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

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TITLE

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RUNNING TITLE

Beetroot juice and performance at altitude.

ABSTRACT

We hypothesized that acute dietary nitrate (NO_3^-) provided as concentrated beetroot juice supplement would improve endurance running performance of well-trained runners in normobaric hypoxia. Ten male runners (mean (SD): sea level $\dot{V}O_2$max 66 (7) $mL \cdot kg^{-1} \cdot min^{-1}$, 10 km personal best 36 (2) min) completed incremental exercise to exhaustion at 4000 m and a 10 km treadmill time trial at 2500 m simulated altitude on separate days, after supplementation with ~7 mmol NO_3^- and a placebo, 2.5 h before exercise. Oxygen cost, arterial oxygen saturation, heart rate and ratings of perceived exertion (RPE) were determined during the incremental exercise test. Differences between treatments were determined using means [95% confidence intervals], paired sample t-tests and a probability of individual response analysis. NO_3^- supplementation increased plasma [nitrite] (NO_3^-, 473 (226) nM vs. placebo, 61 (37) nM, $P < 0.001$) but did not alter time to exhaustion during the incremental test (NO_3^-, 402 (80) s vs. placebo 393 (62) s, $P = 0.5$) or time to complete the 10 km time trial (NO_3^-, 2862 (233) s vs. placebo, 2874 (265) s, $P = 0.6$). Further, no practically meaningful beneficial effect on time trial performance was observed as the 11 [-60 to 38] s improvement was less than the *a priori* determined minimum important difference (51 s), and only three runners experienced a 'likely, probable' performance improvement. NO_3^- also did not alter oxygen cost, arterial oxygen saturation, heart rate or RPE. Acute dietary NO_3^- supplementation did not consistently enhance running performance of well-trained athletes in normobaric hypoxia.

KEY WORDS:

Nitrate, nitrite, nitric oxide, exercise, hypoxia.

INTRODUCTION

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

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

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

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

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

that compared to a placebo, acute ingestion of a commercially available high-nitrate beetroot juice shot (~7 mmol NO_3^-) would statistically (beyond chance) and practically (greater than the minimum important difference in the majority of participants) enhance exercise performance in normobaric hypoxia.

METHODS

Participants

Ten well-trained competitive male runners (mean (SD): age 37 (13) years, height 1.78 (0.06) m, body mass 72 (7) kg, sea level $\dot{V}O_2max$ 66 (7) $mL \cdot kg^{-1} \cdot min^{-1}$, 10 km personal best time 36 (2) min) were recruited using opportunistic sampling methods from local running clubs between January and March 2013. Inclusion criteria detailed: a sub-40 min 10 km run time in the previous 12 months, non-smoking, and no exposure to altitude greater than 1500 m in the previous six months. All participants provided written informed consent. Ethical approval was granted by the Ethics Committee of the School of Sport, Health and Exercise Sciences at Bangor University (reference ID; MSc03-12/13), and the study was registered on www.clinicaltrials.gov (reference ID: NCT01795534).

Design

Participants visited the laboratory on six occasions (**Figure 1**). The first and second visits were used to familiarise participants with the experimental exercise tests, which involved completion of a 10 km treadmill time trial at a simulated 2500 m (FiO_2, 15.4%) and an incremental exercise test to exhaustion at sea level. This incremental exercise test was also used to determine maximal oxygen uptake ($\dot{V}O_{2max}$) at sea level. The study then used a double-blind repeated measures crossover design where participants received either acute beetroot juice ingestion (NO_3^-) or placebo ingestion (PLAC) in a random order. The randomisation was completed by

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

PLEASE INSERT FIGURE 1 NEAR HERE

Supplementation

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

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

Procedures

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

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

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

Incremental exercise test

The chamber was set and maintained at a simulated altitude of 4000 m (ambient oxygen 12.9 (0.1) %). Maximal oxygen uptake was assessed using a continuous incremental exercise test on a motorised treadmill (h/p/cosmos, Nussdorf, Germany) until volitional exhaustion. The test started at 10 $km \cdot h^{-1}$ with a 0% gradient. Increments were subsequently achieved by increasing the treadmill speed by 1 $km \cdot h^{-1}$ every minute until 16 $km \cdot h^{-1}$. Thereafter the gradient was increased by 1% every minute until volitional exhaustion. Following a period of active recovery, where the participant completed light exercise until their heart rate reduced to less than 100 bpm, $\dot{V}O_2max$ was verified by runners returning to the treadmill to complete exercise at an intensity greater than at exhaustion (i.e. 1% greater gradient). Oxygen consumption was recorded continuously throughout exercise by a metabolic cart (Metalyser, Cortex, Leipzig, Germany) with $\dot{V}O_2max$ determined as the highest 30 s average at any given time point. Additionally heart rate by remote transmitter (FT3, Polar, Kempele, Finland), blood oxygen saturation by fingertip pulse oximeter (7500, Nonin Medical Inc., Minnesota, USA) and overall rating of perceived exertion (RPE) by Borg CR100 scale (Borg and Borg 2001), were recorded during the final 15 s of each incremental stage. At exhaustion, blood lactate was also measured via ear lobe capillary sampling and a portable analyser (Lactate Pro, Ark Ray Inc, Kyoto, Japan).

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 $km \cdot h^{-1}$ 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 NO_3^- supplementation, paired samples *t*-tests were used to assess differences
218 between NO_3^- and placebo trials. Magnitude of difference between treatments was calculated
219 as NO_3^- 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 ×

between subject SD, confirmed by discussion with expert coaches, and equivalent to 1.8%). A probability analysis was also undertaken on the primary outcome measure, estimating the likelihood of a true positive response to NO_3^- supplementation (Hopkins 2000). Specifically, using calculations on precision of change provided by Hopkins (2000), for each runner the difference between NO_3^- and placebo trials was assigned one of the following verbal descriptors to describe if NO_3^- supplementation had a positive effect on their individual time trial performance: 'almost certainly not'; 'very unlikely'; 'unlikely, probably not'; 'possibly may'; 'likely probable'; 'very likely'; 'almost certainly'. Data from the incremental test (i.e. physiological parameters such as oxygen uptake) were presented and analysed at maximal exercise capacity (100% altitude specific $\dot{V}O_2max$), and at a submaximal workload (45% altitude specific $\dot{V}O_2max$). In order to investigate NO_2^- response, baseline plasma NO_2^- concentrations and also the difference between NO_3^- and placebo trials' plasma NO_2^- concentrations were correlated (Pearson's *r*) against the difference between NO_3^- and placebo trials for all outcome measures. Finally, *post hoc* independent t-tests were completed to explore if baseline characteristics (age, body mass, $\dot{V}O_2max$, haemoglobin), plasma NO_2^- responses (plasma NO_2^- concentrations on the placebo trial and difference between NO_3- and placebo trials' plasma NO_2- concentrations) or hypoxia responses (average arterial oxygen saturation on the placebo trial) may explain why some individuals improved time trial performance after NO_3^- supplementation.

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

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

RESULTS

The NO_3^- and placebo shots effectively altered the independent variable: 2.5 h after NO_3^- consumption plasma [NO_3^-] and [NO_2^-] were significantly greater than after placebo ([NO_3^-] in the NO_3^- trial, 201.6 (25.9) μM vs. placebo trial, 28.9 (6.4) μM, $P < 0.001$; [NO_2^-] in the NO_3^- trial, 473 (226) nM vs. placebo trial, 61 (37) nM, $P < 0.001$). The runners were considered to be sufficiently well blinded as to which supplement they received on each visit, as the manipulation check indicated that only two participants of ten guessed correctly, two guessed incorrectly, and six were unable to distinguish between the supplements at all.

Incremental exercise test

Acute NO_3^- supplementation did not alter any measured physiological variable or RPE during maximal or submaximal exercise at 4000 m (**Table 1**). No statistical difference was present in any parameter obtained at 100% or 45% of $\dot{V}O_2$max. There was also no practical performance difference in time to exhaustion between trials (NO_3^- – placebo: Δ 1.4%). No correlations were observed between baseline plasma [NO_2^-] or the change in plasma [NO_2^-] with any maximal exercise parameter.

PLEASE INSERT TABLE 1 NEAR HERE

Time Trial

Acute NO_3^- supplementation did not improve 10 km running performance at simulated altitude (2500 m). No statistical difference was observed in time to complete the 10 km time trial (NO_3^-, 2862 (233) s vs. placebo, 2874 (265) s, P = 0.6). Additionally, compared to the *a priori* determined minimum practical important difference of -51 s (1.8%) there was also no practical difference in performance (NO_3^- – placebo: Δ -11 [-60 to 38] s or Δ 0.4%: **Figure 2**). Trends in RPE during the time trial were visually explored but no difference was observed between NO_3^- and placebo.

PLEASE INSERT FIGURE 2 NEAR HERE

Results obtained from the probability analysis suggested that three runners experienced a performance improvement with NO_3^- supplementation labelled 'likely, probable'; one runner experienced impaired performance labelled 'likely, probable'; and the remaining six runners exhibited no strong probability of either improved or impaired performance. Further, no correlation was observed between baseline [NO_2^-] or the change in plasma [NO_2^-] with change in time to complete the 10 km time trial ($r < 0.48$, $P > 0.1$).

Exploratory *post hoc* analyses suggested that runners who improved time trial performance responded to hypoxia with greater arterial desaturation, as indicated by lower arterial oxygen saturation during the placebo time trial (82 (2) vs. 84 (2), P = 0.04). There was however no difference in baseline characteristics (age, body mass, $\dot{V}O_{2max}$, haemoglobin, $P > 0.4$) or plasma NO_2^- responses (plasma NO_2^- concentrations on the placebo trial and difference between NO_3- and placebo trials' plasma NO_2- concentrations, $P > 0.6$) between those runners that did or did not improve time trial performance after NO_3^- supplementation.

DISCUSSION

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

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

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

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

Conclusion

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

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

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CONFLICTS OF INTEREST AND SOURCES OF FUNDING

The authors declare they have no conflicts of interests and the study did not receive funding from external sources to Bangor University. The results of the present study do not constitute endorsement by James White Drinks Ltd.

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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

	NO_3^-	**PLAC**	**NO_3^- - PLAC**	***P***
Time to exhaustion (s)	402 ± 80	393 ± 62	9 [-20 to 38]	0.5
45% $\dot{V}O_2$max				
Speed/Gradient ($km \cdot h^{-1}$ / %)	12 (0) / 0 (0)	12 (0) / 0 (0)	-	-
$\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	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% $\dot{V}O_2$max				
Speed/Gradient ($km \cdot h^{-1}$ / %)	16 (0) / 1 (1)	16 (0) / 1 (1)	-	-
$\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	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] ($mmol \cdot L^{-1}$)	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); NO_3^-, 70ml dietary nitrate (beetroot juice) supplementation; PLAC, placebo supplementation; $\dot{V}O_2$max, maximal oxygen uptake at 4000 m; SpO_2, 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.

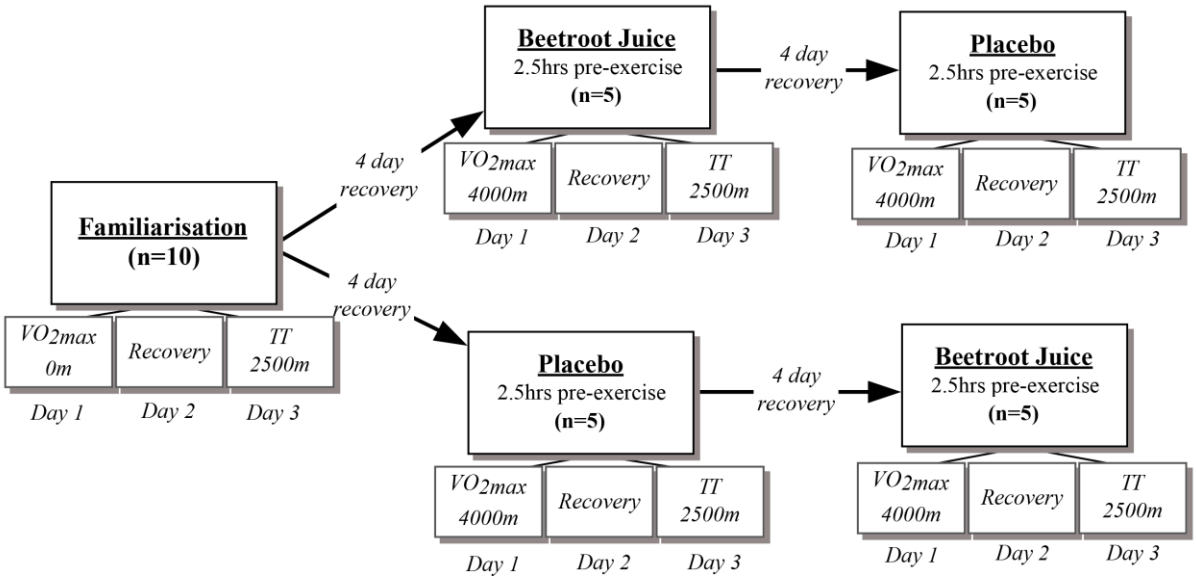

Figure 2: Difference in performance during a simulated altitude (2500 m) 10 km time trial after acute dietary nitrate and placebo supplementation.

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