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ORIGINAL ARTICLE

Dietary nitrate improves sprint performance and cognitive function during prolonged intermittent exercise

Christopher Thompson[1] · Lee J. Wylie[1] · Jonathan Fulford[2] · James Kelly[1] · Matthew I. Black[1] · Sinead T. J. McDonagh[1] · Asker E. Jeukendrup[3] · Anni Vanhatalo[1] · Andrew M. Jones[1]

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Abstract It is possible that dietary nitrate (NO_3^-) supplementation may improve both physical and cognitive performance via its influence on blood flow and cellular energetics.

Purpose To investigate the effects of dietary NO_3^- supplementation on exercise performance and cognitive function during a prolonged intermittent sprint test (IST) protocol, which was designed to reflect typical work patterns during team sports.

Methods In a double-blind randomised crossover study, 16 male team-sport players received NO_3^--rich (BR; 140 mL day^{-1}; 12.8 mmol of NO_3^-), and NO_3^--depleted (PL; 140 mL day^{-1}; 0.08 mmol NO_3^-) beetroot juice for 7 days. On day 7 of supplementation, subjects completed the IST (two 40-min "halves" of repeated 2-min blocks consisting of a 6-s "all-out" sprint, 100-s active recovery and 20 s of rest), on a cycle ergometer during which cognitive tasks were simultaneously performed.

Results Total work done during the sprints of the IST was greater in BR (123 ± 19 kJ) compared to PL (119 ± 17 kJ; $P < 0.05$). Reaction time of response to the cognitive tasks in the second half of the IST was improved in BR compared to PL (BR first half: 820 ± 96 vs. second half: 817 ± 86 ms; PL first half: 824 ± 114 vs. second half: 847 ± 118 ms; $P < 0.05$). There was no difference in response accuracy.

Conclusions These findings suggest that dietary NO_3^- enhances repeated sprint performance and may attenuate the decline in cognitive function (and specifically reaction time) that may occur during prolonged intermittent exercise.

Keywords Nitric oxide · Dietary supplementation · Cognitive performance · Sprint performance

Introduction

Recent investigations have reported improved short-term intermittent running (Wylie et al. 2013a) and rowing (Bond et al. 2012) performance following nitrate-rich beetroot juice supplementation. Since team sports such as football, rugby and hockey involve a similar pattern of repeated sprint exercise, these studies suggest that nitrate (NO_3^-) supplementation may improve physical performance during such activities.

The reduction of NO_3^- to the bioactive nitrite (NO_2^-) and nitric oxide (NO) likely mediate the physiological changes and enhanced exercise capacity that has been reported following dietary NO_3^- supplementation (Bailey et al. 2010a, b; Larsen et al. 2007). This pathway supplements the NO produced by the oxidation of L-arginine and is potentiated in hypoxic conditions (Lundberg and Weitzberg 2010), ensuring that NO is produced across a wide range of O_2 concentrations (Lundberg et al. 2008). NO_3^- supplementation has been shown to modify intracellular calcium handling and increase contractile force production in type II muscle fibres (Hernández et al. 2012). Furthermore, NO_3^- supplementation may result in preferential

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✉ Andrew M. Jones
a.m.jones@exeter.ac.uk

[1] Sport and Health Sciences, University of Exeter, St. Luke's Campus, Heavitree Road, Exeter, UK

[2] NIHR Exeter Clinical Research Facility, University of Exeter, Heavitree Road, Exeter, Devon EX1 2LU, UK

[3] GSSI, Barrington, IL, USA

distribution of blood flow to muscle containing predominantly type II fibres (Ferguson et al. 2013). Given that type II muscle fibres are preferentially recruited during transitions from low to high-intensity exercise (Krustrup et al. 2006), these mechanisms may enhance performance during intermittent exercise.

Cognitive ability, which includes functions of perception, learning, decision-making and communication, is sensitive to alterations in physical demand, mood and arousal (Hogervorst et al. 1996; Reilly and Smith 1986). At very high exercise intensities (>85 % $\dot{V}O_{2peak}$), cognitive task performance deteriorates, with a pronounced detrimental effect on reaction time (Fery et al. 1997). Team sport players are often required to make rapid and appropriate decisions whilst simultaneously exercising at variable intensities. Dietary NO_3^- supplementation has been reported to affect cerebral haemodynamics (Haskell et al. 2011; Rifkind et al. 2007), to improve perfusion to brain areas associated with executive function (Presley et al. 2011), and to enhance neurovascular coupling in response to visual stimuli (Aamand et al. 2013). Given that physical exertion can negatively influence cognitive task performance, it is possible that NO_3^- supplementation may improve aspects of cognitive performance and in particular the speed and/or accuracy of decision-making, during intermittent high-intensity exercise such as occurs in team sports.

Previous studies investigating the influence of NO_3^- supplementation on intermittent exercise performance (Bond et al. 2012; Martin et al. 2014; Wylie et al. 2013a) have employed protocols of short duration which are not representative of team sports. Moreover, no previous study has assessed possible effects of NO_3^- supplementation on cognitive task performance during intermittent exercise. Ideally, the efficacy of NO_3^- supplementation in team sports should be explored in a protocol which not only imitates the physical and cognitive stress, but also the duration typical of team sport match play (e.g. 2 × 40 min 'halves' such as occurs in rugby union and field hockey).

The purpose of this study was to assess the physiological, cognitive and performance effects of dietary NO_3^- supplementation during an exercise protocol which reflects the multiple sprint pattern and metabolic demands of team sport match play. We used a series of alternating cognitive tasks interspersed within an extended (2 × 40 min) and modified version of a previously described intermittent sprint test (IST; Bishop and Claudius 2005) for this purpose. Compared to placebo, we hypothesised that dietary NO_3^- supplementation would result in: (1) a greater total work done during the IST; and (2) a smaller decline in reaction time and response accuracy in the cognitive tests in the second compared to the first half of the IST.

Methods

Subjects

Sixteen male, recreational team-sport players from local field hockey, football and rugby teams (mean ± SD: age 24 ± 5 years, body mass 78.0 ± 7.0 kg, height 1.78 ± 0.60 m, $\dot{V}O_{2max}$ 50 ± 7 mL kg^{-1} min^{-1}) volunteered to participate in the study. None of the subjects were supplementing their diet with any putative ergogenic aid for 6 months prior to the start of the study. Following an explanation of the experimental procedures, associated risks, potential benefits and likely value of possible findings, subjects gave their written informed consent to participate. The study was approved by the Institutional Research Ethics Committee and conformed to the code of ethics of the Declaration of Helsinki.

Experimental design

Subjects visited the laboratory on four separate occasions over a 26–30-day period. On visit 1, subjects completed an incremental exercise test on a cycle ergometer for the determination of $\dot{V}O_{2max}$. On visit 2, subjects completed a familiarisation session in which the first half of the IST and simultaneous cognitive tasks were performed. In a double-blind, randomised, crossover design subjects were then assigned to receive NO_3^--rich beetroot juice (BR) and NO_3^--depleted beetroot juice (PL) for 7 days with a wash-out period of at least 10 days separating the two supplementation periods. On day 7 of each supplementation period, subject reported to the laboratory to perform the full IST.

Experimental visits were scheduled at the same time of day (±2 h). Subjects were asked to maintain their normal dietary and exercise behaviour throughout the study. However, subjects were instructed to record their diet during the 24 h preceding the first IST and to repeat this prior to the second IST. Subjects were also instructed to arrive at the laboratory ≥3 h post-prandial, having avoided strenuous exercise and the consumption of alcohol and caffeine in the 12 h preceding each exercise test. Subjects were requested to refrain from chewing gum and using antibacterial mouthwash for the duration of the study as this inhibits the reduction of NO_3^- to NO_2^- in the oral cavity (Govoni et al. 2008).

Exercise protocols

The subjects completed a ramp incremental cycle exercise test for determination of the $\dot{V}O_{2max}$. Initially, subjects performed 3 min of baseline cycling at 0 W, after which the

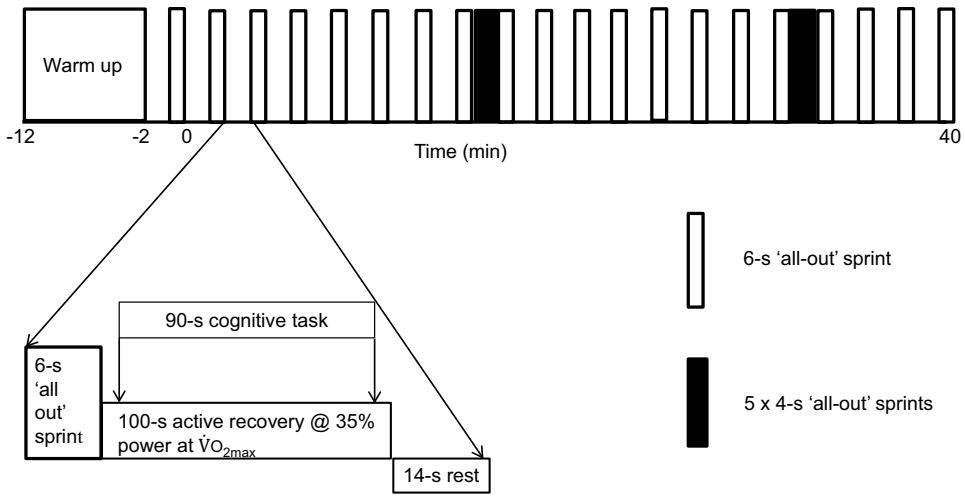

Fig. 1 Schematic of one 'half' of the intermittent sprint test. Each 40-min half was separated into 2-min blocks (6-s 'all-out' sprint, 100 s of active rest and 14 s of passive rest). On two occasions subjects performed 5 × 4 s sprints separated by 16 s of active rest. A 15-min period was given as 'half-time'. Note that the figure is not to scale

work rate was increased at a rate of 30 W/min until the limit of tolerance. The subjects cycled at a self-selected pedal rate (70–90 rpm). The saddle and handle bar height and configuration was recorded and reproduced in subsequent tests. Breath-by-breath pulmonary gas-exchange data were collected continuously during the incremental tests and averaged over consecutive 10-s periods. The $\dot{V}O_{2max}$ was accepted as the highest 30-s mean value attained before the subject's volitional exhaustion in the test. We did not utilise secondary criteria to 'verify' the attainment of $\dot{V}O_{2max}$ because the validity of this approach has been questioned (Poole et al. 2008). The individual power output and $\dot{V}O_2$ data from the ramp test were used to inform exercise intensity during the IST (see below).

The IST (Fig. 1) was based on a motion analysis study of international field hockey (Spencer et al. 2004) and is an extension of a protocol described previously (Bishop and Claudius 2005). The IST was performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands). Although the IST cannot replicate the exact movement patterns encountered in team sports, an advantage to using a cycle ergometer is that, unlike field-based running tests, it permits the simultaneous performance of cognitive tasks (see "Cognitive assessment"). Briefly, the test consisted of a 10-min warm-up followed by two 40-min halves of intermittent exercise separated by 15 min of recovery ("half time"). The warm-up required subjects to cycle for 5 min at 50 % predetermined power output at $\dot{V}O_{2max}$, followed by 1 min at 70 % power output at $\dot{V}O_{2max}$ and 2 min of rest. A practice 2-min block of the IST was then performed followed by a 2-min rest period before the IST began. Each half of the IST was divided into 2-min blocks which consisted of a 6-s all-out sprint, 100 s of active recovery and 14 s of passive recovery. On two occasions during each half, subjects completed blocks of 5 × 4-s all-out sprints separated by 16 s of active recovery. The fixed resistance for the 6- and 4-s all-out sprints was determined using the linear function of the Lode ergometer such that at a cadence of 120 rpm, subjects would achieve 250 % of their ramp test peak power output. The active recovery required the subject to maintain a constant power output of 35 % of $\dot{V}O_{2max}$.

Blood was sampled at rest (baseline), before and after each half, and at 15 min following the completion of the IST. All blood samples were drawn from a cannula (Insyte-WTM, Becton-Dickinson, Madrid, Spain) inserted into the subject's antecubital vein. Blood [lactate] and [glucose], as well as plasma [K^+], [Na^+], [NO_2^-] and [NO_3^-] were analysed in all samples (square brackets denote concentration).

Supplementation

Following familiarisation visits, subjects were allocated in a double-blind, randomised, crossover design to consume concentrated NO_3^--rich beetroot juice (BR; organic beetroot juice; ~6.4 mmol of NO_3^- per 70 mL; Beet it, James White Drinks Ltd., Ipswich, UK) and NO_3^--depleted beetroot juice (PL; organic beetroot juice; ~0.04 mmol NO_3^- per 70 mL; Beet it, James White Drinks Ltd., Ipswich, UK). Subjects consumed supplements for 7 days with ≥10 days washout between conditions. Subjects consumed 1 × 70 mL of supplement each morning and evening for 6 days. On day 7 of each condition, the subjects consumed 2 × 70 mL of supplement and commenced the IST 2.5 h later.

Measurements

Pulmonary gas exchange and heart rate

Heart rate was measured continuously (Polar RS400, Polar Electro Oy, Kempele, Finland). Breath-by-breath

pulmonary gas-exchange data were collected continuously throughout each experimental test (Oxycon Pro, Jaeger, Hoechberg, Germany) and mean $\dot{V}O_2$ was calculated for each half.

Blood analysis

All blood samples were collected into lithium–heparin vacutainers (Becton-Dickinson, New Jersey, USA). 200 μL of blood was immediately extracted and haemolysed in 200 μL of Triton X-100 solution (Triton X-100, Amresco, Salon, OH) before blood [lactate] and [glucose] were measured (YSI 2300, Yellow Springs Instruments, Yellow Springs, OH). The remaining whole blood from each sample was centrifuged at 4000 rpm for 8 min at 4 °C within 2 min of collection. Plasma was immediately extracted and a portion analysed to determine [K^+] and [Na^+] (9180 Electrolyte Analyzer, F. Hoffmann-La Roche, Basel, Switzerland). The remaining plasma was immediately frozen at −80 °C and subsequently analysed for [NO_2^-] and [NO_3^-] using a modification of the chemiluminescence technique described by Wylie et al. (2013b).

Cognitive assessment

Subjects were asked to complete cognitive tasks 15 min before, at 7.5 min during the half-time interval and at 15 min following the IST, as well as during each 100-s active recovery period of the IST. The cognitive tasks were computerised and delivered using E-Prime® 2.0 (Psychology Software Tools, Inc. 2013). Each task was presented on a computer screen positioned at eye level and subjects were instructed to respond using a custom-built handlebar-mounted control button box. The tasks to be completed during each active rest period alternated between Stroop and Decision-Reaction tests. The duration of each cognitive task was 90 s. In total, 18 cognitive tasks were presented during each half; 9 Stroop and 9 Decision-Reaction tests.

Stroop test. The Stroop test has been used widely in previous research because of its ability to assess information processing speed, executive abilities and selective attention (Pachana et al. 2004). A series of text strings were presented consisting of either neutral form 'XXXX' or words representing colours 'RED', 'YELLOW', 'GREEN' or 'BLUE'. Each string was presented in turn against a dark background and in a font colour of red, yellow, green or blue. Subjects were instructed to press the appropriately coloured button on the control box that corresponded to the colour the text was written in. Tasks were presented in a random order evenly distributed between neutral, congruent (the word and font colour matched) and incongruent (the word and font colour did not match) conditions with 12 cases of each presented within every exercise block. Subjects were instructed to respond as quickly and as accurately as possible with reaction time and accuracy recorded.

Decision-Reaction test. Variations of this task have been previously used to study the relationship between nutrient intake and information processing speed (Durlach et al. 2002; Irwin et al. 2013). Black arrows pointing either to the left or right of the screen were presented, either on the left or right of a white background. At the top of the screen a categorisation header was simultaneously presented which read either 'LOCATION' or 'DIRECTION'. In the case of the former, subjects were instructed to press a button on the left or right of the control box dependent upon the location the arrow appeared. In the case of the latter, the choice of appropriate button was based upon the direction the arrow was pointing. For example, an arrow appearing on the left side of the screen with the header of 'LOCATION' prompted subjects to press the left button on the control box irrespective of the direction of the arrow, whereas an arrow pointing to the left appearing with the header 'DIRECTION' prompted subjects to press the left button irrespective of the location of the arrow. Tasks were presented in a random order evenly distributed between congruent (the arrow direction and location matching) and incongruent (the arrow direction and location not matching) conditions with 16 cases of each presented within every exercise block. As with the Stroop test, subject accuracy and reaction times were recorded.

Statistical analysis

Two-way, repeated-measures ANOVAs (supplement × time) were used to analyse the difference in work done, blood and plasma variables and reaction time and response accuracy to the cognitive tasks during the IST as well as the cognitive tasks performed before, at half time and following the IST. Mean HR, $\dot{V}O_2$, total work done, and mean reaction time and response accuracy to the cognitive tasks during each half were analysed using paired-samples *t* tests. Initially, both cognitive tasks were considered together, but in the event of significant differences arising, the Stroop and Decision-Reaction results were assessed separately. If significant differences were subsequently found for either test, further subdivision of results obtained during exercise for that specific test were undertaken examining results from the first, middle and last third of each exercise half. Relationships between differences in plasma [NO_2^-] and differences in total work done were analysed using Pearson product-moment correlation coefficients. Significant main and interaction effects were followed up using simple contrasts. All values are reported as mean ± SD. Statistical significance was accepted at $P < 0.05$.

Results

Plasma [NO_2^-] and [NO_3^-]

There were significant main effects by supplement and time, and an interaction effect on the plasma [NO_2^-] ($P < 0.001$). Averaged across all measurement time points, [NO_2^-] was ~343 % greater in BR compared to PL. At resting baseline, the plasma [NO_2^-] was 360 ± 118 nM in BR and 102 ± 82 nM in PL ($P < 0.001$). Relative to baseline, plasma [NO_2^-] declined following the first half (by 109 ± 126 nM; $P < 0.05$) and second half (by 111 ± 126 nM; $P < 0.05$) of the IST in BR (Fig. 2). However, following a 15-min period of recovery at the end of each half, plasma [NO_2^-] was restored to baseline values in BR (Fig. 2). There was a significant main effect by supplement on plasma [NO_3^-] ($P < 0.05$). Averaged across all measurement time points, [NO_3^-] was ~940 % greater in BR than in PL. At resting baseline, [NO_3^-] was 360 ± 78 μM in BR and 37 ± 60 μM in PL ($P < 0.001$). Relative to baseline, [NO_3^-] rose in BR following the 10-min warm-up (by 40 ± 55 μM; $P < 0.05$), but was unchanged throughout the remainder of the test in BR. In PL, [NO_3^-] was significantly greater than baseline following 15-min recovery at the end of the first ($P < 0.05$) and second ($P < 0.05$) halves (Fig. 2).

IST performance

There was no significant difference in mean $\dot{V}O_2$ between conditions in the first half (BR: 2.57 ± 0.33 vs. PL: 2.60 ± 0.40 L min^{-1}) or the second (BR: 2.57 ± 0.38 vs. PL: 2.56 ± 0.41 L min^{-1}) half of the IST. There were also no differences in blood [lactate], blood [glucose], plasma [Na^+], plasma [K^+] or heart rate at any time point during each half.

Total work done during the sprints of the IST was ~3.5 % greater in BR (123 ± 19 kJ) compared to PL (119 ± 17 kJ; $P < 0.05$). There was a significant interaction effect (supplement by sprint number) on work done during the first (BR: 63 ± 20 vs. PL: 60 ± 18 kJ; $P < 0.05$), but not the second (BR: 60 ± 17 vs. PL: 59 ± 16 kJ; $P > 0.05$) half of the IST. Specifically, subjects completed significantly more work in 5 of 20 first half 6-s sprints in BR compared to PL ($P < 0.05$; Fig. 3). There were no significant differences in total work done between the first and second halves within either condition. There was a negative correlation between the difference in total work done following BR compared to PL and the change in plasma [NO_2^-] from baseline to end-exercise in the first ($r = -0.69$, $P < 0.05$), but not the second ($r = -0.15$) half of the IST.

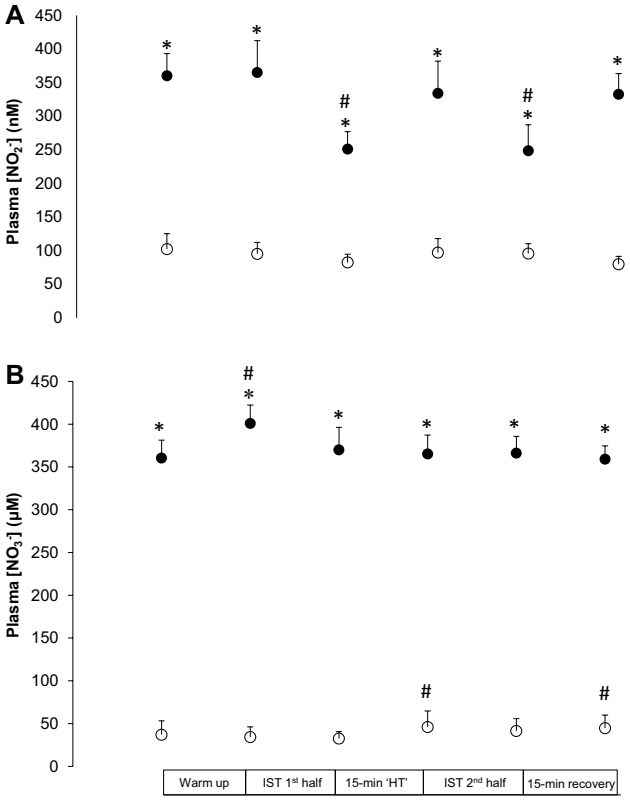

Fig. 2 Plasma [NO_2^-] decreased significantly during each half in BR (*closed circles*) but not in PL (*open circles*) (**a**). In BR (*closed circles*) plasma [NO_3^-] increased by 11 % during the warm-up but remained unchanged relative to baseline throughout the IST (**b**). In PL (*open circles*), [NO_3^-] increased by 24 % during the 15-min half-time at the end of the first half and by 21 % during the 15-min recovery at the end of the second (**b**). *$P < 0.05$ compared to PL; #$P < 0.05$ compared to baseline

Cognitive performance

Reaction time of response to the cognitive tasks in the second half of the IST was shorter in BR (817 ± 86 ms) compared to PL (847 ± 118 ms) ($P < 0.05$; Fig. 4). There was no difference in reaction time in the first half between BR (820 ± 96 ms) and PL (824 ± 114 ms). The mean decline in speed of reaction between the first and second half in PL ($P < 0.05$) was offset in the BR group by ~25 ms corresponding to a 3 % improvement in reaction time ($P < 0.05$). Accuracy of the response was not different between BR and PL (BR: first half: 6.9 ± 6.1 vs. second half: 7.7 ± 7.3; PL first half: 7.3 ± 5.8 vs. second half: 7.1 ± 5.9 incorrect responses; $P > 0.05$; Fig. 4). There were no significant differences in reaction time or response accuracy within conditions in the cognitive tasks performed before, at half time, and following the IST.

Performances in the Decision-Reaction and Stroop tasks were also assessed separately. For the Decision-Reaction

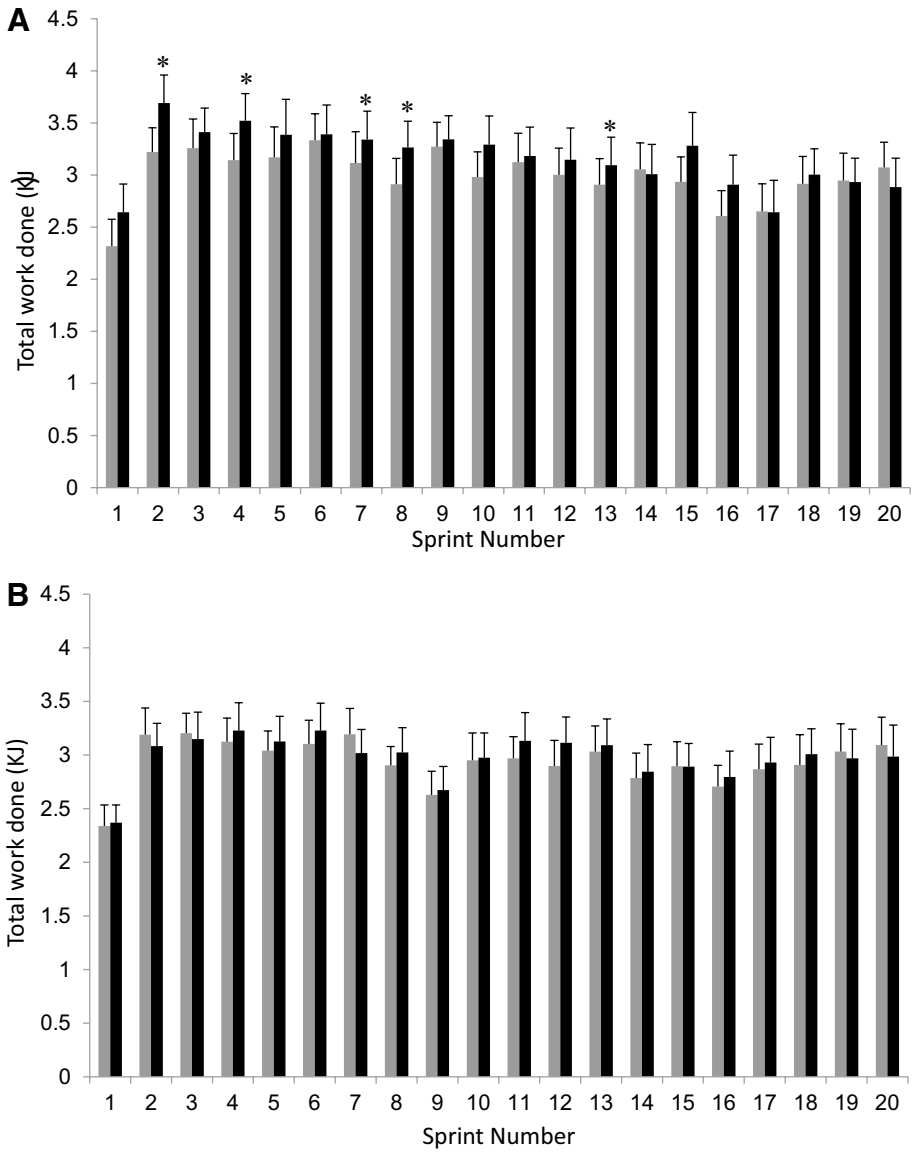

Fig. 3 Total work completed (kJ) for each 6-s sprint during the first (**a**) and the second (**b**) half of the ICT for BR (*black bars*) and PL (*grey bars*). *Error bars* indicate the SE. *Asterisks* denote statistical significance between condition ($P < 0.05$)

task, no significant difference was found between BR and PL for the changes in reaction time between the second and first half of the IST (BR first half: 770 ± 135 vs. second half: 769 ± 128 ms; PL first half: 784 ± 161 vs. second half: 794 ± 156 ms). For the Stroop test, however, there was a significant difference between BR and PL in the changes in reaction time between the second and first half of the IST (BR first half: 869 ± 80 vs. second half: 865 ± 70 ms; PL first half: 864 ± 91 vs. second half: 896 ± 93 ms; $P < 0.05$). The Stroop test analysis was further extended to assess reaction time changes of the IST for the first, middle and last thirds between each half. This analysis revealed a significant difference for the final third of each half of the IST only, with an improvement of 44 ms for the second half relative to the first half for BR relative to PL ($P < 0.05$).

Discussion

There were two original findings to this study. Firstly, 7 days of dietary NO_3^- supplementation in the form of BR improved total work done during an intermittent sprint protocol which was designed to reflect the dynamic work profile of team sport play. Secondly, BR supplementation significantly improved reaction time of response to the cognitive tasks without altering response accuracy. These findings suggest that BR can serve as an effective ergogenic aid in team sports involving repeat sprint–recovery cycles and may also attenuate the decline in cognitive function (specifically decision-making reaction time) that typically occurs over time during prolonged intermittent exercise.

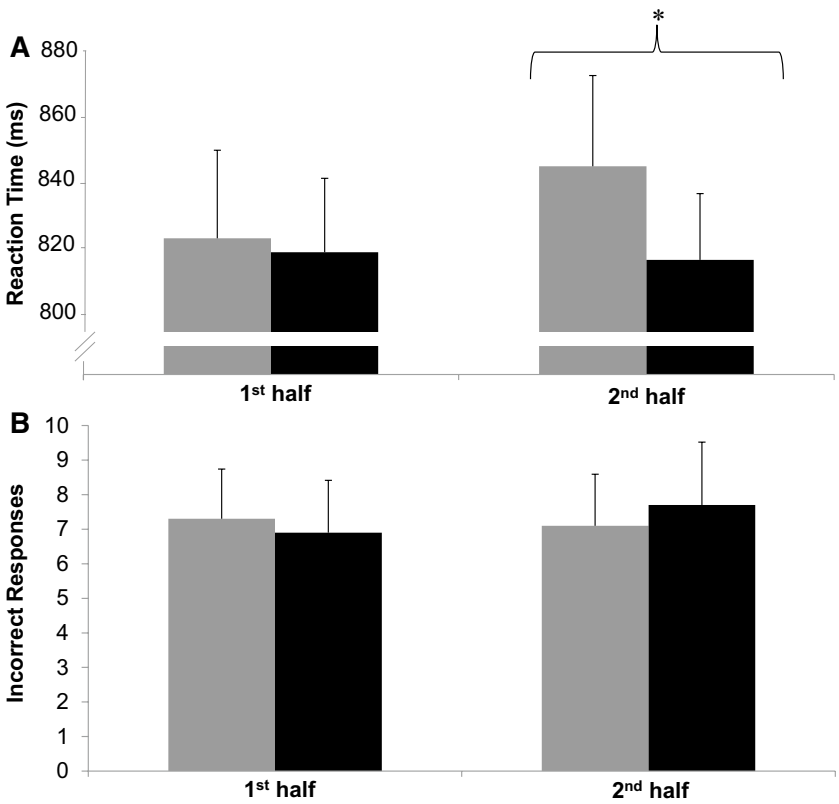

Fig. 4 Combined cognitive task (Stroop and Decision-Reaction) performance in the 1st half vs. 2nd half. **a** Reaction time in the second half was improved in BR (*black bars*) relative to PL (*grey bars*) ($P < 0.05$). **b** There were no differences in accuracy of response between conditions. *Error bars* indicate the SE. *Asterisks* denote statistical significance between conditions ($P < 0.05$)

Influence of dietary nitrate supplementation on repeated sprint performance

Several previous studies have reported that short-term BR supplementation extends time to exhaustion at fixed submaximal exercise intensities (e.g. Bailey et al. 2010a, b; Lansley et al. 2011a) and, more recently, high-intensity intermittent exercise performance (Wylie et al. 2013a). However, the influence of BR supplementation on prolonged intermittent exercise which better reflects the duration and changes of intensity of team sports, i.e. all-out efforts interspersed with extended recovery periods of variable intensity, has not been previously investigated. To assess the utility of BR supplementation in this regard, we used an extension of a previously described intermittent sprint test performed on a cycle ergometer (Bishop and Claudius 2005) in which the repeat sprint–recovery cycles characterise the typical performance profile of team sport athletes (Spencer et al. 2004). A key advantage of the IST is that it permits physical and cognitive challenges to be applied simultaneously in a controlled fashion, although it is acknowledged that the test does not reflect other characteristics of team sports.

It has been reported that NO_3^- supplementation can improve the efficiency of muscular work during submaximal exercise by reducing $\dot{V}O_2$, an effect which may be related to a reduced O_2 cost of mitochondrial ATP resynthesis (Larsen et al. 2011) and/or to a lower ATP cost of muscle contraction (Bailey et al. 2010a). In the present study, $\dot{V}O_2$ in the IST was not different with BR compared to PL. The lack of effect on $\dot{V}O_2$ with BR in the present study may be explained, in part, by the regular fluctuations in exercise intensity (i.e. non-steady-state conditions prevailed), as well as the differences in work done between conditions. The ergogenic basis for the improved sprint performance observed with BR may be better explained by factors unrelated to changes in the efficiency of oxidative metabolism.

The present findings indicate that 7 days of BR supplementation improved total work done in the IST. This is consistent with some (Bond et al. 2012; Wylie et al. 2013a) but not all (Christensen et al. 2013; Martin et al. 2014; Muggeridge et al. 2013) previous findings. The explanation for these contrasting results is unclear; however, these studies differed in terms of the dose and duration of BR supplementation, the nature of the exercise test, and the training status of the participants. Further studies are therefore required to determine the effectiveness of BR ingestion in other situations involving intermittent exercise performance.

Hernández et al. (2012) reported increased expression of the Ca^{2+} handling proteins, calsequestrin and dihydropyridine, in type II skeletal muscle of mice supplemented with sodium nitrate for 7 days. These changes were associated

with increased myoplasmic free Ca^{2+} and improved type II muscle force production at 100 Hz stimulation compared to mice receiving placebo (Hernández et al. 2012). Recently, Haider and Folland (2014) reported enhanced electrically evoked explosive force and peak force production at low frequencies of stimulation in untrained humans following 7 days of BR supplementation. Although voluntary explosive and maximal force production were unaltered by BR, the authors suggested that the subtle changes observed in contractile function may be beneficial for other types of voluntary contractile activity (Haider and Folland 2014). Coggan et al. (2014) have recently reported that acute BR ingestion significantly improved calculated maximal knee extensor power and velocity as measured using isokinetic dynamometry. Given the importance of type II muscle fibres in the performance of high-intensity intermittent exercise (Krustrup et al. 2006), the effects of BR on the contractile function of type II muscle may be an important mechanistic determinant of the improved sprint performance observed in the present study. BR supplementation has also been shown to alter vascular conductance and to elevate microvascular PO_2 in rats (Ferguson et al. 2013, 2014). Specifically, the elevated PO_2 in the microvasculature of type II muscle fibres improves the capillary-myocyte driving pressure for O_2 exchange, thus enhancing oxidative function (Ferguson et al. 2013, 2014). It is possible that elevated O_2 delivery and microvascular PO_2, especially in type II fibres, contributed to the enhanced sprint performance observed with BR compared to PL in the present study.

Influence of dietary nitrate supplementation on cognitive performance

It is well established that, during high-intensity exercise, cognitive abilities diminish and mental fatigue develops (Fery et al. 1997; Iadecola 1993; Reilly and Smith 1986). Given that dietary NO_3^- has been shown to improve neurovascular coupling in response to visual stimuli (Aamand et al. 2013), and also cerebral perfusion (Haskell et al. 2011; Rifkind et al. 2007) specifically to areas responsible for executive function (Presley et al. 2011), we hypothesised that, compared to PL, BR supplementation would improve reaction time and accuracy in the cognitive tasks completed during the IST. Reaction time and response accuracy were measured using cognitive tests validated for the assessment of information processing speed, executive abilities and selective attention (Pachana et al. 2004; Durlach et al. 2002; Irwin et al. 2013).

The results of the present study indicate that BR supplementation attenuated the decline in reaction time in the second half compared to the first half of the IST which was observed with PL. There was no difference in response accuracy between conditions. However, although significant overall improvements in reaction times were observed, when the two cognitive tests employed were examined separately, a significant effect was only observed for the Stroop test. This may arise from the Stroop test placing a greater strain on an individual's limited processing capacity (Besner and Roberts 2005). Thus, BR may protect against fatigue-related reductions in processing capacity when tasks with large cognitive loads are undertaken. From an examination of the temporal changes seen within the Stroop test, it was evident that the greatest improvement in reaction time with BR compared to PL occurred in the final third of each of the two halves of the IST, when any fatigue-related decrement in performance may be expected to be greatest (Fery et al. 1997).

The nitrate–nitrite–NO pathway may be particularly important in hypoxia and at low tissue pH when the activity of the O_2-dependent nitric oxide synthase (NOS) pathway is reduced (Lundberg et al. 2008). BR supplementation may permit greater stability of NO-linked cerebral processes including neurotransmission, vasodilation and neurovascular coupling (Aamand et al. 2013; Iadecola et al. 1999; Rifkind et al. 2007) during situations when NOS activity might be compromised. Indeed, improved regional brain perfusion (Presley et al. 2011) and a reduced cerebral O_2 cost of mental processing without decrements in exercise performance (Thompson et al. 2014) have been reported following NO_3^- supplementation. In light of these earlier findings (Presley et al. 2011; Thompson et al. 2014), it is possible that the difference in response time between BR and PL in the present study may be related to differences in cerebral oxygenation between conditions. It is well known that the hyperventilation that attends high-intensity intermittent exercise will drive down $PaCO_2$ and that this could, in turn, lead to vasoconstriction and compromised cerebral blood flow (Secher et al. 2008). Mechanistically, it is possible that BR acts in opposition to this and facilitates a better maintenance of adequate oxygenation to support cerebral function. From a practical perspective, a novel and potentially important result of the present study is that the decline in cognitive task performance, specifically speed of decision-making, associated with prolonged intermittent exercise, may be offset by BR supplementation.

Influence of dietary nitrate supplementation on plasma $[NO_2^-]$ and $[NO_3^-]$

Seven days of BR supplementation elevated baseline plasma $[NO_2^-]$ and $[NO_3^-]$ by ~350 and ~975 %, respectively. The percentage increases in plasma $[NO_2^-]$ and $[NO_3^-]$ reported herein were approximately double the values reported in some previous studies using NO_3^--rich BR (Bailey et al. 2010a, b; Lansley et al. 2011b; Wylie et al.

2013a). This likely reflects the fact that the dose of NO_3^- administered (~12.8 mmol/day) was also substantially greater than in most previous studies, and supports previous observations on the dose-dependent relationship between NO_3^- supplementation and plasma [NO_2^-] and [NO_3^-] (Wylie et al. 2013b). The effects on cognitive function and sprint performance during intermittent exercise following smaller or larger doses of NO_3^- remain to be investigated.

Interestingly, plasma [NO_2^-] decreased during both the first and second half of the IST in the BR condition, which is consistent with previous findings (Dreissigacker et al. 2010; Wylie et al. 2013a). The difference in plasma [NO_2^-] from baseline to end-exercise was correlated with differences in total work done in the first, but not the second half, of the IST. These findings support the notion that plasma NO_2^- acts as a reservoir for NO production during conditions of low O_2 availability and pH, such as high-intensity intermittent exercise, when NOS activity may be reduced (Lundberg and Weitzberg 2010). However, both absolute and relative changes in plasma [NO_2^-] and [NO_3^-] during exercise and recovery are difficult to interpret due to likely changes in the relative activity of the NOS-dependent and NOS-independent NO production pathways (Kelly et al. 2014). In this regard, plasma [NO_2^-] may be considered to be both a precursor to, and a product of, NO synthesis.

Conclusions

Relative to PL, BR supplementation improved sprint performance during the IST. Moreover, reaction time to the cognitive tasks in the second half of the IST was improved with BR compared to PL with no difference in response accuracy. These findings suggest that BR enhances repeated sprint performance and may attenuate the decline in decision-making reaction time that typically occurs over time during prolonged intermittent exercise which is characteristic of many team sports.

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