European Journal of Sport Science

ISSN: 1746-1391 (Print) 1536-7290 (Online) Journal homepage: http://www.tandfonline.com/loi/tejs20

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To cite this article: K.L. Jonvik, J. Nyakayiru, J.W. Van Dijk, K. Maase, S.B. Ballak, J.M.G. Senden, L.J.C. Van Loon & L.B. Verdijk (2018) Repeated-sprint performance and plasma responses following beetroot juice supplementation do not differ between recreational, competitive and elite sprint athletes, European Journal of Sport Science, 18:4, 524-533, DOI: 10.1080/17461391.2018.1433722

To link to this article: https://doi.org/10.1080/17461391.2018.1433722

Published online: 07 Feb 2018.

European Journal of Sport Science, 2018
Vol. 18, No. 4, 524–533, https://doi.org/10.1080/17461391.2018.1433722

ORIGINAL ARTICLE

Repeated-sprint performance and plasma responses following beetroot juice supplementation do not differ between recreational, competitive and elite sprint athletes

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Abstract
Purpose: There is an ongoing debate whether highly trained athletes are less responsive to the ergogenic properties of nitrate. We assessed the effects of nitrate supplementation on plasma nitrate and nitrite concentrations and repeated-sprint performance in recreational, competitive and elite sprint athletes. *Methods*: In a randomized double-blinded cross-over design, recreational cyclists (n = 20), national talent speed-skaters (n = 22) and Olympic-level track cyclists (n = 10) underwent two 6-day supplementation periods; 140 mL/d nitrate-rich (BR; ~800 mg/d) and nitrate-depleted (PLA; ~0.5 mg/d) beetroot juice. Blood samples were collected and three 30-s Wingate tests were performed. *Results*: Plasma nitrate and nitrite concentrations were higher following BR vs PLA ($P < .001$), with no differences between sport levels (all $P > .10$). Peak power over the three Wingates was not different between BR and PLA (1338 ± 30 vs 1333 ± 30 W; $P = .62$), and there was no interaction between treatment (BR-PLA) and Wingate number (1-2-3; $P = .48$). Likewise, mean power did not differ between BR and PLA ($P = .86$). In contrast, time to peak power improved by ~2.8% following BR vs PLA ($P = .007$). This improvement in BR vs PLA was not different between Wingate 1, 2 and 3. Moreover, the effects of BR vs PLA did not differ between sport levels for any Wingate parameter (all $P > .30$). *Conclusion*: The plasma and repeated-sprint performance responses to beetroot juice supplementation do not differ between recreational, competitive and elite sprint athletes. Beetroot juice supplementation reduces time to reach peak power, which may improve the capacity to accelerate during high-intensity and sprint tasks in recreational as well as elite athletes.

Keywords: *Exercise, fitness, performance, training, nutrition*

Highlights

- Previous work has suggested reduced responsiveness of endurance athletes to nitrate supplementation. We show that both at baseline and following beetroot juice supplementation, plasma nitrate and nitrite concentrations do not differ between recreational, competitive and elite athletes of sprint disciplines.
- The proposed unresponsiveness to nitrate supplementation in elite athletes in unlikely due to differences in plasma responses between athletes of different training status.
- Based on repeated Wingate tests, beetroot juice supplementation did not affect peak or mean power, but the time to reach peak power was reduced.
- An improved time to reach peak power could be relevant to athletes of various sports where a quick acceleration is important, such as high-intensity field sports and sprint disciplines like running, speed-skating, BMX and track cycling.
- Since no differences were observed between recreational, competitive and elite athletes of sprint disciplines for any of the parameters assessed, we suggest that even elite athletes of high-intensity and/or sprint disciplines could benefit from beetroot juice supplementation.

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Introduction

Due to the proposed effects of dietary nitrate on exercise performance, nitrate-rich supplements and foods currently receive considerable attention as ergogenic aids. The effects of dietary nitrate have been attributed to its reduction to nitrite by oral bacteria, which in turn enters the circulation after swallowing, and is then further reduced to nitric oxide (NO), particularly in environments of low oxygen availability and low pH (Lundberg, Weitzberg, & Gladwin, 2008). NO is important for several physiological processes associated with exercise, including the regulation of blood flow and skeletal muscle contraction (Stamler & Meissner, 2001). Over the past decade, dietary nitrate supplementation has been shown to improve oxygen efficiency during submaximal exercise (Bailey et al., 2009; Larsen, Weitzberg, Lundberg, & Ekblom, 2007; Vanhatalo et al., 2010), increase time to exhaustion of high-intensity exercise (Bailey et al., 2009; Breese et al., 2013; Kelly, Vanhatalo, Wilkerson, Wylie, & Jones, 2013) and improve exercise time-trial performance (Cermak, Gibala, & van Loon, 2012; Lansley, Winyard, Bailey, et al., 2011) in recreational to well-trained athletes. Whereas most research on performance effects of dietary nitrate has been on endurance sports, the focus is currently shifting towards intermittent and very high-intensity sports. During high-intensity exercise metabolic by-products accumulate and cause acidosis (Robergs, Ghiasvand, & Parker, 2004), creating an environment that promotes nitrite to NO conversion (Lundberg et al., 2008). It has been suggested that under such circumstances, nitrate supplementation may be more effective in eliciting its ergogenic potential (Callahan, Parr, Hawley, & Burke, 2017; Wylie et al., 2016). In this respect, improvements in intermittent and repeated-sprint performance following beetroot juice supplementation have been observed in recreational athletes (Muggeridge, Sculthorpe, James, & Easton, 2016; Thompson et al., 2015; Thompson et al., 2016; Wylie et al., 2016; Wylie, Mohr, et al., 2013), although this has not been confirmed by all studies (Clifford et al., 2016; Martin, Smee, Thompson, & Rattray, 2014). A potential mechanism for improvement of repeated-sprint performance following beetroot juice supplementation is a decreased fatigue development between sprints associated with reduced PCr cost of force production (Wylie et al., 2016). Given that short rest periods normally lead to an incomplete recovery of muscle PCr and force production, beetroot juice may be most effective for repeated-sprints interspersed by short recovery periods. However, power production *per se* has also been shown to improve following nitrate ingestion (Coggan et al., 2015; Kramer, Baur, Spicer, Vukovich, & Ormsbee, 2016), which could be caused by improved rate of force production (Haider & Folland, 2014) and speed of contraction (Coggan et al., 2015). As such, both a more generalized effect of nitrate ingestion on (peak) power output, as well as more prominent effect in the later sprints of a repeated-sprint protocol could be expected.

It has been suggested that differences in the efficacy of dietary nitrate to improve exercise performance may be related to training status, with highly trained athletes potentially being less responsive to the ergogenic properties of nitrate than recreational athletes (Porcelli et al., 2015). Such unresponsiveness may be explained by higher endogenous NO synthesis, higher habitual dietary nitrate intakes, and optimized exercise training adaptations in highly trained athletes (Jonvik, Nyakayiru, van Loon, & Verdijk, 2015). Recent work by Porcelli et al. (2015) suggests that highly trained endurance athletes have higher baseline plasma nitrate and nitrite concentrations and show less of an increase in plasma concentrations following nitrate ingestion. In contrast, we have shown substantial increases in plasma nitrate and nitrite concentrations after nitrate supplementation in highly trained endurance athletes (Nyakayiru et al., 2016). As such, it remains to be determined what the potential effect of training status is on (changes in) plasma nitrate and nitrite concentrations.

Apart from differences in plasma nitrate or nitrite concentrations, the benefits of nitrate supplementation may also depend on genetic or training effects on muscle oxygenation, mitochondrial function and on muscle fiber type composition (Wylie et al., 2016). It has been suggested that nitrate supplementation may be particularly effective at improving physiological and functional responses in type II (fast-twitch) muscle fibers (Bailey et al., 2015). This could be relevant for short, high-intensity events with greater type II muscle fiber recruitment (Burke, 2017), further explaining the shifted attention in nitrate research towards these activities. Importantly, the suggested blunted performance effect of nitrate in highly trained compared to recreational athletes has only been described for endurance athletes (Porcelli et al., 2015), and the effects of nitrate for elite athletes of sprint disciplines remain unknown.

The current study was aimed to assess and compare plasma nitrate and nitrite concentrations and repeated-sprint performance following beetroot juice supplementation between recreational, competitive and elite athletes of sprint disciplines. We hypothesized that plasma nitrate and nitrite concentrations would not differ between sport levels, but that only the recreational athletes would improve

Table I. Characteristics by sex and sports level.

	Males				Females			
	Total	Recreational	Competitive	Elite	Total	Recreational	Competitive	Elite
N	29	10	14	5	23	10	8	5
Age	22 ± 5	27 ± 6	19 ± 1†	21 ± 2†	26 ± 8	33 ± 7	20 ± 3†	22 ± 3†
Weight	80 ± 9	78 ± 8	78 ± 6	92 ± 7	67 ± 10*	64 ± 8	69 ± 9	71 ± 15
Height	184 ± 6	184 ± 7	183 ± 5	186 ± 7	171 ± 8*	170 ± 7	171 ± 10	171 ± 9
BMI	23.7 ± 2.1	23.1 ± 1.6	23.1 ± 1.7	26.5 ± 1.9	23.1 ± 2.7	22.2 ± 3.3	23.7 ± 1.9	23.9 ± 2.6
FFM	63.4 ± 7.0	59.1 ± 4.1	62.9 ± 3.4	75.2 ± 6.7†,§	46.6 ± 6.4*	42.8 ± 2.4	50.4 ± 6.6	50.4 ± 8.3
LL FFM	22.2 ± 3.0	19.8 ± 1.7	22.7 ± 1.4	26.9 ± 2.3†,§	16.2 ± 3.1*	14.2 ± 2.4	17.9 ± 3.0	18.4 ± 3.9†,§
FFMI	18.7 ± 1.8	17.5 ± 0.93	18.7 ± 0.93	21.7 ± 1.8†,§	15.9 ± 1.4*	14.9 ± 1.2	16.7 ± 0.36†	17.1 ± 1.0†
WG1 Peak (W)	1698 ± 456	1280 ± 147	1721 ± 173†	2470 ± 353†,§	1095 ± 382*	766 ± 103	1270 ± 309†	1473 ± 316†
WG2 Peak (W)	1514 ± 403	1151 ± 145	1532 ± 149†	2189 ± 338†,§	1005 ± 344*	705 ± 96	1161 ± 262†	1355 ± 289†
WG3 Peak (W)	1316 ± 319	1052 ± 136	1377 ± 155†	1674 ± 497†	910 ± 279*	663 ± 90	1098 ± 234†	1105 ± 208†
WG1 Mean (W)	897 ± 161	755 ± 64	898 ± 60†,§	1180 ± 94†,§	623 ± 149*	502 ± 55	678 ± 96†	776 ± 167†
WG2 Mean (W)	791 ± 128	674 ± 62	801 ± 61†,§	996 ± 79†,§	551 ± 104*	466 ± 49	589 ± 64†	658 ± 110†
WG3 Mean (W)	716 ± 97	623 ± 49	741 ± 60†,§	830 ± 95†,§	513 ± 87*	445 ± 52	561 ± 53†	574 ± 98†

Data expressed as means ± SDs. BMI: body mass index, (LL) FFM: (lower limb) fat-free mass, FFMI: fat-free mass index, WG: Wingate. Peak/mean: average for PLA and BR is presented since no differences were observed. Data were analysed using repeated-measures ANOVA, with 'treatment' and 'Wingate' as within-subjects factor, and 'sex' and 'sport level' as between-subjects factors.

*Different from males ($P < .001$).

†Different from recreational ($P < .05$).

§Different from competitive ($P < .05$). No interactions were observed. For (LL) FFM and FFMI there were 8 missing cases (4 male competitive, 3 female competitive and 1 male elite athlete).

repeated-sprint performance, showing an increase in power output in all sprints.

Methods

Subjects

Out of a total of 53 athletes who participated in this study, 52 completed the entire protocol: 10 elite (5 male and 5 female), 22 competitive (14 male and 8 female) and 20 recreational (10 male and 10 female) athletes (Table I). One male subject from the competitive group dropped out during the trial. We included the Dutch Olympic track cycling sprint selection. They were tested in the preparation phase leading up to the European Championship 2015, where five athletes won a medal and two won a medal at the 2016 Olympics. Due to their competitive levels these athletes could be classified as absolute elite sprinters. The second group consisted of regional talent level speed-skaters of sprint distances (mainly 1500 m), competing at the national level (senior or youth), and were tested off-season in May–June 2016. These speed-skaters were cycling the whole off-season and used Wingate tests as the main performance test in daily practice. Due to their competitive level and test cycling sprint experience, these athletes could be classified as competitive sprinters. The final group comprised healthy individuals cycling 1–5 h per week, who were tested in September–November 2016. They had no experience with Wingate testing and were classified as recreational athletes.

After being informed about the purpose and potential risks of the study, all subjects provided written informed consent. The experimental protocol and procedures were approved by the HAN University of Applied Sciences ethical advisory board and the local ethical committee of the Arnhem and Nijmegen region, The Netherlands, and followed the principles of the Declaration of Helsinki (World Medical, 2013).

Study design

In a randomized, double-blinded, placebo-controlled cross-over design, subjects underwent two supplementation periods. Over two 6-day periods, they were provided 140 mL nitrate-rich (BR; ~800 mg/d) and 140 mL nitrate-depleted (PLA; ~0.5 mg/d) beetroot juice per day, both provided by Beet it, James White Drinks Ltd., Ipswich, UK. Randomization was performed in blocks of 4 within each sport level, and resulted in supplementation order BR-PLA for 26 athletes and PLA-BR for the other 26 athletes. The study included three visits over a 4-week period, consisting of a screening and familiarization session (visit 1) and two experimental test days (visits 2–3). The two experimental test days were performed at the same day of the week and time of day for each individual (±1 h). The supplementation periods were interspaced by a 1-week washout.

Experimental protocol

Baseline characteristics were obtained, and DEXA scans were performed to determine whole body and regional fat-free mass (FFM) (Table I). DEXA scans were not performed in 8 of the 52 subjects due to time constraints. During visit 1, subjects were familiarized to the triple Wingate test. First, they underwent a 15-17 min warming up at 1.5 W kg^{-1} including two 3-s sprints. Then, a series of three all-out Wingate tests was performed, interspersed by 4 min recovery periods. During the recovery periods the subjects kept pedalling against a resistance of 50 W. For each subsequent 30-s Wingate, they were notified at the 15 s mark followed by a countdown during the final 5 s. All tests were performed on calibrated Lode Excalibur Sport ergometer bikes (Lode, Groningen, Netherlands) with a standstill start, and applying a fixed load of 8.5% bodyweight (0.83 Nm·kg^{-1}) (Vargas, Robergs, & Klopp, 2014). Load, bike setup and verbal encouragements were kept similar over all test days. Lode Ergometer Manager was used to extract the raw power data measured at 5 Hz.

Following the familiarization visit, subjects started a 6-day beetroot juice supplementation period during which 140 mL of either BR or PLA was ingested each day at breakfast. Two hours after ingestion of the last supplemental dose (on the 6th supplementation day), subjects arrived at the sports facility for the first test day. Prior to the warm-up, a single blood sample was obtained and a gastrointestinal tolerance questionnaire (Trommelen et al., 2017) was administered. Subjects then performed the same warm-up as during the familiarization visit, and the Wingate test was initiated 3 h after ingestion of the supplement. Heart rate was monitored continuously from the last 7min of the warm-up until the end of the Wingate tests. The rate of perceived exertion was obtained immediately after individual termination of the triple Wingate test. Subjects were allowed to drink water ad libitum during test days. After a 1-week washout, the second supplementation period was started and on day 6 of supplementation, the exact same procedures were followed as during the first test day.

Physical activity and dietary standardization

Subjects recorded their dietary intake 36 h prior to the first test day (visit 2). They were subsequently instructed to replicate their dietary intake 36 h prior to the second test day (visit 3). Subjects were asked to avoid caffeine and alcohol for 12 and 24 h prior to each test day, respectively. No restrictions were set for the intake of nitrate-rich foods during the intervention period, to allow for the determination of the additional effect of nitrate supplementation on plasma nitrate and nitrite concentrations and performance outcomes on top of the normal diet (Vanhatalo et al., 2010). However, to prevent any attenuation in the reduction of nitrate to nitrite in the oral cavity by commensal bacteria, subjects were asked to refrain from using any antibacterial mouthwash/toothpaste, chewing gum and tongue-scraping during each day of supplementation (Govoni, Jansson, Weitzberg, & Lundberg, 2008). Supplement logs and training diaries were kept for both intervention periods, to verify compliance and monitor potential period differences.

Plasma analyses

Blood samples were collected in Lithium-Heparin containing tubes for nitrate and nitrite determination, and centrifuged immediately at 1000 *g* for 5 min, at 20 ± 1°C. Aliquots of the plasma were transferred to 2 mL Eppendorf tubes and immediately frozen with dry ice, 5 h prior to being stored in a –80°C freezer for subsequent analysis. Plasma nitrate and nitrite concentrations were determined after reduction to NO using the chemiluminescence technique (NOA; Sievers NOA 280i; Analytix, Durham, UK) as described previously (Jonvik et al., 2016).

Statistical analysis

Power calculations were based on previously reported differences in plasma nitrate responses between moderately and highly trained endurance athletes (Porcelli et al., 2015), and a ~4% improvement of sprint performance in recreational athletes (Thompson et al., 2015; Wylie, Mohr, et al., 2013), with a CV of ~3% (Jaafar et al., 2014). This resulted in an effect size of 2.75 for plasma nitrate and an effect size of 1.33 for peak and mean power. Taking into account a power of 80%, and an alpha level of 0.025 (enabling post-hoc comparisons between recreational and elite athletes), a minimum of 10 athletes should be included per sport level. Although we did not power for potential sex differences within sport levels, we included more athletes in the recreational and competitive groups to provide some insights into potential sex differences. Normality of all data was assessed using Kolmogorov–Smirnov tests. Peak power was defined as the maximum power achieved over 1 s (i.e. average of the 5 highest consecutive 0.2 s values). Mean power was defined as the average power during the entire 30 s of a Wingate. Time to peak power was defined as the time (closest 0.2 s) from start to reach peak power.

Baseline characteristics were analysed using univariate ANOVA with 'sex' and 'sport level' as factors. Peak and mean power were analysed using repeated-measures ANOVA, with 'Wingate' (first, second and third Wingate) and 'treatment' (BR vs PLA) as within-subjects factors. This approach enabled us to assess a potential generalized effect of BR on peak and mean power, as well as a more specific effect towards the later sprints of the repeated-sprint protocol (i.e. 'Wingate x treatment' interaction). 'Sport level' and 'sex' were included as between-subjects factors to assess whether potential treatment effects were different between sport levels or between men and women. Time to peak was analysed non-parametrically. To enable a similar analysis as for peak and mean power, difference in time to peak power between BR and PLA was calculated for each participant and each Wingate, and these data were analysed by Wilcoxon signed-rank, Kruskal–Wallis and Friedman tests. Plasma nitrate was also analysed using non-parametric testing (Wilcoxon signed-rank, Kruskal–Wallis and Mann–Whitney *U* tests). Plasma nitrite, heart rate (mean and maximum per Wingate) and rate of perceived exertion were analysed using repeated-measures ANOVA, with 'treatment' as within-subjects factor, and 'sex' and 'sport level' as between-subjects factors. Statistical significance was set at $P < .05$ and any interaction or main effect was subsequently analysed using a Bonferroni post-hoc test in SPSS 22.0 (IBM Corp., Armonk, USA). Data are presented as means ± SDs, or medians [IQR] where appropriate. For data on group total, estimated marginal means ± SEMs are presented, due to the uneven number of athletes per sport level.

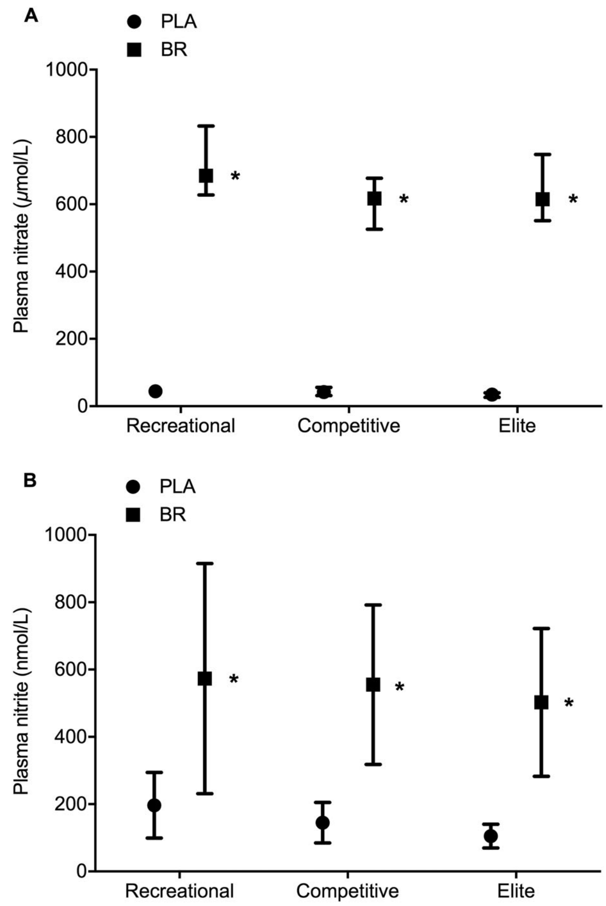

Figure 1. Plasma nitrate (A) and nitrite (B) concentrations for recreational ($n = 20$), competitive ($n = 22$) and elite athletes ($n = 10$) following 6 days of placebo (PLA) or beetroot juice (BR). Plasma nitrate was analysed by Wilcoxon signed-rank, Kruskal–Wallis and Mann–Whitney *U* tests. Values are medians [IQRs]. Plasma nitrite was analysed using repeated-measures ANOVA, with 'treatment' as within-subjects factor, and 'sex' and 'sport level' as between-subjects factors. Values are means ± SDs. *Significantly different from PLA, $P < 0.001$. See text for interaction effects.

Results

Subject characteristics are reported in Table I. Independent of sex, lower limb FFM was higher in elite vs competitive vs recreational athletes (all $P < .05$). Independent of sport level, lower limb FFM was higher in male vs female athletes ($P < .001$). Supplement logs showed 100% compliance by all subjects, and training diaries showed no difference in activity between the two periods.

Plasma nitrate and nitrite concentrations

No differences in plasma nitrate concentrations were observed between sport levels following PLA (recreational: 44[28–68], competitive: 42[32–56], elite athletes: 35[26–40] μmol L^{-1}; $P = .33$). For all groups, plasma nitrate concentrations were significantly higher following BR vs PLA (~16 fold, $P < .001$), but this difference was not dependent on sport level (Figure 1A; $P = .13$). However, despite similar baseline nitrate concentrations (female: 37 [27–50] vs male: 44[32–64] μmol L^{-1}, $P = .13$), the difference in plasma nitrate following BR vs PLA was higher in female vs male athletes (717[557–775] vs 569[495–611] μmol L^{-1}, $P = .007$).

Similarly, plasma nitrite concentrations following PLA were not different between recreational (197 ± 97 nmol·L^{-1}), competitive (145 ± 60 nmol·L^{-1}) and elite athletes (105 ± 35 nmol·L^{-1}). Plasma nitrite was ~fourfold higher following BR vs PLA ($P < .001$, Figure 1B), with no differences between sport levels ($P = .62$). In line with plasma nitrate, an effect of sex was observed for plasma nitrite, with post-hoc analyses showing that for all athletes together, plasma nitrite concentrations were higher in female vs male athletes following BR (756 ± 275 vs 390 ± 132 nmol·L^{-1}, $P < .001$). Following PLA, this sex difference was only apparent in the recreational athletes (256 ± 98 vs 137 ± 51 nmol L^{-1}; $P = .003$), whereas no differences between females and males were observed in competitive (152 ± 48 vs 141 ± 68 nmol L^{-1}; $P = .69$) and elite athletes (103 ± 43 vs 107 ± 31 nmol L^{-1}; $P = .85$). Furthermore, within the female athletes, plasma nitrite concentrations were similar between all sport levels in both PLA and BR. In contrast, within male athletes, plasma nitrite concentrations were higher in competitive athletes compared with both recreational and elite athletes, which was most pronounced following BR supplementation (460 ± 135 vs 325 ± 113 and 325 ± 23 nmol L^{-1}, respectively; $P = .015$).

Wingate performance

The peak and mean power per Wingate for male and female athletes of recreational, competitive and elite sport levels are presented in Table I. Both the peak and mean power significantly declined over the three consecutive Wingates ($P < .001$). Peak and

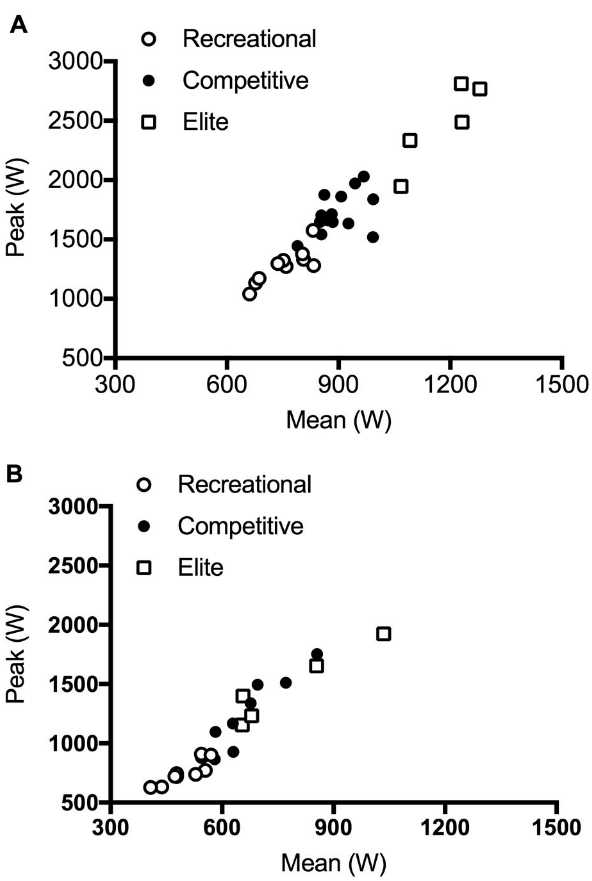

Figure 2. Scatter plot for peak and mean power output of the first Wingate for male (A, $n = 29$) and female (B, $n = 23$) athletes. Output represents each individual's average values for PLA and BR. Markers indicate the recreational, competitive and elite athletes.

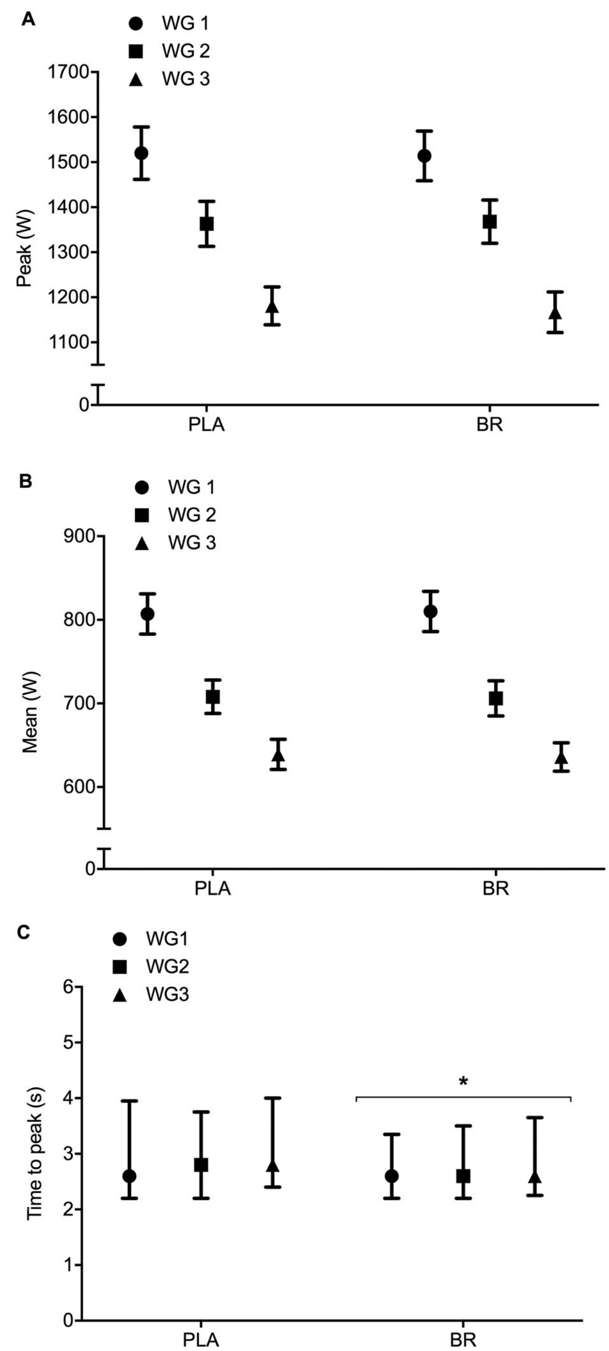

Figure 3. Peak power (A), mean power (B) and time to peak power (C) of the three consecutive Wingates for the total group of 52 athletes following 6 days of placebo (PLA) or beetroot juice (BR). Peak and mean power were analysed using repeated-measures ANOVA, with 'treatment' and 'Wingate' as within-subjects factor, and 'sex' and 'sport level' as between-subjects factors. Values are estimated marginal means ± SEMs. Time to peak was analysed by Wilcoxon signed-rank, Kruskal–Wallis and Friedman tests. Values are medians [IQRs]. *Significantly different from PLA, $P = 0.007$. No interactions were observed.

mean power were higher in male vs female athletes, and higher in elite vs competitive vs recreational athletes ($P < .001$). The peak and mean power of Wingate 1 (average for PLA and BR) for male and female athletes are shown as a scatter plot in Figure 2A and B, respectively, to visualize the differences in power between the different sport levels.

No differences were observed between BR and PLA for peak power over the three Wingates (1338 ± 30 vs 1333 ± 30 W; $P = .62$, Figure 3A), and there was no interaction between treatment (BR vs PLA) and Wingate number (1–2–3; $P = .48$). No effects of sport level ($P = .70$) or sex ($P = .21$) were observed. Likewise, mean power did not differ between BR and PLA over the three Wingates ($P = .86$, Figure 3B), and there was no interaction between treatment (BR vs PLA) and Wingate number (1–2–3; $P = .55$). No effects of sport level ($P = .81$) or sex ($P = .27$) were observed. In contrast, time to peak power improved by ~2.8% following BR vs PLA, with a median difference of −0.2[−1.0 to 0.4] s ($P = .007$, Figure 3C). This effect of BR was not dependent on sport level ($P = .82$) or sex ($P = .39$), and was not different between the three consecutive Wingates ($P = .80$).

RPE, heart rate and GI discomfort

No differences were found for the rate of perceived exertion between BR and PLA (17.4 ± 1.8 vs 17.7 ± 1.8, $P = .13$). For 41 of the 52 athletes heart rates were successfully recorded for all Wingates. No differences between BR and PLA were observed for the mean or maximum heart rate during the consecutive Wingates ($P = .56$ and $P = .46$, respectively). No serious adverse effects were reported. One male athlete from the competitive group was excluded prior to testing due to an allergic reaction to beetroot juice. Gastrointestinal complaints 2 h after supplement ingestion on the test day were only reported by 1 athlete following the PLA trial (diarrhoea) and 1 athlete following the BR trial (fullness and headache).

Discussion

We show that six days of nitrate-rich beetroot juice supplementation substantially increases plasma nitrate and nitrite concentrations, with no differences between recreational, competitive and elite athletes. BR did not affect peak and mean power during repeated Wingate tests. However, time to peak power over the consecutive Wingates was significantly improved by beetroot juice supplementation, independent of sport level.

Given the current discussion whether dietary nitrate may or may not induce similar ergogenic benefits for athletes of different fitness level, we aimed to compare both the plasma and repeated-sprint performance responses to beetroot juice supplementation between recreational, competitive and elite athletes. The three groups were selected based on their very different level of competition in sprint disciplines, and not on their cycling test performance. The elite comprised Olympic sprinters, the competitive comprised national talent level sprinters and the recreational comprised inexperienced non-competitive athletes. The differences observed in peak power output and lower limb FFM (Table I) between the three groups further support this differentiation into recreational, competitive and elite athletes. In the only study that previously compared athletes of low, moderate and high fitness level, Porcelli et al. (2015) reported slightly higher baseline levels as well as an attenuated increase in plasma nitrate concentrations following dietary nitrate ingestion in the highly trained subjects, but no differences in plasma nitrite concentrations. In contrast, we show no differences in plasma nitrate or nitrite concentrations between recreational, competitive and elite athletes both following PLA and BR supplementation. Notably, in the current study we used the gold-standard methodology for determining plasma nitrate and nitrite concentrations and included a much larger group of athletes when compared with previous work (Porcelli et al., 2015). Overall, we report baseline values (i.e. following PLA ingestion) as well as substantial increases in plasma nitrate and nitrite concentrations that are in line with recent literature (Bailey et al., 2009; Jonvik et al., 2016; Lansley, Winyard, Bailey, et al., 2011; Lansley, Winyard, Fulford, et al., 2011; Wylie, Kelly, et al., 2013), and that are independent of athletes' training status. Based on these findings, the proposed unresponsiveness to nitrate supplementation in elite athletes is unlikely due to basal or post-ingestion differences in plasma nitrate and nitrite concentrations between athletes of different training status. On the contrary, we did observe higher plasma nitrate and nitrite concentrations in female vs male athletes, especially following BR supplementation. This is in line with our previous findings in healthy individuals (Jonvik et al., 2016). For now it remains unknown what may cause this sex difference and whether it also could translate to functional differences in the response to nitrate supplementation between men and women.

Despite a substantial increase in plasma nitrate and nitrite concentrations for athletes of all sport levels, BR did not affect peak or mean power during the Wingate tests. It has been suggested that nitrate supplementation may be most beneficial during exercise protocols likely to involve high type II muscle fibre recruitment (Bailey et al., 2015), as may be expected during a repeated Wingate sprint test. However, inconsistent findings have been reported on the effects of beetroot juice on sprint performance. An improved peak power of a single Wingate test has been observed in cross-fit athletes (Kramer et al., 2016), while others found no effect on 7x 30-s sprints in recreational athletes (Wylie et al., 2016). In the same study however, mean power of 24x 6-s sprints improved following beetroot juice. Apart from differences in the training background of the participants, it was argued that the specific test used may be of relevance, and that beetroot juice could particularly improve performance of repeated-sprints with short rests, when muscle PCr and force production is not fully recovered (Wylie et al., 2016). In line, other studies using shorter sprints and shorter recovery periods have found ergogenic effects of beetroot juice (Porcelli et al., 2016; Thompson et al., 2016). Interestingly, Haider and Folland (2014) recently reported an enhanced force production during twitch contractions evoked by supra-maximal nerve stimulation following nitrate supplementation in humans, especially during the rising phase of contraction. Likewise, nitrate supplementation was shown to enhance peak twitch force and early-phase explosive force production but not peak force of high-frequency stimulation in mouse fast-twitch muscle (Hernandez et al., 2012). As such, it could be speculated that the faster recruitment of type II muscle fibres following beetroot juice supplementation mainly affects the initial force production during sprint activities. This would be in line with our observation of a faster time to reach peak power following beetroot juice. Such a benefit during the initial phase of contraction would also fit with the observation that nitrate can improve muscle calcium handling (Wylie et al., 2016), as this would likely elicit the greatest impact during the initial phase of contraction where the calcium saturation normally is incomplete. Alternatively, the improved time to peak power following beetroot juice may also be (partly) explained by an improved reaction time, as was recently reported during repeated sprints in recreational athletes (Thompson et al., 2016).

One of the main findings of the current study is the similar effect of beetroot juice on all Wingate parameters for recreational, competitive and elite athletes. Indeed, the improved time to peak power was not dependent on the training status of the athletes. This finding contradicts the suggested reduced response to nitrate in highly trained compared with less trained athletes (Porcelli et al., 2015). However, this study investigated endurance athletes, as opposed to the sprint athletes of the current study. Since elite athletes of sprint disciplines have higher proportions of type II muscle fibres compared to elite endurance athletes (Tesch, Thorsson, & Kaiser, 1984), we suggest that the sprinters might have a greater response to nitrate supplementation and that even elite athletes of high-intensity and/or sprint disciplines could experience positive effects of beetroot juice supplementation. In line, improved high-intensity short distance performance following beetroot juice supplementation has previously been reported for elite kayak athletes (Peeling, Cox, Bullock, & Burke, 2014). However, more studies in elite athletes of high-intensity or sprint disciplines are needed to establish the ergogenic potential of nitrate supplementation for specific sports, and specify the conditions under which nitrate may be most beneficial.

Clearly there are limitations to the testing of athletes in simulated real-life settings. Due to practical reasons, in the current study athletes from different sport levels were tested during different time periods. Furthermore, detection of small but potentially relevant performance benefits is complicated by the normal day-to-day variations in performance. This was kept to a minimum by using a familiar test setting for the elite and competitive athletes (i.e. Wingate tests on Lode ergometers), as well as including a familiarization trial before the actual test days. Furthermore, by including a relatively large number of subjects, we feel confident that the current findings are representative and truly indicate no effects of BR on peak and mean power during repeated Wingate tests, but an improvement in the time to reach peak power. Obviously it could be questioned to what extent the improved time to peak power represents an actual performance benefit, as it did not lead to an increase in peak or mean power. Importantly though, for many athletes of high-intensity and sprint disciplines, a faster acceleration can be of great relevance. In sprint disciplines, such as short distance running (100, 200 and 400 m), speed-skating (500, 1000 and 1500 m), short track speed-skating, BMX and track cycling, the ability to produce a great(er) concentric power and to generate high(er) velocity during acceleration is key to success (Mero, Komi, & Gregor, 1992). Furthermore, speed and acceleration are essential for high-intensity field sports (Lockie, Murphy, Knight, Jonge, & A, 2011). Potentially, beetroot juice supplementation could result in reaching the top speed faster, which may prove relevant to various sports where a quick acceleration is important. For instance, in track cycling a faster acceleration can improve positioning, whereupon fewer meters have to be made. Furthermore, elite BMX riders continuously search for the optimal strategy to improve time to reach peak power (Rylands, Roberts, Hurst, & Bentley, 2017), as the athlete first at the curve very often is the winner.

Conclusion

In conclusion, the plasma and repeated-sprint performance responses to beetroot juice supplementation do not differ between recreational, competitive and elite athletes. Beetroot juice reduces the time to reach peak power, which may improve the capacity to accelerate during high-intensity and sprint tasks in recreational as well as elite athletes.

AKNOWLEDGEMENTS

We thank Melvin Kantebeen MSc (Sportadviesbureau ConTest) and Wouter van der Ploeg MSc (Gewest Friesland) for the support in performing the Wingate tests. This study was designed by KJ, JN, KM, LvL and LV; data were collected and analyzed by KJ, JWvD, SB and JS; data interpretation and manuscript preparation were undertaken by KJ, JN, JWvD, LvL and LV. All authors approved the final version of the paper, and have no conflicts of interest.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was financially supported by a grant from the Dutch Technology Foundation (Stichting voor de Technische Wetenschappen: [Grant Number 12887]).

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