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ORIGINAL ARTICLE

Dietary nitrate supplementation enhances short but not longer duration running time-trial performance

Oliver Michael Shannon[1] · Matthew John Barlow[1] · Lauren Duckworth[1] · Emily Williams[1] · Georgina Wort[1] · David Woods[1,2] · Mario Siervo[3] · John Paul O'Hara[1]

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Abstract
Purpose This study evaluated the effects of dietary nitrate (NO_3^-) supplementation on physiological functioning and exercise performance in trained runners/triathletes conducting short and longer-distance treadmill running time-trials (TT).
Method Eight trained male runners or triathletes completed four exercise performance tests comprising a 10 min warm up followed by either a 1500 or 10,000 m treadmill TT. Exercise performance tests were preceded 3 h before the exercise by supplementation with either 140 ml concentrated nitrate-rich (~12.5 mmol nitrate) (BRJ) or nitrate-deplete (~0.01 mmol nitrate) (PLA) beetroot juice.
Results BRJ supplementation significantly elevated plasma [NO_2^-] ($P<0.05$). Resting blood pressure and exercise $\dot{V}O_2$ were not significantly different between BRJ and PLA ($P>0.05$). However, post-exercise blood [lactate] was significantly greater in BRJ following the 1500 m TT (6.6 ± 1.2 vs. 6.1 ± 1.5 mM; $P<0.05$), but not significantly different between conditions in the 10,000 m TT ($P>0.05$). Performance in the 1500 m TT was significantly faster in BRJ vs. PLA (319.6 ± 36.2 vs. 325.7 ± 38.8 s; $P<0.05$). Conversely, there was no significant difference in 10,000 m TT performance between conditions ($2643.1\pm324.$ 1 vs. 2649.9 ± 319.8 s, $P>0.05$).
Conclusion Acute BRJ supplementation significantly enhanced 1500 m, but not 10,000 m TT performance. These findings suggest that BRJ might be ergogenic during shorter distance TTs which allow for a high work rate, but not during longer distance TTs, completed at a lower work rate.

Keywords Nitric oxide · Beetroot juice · Dietary supplementation · Exercise performance

Abbreviations

ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BP	Blood pressure
BRJ	Nitrate-rich beetroot juice
Ca^{2+}	Calcium
DTPA	Diethylenetriaminepentaacetic acid
HR	Heart rate
MAP	Mean arterial pressure
NEM	*N*-Ethylmaleimide
NO	Nitric oxide
NO_2^-	Nitrite
NO_3^-	Nitrate
NOS	Nitric oxide synthase
O_2	Oxygen
PCr	Phosphocreatine
PO_2	Partial pressure of oxygen
PLA	Nitrate-deplete beetroot juice
RER	Respiratory exchange ratio
RPE	Ratings of perceived exertion
SWC	Smallest worthwhile change
TT	Time-trial
TTE	Time to exhaustion

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✉ Oliver Michael Shannon
O.Shannon@LeedsBeckett.ac.uk

[1] Research Institute for Sport, Physical Activity, and Leisure, Leeds Beckett University, Leeds LS163QS, UK

[2] Defence Medical Services, Royal Centre for Defence Medicine, Birmingham B152TH, UK

[3] Institute of Cellular Medicine, University of Newcastle, Newcastle upon Tyne NE45PL, UK

$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}O_2$	Oxygen uptake
$\dot{V}O_{2max}$	Maximal oxygen uptake

Introduction

Nitric oxide (NO) is a reactive gaseous signalling molecule that regulates a broad range of physiological processes, including muscle contraction (Reid 1998), mitochondrial respiration (Clerc et al. 2007), vasodilation (Kelm and Schrader 1990), and immune function (Bogdan 2001). NO can be generated by the NO synthase (NOS) family of enzymes from the precursors L-arginine and oxygen (O_2), in a complex reaction that requires the presence of multiple cofactors (Moncada and Higgs 1993). A complementary pathway for NO production has also been elucidated, and entails the reduction of nitrate (NO_3^-) into nitrite (NO_2^-) by oral bacteria (Duncan et al. 1995), and subsequent reduction of NO_2^- into NO throughout the body via multifarious enzymatic and non-enzymatic catalysts (Lundberg and Govoni 2004).

A strong body of evidence indicates that NO_3^- supplementation can improve time to exhaustion (TTE) and/or time-trial (TT) performance during short duration (<30 min) exercise tests, which mandate a high relative (to the peak) work rate (Bailey et al. 2009, 2010; Vanhatalo et al. 2010; Lansley et al. 2011; Cermak et al. 2012a; Porcelli et al. 2014; McMahon et al. 2016), particularly in untrained and moderately-trained individuals ($\dot{V}O_{2max}$ < ~60 ml kg^{-1} min^{-1}). Notably, Lansley et al. (2011) reported a significant and comparable improvement in 4 km (TT duration: ~6 min; performance improvement: 2.8%) and 16.1 km (TT duration: ~ 28 min; performance improvement: 2.7%) cycle ergometry TT performance in moderately trained males ($\dot{V}O_{2max}$ ~56 ml kg^{-1} min^{-1}) consequent to NO_3^- ingestion. Conversely, NO_3^- supplementation has been reported to have a minimal effect on performance during longer duration (>30 min) exercise tests, which mandate a lower work rate (Bescós et al. 2012; Wilkerson et al. 2012; Cermak et al. 2012b; Lane et al. 2014; Glaister et al. 2015).

The precise reasons for this disparity are unclear, but might be related to differences in muscle recruitment patterns between short and longer duration exercise tests. Recent data from murine models suggest that NO_3^- supplementation elevates blood flow and oxygenation (Ferguson et al. 2013, 2015), and increases calcium (Ca^{2+}) handling protein expression and force production (Hernandez et al. 2012) in type II muscle only (for review, see Jones et al. 2016). Therefore, the beneficial effects of NO_3^- supplementation might be restricted to, or most pronounced during, exercise where type II fibers are predominantly recruited (Breese et al. 2013; Bailey et al. 2015; Coggan et al. 2015), such as during short duration exercise where a higher relative work rate can be adopted (Essén 1978; Sale 1987). Additionally, hypoxia and acidosis—cellular conditions particularly prevalent at higher work rates (Richardson et al. 1995; Robergs et al. 2004)—are known to enhance the reduction of NO_2^- into NO (Modin et al. 2001; Cosby et al. 2003; Castello et al. 2006). Consequently, NO_3^- supplementation may be more effective at elevating NO_2^- derived NO, and thus enhancing NO signalling, during short duration exercise tests where a high work rate is adopted, compared with longer duration exercise tests conducted at a lower work rate.

Previous investigations utilising longer duration (>30 min) exercise tests have employed trained subjects (Males: $\dot{V}O_{2max}$ >60 ml kg^{-1} min^{-1}, Females: $\dot{V}O_{2max}$ >50 ml kg^{-1} min^{-1}), who manifest a diminished response to NO_3^- supplementation (Porcelli et al. 2014). This may be explained by the elevated baseline plasma [NO_3^-] and [NO_2^-] in this cohort (Vassalle et al. 2003), although multiple other factors may also be important (Jones 2014). It is therefore unclear whether the reduced effects of NO_3^- supplementation reported during longer vs. shorter duration exercise tests are related to the exercise duration (and requisite work rate) or the high training status of subjects in these investigations (Bescós et al. 2012; Wilkerson et al. 2012; Cermak et al. 2012b; Lane et al. 2014; Glaister et al. 2015). Direct comparison between the effects of NO_3^- supplementation on short and longer duration (< or >30 min) exercise performance where other methodological variables are fixed is necessary, yet presently scarce.

Therefore, the purpose of this investigation was to evaluate the effects of NO_3^- supplementation on short duration high work rate (1500 m; ~5 min) versus longer duration lower work rate (10,000 m; ~45 min) treadmill running TT performance in a group of trained men following an identical NO_3^- supplementation regimen. We hypothesized that NO_3^- supplementation would enhance short, but not longer duration exercise performance.

Methods

Subjects

Eight trained male runners or triathletes (age: 28.3 ± 5.8 years, body mass: 74.7 ± 10.1 kg, height 179.1 ± 2.4 cm, $\dot{V}O_{2max}$: 62.3 ± 8.1 ml kg^{-1} min^{-1}) volunteered to take part in this study. The subjects all had experience of competing in running events. The procedures and any associated risks and benefits of the study were explained to the subjects, after which they gave fully informed written consent.

The study received institutional ethical clearance, and was conducted in accordance with the Declaration of Helsinki.

Experimental design

Each subject visited the laboratory on six separate occasions. On the first visit, subjects completed an incremental exercise test to exhaustion to determine their maximal rate of oxygen uptake ($\dot{V}O_{2max}$) and elucidate suitable sub-maximal warm up exercise intensities for the experimental trials. Following a rest period of approximately 20 min, subjects then completed familiarization of the 1500 m TT. On the second visit to the laboratory, subjects completed familiarization of the 10,000 m TT. The third to sixth visits included each of the following performance trials: (1) 1500 m TT preceded by nitrate-rich beetroot juice (BRJ) supplementation; (2) 1500 m TT preceded by nitrate-deplete beetroot juice (PLA) supplementation; (3) 10,000 m TT preceded by BRJ supplementation; (4) 10,000 m TT preceded by PLA supplementation. Performance trials were conducted in a randomized order, with 140 ml concentrated BRJ (~12.5 mmol nitrate) or PLA (~0.01 mmol nitrate) (Beet It, James White Ltd., Ipswich, UK) administered double blind, 3 h before the start of exercise. The exact NO_3^- content of the beetroot juice was determined via ozone-based chemiluminescence, as previously described (Shannon et al. 2016b). Subjects were asked to complete a food and exercise diary for 24 h prior to the first experimental trial, and used this to replicate their diet and activity as closely as possible for subsequent visits. During this time period, subjects were asked not to perform strenuous exercise, or consume caffeine and alcohol. Subjects were also asked not to consume anything except for water in the 3 h before testing. Antibacterial mouthwash and chewing gum were also avoided throughout the testing period, as these are known to destroy the oral bacteria responsible for nitrate reduction into nitrite (Govoni et al. 2008). Each testing sessions was conducted at the same time of the day to minimise the influence of circadian variance.

Maximal exercise testing

Subjects completed a two-part incremental running test on a motorized treadmill (Woodway, Cranlea, Birmingham, UK) (Jones 2007). The first part of the test comprised five to eight, 3-min sub-maximal stages, separated with 1 min recovery periods. Running speed was increased by 1 km h^{-1} each stage. A finger-tip blood sample was also obtained between stages to determine blood [lactate] (YSI 2300 STAT plus, Yellow Springs, Ohio, USA). At the end of each stage, heart rate (HR) was monitored via a chest worn strap (Polar Electro, Oy, Finland), and ratings of perceived exertion (RPE) was assessed using a 15-point (6–20) scale. A 1% treadmill gradient was applied to replicate the energetic cost of running outdoors (Jones and Doust 1996). Exercise was stopped when blood [lactate] exceeded 4 mM. Subjects then rested for approximately 5 min, after which the second phase of the test commenced. A fixed running speed was applied, equal to the final speed achieved during the first part of the test, minus 2 km h^{-1}. Treadmill gradient was increased by 1% every minute, until volitional exhaustion. An online gas analysis system (MedGraphics Ultima CPX, MGC Diagnostics, MN, USA), calibrated before each trial according to the manufacturer's instructions, was used to monitor expired gas throughout exercise. Expired gas data was used to define $\dot{V}O_{2max}$ (greatest 30 s mean value) and the relevant sub-maximal running speeds for the experimental trials. All subjects were deemed to have attained $\dot{V}O_{2max}$, as they achieved ≥2 of the following criteria: a plateau in $\dot{V}O_2$ in the final exercise stage (Taylor et al. 1955), RER ≥1.15 (Issekutz et al. 1962), HR within 10 b min^{-1} of age-predicted maximum (220—age), RPE ≥19, blood [lactate] ≥8 mM (Midgley et al. 2007).

Performance trials

Approximately 1 week after the 10,000 m familiarization trial, subjects completed the first of four performance trials. All performance trials were conducted over a 3–5-week period, with a minimum of 5 days and maximum of 10 days between visits. These consisted of 10 min of continuous treadmill running as a warm up period, split as 5 min bouts at 45 and 65% $\dot{V}O_{2max}$, respectively. After a 5-min passive rest period, subjects then completed either a 1500 or a 10,000 m TT. Each TT commenced with a 30 s rolling start at a running speed approximating 80% $\dot{V}O_{2max}$, after which subjects could freely adjust their speed. Running speed and time were not visible to subjects during the TT. Subjects were verbally informed of the distance they had covered at 200 m (1500 m TT) or 500 m (10,000 m TT) intervals. The treadmill gradient was set to 1% throughout the exercise (Jones and Doust 1996).

Measurements

Prior to the start of each performance trial, subjects were required to sit for a 10-min period, after which blood pressure (BP) of the brachial artery was measured using an automated sphygmomanometer (Omron Healthcare Ltd., Kyoto, Japan). Four measures were obtained, and the mean value of the final three measurements was used for data analysis. An 8 ml venous blood sample was then collected via venepuncture from a vein in the arm into lithium heparin containing tubes. Blood was immediately centrifuged at 5000 rpm for 3 min. Plasma was extracted into opaque cryotubes (Argos Technologies, IL, USA),

which were pre-treated with 6.5 mM *N*-ethylmaleimide (NEM) and 0.1 mM Diethylenetriaminepentaacetic acid (DTPA) to prevent the interchange between NO metabolites (Nagababu and Rifkind 2010). Cryotubes were immediately placed in a freezer at −80 °C, for later analysis of plasma [NO_2^-] via ozone-based chemiluminescence, as previously described (Shannon et al. 2016b). A blood sample was also collected from the fingertip, to determine blood [lactate] (YSI 2900, Yellow Springs, OH, USA). A further fingertip blood sample was obtained immediately after exercise, and an additional venous blood sample was collected within 5 min of TT completion to monitor the change in plasma [NO_2^-]. HR and RPE were monitored immediately following completion of the TT, as previously described. Expired gas was monitored continuously throughout TT exercise, as previously described.

Data analysis

Data analysis was conducted using SPSS version 22. An α level of $P < 0.05$ was accepted for significance. Between-supplement differences in plasma [NO_2^-], the transient change in pulmonary gas data, blood [lactate], and TT splits and running speed were compared in the 1500 and 10,000 m exercise testing sessions using a two-way (time × condition) repeated measures ANOVA. In the 1500 m condition, pulmonary gas data, TT splits, and running speed were averaged over 200 m intervals to 1400 m, and then during the final 100 m. Alternatively, these data were averaged over 1000 m intervals for the 10,000 m TT. Post-hoc analysis was conducted using *t*-tests with a Bonferroni correction. Between-supplement differences in BP, post-exercise HR and RPE, mean (average during the entire TT) $\dot{V}O_2$, $\dot{V}CO_2$, RER, and TT performance were compared in the 1500 and 10,000 m exercise testing sessions using a paired *t*-test. A statistical spreadsheet was also applied to derive qualitative probabilistic inferences for performance data (Hopkins 2007). Comparisons were made against a smallest worthwhile change (SWC) of 0.5% derived from previously published data (Russell et al. 2004; Shannon et al. 2016a). Verbal descriptors were used to express the chance that the true value of the effect was beneficial, trivial, or harmful, according to the following scale: < 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'. The effect was deemed unclear when an odds ratio of benefit to harm of <66 was identified, corresponding to a 25% chance of benefit and 0.5% risk of harm. Data are presented as mean ± SD, unless otherwise stated.

Results

Plasma [NO_2^-] and blood pressure

Plasma [NO_2^-] data for the 1500 and 10,000 m exercise test protocols is presented in Fig. 1. Pre-exercise plasma [NO_2^-] was significantly elevated in BRJ compared with PLA prior to both the 1500 and 10,000 m TTs ($P < 0.05$). Pre-exercise plasma [NO_2^-] tended to be greater in the 1500 m compared to 10,000 m BRJ condition, and was significantly greater prior to the 1500 m compared with the 10,000 m exercise testing session in PLA ($P < 0.05$). Plasma [NO_2^-] remained significantly elevated in both BRJ conditions post-exercise compared with PLA ($P < 0.05$). In the 1500 m exercise test protocol, post-exercise plasma [NO_2^-] was significantly decreased in BRJ relative to pre-exercise levels ($P < 0.05$). In contrast, plasma [NO_2^-] was not significantly different to pre-exercise levels in PLA ($P > 0.05$). In the 10,000 m exercise test protocol, post-exercise plasma [NO_2^-] was not significantly different to pre-exercise levels in either BRJ ($P > 0.05$) or PLA ($P > 0.05$). Post-exercise plasma [NO_2^-] was not significantly different between the two BRJ conditions, or between the two PLA conditions ($P > 0.05$).

There was no difference in mean arterial blood pressure (MAP) in BRJ compared with PLA prior to the 1500 m (BRJ 92 ± 5 vs. PLA 92 ± 5 mmHg; $P > 0.05$) nor 10,000 m (BRJ 94 ± 6 vs. PLA 94 ± 6 mmHg; $P > 0.05$) exercise test protocols.

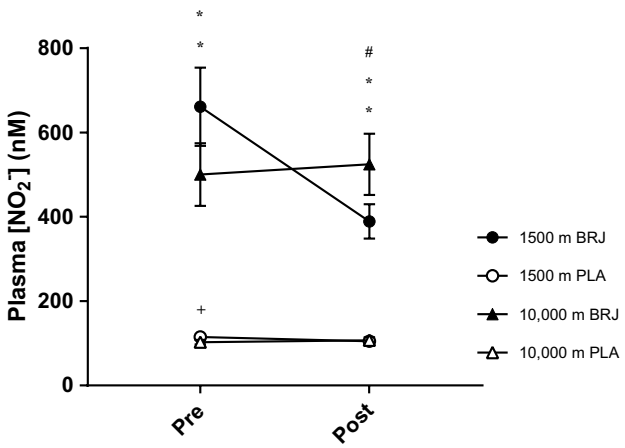

Fig. 1 Group mean ± SEM pre and post-exercise plasma [NO_2^-] in BRJ (closed *circles/triangles*) and PLA (*open circles/triangles*). BRJ significantly elevated plasma [NO_2^-] above PLA in both 1500 and 10,000 m conditions (*) ($P < 0.05$). Pre-exercise plasma [NO_2^-] was significantly greater in 1500 m PLA compared to 10,000 m PLA (+) ($P < 0.05$). Plasma [NO_2^-] significantly decreased in 1500 m BRJ (#) ($P < 0.05$)

Heart rate and ratings of perceived exertion

HR and RPE assessed immediately following the TTs did not differ significantly between BRJ and PLA ($P > 0.05$; Table 1).

Pulmonary gas exchange

Mean pulmonary $\dot{V}O_2$, $\dot{V}CO_2$, and RER data throughout the 1500 and 10,000 m TTs did not differ significantly between BRJ and PLA ($P > 0.05$; Table 1). The transient change in these data at 200 m (1500 m TT) and 1000 m (10,000 m TT) intervals throughout the TT is presented in Fig. 2. These variables did not differ significantly between BRJ and PLA at any time point ($P > 0.05$).

Blood [lactate]

Pre-exercise blood [lactate] was not significantly different between BRJ and PLA in either exercise test protocol ($P > 0.05$). In the 1500 m exercise test protocol, post-exercise blood [lactate] was significantly greater in BRJ compared with PLA (6.6 ± 1.2 vs. 6.1 ± 1.5 mM; $P < 0.05$), however, not different between BRJ and PLA in the 10,000 m exercise test protocol (4.5 ± 1.5 vs. 4.2 ± 0.8 mM; $P > 0.05$).

Time trial performance

Individual performance times and running speed for the 1500 and 10,000 m TT are presented in Fig. 3.

1500 m TT

Completion time was significantly faster in BRJ (319.6 ± 36.2 s) compared with PLA (325.7 ± 38.8 s; $P < 0.05$) for the 1500 m TT. Magnitude based inferences indicated a 'very likely beneficial' effect of BRJ on group mean 1500 m TT performance. Six of eight subjects exceeded the SWC for an improvement in 1500 m TT performance in BRJ vs. PLA (Fig. 3a). There was no significant difference in running speed at any of the 200 m (up to 1400 m) and final 100 m (1400–1500 m) splits ($P > 0.05$, Fig. 3b).

10,000 m TT

There was no significant difference in 10,000 m TT completion time between BRJ (2643.1 ± 324.1 s) compared to PLA (2649.9 ± 319.8 s, $P > 0.05$). Magnitude-based inferences indicated an 'unclear' effect of BRJ on group mean 10,000 m TT performance. However, four of eight subjects exceeded the SWC for an improvement in 10,000 m TT performance in BRJ vs. PLA (Fig. 3c). There was no significant difference in running speed at any of the 1000 m splits ($P > 0.05$, Fig. 3d). However, when the TT was divided into two, 5000 m segments for analysis, subjects had a significantly faster running speed (BRJ 14.0 ± 1.6 vs. PLA 13.7 ± 1.5 km h^{-1}, $P < 0.05$) and quicker time (BRJ 1287.5 ± 153 vs. PLA 1317.6 ± 149.9 s, $P < 0.05$) in the first 5000 m with BRJ compared to PLA. There was no difference in running speed (BRJ 13.3 ± 1.8 vs. PLA 13.5 ± 1.8 km·h^{-1}, $P > 0.05$) nor time (BRJ 1355.6 ± 189.5 vs. PLA 1332.3 ± 173.3 s, $P > 0.05$) during the second 5000 m split. Magnitude based inferences indicated a 'very likely beneficial' effect of BRJ during the first 5000 m, and an 'unclear' effect of BRJ during the second 5000 m of the 10,000 m TT.

Table 1 Mean (±SD) cardiopulmonary variables and perceived exertion during the 1500 and 10,000 m TTs with BRJ and PLA supplementation

	1500 m		10,000 m	
	PLA	BRJ	PLA	BRJ
HR (b·min^{-1})	187 ± 8	187 ± 4	180 ± 12	182 ± 13
RPE	19 ± 1	19 ± 1	19 ± 1	19 ± 1
$\dot{V}O_2$ (ml·kg^{-1}·min^{-1})	53.9 ± 6.9	53.4 ± 6.8	48.6 ± 6.3	49.0 ± 6.0
$\dot{V}CO_2$ (ml·kg^{-1}·min^{-1})	61.8 ± 10.5	61.1 ± 11.2	50.2 ± 6.5	52.7 ± 8.4
RER	1.15 ± 0.10	1.15 ± 0.10	1.03 ± 0.10	1.08 ± 0.10

Discussion

The main finding of the present investigation was that acute supplementation with BRJ significantly enhanced 1500 m treadmill TT performance, but not 10,000 m treadmill TT performance. This suggests that BRJ supplementation might improve performance in trained males conducting shorter distance TTs which allow for a high work rate, but not necessarily during longer distance TTs where a lower work rate is mandated.

Effects of BRJ on plasma [NO_2^-] and blood pressure

In agreement with the findings of a number of earlier investigations (e.g. Bailey et al. 2009, 2010; Vanhatalo et al. 2010; Lansley et al. 2011; Cermak et al. 2012a; Wylie et al. 2013; Muggeridge et al. 2014; Shannon et al. 2016b; McMahon et al. 2016), BRJ supplementation significantly elevated pre-exercise plasma [NO_2^-], which is a highly sensitive marker of NO bioavailability (Lundberg et al. 2008). Interestingly, despite close replication of diet

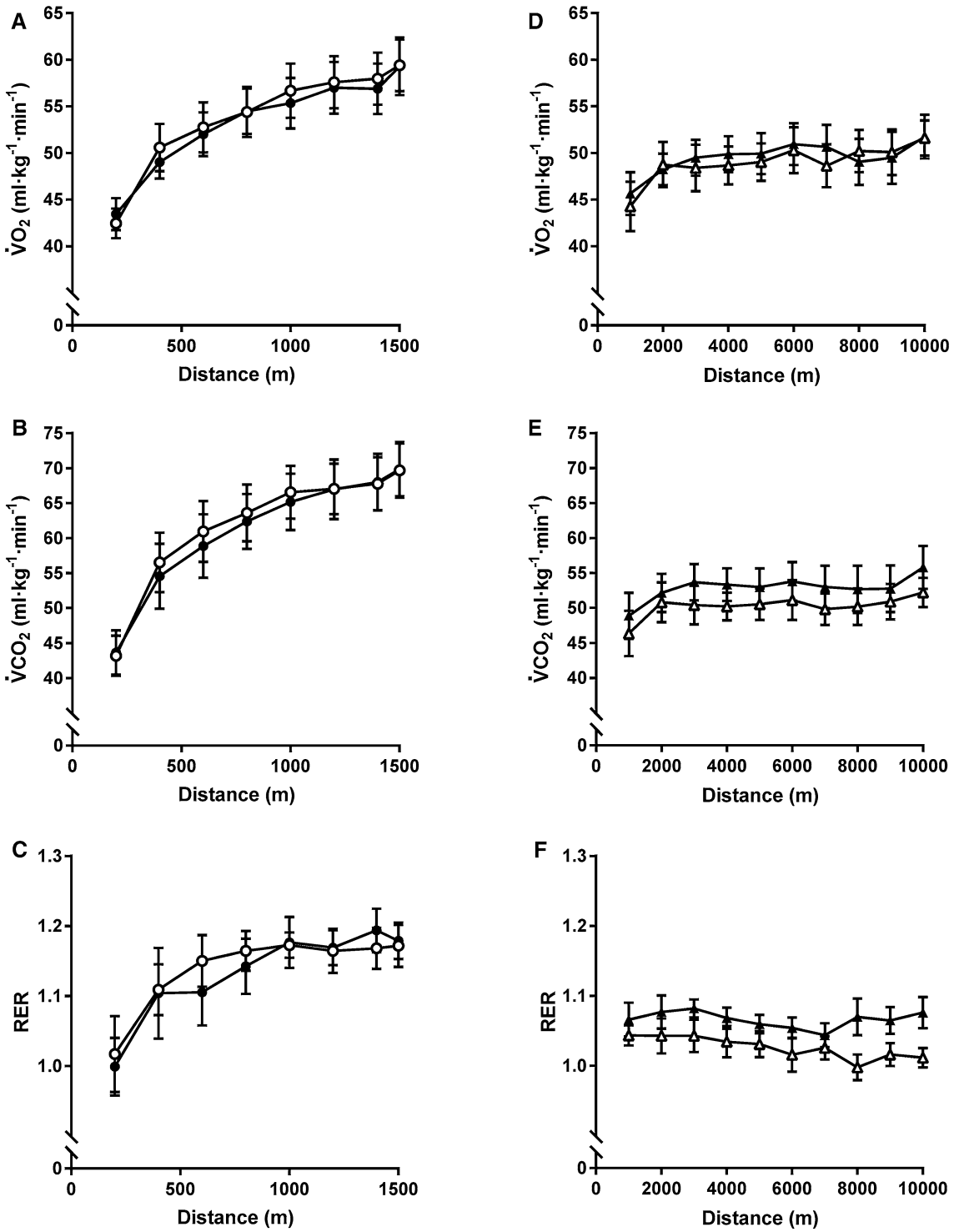

Fig. 2 The transient changes in pulmonary gas-exchange data with BRJ (*closed circles*/*triangles*) and PLA (*open circles*/triangles) throughout the 1500 m (**a**–**c**) and 10,000 m (**d**–**f**) TTs. Data for the 1500 m TT represents average values over 200 m intervals up to 1400 m, and during the final 100 m. Data for the 10,000 m TT represents average values over 1000 m intervals

and exercise for 24 h preceding each trial, administration of identical batch checked supplements, and a consistent time-frame for post-supplement blood sampling, there was some variation in pre-exercise plasma [NO_2^-] between the two BRJ conditions, and between the two PLA conditions. Given pre-exercise plasma [NO_2^-] was

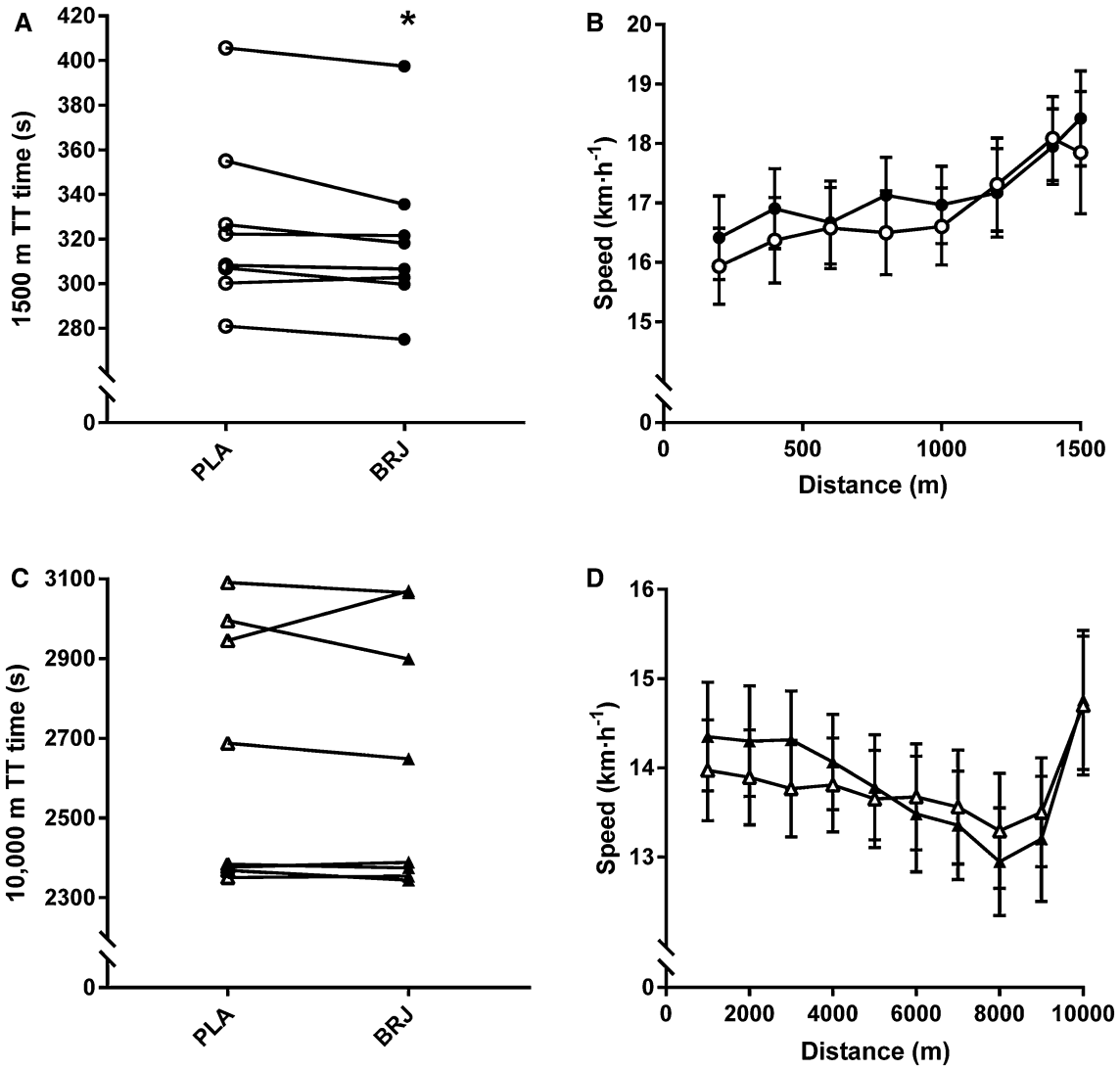

Fig. 3 Individual performance times and running speed during the 1500 and 10,000 m TT tests. Performance in the 1500 m TT was significantly faster (*asterisk*) in BRJ (*filled circles*) compared with PLA (*open circles*) (**a**) ($P < 0.05$). There was no significant difference in 10,000 m TT performance between BRJ (*filled triangles*) and PLA (*open triangles*) (**c**). Running speed did not significantly differ between BRJ (*closed circles/triangles*) and PLA (*open circles/triangles*) in the 1500 m (**b**) and 10,000 m (**d**) TT tests

~5–6-fold greater in both BRJ conditions compared to PLA, and remained elevated above PLA post-exercise, it is likely that the difference in the performance response to BRJ between 1500 and 10,000 m exercise test protocols is not attributable to insufficient availability of NO_2^- as a 'substrate' for NO generation in the 10,000 m BRJ trial. Plasma [NO_2^-] did not significantly decrease post-exercise in the BRJ 10,000 m condition, yet declined significantly in the BRJ 1500 m condition. This suggests that it is differences in the 'use' rather than the availability of NO_2^- that is important. This might be related to greater acidosis and hypoxia, and thus NO_2^- reduction (Modin et al. 2001; Cosby et al. 2003; Castello et al. 2006), in the 1500 vs. 10,000 m conditions, as a consequence of the relatively greater work rate adopted (~86 vs. 78% $\dot{V}O_{2max}$; ~6.5 vs. 4.5 mM blood [Lactate]).

The increase in plasma [NO_2^-] with BRJ did not translate into a significant reduction in BP, as evident by similar MAP values between conditions. Previous investigations indicate that the magnitude of the decline in BP typically observed with NO_3^- supplementation is related to the initial BP of the subject (Ashworth et al. 2015). Thus, it is possible that the relatively low BP in these subjects minimized the effects of NO_3^- supplementation on this parameter.

Effects of BRJ on 1500 m TT performance

In the cohort of trained men recruited for this study, it was hypothesized that BRJ would enhance 1500 m TT performance. In agreement with previous literature and our experimental hypothesis, BRJ supplementation significantly enhanced 1500 m running performance, with a mean improvement of 1.9%—an effect deemed 'very likely beneficial' via magnitude based inferential statistics. These data are comparable to previous studies which have demonstrated that the ingestion of dietary NO_3^- significantly enhances short duration (<30 min) TTE and/or TT performance in untrained and moderately-trained individuals ($\dot{V}O_{2max}$ < ~60 ml kg^{-1} min^{-1}) (Bailey et al. 2009, 2010; Vanhatalo et al. 2010; Lansley et al. 2011; Cermak et al. 2012a, b; Porcelli et al. 2014). In contrast, limited effects of NO_3^- supplementation have been reported for similar duration exercise tests in individuals with a greater training status (i.e. well-trained and elite athletes) ($\dot{V}O_{2max}$ >70 $ml{\cdot}kg^{-1}{\cdot}min^{-1}$) (Peacock et al. 2012; Bescós et al. 2012; Christensen et al. 2013; Porcelli et al. 2014; Sandbakk et al. 2015). These highly trained athletes manifest a host of different characteristics likely to diminish their responsiveness to NO_3^- supplementation (for review, see Jones 2014), including elevated endogenous NO_3^- and NO_2^- reserves (Vassalle et al. 2003).

Regular splits were obtained throughout TTs, which allowed us to compare the pacing strategy between BRJ and PLA conditions, and identify the points at which BRJ might have enhanced running speed. Although there were no significant differences in running speed at any of the splits, subjects tended to run faster during the first 1000 m of the 1500 m TT in BRJ compared with PLA, such that the majority of time 'gained' in BRJ occurred during this period. Subjects achieved similar running speeds between 1000 and 1400 m, although this represented a greater percentage of average pace in PLA which could not be maintained. Consequently, subjects were typically unable to employ a sprint finish in PLA, unlike in BRJ. The apparent benefits of BRJ during the first and last phases of the 1500 m TT could be of tactical advantage during competition.

A range of mechanisms might explain the ergogenic effect of NO_3^- supplementation on 1500 m TT performance observed here. Multiple previous studies have reported a reduction in the O_2 cost of exercise with NO_3^- supplementation (Larsen et al. 2007; Bailey et al. 2009; Lansley et al. 2011; Cermak et al. 2012a; Wylie et al. 2013; Pawlak-Chaouch et al. 2016), consequent to either an improvement in the efficiency of mitochondrial respiration (Larsen et al. 2011) and/or muscle contraction (Bailey et al. 2010). However, this mechanism cannot explain the improvement in performance observed in this study, as BRJ supplementation did not reduce O_2 consumption during exercise. Instead, the ergogenic effect of BRJ may be better explained by other physiological changes. Interestingly, recent murine model studies have identified effects of NO_3^- which appear to be selective to type II muscle (for review, see Jones et al. 2016), and may be of mechanistic relevance in the present study given the likely high recruitment of type II fibers during the 1500 m TT (Essén 1978; Sale 1987). NO_3^- supplementation was reported to augment blood flow and thus O_2 delivery to type II muscle in exercising rats (Ferguson et al. 2013, 2015). It was suggested that these local blood flow changes might be beneficial by increasing the microvascular partial pressure of O_2 (PO_2), and concomitantly decreasing muscle metabolic perturbations (e.g. PCr degradation, ADP and Pi accumulation)—effects previously demonstrated to extend TTE with NO_3^- supplementation in humans (Bailey et al. 2010; Vanhatalo et al. 2011). The enhanced 1500 m exercise performance subsequent to BRJ in the present study may thus, at least partly, be underpinned by changes to type II muscle blood flow and O_2 delivery. Additionally, Hernandez et al. (2012) observed an increased expression, and therefore content, of the Ca^{2+} handling protein calsequestrin 1 and the dihydropyridine receptor. These effects, which were reported in predominantly type II muscle, were accompanied by an increased muscle force production. Subsequent studies have confirmed the beneficial effect of NO_3^- on force production in humans (Haider and Folland 2014; Coggan et al. 2015). It is therefore possible that elevated type II muscle Ca^{2+} handling and muscle contractile function may have further contributed to the improved 1500 m performance observed in the present investigation. Furthermore, several recent studies have observed improvements in physiological functioning and exercise performance in situations where type II muscle recruitment is likely to be high (Breese et al. 2013; Bailey et al. 2015; Coggan et al. 2015), which further supports the findings of this investigation.

Effects of BRJ on 10,000 m TT performance

There was no significant difference in 10,000 m TT performance between BRJ and PLA in this study. Magnitude based inferences also indicated an 'unclear' practical effect of BRJ on 10,000 m TT performance. Nevertheless, four subjects exceeded the SWC in performance following BRJ in the present study, supporting the notion of a responder vs. non-responder phenomenon (Wilkerson et al. 2012).

Interestingly, subjects appeared to adopt disparate pacing strategies in BRJ and PLA conditions. In BRJ, subjects ran the first 5000 m significantly faster, yet the pace gradually declined throughout the TT before a final sprint finish. Conversely, in PLA subjects adopted a more

even paced strategy prior to a similar paced sprint finish. While it is important to acknowledge that overall 10,000 m performance was no different between conditions, the faster first 5000 m with BRJ might offer tactical advantages in competition. In contrast, Wilkerson et al. (2012) observed no difference in power output during the earlier stages of a 50 mile (total exercise duration: >2 h) cycle ergometry TT, yet the final 10 miles was faster with NO_3^- supplementation. Together, these findings might suggest a potential application of BRJ during higher work rate periods within a longer duration event, perhaps specifically those which require greater type II muscle recruitment or markedly decrease the muscle pH and PO_2 and hence, augment NO_2^- reduction into NO (Modin et al. 2001; Cosby et al. 2003; Castello et al. 2006). This might include facilitating a fast start or finish during competition, or responding to a surge by another competitor. Investigations into the performance effects of NO_3^- supplementation during 'real world' competition would provide interesting insight.

The effects of NO_3^- supplementation are likely determined by an interaction between multiple variables, rather than individual factors, and hence the results of this study cannot easily be generalised to other populations or experimental conditions. Aerobic fitness strongly moderates the effects of NO_3^- supplementation (Porcelli et al. 2014), such that the beneficial effects of NO_3^- supplementation on 1500 m TT performance might not be applicable to individuals with a higher training status (Boorsma et al. 2014). Likewise, it is possible that untrained individuals may derive a beneficial effect of NO_3^- supplementation during longer duration (>30 min) lower work rate exercise tests, which remains to be explored. Additionally, there is some evidence to suggest that chronic dosing might elicit more robust physiological and performance changes (Vanhatalo et al. 2010). Therefore, it cannot be ruled out that NO_3^- supplementation may be beneficial for longer duration lower work rate exercise tests or in trained individuals when more protracted supplementation strategies are applied. Future investigations exploring the potential interaction between these and other variables are warranted, to help elucidate the precise conditions where NO_3^- supplementation might be beneficial.

In conclusion, the results of the present study suggest that acute BRJ supplementation increases plasma [NO_2^-], and enhances 1500 m treadmill TT performance in trained men. In contrast, BRJ supplementation did not significantly enhance 10,000 m TT performance. These findings provide important insight into the specific conditions where BRJ supplementation may be ergogenic, and evidence that the ergogenic effect of NO_3^- supplementation is moderated by the duration and requisite work rate of a TT.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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