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REVIEW

Effectiveness of beetroot juice derived nitrates supplementation on fatigue resistance during repeated-sprints: a systematic review

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ABSTRACT

In recent decades, the search for non-doping substances that enhance sports performance has increased. Ergogenic aids such as beetroot juice (BRJ) and BRJ rich in nitrates (NO_3^-) are widely used to cause physiological benefits that may lead to physical improvements. Therefore, the purpose of this systematic review was to explore the knowledge reported to date on the NO_3^- derived from BRJ intake effect on fatigue resistance during repeated sprints. A digital search was conducted following systematic review guidelines, and 18 studies met inclusion criteria from a total of 209 articles. In a third of the selected studies, the consumption of beet juice rich in NO_3^- contributes to an increase in nitrites in plasma that led to the rise in peak power, mean power, number of sprint repetitions, total work and time to task failure, and causes a decrease in fatigue index and sprints times. Some different dose has been proposed for both chronic and acute protocols. It seems that a chronic intake of ~5–6 mmol of NO_3^- in 70 ml of BRJ, twice a day, for a minimum of 3–6 days could lead to a fatigue resistance improvement during repeated sprints. Besides, acute intake of NO_3^- 2.5–3 h before physical exertion or a dose of 250 ml/d to 500 ml/d of BRJ could lead to similar effective results. This systematic review presents some improvements (1.2–5.38%) in fatigue resistance during repeated sprints when consuming BRJ derived NO_3^-. The in-field practical meaning of these results should be explored.

KEYWORDS
Sports; intake; ergogenic aids; anaerobic exercise; power

Introduction

Nowadays, in elite sport, given the scientific and technological advances, the level between rivals is similar. This knowledge leads to a situation where the margins for a victory become increasingly narrow and motivate sports scientists, athletes, and coaches to seek new alternatives to enhance performance and achieve success. One of these possibilities is ergogenic aids, useful to improve performance indicators that boost the athlete's physiological and physical attributes (Slater, Sygo, and Jorgensen 2019). Food supplements provide the necessary aid to generate a sports performance improvement; indeed, this is why the consumption trend of organic food containing ingredients that collaborate in vital physiological processes has become popular (Sellami et al. 2018).

One of the most recently studied supplements are beets; in this regard, beetroot juice (BRJ) contains potassium, betaine, sodium, magnesium, vitamin C, and nitrates (NO_3^-) (Ormsbee, Lox, and Arciero 2013). NO_3^- are natural compounds that resulted from the nitrogen cycle; they are present in soils, food, and water. When NO_3^- gets in contact with anaerobic bacteria in the oral cavity (Duncan et al. 1995), it converts in nitrite (NO_2^-). Finally, in the bloodstream, NO_2^- lacks oxygen, and it is catabolized in nitric oxide (NO) (Lundberg and Govoni 2004). NO is fundamental in some cardiovascular and metabolic processes as blood flow regulation, muscle contractility improvements, glucose regulation, calcium homeostasis, biogenesis, and mitochondrial respiration (Moreno, Soto, and González 2015; Muggeridge et al. 2014). That is why during exercise the NO_3^- intake leads to cardiovascular (Domínguez et al. 2017) and metabolic (Pawlak-Chaouch et al. 2016) improvements resulting in performance-enhancing in aerobic (McMahon, Leveritt, and Pavey 2017), anaerobic (Lane et al. 2014) and intermittent high-intensity exercises (Domínguez et al. 2018).

The oxygen cost decrease due to NO action contributes to an increase in exercise tolerance (Pardo and Zarazaga 2013) and fatigue delaying (Boorsma, Whitfield, and Spriet

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2014), two essential factors when exercising at high intensities (Domínguez et al. 2017). This NO_3^- intake effect could have a significant impact on sports like soccer (Moher et al. 2015; Moher et al. 2009), basketball (Fox, Stanton, and Scanlan 2018; Pino-Ortega et al. 2020), rugby (Read et al. 2017), triathlon (Jakeman, Adamson, and Babraj 2012), cycling (Hebisz et al. 2019), among others in which the ability to resist repeated sprints is essential. These sports required to execute maximal or near-maximal actions repeatedly (e.g. sprints, actions <10 s) with brief recoveries intervals (60–300 s) to allow an optimal recovery of sprints performance (Girard, Mendez-Villanueva, and Bishop 2011).

Fatigue during repeated-sprint is caused by both neuronal and muscular factors leading to a decrease in maximal power output. While different strategies are used to improve the potential factors that limit the ability to execute repeated-sprints, scientific researchers continue to explore new recommendations (Bishop, Girard, and Mendez-Villanueva 2011). In these cases, the potential positive effect of BRJ derived NO_3^- intake on fatigue resistance during repeated-sprints stills controversial (Martin et al. 2014). Due to opposite opinions regarding the effectiveness of NO_3^- consumption and the lack of clarity regarding the adequate dosage to achieve an effect on physical performance during repeated high-intensity efforts, the present systematic review aimed to explore the scientific knowledge reported to date on the BRJ derived NO_3^- intake effect on fatigue resistance during repeated-sprints. The information that resulted from this review may help to develop new NO_3^- intake strategies to improve fatigue resistance during high-intensity efforts in sports with a high component of repeated intensity actions.

Methods

A systematic review was performed following the Preferred Reporting Guidelines for Systematic Reviews and Meta-analyzes (PRISMA) (Moher et al. 2015; Moher et al. 2009). Two authors (DR-V and J.M-R) independently review studies based on risk-of-bias questions using a 4-point scale ranging from low to high risk-of-bias options. Discrepancies between authors were resolved using consensus. The internal quality of each study was assessed using the Office of Health Assessment and Translation (OHAT) Risk of Bias Rating Tool (OHAT 2015).

Source of information

A literature search was performed at electronic databases: PubMed (MEDLINE), Academic Search, Science Direct (EMBASE), Scopus, Web of Science (WoS), SportDiscus, and Dialnet. The Boolean phrases used as search descriptors were: "beetroot AND/OR sprint," "nitrites AND/OR sprint" and "nitrates AND/OR sprint." All references were extracted and imported into an open-source research tool (5.0.64, Zotero, USA) to systematize studies. A repeated-sprint exercise was defined based on previous literature, and it was considered to select included studies in the present systematic review (Girard, Mendez-Villanueva, and Bishop 2011).

Selection of information

The following inclusion criteria was considered: studies containing keywords in title or abstract, experimental designs in humans, studies published from 2010 to 2020, studies exploring the effects of NO_3^- derived from BRJ on the repeated-sprint performance test, and studies initially written in English language.

The quality of the method (risk of bias) analysis was performed following the PEDro scale (Verhagen et al. 1998). Two different researchers screened the studies and gave a final score; if there was a difference in the criteria, a third researcher made a final decision. Those studies with a final score of 5/10 were included in this review.

Figure 1 shows the results of the risk of bias screening of the selected studies. Only one article obtained the total score of 11 pts (5.55%), five studies obtained 10 pts (27.77%), four researches 9pts (22.22%), three articles 8 pts (16.1%), one article received 7 pts (5.55%), two studies 6pts (11.11%), and two were scored by 5pts (5.55%). The higher the scores represented lower the risk of bias.

Data collection and extraction

Two different authors performed the selection of the studies following the PRISMA protocol (see Figure 2). Specific exclusion criteria was used to discard studies with low quality or irrelevant to the primary purpose of this systematic review as no experimental designs, duplicates, language limitations, different evaluation methods, full text not available, book chapters, abstracts, studies involving factors other than sports as cardiovascular diseases and similar medical and therapeutic interventions, and articles with severe lack of information.

The protocol followed for the selection of the studies was: a. an identification of potential studies, b. duplicates elimination, c. title, abstract, and year analysis, d. quality of method and relevance with the objective of the review wasl also analyzed, and e. selected studies were explored in full text. Finally, e. those studies with a lack of information were excluded.

The selected final studies were analyzed, and the following data was extracted using a descriptive table (see Table 1): authors, year of publication, sample size, sample characteristics, study design, repeated-sprint test used as performance reference, results and the percentage of improvement and studies outcomes related to the effects of BRJ derived NO_3^- intake.

Results

Table 1 shows the compilation of the analysis of the eighteen selected studies for this systematic review; it describes the main methodological aspects and the primary repeated-sprint performance-related outcomes.

Sample characteristics

A total of 209 studies were analyzed, and finally, only 18 studies managed to meet the inclusion criteria in terms of BRJ derived NO_3^- supplementation and assessment of a repeated-

PEDro Scale Item	1	2	3	4	5	6	7	8	9	10	11	Total Score
Christensen, Nyberg & Bangsbo (2013)	√	·	√	√	√	·	·	√	·	√	√	7
Muggeridge et al. (2013)	√	√	√	·	·	√	√	√	√	√	√	9
Martin, Smee, Thompson & Rattray (2014)	·	√	√	√	√	√	√	√	√	√	√	10
Kokkinoplitis & Chester (2014)	√	√	√	·	√	√	√	√	√	√	√	10
Thompson et al. (2015)	√	√	√	·	√	·	·	·	·	·	√	5
Buck et al. (2015)	√	√	√	√	√	·	·	√	·	√	√	8
Wylie et al. (2016)	√	√	√	·	√	√	√	√	√	√	√	10
Thompson et al. (2016)	·	√	·	√	·	·	·	√	√	√	√	6
Clifford et al. (2016)	√	√	√	√	·	·	·	√	√	√	√	8
Thompson et al. (2017)	√	·	·	√	·	·	·	√	√	√	·	6
Muggeridge et al. (2017)	√	√	√	√	·	·	√	√	√	√	√	9
Jonvik, Thompson, Wylie & Vanhatalo (2018)	√	√	√	·	√	√	·	√	·	√	√	8
Jonvik et al. (2018)	√	√	√	√	√	√	·	√	√	√	√	10
Cuenca et al. (2018)	√	√	√	√	√	√	√	√	√	√	√	11
Thompson et al. (2018)	√	·	·	√	·	·	·	√	√	√	·	5
Kent et al (2019)	√	·	√	√	√	√	·	√	√	√	√	9
Pawlak-Chaouch (2019)	√	√	√	√	√	·	·	√	√	√	√	9
Reynolds et al. (2020)	√	√	√	√	√	√	·	√	√	√	√	10

PEDro scale to assess the risk of bias.

Figure 1. PEDro scale to assess the risk of bias.

sprint test. From 337 total participants, 110 were women (32.64%), and 227 were men (67.36%). A total of 61.43% of the participants were team sports practitioners and 38.57% were from individual sports.

Study design

From the 18 investigations, 100% were categorized as randomized studies, 13 (72.2%) were double-blinded, 11 (61.12%) cross-over, and 7 (38.8%) independent groups. A total of 17 (94.4%) studies used placebo groups and three (16.6%) control groups. Additionally, 10 (55.55%) published researches used BRJ NO_3^--depleted placebo, and four (22.22%) selected other beverages as blackcurrant, mixed fruits, tomato, and the rest four articles (22.22%) preferred gels or other NO_3^--depleted substances. The other six (33.3%) studies involved other conditions additionally from BRJ intake (e.g. hypoxia, sprint training, potassium, or sodium phosphate intake).

From the total 18 studies, 11 (61.11%) trials used a chronic BRJ consumption twice a day for three days to four weeks; and 14 (77.77%) prescribed an acute BRJ intake 2–3 h prior tests.

Dose used

The preferred NO_3^- dose was twice a day for 4–6 days, 70 mL with ∼5–6.5 mmol of NO_3^-, for a total 14 (77.78%) studies, other four (22.22%) trials used gels (∼8 mmol), 500 mL (0.5 g of NO_3^-) or 250 mL (≥143 mg of NO_3^-). The most used placebo was a 70 mL shot with a total of ∼0.003–0.5 mg/d of NO_3^-.

Repeated-sprint tests used

A total of 11 (61.11%) studies used a cycle ergometer to perform sprint tests, four (22.22%) selected running field-based tests, and other researches selected swimming (5.55%), kayaking (5.55%) or a treadmill (5.55%) to perform the repeated-sprint assessments.

Tests consisted of one to four series with six to 20 bounts of six to 20 s or alternative 15–40 m sprints. In four cases, a

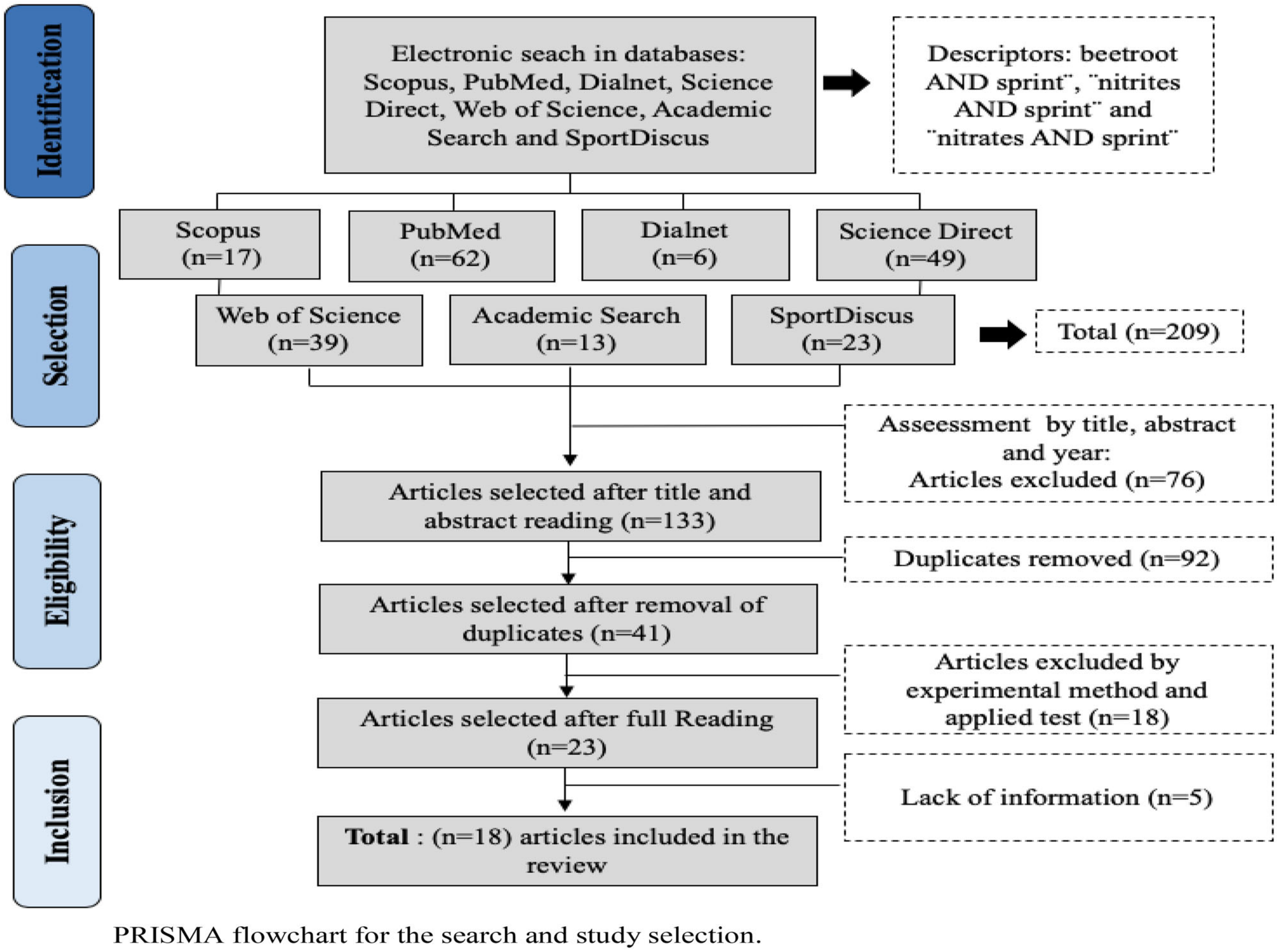

Figure 2. PRISMA flowchart for the search and study selection.

repeated-sprints until volitional fatigue was selected to assess fatigue resistance.

Variables assessed

A total of seven (%) studies used peak power (W) as sprint performance reference, six (%) selected mean power (W), four (22.22%), sprint time (s), two (11.11%) time to task failure, two (11.11%) repetitions (n), two (11.11%) fatigue index (%) and two (11.11%) total work (kJ).

Studies outcome

From those studies that used mean power as sprint performance reference, only two (11.11%) presented a significant difference when comparing the BRJ group vs. placebo or control groups. Two studies presented differences in peak power (11.11%), two in mean power (11.11), one in sprint time (5.55%), one in time to task failure (5.55%) and two in total work total (11.11%). In consequence, a total of seven (38.88%) studies reported any improvement when consuming NO_3^- derived from BRJ.

Finally, percentages of improvement after BRJ intake were as follows: total work (3.35%), peak power (3.75%), mean power output (4.57%), time to peak power (2.8%), and reduction in sprint time (1.78%).

Discussion

This study aimed to explore the scientific knowledge reported to date on the BRJ derived NO_3^- intake effect on fatigue resistance during repeated-sprints. This systematic review found that a third of the studies analyzed presented significant changes when consuming BRJ derived NO_3^- compared to placebo or control groups. The chronic (~5–6 mmol of NO_3^- in 70 ml of BRJ, twice a day, for a minimum of 3–6 days) and acute (2.5–3 h before physical exertion or a dose of 250 ml/d to 500 ml/d) NO_3^- intake could contribute to the improvement of variables related to fatigue resistance and performance during repeated-sprints. Improvements were found in total work (3.35%) (Thompson et al. 2015), peak power (3.75%) (Cuenca et al. 2018), mean power output (4.57%) (Cuenca et al. 2018; Wylie et al. 2016), time to peak power (2.8%) (Jonvik, Nyakayiru, et al. 2018), and reduction in sprint time (1.78%) (Buck et al. 2015; Thompson et al. 2016). The potential practical application of these results must be analized in future in-field trials. Future research may explore if the magnitude of these improvements are meaningful during in-field trials and consequently if the BRJ derived NO_3^-

Table 1. Description of the number of participants and their characteristics, design of the study, sprint tests, results, and outcomes.

Author	Participants	Study design	Repeated-sprint test	Performance reference	Results (% of improvement)	Studies Outcome
(Christensen, Nyberg, and Bangsbo 2013)	n = 10 ♂ Elite Cyclists	Randomized, cross-over: Chronic intake (6d) BRJ: 6d, 500mL, (0.5g of NO_3^-) PLA: 6d x 500ml/d (Apple and Blackcurrant)	Repeated-sprint test (cycle ergometer): 6 x 20s sprints : 100s active recovery	Average peak power: BRJ = 746 ± 111 W PLA = 745 ± 121 W Mean power: BRJ= 630 ± 84 W PLA= 630 ± 92 W	Average peak power: 0.13% Mean power: 0%	Average peak power: BRJ = PLA ($p > 0.05$) Mean power: BRJ = PLA ($p > 0.05$)
(Muggeridge et al. 2013)	n= 8 ♂ Kayakers	Randomized cross-over: acute (3h prior test) BRJ: 70mL (~5 mmol of NO_3^-) PLA: ~0.01 mmol of NO_3^-) (Tomato juice)	Maximal effort sprints (kayaking): 5x 10s sprints: 50s active recovery	Peak power output: BRJ = 404 ± 24 W PLA= 420 ± 23 W	Peak power output: −3.81%	Peak power output: BRJ = PLA ($p = .590$) Total work: BRJ = PLA ($p = .667$) Fatigue index: BRJ = PLA ($p = .555$)
(Martin et al. 2014)	n = 16 (♀ = 9; ♂ = 7) Moderate trained (Team Sports)	Double-blind, randomized cross-over: acute intake 2h prior test BRJ: 70mL (0.3g of NO_3^-) PLA: 70mL (BRJ without NO_3^-)	High-intensity intermittent sprint test (cycle ergometer): 8s sprints: 30s active recovery (until volitional fatigue, unable to pedal at 100rpm, or >45 sprints)	Number of sprints completed: BRJ= 13 ± 5 sprints PLA= 15 ± 6 Total Work: BRJ= 49.2 ± 24.2 kJ PLA= 57.8 ± 34.0 Peak Power: BRJ= 447 ± 104 W PLA = 444 ± 117 W	Number of sprints completed: −13.33% Total Work: −14.88% Peak Power: 0.68%	Number of sprints completed: PLA > BRJ ($p= 0.005$) Total work: PLA > BRJ ($p= 0.027$) Peak power: BRJ = PLA ($p > 0.05$) Mean Power: BRJ = PLA ($p > 0.05$)
(Kokkinoplitis and Chester 2014)	n = 7 ♂ Healthy	Double-blind, Cross-over: acute intake BRJ: 70mL (0.4g of NO_3^-) PLA: 70mL (Blackcurrant) CTL: no intake	Repeated high-intensity sprints (treadmill): 5x6s sprints : 30s active recovery	Mean power output: BRJ= 4133.5 ± 674.4 W PLA= 3938.3 ± 603.1 W CTL= 4013.7 ± 572.9 W	Mean power output: BRJ vs. PLA= 4.96% BRJ vs. CTL= 2.98%	Mean power output: BRJ = PLA = CTL ($p > 0.05$)
(Thompson et al. 2015)	n = 16 ♂ Team Sports (Field hockey, soccer, rugby)	Double-blind, randomized cross-over: Chronic (2 times/d) and acute (2.5h prior test) BRJ: 7d, 70mL (~6.4 mmol of NO_3^-) PLA: 6d, 70mL (~0.04 mmol NO_3^-)	Prolonged intermittent sprint test (cycle ergometer): 2min x 6s sprints (2 x 45min): 100s active + 14s passive recovery	Total work (1st 45min): BRJ= 63 ± 20 kJ PLA: 60 ± 18 kJ Total work (2nd 45min): BRJ= 60 ± 17kJ PLA: 59 ± 16 kJ	Total work (1st 45min): 5% Total work (2nd 45min): 1.69%	Total work (1st 45min): BRJ > PLA ($p < 0.05$) Total work (2nd 45min): BRJ = PLA ($p > 0.05$)
(Buck et al. 2015)	n = 13 ♀ Team Sports (Basketball, soccer, and netball)	Randomized, double-blind: chronic 6d and acute 3h prior test BRJ + SP: acute 70mL (6mmol of NO_3^-) + 6d, 300mL/d (4 × 12.5 mg/kg of $H_{24}Na_3O_{16}P$) BRJ: acute 70mL	Repeated-sprint ability test (running): 3x 6x20m : 25s active recovery between each sprint	Total sprint time (1st set): BRJ + SP= 22.66 ± 1.60s BRJ= 22.95 ± 1.61s SP= 21.94 ± 1.20s PLA= 22.95 ± 1.45s Total sprint time (2nd set):	Total sprint time (1st set): BRJ vs. SP= −3.28% Total sprint time (2nd set): BRJ + SP vs. PLA= 2.03%	Total sprint time (1st set): SP < PLA ($p= 0.02$) SP < BRJ ($p= 0.02$) Total sprint time (2nd set): SP < PLA ($p= 0.02$) SP < BRJ ($p= 0.02$) SP + BRJ < PLA (p=

(*continued*)

Table 1. Continued

		(6mmol of NO_3^-) SP: 6d, 300mL/d (4 × 12.5 mg/kg of $H_{24}Na_3O_{16}P$) PLA: 70mL (~0.04 mmol of NO_3^-)		BRJ + SP= 23.07 ± 1.70s BRJ= 23.37 ± 1.74s SP= 22.25 ± 1.44s PLA= 23.55 ± 1.43s Total sprint time (3rd set): BRJ + SP= 23.11 ± 1.79s BRJ= 23.36 ± 1.76s SP= 22.53 ± 1.48s PLA= 23.48 ± 1.43s		0.05) Total sprint time (3rd set): SP < PLA (p= 0.02)
(Wylie et al. 2016)	n = 10 ♂ Team Sports	Randomized, double-blind, cross-over: chronic and acute (2.5 prior test) intake BRJ: 6d, 70mL/d (~4.1 mmol of NO_3^-) (acute intake 2x70mL) PLA: 6d, 70mL/d (~0.04 mmol of NO_3^-) (acute intake 2x70mL)	All-out sprints (cycle ergometer): Set 1: 24x6s: 24s passive recovery Set 2: 7x30s: 30s passive recovery Set 3: 6x60s: 60s passive recovery	Peak Power output (set 1): BRJ= 792 ± 159 W PLA= 782 ± 154 W Mean power output (set 1): BRJ= 568 ± 136 W PLA= 539 ± 136 W Peak Power output (set 2): BRJ= 768 ± 157 PLA= 776 ± 142 W Mean power output (set 2): BRJ= 558 ± 95 W PLA= 562 ± 94 W Mean Power output (set 3): BRJ= 374 ± 57 W PLA= 375 ± 59 W	Peak Power output (set 1): 1.28% Mean power output (set 1): 5.38% Peak Power output (set 2): −1.03% Mean power output (set 2): −0.71 Mean Power output (set 3): 0.27%	Peak Power output (set 1): BRJ = PLA ($p > 0.05$) Mean power output (set 1): BRJ > PLA ($p < 0.05$) Peak Power output (set 2): BRJ = PLA ($p > 0.05$) Mean power output (set 2): BRJ = PLA ($p > 0.05$) Mean power output (set 3): BRJ = PLA ($p > 0.05$)
(Thompson et al. 2016)	n = 36 ♂ Team sports (Soccer, Rugby, and Hockey)	Double-blind, randomized, cross-over: chronic (5d) and acute (2.5h prior test) intake BRJ: 5d, 70mL/d (6.4mmol) of NO_3^-) PLA: 5d 70mL/d (0.04mmol of NO_3^-)	Repeated-sprint ability test (running): 5x20m sprints : 30s active between each sprint	Mean sprint time (0-20m): BRJ= 3.98 ± 0.18s PLA= 4.03 ± 0.19s Mean sprint time (0-5m): BRJ= 1.73 ± 0.09s PLA= 1.77 ± 0.09s Mean sprint time (0-10m): BRJ= 2.53 ± 0.12s PLA= 2.57 ± 0.12s	Mean sprint time (0-20m): 1.2% Mean sprint time (0-5m): 2.3% Mean sprint time (0-10m): 1.6%	Mean sprint time (0-20m): BRJ < PLA ($p < 0.05$) Mean sprint time (0-5m): BRJ < PLA ($p < 0.05$) Mean sprint time (0-10m): BRJ < PLA ($p < 0.05$)
(Clifford et al. 2016)	n = 20 ♂ Team Sports (soccer, rugby, basketball, handball)	Randomize, independent groups: chronic intake (3d) BRJ: 250 mL (≥143 mg of NO_3^-) PLA: 250 mL (mixed fruit juice with a trace of NO_3^-)	Repeated-sprint test (cycle ergometer): 20x30m: 30s recovery between each sprint	Average Sprint test: BRJ= 4.65 ± 0.25s PLA= 4.70 ± 0.15s Fastest sprint test: BRJ= 4.41 ± 0.23s PLA= 4.48 ± 0.14s Fatigue index: BRJ= 5.60 ± 2.13% PLA= 4.91 ± 1.51%	Average Sprint test: −1.06% Fastest sprint test: −1.56% Fatigue index: 14%	Average Sprint test: BRJ = PLA ($p > 0.05$) Fastest sprint test: BRJ = PLA ($p > 0.05$) Fatigue index: BRJ = PLA ($p > 0.05$)
(Thompson et al. 2017)	n = 36 (♀ = 18; ♂ = 18) Healthy/active, team sports, and endurance	Double-blind of independent groups: chronic (4wk) and acute (2.5h prior test) intake	Sprint interval training (cycle ergometer): Train: 4-5 x 30s all-out sprints: 4min recovery	Time to task failure (Pre-post improvement): BRJ= 163 ± 144 s BRJ + Train= 170 ± 90	Time to task failure (Pre-post improvement): 0%	Time to task failure (Pre-post improvement): BRJ + Train: pre > post ($p < 0.05$)

Table 1. Continued

		BRJ: 4wk, 2x 70mL/d (~6.4 mmol of NO_3^-) BRJ + Train: 4wk, 2x 70mL/d (~6.4 mmol of NO_3^-) PLA + Train: 4wk, 2x 70mL/d (~0.04 mmol of NO_3^-)	between each sprint Severe-intensity exercise test: Time to task failure at a personalized work rate	s PLA + Train = no change		PLA + Train: pre > post ($p < 0.05$) BRJ: pre = post ($p > 0.05$) BRJ + Train = PLA + Train ($p > 0.05$)
(Muggeridge et al. 2017)	n= 27 ♂ Untrained	Randomized, independent-groups: acute (2.5h prior test) BRJ + Train: ~8 mmol of NO_3^- in gel PLA + Train: ~0.1 mmol of NO_3^- in gel CTL: no intake	Sprint interval training (cycle ergometer): 3/wk, 4-6 x 15s all-out sprints: 4min active recovery	Power peak: BRJ + Train= 8.7% PLA + Train= 4.7% CTL = NR	Power peak: BRJ + Train vs. PLA + Train= 85.1% BRJ + Train vs. CTL= 0%	Power peak: BRJ + Train = PLA + Train = CTL
(Jonvik, Nyakayiru, et al. 2018a)	n = 52 (♀ = 29; ♂ = 23) Cyclists, speed-skaters and track cyclist	Randomized double-blinded cross-over: chronic (2x6d) and acute intake (3h) BRJ: 2x6d, 140mL/d (~800 mg/d of NO_3^-) PLA: 2x6d, 140 mL/d (~0.5 mg/d of NO_3^-)	30s Wingate sprint test (cycle ergometer): All-out 30s: 4min recovery between each sprint	Peak power: BRJ= 1338 ± 30 W PLA= 1333 ± 30 W Time to peak power: BRJ improved by ~2.8% vs. PLA	Peak power: 0.38% Time to peak power: 2.8%	Peak Power: BRJ = PLA ($p > 0.05$) Time to Peak power: BRJ < PLA ($p = 0.007$)
(Jonvik, Nyakayiru, et al. 2018b)	n= 14♀ Waterpolo	Double-blinded, randomized, cross-over: Chronic (6d) BRJ: 6d, 140mL/d (~800 mg/day of NO_3^-) PLA: 6d, 140mL/d (~0.5 mg/d of NO_3^-)	Intermittent sprints test (swimming): 16x 15m (4 × 4 block): 30s recovery between blocks	Time: BRJ= 316.0 ± 7.9s PLA= 316.3 ± 6.9s	Time: 0%	Time: BRJ = PLA ($p = 0.73$)
(Cuenca et al. 2018)	n = 15 ♀ Recreational	Randomized cross-over, double-blind: acute (3h prior test) BRJ: 70mL (~6.4 mmol of NO_3^-) PLA: 70mL(~0.04 mmol of NO_3^-)	30s Wingate sprint test (running): All-out 30s: 4min recovery between each sprint	Peak power output: BRJ= 848 ± 134 W PLA= 881 ± 135 W Mean power output: BRJ= 641 ± 91 W PLA= 666 ± 100 W	Peak power output: 3.75% Mean power output: 3.75%	Peak power output: BRJ > PLA ($p= 0.049$) Mean power output: BRJ > PLA ($p= 0.023$)
(Thompson et al. 2018)	n= 30 (♀ = 12; ♂ = 18) Team sports and recreational athletes	Double-blind, independent-groups: chronic (4wk) and acute (2.5h prior test) intake Train: No intake BRJ + Train: 4wk, 2x 70mL/d (~6.4 mmol of NO_3^-) Train + K+BRJ: 4wk, 2x 70mL/d (~6.4 mmol of NO_3^-)	Sprint interval training (cycle ergometer): Train: 3-4/wk x 30s all-out sprints: 4min recovery between each sprint Severe-intensity exercise test: Time to task failure at a personalized work rate	Time to task failure (Pre-post improvement): BRJ + Train= 71% increase Train= 47% increase Train + K+BRJ= 42% increase	Time to task failure (Pre-post improvement): BRJ + Train vs. Train= 51.06% BRJ + Train vs. Train + K+BRJ= 55%	Time to task failure (Pre-post improvement): BRJ + Train > Train > Train + K+BRJ ($p < 0.05$)
(Kent et al. 2019)	n= 12 ♂ Team Sports	Double-blind, repeated-measures, counter-balanced: acute (2h prior tests) BRJ (Hypoxia): 2 × 70	Repeated-sprints efforts (cycle ergometer): 4x 9x4s: 16s active and 16s passive recovery	Peak power: BRJ (Hypoxia)= 1185 ± 172 W PLA (Normoxia)= 1214 ± 179 W	Peak power: BRJ vs. PLA= −2.86% Mean power: BRJ vs. PLA= −4.5%	Peak power: BRJ = PLA ($p > 0.05$) Mean power:

(continued)

Table 1. Continued

		mL (6.45 mmol NO_3^-) PLA (Normoxia): 2 × 70 mL (traces NO_3^-) PLA (Hypoxia): 2 × 70 mL (traces NO_3^-)	(3min recovery between sets)	PLA (Hypoxia)= 1220 ± 136 W Mean power: BRJ (Hypoxia)= 807 ± 144 W PLA (Normoxia)= 829 ± 144 W PLA (Hypoxia)= 845 ± 106 W		BRJ = PLA ($p > 0.05$)
(Pawlak-Chaouch et al. 2019)	n = 11♂ Elite endurance	Randomized, single-blind, placebo-controlled, and cross-over: chronic (3d) BRJ: 340 mg/d of NO_3^- PLA: <2.5mg/d of NO_3^-	supramaximal intensity intermittent sprints (cycle ergometer): 15s (170%) : 30s passive recovery between sprints	Number of repetitions: BRJ= 13.9 ± 4.0 reps PLA= 14.2 ± 4.5 reps	Number of repetitions: 2.1%	Number of repetitions: BRJ = PLA ($p > 0.05$)
(Reynolds et al. 2020)	n= 16 ♂ Team sports	randomized, double-blind: Acute (3h prior test) BRJ: 70mL (∼6 mmol of NO_3^-) PLA: 70mL (∼0.0034 mmol of NO_3^-)	Repeated-sprint performance (running): 10 × 40 m shuttle sprints: 30 s recovery between each sprint	Repeated-sprint performance: BRJ= 5.31 ± 2.49% PLA= 5.71 ± 2.61%	Repeated-sprint performance: −7%	Repeated-sprint performance: BRJ = PLA ($p = 0.337$) Total Sprint time: BRJ = PLA ($p = 0.806$)

Note: d = day (s), wk = week (s), BRJ = beetroot juice, PLA = placebo, CTL = control, NO_3^-= nitrates, W = watts, SP = sodium phosphate, K = potassium.

supplementation causes a significant performance enhancement (Haugen and Buchheit 2016).

Fatigue during repeated-sprints

Fatigue during repeated-sprints is defined as a reduction in maximal power output or speed during exercising (Girard, Mendez-Villanueva, and Bishop 2011). Also, fatigue has a task-dependent factor so that it could differ between different kinds of exercises (e.g. cycling, running, swimming). Consequently, it depends on types of muscle contractions and the magnitude of muscle activation (e.g. concentric vs. excentric, upper-body vs. lower-body) (Bishop 2012).

Some structural and functional particularities of the specific muscle group involved in cycling vs. running could influence fatigue during repeated-sprints (Carpes, Mota, and Faria 2010). The effects of NO_3^- supplementation should be analyzed with caution when performing different types of exercises. Evidence suggests that cycling presents lower demands at the neuromuscular and physiological levels, and this may impact the magnitude of the NO_3^- intake effects (Millet, Vleck, and Bentley 2009). Considering the differences in the distribution of blood flow when performing repeated-sprints testing in cycle ergometer or during running, the cardiovascular effects of NO_3^- could be different and needs future more in-depth analysis.

Fatigue during repeated-sprints is multifactorial, and it could be expressed from neural factors (e.g. inadequate motor command in the prefrontal cortex) to muscular and mechanical elements (e.g. intramuscular accumulation of metabolites) (Girard, Mendez-Villanueva, and Bishop 2011). At the muscle level, some limitations on energy and oxygen supply could be critical factors leading to fatigue (Bishop 2012). In this sense, NO_3^- supplementation could cause physiological benefits as fatigue delay through a decrease in oxygen cost and consumption, due to higher efficiency in adenosine triphosphate (ATP) production and lower ATP consumption (Olsson et al. 2019). These effects on energy cost may lead to better repeated-sprint performance allowing to continue the task in a proper manner and with higher recovery rates (Bishop 2012).

Nitrate benefits on fatigue

The NO_3^--NO_2^--NO pathway mediates several physiological effects as blood flow and oxygen transport improvements. Vasodilation leads to hyperemia in the active muscle as a crucial extra muscular result of BRJ intake (Hoon et al. 2015). NO_3^- intake improves muscule fatigue resistance and attenuates the decline in contractile force (Hoon et al. 2015). NO_3^- when consumed in BRJ generates a positive effect on voluntary contraction and strength (Wylie et al. 2016), this since resulted NO_2^- helps in the type II muscle fibers depolarization and increases Ca^{2+} myoplasm concentrations, generating an increase in actin-myosin bridges to assist in muscle contraction (Bailey et al. 2015).

The process that NO_3^- followed by oxidizing and turning into NO is capable of increase strength and power during muscle contraction. The NO has a fundamental role as an ergogenic benefit by improving energy production and reducing exercise-induced fatigue (Bailey et al. 2010; Buck et al. 2015; Cuenca et al. 2018; Lansley et al. 2011). By consuming BRJ rich in NO_3^-, higher output and maximum power rate are given in response (Jonvik, Nyakayiru, et al. 2018), in addition to a decrease in reaction time. These responses are crucial in the performance of disciplines in which acceleration has a particular influence in success as team and opposition sports (e.g. basketball, soccer) (Pino-Ortega et al. 2020; Svilar, Castellano, and Jukic 2018).

BRJ also contains a large number of antioxidants, suppressing the accumulations of leucocytes (Jädert et al. 2012) that are the primary producers of reactive oxygen species after exercise, and that cause muscle damage (Nikolaidis et al. 2008). Other compounds as the betalains attenuate muscle damage due to some analgesic and anti-inflammatory effects. The same mechanism can mitigate the intensity of muscle pain and collaborate in the recovery process (Pietrzkowski et al. 2014). A previous study showed that there is a reduction in pain trigger points when consuming BRJ for five days, compared to a single intake (Pietrzkowski et al. 2009); this may suggests that long-term consumption of NO_3^- generates greater analgesic effects (Clifford et al. 2016). That is why BRJ can lead to a decrease in muscle fatigue, although they're still some doubt on this behalf, and more evidence is needed to develop a more definite statement.

Dosage

Like any other ergogenic aid, it couldn't recommend an optimal dose. The dose depends on factors such as the level of physical condition, type of sport, accessibility, the magnitude of the prescription, and duration, among other factors that consider the individuality of each athlete.

A recent study found that only five products among 24 different commercials BRJs provided $\geq$5 mmol NO_3^-/serving (Gallardo and Coggan 2019), which appears to be the dosage needed to improve exercise performance (Peeling et al. 2015; Wylie et al. 2013). This evidence suggested that basic recommendations must be followed when using BRJ derived NO_3^- as a supplement.

Some studies recommend high doses (8–16 mmol) of NO_3^- due to its effect on the increase of NO_2^- in plasma levels and future increase of NO. This systematic review, regarding fatigue resistance during repeated-sprints, found that the magnitude of the doses would depend on the duration of the supplementation. Some differences between acute and chronic supplementations were found.

Chronic NO_3^- intake should be performed at least twice a day for 4–6 days at a concentration of ~5–6.5 mmol of NO_3^- (Cuenca et al. 2018; Jones et al. 2018; Jonvik, Nyakayiru, et al. 2018; Kent et al. 2019; Reynolds et al. 2020); the preferred dosage was 70 mL of BRJ in the morning and 70 mL in the evening. On the other hand, acute intake should be performed two to three hours before the training and competition in a single dose of 70 mL of BRJ with ~5–6.5 mmol of NO_3^- (Kokkinoplitis and Chester

2014; Muggeridge et al. 2013; Thompson et al. 2015). Future research should be performed using different doses magnitude and duration due to the trivial to small differences found in the analyzed articles.

Despite these recommendations, based on the presented evidence, there is a significant limitation of the studies regarding the supplementation of NO_3^-. As other well-know dietary supplements, the doses are usually prescribed relative to the weight of the athlete (Jeukendrup 2014; Naderi et al. 2016), in consequence, the NO_3^- should be intake per kilogram of weight instead of a single homogeneous dose as selected in all the studies included in the present review. This dose's personalization effects should be explored in future research.

Limitations

While this study presents some evidence on the BRJ derived NO_3^- intake effects on fatigue resistance during repeated-sprints, these findings must be seen in the light of some limitations. Due to the wide range and heterogeneity of the repeated-sprint test used, it is difficult to interpret the results of the studies fully. There are clear differences in the distances, times, type of exercise, and participant's fitness level, which makes it difficult to compare results and to report the real effect of BRJ on fatigue resistance. Also, there were some difficulties in the interpretation of the actual impact of BRJ derived NO_3^- due to the varied design protocols of studies.

Practical applications

Chronic intake of ∼5–6 mmol of NO_3^- in 70 ml of BRJ, twice a day, for a minimum of three to six days, could lead to a fatigue resistance improvement during repeated sprints under controlled situations. Besides, acute intake of NO_3^- 2.5–3 h before physical exertion or a dose of 250 ml/d to 500 ml/d of BRJ could lead to similar results.

The benefits of chronic and acute BRJ rich in NO_3^- could be useful in sports in which explosive actions and repeated high-intensity intermittent efforts are crucial for general performance as basketball, handball, soccer, cycling, among others. Despite the potential use of BRJ rich in NO_3^- in elite and amateur sports, it should be analyzed if these physiological improvements are significant enough to be applied under in-field conditions. Some considerations regarding dosage, intake duration, and some supplementation timing factors should be considered when prescribing this kind of ergogenic aid.

The consumption of beet juice has direct repercussions on the body, and one of them is the change of color in the urine; this is an aspect that generates complexity in the application of double-blind trials. Besides, there are some latent risks if NO_3^- daily requirements are exceeded (Braakhuis and Hopkins 2015; Poortmans, Gualano, and Carpentier 2015; Zamani et al. 2020).This issue should be addressed by the scientific community interested in developing research protocols involving BRJ intake.

Other practical limitations, is that the use of mouthwash or other similar substances must be suspended during the consumption of NO_3^- since it may lead to difficulties in its absorption process. The contact of NO_3^- with anaerobic bacteria located in the saliva is necessary to convert it to NO_3^- so that the effect is presented without difficulty. Other particular practical application details of the BRJ, such as its sweet and earthy flavor, are essential factors to consider when administered to an athlete as a supplement.

Conclusions

By consuming BRJ rich in NO_3^-, a series of physiological benefits are generated and may impact some sport performance indicators as fatigue resistance in repeated-sprints. Some improvements in total work (3.35%), peak power (3.75%) and mean power output (4.57%), time to peak power (2.8%), and reduction in sprint time (1.78%) are examples of how NO_3^- intake have some potential to enhance repeated-sprints performance. Future in-field trials should be performed in order to confirm if the magnitude of these improvements are practically meaningful.

These effects may differ on NO_3^- dose, intake duration (e.g. chronic or acute), type of exercise (e.g. cycling vs. running) and muscle contraction (e.g. eccentric vs. concentric), fatigue-related variables chosen as reference (e.g. power output vs. number of repetitions), anaerobic performance levels.

Despite a third of the studies presented significant results when consuming BRJ rich in NO_3^-, due to the vast range of reported possibilities regarding the "adequate dose" when using this supplements, these results should be taken with caution. Researchers and practitioners may delve into the real magnitude of the performance improvements and its potential impact on the field when training and competing. Besides, the potential benefits of NO_3^- should be balanced against the latent risk of exceeding NO_3^- daily requirements.

Future research may dig deeper into the effect of more extended NO_3^- intake protocols, higher dosing, and combination with other supplements. Considering the impact of NO_3^- on fatigue, it could be interesting to explore the effects of its intake on inflammation and as a between efforts recovery aid. This research should be developed considering the potential risks of exceeding acceptable NO_3^- daily intake.

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