



An acute dose of inorganic dietary nitrate does not improve high-intensity, intermittent exercise performance in temperate or hot and humid conditions

Kieran Smith^{1,3} · David J. Muggeridge² · Chris Easton⁴ · Mark D. Ross¹

Received: 10 October 2018 / Accepted: 21 December 2018
© The Author(s) 2019

Abstract

Purpose Dietary nitrate (NO_3^-) has repeatedly been shown to improve endurance and intermittent, high-intensity events in temperate conditions. However, the ergogenic effects of dietary NO_3^- on intermittent exercise performance in hot conditions have yet to be investigated.

Methods In a randomised, counterbalanced, double-blind crossover study, 12 recreationally trained males ingested a nitrate-rich beetroot juice shot (BRJ) (6.2 mmol NO_3^-) or a nitrate-depleted placebo (PLA) (<0.004 mmol NO_3^-) 3 h prior to an intermittent sprint test (IST) in temperate (22 °C, 35% RH) and hot conditions (30 °C, 70% RH). The cycle ergometer IST consisted of twenty maximal 6 s sprints interspersed by 114 s of active recovery. Work done, power output, heart rate and RPE were measured throughout; tympanic temperature was measured prior to and upon completion.

Results There were no significant effects of supplement on sprint performance in either temperate or hot, humid conditions ($p > 0.05$). There was a reduced peak (BRJ: $659 \pm 100\text{W}$ vs. PLA: $693 \pm 139\text{W}$; $p = 0.056$) and mean power (BRJ: $543 \pm 29\text{W}$ vs. PLA: $575 \pm 38\text{W}$; $p = 0.081$) following BRJ compared to PLA in the hot and humid condition, but this was not statistically significant. There was no effect of supplement on total work done irrespective of environmental condition. However, ~75% of participants experienced performance decreases following BRJ in the hot and humid environment. No differences were observed between trials for tympanic temperature measured at the conclusion of the exercise trial.

Conclusion In conclusion, an acute dose of inorganic dietary NO_3^- does not improve repeated-sprint performance in either temperate, or hot and humid conditions.

Keywords Nitrate · Exercise · Heat · High-intensity · Beetroot juice · Heat · Humidity

Abbreviations

BRJ Beetroot juice

HR Heart rate

IST Intermittent sprint test

NO Nitric oxide

NO_2^- Nitrite

NO_3^- Nitrate

PLA Placebo

RER Respiratory exchange ratio

RPE Rating of perceived exertion

T_C Core temperature

T_{sk} Skin temperature

TT Time trial

T_{TYMP} Tympanic temperature

Communicated by Michael Lindinger.

✉ Mark D. Ross
M.Ross@napier.ac.uk

¹ School of Applied Sciences, Edinburgh Napier University, Sighthill Campus, Edinburgh, UK

² Active Health Exercise Laboratory, Division of Biomedical Sciences, Institute of Health Research and Innovation, University of the Highlands and Islands, Inverness, UK

³ Institute of Cellular Medicine, Faculty of Medical Sciences, Newcastle University, Newcastle upon Tyne, UK

⁴ Institute for Clinical Exercise and Health Science, University West of Scotland, Hamilton, UK

Introduction

Nitric oxide (NO) is a gaseous signalling compound associated with a plethora of physiological effects including modulating contractile properties of skeletal muscle (Ferguson et al. 2013), mitochondrial efficiency (Clerc et al.

2007; Heinonen et al. 2011) and peripheral/cutaneous blood flow (Lundberg et al. 2008). Circulating NO in the blood is short-lived and rapidly oxidised to nitrite (NO_2^-) and nitrate (NO_3^-). NO_3^- is also known to be stored within skeletal muscle (Piknova et al. 2015) and the skin. Collectively they may act as a reservoir to ensure NO bioactivity is available during conditions of low pO_2 (Lundberg et al. 2008), such as during intense physical exercise.

Dietary NO_3^- has been shown to be effective at increasing circulating plasma NO_2^- and NO_3^- that coincides with improvement in indices of performance during cycling time trials (TT) (Cermak et al. 2012a; Lansley et al. 2011; Muggeridge et al. 2014), supra-maximal intensity cycling (Aucouturier et al. 2015) and explosive running (Sandbakk et al. 2015). This has been attributed to a reduced ATP cost during muscular contractions (Bailey et al. 2010) and potentially reduced $\dot{V}\text{O}_2$ for mitochondrial ATP resynthesis, although the latter has failed to be confirmed more recently (Whitfield et al. 2015). However, some studies show that inorganic dietary NO_3^- has been ineffective at improving performance (Cuenca et al. 2018; Sandbakk et al. 2015; Cermak et al. 2012b), which could be attributed to altered oral microbiota important for the initial conversion of NO_3^- to NO_2^- (Burleigh et al. 2018), chronic vs. acute dosages (Vanhatalo et al. 2010; Boorsma et al. 2014) and the level of athlete investigated, with those towards elite showing less of an ergogenic aid of nitrate than less-trained individuals (Porcelli et al. 2015).

NO-mediated physiological signalling following NO_3^- supplementation is potentiated as the O_2 (Castello et al. 2006) and pH (Modin et al. 2001) tension declines, therefore, NO_3^- should in theory be more effective in high-intensity exercise as it creates favourable physiological conditions for NO production (Richardson et al. 1995). Dietary NO_3^- supplementation has been reported to elevate skeletal muscle O_2 delivery (Ferguson et al. 2013) and enhance sarcoplasmic calcium handling in fast twitch type II muscle fibres (Hernandez et al. 2012) translating to increased force production (Coggan et al. 2015). As such, high-intensity physical activities are more likely to increase NO synthesis from stored NO_3^- reservoirs, and thus, improve performance (Wylie et al. 2016).

Exercise in the heat poses a formidable challenge to the body's ability to control its internal environment through heat gain from external temperatures and high rates of metabolic heat production (Maughan and Shirreffs 2004). Given that cutaneous vasodilation is critical for the maintenance of a stable core temperature (T_c), the role of dietary NO_3^- supplementation in the heat warrants investigation. Indeed, the effect of dietary NO_3^- supplementation on exercise performance in heat has recently been investigated in one non-athletic population (Kuennen et al. 2015) and in three studies of well-trained cyclists (Kent et al. 2018a, b;

McQuillan et al. 2018). Following a moderate dose of inorganic dietary NO_3^- ($8.3 \text{ mmol NO}_3^- \cdot \text{d}^{-1}$) for 6 days, Kuennen et al. (2015) observed a reduced O_2 cost of a 45 min loaded march in a hot and humid environment compared to a PLA. Interestingly, it was shown that dietary NO_3^- supplementation increased subject's T_c , a finding that was later replicated during a 4 km cycling TT in hot conditions (McQuillan et al. 2018). This may be due to elevated gastrointestinal blood perfusion, which may enhance thermal transfer during exercise in the heat. Additionally, the improved workload of the skeletal muscles could cause a subsequent 'overspill' of metabolic heat. However, this has most recently been disputed, where dietary NO_3^- regimens have not influenced cycling TT performance (Kent et al. 2018b) or thermoregulatory responses in young adults (Amano et al. 2018) and elite cyclists (Kent et al. 2018a).

The prospective notion that dietary NO_3^- supplementation alters heat tolerance is yet to be fully understood, where its effect on intermittent, sporting performance in trained individuals is yet to be investigated in hot conditions. As such, this investigation aimed to investigate whether an acute dose of inorganic dietary NO_3^- would elicit performance benefits in recreationally trained males during an intermittent high-intensity exercise cycling protocol in temperate as well as in hot and humid conditions, with a potential improvement in performance resulting from an enhanced tissue and skin perfusion, resulting in enhanced O_2 delivery, and heat dissipation. It was hypothesised that high-intensity, intermittent performance (mean and peak power; total work done) in the heat would improve following dietary NO_3^- supplementation compared to a placebo in both conditions.

Materials and methods

Participants

Twelve recreationally trained male university students (22 ± 4 years, 1.81 ± 0.06 cm, 80.43 ± 5.84 kg, $46.11 \pm 6.42 \text{ ml kg}^{-1} \text{ min}^{-1}$) volunteered to participate in the study. All participants had a history of competing at a high standard of team sports and had been training ≥ 2 times per week for at least 1 year. Participants gave their written consent prior to participation and all risks and potential benefits were fully explained prior to. The procedures employed in this study and risks were accepted in adherence to Edinburgh Napier University's ethical committee and conformed to the code of ethics of the Declaration of Helsinki.

Experimental design

Participants reported to the laboratory on five separate occasions. During the first visit, participants performed a ramp incremental test for assessment of $\dot{V}O_2$ peak (see “Assessment of peak oxygen uptake”). After 20 min of recovery, participants then performed 10 min of the intermittent sports test (IST) in temperate conditions for individual gear calibration for the subsequent incremental exercise tests using a magnetically braked cycle ergometer (Velotron Pro, RacerMate Inc, USA). The 10 min IST required participants to perform five 2 min blocks (114 s of active recovery cycling at 100W maintaining 60 rpm and 6 s maximal sprint). Participants were asked during this session if they felt they could replicate this intensity for the full 40 min IST, following their response amendments were made to their gearing for the active recovery and maximal effort bouts.

Following completion of the preliminary testing, participants were assigned in a randomised, counterbalanced, double-blind, crossover experimental design to receive either an acute dose of NO_3^- -rich beetroot juice shot (BRJ: 6.2 mmol NO_3^-) or a NO_3^- -depleted placebo (PLA: <0.004 mmol NO_3^-), which they would ingest 3 h prior to the IST in temperate (22 °C, 35% RH) and hot conditions (30 °C, 70% RH). This dose of BRJ has been shown previously to improve exercise performance if ingested 2.5–3 h prior to exercise (Thompson et al. 2014; Hoon et al. 2014; Lansley et al. 2011). Randomisation was performed using an online programme, blinded to the researchers. At least 4–7 days separated each IST allowing for optimal recovery and supplement washout for circulating plasma NO_3^- ($[\text{NO}_3^-]$) and $[\text{NO}_2^-]$ levels to return to baseline (Wylie et al. 2013).

Prior to participation, all participants were instructed to fill out a food-screening questionnaire, detailing how often they ate certain foods and in what portion size. Participants were also asked to record their food intake 24 h prior to testing and were instructed to try and replicate this before subsequent sessions. All participants were given information regarding what foods contain the highest amount of $\text{NO}_3^- \cdot \text{g}^{-1}$ and to avoid consuming in high doses for the duration of the testing period. Participants were instructed to arrive to the laboratory in a fully rested, hydrated state at least 3 h postprandial and were advised to avoid any strenuous activity in the 24 h preceding each testing sessions. Caffeine and alcohol were to be refrained from consumption 6 h and 24 h, respectively, before each laboratory visit. Participants were also asked to abstain from antibacterial mouthwash and chewing gum use around supplement ingestion and experimental trials as these products have been previously shown to blunt the reduction of NO_3^- to NO_2^- in the oral cavity (Govoni et al. 2008). Testing all took place at the same time of day (± 3 h).

Assessment of peak oxygen uptake

A $\dot{V}O_2$ peak test to volitional exhaustion was performed on a Velotron Pro (RacerMate Inc, USA) cycle ergometer using a breath-by-breath gas analyser (CPX Jaeger, Germany), which monitored $\dot{V}O_2$, $\dot{V}CO_2$, and respiratory exchange ratio (RER). Participants warmed up for 5 min, cycling at an initial power output of 60W at 60–80 rpm. Following the warm up, in 1-min increments, resistance was increased by 30W until participants could no longer complete the 1-min step at 60–80 rpm or when they felt they could go on no further. $\dot{V}O_2$ peak was taken as the highest mean-value attained during the final 30 s of exercise. HR was monitored throughout (Polar RS400 Heart Rate Monitors, Polar, Finland).

Intermittent sport test (IST)

The IST was based on a motion analysis study of international field hockey players (Spencer et al. 2004) and is an abstract of the protocol previously described by Bishop and Claudius (Bishop and Claudius 2005). The IST, like the familiarisation and $\dot{V}O_2$ peak session was performed on a Velotron Pro (Racer Mate, USA) cycle ergometer. All IST sessions took place in an environmental chamber (Weiss Gallenkamp, UK) in both temperate (22 °C, 35% RH) and hot and humid conditions (30 °C, 70% RH). Mean and peak power, work done, HR, and RPE were recorded after every sprint of the IST. Fatigue index per sprint was determined as: (maximum power – minimum power)/maximum power. Participant tympanic temperature (T_{TYMP}) was measured upon commencement and immediately upon completion of the IST using a thermometer placed in the cavity of the ear (Braun IRT 4520, Braun ThermoScan, Germany).

Before the onset of the IST, a standardised warm up was completed comprising of cycling for 5-min at 100W at 60 rpm followed by a 2 min practice block of the IST. The 40 min IST replicates the duration of ‘one half’ of a rugby or hockey match, which was broken down into twenty \times 2 min blocks consisting of a maximal 6 s sprint followed by 114 s active recovery. Participants were able to drink water *ad libitum*. The fixed resistance during the active recovery and maximum effort sprints were individually determined during the familiarisation session.

Supplementation

Participants were randomly allocated in a crossover manner to consume either NO_3^- -rich BRJ (6.2 mmol NO_3^- per 70 ml; Beet it, James White Drinks Ltd, United Kingdom) or a nitrate-depleted PLA (<0.004 mmol NO_3^- per 70 ml; Beet it, James White Drinks Ltd) shot identical in appearance and taste, administered in a double-blind fashion. Participants consumed their supplements 3 h prior to either the IST.

Three hours prior to testing was chosen as pharmacokinetic data suggest that $[\text{NO}_2^-]$ will be at its peak after a single dose of BRJ (Webb et al. 2008).

Statistical analysis

All data were assessed for normal distribution. Data that were not normally distributed were logarithmically transformed (Log10). Paired sample *T*-tests were performed to compare the mean values of HR, delta T_{TYMP} , peak power, mean power and mean work done per sprint and total work done during the IST between supplements (BRJ vs. PLA). The effect of inorganic dietary NO_3^- on work done, power output, HR, RPE over the duration of the IST were analysed by a two-way repeated measures analysis of variance (ANOVA; time/sprint \times condition). Cohen's effect size (*d*) was calculated and expressed as: small effect >0.2 ; medium effect >0.5 ; large effect >0.8 . Inferential statistical analysis was conducted using the software package IBM SPSS Statistics (IBM Corp, USA). Data are presented as mean \pm standard deviation (SD) unless stated otherwise. Significance was set at $\alpha \leq 0.05$.

Results

Physiological and perceptual responses

Upon termination of the IST, there were no differences in T_{TYMP} between BRJ and PLA in both temperate (BRJ: 35.8 ± 0.8 °C vs. PLA: 35.9 ± 0.5 °C, $p=0.78$) and in the heat (BRJ: 37.3 ± 0.6 °C vs. PLA: 37.2 ± 0.6 °C, $p=0.93$). Similarly, the increase in T_{TYMP} following the IST was not different between supplements (temperate: ΔBRJ : 0.57 ± 1.1 °C vs ΔPLA : 0.68 ± 0.33 °C; $p=0.74$; heat: ΔBRJ : 1.49 ± 0.61 °C vs ΔPLA : 1.38 ± 0.7 °C; $p=0.37$). There were also no differences in HR or RPE between supplements during the IST temperate (HR-BRJ: 151 ± 14 bpm vs. PLA: 151 ± 12 bpm; $p=0.94$; RPE-BRJ: 14 ± 1 vs. PLA: 14 ± 2 , $p=0.99$). and in hot, humid conditions (HR-BRJ: 152 ± 17 bpm vs. PLA: 152 ± 16 bpm; $p=0.41$; RPE-BRJ: 14 ± 1 vs. PLA: 14 ± 1 , $p=0.74$).

Intermittent exercise performance

There was no effect of dietary NO_3^- ingestion on IST performance measures in temperate conditions (mean power production; BRJ: $562 \pm 120\text{W}$, PLA: $571 \pm 124\text{W}$, $p=0.433$; total work done: BRJ: 67.44 ± 14.39 kJ, PLA: 68.46 ± 15.07 kJ, $p=0.447$; Fig. 1). Mean power produced per sprint and total work done was reduced in BRJ than PLA in the heat, but these were not statistically significant differences (mean power production; BRJ:

$543 \pm 29\text{W}$, PLA: $575 \pm 39\text{W}$, $p=0.081$; total work done: BRJ: 66.07 ± 10.84 kJ, PLA: 69.74 ± 15.13 kJ, $p=0.101$; Fig. 2). There was a trend for dietary NO_3^- supplementation to reduce mean peak power production during the IST in the heat which neared statistical significance ($p=0.056$; $d=0.28$) compared to the PLA (Fig. 2). On average, peak power production in the heat was $\sim 6\%$ lower following BRJ ($659 \pm 100\text{W}$) compared to PLA ($683 \pm 139\text{W}$) (Fig. 2a, b).

There were no significant condition and sprint interactions for mean power production in both temperate ($F_{(19, 209)}=0.476$, $p=0.971$; Fig. 3a) and hot ($F_{(19, 209)}=1.147$, $p=0.306$; Fig. 3b) conditions. There was a trend for a lower mean power production per sprint following the BRJ ($543 \pm 29\text{W}$) supplement compared to the PLA in the hot condition ($575 \pm 38\text{W}$; $p=0.081$; $d=0.34$) (Fig. 2b).

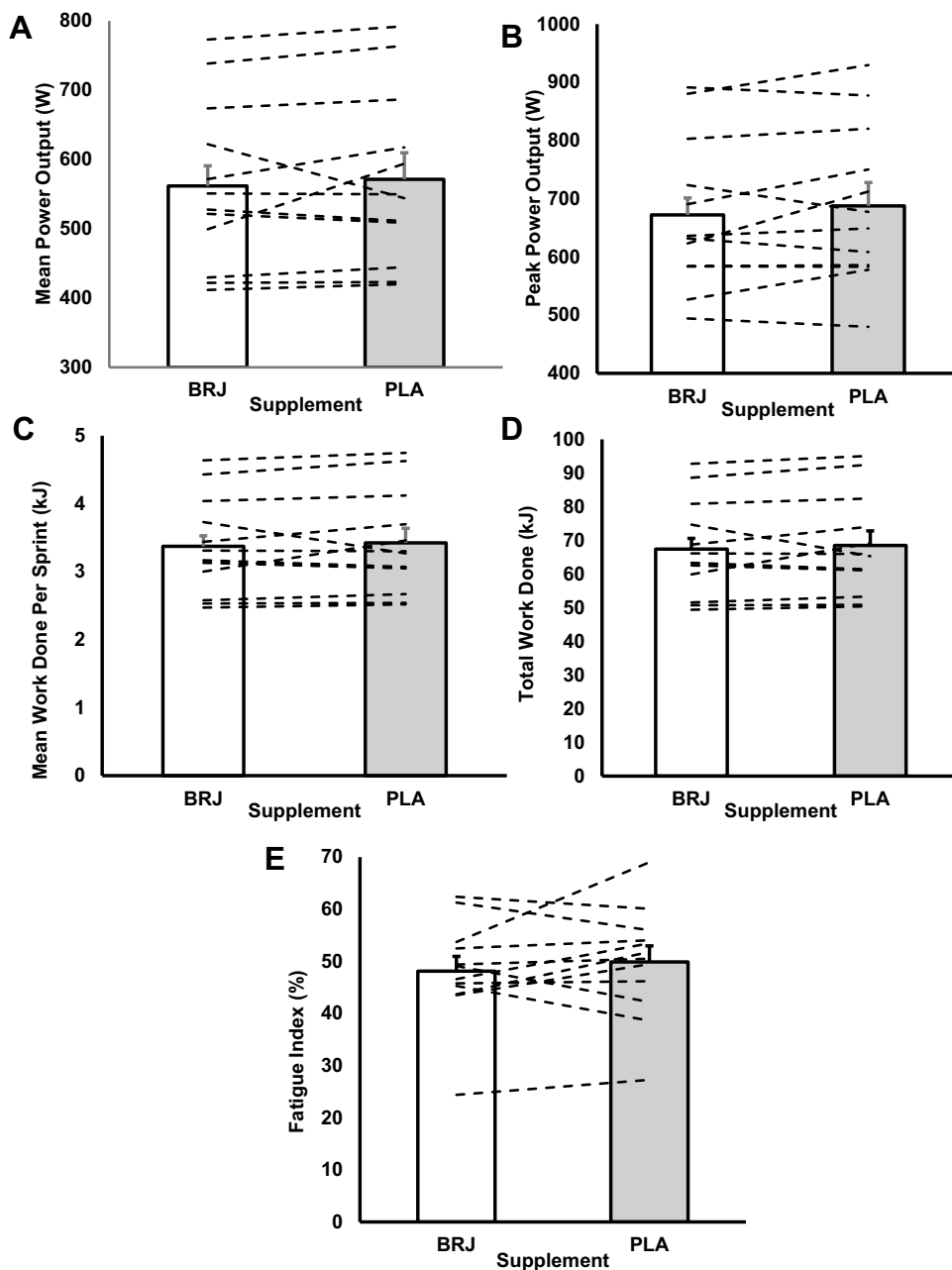
Likewise, no condition \times sprint interaction effect was shown within mean work done for both temperate ($F_{(19, 209)}=0.498$, $p=0.963$; Fig. 4a) and hot conditions ($F_{(19, 209)}=1.062$, $p=0.392$; Fig. 4b). Mean work done per sprint was not different between supplements in either temperate ($p=0.45$, $d=0.07$; Fig. 1c) or hot conditions ($p=0.12$, $d=0.26$; Fig. 2c). BRJ did not influence total work done completed over the IST (temperate-BRJ: 67.44 ± 14.39 kJ vs. PLA: 68.46 ± 15.07 kJ) ($p=0.447$, $d=0.07$; Fig. 1d; hot-BRJ: 66.07 ± 10.84 kJ vs. PLA: 69.74 ± 15.13 kJ) ($p=0.101$, $d=0.28$; Fig. 2d). In addition, there was no difference in fatigue index between supplements (temperate-BRJ: $48.14 \pm 9.77\%$ vs. PLA: $49.89 \pm 10.67\%$; $p=0.38$ Fig. 1e; hot-BRJ: $50.51 \pm 9.20\%$ vs. PLA: $50.49 \pm 13.51\%$; $p=0.99$ Fig. 2e).

Discussion

This is the first study to investigate the effect of dietary NO_3^- supplementation on intermittent, high-intensity performance in both temperate as well as hot and humid conditions. Dietary NO_3^- did not influence cardiovascular, perceptual or thermoregulatory responses to the exercise protocol, and appeared to impair some indices of performance, however this was only in the hot and humid condition, and was not statistically significant. This contrasts with previous research in temperate conditions which typically demonstrates that NO_3^- is ergogenic for high-intensity intermittent exercise performance (Thompson et al. 2015, 2016; Wylie et al. 2013, 2016), with only one other study showing no effect of an acute NO_3^- dose on intermittent exercise performance (Martin et al. 2014).

The present investigation included 12 recreationally trained males, where following the ingestion of ~ 6 mmol NO_3^- , there was a trend for lower peak power ($p=0.056$) and mean power production per sprint ($p=0.081$) compared

Fig. 1 Mean peak power output (a), mean power production (b) and mean work done (c) produced per sprint during the intermittent sprint test (IST) in temperate conditions after ingesting either nitrate-rich beetroot juice (BRJ) or placebo (PLA). **d** Illustrates total work done across the twenty 6 s sprints during the IST and **e** represents fatigue index between trials. Dashed lines represent individual participant response. Data are presented as mean \pm SEM

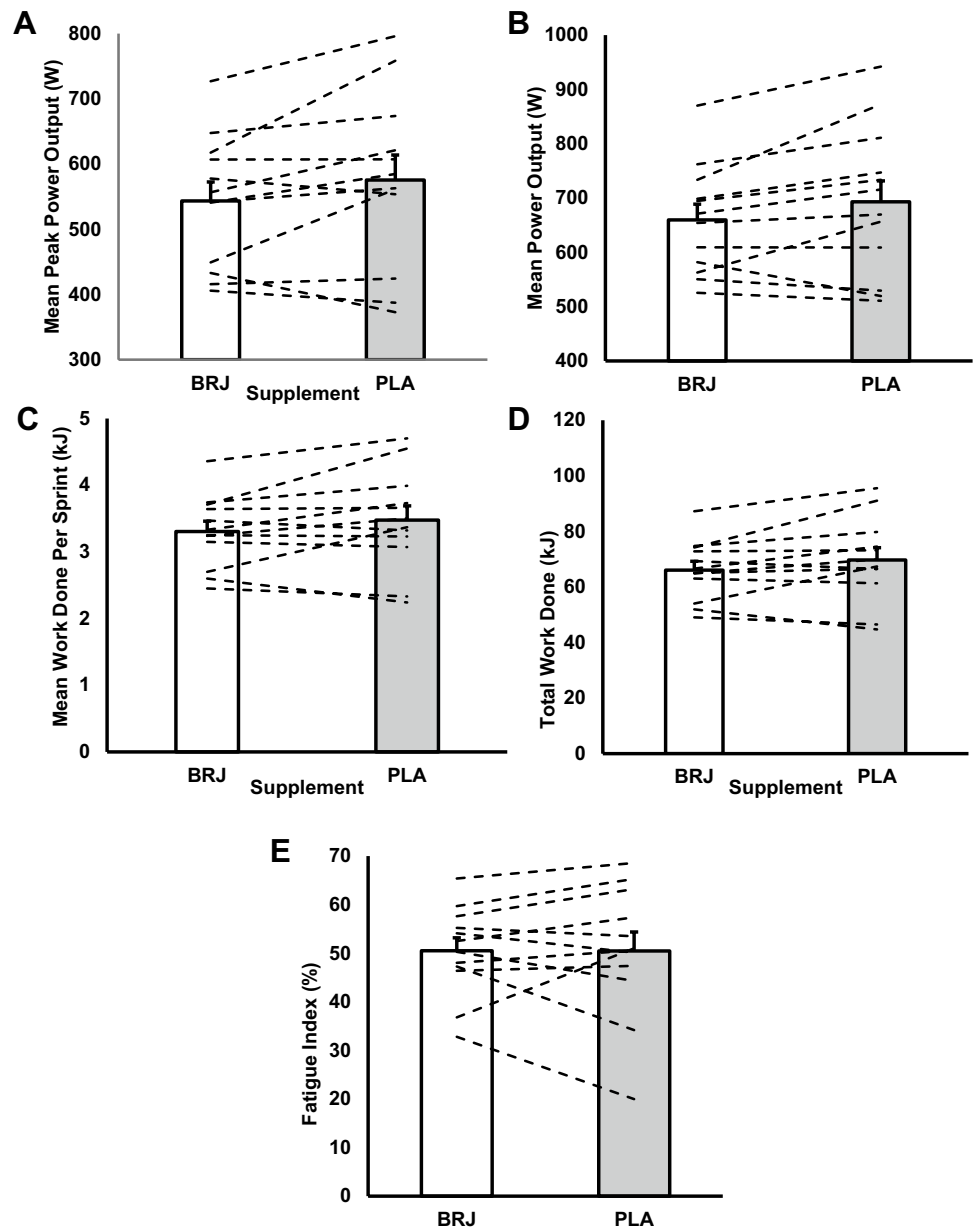


to the PLA trial in the hot condition only, with no such trend in temperate conditions. The reduction in power output with nitrate in the heat was observed in 8 out of the 12 participants, with the remaining four showing either no change, or slight improvement in power output (example figure provided; Fig. 5a–d). This is the first investigation to reveal such potential negative results following dietary NO_3^- supplementation within a recreationally trained population, and appears to be only present in hot and humid conditions. In fact, lower doses of NO_3^- (5–6 mmol NO_3^-) have produced favourable improvements in mean and peak power production during a 30 s Wingate test (Cuenca et al. 2018; Dominguez et al. 2017) and in cycling TT performances

in both simulated altitude (Muggeridge et al. 2014) and normoxic conditions (Lansley et al. 2011). Speculatively, disparities between studies may be explained by the environmental conditions, where exercise in heat increases sympathetic nervous activity (Drust et al. 2005) influencing muscle metabolism (Febbraio et al. 1994) and vascular control (Johnson 2010).

Increases in muscle temperature can improve cross-bridge cycling rates (Karatzafieri et al. 2004) and sprint performance through enhancements in muscle fibre conductance (Girard et al. 2013; Gray et al. 2006). When recovery periods are long enough to allow for complete recovery between short duration sprints and in the absence of hyperthermia,

Fig. 2 Mean peak power output (a), mean power production (b) and mean work done (c) produced per sprint during the intermittent sprint test (IST) in the heat after ingesting either nitrate-rich beetroot juice (BRJ) or placebo (PLA). **d** Illustrates total work done across the twenty 6 s sprints during the IST and **e** represents Fatigue Index between trials. Dashed lines represent individual participant response. Data are presented as mean \pm SEM



there is little evidence to suggest hyperthermic conditions are detrimental to repeated-sprint performance compared to temperate conditions (Almudehki et al. 2012; Girard et al. 2013). Interestingly, we show that peak power production was lower following the BRJ supplement compared to PLA, nearing statistical significance ($p=0.056$). Given type II muscle fibres are extensively recruited during shorter sprints compared to longer maximal efforts (Casey et al. 1996; Gray et al. 2008) and the known preferential NO_3^- -treatment fibre effects (Jones et al. 2016), such as preferential increases in blood flow to type II fibres (Ferguson et al. 2013), our findings are in stark contrast to previous literature within temperate environments (Thompson et al. 2015, 2016; Wylie et al. 2013, 2016), but the addition of a heat stress,

as provided in our study, may compromise the ergogenic impact of inorganic NO_3^- on performance.

It has been reported that dietary NO_3^- supplementation augments an increase in T_c during exercise in the heat (Kuennen et al. 2015; McQuillan et al. 2018). The authors postulate that these effects may be specifically induced in metabolically active muscles, overriding the sympathetic vascular response in the skin that allows redistribution of blood flow to dissipate heat from the body (Crandall and Gonzalez-Alonso 2010). Whilst we displayed that T_{TYMP} rose to a similar extent in the BRJ and PLA conditions, it is plausible this may not fully represent the thermoregulatory responses our subjects may have experienced. Indeed, T_{TYMP} has been revealed to underestimate T_c during exercise

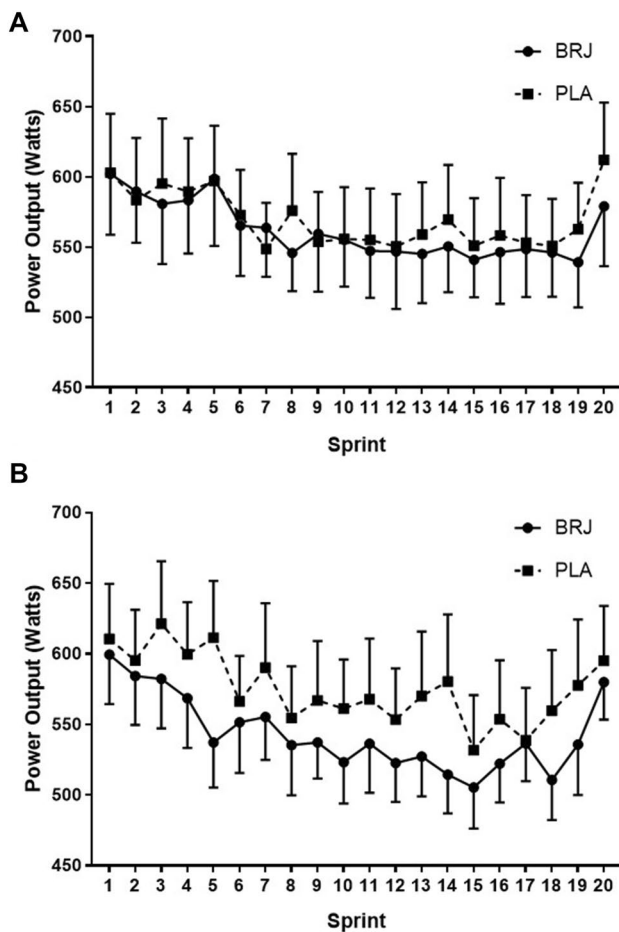


Fig. 3 Mean power output during the intermittent sprint test (IST) following the nitrate-rich beetroot juice (BRJ; solid) and placebo (PLA; dashed) supplements in temperate (a) and hot and humid (b) conditions. Data are presented as mean \pm SEM

in heat (Huggins et al. 2012). As such, T_{TYMP} measurements in this study may not accurately reflect any changes in T_c in our experiment. Increases in T_c during hyperthermic exercise creates a simultaneous demand for blood flow between active skeletal tissues, the skin and vital organs (Kent et al. 2018a); thus, influencing muscle metabolism and oxidative function (Febbraio et al. 1994), and subsequently limiting exercise performance (Drust et al. 2005).

Following local and whole-body heating, BRJ increases cutaneous vasodilation through NO-induced vasodilation despite not influencing skin blood flow suggesting no improved thermoregulatory benefit (Keen et al. 2015; Levitt et al. 2015). However, it has been reported that NO-dependent cutaneous vasodilation is diminished during high-intensity exercise in heat (Fujii et al. 2014). Given power output and total work done was lower in 19 out of the 20 sprints during the BRJ trial compared to the PLA in the heat (Figs. 3b, 4b), blood flow may have been preferentially distributed to other surrounding tissues or other neural

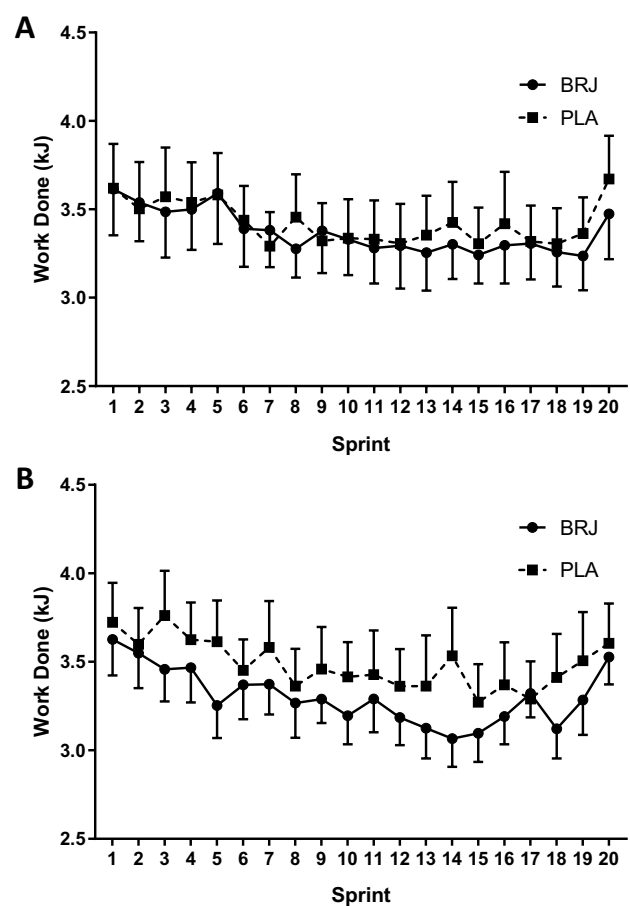


Fig. 4 Mean Work Done per Sprint during the intermittent sprint test (IST) following the nitrate-rich beetroot juice (BRJ; solid) and placebo (PLA; dashed) supplements in temperate (a) and hot and humid (b) conditions. Data are presented as mean \pm SEM

thermoregulatory factors were at work (Drust et al. 2005; Febbraio et al. 1994). However, neither T_c , T_{sk} , peripheral nor muscle blood flow were measured in the present investigation leaving this open for future debate.

Considerations

Larger or loaded dosages of inorganic dietary NO_3^- have been consistently shown to improve repeated-sprint performance of short durations (Thompson et al. 2015; Wylie et al. 2013, 2016)—a hallmark of invasion team sports (Mohr et al. 2003; Spencer et al. 2004). However, we showed that the ingestion of dietary NO_3^- 3 h prior to an IST in heat non-significantly reduced performance by 4–6%, which may represent a substantial performance detriment on the field of play. Despite this, our lack of benefit in the temperate conditions may be due to insufficient dose for this exercise mode, however, this dose did still correspond to a small reduction in performance indices in intermittent sprint activity in hot

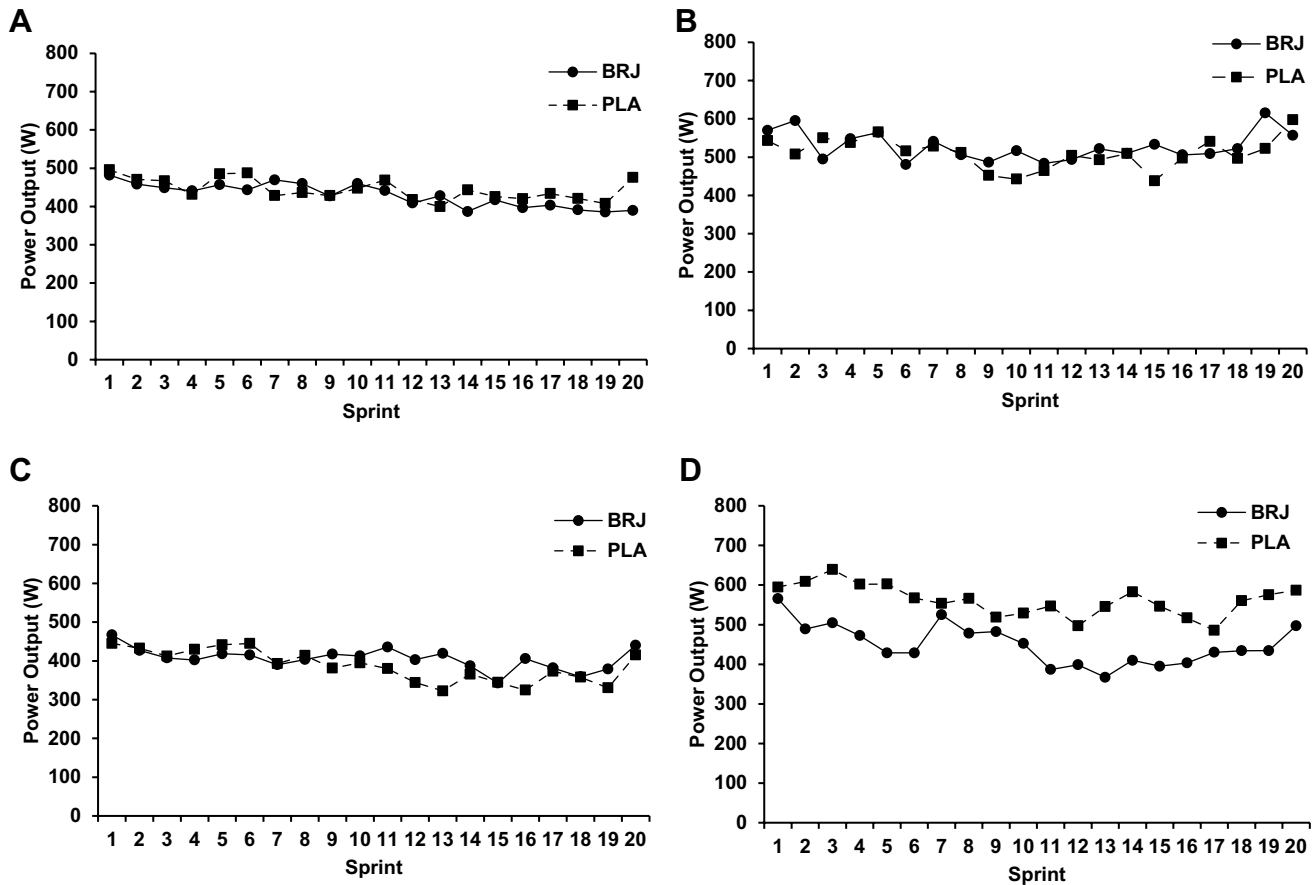


Fig. 5 Mean Power Output during the intermittent sprint test (IST) following the nitrate-rich beetroot juice (BRJ; solid) and placebo (PLA; dashed) supplements in different participants. Example of one of four participants who showed little or no change in performance

indices in the IST in temperate (a) and hot, humid conditions (c), and one example of the 8 participants who displayed decrements in performance in IST with BRJ supplementation (temperate: b, hot, humid: d)

and humid conditions, as seen in this study. While these data seem highly relevant for competitive team sport athletes, they must be interpreted with caution. Our analysis was conducted on a small sample ($n = 12$) and differences between BRJ and PLA conditions were small and did not reach statistical significance, despite observing trends for impaired performance in the heat with BRJ supplementation. However, our findings of a potential negative impact of BRJ on performance in the heat, along with an absence of such negative impacts in ambient conditions, we can suggest that dietary NO_3^- may impair high-intensity exercise performance in a recreationally trained population.

In addition, our measures of thermoregulation (T_{TYP}) are insufficient to fully understand the impact of nitrate on thermoregulation in hot and humid environmental conditions. Therefore future studies should employ more accurate measures of thermoregulatory strain, such as T_c , T_{sk} , sweat rate, muscle and skin blood flow. As a result of the small sample size, and the insufficient thermoregulatory measures, we are unable to specifically determine the physiological

mechanisms that underpin this potential negative impact of BRJ on high-intensity exercise in the heat.

Conclusions

This study demonstrated that relative to the PLA, BRJ does not offer any beneficial aid to high-intensity, repeated-sprint performance in both ambient and hot conditions, but may be detrimental in the heat as demonstrated in our performance indices. However, with the trend of an acute dose potentially being ergolytic in hot and humid environments, the more common dietary NO_3^- supplementary regimes of loading are postulated to be detrimental to repeated-sprint performance in heat through alterations in thermoregulatory responses and/or reductions in skeletal muscle blood flow. As such, we do not recommend athletes ingest dietary NO_3^- supplements prior to high-intensity exercise in the heat.

Author contributions KS, MR designed the study. KS undertook data collection. KS, MR, DM analysed the data. KS, MR wrote the manuscript. KS, MR, DM, CE reviewed the data and the manuscript. All authors read and approved of the manuscript.

Funding No funding was received for this project.

Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest.

OpenAccess This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Almudehki F, Girard O, Grantham J, Racinais S (2012) Hot ambient conditions do not alter intermittent cycling sprint performance. *J Sci Med Sport* 15(2):148–152. <https://doi.org/10.1016/j.jsams.2011.07.009>
- Amano T, Okushima D, Breese BC, Bailey SJ, Koga S, Kondo N (2018) Influence of dietary nitrate supplementation on local sweating and cutaneous vascular responses during exercise in a hot environment. *Eur J Appl Physiol* 118(8):1579–1588. <https://doi.org/10.1007/s00421-018-3889-9>
- Aucouturier J, Boissiere J, Pawlak-Chaouch M, Cuvelier G, Gamelin FX (2015) Effect of dietary nitrate supplementation on tolerance to supramaximal intensity intermittent exercise. *Nitric Oxide* 49:16–25. <https://doi.org/10.1016/j.niox.2015.05.004>
- Bailey SJ, Fulford J, Vanhatalo A, Winyard PG, Blackwell JR, DiMenna FJ, Wilkerson DP, Benjamin N, Jones AM (2010) Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans. *J Appl Physiol* 109(1):135–148. <https://doi.org/10.1152/jappphysiol.00046.2010>
- Bishop D, Claudius B (2005) Effects of induced metabolic alkalosis on prolonged intermittent-sprint performance. *Med Sci Sports Exerc* 37(5):759–767
- Boorsma R, Whitfield R, Spriet LL (2014) Beetroot Juice supplementation does not improve performance of elite 1500-m runners. *Med Sci Sports Exerc* 46(12):2326–2334. <https://doi.org/10.1249/mss.0000000000000364>
- Burleigh MC, Liddle L, Monaghan C, Muggeridge DJ, Sculthorpe N, Butcher JP, Henriquez FL, Allen JD, Easton C (2018) Salivary nitrite production is elevated in individuals with a higher abundance of oral nitrate-reducing bacteria. *Free Radic Biol Med* 120:80–88. <https://doi.org/10.1016/j.freeradbiomed.2018.03.023>
- Casey A, Constantin-Teodosiu D, Howell S, Hultman E, Greenhaff PL (1996) Metabolic response of type I and II muscle fibers during repeated bouts of maximal exercise in humans. *Am J Physiol* 271(1 Pt 1):E38–E43. <https://doi.org/10.1152/ajpenn.1996.271.1.E38>
- Castello PR, David PS, McClure T, Crook Z, Poyton RO (2006) Mitochondrial cytochrome oxidase produces nitric oxide under hypoxic conditions: implications for oxygen sensing and hypoxic signaling in eukaryotes. *Cell Metab* 3(4):277–287. <https://doi.org/10.1016/j.cmet.2006.02.011>
- Cermak NM, Gibala MJ, van Loon LJ (2012a) Nitrate supplementation's improvement of 10-km time-trial performance in trained cyclists. *Int J Sport Nutr Exerc Metab* 22(1):64–71. <https://doi.org/10.1123/ijsnem.22.1.64>
- Cermak NM, Res P, Stinkens R, Lundberg JO, Gibala MJ, van Loon LJ (2012b) No improvement in endurance performance after a single dose of beetroot juice. *Int J Sport Nutr Exerc Metab* 22(6):470–478. <https://doi.org/10.1123/ijsnem.22.6.470>
- Clerc P, Rigoulet M, Leverve X, Fontaine E (2007) Nitric oxide increases oxidative phosphorylation efficiency. *J Bioenerg Biomembr* 39(2):158–166. <https://doi.org/10.1007/s10863-007-9074-1>
- Coggan AR, Leibowitz JL, Spearie CA, Kadkhodayan A, Thomas DP, Ramamurthy S, Mahmood K, Park S, Waller S, Farmer M, Peterson LR (2015) Acute dietary nitrate intake improves muscle contractile function in patients with heart failure: a double-blind, placebo-controlled, randomized trial. *Circ Heart Fail* 8(5):914–920. <https://doi.org/10.1161/CIRCHEARTFAILURE.115.002141>
- Crandall CG, Gonzalez-Alonso J (2010) Cardiovascular function in the heat-stressed human. *Acta Physiol (Oxf)* 199(4):407–423. <https://doi.org/10.1111/j.1748-1716.2010.02119.x>
- Cuenca E, Jodra P, Perez-Lopez A, Gonzalez-Rodriguez LG, Fernandes da Silva S, Veiga-Herreros P, Dominguez R (2018) Effects of beetroot juice supplementation on performance and fatigue in a 30-s all-out sprint exercise: a randomized, double-blind cross-over study. *Nutrients* 10(9):1222. <https://doi.org/10.3390/nu10091222>
- Dominguez R, Garnacho-Castano MV, Cuenca E, Garcia-Fernandez P, Munoz-Gonzalez A, de Jesus F, Lozano-Estevan MDC, Fernandes da Silva S, Veiga-Herreros P, Mate-Munoz JL (2017) Effects of beetroot juice supplementation on a 30-s high-intensity inertial cycle ergometer test. *Nutrients* 9(12):1360. <https://doi.org/10.3390/nu9121360>
- Drust B, Rasmussen P, Mohr M, Nielsen B, Nybo L (2005) Elevations in core and muscle temperature impairs repeated sprint performance. *Acta Physiol Scand* 183(2):181–190. <https://doi.org/10.1111/j.1365-201X.2004.01390.x>
- Febbraio MA, Snow RJ, Hargreaves M, Stathis CG, Martin IK, Carey MF (1994) Muscle metabolism during exercise and heat stress in trained men: effect of acclimation. *J Appl Physiol* (1985) 76(2):589–597. <https://doi.org/10.1152/jappphysiol.1994.76.2.589>
- Ferguson SK, Hirai DM, Copp SW, Holdsworth CT, Allen JD, Jones AM, Musch TI, Poole DC (2013) Impact of dietary nitrate supplementation via beetroot juice on exercising muscle vascular control in rats. *J Physiol* 591(2):547–557. <https://doi.org/10.1113/jphysiol.2012.243121>
- Fujii N, McGinn R, Stapleton JM, Paull G, Meade RD, Kenny GP (2014) Evidence for cyclooxygenase-dependent sweating in young males during intermittent exercise in the heat. *J Physiol* 592(23):5327–5339. <https://doi.org/10.1113/jphysiol.2014.280651>
- Girard O, Bishop DJ, Racinais S (2013) Hot conditions improve power output during repeated cycling sprints without modifying neuromuscular fatigue characteristics. *Eur J Appl Physiol* 113(2):359–369. <https://doi.org/10.1007/s00421-012-2444-3>
- Govoni M, Jansson EA, Weitzberg E, Lundberg JO (2008) The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash. *Nitric Oxide* 19(4):333–337. <https://doi.org/10.1016/j.niox.2008.08.003>
- Gray SR, De Vito G, Nimmo MA, Farina D, Ferguson RA (2006) Skeletal muscle ATP turnover and muscle fiber conduction velocity are elevated at higher muscle temperatures during maximal power output development in humans. *Am J Physiol Regul Integr Comp Physiol* 290(2):R376–R382. <https://doi.org/10.1152/ajpregu.00291.2005>
- Gray SR, Soderlund K, Ferguson RA (2008) ATP and phosphocreatine utilization in single human muscle fibres during the development of maximal power output at elevated muscle temperatures.

- J Sports Sci 26(7):701–707. <https://doi.org/10.1080/02640410701744438>
- Heinonen I, Saltin B, Kemppainen J, Sipila HT, Oikonen V, Nuutila P, Knuuti J, Kalliokoski K, Hellsten Y (2011) Skeletal muscle blood flow and oxygen uptake at rest and during exercise in humans: a pet study with nitric oxide and cyclooxygenase inhibition. *Am J Physiol Heart Circ Physiol* 300(4):H1510–H1517. <https://doi.org/10.1152/ajpheart.00996.2010>
- Hernandez A, Schiffer TA, Ivarsson N, Cheng AJ, Bruton JD, Lundberg JO, Weitzberg E, Westerblad H (2012) Dietary nitrate increases tetanic $[Ca^{2+}]_i$ and contractile force in mouse fast-twitch muscle. *J Physiol* 590(15):3575–3583. <https://doi.org/10.1113/jphysiol.2012.232777>
- Hoon MW, Jones AM, Johnson NA, Blackwell JR, Broad EM, Lundy B, Rice AJ, Burke LM (2014) The effect of variable doses of inorganic nitrate-rich beetroot juice on simulated 2000-m rowing performance in trained athletes. *Int J Sports Physiol Perform* 9(4):615–620. <https://doi.org/10.1123/ijspp.2013-0207>
- Huggins R, Glaviano N, Negishi N, Casa DJ, Hertel J (2012) Comparison of rectal and aural core body temperature thermometry in hyperthermic, exercising individuals: a meta-analysis. *J Athl Train* 47(3):329–338. <https://doi.org/10.4085/1062-6050-47.3.09>
- Johnson JM (2010) Exercise in a hot environment: the skin circulation. *Scand J Med Sci Sports* 20(Suppl 3):29–39. <https://doi.org/10.1111/j.1600-0838.2010.01206.x>
- Jones AM, Ferguson SK, Bailey SJ, Vanhatalo A, Poole DC (2016) Fiber type-specific effects of dietary nitrate. *Exerc Sport Sci Rev* 44(2):53–60. <https://doi.org/10.1249/JES.0000000000000074>
- Karatzafieri C, Chinn MK, Cooke R (2004) The force exerted by a muscle cross-bridge depends directly on the strength of the actomyosin bond. *Biophys J* 87(4):2532–2544. <https://doi.org/10.1529/biophysj.104.039909>
- Keen JT, Levitt EL, Hodges GJ, Wong BJ (2015) Short-term dietary nitrate supplementation augments cutaneous vasodilatation and reduces mean arterial pressure in healthy humans. *Microvasc Res* 98:48–53. <https://doi.org/10.1016/j.mvr.2014.12.002>
- Kent GL, Dawson B, Cox GR, Abbiss CR, Smith KJ, Croft KD, Lim ZX, Eastwood A, Burke LM, Peeling P (2018a) Effect of dietary nitrate supplementation on thermoregulatory and cardiovascular responses to submaximal cycling in the heat. *Eur J Appl Physiol* 118(3):657–668. <https://doi.org/10.1007/s00421-018-3809-z>
- Kent GL, Dawson B, Cox GR, Burke LM, Eastwood A, Croft KD, Peeling P (2018b) Dietary nitrate supplementation does not improve cycling time-trial performance in the heat. *J Sports Sci* 36(11):1204–1211. <https://doi.org/10.1080/02640414.2017.1364404>
- Kuennen M, Jansen L, Gillum T, Granados J, Castillo W, Nabiyyar A, Christmas K (2015) Dietary nitrate reduces the O_2 cost of desert marching but elevates the rise in core temperature. *Eur J Appl Physiol* 115(12):2557–2569. <https://doi.org/10.1007/s00421-015-3255-0>
- Lansley KE, Winyard PG, Bailey SJ, Vanhatalo A, Wilkerson DP, Blackwell JR, Gilchrist M, Benjamin N, Jones AM (2011) Acute dietary nitrate supplementation improves cycling time trial performance. *Med Sci Sports Exerc* 43(6):1125–1131. <https://doi.org/10.1249/MSS.0b013e31821597b4>
- Levitt EL, Keen JT, Wong BJ (2015) Augmented reflex cutaneous vasodilatation following short-term dietary nitrate supplementation in humans. *Exp Physiol* 100(6):708–718. <https://doi.org/10.1113/EP085061>
- Lundberg JO, Weitzberg E, Gladwin MT (2008) The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nat Rev Drug Discov* 7(2):156–167. <https://doi.org/10.1038/nrd2466>
- Martin K, Smee D, Thompson KG, Rattray B (2014) No improvement of repeated-sprint performance with dietary nitrate. *Int J Sports Physiol Perform* 9(5):845–850. <https://doi.org/10.1123/ijspp.2013-0384>
- Maughan R, Shirreffs S (2004) Exercise in the heat: challenges and opportunities. *J Sports Sci* 22(10):917–927. <https://doi.org/10.1080/02640410400005909>
- McQuillan JA, Casadio JR, Dulson DK, Laursen PB, Kilding AE (2018) The effect of nitrate supplementation on cycling performance in the heat in well-trained cyclists. *Int J Sports Physiol Perform* 13(1):50–56. <https://doi.org/10.1123/ijspp.2016-0793>
- Modin A, Bjerne H, Herulf M, Alving K, Weitzberg E, Lundberg JO (2001) Nitrite-derived nitric oxide: a possible mediator of ‘acidic-metabolic’ vasodilation. *Acta Physiol Scand* 171(1):9–16. <https://doi.org/10.1046/j.1365-201X.2001.00771.x>
- Mohr M, Krstrup P, Bangsbo J (2003) Match performance of high-standard soccer players with special reference to development of fatigue. *J Sports Sci* 21(7):519–528. <https://doi.org/10.1080/0264041031000071182>
- Muggeridge DJ, Howe CC, Spendiff O, Pedlar C, James PE, Easton C (2014) A single dose of beetroot juice enhances cycling performance in simulated altitude. *Med Sci Sports Exerc* 46(1):143–150. <https://doi.org/10.1249/MSS.0b013e3182a1dc51>
- Piknova B, Park JW, Swanson KM, Dey S, Noguchi CT, Schechter AN (2015) Skeletal muscle as an endogenous nitrate reservoir. *Nitric Oxide* 47:10–16. <https://doi.org/10.1016/j.niox.2015.02.145>
- Porcelli S, Ramaglia M, Bellistri G, Pavei G, Pugliese L, Montorsi M, Rasica L, Marzorati M (2015) Aerobic fitness affects the exercise performance responses to nitrate supplementation. *Med Sci Sports Exerc* 47(8):1643–1651. <https://doi.org/10.1249/MSS.0000000000000577>
- Richardson RS, Noyszewski EA, Kendrick KF, Leigh JS, Wagner PD (1995) Myoglobin O_2 desaturation during exercise. Evidence of limited O_2 transport. *J Clin Invest* 96(4):1916–1926. <https://doi.org/10.1172/JCI118237>
- Sandbakk SB, Sandbakk O, Peacock O, James P, Welde B, Stokes K, Bohlke N, Tjonna AE (2015) Effects of acute supplementation of L-arginine and nitrate on endurance and sprint performance in elite athletes. *Nitric Oxide* 48:10–15. <https://doi.org/10.1016/j.niox.2014.10.006>
- Spencer M, Lawrence S, Rechichi C, Bishop D, Dawson B, Goodman C (2004) Time-motion analysis of elite field hockey, with special reference to repeated-sprint activity. *J Sports Sci* 22(9):843–850. <https://doi.org/10.1080/02640410410001716715>
- Thompson KG, Turner L, Prichard J, Dodd F, Kennedy DO, Haskell C, Blackwell JR, Jones AM (2014) Influence of dietary nitrate supplementation on physiological and cognitive responses to incremental cycle exercise. *Respir Physiol Neurobiol* 193(0):11–20. <https://doi.org/10.1016/j.resp.2013.12.015>
- Thompson C, Wylie LJ, Fulford J, Kelly J, Black MI, McDonagh ST, Jeukendrup AE, Vanhatalo A, Jones AM (2015) Dietary nitrate improves sprint performance and cognitive function during prolonged intermittent exercise. *Eur J Appl Physiol* 115(9):1825–1834. <https://doi.org/10.1007/s00421-015-3166-0>
- Thompson C, Vanhatalo A, Jell H, Fulford J, Carter J, Nyman L, Bailey SJ, Jones AM (2016) Dietary nitrate supplementation improves sprint and high-intensity intermittent running performance. *Nitric Oxide* 61:55–61. <https://doi.org/10.1016/j.niox.2016.10.006>
- Vanhatalo A, Bailey SJ, Blackwell JR, DiMenna FJ, Pavey TG, Wilkerson DP, Benjamin N, Winyard PG, Jones AM (2010) Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate-intensity and incremental exercise. *Am J Physiol Regul Integr Comp Physiol* 299(4):R1121–R1131. <https://doi.org/10.1152/ajpregu.00206.2010>
- Webb AJ, Patel N, Loukogeorgakis S, Okorie M, Aboud Z, Misra S, Rashid R, Miall P, Deanfield J, Benjamin N, MacAllister R,

- Hobbs AJ, Ahluwalia A (2008) Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite. *Hypertension* 51(3):784–790. <https://doi.org/10.1161/HYPERTENSIONAHA.107.103523>
- Whitfield J, Ludzki A, Heigenhauser GJF, Senden JMG, Verdijk LB, van Loon LJC, Spriet LL, Holloway GP (2015) Beetroot juice supplementation reduces whole body oxygen consumption but does not improve indices of mitochondrial efficiency in human skeletal muscle. *J Physiol*. <https://doi.org/10.1113/JP270844>
- Wylie LJ, Mohr M, Krstrup P, Jackman SR, Ermotadis G, Kelly J, Black MI, Bailey SJ, Vanhatalo A, Jones AM (2013) Dietary nitrate supplementation improves team sport-specific intense intermittent exercise performance. *Eur J Appl Physiol* 113(7):1673–1684. <https://doi.org/10.1007/s00421-013-2589-8>
- Wylie LJ, Bailey SJ, Kelly J, Blackwell JR, Vanhatalo A, Jones AM (2016) Influence of beetroot juice supplementation on intermittent exercise performance. *Eur J Appl Physiol* 116(2):415–425. <https://doi.org/10.1007/s00421-015-3296-4>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.