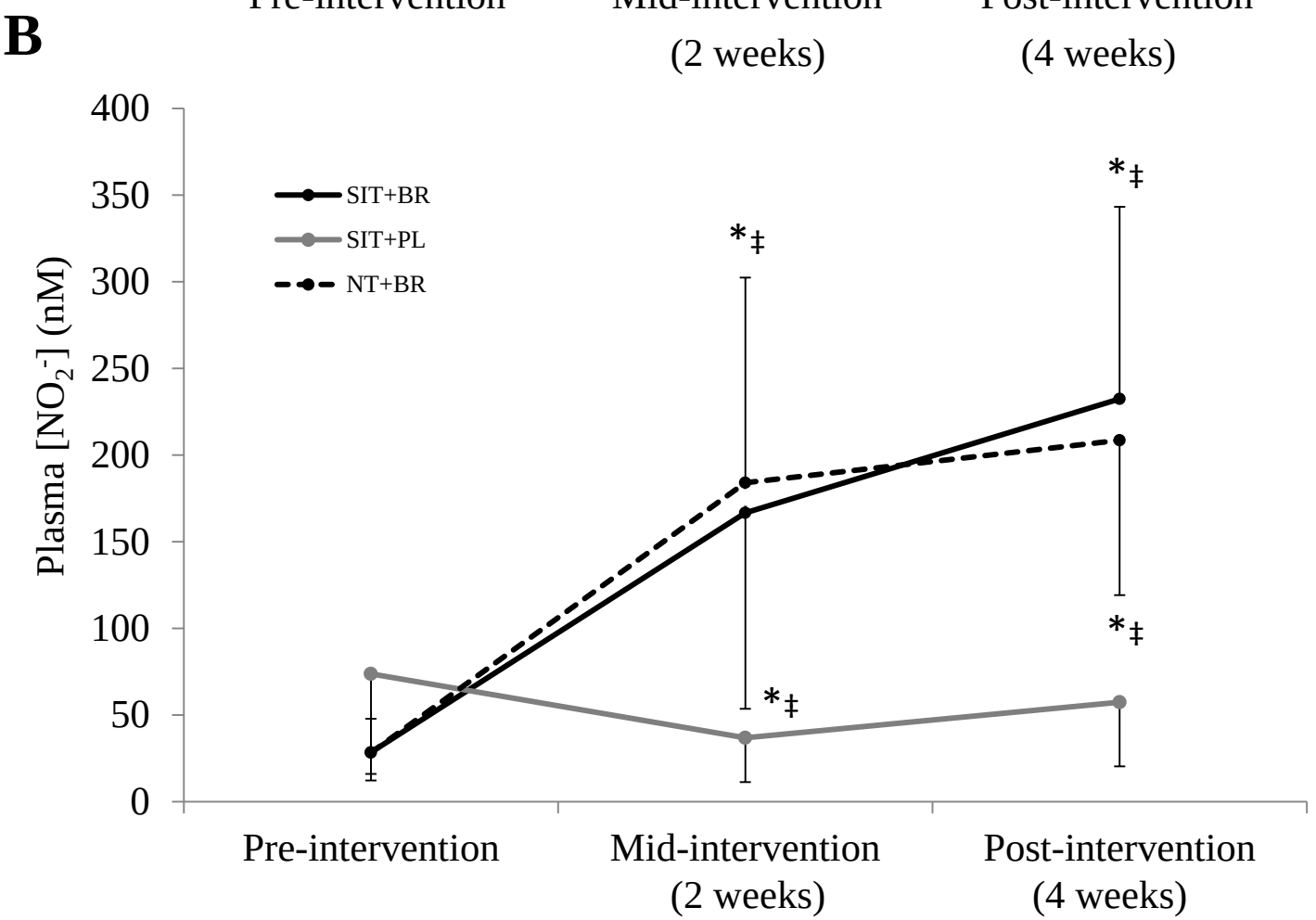

B
400
350
300
250
200
150
100
50
0
Plasma [NO2-] (nM)
SIT+BR
SIT+PL
NT+BR
*‡
*‡
*‡
*‡
Pre-intervention
Mid-intervention
(2 weeks)
Post-intervention
(4 weeks)

Figure 2

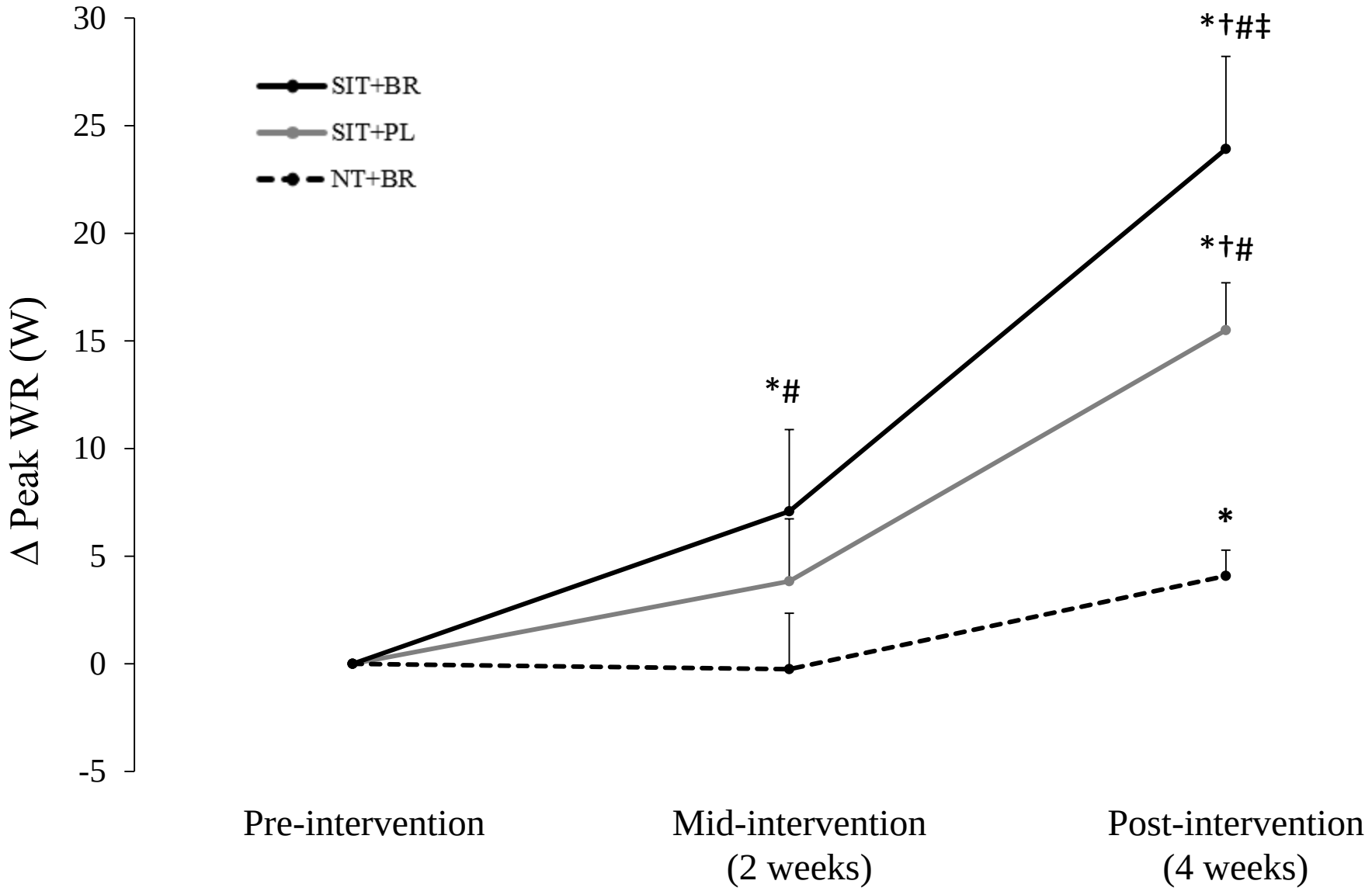

30
25
20
15
10
5
0
-5
Δ Peak WR (W)
SIT+BR
SIT+PL
NT+BR
*†#‡
*†#
*#
*
Pre-intervention
Mid-intervention
(2 weeks)
Post-intervention
(4 weeks)

Figure 3

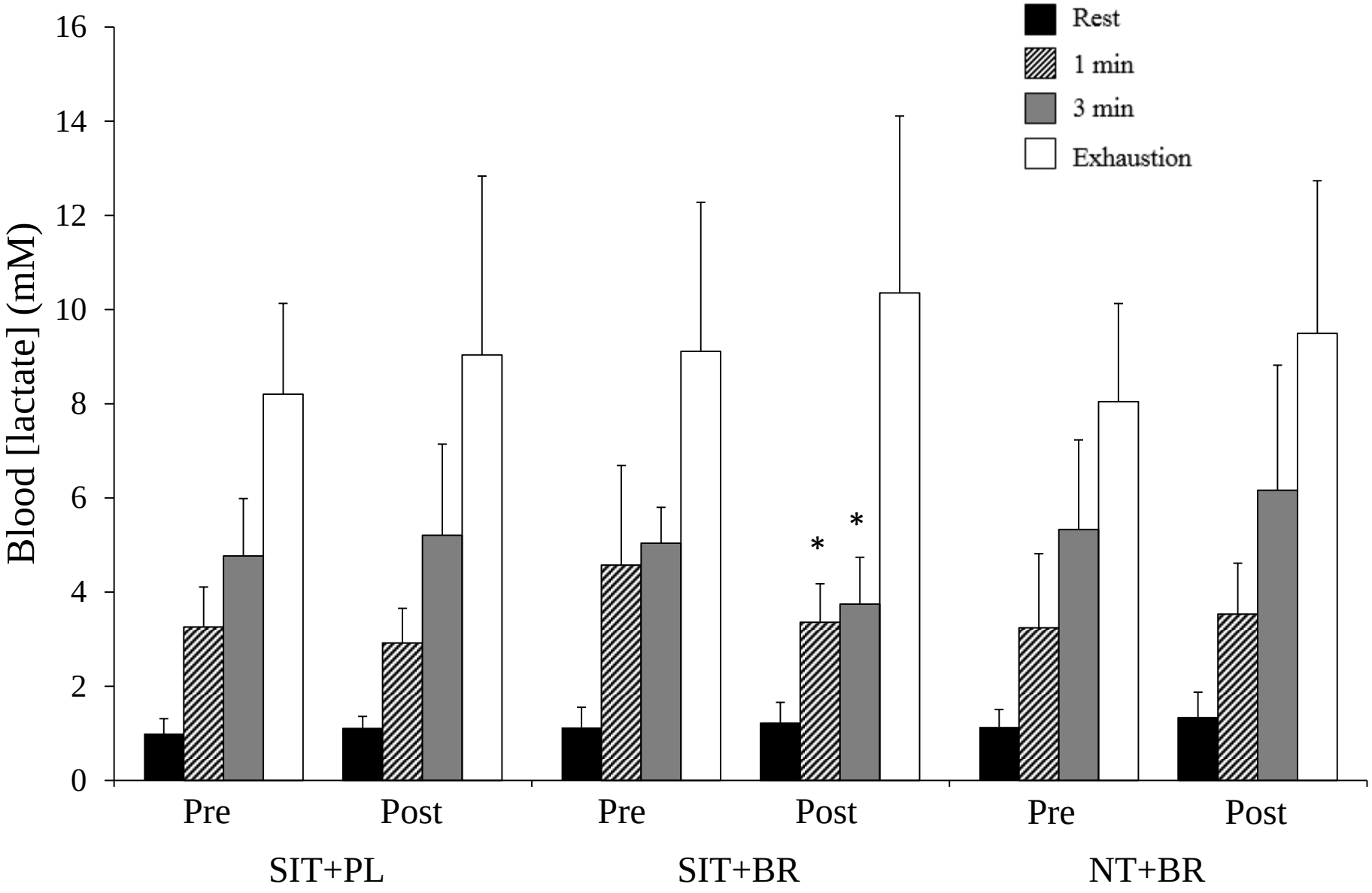
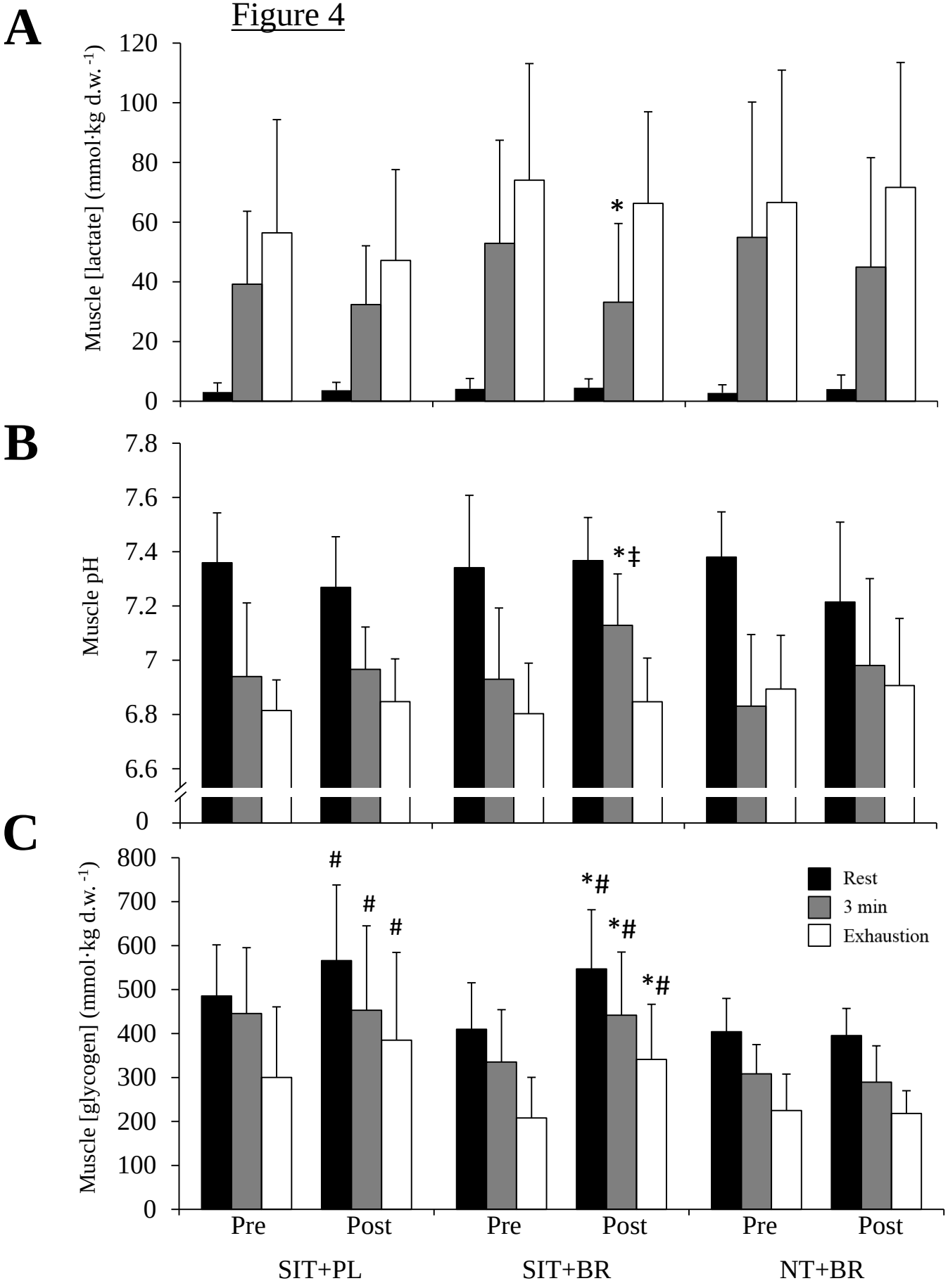

Rest
1 min
3 min
Exhaustion
Blood [lactate] (mM)
16
14
12
10
8
6
4
2
0
*
*
Pre
Post
Pre
Post
Pre
Post
SIT+PL
SIT+BR
NT+BR

Figure 4


A
Muscle [lactate] (mmol·kg d.w.-1)
120
100
80
60
40
20
0
*
B
Muscle pH
7.8
7.6
7.4
7.2
7
6.8
6.6
0
*‡
C
Muscle [glycogen] (mmol·kg d.w.-1)
800
700
600
500
400
300
200
100
0
#
*#
Rest
3 min
Exhaustion
Pre
Post
SIT+PL
SIT+BR
NT+BR