



Beetroot Juice Does Not Enhance Altitude Running Performance in Well-Trained Athletes

Arnold, J.T.; Oliver, S.J.; Lewis-Jones, T.M.; Wylie, L.J.; Macdonald, J.H.

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1 **TITLE**

2 Beetroot Juice Does Not Enhance Altitude Running Performance in Well-Trained Athletes

3

4 **AUTHORS**

5 1. Josh Timothy Arnold^{1,2}, josh.t.arnold@gmail.com

6 2. Samuel James Oliver¹, s.j.oliver@bangor.ac.uk

7 3. Tammy Maria Lewis-Jones¹, tammylewisjones@aol.com

8 4. Lee John Wylie³, ljw221@exeter.ac.uk

9 5. Jamie Hugo Macdonald¹, j.h.macdonald@bangor.ac.uk

10

11 **AFFILIATIONS**

12 ¹School of Sport, Health and Exercise Sciences, Bangor University.

13 ²Centre for Health, Exercise and Sport Science, Southampton Solent University.

14 ³Sport and Health Sciences, College of Life and Environmental Sciences, University of Exeter.

15

16 **CORRESPONDENCE**

17 Samuel James Oliver, PhD

18 Extremes Research Group, School of Sport, Health and Exercise Sciences

19 Bangor University, George Building, Bangor,

20 Gwynedd, LL57 2PZ

21 s.j.oliver@bangor.ac.uk; + 44 1248 383965

22

23 **RUNNING TITLE**

24 Beetroot juice and performance at altitude.

25 **ABSTRACT**

26 We hypothesized that acute dietary nitrate (NO_3^-) provided as concentrated beetroot juice
27 supplement would improve endurance running performance of well-trained runners in
28 normobaric hypoxia. Ten male runners (mean (SD): sea level $\dot{V}\text{O}_2\text{max}$ 66 (7) $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, 10
29 km personal best 36 (2) min) completed incremental exercise to exhaustion at 4000 m and a 10
30 km treadmill time trial at 2500 m simulated altitude on separate days, after supplementation
31 with ~ 7 mmol NO_3^- and a placebo, 2.5 h before exercise. Oxygen cost, arterial oxygen
32 saturation, heart rate and ratings of perceived exertion (RPE) were determined during the
33 incremental exercise test. Differences between treatments were determined using means [95%
34 confidence intervals], paired sample t-tests and a probability of individual response analysis.
35 NO_3^- supplementation increased plasma [nitrite] (NO_3^- , 473 (226) nM vs. placebo, 61 (37) nM,
36 $P < 0.001$) but did not alter time to exhaustion during the incremental test (NO_3^- , 402 (80) s vs.
37 placebo 393 (62) s, $P = 0.5$) or time to complete the 10 km time trial (NO_3^- , 2862 (233) s vs.
38 placebo, 2874 (265) s, $P = 0.6$). Further, no practically meaningful beneficial effect on time
39 trial performance was observed as the 11 [-60 to 38] s improvement was less than the *a priori*
40 determined minimum important difference (51 s), and only three runners experienced a ‘likely,
41 probable’ performance improvement. NO_3^- also did not alter oxygen cost, arterial oxygen
42 saturation, heart rate or RPE. Acute dietary NO_3^- supplementation did not consistently enhance
43 running performance of well-trained athletes in normobaric hypoxia.

44

45 **KEY WORDS:**

46 Nitrate, nitrite, nitric oxide, exercise, hypoxia.

47 **INTRODUCTION**

48 Exposure to altitude has a profound negative effect on exercise performance because reduced
49 partial pressure of ambient oxygen causes arterial oxygen desaturation, tissue hypoxia and
50 disturbed muscle metabolism (Modin et al. 2001). Increasing dietary nitrate via beetroot
51 supplementation (NO_3^-) is an increasingly popular strategy to improve exercise capacity at sea
52 level (Hoon et al. 2013). As conjectured by previous publication, NO_3^- supplementation may
53 be particularly effective at altitude due to its ‘oxygen sparing effect’ whereby whole body
54 oxygen utilisation is reduced during submaximal exercise (Larsen et al. 2007; Bailey et al.
55 2009; Vanhatalo et al. 2010; Lansley et al. 2011). The mechanism by which NO_3^-
56 supplementation has this effect is not completely understood but is likely related to increased
57 plasma nitrite (NO_2^-) concentration and nitric oxide (NO) production.

58

59 As a physiological signalling molecule, NO plays a key role in the regulation of blood flow,
60 mitochondrial respiration and biogenesis, muscle contractility, and glucose and calcium
61 homeostasis (Stamler and Meissner 2001). New evidence also suggests a high NO
62 bioavailability is characteristic of successful adaptation to altitude (Levett 2011). This notion
63 is supported by increased concentrations of expired NO and plasma NO_2^- observed in Tibetan
64 highlanders (Beall et al. 2001; Erzurum et al. 2007). Theoretically, compared with sea level,
65 dietary NO_3^- supplementation at altitude may be more beneficial as the reduction process of
66 NO_3^- to NO is enhanced under acidic (Modin et al. 2001) and hypoxic conditions (Castello et
67 al. 2006), whereas the endogenous L-arginine NO synthase (oxygen dependent) pathway is
68 suppressed.

69

70 Studies investigating the effects of dietary NO_3^- supplementation on exercise at high altitude or
71 normobaric hypoxia are limited, but in general support beneficial effects (Vanhatalo et al. 2011;

72 Masschelein et al. 2012; Muggeridge et al. 2014). Specifically, Masschelein and colleagues
73 (2012) showed partial restoration of oxygen delivery and utilisation in hypoxia, reporting
74 increased arterial and muscle oxygenation during exercise after NO_3^- ingestion compared with
75 a placebo. Additionally, this and another study (Vanhatalo et al. 2011) demonstrated that dietary
76 NO_3^- supplementation improves exercise capacity in hypoxia, as time-to-exhaustion on an
77 incremental cycling test (Masschelein et al. 2012) and leg extension exercise (Vanhatalo et al.
78 2011) were longer after NO_3^- was ingested. Unfortunately, these studies only demonstrated
79 statistically significant differences between NO_3^- consumption compared with a placebo, of
80 which the practical performance benefit remained unclear. To address this issue, Muggeridge
81 and colleagues (2014) investigated the benefit of NO_3^- ingestion on trained cyclists during a 16
82 km time trial at 2500m, and found both a statistically significant and practically meaningful
83 (2.2%) improvement in performance time. Although these results draw attention to the potential
84 endurance performance benefits of NO_3^- supplementation, there is a requirement for further
85 studies that investigate acute supplementation protocols, in well-trained athletes, using
86 practically relevant outcome measures, to determine if NO_3^- supplementation can enhance
87 athletic performance such as endurance running capacity in hypoxia (Hoon et al. 2013; Jones
88 2013). In addition, anecdotal reports obtained from national level altitude training camps
89 indicate the possibility of responders and non-responders to ergogenic supplements including
90 NO_3^- , but scientific evidence is lacking to support this observation.

91
92 With an increasing number of athletic camps and competitive running events now held at
93 altitude each year, well trained runners are increasingly utilising NO_3^- supplements despite
94 minimal evidence of their ergogenic effect. The current investigation therefore aimed to assess
95 the influence of acute NO_3^- ingestion, via beetroot juice, upon endurance running performance
96 and exercise tolerance at moderate altitude, in a well-trained population. It was hypothesised

97 that compared to a placebo, acute ingestion of a commercially available high-nitrate beetroot
98 juice shot (~7 mmol NO₃⁻) would statistically (beyond chance) and practically (greater than the
99 minimum important difference in the majority of participants) enhance exercise performance
100 in normobaric hypoxia.

101

102 **METHODS**

103 *Participants*

104 Ten well-trained competitive male runners (mean (SD): age 37 (13) years, height 1.78 (0.06)
105 m, body mass 72 (7) kg, sea level $\dot{V}O_{2max}$ 66 (7) mL·kg⁻¹·min⁻¹, 10 km personal best time 36
106 (2) min) were recruited using opportunistic sampling methods from local running clubs between
107 January and March 2013. Inclusion criteria detailed: a sub-40 min 10 km run time in the
108 previous 12 months, non-smoking, and no exposure to altitude greater than 1500 m in the
109 previous six months. All participants provided written informed consent. Ethical approval was
110 granted by the Ethics Committee of the School of Sport, Health and Exercise Sciences at
111 Bangor University (reference ID; MSc03-12/13), and the study was registered on
112 www.clinicaltrials.gov (reference ID: NCT01795534).

113

114 *Design*

115 Participants visited the laboratory on six occasions (**Figure 1**). The first and second visits were
116 used to familiarise participants with the experimental exercise tests, which involved completion
117 of a 10 km treadmill time trial at a simulated 2500 m (FiO₂, 15.4%) and an incremental exercise
118 test to exhaustion at sea level. This incremental exercise test was also used to determine
119 maximal oxygen uptake ($\dot{V}O_{2max}$) at sea level. The study then used a double-blind repeated
120 measures crossover design where participants received either acute beetroot juice ingestion
121 (NO₃⁻) or placebo ingestion (PLAC) in a random order. The randomisation was completed by

122 JHM using www.randomization.com. A minimum four-day wash out was used between
123 supplementations to ensure circulating NO_3^- and NO_2^- concentrations returned to basal levels
124 (Wylie et al. 2013). During each supplementation period participants visited the laboratories on
125 two occasions. The first visit consisted of an incremental exercise test to exhaustion on a
126 treadmill at a simulated 4000 m (FiO_2 , 12.8%). This relatively high altitude was chosen to
127 maximise hypoxemia and thus potentiate any physiological effects of NO_3^- supplementation
128 (enhanced production of NO via exogenous NO_3^- reduction occurs in hypoxic conditions
129 (Castello et al. 2006)). The second visit consisted of a 10 km treadmill time trial at a simulated
130 2500 m (FiO_2 , 15.4%), which directly tested moderate altitude endurance performance as
131 required for events such as the Trans Alps Run, Tour de France, Pikes Peak Marathon and
132 training camps (Wilber 2004).

133

134 *PLEASE INSERT FIGURE 1 NEAR HERE*

135

136 *Supplementation*

137 Supplementation consisted of either a single 70 mL concentrated shot of beetroot juice (~7
138 mmol NO_3^- , Beet It SportTM, James White Drinks Ltd, Ipswich, UK) or a NO_3^- depleted placebo
139 shot that was identical in appearance, taste and texture (~0.003 mmol NO_3^- , James White Drinks
140 Ltd, Ipswich, UK). Placebo shots were created by passing the NO_3^- active beetroot juice through
141 a Purolite A520E NO_3^- selective ion exchange resin before pasteurisation (Lansley et al. 2011).
142 Supplements were ingested under experimenter supervision 2 h before visits three to six, which
143 was 2.5 h before each exercise test. Shots were packaged in identical coded containers by James
144 White Drinks and were distributed by JHM to participants, ensuring blinding of participants
145 and observers (JTA, TLJ, SJO). To ensure that the placebo had been theoretically effective, a

146 manipulation check was conducted after each visit, asking participants to guess what
147 intervention (NO_3^- or placebo) they had received.

148

149 *Procedures*

150 One week before testing, participants were fully briefed with regards to the study aims and
151 design. A list of high NO_3^- foodstuffs to avoid throughout the study was presented to each
152 participant in an attempt to isolate supplemented NO_3^- as a cause of any potential effect.
153 Participants were asked to not increase or decrease training load throughout the study.
154 Furthermore, twenty four hours before the first familiarisation session, each participant was
155 asked to produce a diet and activity diary and to repeat these recorded behaviours in the twenty
156 four hours prior to all trials. Participants were also allocated drinking water equal to $35 \text{ mL}\cdot\text{kg}^{-1}$
157 ¹ of body mass to be consumed in the 24 hours prior to each visit. Participants were asked to
158 abstain from the use of any chewing gum or antibacterial mouthwashes as this has previously
159 shown to lessen the reduction of NO_3^- to NO_2^- by commensal bacteria within the oral cavity
160 (Govoni et al. 2008). These actions were then repeated for subsequent visits.

161

162 Each participant completed all exercise tests at the same time of day. At the start of each visit
163 body mass was measured and urine and capillary blood samples were obtained to ensure runners
164 were euhydrated (urine specific gravity less than 1.020, refractometer Atago, Japan (Oppliger
165 et al. 2005)) and had normal hemoglobin (greater than $13.5 \text{ g}\cdot\text{dL}^{-1}$, Hemocue Ltd, Derbyshire,
166 UK). After, a resting venous blood sample was obtained by venepuncture into a lithium-heparin
167 tube (Monovette Lithium Heparin, Sarstedt, Leicester, UK). This blood sample was placed in
168 a centrifuge and spun at 4000 rpm at 4°C for 10 min within 3 min of collection. Immediately
169 after the centrifugation, plasma was aspirated into eppendorfs and frozen at -80°C for a
170 standardised time period prior to subsequent analysis of NO availability (NO_2^- and NO_3^-

171 concentration) as per Wylie et al. (2013). All subsequent data collection was conducted in a
172 temperature and humidity controlled normobaric hypoxic environmental chamber (Hypoxico
173 Inc., The Altitude Centre, London, UK, 20.0 (0.1) °C, 40 (3) %).

174

175 *Incremental exercise test*

176 The chamber was set and maintained at a simulated altitude of 4000 m (ambient oxygen 12.9
177 (0.1) %). Maximal oxygen uptake was assessed using a continuous incremental exercise test on
178 a motorised treadmill (h/p/cosmos, Nussdorf, Germany) until volitional exhaustion. The test
179 started at 10 km·h⁻¹ with a 0% gradient. Increments were subsequently achieved by increasing
180 the treadmill speed by 1 km·h⁻¹ every minute until 16 km·h⁻¹. Thereafter the gradient was
181 increased by 1% every minute until volitional exhaustion. Following a period of active
182 recovery, where the participant completed light exercise until their heart rate reduced to less
183 than 100 bpm, $\dot{V}O_{2\max}$ was verified by runners returning to the treadmill to complete exercise
184 at an intensity greater than at exhaustion (i.e. 1% greater gradient). Oxygen consumption was
185 recorded continuously throughout exercise by a metabolic cart (Metalyser, Cortex, Leipzig,
186 Germany) with $\dot{V}O_{2\max}$ determined as the highest 30 s average at any given time point.
187 Additionally heart rate by remote transmitter (FT3, Polar, Kempele, Finland), blood oxygen
188 saturation by fingertip pulse oximeter (7500, Nonin Medical Inc., Minnesota, USA) and overall
189 rating of perceived exertion (RPE) by Borg CR100 scale (Borg and Borg 2001), were recorded
190 during the final 15 s of each incremental stage. At exhaustion, blood lactate was also measured
191 via ear lobe capillary sampling and a portable analyser (Lactate Pro, Ark Ray Inc, Kyoto,
192 Japan).

193

194

195

196 ***Time Trial***

197 The chamber was set and maintained at a simulated altitude of 2500 m (ambient oxygen 15.4
198 (0.1) %). After runners had completed a standardised warm up of 3 min at 10 km·h⁻¹ they
199 completed a 10 km time trial on a treadmill. Treadmill gradient was set to 1% to better replicate
200 the physiological demands of outside running (Jones and Doust 1996). Runners were instructed
201 to complete the distance as quickly as possible. During the time trial runners were blinded to
202 the elapsed time and speed of the treadmill. Verbal prompts at kilometre intervals were provided
203 to replicate distance markers during running race competitions. Runners self-selected their
204 running speed throughout the time trial. Differentiated RPE (legs, chest and overall) was
205 recorded at the completion of each time trial kilometre to assess trends in pacing. The reliability
206 of this 10 km time trial protocol at 2500 m simulated altitude was assessed in six similarly
207 trained runners to be 3.9 (1.0) % (within subjects coefficient of variation), across three time
208 trials each separated by seven days. The within subjects coefficient of variation of the second
209 and third time trial alone was assessed to be 2.1 (1.4) %.

210

211 ***Data Analysis***

212 The primary outcome measure was time to complete the 10 km treadmill time trial. All data
213 extraction was completed whilst experimenters were blinded; only statistical analyses were
214 completed un-blinded. Data are presented as means (SD) or [95% confidence interval].
215 Inferential statistical analysis was conducted using the software package SPSS (version 20,
216 IBM, Portsmouth, UK). Statistical significance was set at $P \leq 0.05$. To evaluate the statistical
217 significance of NO₃⁻ supplementation, paired samples *t*-tests were used to assess differences
218 between NO₃⁻ and placebo trials. Magnitude of difference between treatments was calculated
219 as NO₃⁻ minus placebo trial and for the primary outcome measure compared to a minimal
220 practical important difference determined as 51 s (Cohen's smallest important effect: $0.2 \times$

221 between subject SD, confirmed by discussion with expert coaches, and equivalent to 1.8%). A
222 probability analysis was also undertaken on the primary outcome measure, estimating the
223 likelihood of a true positive response to NO_3^- supplementation (Hopkins 2000). Specifically,
224 using calculations on precision of change provided by Hopkins (2000), for each runner the
225 difference between NO_3^- and placebo trials was assigned one of the following verbal descriptors
226 to describe if NO_3^- supplementation had a positive effect on their individual time trial
227 performance: ‘almost certainly not’; ‘very unlikely’; ‘unlikely, probably not’; ‘possibly may’;
228 ‘likely probable’; ‘very likely’; ‘almost certainly’. Data from the incremental test (i.e.
229 physiological parameters such as oxygen uptake) were presented and analysed at maximal
230 exercise capacity (100% altitude specific $\dot{V}\text{O}_{2\text{max}}$), and at a submaximal workload (45%
231 altitude specific $\dot{V}\text{O}_{2\text{max}}$). In order to investigate NO_2^- response, baseline plasma NO_2^-
232 concentrations and also the difference between NO_3^- and placebo trials’ plasma NO_2^-
233 concentrations were correlated (Pearson’s r) against the difference between NO_3^- and placebo
234 trials for all outcome measures. Finally, *post hoc* independent t-tests were completed to explore
235 if baseline characteristics (age, body mass, $\dot{V}\text{O}_{2\text{max}}$, haemoglobin), plasma NO_2^- responses
236 (plasma NO_2^- concentrations on the placebo trial and difference between NO_3^- and placebo
237 trials’ plasma NO_2^- concentrations) or hypoxia responses (average arterial oxygen saturation
238 on the placebo trial) may explain why some individuals improved time trial performance after
239 NO_3^- supplementation.

240

241 For the primary outcome, sample size estimation was completed using both statistical
242 significance and magnitude based inference methods (Hopkins 2006). Data on expected
243 reliability of the 10 km time trial between two trials after a familiarisation trial was obtained
244 from a pilot study on six well trained athletes: the Pearson’s correlation coefficient was 0.98,
245 the between subject SD was 255 s, and the typical error was 33 s. The minimum practical

246 important difference was therefore set at 51 s. Using the magnitude based inference method
247 and maximum chances of Type I and Type II clinical errors of 0.5 and 25% respectively, six
248 participants were estimated as required to detect a difference in means in a post-only
249 crossover trial. Using the statistical significance method and maximum rates of Type I and
250 Type II statistical errors of 5 and 20%, respectively, nine participants were required.

251

252 **RESULTS**

253 The NO_3^- and placebo shots effectively altered the independent variable: 2.5 h after NO_3^-
254 consumption plasma $[\text{NO}_3^-]$ and $[\text{NO}_2^-]$ were significantly greater than after placebo ($[\text{NO}_3^-]$ in
255 the NO_3^- trial, 201.6 (25.9) μM vs. placebo trial, 28.9 (6.4) μM , $P < 0.001$; $[\text{NO}_2^-]$ in the NO_3^-
256 trial, 473 (226) nM vs. placebo trial, 61 (37) nM, $P < 0.001$). The runners were considered to
257 be sufficiently well blinded as to which supplement they received on each visit, as the
258 manipulation check indicated that only two participants of ten guessed correctly, two guessed
259 incorrectly, and six were unable to distinguish between the supplements at all.

260

261 ***Incremental exercise test***

262 Acute NO_3^- supplementation did not alter any measured physiological variable or RPE during
263 maximal or submaximal exercise at 4000 m (**Table 1**). No statistical difference was present in
264 any parameter obtained at 100% or 45% of $\dot{V}\text{O}_{2\text{max}}$. There was also no practical performance
265 difference in time to exhaustion between trials (NO_3^- – placebo: Δ 1.4%). No correlations were
266 observed between baseline plasma $[\text{NO}_2^-]$ or the change in plasma $[\text{NO}_2^-]$ with any maximal
267 exercise parameter.

268

269 *PLEASE INSERT TABLE 1 NEAR HERE*

270

271 ***Time Trial***

272 Acute NO₃⁻ supplementation did not improve 10 km running performance at simulated altitude
273 (2500 m). No statistical difference was observed in time to complete the 10 km time trial (NO₃⁻
274 , 2862 (233) s vs. placebo, 2874 (265) s, $P = 0.6$). Additionally, compared to the *a priori*
275 determined minimum practical important difference of -51 s (1.8%) there was also no practical
276 difference in performance (NO₃⁻ – placebo: $\Delta -11 [-60 \text{ to } 38]$ s or $\Delta 0.4\%$: **Figure 2**). Trends in
277 RPE during the time trial were visually explored but no difference was observed between NO₃⁻
278 and placebo.

279

280 *PLEASE INSERT FIGURE 2 NEAR HERE*

281

282 Results obtained from the probability analysis suggested that three runners experienced a
283 performance improvement with NO₃⁻ supplementation labelled ‘likely, probable’; one runner
284 experienced impaired performance labelled ‘likely, probable’; and the remaining six runners
285 exhibited no strong probability of either improved or impaired performance. Further, no
286 correlation was observed between baseline [NO₂⁻] or the change in plasma [NO₂⁻] with change
287 in time to complete the 10 km time trial ($r < 0.48$, $P > 0.1$).

288

289 Exploratory *post hoc* analyses suggested that runners who improved time trial performance
290 responded to hypoxia with greater arterial desaturation, as indicated by lower arterial oxygen
291 saturation during the placebo time trial (82 (2) vs. 84 (2), $P = 0.04$). There was however no
292 difference in baseline characteristics (age, body mass, $\dot{V}O_{2\max}$, haemoglobin, $P > 0.4$) or plasma
293 NO₂⁻ responses (plasma NO₂⁻ concentrations on the placebo trial and difference between NO₃-
294 and placebo trials’ plasma NO₂- concentrations, $P > 0.6$) between those runners that did or did
295 not improve time trial performance after NO₃⁻ supplementation.

296 **DISCUSSION**

297 The current study aimed to assess the influence of NO_3^- supplementation upon endurance
298 running performance at altitude in well-trained runners. The principal finding contradicted the
299 hypothesis: acute NO_3^- supplementation did not enhance endurance running performance in
300 normobaric hypoxia. Specifically, no statistical or practical difference in 10 km time trial
301 running performance was observed between NO_3^- and placebo trials, whilst probability analysis
302 of individual responses suggested only three of ten participants had a “likely, probably”
303 increase in performance. In addition, no significant differences were seen in any measured
304 physiological or perceptual parameters or time to exhaustion during an incremental treadmill
305 test in normobaric hypoxia. These findings contrast those of other investigations conducted in
306 hypoxia that have suggested positive effects of NO_3^- supplementation on time to exhaustion
307 (Vanhatalo et al. 2011; Masschelein et al. 2012) and time trial performance (Muggeridge et al.
308 2014).

309

310 It is unlikely that the acute nitrate dose of 7 mmol NO_3^- administered in the present study was
311 simply insufficient to cause an effect. In a previous dose response study completed in normoxia,
312 time to exhaustion was improved after acute NO_3^- supplementation equal to 8 mmol of dietary
313 NO_3^- (Wylie et al. 2013). The positive effects in hypoxia on exercise tolerance previously
314 observed by Vanhatalo et al. (2011) and Masseurchelein et al. (2012) and on exercise performance
315 by Muggeridge et al. (2014) were achieved with NO_3^- doses that ranged from smaller (5 mmol)
316 to larger (9 mmol acutely and 5 mmol once daily for six days) doses than used in the present
317 investigation. Considering that suppression of the endogenous L-arginine NO synthase (oxygen
318 dependent) pathway occurs in hypoxia (Castello et al. 2006), suggesting a greater reliance on
319 reduction of NO_3^- to NO (potentially reducing the required dose to have a physiological effect),

320 the non-significant finding following dietary supplementation of NO_3^- in the present study
321 remains surprising.

322

323 Theoretically the negative finding of the current investigation may be explained by the well-
324 trained status of the participants recruited (Hoon et al. 2013). Sea level studies have shown that
325 the beneficial effects of NO_3^- supplementation on exercise performance may be reduced in well-
326 trained athletes (Wilkerson et al. 2012), and thus well trained athletes may require longer
327 periods of supplementation to elicit an ergogenic effect (Cermak *et al.*, 2012a, 2012b). Well-
328 trained athletes have greater resting plasma NO_3^- concentrations (Jungersten et al. 1997),
329 greater presence of NO synthase (Green et al. 2004), and experience less severe localised
330 hypoxia and acidosis in the muscle compared to untrained populations (Wilkerson et al. 2012).
331 Such adaptations allow more NO to be derived from the endogenous NO synthase pathway,
332 and place less reliance on NO_3^- supplementation as a means to maintain adequate NO
333 concentrations. However we hypothesized that such adaptations in well-trained athletes would
334 be outweighed by the deleterious effects of hypoxia, allowing a benefit to be observed from
335 acute nitrate supplementation even in well-trained athletes. Unfortunately the current findings
336 do not support this hypothesis. As comparison of training status of participants between studies
337 completed in hypoxia is difficult (Masschelein et al. 2012; Muggeridge et al. 2014; Vanhatalo
338 et al. 2011), and because completing correlational analyses between baseline fitness or baseline
339 NO bioavailability and response to supplementation is problematic in homogenous groups such
340 as recruited herein, an important future direction for research in this area is to investigate the
341 moderating effect of training status in response to NO_3^- supplementation.

342

343 It is also possible that the effects of NO_3^- on exercise performance in hypoxia may in part be
344 dependent upon exercise mode, duration and intensity. Some previous investigations have

345 utilised exercise protocols that are arguably less ecologically valid, over-estimating ergogenic
346 effects of any intervention (Masschelein et al. 2012; Vanhatalo et al. 2011). In fact even within
347 sea level studies that have specifically assessed performance through practically relevant time
348 trial testing, the results of NO_3^- supplementation remain mixed (Hoon et al. 2013). Perhaps of
349 greatest relevance is the study by Muggeridge and colleagues (2014) that utilised a cycling time
350 trial in hypoxia, which revealed positive effects of NO_3^- supplementation. Of interest, the
351 utilised time trial was noticeably shorter in duration than the test used in the current study (28
352 vs. 48 min). Possibly the effect size of NO_3^- supplementation is reduced in longer duration
353 activities (Wilkerson et al. 2012). The mechanism remains unknown, but during shorter
354 duration exercise more type II muscle fibres are recruited, and recent findings suggest the
355 effects of NO_3^- are perhaps preferential to type II fibres (Hernandez et al. 2012; Ferguson et al.
356 2013).

357

358 Whilst these mechanistic explanations are speculative, detailed analysis within the present
359 study of individual responses clearly show that the performance benefit of NO_3^-
360 supplementation is very variable. A probability analysis addressing the true likelihood of
361 individual responses to NO_3^- supplementation suggested that three participants experienced a
362 'likely/probable' improvement in performance when supplemented with NO_3^- , one participant
363 experienced a 'likely/probable' decrease in performance, whilst the remaining participants had
364 no strong probability of either enhanced or impaired performance. The reason for the improved
365 performance in some but not all individuals is of particular interest. A placebo effect can be
366 excluded as all three participants with improved performance could not differentiate which
367 supplement they were taking before each time trial. Exploratory *post hoc* analysis suggested
368 that NO_3^- supplementation improved time trial performance in those runners that had the
369 greatest arterial desaturation in hypoxia. As this exploratory *post hoc* analysis was completed

370 in small numbers, future studies are required to confirm whether individual susceptibility to
371 hypoxia moderates performance benefits of NO_3^- supplementation. Future studies are also
372 required to provide sufficient data for meta-analyses, before NO_3^- can be accepted as an
373 ergogenic aid in hypoxia.

374

375 Criticisms of the current work include the use of well-trained athletes. Difficulties surrounding
376 physiological testing of trained populations include other training and competition
377 commitments. In order to control for such variables, athletes were encouraged to maintain
378 consistent training load during the study; however compliance was only confirmed by
379 inspection of training diaries. Nevertheless the consistency in which these athletes were able to
380 complete the 10 km time trial, as shown by the acceptable reliability results, suggests that any
381 effect of other training or competition exercise was minimal on the time trial results of this
382 study. The acute exposure to hypoxia may be considered another limitation, as the influence of
383 nitrate supplementation on exercise during longer exposures to hypoxia is unknown. However,
384 as many athletes do not have adequate time to acclimatize to altitude before training or
385 competition, the moderate altitude used for the time trial (2500 m) is typical of that experienced
386 by athletes.

387

388 ***Conclusion***

389 This investigation was unable to provide evidence for either a statistically significant or
390 practically beneficial effect of acute NO_3^- supplementation on 10km running performance or
391 exercise tolerance in a maximal incremental test (both completed in normobaric hypoxia).
392 These results contradict previous studies, most likely due to the inter-individual response to
393 acute dietary NO_3^- supplementation that was observed in the present investigation. Further
394 investigation of the mechanistic reasons for inter-individual responses to supplementation is

395 thus required before NO_3^- supplementation can be accepted as an effective ergogenic aid in
396 hypoxia.

397

398

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402

403 **CONFLICTS OF INTEREST AND SOURCES OF FUNDING**

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TABLES

Table 1. Time to exhaustion and other psychophysiological responses at submaximal and maximal exercise intensities during an incremental treadmill exercise test at simulated altitude (4000 m) after acute dietary nitrate and placebo supplementation

	NO₃⁻	PLAC	NO₃⁻ - PLAC	P
Time to exhaustion (s)	402 ± 80	393 ± 62	9 [-20 to 38]	0.5
45% $\dot{V}O_{2max}$				
Speed/Gradient (km·h ⁻¹ / %)	12 (0) / 0 (0)	12 (0) / 0 (0)	-	-
$\dot{V}O_2$ (mL·kg ⁻¹ ·min ⁻¹)	26 (2)	26 (2)	0 [-1 to 1]	0.7
SpO ₂ (%)	78 (3)	77 (5)	1 [-5 to 3]	0.6
Heart rate (bpm)	136 (13)	134 (12)	1 [-9 to 6]	0.7
Rating of perceived exertion	24 (14)	25 (14)	-1 [-6 to 7]	0.8
100% $\dot{V}O_{2max}$				
Speed/Gradient (km·h ⁻¹ / %)	16 (0) / 1 (1)	16 (0) / 1 (1)	-	-
$\dot{V}O_2$ (mL·kg ⁻¹ ·min ⁻¹)	48 (4)	48 (5)	0 [-2 to 1]	0.8
SpO ₂ (%)	74 (3)	74 (4)	1 [-2 to 3]	0.7
Heart rate (bpm)	155 (12)	158 (26)	-3 [-19 to 12]	0.7
Rating of perceived exertion	79 (27)	79 (30)	0 [-6 to 6]	1.0
[Blood lactate] (mmol·L ⁻¹)	8.8 (2.0)	8.3 (3.0)	0.5 [-1.2 to 2.1]	0.6

Data are mean (SD) or mean difference [95% confidence interval]; significance determined by paired samples t-test (n = 10); NO₃⁻, 70ml dietary nitrate (beetroot juice) supplementation; PLAC, placebo supplementation; $\dot{V}O_{2max}$, maximal oxygen uptake at 4000 m; SpO₂, arterial oxygen saturation; whole-body rating of perceived exertion by Borg CR100 scale.

FIGURE CAPTIONS

Figure 1: Schematic representation of research design

n, number of participants; $\dot{V}O_{2max}$, maximal oxygen uptake incremental exercise test; TT, 10 km time trial.

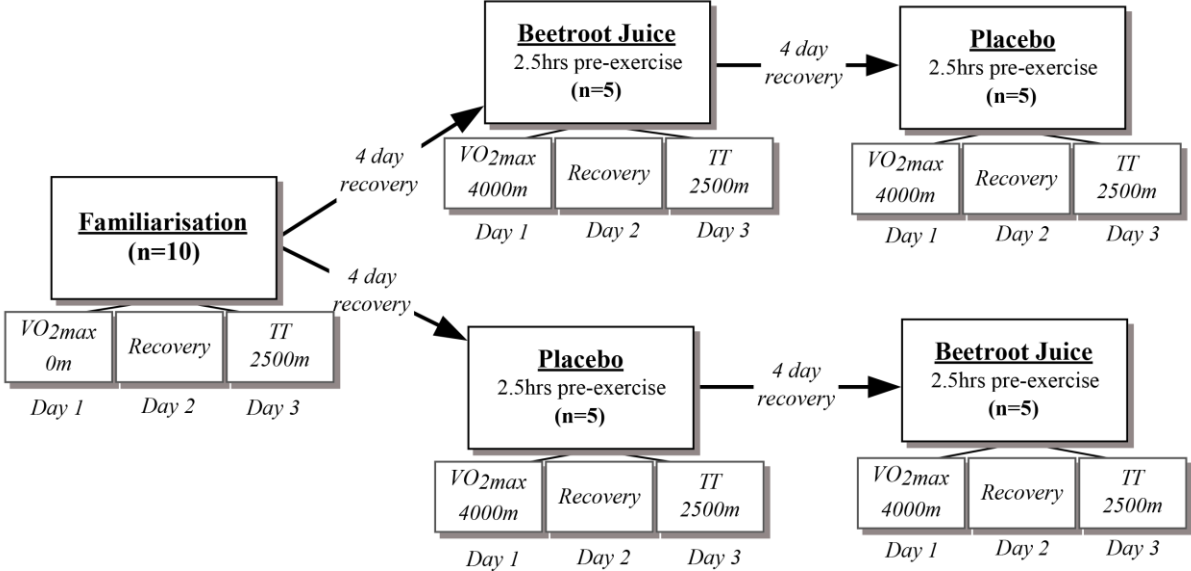


Figure 2: Difference in performance during a simulated altitude (2500 m) 10 km time trial after acute dietary nitrate and placebo supplementation.

NO_3^- , 70 ml dietary nitrate (beetroot juice) supplementation; PLAC, placebo supplementation; horizontal lines = mean response [95% confidence interval]; dots = individual runner responses. The negative values indicate runners that completed the time trial sooner when supplemented with dietary nitrate than placebo

