

1 **Dietary nitrate supplementation attenuates the reduction in**
2 **exercise tolerance following blood donation**

3

4 *Original Article*

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13 **Running head:** Nitrate, blood donation and exercise performance

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29 **ABSTRACT**

30 We tested the hypothesis that dietary nitrate-rich beetroot juice (BR) supplementation could
31 partially offset deteriorations in O₂ transport and utilization, and exercise tolerance, after
32 blood donation. Twenty-two healthy volunteers performed moderate-intensity and ramp
33 incremental cycle exercise tests prior to and following the withdrawal of ~450 mL of whole
34 blood. Before donation, all subjects consumed 7 x 70 mL of nitrate-depleted beetroot juice
35 shots (PL) in the 48 h preceding the exercise tests. During the 48 h after blood donation,
36 subjects consumed 7 shots of either BR (each containing 6.2 mmol nitrate; *n*=11) or PL
37 (*n*=11) before repeating the exercise tests. [Hemoglobin] and hematocrit were reduced by ~8-
38 9% following blood donation (*P*<0.05), with no difference between the BR and PL groups.
39 When compared with pre-donation, steady-state $\dot{V}O_2$ during moderate-intensity exercise was
40 ~4% lower post-donation in BR (*P*<0.05) but was unchanged in PL. The ramp test peak
41 power decreased from pre-donation (PL: 341 ± 70 vs. BR: 331 ± 68 W) to post-donation (PL:
42 324 ± 69 vs. BR: 322 ± 66 W) in both groups (*P*<0.05). However, the decrement in
43 performance was significantly less in BR (2.7%) compared with PL (5.0%; *P*<0.05). Nitrate
44 supplementation reduced the O₂ cost of moderate-intensity exercise and attenuated the
45 decline in ramp incremental exercise performance following blood donation. These results
46 have implications for improving functional capacity following blood loss.

47

48 **New and Noteworthy:** Dietary nitrate supplementation with beetroot juice lowered the O₂
49 cost of moderate-intensity exercise, better preserved muscle oxygenation and attenuated the
50 decline in incremental exercise test performance following donation of 450 mL whole blood.
51 These results have implications for improving functional capacity following blood loss.

52

53 **Key words:** blood withdrawal; beetroot juice; O₂ transport; O₂ uptake; exercise performance;
54 nitric oxide

55 INTRODUCTION

56 The peak rate of pulmonary oxygen uptake ($\dot{V}O_{2\text{peak}}$) is an important determinant of exercise
57 capacity and is influenced by the interaction of several central and peripheral factors (6, 53,
58 64). $\dot{V}O_{2\text{peak}}$ and exercise performance can be altered by manipulating the capability of the
59 cardiovascular system to transport O₂ to contracting skeletal muscles during exercise (5, 11,
60 18, 51, 57, 67). For example, interventions involving the infusion of erythrocytes (18, 19) or
61 the stimulation of erythropoiesis (57, 67) to enhance hemoglobin concentration ([Hb]),
62 increase $\dot{V}O_{2\text{peak}}$ during maximal exercise. Conversely, limiting O₂ transport to working
63 muscle by reducing [Hb] via whole blood withdrawal consistently results in a lowered
64 $\dot{V}O_{2\text{peak}}$ (11, 18, 47, 54). During sub-maximal exercise, however, Panebianco et al. (47)
65 reported no change in $\dot{V}O_2$ at two and seven days post 450 mL blood donation, despite
66 significant reductions in [Hb]. Compensatory adjustments in cardiovascular control, such as
67 increases in heart rate (HR) and cardiac output (\dot{Q}), offset the lower [Hb] and enable muscle
68 O₂ delivery to be maintained during low-intensity exercise after blood donation (19, 27, 51).

69

70 The gaseous physiological signaling molecule, nitric oxide (NO), plays a key role in the
71 regulation of vascular tone. NO can be synthesised via the oxidation of L-arginine in a
72 reaction catalysed by the NO synthases (NOS; 32) or it can be produced via the reduction of
73 nitrate (NO₃⁻) to nitrite (NO₂⁻) and subsequently NO (8). Recently, dietary NO₃⁻
74 supplementation has been employed to augment plasma [NO₂⁻] and the potential for O₂-
75 independent NO synthesis (4, 38, 65). This NO₃⁻-NO₂⁻-NO pathway may be particularly
76 important when NOS activity is compromised (20, 42), O₂ availability is limited (14, 25, 34,

77 35) and pH is low (44). Limitations in systemic O₂ transport can result in tissue hypoxia and
78 greater metabolic perturbation (41, 60), which can contribute to reduced exercise tolerance
79 (1), as is commonly observed at altitude (2) and in a number of disease states (35, 68). There
80 is evidence to suggest that NO and NO₂⁻ can combat an insufficient muscle O₂ supply by
81 increasing muscle blood flow via hypoxia-induced vasodilatation (13, 61). Therefore, it is
82 possible that dietary NO₃⁻ supplementation could ameliorate deteriorations in exercise
83 performance when ‘normal’ O₂ availability is reduced, during for example, high-intensity
84 exercise, in hypobaric hypoxia or after blood donation.

85

86 We and others have reported that, in healthy subjects, dietary NO₃⁻ supplementation can
87 significantly impact the physiological responses to exercise (4, 15, 38, 59). Specifically, a
88 reduction in the O₂ cost of moderate-intensity exercise has been reported after
89 supplementation with both sodium NO₃⁻ (38, 39, 40) and NO₃⁻-rich beetroot juice (BR; 3, 4,
90 15, 59, 69). In addition, a significantly increased time to task failure (TTF), indicating
91 improved exercise tolerance, has been reported following BR ingestion when recreationally-
92 active, but not highly trained, subjects completed severe-intensity (3, 4, 37) and ramp
93 incremental exercise (59). These alterations may be due to a NO₂⁻ or NO-related reduction in
94 the ATP cost of muscle contraction (3), greater mitochondrial efficiency (40), changes in
95 muscle redox status (66), and/or enhanced muscle blood flow, particularly to type II fibres
96 (21, 22). Such changes could be particularly advantageous after whole blood withdrawal
97 when [Hb] is reduced and O₂ transport is challenged (11, 18, 54). Indeed, BR
98 supplementation has been shown to reduce muscle metabolic perturbation during exercise in
99 normobaric hypoxia and to restore exercise tolerance and oxidative function to the values
100 observed in normoxia (60, 61). In addition, it has been reported that, when the fraction of
101 inspired O₂ is lowered to 11-13%, BR supplementation can improve muscle oxygenation

102 status (43), reduce $\dot{V}O_2$ during sub-maximal exercise (34, 46), and enhance TTF during
103 incremental exercise (43). BR supplementation has also been reported to increase arterial O_2
104 saturation following dynamic apnea (i.e., breath-hold diving), which supports an O_2 sparing
105 effect of NO_3^- ingestion (48). Collectively, these studies suggest that NO_3^- ingestion may
106 enhance the physiological response to exercise when O_2 availability is limited, by sparing
107 muscle O_2 demand and/or better preserving muscle O_2 supply. However, it is not known
108 whether the reductions in O_2 carrying capacity and exercise performance subsequent to the
109 withdrawal of whole blood can be offset by BR supplementation. If so, this may have
110 important implications for clinical conditions in which [Hb] is lowered, for example in
111 anemia, following surgery or involuntary blood loss, or in athletes wishing to donate blood
112 without compromising training.

113

114 The purpose of the present study was to determine whether 48 h of BR supplementation
115 following 450 mL of whole blood withdrawal alters the physiological responses to sub-
116 maximal and maximal intensity cycle exercise. It was hypothesized that BR supplementation
117 would lower the O_2 cost of moderate-intensity exercise, improve muscle oxygenation status,
118 and attenuate the expected reduction in TTF during ramp incremental exercise following
119 blood donation.

120

121 **METHODS**

122 *Subjects*

123 Twenty-two recreationally active and pre-registered National Health Service (NHS) blood
124 donors (males, $n = 14$; females, $n = 8$) volunteered to participate in this study, which was
125 approved by the Institutional Research Ethics Committee and conformed to the ethical
126 principles of the Declaration of Helsinki. None of the subjects were tobacco smokers or

127 habitual users of dietary supplements. All subjects provided written informed consent prior to
128 the commencement of the study, after the experimental procedures, associated risks and
129 potential benefits of participation had been explained.

130

131 Subjects were instructed to arrive at the laboratory in a rested and fully hydrated state, at least
132 3 h postprandial, and to avoid strenuous exercise in the 24 h preceding each visit. In addition,
133 subjects were asked to avoid alcohol consumption, chewing gum and antibacterial
134 mouthwash throughout each supplementation period and to avoid caffeine intake in the 3 h
135 preceding each laboratory visit. Each subject recorded habitual diet and exercise undertaken
136 during the first supplementation period and were asked to replicate these habits during the
137 second supplementation period. Prior to data collection, subjects were fully familiarized with
138 the exercise testing procedures. This minimized any possible learning effects during the
139 study. Exclusion criteria were the presence of known cardiovascular disease, hypertension
140 and anemia, the use of antihypertensive medication and antibiotics, and having major surgery
141 or giving blood within 6 months of the study commencing.

142

143 *Experimental Overview*

144 Subjects were asked to report to the laboratory on three separate occasions over a ten day
145 period. The first visit included a 5 min bout of moderate-intensity cycle exercise at 80 W,
146 followed by a ramp incremental test to task failure with no dietary supplementation. This
147 served as the pre-intervention familiarization test. Hematocrit (Hct), [Hb], plasma [NO₃⁻] and
148 [NO₂⁻], pulmonary $\dot{V}O_2$ dynamics, muscle oxygenation status, HR, blood lactate
149 concentration ([lactate]), blood glucose concentration ([glucose]) and TTF during ramp
150 incremental exercise were measured during the first visit and repeated during each visit to the
151 laboratory. Prior to visit 2, subjects consumed 7 shots of NO₃⁻-depleted beetroot juice (PL)

152 over ~48 h. On the final day of supplementation, subjects completed the same moderate-
153 intensity exercise bout and ramp incremental test on a cycle ergometer as was performed at
154 pre-intervention. Two days before the final visit to the lab, subjects attended a National
155 Health Service (NHS) blood donation clinic. Each subject lay supine on a bed before ~450
156 mL of whole blood was drawn from an antecubital vein over a 15 min period. The blood
157 withdrawal was performed by the NHS as part of the national blood donation service.
158 Following blood donation, each subject was randomly assigned, in a double-blind, placebo
159 controlled fashion to consume 7 shots of either NO₃⁻-rich beetroot juice (BR; $n = 11$; mean \pm
160 SD; females, $n = 4$: age 23 ± 3 years, body mass 67 ± 4 kg, height 1.76 ± 0.05 m; males, $n =$
161 7 : age 26 ± 5 years, body mass 81 ± 12 kg, height 1.80 ± 0.10 m) or NO₃⁻-depleted beetroot
162 juice as a placebo (PL; $n = 11$; mean \pm SD; females, $n = 4$: age 22 ± 3 years, body mass $77 \pm$
163 11 kg, height 1.75 ± 0.10 m; males, $n = 7$: age 28 ± 7 years, body mass 77 ± 8 kg, height 1.79
164 ± 0.10 m) over the next ~48 h. Visit 3 occurred on the final day of supplementation with the
165 exercise tests conducted 2 h following final supplement ingestion. All tests were performed at
166 the same time of day (± 2 h) to minimise diurnal variation on the physiological variables
167 under investigation.

168

169 *Exercise tests*

170 During the first visit to the laboratory subjects performed a short bout of low-intensity
171 exercise at 80 W, followed by a ramp incremental exercise test to task failure on an
172 electrically-braked cycle ergometer (Lode Excalibur Sport, Gronigen, The Netherlands) for
173 determination of $\dot{V}O_{2peak}$ and gas exchange threshold (GET). The protocol began with 3 min
174 of 'unloaded' baseline cycling at 20 W, followed by 5 min at 80 W and 10 min of passive
175 rest. Subsequently, 3 min of baseline cycling at 20 W was performed and then the power
176 output was increased linearly by $30 \text{ W}\cdot\text{min}^{-1}$ until the subject was unable to continue. The

177 subjects cycled at a self selected cadence (~80 rpm), and this cadence, along with saddle and
178 handle bar configuration, was recorded and replicated for subsequent tests. Pulmonary gas
179 exchange was measured breath-by-breath and averaged into 10-s bins. $\dot{V}O_{2peak}$ was taken as
180 the highest 30-s mean value attained during the test. The GET was determined as described
181 previously (59). The work rate that would require 80% of the GET (moderate-intensity
182 exercise) was calculated, taking into account the mean response time for $\dot{V}O_2$ during ramp
183 exercise (59).

184

185 Subjects returned to the laboratory on two further occasions. The second visit was preceded
186 by PL supplementation ($n = 22$) and the third visit, ~48 h post blood donation, was preceded
187 by 2 days of either BR ($n = 11$) or PL ($n = 11$) supplementation. The final visit was
188 conducted 48 h post donation to allow restoration of total blood volume (23) and to minimize
189 the risk of a syncopal episode occurring during maximal exercise. On each of these two
190 laboratory visits, subjects completed a single 5-min bout of moderate-intensity exercise (at 80
191 % of the GET) and a ramp incremental test to task failure, separated by 10 min of passive
192 rest. The incremental test was terminated when cadence fell more than 10 rpm below the
193 chosen cadence, despite strong verbal encouragement. TTF was recorded to the nearest
194 second and the power output achieved at the point of test termination was recorded as the
195 peak power output (PPO). Feedback on performance was only provided once all
196 experimentation for the entire study had been completed.

197

198 *Measurements*

199 During each visit to the laboratory, a venous blood sample (~4 mL) was drawn from an
200 antecubital vein into lithium-heparin tubes (Vacutainer, Becton-Dickinson, NJ, USA) and
201 centrifuged for 10 min at 3000 g and 4°C, within 2 min of collection. Subsequently, the

202 plasma was extracted and frozen at -80°C for later determination of $[\text{NO}_3^-]$ and $[\text{NO}_2^-]$ using
203 a modified chemiluminescence technique (7) as previously described (69). Blood samples
204 from a pre-warmed fingertip were collected into four $30\ \mu\text{l}$ heparinized microhematocrit tubes
205 (Hawksley and Sons Ltd, Lancing, Sussex, England) which underwent microcentrifugation
206 for 1 min for the determination of Hct (1560 Micro-haematocrit reader, Hawksley and Sons
207 Ltd, Lancing, Sussex, England). In addition, blood from the same fingertip was collected into
208 four microcuvettes for determination of [Hb] (HemoCue AB, Ängelholm, Sweden).

209

210 Pulmonary gas exchange and ventilation were measured breath-by-breath throughout all
211 exercise tests. Subjects wore a nose clip and breathed through a mouthpiece and impeller
212 turbine assembly (Jaeger Triple V). The inspired and expired gas volume and gas
213 concentration signals were sampled continuously at 100 Hz, with the latter using
214 paramagnetic (O_2) and infrared (carbon dioxide; CO_2) analyzers (Oxycon Pro, Jaeger,
215 Hoechberg, Germany) via a capillary line connected to the mouthpiece. These analyzers were
216 calibrated before each test with gases of known concentration, and the turbine volume
217 transducer was calibrated using a 3-litre syringe (Hans Rudolph, Kansas City, MO, USA).
218 The volume and concentration signals were time-aligned by accounting for the delay in
219 capillary gas transit and analyzer rise time relative to the volume signal. Pulmonary O_2
220 uptake ($\dot{V}\text{O}_2$), CO_2 output ($\dot{V}\text{CO}_2$), minute ventilation ($\dot{V}\text{E}$) and respiratory exchange ratio
221 (RER) were calculated and displayed breath-by-breath. HR was measured at rest and during
222 all cycle tests using short-range radiotelemetry (Polar S610, Polar Electro Oy, Kempele,
223 Finland). A fingertip blood sample was collected into a capillary tube over the 20 s preceding
224 the step transition in work rate to moderate-intensity exercise and the incremental test.
225 Capillary samples were also collected during the final 20 s of the moderate-intensity exercise
226 bout and following exhaustion in the ramp test. These samples were analyzed within 60 s of

227 collection to determine blood [lactate] (YSI 2300, Yellow Springs Instruments, Yellow
228 Springs, OH, USA).

229

230 The oxygenation status of the *m. vastus lateralis* of the right leg was monitored using near-
231 infrared spectroscopy (NIRS; model NIRO 300, Hamamatsu Photonics KK, Hiugashi-ku,
232 Japan). Four different wavelength laser diodes provided the light source (776, 826, 845 and
233 905 nm) and a photomultiplier tube in the spectrometer was used to detect the light returning
234 from the tissue. The intensity of incident and transmitted light was recorded continuously
235 throughout exercise at 2 Hz and used to estimate the change in concentration from baseline
236 for oxygenated, deoxygenated, and total tissue Hb and myoglobin. The NIRS data therefore
237 represent a relative change based on the optical density measured in the first data point
238 collected. The deoxyhemoglobin concentration ([HHb]) was assumed to represent the balance
239 between local O₂ supply and utilization and therefore to provide an estimate of changes in O₂
240 extraction within the field of interrogation (28, 36). Prior to the cycling exercise, the right leg
241 was cleaned and shaved around the belly of the muscle, the probes were placed in the holder
242 and attached to the skin with an adhesive 20 cm above the fibular head. An elastic bandage
243 was wrapped around the subject's leg to secure the holder and wires in place and to minimize
244 the possibility of extraneous light influencing the signal. Pen marks were made around the
245 probe holder to allow for precise reproduction of the position of the probe in subsequent tests.
246 The probe gain was set at rest with the subject in a seated position and the leg extended at
247 down stroke on the cycle ergometer. NIRS data were collected continuously throughout the
248 moderate-intensity and incremental exercise tests.

249

250 *Supplementation*

251 After completion of the familiarization test, subjects consumed 7 shots of NO_3^- -depleted
252 beetroot juice (PL; beetroot juice containing ~ 0.04 mmol NO_3^- per 70 mL; Beet It Sport
253 Stamina Shot, James White Drinks, Ltd., Ipswich, UK) over ~ 48 h before completing the pre-
254 donation control trial (PL-Pre and BR-Pre for the PL and BR groups, respectively). This was
255 done in order to control for the antioxidants and polyphenols that exist in both the NO_3^- -rich
256 and NO_3^- -depleted beverages. The PL was created by passing NO_3^- -rich BR through a
257 Purolite A520E ion-exchange resin which selectively removes NO_3^- (37). After blood
258 donation, subjects were randomly assigned, in a double-blind, placebo-controlled fashion, to
259 consume 7 shots of either NO_3^- -rich (BR; beetroot juice containing ~ 6.2 mmol NO_3^- per 70
260 mL; Beet It Sport Stamina Shot, James White Drinks, Ltd., Ipswich, UK; $n = 11$) or NO_3^- -
261 depleted beetroot juice (PL; beetroot juice containing ~ 0.04 mmol NO_3^- per 70 mL; Beet It,
262 James White Drinks, Ltd., Ipswich, UK; $n = 11$) over ~ 48 h (PL-Post and BR-Post for the PL
263 and BR groups, respectively). During both supplementation periods subjects were instructed
264 to consume 2 x 70 mL of the beverage in the evening (~ 7 p.m.) two days prior to testing, and
265 1 x 70 mL in the morning (~ 10 a.m.) and 1 x 70 mL in the evening (~ 7 p.m.) one day prior to
266 testing. On each experimental day, subjects consumed a further 2 x 70 mL, 2 h prior to testing
267 and 1 x 70 mL on arrival at the laboratory. The supplementation periods were separated by a
268 mean of 8 days (BR: 7 ± 5 days, PL: 9 ± 5 days).

269

270 *Data Analyses*

271 The breath-by-breath $\dot{V}\text{O}_2$ data collected during the exercise tests were initially examined to
272 exclude errant breaths caused by, for example, coughing, swallowing and sighing, and those
273 values lying more than four standard deviations (SDs) from the local mean were removed.
274 $\dot{V}\text{O}_{2\text{baseline}}$ was defined as the mean $\dot{V}\text{O}_2$ measured over the last 60 s of baseline cycling and
275 end-exercise $\dot{V}\text{O}_2$ was defined as the mean $\dot{V}\text{O}_2$ measured over the last 30 s of exercise. The

276 baseline and end-exercise $\dot{V}CO_2$, RER, $\dot{V}E$ and HR values were calculated in the same
277 manner.

278

279 To provide information on muscle oxygenation, the changes in [HHb] and the tissue
280 oxygenation index (TOI; calculated as the fraction of oxygenated [Hb] compared to total
281 [Hb]) during moderate-intensity exercise were assessed at baseline (60 s preceding the
282 transition to moderate-intensity exercise), in 10 s time bins surrounding 60 s, 120 s, 240 s,
283 and at end-exercise (mean response over the final 30 s of exercise). During ramp incremental
284 exercise, the changes in [HHb] and TOI were assessed at baseline, in 10 s time bins
285 surrounding 120 s, 240 s, 360 s and at task failure.

286

287 Blood lactate accumulation (Δ blood [lactate]) was calculated as the difference between
288 blood [lactate] at end-exercise and blood [lactate] at baseline. Similarly, the change in blood
289 glucose concentration (Δ blood [glucose]) was calculated as the difference between blood
290 [glucose] at end-exercise and blood [glucose] at baseline.

291

292 *Statistical Analyses*

293 Differences in Hct, [Hb], plasma $[NO_3^-]$ and $[NO_2^-]$, pulmonary $\dot{V}O_2$ dynamics, HR, blood
294 [lactate], NIRS-derived variables and TTF were assessed using a mixed model ANOVA.
295 Significant main and interaction effects were further explored using Fisher's LSD.
296 Independent t-tests were used to assess the relative change between the BR and PL treatment
297 groups. Pearson's product moment correlation coefficient was used to explore relationships
298 between changes in [Hb] and Hct and changes in TTF. Statistical analyses were performed
299 using SPSS version 19.0 (Chicago, IL, USA). Data are presented as mean \pm SD, unless
300 otherwise stated. Statistical significance was accepted at $P < 0.05$.

301

302 **RESULTS**

303 Subjects' self-reported adherence to the supplementation regimen prior to and post blood
304 donation was 100%. All subjects reported that their physical activity and dietary patterns
305 were similar throughout each of the supplementation periods. The ingestion of BR and PL
306 supplements were well tolerated and no negative side effects were reported. Subjects did,
307 however, report beeturia (red-stained urine).

308

309 *[Hb] and Hct*

310 The group mean [Hb] and Hct data prior to and following blood donation and BR or PL
311 ingestion are displayed in Table 1. There was a significant main effect by time for both [Hb]
312 and Hct ($P<0.01$) but no main effect by group and no interaction effect ($P>0.05$). Prior to
313 donation, [Hb] and Hct were not different between the BR and PL treatment groups. [Hb] and
314 Hct were both significantly reduced from pre to post donation ($P<0.05$), with no differences
315 between PL and BR groups ($P>0.05$).

316

317 *Plasma $[NO_3^-]$ and $[NO_2^-]$*

318 The group mean plasma $[NO_3^-]$ and $[NO_2^-]$ pre and post blood donation in the BR and PL
319 groups are shown in Table 1. There was a significant main effect by time and group and an
320 interaction effect on plasma $[NO_3^-]$ and $[NO_2^-]$ ($P<0.01$). Prior to blood donation, neither
321 plasma $[NO_3^-]$ nor $[NO_2^-]$ were different between groups ($P>0.05$). Following blood
322 donation, there was a substantial increase in plasma $[NO_3^-]$ and $[NO_2^-]$ in the BR group
323 ($P<0.05$). A small (~11%) rise in plasma $[NO_3^-]$ ($P<0.05$) was also observed in the PL group
324 but there was no change in plasma $[NO_2^-]$ ($P>0.05$).

325

326 $\dot{V}O_2$ response to moderate-intensity and incremental exercise

327 *Moderate-intensity exercise*

328 The pulmonary gas exchange and ventilatory responses to moderate-intensity exercise pre
329 and post blood donation in PL and BR groups are reported in Table 2 and the group mean
330 $\dot{V}O_2$ response profiles in BR and PL groups pre and post blood donation are shown in Figure
331 1. There was a significant main effect by time ($P<0.01$) but no main effect by condition and
332 no interaction effect ($P>0.05$) for the $\dot{V}O_2$ measured during the baseline cycling period and at
333 end-exercise. Prior to donation, there were no differences in baseline or end-exercise $\dot{V}O_2$
334 between BR and PL groups ($P>0.05$). Follow-up tests revealed that both baseline $\dot{V}O_2$
335 ($P<0.01$) and end-exercise $\dot{V}O_2$ ($P<0.05$) were reduced in the BR group post-donation
336 compared with pre-donation.

337

338 The $\dot{V}CO_2$, $\dot{V}E$, RER, blood [lactate] and blood [glucose] data during moderate-intensity
339 exercise are reported in Table 2. Prior to donation, there were no differences in these
340 variables at baseline or at end-exercise between the BR and PL groups ($P>0.05$) and there
341 were no significant main effects by condition or time and no interaction effects ($P>0.05$).

342

343 *Ramp incremental exercise*

344 The effects of blood donation and BR and PL supplementation on the ramp incremental test
345 parameters are reported in Table 3 and illustrated in Figures 2 and 3.

346

347 There was a significant main effect by time on $\dot{V}O_{2peak}$ ($P<0.05$), but no main effect by
348 condition or an interaction effect ($P>0.05$). There were no differences between the groups at
349 baseline ($P>0.05$). Follow-up tests indicated that, from pre to post donation, there was a
350 significant reduction ($0.19 \text{ L}\cdot\text{min}^{-1}$; $\sim 5\%$) in $\dot{V}O_{2peak}$ in the PL group ($P<0.05$) but not in the

351 BR group ($0.12 \text{ L}\cdot\text{min}^{-1}$; $\sim 3\%$; $P > 0.05$). There was a significant main effect by time and an
352 interaction effect ($P < 0.05$) but no main effect by condition ($P > 0.05$) for PPO and TTF. Post
353 hoc tests revealed a significant reduction in PPO and TTF in both PL and BR groups from pre
354 to post donation ($P < 0.01$). There were no differences in PPO or TTF between the groups
355 prior to blood donation ($P > 0.05$). However, the reduction in PPO and TTF following blood
356 donation was more pronounced in PL compared with BR (5% vs. 3%; $P < 0.05$). The change
357 in [Hb] and Hct from pre to post donation was correlated with the change in TTF during ramp
358 incremental exercise in PL ($r = 0.58$; $P = 0.06$, and $r = 0.70$; $P < 0.05$, respectively) but not BR
359 ($r = -0.10$; $P > 0.05$ and $r = -0.41$; $P > 0.05$, respectively).

360

361 There was a significant interaction effect, but no main effects by time or group, for peak
362 $\dot{V}\text{CO}_2$. Specifically, peak $\dot{V}\text{CO}_2$ was reduced in the PL group ($P < 0.05$), but was unaffected
363 in the BR group ($P > 0.05$). There was no main effect by time or condition nor an interaction
364 effect for peak $\dot{V}\text{E}$ ($P > 0.05$). There was a significant main effect by time and an interaction
365 effect for peak RER ($P < 0.05$). Despite no difference at baseline, post hoc tests revealed an
366 increase in peak RER in the BR group from pre to post donation ($P < 0.01$).

367

368 *NIRS measurements*

369 *Moderate-intensity exercise*

370 There were no differences for total Hb (THb) between or within conditions during
371 the moderate-intensity exercise bout. The [HHb] and TOI values measured during moderate-
372 intensity exercise are reported in Table 4. There were no main effects by condition or time
373 and no interaction effect for baseline [HHb] ($P > 0.05$). There was a significant main effect by
374 time for [HHb] from pre to post donation at 60 s, 120 s, 240 s and end-exercise ($P < 0.05$), but
375 no main effect by condition or an interaction effect at any time point ($P > 0.05$). Post hoc tests

376 revealed a trend toward an increase in [HHb] in the PL group, but not the BR group, from pre
377 to post donation at 120 s and 240 s of moderate exercise ($P<0.10$). There were no main
378 effects by time or interaction effects for TOI at 60 s, 120 s, 240 s and end-exercise ($P>0.05$).
379 However, there was a trend toward a main effect by condition for all time points ($P<0.10$).
380 Follow-up tests revealed that blood donation resulted in reductions in TOI in the PL group at
381 60 s, 120 s and 240 s during moderate exercise, respectively ($P<0.05$; Table 4).

382

383 *Ramp incremental exercise*

384 There were no differences for THb between or within conditions during ramp incremental
385 exercise. The [HHb] and TOI values measured during ramp incremental exercise are reported
386 in Table 4 and the [HHb] profile is shown in Figure 4. There was a significant main effect by
387 time ($P<0.05$) but no main effect by condition or an interaction effect ($P>0.05$) for [HHb] at
388 120 s and 240 s during ramp incremental exercise. Post hoc tests showed that [HHb]
389 increased from pre to post donation at 240 s in PL ($P<0.05$) but not BR ($P>0.05$; Table 4).
390 There was a significant main effect by time ($P<0.05$) and a trend for an interaction effect for
391 [HHb] at 360 s ($P<0.10$) and at end-exercise ($P<0.05$) during the incremental exercise test.
392 Post hoc tests revealed that [HHb] increased significantly from pre to post donation in the PL
393 group at both 360 s and end-exercise ($P<0.05$; Table 4). The change in [HHb] from pre to
394 post donation was higher in PL versus BR at end-exercise ($P<0.05$) and tended to be higher at
395 360 s ($P<0.10$).

396

397 **DISCUSSION**

398 The principal original findings in this study, consistent with our hypotheses, were that NO_3^- -
399 rich beetroot juice ingestion lowered the O_2 cost of moderate-intensity exercise, better
400 preserved muscle oxygenation during moderate and ramp incremental exercise and attenuated

401 the reduction in ramp incremental exercise test performance and $\dot{V}O_{2\text{peak}}$ following blood
402 donation. These results indicate that dietary NO_3^- supplementation can ameliorate decrements
403 in exercise performance in a situation (i.e. reduction in blood O_2 -carrying capacity) which
404 would be expected to compromise physiological function during exercise.

405

406 *Effects of blood donation on [Hb] and Hct*

407 The standard NHS blood bank donation (~450mL) reduced [Hb] and Hct by a similar
408 magnitude in the PL and BR groups. These results concur with previous studies that have
409 investigated the influence of whole blood withdrawal on [Hb]. For example, Gordon et al.
410 (27) and Mora-Rodriguez et al. (45) reported ~8% and ~7% reductions in [Hb], 24 and 48 h
411 post blood donation, respectively. The ~8% reduction in Hct in the present study is also
412 similar to the values reported by Burnley et al. (11) and Gordon et al. (27) who reported a ~7-
413 8% decrease in Hct one day after 450 mL blood donation. The reduction in blood O_2 carrying
414 capacity, secondary to the lower [Hb] and Hct, can result in a reduction in muscle O_2 delivery
415 and muscle O_2 diffusing capacity during maximal exercise, with significant implications for
416 exercise performance (5, 11, 18, 47, 54).

417

418 *Effects of nitrate supplementation on plasma $[\text{NO}_3^-]$ and $[\text{NO}_2^-]$*

419 The ingestion of NO_3^- -rich BR significantly elevated plasma $[\text{NO}_3^-]$ and $[\text{NO}_2^-]$ when
420 compared with baseline values. These findings are in agreement with earlier studies which
421 also examined the influence of BR supplementation in young, healthy subjects (4, 34, 69).
422 A small but significant rise in plasma $[\text{NO}_3^-]$ was also noted in the PL group post
423 donation. This may be explained by a slight hemoconcentration or an upregulation in NOS
424 activity consequent to the reduction in whole body iron concentration after donating blood
425 (62). Plasma $[\text{NO}_2^-]$ rose by ~800% in the BR group from pre to post donation, suggesting

426 appreciably enhanced NO bioavailability. Numerous other studies have also reported
427 increases in plasma $[\text{NO}_2^-]$ after BR supplementation, but the percentage increases
428 attained were approximately half of those reported in this study (56, 69). This finding is
429 likely a result of the higher dose of NO_3^- ingested (~43 mmol over 48 h) when compared
430 with previous short-term BR supplementation studies. Interestingly, unlike in some earlier
431 studies (4, 38, 59, 69), BR supplementation did not reduce resting blood pressure (BP)
432 despite the elevated plasma $[\text{NO}_2^-]$ (mean arterial pressure, pre- vs. post-donation: 81 ± 7
433 vs. 80 ± 7 mmHg). Similar BP values pre- vs. post-donation in the PL group indicates that
434 total blood volume was restored 48 h following blood donation. The lack of effect of BR
435 on BP in the present study may be related to the relatively low baseline BP values of the
436 study participants (115/64 mmHg) and the relatively large number of female participants.
437 It has been reported that females are less sensitive than males to the influence of NO_3^-
438 supplementation on BP and that the extent of BP reduction with NO_3^- supplementation is
439 correlated with the baseline BP (33).

440

441 *Effects of blood donation and nitrate supplementation on the physiological responses to*
442 *moderate-intensity exercise*

443 The $\dot{V}\text{O}_2$ during both the unloaded baseline period and in the steady state of moderate-
444 intensity exercise was significantly reduced (by ~4%) in the BR group, but not the PL group,
445 after blood donation. A similar reduction in the O_2 cost of moderate-intensity exercise has
446 been reported by Bailey et al. (4) after six days of non-concentrated NO_3^- -rich BR ingestion
447 and by Larsen et al. (38) after three days of NaNO_3 supplementation. The present findings
448 are consistent with those of Kelly et al. (34) who observed that, in hypoxia, BR
449 supplementation resulted in a decrease in both baseline and steady-state $\dot{V}\text{O}_2$ when compared
450 with placebo. It has also been reported that acute (46) and 6 days (43) BR ingestion resulted

451 in significant reductions in $\dot{V}O_2$ during submaximal cycling exercise in hypoxia (15% and
452 11% O_2 , respectively). Acute BR supplementation has also been reported to better preserve
453 arterial O_2 saturation following dynamic apnea (48).

454

455 The lowering of the O_2 cost of submaximal exercise after NO_3^- supplementation may be due
456 to a number of mechanisms, including a reduction in the ATP cost of muscle force production
457 (4) and/or an improvement in mitochondrial efficiency (40) and/or changes in redox
458 signalling (66). In addition to changes in muscle contractile or metabolic efficiency, muscle
459 O_2 delivery or its intramuscular distribution may be altered following NO_3^- supplementation
460 (21, 22). Exercise, particularly in hypoxia or under conditions that may limit O_2 carrying
461 capacity, such as blood donation, acts as a potent stimulus for vasodilatation and delivery of
462 O_2 to working muscle (12, 13). Both NO and O_2 compete for the binding site at cytochrome-
463 *c* oxidase (COX) in the mitochondrial electron transport chain (9). An elevation in NO
464 availability via NO_3^- supplementation, perhaps especially in conditions limiting O_2 delivery,
465 increases the likelihood of NO binding to COX and therefore inhibiting O_2 consumption at
466 the mitochondrion (10). As a result, NO may modify the intramuscular distribution of O_2 and
467 improve the oxygenation status of muscle fibres that are situated further away from the
468 capillaries (29, 55, 63). Compared to placebo, BR supplementation has been reported to
469 enable a greater maximal rate of mitochondrial ATP resynthesis (Q_{max}) and result in faster
470 muscle phosphocreatine recovery kinetics following exercise in hypoxia (60, 61), indicating
471 improved muscle O_2 availability at least in the immediate post-exercise period (61).

472

473 In the present study, TOI was significantly reduced and [HHb] tended to be higher during
474 moderate-intensity exercise post- compared to pre-donation in the PL group, suggesting that
475 muscle O_2 availability was lower and a greater muscle fractional O_2 extraction was necessary
476 to achieve the required $\dot{V}O_2$ (24, 36). These changes were attenuated in the BR group,

477 consistent with our hypothesis that BR supplementation would better preserve muscle
478 oxygenation during moderate-intensity exercise when compared with PL. These results are
479 consistent with Masschelein et al. (43) who reported that BR resulted in a greater muscle TOI
480 and lower [HHb] during submaximal exercise in normobaric hypoxia. Collectively, these
481 studies indicate that under conditions which may impair blood O₂ carrying capacity, such as
482 following blood donation (present study) or in normobaric hypoxia (43), BR ingestion
483 promotes a better matching between muscle O₂ delivery and O₂ demand, i.e. less O₂
484 extraction is required for the same moderate-intensity work rate, perhaps due to the lower
485 exercise $\dot{V}O_2$ (34) or to preferential alterations in muscle perfusion (21, 22, 61). An increased
486 ratio of O₂ delivery to O₂ consumption at a given work rate would be expected to retard the
487 rate of fatigue development and to improve exercise performance.

488

489 *Effects of blood donation and nitrate supplementation on the physiological responses to*
490 *incremental exercise*

491 As expected, blood donation and the associated reduction in O₂ carrying capacity resulted in
492 a significant reduction in PPO and TTF during ramp incremental exercise. Panebianco et al.
493 (47) also reported a significant reduction in PPO during incremental exercise, 2 days post
494 blood donation. An important original finding in the present study was that ingestion of BR in
495 the 48 hours post blood donation partly negated the decrement in performance when
496 compared with PL. Specifically, the reduction in PPO and TTF following blood donation was
497 significantly more pronounced in the PL group compared with BR. Interestingly, the
498 reduction in TTF in the PL group was quite well correlated with the reduction in [Hb] ($r =$
499 $0.58, P=0.06$) and Hct ($r = 0.70, P<0.05$) following blood donation, whereas in the BR group,
500 the correlations were weaker and non-significant ([Hb]: $r = -0.10$; Hct: $r = -0.41$; both
501 $P>0.05$), implying that BR supplementation compensated for the lower [Hb] and Hct. These

502 findings are consistent with those of Masschelein et al. (43) who reported that, compared to
503 PL, BR ingestion significantly attenuated the reduction in TTF when incremental exercise
504 was performed in hypoxia.

505

506 $\dot{V}O_{2\text{peak}}$ was reduced by 5% from pre to 48 h post donation in the PL group. Similarly,
507 Burnley et al. (11) reported a 4% decrease in $\dot{V}O_{2\text{peak}}$ during severe-intensity exercise 24 h
508 following blood donation. This reduction was proportional to the reduced [Hb] and thus the
509 ability to deliver O_2 to the working skeletal muscle during maximal exercise. In the present
510 study, the reduced $\dot{V}O_{2\text{peak}}$ in the PL group following blood donation occurred in conjunction
511 with an increased muscle [HHb], which may be interpreted as an increase in muscle
512 fractional O_2 extraction in an (ultimately unsuccessful) attempt to offset the effects of a
513 reduced [Hb] and lower muscle O_2 delivery (51, 54). In contrast, $\dot{V}O_{2\text{peak}}$ and [HHb] during
514 the incremental test were not significantly altered by blood donation in the BR group. These
515 results may indicate that the O_2 sparing effect of BR ingestion (Figure 2B), coupled perhaps
516 with altered perfusion distribution (21, 22, 61), enabled muscle oxygenation to be better
517 preserved during incremental exercise, such that an increased muscle fractional O_2 extraction
518 was not mandated to achieve a given $\dot{V}O_{2\text{peak}}$. Ferguson et al. (21, 22) have reported that, in
519 rats, BR supplementation can enhance vascular conductance and blood flow to working
520 muscle and elevate the microvascular partial pressure of O_2 ($PO_{2\text{mv}}$), particularly in type II
521 fibres. If similar effects occur in humans, this may enhance the blood-myocyte O_2 exchange
522 gradient during higher intensity exercise, better preserving muscle oxygenation status,
523 homeostasis and performance. It is also possible that a portion of the preserved ramp
524 incremental test performance following blood donation with BR compared to PL may be
525 attributable to effects of NO_3^- on muscle contractile function (50), perhaps particularly in
526 type II fibers (31).

527

528 The mechanistic bases for the positive effects of BR ingestion on vascular and metabolic
529 function in this and other situations warrants further investigation. In particular, while it is
530 widely believed that the effects may be attributed to greater NO bioavailability or bioactivity,
531 it is presently unclear precisely how this NO pool is stored and transported. NO is a highly
532 reactive molecule with a short-half life *in vivo* and its rapid reaction with, for example, O₂ or
533 heme proteins (30) suggests that the free transport of NO may be limited in plasma and
534 within cells. It has been proposed that NO₂⁻ itself represents a principal means of 'NO'
535 storage and transport, with the one electron reduction of NO₂⁻ to NO in blood and other
536 tissues being facilitated, amongst many other factors including xanthine oxidoreductase, by
537 deoxyhemoglobin and deoxymyoglobin, which will naturally be present in greater abundance
538 in contracting skeletal muscle (16, 42). However, BR ingestion likely also increases the
539 production and storage of other reactive nitrogen species. In particular, low molecular weight
540 thiol groups may react with nitrogen oxides to yield s-nitrosothiol species (SNOs) which can
541 be transported in the blood as s-nitrosohemoglobin (HbSNO) (17). It has recently been
542 reported that the reduction in blood pressure following NO₃⁻ or NO₂⁻ ingestion in a rat model
543 of hypertension was more closely related to plasma [s-nitrosothiol] than to plasma [NO₂⁻]
544 (49) and that s-nitrosothiol bioactivity derived through βCys93 may be essential for hypoxic
545 vasodilation by erythrocytes (70). In contrast, in humans, Gladwin et al. (26) reported a
546 significant arterial-venous NO₂⁻ gradient during forearm exercise and concluded that SNOs
547 and HbSNO do not play a significant role in the regulation of vascular tone. The role of
548 SNOs and HbSNO in the physiological effects of nitrate ingestion in humans remains to be
549 clarified. Equally, the precise mechanisms by which an elevation of tissue [NO₂⁻] following
550 NO₃⁻ ingestion influences metabolic and vascular control at rest and during exercise remains
551 unclear. While it is possible that NO₂⁻ itself is bioactive (58), unresolved questions include

552 the triggers and time course for the possible reduction of NO_2^- to NO, and the nature of both
553 NO transport to, and storage within, biological targets. Resolution of these issues will likely
554 require synthesis of experimental data deriving from ‘competing’ hypotheses.

555

556 *Perspectives*

557 This study has shown for the first time that despite a significant reduction in [Hb] post blood
558 withdrawal, BR supplementation lowered the O_2 cost of moderate-intensity exercise, better
559 preserved muscle oxygenation during moderate-intensity and ramp incremental exercise, and
560 attenuated the reduction in $\dot{V}\text{O}_{2\text{peak}}$ and incremental exercise test performance. These results
561 may have significant implications for athletes who wish to give blood without significant
562 detriment to training, individuals with clinical conditions which reduce blood O_2 carrying
563 capacity, such as anemia, and in conditions resulting in acute blood loss such as surgery or
564 military combat. In this context, it is of interest that transfusion of stored blood may impair
565 vasodilatory capacity, an effect that might be linked to the loss of NO bioavailability that
566 occurs during blood storage (17, 52). Treating banked blood to better maintain NO stores
567 might lead to improved functional outcomes following transfusion. In conclusion, BR
568 supplementation attenuates the decline in functional capacity arising from blood donation.

569

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574

575 **REFERENCES**

576

577 1. Allen DG, Lamb GD, Westerblad H. Skeletal muscle fatigue: cellular mechanisms. *Physiol*
578 *Rev* 88: 287-332, 2008.

579

580 2. Amann M, Calbet JA. Convective oxygen transport and fatigue. *J Appl Physiol* 104: 861-
581 870, 2008.

582

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

586

587 4. Bailey SJ, Winyard P, Vanhatalo A, Blackwell JR, DiMenna FJ, Wilkerson DP, Tarr J,
588 Benjamin N, Jones AM. Dietary nitrate supplementation reduces the O₂ cost of low-intensity
589 exercise and enhances tolerance to high-intensity exercise in humans. *J Appl Physiol*, 106:
590 1144-1155, 2009.

591

592 5. Balke B, Grillo GP, Konecci EB, Luft UC. Work capacity after blood donation. *J Appl*
593 *Physiol* 7: 231-238, 1954.

594

595 6. Bassett DR, Howley ET. Limiting factors for maximum oxygen uptake and determinants
596 of endurance performance. *Med Sci Sports Exerc* 32: 70-84, 2000.

597

- 598 7. Bateman RM, Ellis CG, Freeman DJ. Optimization of nitric oxide chemiluminescence
599 operating conditions for measurement of plasma nitrite and nitrate. *Clin Chem* 48: 2020-
600 2027, 2002.
- 601
- 602 8. Benjamin N, O'Driscoll F, Dougall H, Duncan C, Smith S, Golden M, McKenzie H.
603 Stomach NO synthesis. *Nature* 368: 502, 1994.
- 604
- 605 9. Brown GC. Regulation of mitochondrial respiration by nitric oxide inhibition of
606 cytochrome *c* oxidase. *Biochem Biophys Acta* 1504: 46-57, 2001.
- 607
- 608 10. Brown GC, Cooper C. Nanomolar concentrations of nitric oxide reversibly inhibit
609 synaptosomal respiration by competing with oxygen at cytochrome oxidase. *FEBS Letter*
610 356: 295-298, 1994.
- 611
- 612 11. Burnley M, Roberts CL, Thatcher R, Doust JH, Jones AM. Influence of blood donation
613 on O₂ uptake on-kinetics, peak O₂ uptake and time to exhaustion during severe-intensity
614 cycle exercise in humans. *Exp Physiol* 91: 499-509.
- 615
- 616 12. Calbet JA, Rådegran G, Boushel R, Saltin B. On the mechanism that limit oxygen uptake
617 during exercise in acute and chronic hypoxia: role of muscle mass. *J Physiol* 587: 477-490,
618 2009.
- 619
- 620 13. Casey DP, Madery BD, Curry TB, Eisenach JH, Wilkins BW, Joyner MJ. Nitric oxide
621 contributes to the augmented vasodilation during hypoxic exercise. *J Physiol* 588: 373-385,
622 2010.

623

624 14. Castello PR, David PS, McClure T, Crook Z, Poyton RO. Mitochondrial cytochrome
625 oxidase produces nitric oxide under hypoxic conditions: implications for oxygen sensing and
626 hypoxic signalling in eukaryotes. *Cell Metab* 3: 277-287, 2006.

627

628 15. Cermak NM, Gibala MJ, van Loon LJC. Nitrate supplementation's improvement of 10-
629 km time trial performance in trained cyclists. *Int J Sport Nutr Exerc Metab* 22: 64-71, 2012.

630

631 16. Cosby K, Partovi KS, Crawford JH, Patel RP, Reiter CD, Martyr S, Yang BK, Waclawiw
632 MA, Zalos G, Xu X, Huang KT, Shields H, Kim-Shapiro DB, Schechter AN, Cannon RO
633 3rd, Gladwin MT. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the
634 human circulation. *Nat Med* 9: 1498-1505, 2003.

635

636 17. Doctor A, Stamler JS. Nitric oxide transport in blood: a third gas in the respiratory
637 cycle. *Compr Physiol* 1: 541-568, 2011.

638

639 18. Ekblom B, Goldbarg AN, Gullbring B. Response to exercise after blood loss and
640 reinfusion. *J Appl Physiol* 33: 175-180, 1972.

641

642 19. Ekblom B, Wilson G, Astrand PO. Central circulation during exercise after venesection
643 and reinfusion of red blood cells. *J Appl Physiol* 81: 379-383, 1976.

644

645 20. Ferguson SK, Glean AA, Holdsworth CT, Wright JL, Fees AJ, Colburn TD, Stabler T,
646 Allen JD, Jones AM, Musch TI, Poole DC. Skeletal muscle vascular control during exercise:

647 impact of nitrite infusion during nitric oxide synthase inhibition in healthy rats. *J Cardiovasc*
648 *Pharmacol Ther* 21: 201-208, 2016.

649

650 21. Ferguson SK, Hirai DM, Copp SW, Holdsworth CT, Allen JD, Jones AM, Musch TI,
651 Poole DC. Effects of nitrate supplementation via beetroot juice on contracting rat skeletal
652 muscle microvascular oxygen pressure dynamics. *Respir Physiol Neurobiol* 187: 250-255,
653 2013.

654

655 22. Ferguson SK, Hirai DM, Copp SW, Holdsworth CT, Allen JD, Jones AM, Musch TI,
656 Poole DC. Impact of dietary nitrate supplementation via beetroot juice on exercising muscle
657 vascular control in rats. *J Physiol* 59: 547-557, 2013.

658

659 23. Fernández-Real JM, Peñarroja G, Castro A, García-Braado F, López-Bermejo A, Ricart
660 W. Blood letting in high-ferritin type 2 diabetes: effects on vascular reactivity. *Diabetes Care*
661 25: 2249-2255, 2002.

662

663 24. Ferreira LF, Koga S, Barstow TJ. Dynamics of noninvasively estimated microvascular O₂
664 extraction during ramp exercise. *J Appl Physiol* 103: 1999-2004, 2007.

665

666 25. Giraldez RR, Panda A, Xia Y, Sanders SP, Zweier JL. Decreased nitric-oxide synthase
667 activity causes impaired endothelium-dependent relaxation in the postischemic heart. *J Biol*
668 *Chem* 272: 21420-21426, 1997.

669

670 26. Gladwin MT, Shelhamer JH, Schechter AN, Pease-Fye ME, Waclawiw MA, Panza JA,
671 Ognibene FP, Cannon RO 3rd. Role of circulating nitrite and S-nitrosohemoglobin in the

672 regulation of regional blood flow in humans. *Proc Natl Acad Sci U S A* 97: 11482-11487,
673 2000.

674

675 27. Gordon D, Marshall K, Connell A, Barnes RJ. Influence of blood donation on oxygen
676 uptake kinetics during moderate and heavy intensity cycle exercise. *Int J Sports Med* 31: 298-
677 303, 2010.

678

679 28. Grassi B, Pogliaghi S, Rampichini S, Quaresima V, Ferrari M, Marconi C, Cerretelli P.
680 Muscle oxygenation and pulmonary gas exchange kinetics during cycling exercise on-
681 transitions in humans. *J Appl Physiol* 95: 149-158, 2003.

682

683 29. Hagen T, Taylor CT, Lam F, Moncada S. Redistribution of intracellular oxygen in
684 hypoxia by nitric oxide: effect of HIF1 α . *Science* 302: 1975-1978, 2003.

685

686 30. Hakim TS, Sugimori K, Camporesi EM, Anderson G. Half-life of nitric oxide in aqueous
687 solutions with and without haemoglobin. *Physiol Meas* 17: 267-77, 1996.

688

689 31. Hernández A, Schiffer TA, Ivarsson N, Cheng AJ, Bruton JD, Lundberg JO, Weitzberg E,
690 Westerblad H. Dietary nitrate increases tetanic [Ca²⁺]_i and contractile force in mouse fast-
691 twitch muscle. *J Physiol* 590: 3575-3583, 2012.

692

693 32. Ignarro LJ. Endothelium-derived nitric oxide: actions and properties. *FASEB J* 3: 31-36,
694 1989.

695

- 696 33. Kapil V, Milsom AB, Okorie M, Maleki-Toyserkani S, Akram F, Rehman F, Arghandawi
697 S, Pearl V, Benjamin N, Loukogeorgakis S, Macallister R, Hobbs AJ, Webb AJ, Ahluwalia
698 A. Inorganic nitrate supplementation lowers blood pressure in humans: role for nitrite-derived
699 NO. *Hypertension* 56: 274-281, 2010.
- 700
- 701 34. Kelly J, Vanhatalo A, Bailey SJ, Wylie LJ, Tucker C, List S, Jones AM. Dietary nitrate
702 supplementation: effects on plasma nitrite and pulmonary O₂ uptake dynamics during
703 exercise in hypoxia and normoxia. *Am J Physiol Regul Integr Compar Physiol* 307: R920-
704 R930, 2014.
- 705
- 706 35. Kenjale AA, Ham KL, Stabler T, Robbins JL, Johnson JL, VanBruggen M, Privette G,
707 Yim E, Kraus WE, Allen JD. Dietary nitrate supplementation enhances exercise performance
708 in peripheral arterial disease. *J Appl Physiol* 110: 1582-1591, 2011.
- 709
- 710 36. Koga S, Kano Y, Barstow TJ, Ferreira LF, Ohmae E, Sudo M, Poole DC. Kinetics of
711 muscle deoxygenation and microvascular PO₂ during contractions in rat: comparison of
712 optical spectroscopy and phosphorescence-quenching techniques. *J Appl Physiol* 112: 26-32,
713 2012.
- 714
- 715 37. Lansley KE, Winyard PG, Fulford J, Vanhatalo A, Bailey SJ, Blackwell JR, DiMenna FJ,
716 Gilchrist M, Benjamin N, Jones AM. Dietary nitrate supplementation reduces the O₂ cost of
717 walking and running: a placebo-controlled study. *J Appl Physiol* 110: 591-600, 2011.
- 718
- 719 38. Larsen FJ, Weitzberg E, Lundberg JO, Ekblom B. Effects of dietary nitrate on oxygen
720 cost during exercise. *Acta Physiol* 191: 59-66, 2007.

721

722 39. Larsen FJ, Weitzberg E, Lundberg JO, Ekblom B. Dietary nitrate reduces maximal
723 oxygen consumption while maintaining work performance in maximal exercise. *Free Rad*
724 *Biol Med* 48: 342-347, 2010.

725

726 40. Larsen FJ, Schiffer TA, Borniquel S, Sahlin K, Ekblom B, Lundberg JO, Weitzberg E.
727 Dietary inorganic nitrate improves mitochondrial efficiency in humans. *Cell Metab* 13: 149-
728 159, 2011.

729

730 41. Linnarsson D, Karlsson J, Fagraeus L, Saltin B. Muscle metabolites and oxygen deficit
731 with exercise in hypoxia and hyperoxia. *J Appl Physiol* 36: 399-402, 1974.

732

733 42. Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in
734 physiology and therapeutics. *Nat Rev Drug Disc* 7: 156-167, 2008.

735

736 43. Masschelein E, Van Thienen R, Wang X, Van Schepdael A, Thomis M, Hespel P. Dietary
737 nitrate improves muscle but not cerebral oxygenation status during exercise in hypoxia. *J*
738 *Appl Physiol* 113: 736-745, 2012.

739

740 44. Modin A, Björne H, Herulf M, Alving K, Weitzberg E, Lundberg JO. Nitrite-derived
741 nitric oxide: a possible mediator of 'acidic-metabolic' vasodilation. *Acta Physiol Scand* 171:
742 9-16, 2001.

743

744 45. Mora-Rodriguez R, Aguado-Jimenez R, Del Coso J, Estevez E. A standard blood bank
745 donation alters the thermal and cardiovascular responses during subsequent exercise.
746 *Transfusion* 52: 2339-2347, 2012.

747

748 46. Muggeridge DJ, Howe CCF, Spendiff O, Pedlar C, James PE, Easton C. A single dose of
749 beetroot juice enhances performance in simulated altitude. *Med Sci Sports Exerc* 46: 143-150,
750 2014.

751

752 47. Panebianco RA, Stachenfeld N, Copan NL, Gleim GM. Effects of blood donation on
753 exercise performance in competitive cyclists. *Am Heart J* 130: 838-840, 1995.

754

755 48. Patrician A, Schagatay E. Dietary nitrate enhances arterial oxygen saturation after
756 dynamic apnea. *Scand J Med Sci Sports* doi: 10.1111/sms.12684. [Epub ahead of print].

757

758 49. Pinheiro LC, Amaral JH, Ferreira GC, Portella RL, Ceron CS, Montenegro MF, Toledo
759 JC Jr, Tanus-Santos JE. Gastric S-nitrosothiol formation drives the antihypertensive effects of
760 oral sodium nitrite and nitrate in a rat model of renovascular hypertension. *Free Radic Biol*
761 *Med* 87: 252-262, 2015.

762

763 50. Rimer EG, Peterson LR, Coggan AR, Martin JC. Acute dietary nitrate supplementation
764 increases maximal cycling power in athletes. *Int J Sports Physiol Perform* 2015 [Epub ahead
765 of print].

766

767 51. Roach RC, Koskolou MD, Calbet JA, Saltin B. Arterial O₂ content and tension in
768 regulation of cardiac output and leg blood flow during exercise in humans. *Am J Physiol* 276:
769 H438-445, 1999.
770

771 52. Roback JD, Neuman RB, Quyyumi A, Sutliff R. Insufficient nitric oxide bioavailability: a
772 hypothesis to explain adverse effects of red blood cell transfusion. *Transfusion* 51: 859-866,
773 2011.
774

775 53. Saltin B, Calbet JA. Point: in health and in a normoxic environment, VO₂ max is limited
776 primarily by cardiac output and locomotor muscle blood flow. *J Appl Physiol* 100: 744-5,
777 2006.
778

779 54. Schaffartzik W, Barton ED, Poole DC, Tsukimoto K, Hogan MC, Bebout DE, Wagner
780 PD. Effect of reduced haemoglobin concentration on leg oxygen uptake during maximal
781 exercise in humans. *J Appl Physiol* 75: 491-498, 1993.
782

783 55. Thomas DD, Liu X, Kantrow SP, Lancaster JR. The biological lifetime of nitric oxide:
784 implications for the perivascular dynamics of NO and O₂. *Proc Nat Acad Sci* 98: 355-360,
785 2001.
786

787 56. Thompson C, Wylie LJ, Fulford J, Kelly J, Black MI, McDonagh STJ, Jeukendrup AE,
788 Vanhatalo A, Jones AM. Dietary nitrate improves sprint performance and cognitive function
789 during prolonged intermittent exercise. *Eur J Appl Physiol*, 115: 1825-1834, 2015.
790

791 57. Thomsen JJ, Rentsch RL, Robach P, Calbet JA, Boushel R, Rasmussen P, Juel C, Lundby
792 C. Prolonged administration of recombinant human erythropoietin increases submaximal
793 performance more than maximal aerobic capacity. *Eur J Appl Physiol* 101: 481-486, 2007.
794

795 58. van Faassen EE1, Bahrami S, Feelisch M, Hogg N, Kelm M, Kim-Shapiro DB, Kozlov
796 AV, Li H, Lundberg JO, Mason R, Nohl H, Rassaf T, Samouilov A, Slama-Schwok A, Shiva
797 S, Vanin AF, Weitzberg E, Zweier J, Gladwin MT. Nitrite as regulator of hypoxic signaling
798 in mammalian physiology. *Med Res Rev* 29: 683-741, 2009.

799 59. Vanhatalo A, Bailey SJ, Blackwell JR, DiMenna FJ, Pavey TG, Wilkerson DP, Benjamin
800 N, Winyard P, Jones AM. Acute and chronic effects of dietary nitrate supplementation on
801 blood pressure and the physiological responses to moderate-intensity and incremental
802 exercise. *Am J Physiol* 299: R1121-R1131, 2010.

803

804 60. Vanhatalo A, Fulford J, Bailey SJ, Blackwell JR, Winyard PG, Jones AM. Dietary nitrate
805 reduces muscle metabolic perturbation and improves exercise tolerance in hypoxia. *J Physiol*
806 589: 5517-5528, 2011.

807

808 61. Vanhatalo A, Jones AM, Blackwell JR, Winyard PG, Fulford J. Dietary nitrate
809 accelerates postexercise muscle metabolic recovery and O₂ delivery in hypoxia. *J Appl*
810 *Physiol* 117: 1460-1470, 2014.

811

812 62. Van Jaarsveld H, Pool GF. Beneficial effects of blood donation on high density
813 lipoprotein concentration and the oxidative potential of low density lipoprotein.
814 *Atherosclerosis* 161: 395-402, 2002.

815

816 63. Victor VM, Nuñez C, D'Ocón P, Taylor CT, Esplugues JV, Moncada S. Regulation of
817 oxygen distribution in tissues by endothelial nitric oxide. *Circ Res* 104: 1178-1183, 2009.

818

819 64. Wagner PD. Determinants of maximal oxygen transport and utilization. *Ann Rev Physiol*
820 58: 21-50, 1996.

821

822 65. Webb AJ, Patel N, Loukogeorgakis S, Okorie M, Aboud Z, Misra S, Rashid R, Miall P,
823 Deanfield J, Benjamin N, MacAllister R, Hobbs AJ, Ahluwalia A. Acute blood pressure
824 lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to
825 nitrite. *Hypertension* 51: 784-790, 2008.

826

827 66. Whitfield J, Ludzki A, Heigenhauser GJ F, Senden JMG, Verdijk LB, van Loon LJC,
828 Spriet LL, Holloway GP. Beetroot juice supplementation reduces whole body oxygen
829 consumption but does not improve indices of mitochondrial efficiency in human skeletal
830 muscle. *J Physiol* 594: 421-435, 2016.

831

832 67. Wilkerson DP, Rittweger J, Berger NJA, Naish PF, Jones AM. Influence of recombinant
833 human erythropoietin treatment on pulmonary O₂ uptake kinetics during exercise in humans.
834 *J Physiol* 568: 639-652, 2005.

835

836 68. Wilson JR, Martin JL, Schwartz D, Ferraro N. Exercise intolerance in patients with
837 chronic heart failure: role of impaired nutritive flow to skeletal muscle. *Circulation* 69:1079-
838 87, 1984.

839

840 69. Wylie LJ, Kelly J, Bailey SJ, Blackwell JR, Skiba PF, Winyard PG, Jeukendrup AE,
841 Vanhatalo A, Jones AM. Beetroot juice and exercise: pharmacodynamics and dose-response
842 relationships. *J Appl Physiol* 115: 325-336, 2013.

843

844 70. Zhang R, Hess DT, Qian Z, Hausladen A, Fonseca F, Chaube R, Reynolds JD, Stamler
845 JS. Hemoglobin β Cys93 is essential for cardiovascular function and integrated response to
846 hypoxia.. 112: 6425-6430, 2015.

847

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849 **FIGURE LEGENDS**

850 **Figure 1:** Pulmonary oxygen uptake ($\dot{V}O_2$) response following BR and PL supplementation
851 prior to and following blood donation during a step increment to a moderate-intensity work
852 rate. Responses prior to blood donation are shown as solid, filled circles, while responses post
853 blood donation are shown as open, unfilled circles. The dotted vertical line represents the
854 abrupt imposition of the moderate work rate from a baseline of 'unloaded' cycling. *A:* Group
855 mean $\dot{V}O_2$ response to moderate-intensity exercise following PL ingestion. *B:* Group mean
856 $\dot{V}O_2$ response to moderate-intensity exercise following BR ingestion. *C:* Steady state $\dot{V}O_2$
857 following PL and BR supplementation relative to pre blood donation baseline. The O_2 cost of
858 moderate-intensity exercise was reduced following BR supplementation and blood donation
859 compared with pre donation values, * $P < 0.05$.

860

861 **Figure 2:** Group mean pulmonary $\dot{V}O_2$ response to incremental exercise prior to blood
862 donation and following BR and PL supplementation after blood donation. Responses prior to
863 blood donation are shown as solid, filled circles, while responses post blood donation are
864 shown as open, unfilled circles. The dotted vertical line represents the onset of the ramp
865 incremental test from a baseline of 'unloaded' cycling. The $\dot{V}O_{2peak}$ was reduced in the PL
866 group (*= $P < 0.05$), but not the BR group, after blood donation. TTF was reduced in both
867 groups post donation ($\# = P < 0.05$), however, the reduction in TTF was greater in the PL
868 group when compared with the BR group ($\$ = P < 0.05$).

869

870 **Figure 3.** Group mean time to task failure (TTF) in the ramp incremental test prior to and
871 post blood donation, following BR and PL supplementation. Responses prior to blood
872 donation are shown as solid, filled bars, while responses post donation are shown as open,

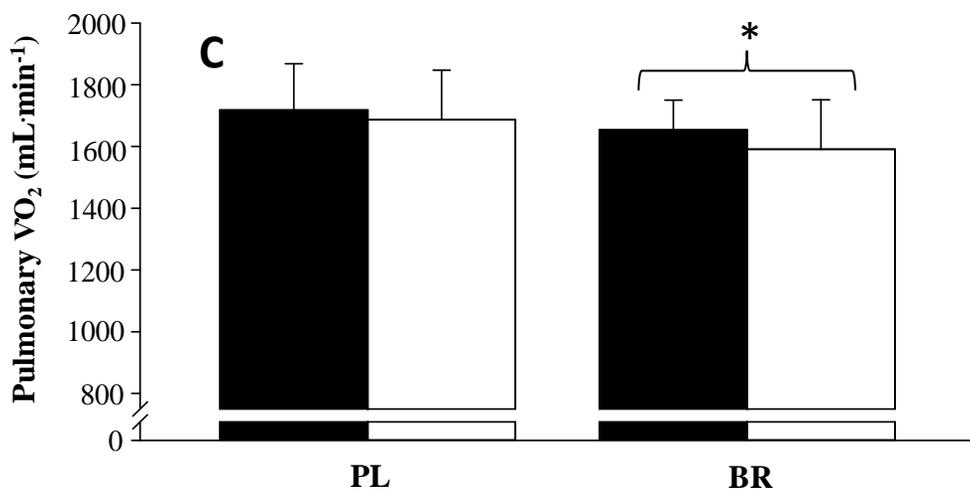
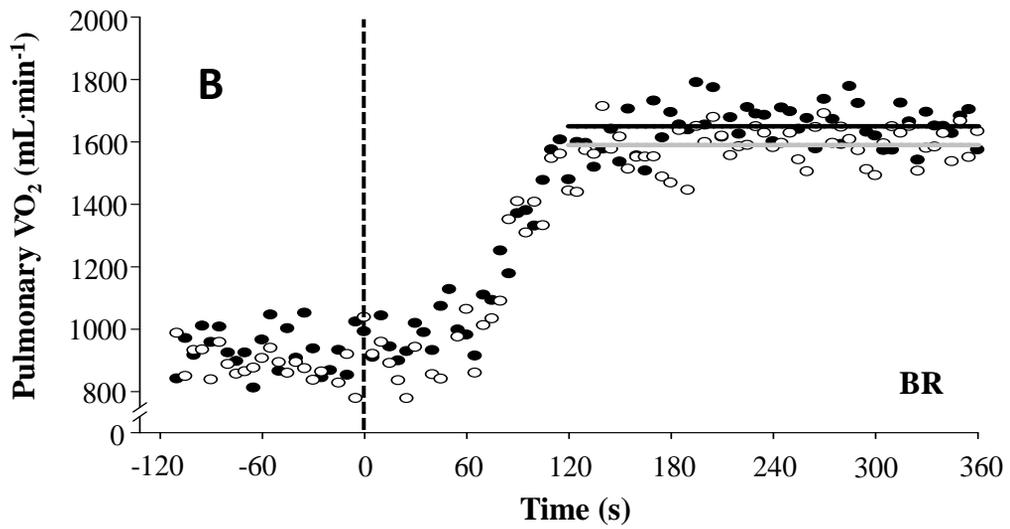
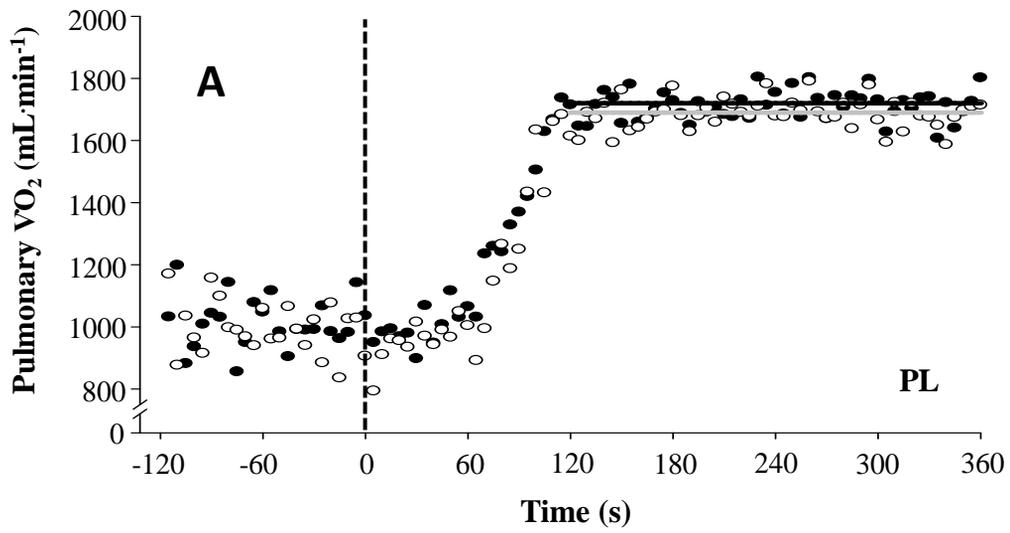
873 unfilled bars. The TTF was reduced in both groups post donation ($*=P<0.05$); however, the
874 reduction in TTF was greater in the PL group when compared with the BR group ($^{\#}=P<0.05$).

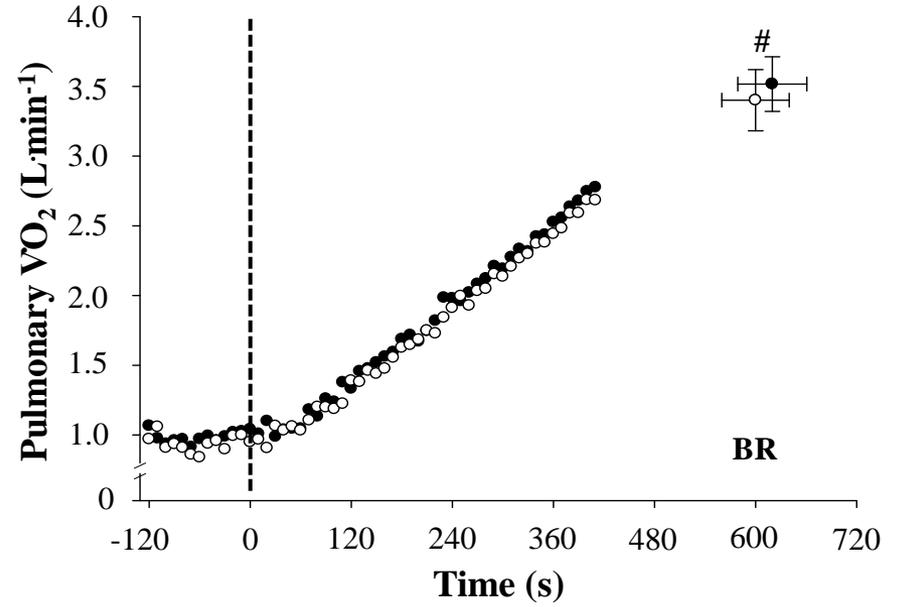
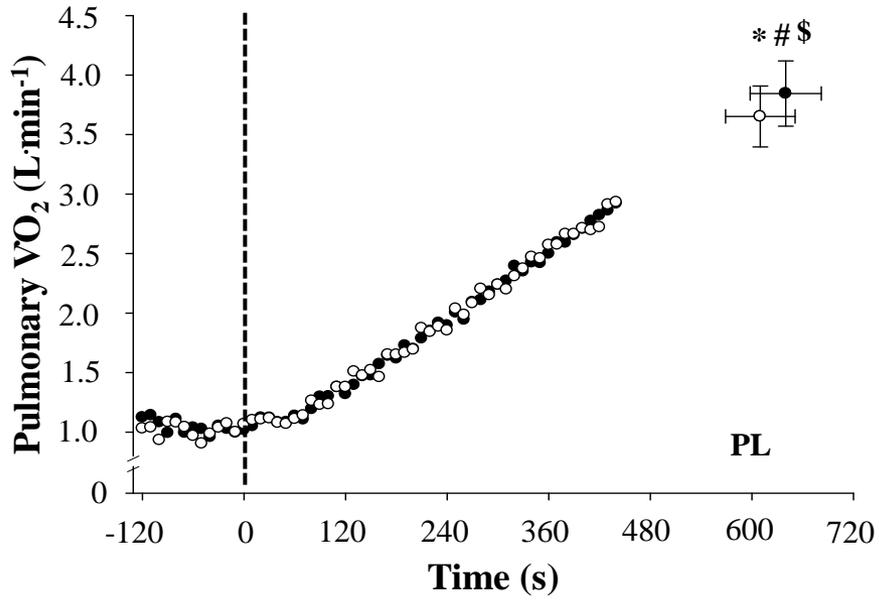
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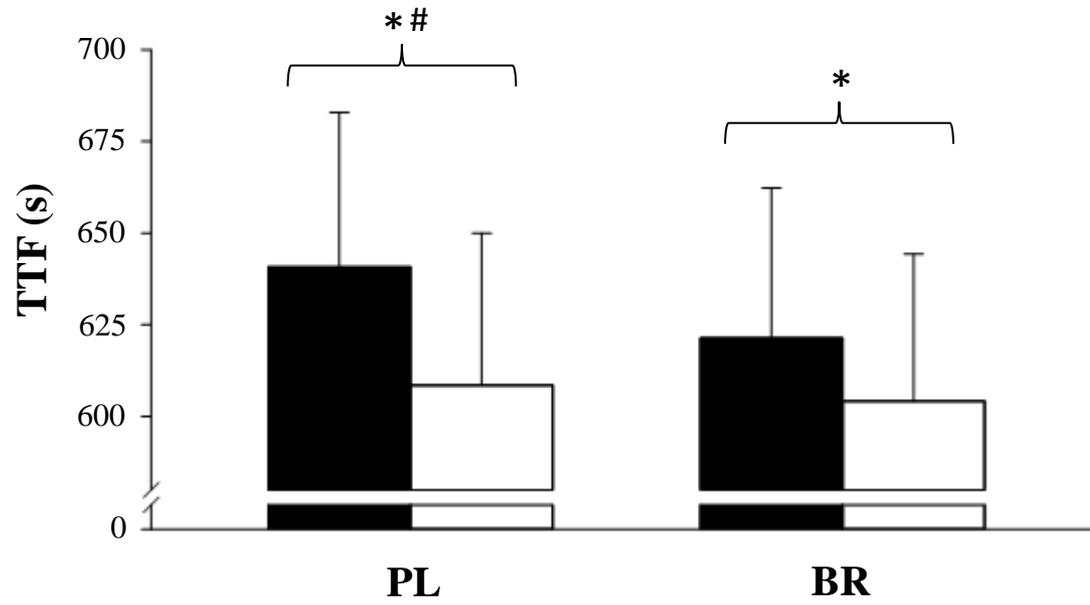
876 **Figure 4.** Group mean changes in deoxyhaemoglobin ([HHb]) prior to and post blood
877 donation, following BR and PL ingestion. Responses prior to blood donation are shown as
878 solid, filled circles, while responses post blood donation are shown as open, unfilled circles.
879 The dotted vertical line represents the onset of the ramp incremental test from a baseline of
880 'unloaded' cycling. [HHb] increased significantly from pre to post donation in the PL group
881 at 360 s and end-exercise ($*=P<0.05$). [HHb] was not altered from pre to post donation in the
882 BR group. TTF was reduced in both groups post donation ($^{\#}=P<0.05$), however, the
883 reduction in TTF was greater in the PL group when compared with the BR group ($^{\$}=$
884 $P<0.05$).

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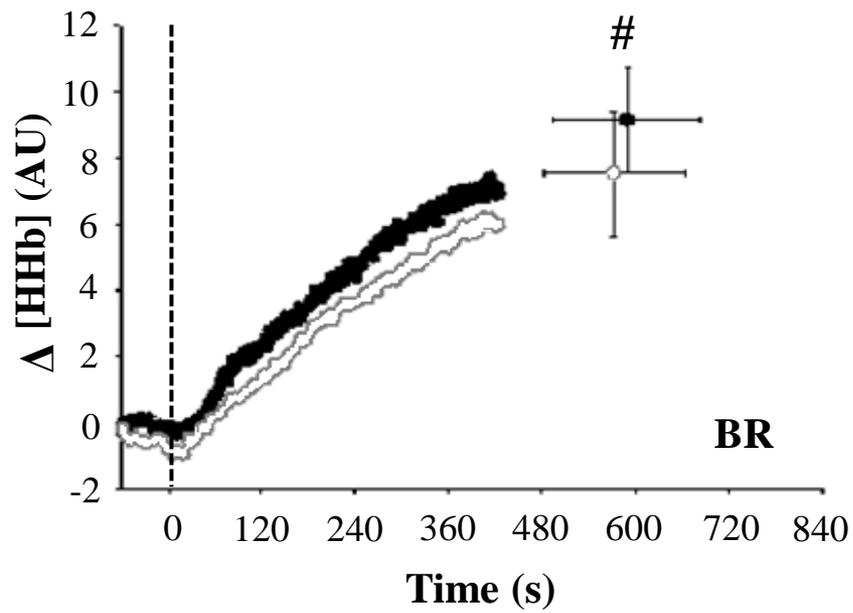
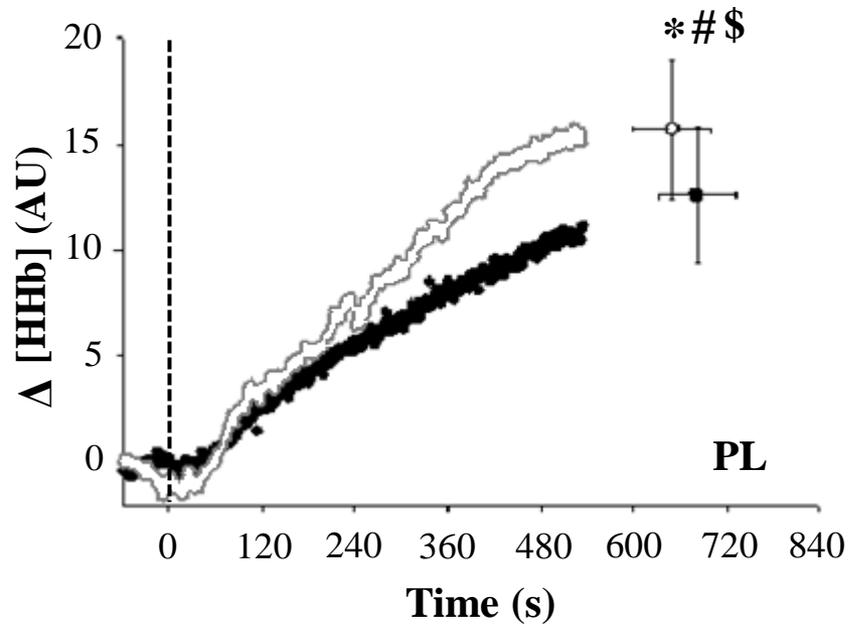


Table 1: Blood pressure, resting heart rate, plasma nitrate and nitrite concentrations, hemoglobin concentration and hematocrit prior to and following blood donation in the PL and BR groups.

	PL		BR	
	Pre	Post	Pre	Post
Blood pressure (mmHg)				
<i>Systolic</i>	119 ± 7	118 ± 9	115 ± 11	113 ± 11*
<i>Diastolic</i>	69 ± 7	67 ± 7	64 ± 7	63 ± 7
<i>Mean Arterial</i>	86 ± 6	84 ± 8	81 ± 7	80 ± 7
Resting HR (b·min⁻¹)	62 ± 9	66 ± 9	66 ± 11	71 ± 10*
Plasma [NO₃⁻] (µM)	45 ± 11	50 ± 14*	47 ± 17	845 ± 350* [§]
Plasma [NO₂⁻] (nM)	73 ± 18	72 ± 21	81 ± 29	619 ± 363* [§]
[Hb] (g·L⁻¹)	149 ± 12	132 ± 18*	148 ± 15	137 ± 19*
Hct (%)	45 ± 2	41 ± 4*	45 ± 3	42 ± 5*

Values are mean ± SD. PL, Placebo group; BR, Nitrate group; Pre, pre-donation; Post, post-donation ; HR, heart rate; [NO₂⁻], nitrite concentration; [NO₃⁻], nitrate concentration; [Hb], hemoglobin concentration; Hct, hematocrit. *Significantly different from pre in the same condition ($P < 0.05$). [§]Significantly different from post supplementation value in the PL group ($P < 0.05$).

Table 2: Ventilatory and gas exchange dynamics, and blood lactate and glucose concentrations during moderate-intensity exercise prior to and following blood donation in the PL and BR groups

	PL		BR	
	Pre	Post	Pre	Post
$\dot{V}O_2$ (L·min⁻¹)				
<i>Baseline</i>	1.01 ± 0.17	0.97 ± 0.20	0.96 ± 0.20	0.87 ± 0.21 [#]
<i>End exercise</i>	1.72 ± 0.50	1.69 ± 0.53	1.65 ± 0.32	1.59 ± 0.34 [#]
$\dot{V}CO_2$ (L·min⁻¹)				
<i>Baseline</i>	0.88 ± 0.19	0.86 ± 0.19	0.89 ± 0.19	0.81 ± 0.19 [#]
<i>End exercise</i>	1.60 ± 0.52	1.56 ± 0.50	1.53 ± 0.29	1.54 ± 0.29
RER				
<i>Baseline</i>	0.88 ± 0.08	0.90 ± 0.06	0.89 ± 0.05	0.92 ± 0.09
<i>End exercise</i>	0.94 ± 0.06	0.93 ± 0.06	0.93 ± 0.04	0.96 ± 0.06 [#]
$\dot{V}E$ (L·min⁻¹)				
<i>Baseline</i>	25 ± 5	24 ± 5	24 ± 5	22 ± 5 [#]
<i>End exercise</i>	42 ± 11	40 ± 11	38 ± 6	38 ± 6
Δ Blood [lactate] (mM)	0.0 ± 0.3	0.1 ± 0.4	0.1 ± 0.3	0.1 ± 0.4
Δ Blood [glucose] (mM)	0.1 ± 0.7	- 0.2 ± 0.7	0.00 ± 0.3	0.1 ± 0.5

Values are mean ± SD. PL, Placebo group; BR, Nitrate group; Pre, pre-donation; Post, post-donation; [Bla], blood lactate concentration; [glu], blood glucose concentration; HR, heart rate. [#]Significantly different from pre in the same condition ($P < 0.05$).

Table 3: Physiological responses to ramp incremental exercise prior to and following blood donation in the PL and BR groups.

	PL		BR	
	Pre	Post	Pre	Post
$\dot{V}O_2$ peak (L·min ⁻¹)	3.84 ± 0.91	3.65 ± 0.85*	3.52 ± 0.65	3.40 ± 0.73
$\dot{V}O_2$ peak (mL·kg ⁻¹ ·min ⁻¹)	49.9 ± 11.0	47.4 ± 10.0*	46.6 ± 6.0	44.9 ± 6.0
Peak power (W)	341 ± 70	324 ± 69*	331 ± 68	322 ± 66*
GET (L·min ⁻¹)	1.76 ± 0.40	1.68 ± 0.43	1.64 ± 0.44	1.63 ± 0.44
GET (W)	117 ± 29	109 ± 27	116 ± 35	112 ± 24
$\dot{V}CO_2$ peak (L·min ⁻¹)	4.69 ± 1.12	4.44 ± 0.97*	4.26 ± 0.68	4.36 ± 0.77
RER peak	1.22 ± 0.06	1.22 ± 0.05	1.22 ± 0.06	1.29 ± 0.06*
$\dot{V}E$ peak (L·min ⁻¹)	156 ± 44	150 ± 43*	134 ± 28	137 ± 32
HRpeak (b·min ⁻¹)	177 ± 16	181 ± 9	178 ± 12	179 ± 10
Δ Blood [lactate] (mM)	6.1 ± 1.4	5.5 ± 1.2	6.1 ± 1.9	6.8 ± 2.5
Δ Blood [glucose] (mM)	-0.2 ± 0.7	0.0 ± 1.1	-0.2 ± 0.4	0.0 ± 1.1

Values are mean ± SD. PL, Placebo group; BR, Nitrate group; Pre, pre-donation; Post, post-donation; GET, Gas exchange threshold; [Bla], blood lactate concentration; [glu], blood glucose concentration; HR, heart rate. *Significantly different from pre in the same condition ($P < 0.05$).

Table 4: Near-infrared spectroscopy-derived [HHb] and TOI dynamics during moderate-intensity and ramp incremental exercise prior to and following blood donation in the PL and BR groups.

	PL		BR	
	Pre	Post	Pre	Post
<i>Moderate-intensity exercise</i>				
[HHb]				
<i>Baseline (AU)</i>	-4.4 ± 3.0	-2.3 ± 3.1	-3.1 ± 3.7	-1.9 ± 2.5
<i>60 s (AU)</i>	-1.2 ± 2.3	2.3 ± 5.0	-0.1 ± 5.0	0.6 ± 3.9
<i>120 s (AU)</i>	-0.9 ± 3.0	3.5 ± 6.2	-0.1 ± 4.9	1.0 ± 3.7
<i>240 s (AU)</i>	-0.7 ± 3.9	2.3 ± 5.2	0.1 ± 4.9	1.1 ± 3.6
<i>End (AU)</i>	0.0 ± 4.4	2.5 ± 4.9	0.0 ± 4.9	1.0 ± 3.4
TOI				
<i>Baseline (%)</i>	65.3 ± 3.4	63.4 ± 3.3*	68.2 ± 4.3	70.1 ± 5.8
<i>60 s (%)</i>	61.9 ± 4.9	57.7 ± 5.0*	64.6 ± 6.5	65.6 ± 8.5
<i>120 s (%)</i>	61.9 ± 4.8	57.1 ± 5.7*	64.8 ± 6.1	65.6 ± 8.8
<i>240 s (%)</i>	60.7 ± 6.6	58.1 ± 4.8*	64.8 ± 6.5	65.8 ± 8.9
<i>End (%)</i>	61.4 ± 6.4	57.8 ± 5.0	65.3 ± 6.3	65.8 ± 8.9
<i>Ramp incremental exercise</i>				
[HHb]				
<i>Baseline (AU)</i>	-6.2 ± 4.1	-3.4 ± 3.6	-5.1 ± 4.1	-2.6 ± 2.5
<i>120 s (AU)</i>	-3.3 ± 5.4	-0.1 ± 5.0	-2.7 ± 5.0	-0.7 ± 3.3
<i>240 s (AU)</i>	-0.8 ± 6.2	3.3 ± 5.8*	-0.6 ± 5.8	1.4 ± 4.4
<i>360 s (AU)</i>	2.0 ± 9.4	7.3 ± 9.1*	1.5 ± 6.6	3.4 ± 5.8
<i>End (AU)</i>	6.2 ± 11.3	12.8 ± 10.1*	3.8 ± 7.6	5.3 ± 7.2
TOI				
<i>Baseline (%)</i>	66.5 ± 3.9	67.3 ± 7.1	71.5 ± 3.9	72.5 ± 4.7
<i>120 s (%)</i>	63.3 ± 5.1	64.6 ± 8.6	68.6 ± 5.5	69.5 ± 6.9
<i>240 s (%)</i>	60.8 ± 6.5	60.7 ± 9.2	65.8 ± 7.5	65.9 ± 9.7
<i>360 s (%)</i>	57.3 ± 11.5	55.4 ± 12.3	61.9 ± 8.6	61.7 ± 11.4
<i>End (%)</i>	49.5 ± 12.6	47.6 ± 14.9	57.1 ± 7.0	57.2 ± 10.9

Values are mean ± SD. PL, Placebo group; BR, Nitrate group; Pre, pre-donation; Post, post-donation; [HHb], deoxygenated haemoglobin concentration; TOI, tissue oxygenation index; AU, arbitrary units. *Significantly different from pre in the same condition ($P < 0.05$).