Shannon OM, Duckworth L, Barlow MJ, Woods D, Lara J, Siervo M, O'Hara JP.

Dietary nitrate supplementation enhances high-intensity running performance in moderate normobaric hypoxia, independent of aerobic fitness.

***Nitric Oxide: Biology and Chemistry* 2016, 59, 63-70.**

Title Dietary nitrate supplementation enhances high-intensity running performance in moderate normobaric hypoxia, independent of aerobic fitness

Authors Oliver Michael Shannon[a], Lauren Duckworth[a], Matthew John Barlow[a], David Woods[ab], Jose Lara[c], Mario Siervo[d], John Paul O'Hara[a]

Affiliations [a] Research Institute for Sport, Physical Activity, and Leisure, Leeds Beckett University, Leeds, LS163QS, United Kingdom. [b] Defence Medical Services, Royal Centre for Defence Medicine, Birmingham, B152TH United Kingdom. [c] Department of Applied Sciences, Northumbria University, Newcastle upon Tyne, NE18ST, United Kingdom.[d] Institute for Aging and Health, University of Newcastle, Newcastle upon Tyne, NE45PL, United Kingdom

Corresponding Author Oliver Michael Shannon (O.Shannon@LeedsBeckett.ac.uk)

Research Institute for Sport, Physical Activity, and Leisure, Leeds Beckett University, Leeds, LS6 3QS, United Kingdom

Abstract

Nitrate-rich beetroot juice (BRJ) increases plasma nitrite concentration, lowers the oxygen cost ($\dot{V}O_2$) of steady-state exercise and improves exercise performance in sedentary and moderately-trained, but rarely in well-trained individuals exercising at sea-level. BRJ supplementation may be more effective in a hypoxic environment, where the reduction of nitrite into nitric oxide (NO) is potentiated, such that well-trained and less well-trained individuals may derive a similar ergogenic effect. We conducted a randomised, counterbalanced, double-blind placebo controlled trial to determine the effects of BRJ on treadmill running performance in moderate normobaric hypoxia (equivalent to 2500 m altitude) in participants with a range of aerobic fitness levels. Twelve healthy males ($\dot{V}O_{2max}$ ranging from 47.1 - 76.8 $ml \cdot kg^{-1} \cdot min^{-1}$) ingested 138 ml concentrated BRJ (~ 15.2 mmol nitrate) or a nitrate-deplete placebo (PLA) (~ 0.2 mmol nitrate). Three hours later, participants completed steady-state moderate intensity running, and a 1500 m time-trial (TT) in a normobaric hypoxic chamber (F_IO_2 ~15 %). Plasma nitrite concentration was significantly greater following BRJ versus PLA 1 hour post supplementation, and remained higher in BRJ throughout the testing session ($p < 0.01$). Average $\dot{V}O_2$ was significantly lower (BRJ: 18.4 ± 2.0, PLA: 20.4 ± 12.6 $ml \cdot kg^{-1} \cdot min^{-1}$; $p = 0.002$), whilst arterial oxygen saturation (S_aO_2) was significantly greater (BRJ: 88.4 ± 2.7, PLA: 86.5 ± 3.3 %; $p < 0.001$) following BRJ. BRJ improved TT performance in all 12 participants by an average of 3.2 % (BRJ: 331.1 ± 45.3 vs. PL: 341.9 ± 46.1 s; $p < 0.001$). There was no apparent relationship between aerobic fitness and the improvement in performance following BRJ ($r^2 = 0.05$, $p > 0.05$). These findings suggests that a high nitrate dose in the form of a BRJ supplement may improve running performance in individuals with a range of aerobic fitness levels conducting moderate and high-intensity exercise in a normobaric hypoxic environment.

Key words: Nitric Oxide, Nitrate, Exercise performance, Hypoxia

1. Introduction

Supplementation with dietary nitrate via nitrate-rich foods or nitrate salts elicits an array of potentially beneficial physiological changes. These include, but are not restricted to: lower blood pressure [1–3], reduced O_2 consumption ($\dot{V}O_2$) during steady-state exercise [4–6], attenuated muscle metabolic perturbations [7,8], and enhanced muscle force production [9,10]. These effects are not directly attributable to the nitrate anion, which is relatively inert [11]. Instead, the benefits of nitrate supplementation appear to be related to an increased production of the multifunctional signalling molecule nitric oxide (NO). Ingested nitrate is reduced to nitrite by symbiotic bacteria residing predominantly on the dorsal surface of the tongue [12,13]. A portion of the nitrite is converted into NO in the acidic environment of the stomach [14,15], but the majority enters systemic circulation where it may be reduced to NO and other bioactive nitrogen oxides in the blood and tissue via various nitrite reductases [11].

Increasing NO bioavailability via nitrate supplementation has been reported to improve exercise time to exhaustion (TTE) or time-trial (TT) performance in the majority [5,16–18], but not all [23,24] previous investigations in untrained and moderately-trained individuals. Conversely, well-trained participants ($\dot{V}O_{2max} > 60\ ml \cdot kg^{-1} \cdot min^{-1}$) are typically less responsive to dietary nitrate supplementation [17,19–21,25–27], even when very high nitrate doses (~ 19.5 mmol) are administered [28]. Highly-trained individuals are known to possess elevated baseline nitrate/ nitrite reserves [29], may habitually consume large amounts of nitrate, subsequent to their increased calorie consumption [30], and exhibit greater presence and activity of the NOS enzymes, relative to the untrained [31]. Such differences may reasonably be expected to lessen the ergogenic effect of nitrate supplementation [30].

71 The reduction of nitrite to NO, and hence the capacity to influence NO mediated physiological
72 processes, is potentiated when muscle pH [32] and PO_2 [33] decline. Conversely, the L-
73 arginine NOS pathway becomes dysfunctional with low O_2 tensions and pH [33]. Such cellular
74 conditions are likely to be especially prevalent during exercise in a hypoxic environment [8,34],
75 such as experienced on ascent to terrestrial altitude. This suggests that nitrate supplementation
76 may be more likely to enhance NO generation and therefore improve performance in hypoxia
77 relative to normoxia. Moreover, an improvement in mitochondrial [35] and/ or muscle
78 contractile efficiency [7] subsequent to nitrate supplementation which reduces $\dot{V}O_2$ is likely to
79 be especially advantageous when O_2 availability is low. This is supported by the findings of a
80 recent study, in which nitrate supplementation improved tolerance to severe intensity cycling
81 in hypoxia (F_IO_2 13.1 %) but not normoxia [36]. However, in other studies, nitrate
82 supplementation has been shown both to improve [8,22,37] and have no effect [38–40] on
83 hypoxic exercise performance. These equivocal findings may be attributed to differences in
84 the training status of participants, exercise protocol, supplementation protocol, and hypoxic
85 stimuli applied, and warrant further exploration.
86
87 Evidence from murine models suggests that the effects of dietary nitrate supplementation may
88 be fibre type specific, increasing muscle perfusion and oxygenation [41,42], and enhancing
89 calcium handling and force production [9] in type II muscle fibres only, as may be recruited
90 during high-intensity exercise (for review, see Jones et al. [43]). Moreover, nitrate
91 supplementation has been demonstrated to elicit beneficial effects when the contribution of
92 type II muscle towards force generation is high, including at fast but not slow muscle
93 contraction frequencies [44], and during the transition from moderate to severe but not
94 unloaded to moderate intensity exercise [45].
95

It is possible that nitrate supplementation is especially effective when conducting high-intensity exercise in a hypoxic environment, such that individuals across a range of aerobic fitness levels may derive a similar performance enhancing benefit from dietary nitrate supplementation. This has considerable relevance, given the widespread popularity of travel to high-altitude (i.e. hypobaric hypoxia) each year for recreational (e.g. hiking, skiing, mountaineering) and sporting (e.g. training camps, high-altitude running events, and cycle mountain stages) purposes in individuals with a range of different fitness levels. Therefore, the purpose of this study was to assess the effect of nitrate supplementation on physiological functioning and TT performance in moderate normobaric hypoxia (equivalent to 2500 m) in individuals with varying aerobic fitness levels (VO_{2max}). We hypothesised that: (1) dietary nitrate supplementation would enhance exercise performance in hypoxia and (2) this effect would be consistent across a range of aerobic fitness levels.

2. Methods

2.1 Participants

Twelve healthy men aged 24.4 ± 4.3 years, with a body mass of 76.4 ± 9.6 kg, stature of 181.6 ± 6.4 cm, and maximal O_2 uptake ($\dot{V}O_{2max}$) at sea-level (normoxia) of 62.1 ± 9.3 $ml \cdot kg^{-1} \cdot min^{-1}$ (range: 47.1 to 76.8 $ml \cdot kg^{-1} \cdot min^{-1}$) and simulated altitude (2500 m) of 52.2 ± 7.5 $ml \cdot kg^{-1} \cdot min^{-1}$ (range: 40.8 to 61.8 $ml \cdot kg^{-1} \cdot min^{-1}$) volunteered to take part in this study. Participants were recruited across a range of aerobic fitness levels to assess the relationship between $\dot{V}O_{2max}$ and the change in hypoxic exercise performance consequent to nitrate supplementation. The participant cohort comprised six competitive runners / triathletes (all with experience racing over 1500 m), four individuals who regularly took part in recreational sport but were not competitive athletes, and two individuals who were physically active, yet did not take part in any sport. The study received institutional ethical approval and was conducted in accordance

with the Declaration of Helsinki. Participants provided written informed consent prior to testing.

2.2 Overview

Participants visited the laboratory on five occasions within a six week period. On the first visit to the laboratory, participants completed an incremental running test to volitional exhaustion in normoxia to determine $\dot{V}O_{2max}$. The values obtained were used to define training status. Each subsequent session involved exercise in a normobaric hypoxic chamber (TISS, Alton, UK, and Sporting Edge, Sherfield on Loddon, UK) situated at ~ 113 m above sea-level at a simulated altitude of 2500 m (F_IO_2 ~ 15.0 %). F_IO_2 was adjusted daily based around fluctuations in barometric pressure, and accounting for 47 mmHg water vapour pressure [46]. Ambient air temperature and relative humidity were maintained at 20 °C and 50 % respectively. On the second visit to the laboratory, participants completed an incremental running test in hypoxia, to define altitude specific $\dot{V}O_{2max}$, and elucidate suitable relative exercise intensities for the experimental trials. The third visit involved a familiarisation session, replicating the experimental trials but without any intervention. The fourth and fifth visits constituted the experimental trials. Experimental trials were preceded by the consumption of 138 ml concentrated nitrate-rich (BRJ) (~ 15.2 mmol nitrate) or nitrate-deplete (PLA) (~ 0.2 mmol nitrate) beetroot juice (BEET IT, James White Drinks Ltd., Ipswich, UK), administered in a randomised double-blind cross-over design three hours before exercise. Participants were asked to abstain from intense exercise, alcohol, and caffeine for 24 hours prior to each trial. Antibacterial mouthwash and chewing gum, known to ablate the oral bacteria responsible for nitrate reduction into nitrite, was also avoided [47].

146 **2.3. Preliminary trials**

147 A two-part incremental running test was conducted on a motorised treadmill (Woodway,
148 Cranlea, Birmingham, UK) [48], once in normoxia and once in normobaric hypoxia.
149 Participants completed five to eight sub-maximal stages lasting three minutes, separated with
150 one minute recovery periods. Starting speed was determined based around perceived
151 participant fitness. Running speed was increased by 1 $km \cdot h^{-1}$ each stage, and a 1 % treadmill
152 gradient was applied to approximate the energetic cost of outdoor running [49]. Finger-tip
153 blood samples were obtained between stages to determine blood lactate concentration (YSI
154 2300 STAT plus, Yellow Springs, Ohio). Exercise was ceased when blood lactate
155 concentration reached 4 mM. The second phase of the test commenced following 5 minutes
156 recovery. A fixed running speed equal to the final speed obtained during the first part of the
157 test, minus 2 $km \cdot h^{-1}$ was applied. The gradient was increased by 1 % every minute, until
158 volitional exhaustion. Respiratory variables were monitored continuously via an online gas
159 analysis system, calibrated before each trial according to the manufacturer's instructions
160 (MedGraphics Ultima CPX, MGC Diagnostics, MN, USA).

161

162 **2.4. Experimental trials**

163 **2.4.1. Protocol**

164 On the morning of each experimental session, participants arrived at the laboratory between 7
165 and 9 am following an overnight fast. A cannula was fitted by a trained phlebotomist into a
166 vein in the arm to enable repeated blood sampling. Participants then ingested BRJ or PLA
167 within a 5 minute period and consumed a standardised breakfast (360 kcal, carbohydrates 62
168 %, fat 22 %, protein 16 %) within a 10 minute period. They then rested in normoxia for 2.5
169 hours, during which time water was permitted *ad libitum*. Participants then entered the hypoxic
170 chamber, where they rested for a further 30 minutes. Exercise commenced 3 hours post-

supplementation, and included 2 x 15 minute bouts of steady-state running at 45 % and 65 % altitude-specific $\dot{V}O_{2max}$, each followed by a 5 minute recover period. A 1500 m TT then commenced. Participants ran at a speed approximating 80 % altitude-specific $\dot{V}O_{2max}$ for 30 seconds as a 'rolling start', before the TT commenced. Participants were asked to run the 1500 m TT as fast as possible. Running speed and time were not visible during the TT, although participants were informed of the distance they had covered at 200 m intervals. The treadmill gradient was set to 1 % throughout exercise [49]. We have previously demonstrated excellent reliability of this performance test (CV < 1 %)[50]. Participants rested in normobaric hypoxia for 10 minutes following TT, after which they were free to leave.

2.4.2. Measurements

Ten blood samples were drawn throughout each experimental trial, comprising two, 4 ml lithium heparin containing vacutainers (Becton Dickinson, Plymouth, UK) for later determination of plasma nitrate and nitrite. Measurement points included pre-supplementation, 30, 60, 90, 120, 150 (pre-hypoxic exposure), and 180 (pre-exercise) minutes post-supplementation, following each bout of steady-state exercise, and immediately post-TT. Blood pressure (BP) of the brachial artery was measured using an automated sphygmomanometer (Omron Healthcare Ltd., Kyoto, Japan) pre-supplementation, pre-hypoxic exposure, pre-exercise, and 5 minutes post-TT. Mean arterial pressure (MAP) was calculated as 1/3·systolic pressure + 2/3·diastolic pressure [51]. At the same time points, a measure of exhaled NO was also recorded, using a hand-held electrochemical analyser (NObreath, Bedfont Scientific Ltd., UK). Four measures were obtained for BP and exhaled NO, and the mean value of the final three measurements was used for data analysis. Heart rate (HR) was measured via a chest worn heart-rate monitor strap (Polar Electro, Oy, Finland) pre-supplementation, pre-hypoxic exposure, pre-exercise, during the final 2 minutes of each steady-state exercise bout,

and immediately post-TT. Arterial O_2 saturation (S_aO_2) was monitored via pulse oximetery (Nellcor, Medtronic, Minneapolis, MN) pre-hypoxic exposure, pre-exercise, during the final 2 minutes of each steady-state exercise bout, and immediately post-TT. Expired gas was monitored as previously described for 10 minutes pre-exercise, and during steady-state exercise. Data obtained during the final 5 minutes of rest and each steady-state exercise bout was averaged and used for subsequent analysis. Perceptions of exertion were also monitored during the final 2 minutes of each steady-state exercise bout and immediately post-TT using a 15 point (6 – 20) ratings of perceived exertion (RPE) scale [52].

2.5. Assessment of NO blood metabolites

2.5.1. Blood handling

Blood samples were centrifuged at 5000 rpm for 3 minutes immediately post-collection. Plasma (1 ml) was drawn from each vacutainer into opaque cryotubes (Argos Technologies, IL, USA), each containing 6.5 mM N-ethylmaleimide (NEM) and 0.1 mM Diethylenetriaminepentaacetic acid (DTPA). NEM and DTPA were added to prevent the interchange between NO metabolites [53]. Cryotubes were immediately placed in a freezer at – 80 ˚C, for later analysis of nitrate and nitrite. 1 ml was also extracted from each 70 ml beetroot juice 'shot' prior to administration, and frozen in untreated opaque cryotubes at – 80 ºC for subsequent determination of nitrate concentration.

2.5.2. Ozone-based chemiluminescence

Plasma nitrate and nitrite concentration, and the nitrate content of administered beetroot juice were measured by ozone-based chemiluminescence as per the manufacturer's instructions (Sievers NOA 280i, Analytix, UK). Briefly, nitrite was determined by addition of samples to 0.17M sodium iodide in glacial acetic acid under nitrogen at room temperature. Sodium iodide

in acetic acid has the capacity to convert nitrite to NO, but is unable to reduce any higher oxides of nitrogen such as nitrate and thus is relatively specific for nitrite. To obtain concentration of total plasma nitrogen oxides (NOx), we used the same apparatus with a stronger reducing agent vanadium chloride (0.1M) in hydrochloric acid (1M) at 95 °C. These stronger conditions reduce the sum of all nitrogen oxides, which is predominantly nitrate (μM) but also includes both nitrite (nM) and nitrosothiols (nM). Nitrate concentration was calculated by subtraction of the nitrite from NOx.

2.6. Statistical analysis

Data analysis was conducted using SPSS version 22. An α level of $p < 0.05$ was accepted for significance. Normality was assessed using the Shapiro-Wilk test. Non-normal data was log-transformed (log_{10}) prior to analysis. Physiological data was compared between trials using a two-way (time and condition) ANOVA. To adjust for asphericity, the Greinhouse Geisser correction was applied for $\varepsilon < 0.75$, and the Huynh-Feldt correction was adopted for $\varepsilon > 0.75$. Post-hoc analysis was conducted using t-tests with a Bonferroni adjustment. A paired t-test was used to compare TT performance between conditions. The square of Pearson's correlation coefficient (r^2) was used to explicate the relationship between variables. A statistical spreadsheet was also used to derive a qualitative probabilistic inference for performance data [54]. One of the following verbal descriptors was assigned to describe the likelihood of a practically beneficial effect of BRJ on performance: < 0.5 %, 'almost certainly not'; 0.5 – 5 %, 'very unlikely not'; 5 – 25 %, 'unlikely'; 25 – 75 %, 'possibly'; 75 – 95 %, 'likely'; '95-99.5 %', 'very likely'; > 99.5 %, 'almost certainly'. Data are presented as means ± SD, unless otherwise stated.

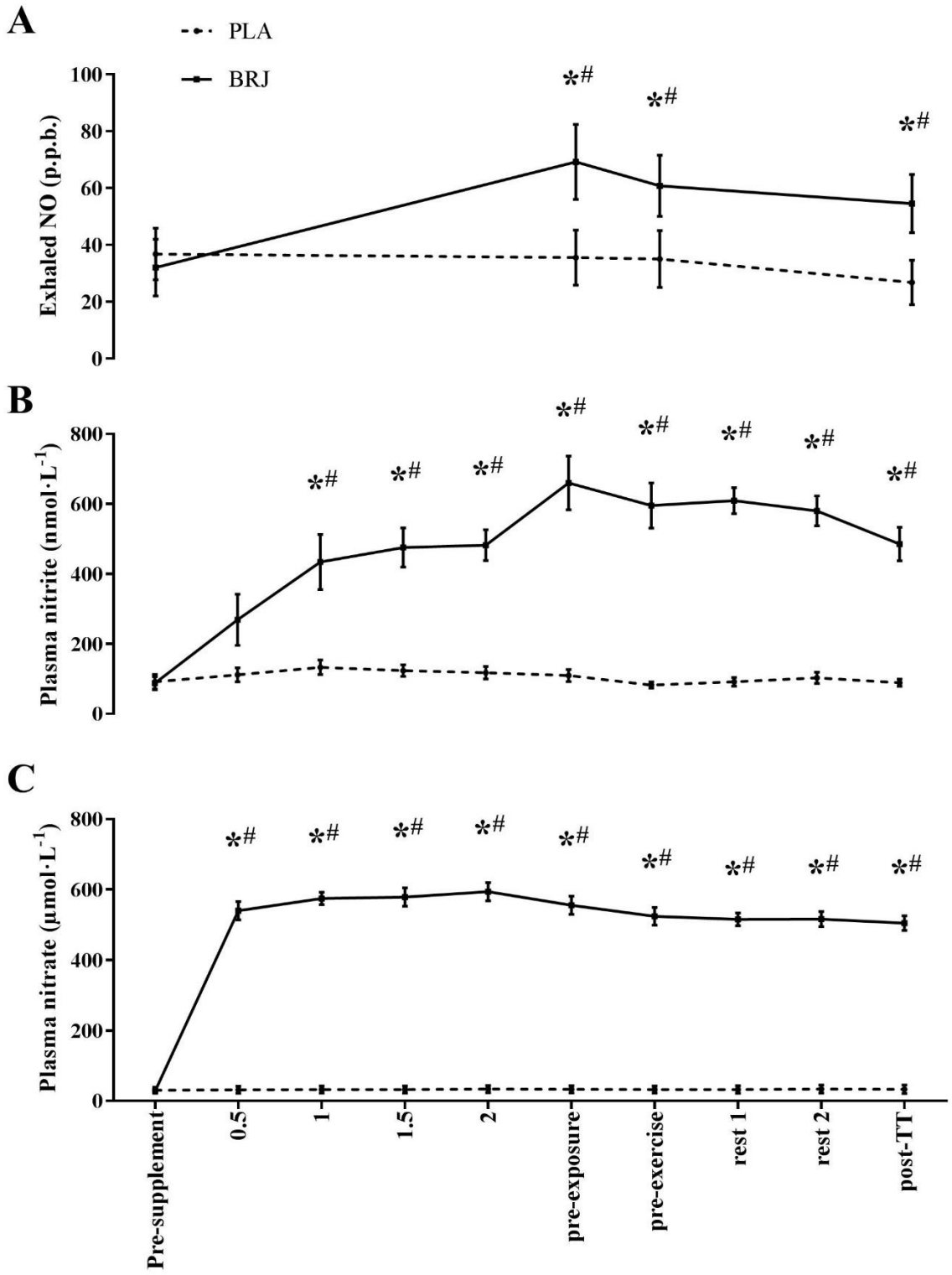
3. Results

3.1. Plasma nitrate and nitrite and exhaled NO

Plasma nitrate, nitrite and exhaled NO data are presented in Figure 1. Pre-supplementation plasma nitrate concentration was no different between BRJ (28.8 ± 14.3 $\mu mol \cdot L^{-1}$) and PLA (30.1 ± 29.4 $\mu mol \cdot L^{-1}$) ($p > 0.05$). There was a marked increase in plasma nitrate concentration (i.e. a condition effect) in BRJ (493.0 ± 174.4 $\mu mol \cdot L^{-1}$) versus PLA (32.5 ± 34.6 $\mu mol \cdot L^{-1}$, $p < 0.01$). Significant effects for time ($p < 0.001$) and time * condition interaction effects ($p < 0.001$) were observed. Post-hoc analysis revealed significantly elevated plasma nitrate concentration in BRJ compared to PLA and pre-supplementation baseline values, 30 minutes post-supplementation, and at all subsequent measurement points ($p < 0.001$). Peak plasma nitrate concentration occurred on average approximately 2 hours post-supplementation (BRJ = 594.1 ± 90.5 vs. PLA = 33.9 ± 35.6 $\mu mol \cdot L^{-1}$, $p < 0.001$). Plasma nitrate concentration was unchanged relative to pre-supplementation in PLA ($p > 0.05$).

Pre-supplementation plasma nitrite concentration did not differ between BRJ (87.5 ± 65.7 $nmol \cdot L^{-1}$) and PLA (91.7 ± 70.8 $nmol \cdot L^{-1}$) ($p > 0.05$). Conversion of nitrate into nitrite was evident, with a significant effect of condition on plasma nitrite concentration (BRJ: 468.0 ± 252.5 vs. 105.4 ± 57.5 $nmol \cdot L^{-1}$, $p < 0.001$). Significant effects for time ($p < 0.001$) and time * condition interaction effects ($p < 0.001$) were observed. Post-hoc analysis revealed that plasma nitrite was significantly greater in BRJ versus PLA and pre-supplementation baseline values, 1 hour post-supplementation ($p < 0.01$), and remained higher in BRJ across all subsequent measurement points ($p < 0.001$). Peak plasma nitrite concentration occurred on average approximately 2.5 hours post-supplementation (BRJ = 660.4 ± 265.0 vs. PLA = 109.7 ± 61.0 $nmol \cdot L^{-1}$, $p < 0.001$). Plasma nitrite concentration was unchanged relative to pre-supplementation values in PLA ($p > 0.05$).

Pre-supplementation concentration of exhaled NO was no different between BRJ (32.1 ± 34.7 p.p.b.) and PLA (36.8 ± 31.5 p.p.b.) ($p > 0.05$). There was a significant condition effect on exhaled NO (BRJ = 54.1 ± 39.8 vs. PLA = 33.6 ± 31.1 p.p.b., $p = 0.002$). Significant effects for time ($p = 0.021$) and time * condition interaction effects ($p = 0.002$) were observed. Post-hoc analysis revealed exhaled NO was significantly elevated in BRJ compared with PLA and pre-supplementation values at all post-supplementation measurement points ($p < 0.05$), but were unchanged in PLA ($p > 0.05$).



296

297 **Figure 1** Exhaled NO (A), Plasma nitrite (B) and plasma nitrate (C) throughout experimental trials in the placebo (PLA) (dashed lines) and
298 nitrate-rich beetroot juice (BRJ) (solid lines) conditions. Values are mean ± SEM. * Time points significantly different between BRJ and
299 PLA conditions ($p < 0.05$). # Time points significantly different from baseline in the BRJ condition.

300

3.2. Perceived exertion and cardio-respiratory variables

Data for RPE, HR, MAP, $\dot{V}O_2$, and S_aO_2 is presented in Table 1. There was a significant condition effect for RPE (BRJ: 13 ± 4 vs. PLA: 14 ± 4, $p = 0.037$), and a clear effect of time on RPE ($p < 0.001$), although no time * condition interaction effects were observed ($p = 0.152$). There was a significant time effect on HR ($p < 0.001$), but no condition ($p = 0.495$) or time * condition interaction effects ($p = 0.383$) were observed. MAP showed a significant effect of condition (BRJ: 83 ± 7 vs. PLA: 86 ± 7 mmHg, $p = 0.006$), but no time ($p = 0.187$) or time * condition interaction effects ($p = 0.646$). There was a significant effect of condition on $\dot{V}O_2$ (BRJ: 18.4 ± 12.0 vs. 20.4 ± 12.6 $ml \cdot kg^{-1} \cdot min^{-1}$, $p = 0.002$). Likewise, there were significant effects of time ($p < 0.001$) and time * condition interaction effects ($p = 0.005$) on $\dot{V}O_2$. Post-hoc analysis revealed significantly lower $\dot{V}O_2$ in BRJ during exercise at 45 % ($p = 0.014$) and 65 % ($p = 0.002$) $\dot{V}O_{2max}$. There was a significant effect of condition (BRJ: 88 ± 3 vs. PLA: 87 ± 3 %, $p < 0.001$), time ($p < 0.001$) and time * condition interaction effects ($p = 0.034$) on S_aO_2. Post-hoc analysis revealed significant differences in S_aO_2 during the pre-exercise rest period ($p = 0.012$) and during exercise at 45 % $\dot{V}O_{2max}$ ($p = 0.003$). There was no significant relationship between the reduction in $\dot{V}O_2$ and increase in S_aO_2 consequent to BRJ ($r^2 = 0.13$, $p = 0.242$).

Table 1 Ratings of perceived exertion, heart rate, mean arterial blood pressure, oxygen consumption, and arterial oxygen saturation throughout experimental trials in the placebo (PLA) and nitrate-rich beetroot juice (BRJ) conditions

Variable and condition	Arrival	Pre-exposure	Pre-exercise	45 % $\dot{V}O_{2max}$	65 % $\dot{V}O_{2max}$	TT
RPE						
PLA				10 ± 1	12 ± 1	19 ± 1
BRJ[a]				9 ± 1	12 ± 1	19 ± 1
HR ($b \cdot min^{-1}$)						
PLA	61 ± 8	60 ± 9	58 ± 9	107 ± 16	146 ± 13	183 ± 9
BRJ	58 ± 9	60 ± 8	59 ± 7	105 ± 18	144 ± 12	183 ± 8
MAP (mmHg)						
PLA	87 ± 8	86 ± 5	85 ± 7			85 ± 10
BRJ[a]	87 ± 10	81 ± 10	83 ± 7			82 ± 5
VO_2 ($ml \cdot kg^{-1} \cdot min^{-1}$)						
PLA			4.8 ± 1.0	21.4 ± 6.0	33.7 ± 4.3	
BRJ[a]			4.5 ± 0.7	19.2 ± 5.9[b]	31.4 ± 3.9[b]	
S_aO_2 (%)						
PLA		98 ± 1	91 ± 3	85 ± 2	81 ± 3	78 ± 7
BRJ[a]		97 ± 2	94 ± 2[b]	88 ± 2 [b]	83 ± 2	80 ± 6

[a] denotes significant difference overall (condition effect) versus PLA
[b] denotes significant difference (time * condition interaction effect) versus PLA

3.5. TT performance

BRJ improved 1500 m TT performance by 3.2 % (BRJ = 331.1 ± 45.3 s) versus PLA (341.9 ± 46.1 s, $p < 0.001$). Magnitude based inferences [54] indicated that the true value of the effect would 'almost certainly' be practically beneficial to an athlete. All 12 participants completed the TT quicker following BRJ ingestion (Figure 2.), with the range of improvement between 1.1 and 20.2 s. There was no significant correlation between $\dot{V}O_{2max}$ measured at sea-level ($r^2 = 0.05$; $p = 0.48$) nor simulated-altitude ($r^2 = 0.05$, $p = 0.46$) and the change in performance following BRJ supplementation. The observed r^2 value indicates that 5 % of the 1500 m TT variability can be explained in terms of differences in $\dot{V}O_{2max}$. There was no significant

correlation between the change in VO_2 ($r^2 = 0.04$, $p = 0.84$) nor S_aO_2 ($r^2 = 0.001$, $p = 0.90$) and the change in performance following BRJ.

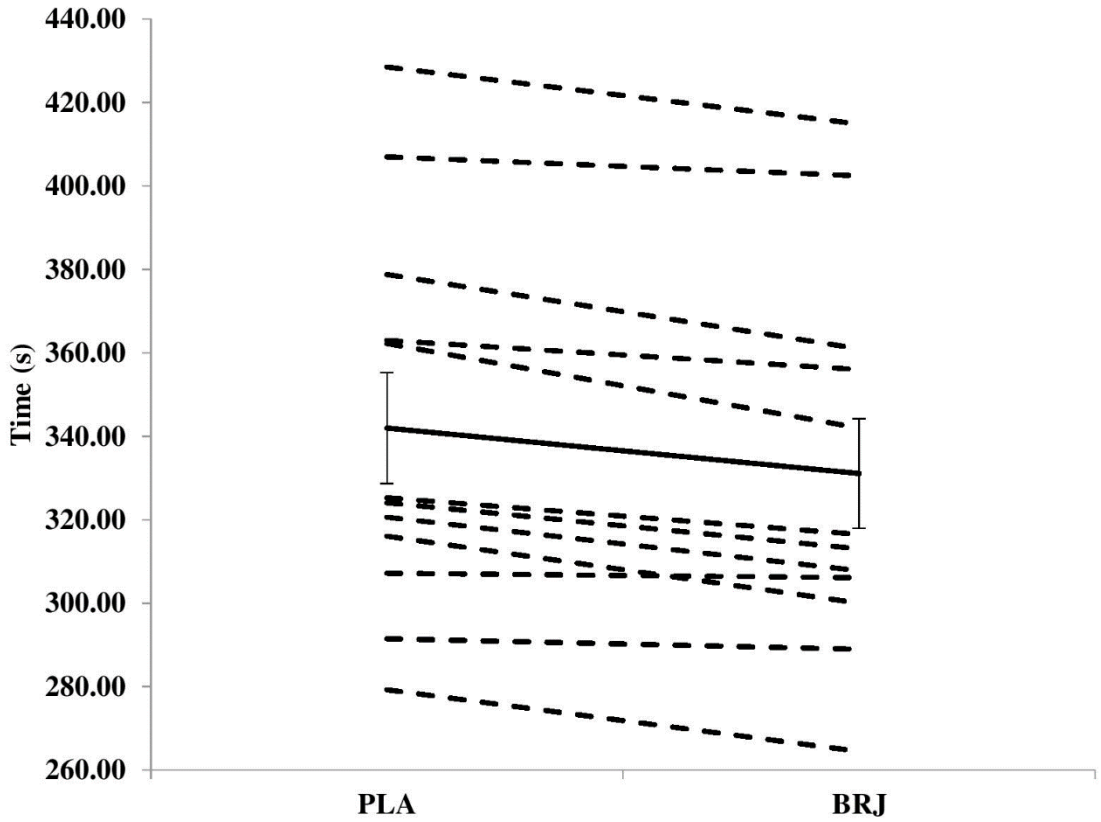


Figure 2 1500 m TT performance following ingestion of placebo (PLA) or nitrate-rich beetroot juice (BRJ).

4. Discussion

Nitrate-rich beetroot juice has emerged as a popular ergogenic aid, although previous research suggests that this supplement has limited effects in well-trained individuals ($\dot{V}O_{2max}$ > 60 $ml \cdot kg^{-1} \cdot min^{-1}$). In contrast, the results of this study suggest that nitrate-rich beetroot juice may reduce the O_2 cost of exercise, elevate S_aO_2, and enhance 1500 m TT performance in individuals across a range of different fitness levels exercising in moderate-normobaric hypoxia. These data have relevance for the thousands of recreational and competitive athletes ascending to altitude each year for sporting purposes.

357 **4.1. Plasma nitrate, nitrite, and exhaled NO**

358 Consistent with previous investigations, plasma nitrate and nitrite, and exhaled NO
359 concentrations were significantly elevated following dietary nitrate supplementation
360 [1,2,5,8,55,22,39,38], signifying an increase in the 'substrates' available for NO generation via
361 the nitrate-nitrite-NO pathway [11]. Peak plasma nitrite concentration was 602 % higher in
362 BRJ versus PLA, similar to Arnold et al. [38] (775 %) (~ 7 mmol nitrate), but considerably
363 greater than reported by Muggeridge et al. [22] (134 %) (~ 5 mmol nitrate) and Vanhatalo et
364 al. [8] (150 %) (~ 9.3 mmol). Importantly, all participants increased plasma nitrite
365 concentration by substantially greater than 30 % - a cut off proposed by Wilkerson [20] for
366 identifying 'non-responders'. This may, in part, be a consequence of the high nitrate dose
367 administered, and may help explain the consistent ergogenic effect observed in this study.
368 Nevertheless, there was substantial inter-individual variability in baseline plasma nitrite
369 concentration (range: 40 – 300 $nmol \cdot L^{-1}$), the magnitude of the increase in plasma nitrite
370 concentration post-supplementation (Δ range: 160 – 1335 $nmol \cdot L^{-1}$), and the time at which
371 peak plasma nitrite concentration occurred (1 – 3 hours post-supplementation). A variable
372 response to nitrate supplementation has been well-reported in the literature [56]. Inter-
373 individual differences could not be explained by participant aerobic fitness in this study.
374 Genetic factors, chronobiology, variations in the quantity and/or taxa of oral nitrate reducing
375 bacteria, and study protocol variables may be important in explaining this phenomenon, and
376 require further investigation [56].

377

378 **4.2. Effect of nitrate supplementation on cardiorespiratory variables and RPE**

379 In the present study, $\dot{V}O_2$ did not differ significantly between BRJ and PLA during rest in
380 hypoxia, but was significantly lower in BRJ versus PLA during steady-state exercise. These
381 findings are in line with most previous investigations [4,5,22,37], and similar to the results of

382 a recent meta-analysis [6]. The physiological mechanisms underlying the decreased $\dot{V}O_2$
383 subsequent to nitrate supplementation have not been fully elucidated, but may be accounted for
384 by: a) an improvement in the efficiency of mitochondrial respiration [35] and/or b) enhanced
385 efficiency of muscle force production [7].
386
387 Larsen and co-workers [35] reported an improvement in the phosphate/O_2 (P/O) ratio (i.e. the
388 amount of ATP produced per mole O_2 consumed) in mitochondria harvested from the vastus
389 lateralis of healthy men following three days supplementation with sodium nitrate (0.1
390 $mmol \cdot kg^{-1} \cdot d^{-1}$). This effect was attributed to decreased proton leak during oxidative
391 phosphorylation, and was associated with a reduced expression of the ATP/ADP translocase
392 protein (ANT) and a tendency towards downregulation of uncoupling protein 3 (UCP3). The
393 change in the mitochondrial P/O ratio correlated with the reduced *in vivo* $\dot{V}O_2$ measured during
394 moderate-intensity cycle ergometry. In contrast to the findings of Larsen et al. [47] with
395 sodium nitrate, a recent investigation by Whitfield and colleagues [57] reported no change in
396 mitochondrial efficiency despite a reduced $\dot{V}O_2$, following seven days supplementation with
397 nitrate-rich beetroot juice (26 $mmol \cdot d^{-1}$). There is presently no explanation as to why sodium
398 nitrate and nitrate-rich beetroot juice may have different effects on the mitochondria, and direct
399 comparison is therefore warranted to rule out other methodological differences [58].
400 Nevertheless, alterations in mitochondrial efficiency are not necessarily required to explain the
401 reduction in $\dot{V}O_2$ reported in this study following BRJ supplementation.
402
403 Bailey et al. [7] reported reduced intramuscular PCr, ADP, and P_i perturbations and lower $\dot{V}O_2$
404 during leg extension exercise following nitrate-rich beetroot juice supplementation (5.1
405 $mmol \cdot d^{-1}$ x 6 days), reflecting a lower ATP turnover for a given work rate. It was suggested
406 that the decreased ATP turnover occurring after nitrate supplementation might be related to a

407 reduced ATP requirement for actin-myosin cross-bridge cycling and/or calcium handling,
408 given previous reports that NO slows actin-myosin cross-bridge cycling [59,60] and inhibits
409 calcium-ATPase activity [61,62]. Interestingly, a recent murine model investigation reported
410 greater force production, and increased expression of the calcium handling protein
411 calsoquestrin 1 and the dihydropyridine receptor in type II muscle fibres following nitrate
412 supplementation [9]. It was speculated that such effects in humans may allow muscle
413 activation at a lower frequency for an equivalent force production, lowering motor unit
414 recruitment and concomitantly the ATP cost of exercise.
415
416 In the present study, S_aO_2 was significantly elevated overall in BRJ compared to PLA. These
417 findings are similar to those of Masschelein and colleagues [37] who reported a significant
418 increase in S_aO_2 during cycle ergometry exercise in severe hypoxia (F_IO_2 11 %) following 6
419 days nitrate supplementation (~ 5 mmol·d^{-1}). Tissue oxygenation in the vastus lateralis was
420 also elevated following nitrate supplementation in that study. These findings are supportive of
421 the notion that nitrate supplementation may reduce muscle O_2 consumption during exercise in
422 hypoxia, presumably via the aforementioned mitochondrial [35] and/or muscle [7] alterations
423 that also manifest as a reduced $\dot{V}O_2$. Interestingly, Arnold et al. [38] found that runners
424 identified as 'responders' to nitrate supplementation during a 10,000 m TT in moderate hypoxia
425 (F_IO_2 15.4 %) typically experienced a lower S_aO_2 during the placebo TT than 'non-responders'
426 (82 vs 84 %). This suggests that the S_aO_2 response to hypoxia may moderate the performance
427 effects of nitrate supplementation, possibly due to greater nitrite reduction into NO at lower O_2
428 tensions [33]. In this study, there was no apparent relationship between average nor post-TT
429 S_aO_2 values and the percentage improvement in performance (r^2 = 0.05). However, S_aO_2
430 reached lower values in the present investigation (post TT PLA: 77.8 ± 6.9 %) than observed

by Arnold et al. [38], presumably due to the shorter higher-intensity TT, which may be important.

Interestingly, RPE was significantly lower overall in BRJ versus PLA, suggesting reduced overall physiological 'strain' [52], which may in part be reflective of the lower $\dot{V}O_2$ and/or elevated S_aO_2 with BRJ. In normoxia, Murphy and colleagues [63] reported lower RPE during the first mile of a 5 km running TT, although others have reported no effect of nitrate supplementation on RPE during sub-maximal and maximal exercise in hypoxia following nitrate supplementation [37,38].

4.3. Effect of nitrate supplementation on running performance in hypoxia

The main finding of the present study was that BRJ improved 1500 m running performance in moderate normobaric hypoxia by 3.2 % versus PLA. Magnitude based inferences suggested an 'almost certain' chance that the true value of the effect would be practically beneficial. In agreement with our hypothesis, there was no apparent relationship between the change in exercise performance post-BRJ supplementation and $\dot{V}O_{2max}$ measured in normoxia nor hypoxia, suggesting a similar effect of BRJ on TT performance across a range of fitness levels under these experimental conditions.

A number of previous investigations have confirmed the beneficial effect of nitrate supplementation on TTE [8,24,37] and TT performance [22] in hypoxia. Conversely, studies in well-trained individuals exercising in hypoxia have typically found no effect of nitrate supplementation [38–40]. Well-trained individuals exhibit increased presence and activity of the NOS enzymes [31]; possess higher baseline nitrate/nitrite concentration [29]; may habitually consume high levels of nitrate as a consequence of their large daily energy intake

[30]; and experience lower tissue acidosis and hypoxia [20] relative to the untrained. Moreover, a recent investigation in normoxia observed an inverse correlation between aerobic fitness and the response to nitrate supplementation [17]. It is therefore reasonable to assume that adaptations elicited by endurance training may blunt the response to nitrate supplementation. Nevertheless, our results suggest a high training status does not necessarily preclude an ergogenic effect of nitrate supplementation. Instead, it is suggested that individuals across a spectrum of aerobic fitness levels may derive similar performance enhancing benefits of nitrate supplementation under specific experimental conditions, including a hypoxic exercise environment, short-duration high-intensity TT protocol, and high nitrate dose.

Considering generation of NO via the L-arginine NOS pathway is supressed in hypoxia, yet the nitrate-nitrite-NO pathway is potentiated as O_2 tensions fall [11,33], it is likely that nitrate supplementation is more effective in hypoxia than normoxia. Supportive evidence is provided by Kelly et al. [36], who subjected healthy male participants to identical exercise regimes preceded by the same nitrate supplement strategy (8.4 $mmol \cdot d^{-1}$ x 3 days), but with exercise varied between normoxia and hypoxia (F_IO_2 13.1 %). Nitrate supplementation had no effect on severe-intensity cycling TTE in normoxia, but improved TTE by ~ 8.6 % in hypoxia relative to placebo. Further, there is evidence from murine model investigations that nitrate supplementation may enhance tissue blood flow [41,42] and muscle contractile function [9] preferentially in type II muscle fibres. It is therefore possible that nitrate supplementation is more effective during shorter more high-intensity exercise, during which these muscle fibres are more heavily recruited [9,43–45,64], or else in individuals with greater distribution of type II muscle fibres [43]. The time-trial duration employed in this study (< 6 minutes) was considerably less than other investigations (~ 17 - 48 minutes) which have not observed an ergogenic effect of nitrate supplementation in hypoxia [38–40], and may be important. Thus,

481 the combination of a hypoxic exercise environment and high-intensity TT may have maximised
482 the effects of nitrate supplementation.
483
484 Finally, there is some evidence of a dose-response to nitrate supplementation [55,65,66], and
485 it is possible that well-trained athletes may require a high nitrate dose to appreciably alter
486 plasma nitrite concentration relative to untrained individuals [30]. Therefore, the high nitrate
487 dose administered in this study relative to investigations which have not observed an effect of
488 nitrate supplementation (~ 5 – 7 mmol) [38–40] may have also contributed towards the
489 consistent ergogenic effect observed here.
490
491 **4.4. Strengths and limitations**
492 The current study has several strengths. In particular, the broad spectrum of participant aerobic
493 fitness levels allowed us to assess the relationship between $\dot{V}O_{2max}$ and the improvement in
494 hypoxic exercise performance following nitrate supplementation. Nevertheless, none of our
495 participants were elite athletes nor entirely sedentary, and it is possible that individuals outside
496 of the present fitness range may respond differently to nitrate supplementation. Moreover, we
497 only included healthy male participants, and further research is warranted in other populations
498 who may respond differently to nitrate supplementation [67]. A further strength of this
499 investigation was that the consumption of nitrate-rich foods was not restricted during the study
500 period. This approach, first employed by Vanhatalo and colleagues [51] and later adopted in
501 several subsequent investigations [25,68], preserves ecological validity and demonstrates that
502 nitrate supplementation can alter physiological functioning and exercise performance when
503 participants are consuming their normal diet.
504
505

4.5. Conclusion

The present study reported an increase in plasma nitrite concentration, reduction in steady-state $\dot{V}O_2$, elevation in S_aO_2, and enhancement of 1500 m running performance in normobaric hypoxia following supplementation with dietary nitrate. Further, this effect did not appear to be related to the aerobic fitness of participants. This suggests that individuals across a range of different aerobic fitness levels may derive a similar performance enhancing benefits of dietary nitrate when consuming a high nitrate dose, and conducting moderate and high-intensity exercise in a hypoxic environment.

Acknowledgements

The authors would like to thank Ashley Grindrod, George Hinson, and Rachael Bradley for their assistance with data collection.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

551 **References:**

552 [1] F.J. Larsen, B. Ekblom, K. Sahlin, J.O. Lundberg, E. Weitzberg, Effects of dietary nitrate
553 on blood pressure in healthy volunteers, N. Engl. J. Med. 355 (2006) 2792–2793.
554

555 [2] A.J. Webb, N. Patel, S. Loukogeorgakis, M. Okorie, Z. Aboud, S. Misra, R. Rashid, P.
556 Miall, J. Deanfield, N. Benjamin, R. MacAllister, A.J. Hobbs, A. Ahluwalia, Acute blood
557 pressure lowering, vasoprotective and anti-platelet properties of dietary nitrate via
558 bioconversion to nitrite, Hypertension. 51 (2008) 784–790.
559

560 [3] M. Siervo, J. Lara, I. Ogbonmwan, J.C. Mathers, Inorganic nitrate and beetroot juice
561 supplementation reduces blood pressure in adults: a systematic review and meta-analysis,
562 J. Nutr. 143 (2013) 818–826.
563

564 [4] F.J. Larsen, E. Weitzberg, J.O. Lundberg, B. Ekblom, Effects of dietary nitrate on oxygen
565 cost during exercise, Acta Physiol. 191 (2007) 59–66.
566

567 [5] S.J. Bailey, P. Winyard, A. Vanhatalo, J.R. Blackwell, F.J. DiMenna, D.P. Wilkerson, J.
568 Tarr, N. Benjamin, A.M. Jones, Dietary nitrate supplementation reduces the O2 cost of
569 low-intensity exercise and enhances tolerance to high-intensity exercise in humans, J.
570 Appl. Physiol. 107 (2009) 1144–1155.
571

572 [6] M. Pawlak-Chaouch, J. Boissière, F.X. Gamelin, G. Cuvelier, S. Berthoin, J. Aucouturier,
573 Effect of dietary nitrate supplementation on metabolic rate during rest and exercise in
574 human: a systematic review and a meta-analysis, Nitric Oxide. 53 (2016) 65–76.

[7] S.J. Bailey, J. Fulford, A. Vanhatalo, P.G. Winyard, J.R. Blackwell, F.J. DiMenna, D.P. Wilkerson, N. Benjamin, A.M. Jones, Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans, J. Appl. Physiol. 109 (2010) 135–148.

[8] A. Vanhatalo, J. Fulford, S.J. Bailey, J.R. Blackwell, P.G. Winyard, A.M. Jones, Dietary nitrate reduces muscle metabolic perturbation and improves exercise tolerance in hypoxia, J. Physiol. 589 (2011) 5517–5528.

[9] A. Hernandez, T.A. Schiffer, N. Ivarsson, A.J. Cheng, J.D. Bruton, J.O. Lundberg, E. Weitzberg, H. Westerblad, Dietary nitrate increases tetanic [Ca2+] and contractile force in mouse fast-twitch muscle, J. Physiol. 590 (2012) 3575–3583.

[10] G. Haider, J.P. Folland, Nitrate supplementation enhances the contractile properties of human skeletal muscle, Med. Sci. Sports Exerc. 46 (2014) 2234–2243.

[11] J.O. Lundberg, E. Weitzberg, M.T. Gladwin, The nitrate–nitrite–nitric oxide pathway in physiology and therapeutics, Nat. Rev. Drug Discov. 7 (2008) 156–167.

[12] C. Duncan, H. Dougall, P. Johnston, S. Green, R. Brogan, C. Leifert, L. Smith, M. Golden, N. Benjamin, Chemical generation of nitric oxide in the mouth from the enterosalivary circulation of dietary nitrate, Nat. Med. 1 (1995) 546–551.

598 [13] J.J. Doel, N. Benjamin, M.P. Hector, M. Rogers, R.P. Allaker, Evaluation of bacterial
599 nitrate reduction in the human oral cavity, Eur. J. Oral Sci. 113 (2005) 14–19.
600
601 [14] N. Benjamin, F. O'Driscoll, H. Dougall, C. Duncan, L. Smith, M. Golden, H. McKenzie,
602 Stomach NO synthesis, Nature. 368 (1994) 502.
603
604 [15] J.O. Lundberg, E. Weitzberg, J.M. Lundberg, K. Alving, Intragastric nitric oxide
605 production in humans: measurements in expelled air, Gut. 35 (1994) 1543–1546.
606
607 [16] N.M. Cermak, M.J. Gibala, L.J. van Loon, Nitrate supplementation's improvement of 10-
608 km time-trial performance in trained cyclists, Int. J. Sport Nutr. Exerc. Metab. 22 (2012)
609 64–71.
610
611 [17] S. Porcelli, M. Ramaglia, G. Bellistri, G. Pavei, L. Pugliese, M. Montorsi, L. Rasica, M.
612 Marzorati, Aerobic fitness affects the exercise performance responses to nitrate
613 supplementation, Med. Sci. Sports Exerc. 47 (2014) 1643–51.
614
615 [18] M.W. Hoon, N.A. Johnson, P.G. Chapman, L.M. Burke, others, The effect of nitrate
616 supplementation on exercise performance in healthy individuals: a systematic review and
617 meta-analysis, Int. J. Sport Nutr. Exerc. Metab. 23 (2013) 522–532.
618
619 [19] N.M. Cermak, P. Res, R. Stinkens, J.O. Lundberg, M.J. Gibala, J.C. van Loon L, No
620 improvement in endurance performance after a single dose of beetroot juice, Int. J. Sport
621 Nutr. Exerc. Metab. 22 (2012) 470–478.

622 [20] D.P. Wilkerson, G.M. Hayward, S.J. Bailey, A. Vanhatalo, J.R. Blackwell, A.M. Jones,
623 Influence of acute dietary nitrate supplementation on 50 mile time trial performance in
624 well-trained cyclists, Eur. J. Appl. Physiol. 112 (2012) 4127–4134.
625
626 [21] R. Bescós, V. Ferrer-Roca, P.A. Galilea, A. Roig, F. Drobnic, A. Sureda, M. Martorell,
627 A. Cordova, J.A. Tur, A. Pons, Sodium nitrate supplementation does not enhance
628 performance of endurance athletes, Med. Sci. Sports Exerc. 44 (2012) 2400–2409.
629
630 [22] D.J. Muggeridge, C.C.F. Howe, O. Spendiff, C. Pedlar, P.E. James, C. Easton, A single
631 dose of beetroot juice enhances cycling performance in simulated altitude, Med. Sci.
632 Sports Exerc. 46 (2014) 143–150.
633
634 [23] D.J. Muggeridge, C.C. Howe, O. Spendiff, C. Pedlar, P.E. James, C. Easton, The effects
635 of a single dose of concentrated beetroot juice on performance in trained flatwater
636 kayakers, Int. J. Sport Nutr. Exerc. Metab. 23 (2013) 498–506.
637
638 [24] J. Kelly, A. Vanhatalo, S.J. Bailey, L.J. Wylie, C. Tucker, S. List, P.G. Winyard, A.M.
639 Jones, Dietary nitrate supplementation: effects on plasma nitrite and pulmonary O2
640 uptake dynamics during exercise in hypoxia and normoxia, Am. J. Physiol. Regul. Integr.
641 Comp. Physiol. 307 (2014) 920–930.
642
643 [25] O. Peacock, A.E. Tjønna, P. James, U. Wisløff, B. Welde, N. Böhlke, A. Smith, K. Stokes,
644 C. Cook, Ø. Sandbakk, Dietary nitrate does not enhance running performance in elite
645 cross-country skiers, Med. Sci. Sports Exerc. 44 (2012) 2213–2219.

[26] P.M. Christensen, M. Nyberg, J. Bangsbo, Influence of nitrate supplementation on VO_2 kinetics and endurance of elite cyclists, Scand. J. Med. Sci. Sports. 23 (2013) e21-31.

[27] S.C. Lane, J.A. Hawley, B. Desbrow, A.M. Jones, J.R. Blackwell, M.L. Ross, A.J. Zemski, L.M. Burke, Single and combined effects of beetroot juice and caffeine supplementation on cycling time trial performance, Appl. Physiol. Nutr. Metab. 39 (2014) 1050–1057.

[28] R.K. Boorsma, J. Whitfield, L.L. Spriet, Beetroot juice supplementation does not improve performance of elite 1500-m runners, Med. Sci. Sports Exerc. 46 (2014) 2326–2334.

[29] C. Vassalle, V. Lubrano, C. Domenici, A. L'Abbate, Influence of chronic aerobic exercise on microcirculatory flow and nitric oxide in humans, Int. J. Sports Med. 24 (2003) 30–35.

[30] A.M. Jones, Influence of dietary nitrate on the physiological determinants of exercise performance: a critical review, Appl. Physiol. Nutr. Metab. 39 (2014) 1019–1028.

[31] G.K. McConell, S.J. Bradley, T.J. Stephens, B.J. Canny, B.A. Kingwell, R.S. Lee-Young, Skeletal muscle nNOS mu protein content is increased by exercise training in humans, Am. J. Physiol. Regul. Integr. Comp. Physiol. 293 (2007) R821-828.

668 [32] A. Modin, H. Björne, M. Herulf, K. Alving, E. Weitzberg, J.O. Lundberg, Nitrite-derived
669 nitric oxide: a possible mediator of "acidic-metabolic" vasodilation, Acta Physiol. Scand.
670 171 (2001) 9–16.
671
672 [33] P.R. Castello, P.S. David, T. McClure, Z. Crook, R.O. Poyton, Mitochondrial cytochrome
673 oxidase produces nitric oxide under hypoxic conditions: implications for oxygen sensing
674 and hypoxic signalling in eukaryotes, Cell Metab. 3 (2006) 277–287.
675 [34] R.S. Richardson, B. Grassi, T.P. Gavin, L.J. Haseler, K. Tagore, J. Roca, P.D. Wagner,
676 Evidence of O2 supply-dependent VO2 max in the exercise-trained human quadriceps, J.
677 Appl. Physiol. 86 (1999) 1048–1053.
678
679 [35] F.J. Larsen, T.A. Schiffer, S. Borniquel, K. Sahlin, B. Ekblom, J.O. Lundberg, E.
680 Weitzberg, Dietary inorganic nitrate improves mitochondrial efficiency in humans, Cell
681 Metab. 13 (2011) 149–159.
682
683 [36] J. Kelly, A. Vanhatalo, D.P. Wilkerson, L.J. Wylie, A.M. Jones, Effects of nitrate on the
684 power–duration relationship for severe-intensity exercise, Med. Sci. Sports Exerc. 45
685 (2013) 1798–1806.
686
687 [37] E. Masschelein, R. Van Thienen, X. Wang, A. Van Schepdael, M. Thomis, P. Hespel,
688 Dietary nitrate improves muscle but not cerebral oxygenation status during exercise in
689 hypoxia, J. Appl. Physiol. 113 (2012) 736–745.
690

691 [38] J.T. Arnold, S.J. Oliver, T.M. Lewis-Jones, L.J. Wylie, J.H. Macdonald, Beetroot juice
692 does not enhance altitude running performance in well-trained athletes, Appl. Physiol.
693 Nutr. Metab. 40 (2015) 590-595.
694
695 [39] K.E. MacLeod, S.F. Nugent, S.I. Barr, M.S. Koehl, B.C. Sporer, M.J. MacInnis, Acute
696 beetroot juice supplementation does not improve cycling performance in normoxia or
697 moderate hypoxia, Int. J. Sport Nutr. Exerc. Metab. 25 (2015) 359-366.
698
699 [40] N. Bourdillon, J.-L. Fan, B. Uva, H. Müller, P. Meyer, B. Kayser, Effect of oral nitrate
700 supplementation on pulmonary hemodynamics during exercise and time trial performance
701 in normoxia and hypoxia: a randomized controlled trial, Front. Physiol. 6 (2015) 288.
702
703 [41] S.K. Ferguson, D.M. Hirai, S.W. Copp, C.T. Holdsworth, J.D. Allen, A.M. Jones, T.I.
704 Musch, D.C. Poole, Impact of dietary nitrate supplementation via beetroot juice on
705 exercising muscle vascular control in rats, J. Physiol. 591 (2013) 547–557.
706
707 [42] S.K. Ferguson, C.T. Holdsworth, J.L. Wright, A.J. Fees, J.D. Allen, A.M. Jones, T.I.
708 Musch, D.C. Poole, Microvascular oxygen pressures in muscles comprised of different
709 fiber types: Impact of dietary nitrate supplementation, Nitric Oxide. 48 (2015) 38–43.
710
711 [43] A.M. Jones, S.K. Ferguson, S.J. Bailey, A. Vanhatalo, D.C. Poole, Fiber-type specific
712 effects of dietary nitrate, Exerc. Sport Sci. Rev. 44 (2016) 53–60.
713
714

[44] S.J. Bailey, R.L. Varnham, F.J. DiMenna, B.C. Breese, L.J. Wylie, A.M. Jones, Inorganic nitrate supplementation improves muscle oxygenation, O2 uptake kinetics, and exercise tolerance at high but not low pedal rates, J. Appl. Physiol. 118 (2015) 1396–1405.

[45] B.C. Breese, M.A. McNarry, S. Marwood, J.R. Blackwell, S.J. Bailey, A.M. Jones, Beetroot juice supplementation speeds O2 uptake kinetics and improves exercise tolerance during severe-intensity exercise initiated from an elevated metabolic rate, Am. J. Physiol. Regul. Integr. Comp. Physiol. 305 (2013) R1441-1450.

[46] J. Conkin, Ph2o and simulated hypobaric hypoxia, Aviat. Space Environ. Med. 82 (2011) 1157–1158.

[47] M. Govoni, E.A. Jansson, E. Weitzberg, J.O. Lundberg, The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash, Nitric Oxide. 19 (2008) 333–337.

[48] A.M. Jones, Middle- and long-distance running, in: E.M. Winter, A.M. Jones, R. Davison, P.D. Bromley, T.H. Mercer (Eds.), Sport Exerc. Physiol. Test. Guidel. Vol. - Sport Test. Br. Assoc. Sport Exerc. Sci. Guide, Routledge, London, UK, 2007.

[49] A.M. Jones, J.H. Doust, A 1% treadmill grade most accurately reflects the energetic cost of outdoor running, J. Sports Sci. 14 (1996) 321–327.

739 [50] O. Shannon, M. Barlow, L. Duckworth, D.R. Woods, T. Barker, A. Grindrod, A. Griffiths,
740 J.P. O'Hara, The reliability of a pre-loaded treadmill time-trial in moderate normobaric
741 hypoxia, Int. J. Sports Med. (2016) [Epub ahead of print].
742
743 [51] A. Vanhatalo, S.J. Bailey, J.R. Blackwell, F.J. DiMenna, T.G. Pavey, D.P. Wilkerson, N.
744 Benjamin, P.G. Winyard, A.M. Jones, Acute and chronic effects of dietary nitrate
745 supplementation on blood pressure and the physiological responses to moderate-intensity
746 and incremental exercise, AJP Regul. Integr. Comp. Physiol. 299 (2010) R1121–R1131.
747
748 [52] G.A. Borg, Psychophysical bases of perceived exertion, Med. Sci. Sports Exerc. 14 (1982)
749 377–381.
750
751 [53] E. Nagababu, J.M. Rifkind, Measurement of plasma nitrite by chemiluminescence, in:
752 R.M. Uppu, S.N. Murthy, W.A. Pryor, N.L. Parinandi (Eds.), Free Radic. Antioxid.
753 Protoc., Humana Press, Totowa, NJ, 2010: pp. 41–49.
754
755 [54] W.G. Hopkins, A spreadsheet for deriving a confidence interval, mechanistic inference
756 and clinical inference from a P value, Sportscience. 11 (2007) 16–20.
757
758 [55] L.J. Wylie, J. Kelly, S.J. Bailey, J.R. Blackwell, P.F. Skiba, P.G. Winyard, A.E.
759 Jeukendrup, A. Vanhatalo, A.M. Jones, Beetroot juice and exercise: pharmacodynamic
760 and dose-response relationships, J. Appl. Physiol. 115 (2013) 325–336.
761

762 [56] P.E. James, G.R. Willis, J.D. Allen, P.G. Winyard, A.M. Jones, Nitrate pharmacokinetics:
763 Taking note of the difference, Nitric Oxide. 48 (2015) 44–50.
764
765 [57] J. Whitfield, A. Ludzki, G.J.F. Heigenhauser, J.M.G. Senden, L.B. Verdijk, L.J.C. van
766 Loon, L.L. Spriet, G.P. Holloway, Beetroot juice supplementation reduces whole body
767 oxygen consumption but does not improve indices of mitochondrial efficiency in human
768 skeletal muscle, J. Physiol. 594 (2015) 421–435.
769
770 [58] G.J. Kemp, Beetroot juice supplementation reduces the oxygen cost of exercise without
771 improving mitochondrial efficiency: but how?, J. Physiol. 594 (2016) 253–253.
772
773 [59] S. Galler, K. Hilber, A. Göbesberger, Effects of nitric oxide on force-generating proteins
774 of skeletal muscle, Pflüg. Arch. Eur. J. Physiol. 434 (1997) 242–245.
775
776 [60] L.M. Heunks, M.J. Cody, P.C. Geiger, P.N. Dekhuijzen, G.C. Sieck, Nitric oxide impairs
777 Ca2+ activation and slows cross-bridge cycling kinetics in skeletal muscle, J. Appl.
778 Physiol. 91 (2001) 2233–2239.
779
780 [61] T. Ishii, O. Sunami, N. Saitoh, H. Nishio, T. Takeuchi, F. Hata, Inhibition of skeletal
781 muscle sarcoplasmic reticulum Ca2+-ATPase by nitric oxide, FEBS Lett. 440 (1998)
782 218–222.
783

[62] R.I. Viner, T.D. Williams, C. Schöneich, Nitric oxide-dependent modification of the sarcoplasmic reticulum Ca-ATPase: localization of cysteine target sites, Free Radic. Biol. Med. 29 (2000) 489–496.

[63] M. Murphy, K. Eliot, R.M. Heuertz, E. Weiss, Whole beetroot consumption acutely improves running performance, J. Acad. Nutr. Diet. 112 (2012) 548–552.

[64] A.R. Coggan, J.L. Leibowitz, A. Kadkhodayan, D.P. Thomas, S. Ramamurthy, C.A. Spearie, S. Waller, M. Farmer, L.R. Peterson, Effect of acute dietary nitrate intake on maximal knee extensor speed and power in healthy men and women, Nitric Oxide. 48 (2015) 16–21.

[65] L.J. Wylie, J. Ortiz de Zevallos, T. Isidore, L. Nyman, A. Vanhatalo, S.J. Bailey, A.M. Jones, Dose-dependent effects of dietary nitrate on the oxygen cost of moderate-intensity exercise: Acute vs. chronic supplementation, Nitric Oxide. 57 (2016) 30–39.

[66] M.W. Hoon, A.M. Jones, N.A. Johnson, J.R. Blackwell, E.M. Broad, B. Lundy, A.J. Rice, L.M. Burke, The effect of variable doses of inorganic nitrate-rich beetroot juice on simulated 2,000 m rowing performance in trained athletes, Int. J. Sports Physiol. Perform. 9 (2013) 615–620.

[67] A.R. Coggan, D. Mikhalvkova, K. Mahmood, I. Bole, J. Leibowitz, A. Kadkhodayan, S. Park, D. Thomas, D. Thies, L.R. Peterson, Dietary nitrate (NO3−)-induced increases in skeletal muscle contractile function: high vs. low responders, FASEB J. 30 (2016) 1245.

[68] K.E. Lansley, P.G. Winyard, S.J. Bailey, A. Vanhatalo, D.P. Wilkerson, J.R. Blackwell, M. Gilchrist, N. Benjamin, A.M. Jones, Acute dietary nitrate supplementation improves cycling time trial performance, Med. Sci. Sports Exerc. 43 (2011) 1125–1131.