

Acute Dose of Beet Root Juice Does Not Improve Endurance Performance in Elite Triathletes

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Abstract

The aim of this study was to investigate whether consuming a high acute dose of NO_3 -rich beetroot juice (BRJ) results in a significant (A) increase in plasma nitrite (NO_2^{-}), and; (B) improvement in exercise performance during a 30 min time trial (TT) among competitive elite triathletes. Eight triathletes (4 females, 4 males; age 22 ± 0.9 yrs) participated in this study. (A) Baseline (PRE) venous blood samples were taken. Subjects then consumed 210 mL (19.4 mmol NO_3^{-}) of BRJ and a second blood sample was taken 2.5 hr after consumption (POST) and blood samples were analyzed for [NO_2^{-}]. (B) Seven days later, in a randomized, single-blinded, crossover design, subjects completed two laboratory-based 30 min TT separated by 7 days. 2.5 hr prior the TT, subjects consumed either BRJ or a nitrate-free BRJ placebo (PL). (A) Plasma [NO_2^{-}] did not significantly change from baseline values after consuming 210 mL of BRJ (PRE: 1.62 ± 0.17 uM, POST: 1.76 ± 0.15 uM). (B) In response to BRJ supplementation, there was no significant difference over the TT in mean power output (BRJ: 4.19 ± 0.2 W/kg, PL: 4.23 ± 0.2 W/kg), distance covered (BRJ: 16.1 ± 0.6 km, PL: 16.2 ± 0.7 km), or average speed (PL: 32.4 ± 1.4; BRJ: 32.6 ± 1.3 kph). Further, when the subjects were separated into BRJ 'responders' and 'non-responders' we did not observe statistically significant changes in any of the performance measures between the PL and BRJ supplementation. In conclusion, there is no ergogenic benefit to supplementing elite athletes with an acute dose of BRJ prior to a high intensity middle-distance cycling competition.

Keywords: Beetroot; Cycling; Ergogenic aids; Time trial; Elite athletes

Introduction

Supplements thought to increase nitric oxide (NO) have received considerable attention as an ergogenic aid in both the scientific community and among athletes. NO is a signaling molecule which has a number of physiological roles in the body, including the regulation of vascular tone, immune function, neurotransmission, muscle contractibility, and energy metabolism [1,2]. Nitrite (NO_2^{-}) is a bioactive source of NO and levels in the plasma can be increased by consuming foods that are high in nitrate (NO_3^{-}) , such as leafy green vegetables and beetroot.

Improvements in whole body exercise efficiency, tolerance, and performance have been reported in response to NO_3^- -rich BRJ supplementation. More specifically, the volume of oxygen (VO_2) consumed at any given rate of work is reduced; or alternately, the power output (PO) is increased for any sustainable metabolic rate of oxygen utilization [3-5]. Research has suggested that the increase in exercise efficiency may be due to the reduction in VO_2 ; which may be the result of the decreased energy cost of muscle force production and/or the increased adenosine triphosphate production per unit oxygen consumed [6]. Exercise tolerance has also been reported to be improved with NO_3^- supplementation. In fact, exercise tolerance during intense cycling has been reported to increase by 3 to 25% during time-to-exhaustion tests [2,3,7,8]. Performance, as determined by a cycling time trial (TT) test was also shown to improve by 1.2 to 2.8% in moderately trained subjects [3,9].

Although this performance improvement may seem insignificant, researchers have suggested that an increased performance of 0.6% for an elite athlete is sizable enough to be of benefit [10]. However, the majority of researches reporting exercise benefits of NO3⁻ supplementation have been conducted on moderately/recreationally active subjects, while findings are less clear in populations of elite

athleticism [11]. The majority of studies in trained athletes have reported no exercise benefits in response to NO₃⁻ supplementation [4,12-15], while several studies have reported benefits [3,4,16,17]. It has been speculated that exercise benefits in trained athletes may be blunted with NO3⁻ supplementation since these individuals may have no need for further enhancement of their cardiovascular system as it is nearing physiological capacity [18]. In addition, exercise duration and intensity may be partly responsible for the equivocal results, as the ergogenic effects of BRJ supplementation on exercise performance may be greater during high intensity exercise where a higher number of type IIb muscle fibers are activated [19]. The elevated hypoxic and acidic environment may potentiate both the conversion of NO₂ to NO and the associated physiological benefits of higher NO plasma levels [19]. Research by Muggeridge et al. [16] has demonstrated that a relatively low acute dose (5 mmol) of BRJ during normobaric hypoxia conditions significantly reduced the O, cost and enhanced cycling performance by 2.2% during a high altitude (2500 m) cycling trial (16.1 m) among trained cyclists. However, at conditions of normal altitude, low doses of BRJ generally have not been found to elicit performance benefits in trained athletes.

The effectiveness of supplementation, in terms of performance benefits, may be reduced in athletic populations due to higher baseline

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plasma levels of NO⁻ in comparison to non-athletic groups [20,21]. Increasing the dose may be required in elite populations to elicit similar performance benefits as reported in moderately trained groups [3,9,12,17,22,23]. In fact, Lansley et al. [3] reported a decrease in time to complete a 4 km TT by 2.8% and a 16.1 km TT by 2.7% when supplementing trained male cyclists with 6.2 mmol NO, BRJ 2.5 hr prior to exercise. Further, a 4.5 to 6% increase in PO was also observed in the 4 km TT. Another recent study reported that a single dose of 4.3 mmol NO₃ had no effect on simulated 2000 m rowing performance, whereas a dose of 12.9 mmol NO, resulted in improved rowing performance [17]. It has also been reported that variability exists among populations of elite athletes; with some athletes experiencing increases in plasma [NO₂⁻] and performance while others do not experience plasma [NO₂⁻] increases or performance improvements. For this reason, Wilkerson et al. [24] has proposed that, based on the plasma NO₂⁻ response, athletes should be separated into two groups; 'responders' (>group mean of plasma [NO₂]) and 'non-responders' (<group mean of plasma [NO₂]) to BRJ supplementation.

Dose, exercise intensity, and individual response are important factors to take into consideration when supplementing elite athletes with an acute dose of NO_3^{-} . Therefore, the purpose of this study was to investigate whether an acute high dose of NO_3^{-} rich BRJ (210 mL (19.4 mmol NO_3^{-}) in comparison to an NO_3^{-} -free placebo (PL), would A) increase plasma $[NO_2^{-}]$, and; B) improve physical performance (i.e., distance, PO, speed) of elite triathletes competing in the World Triathlon Series (WTS) circuit during a high intensity, middle distance cycling performance (30 min TT). We also aim to identify whether each athlete is a 'responder' or a 'non-responder' to BRJ supplementation.

Materials and Methods

Subjects

Eight trained triathletes (4 females, 4 males) participated in the study (females: age 22 ± 0.9 yrs; height 168 ± 4 cm; weight 61.5 ± 1.4 kg; functional threshold power 221 ± 12 watt (W); males: age 23 ± 0.9 yrs; height $182 \pm$ cm; weight 75.4 ± 1.1 kg; functional threshold power 331 ± 13 W).All of the subjects were National level triathletes and compete internationally in the World Triathlon Series races and elite World Championships. Subjects trained for an average of 21 ± 3 hr per week. The Research Ethics Board of the Canadian Sport Institute Ontario (Toronto, ON) granted ethics approval of all procedures of the study. Subjects were informed both verbally and in writing of the experimental protocol and potential risks before giving their verbal and written consent to participate.

Dietary and training standardization

All subjects were instructed to keep a food/drink log in the 24 hr preceding their first experimental visit and were instructed to replicate their food/drink consumption and physical activity in the 24 hr preceding all testing days. Subjects generally consumed diets high in CHO which ensured that glycogen supply would not be limited during the performance trials. Subjects were instructed to avoid drinking caffeine 24 hr prior to each test, as caffeine has been shown to induce performance enhancing effects [22]. Subjects also refrained from using antibacterial mouthwash and chewing gum 24 hr prior to each test since these have been shown to kill bacteria responsible for converting nitrate to nitrite in the mouth, thus reducing nitrite bioavailability [25]. During the study each athlete maintained the same training load which was verified by analyzing each athlete's training log. Further, all subjects were very familiar with the 30 min TT test and the equipment used since all had previously participated in workouts using power trainers for TT practice.

Part A: Determining plasma nitrite response

All subjects reported to the laboratory on one occasion to determine the plasma NO₃⁻/NO₃⁻ response of a high dose of BRJ. Subjects arrived at 9 am after having their normal breakfast which was recorded and repeated each trial morning. Upon arrival to the laboratory, a baseline venous blood sample (PRE) was obtained for the measurement of resting plasma [NO₂⁻]. Subjects then consumed 210 mL (19.4 mmol NO₃) of concentrated BRJ (Beet It, James White Drinks, Ipswich, UK) and a second venous blood sample was taken 2.5 hr after consumption (POST). Subjects engaged in passive rest between PRE and POST blood sampling. Venous blood samples were collected in EDTA treated tubes and centrifuged with the supernatant analyzed for total [NO,⁻] via a NO₂⁻/NO₂⁻ fluorometric assay (No.780051, Caymen Chemical Company, Ann Arbor, MI, USA). Athletes were separated into two groups based on their plasma [NO,⁻]) response: 1) 'responders', defined as a plasma value > group mean, and; 2) 'non-responders' defined as a plasma value < group mean.

Part B: Performance trials

In following, subjects arrived to their training location for two performance trials each 7 days apart. During the performance trial, subjects completed a 30 min cycling time trial (TT) with the goal being to cycle the furthest distance in 30 min. Performance trials were conducted using the subject's racing bike mounted on a calibrated power trainer (Wahoo Fitness KICKR, Atlanta, GA, USA). The Wahoo KICKR was calibrated before each ride and after the warm-up using the spindown procedure set forth by Wahoo Fitness. The spindown served two functions: 1) to determine the power required to overcome friction in the bearings and belt, and 2) set the zero-offset on the strain gauge.

In a randomized, single blind, crossover design, subjects consumed either 210 mL of BRJ (19.4 mmol NO₃; Beet-it, James White Drinks, Ipswich, UK) or the same volume of a NO₃⁻ -free BRJ (0.065 mmol NO₃⁻ /70 mL; Beet-it Nitrate-free Placebo (PL), James White Drinks, Ipswich, UK) 2.5 hr prior to the start of the TT. The BRJ and PL supplements were identical in packaging, appearance, taste, and smell. Prior to the TT, subjects underwent a 20 min cycling warm-up of choice which was replicated for the second trial. PO and revolutions per minute (RPM) were recorded throughout. Total distance and average speed were recorded at the end of the TT. A capillary blood sample was obtained at rest, 10, 20, and 30 min of the TT and analyzed for blood lactate concentration (Lactate Pro Analyzer). Heart rate (HR) and rating of perceived exertion (RPE) were collected every 10 min throughout the TT. Athletes were motivated with monetary incentives for completing the furthest cumulative distance over the two trials. The trials were randomized and separated by 7 days. One female was removed from Part B of the study due to illness.

Statistical analysis

To determine the differences between treatment (PL, 210 mL BRJ) and plasma $[NO_2^{-1}]$ and performance variables (mean distance, power and speed) Student's paired *t* tests were performed. Lactate, heart rate, and RPE variables were analyzed at four time points (0, 10, 20, 30 min) during the 30 min TT using the repeated measures ANOVA. Statistical tests were performed using GraphPad Prism (Version 6.0f, La Jolla, CA, USA). Statistical significance was accepted when P < 0.05. All data are mean \pm SD.

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Results

Part A: Plasma [NO₃/NO₂]

Baseline plasma NO₃⁻ significantly increased 2.5 h after consuming 210 mL of concentrated BRJ compared to pre-supplementation values (PRE: 45.6 ± 7.4 μ M, POST: 461.2 ± 97 μ M; p < 0.01; Table 1). However, NO₂⁻ content did not significantly change from baseline (PRE: 1.62 ± 0.17 μ M, POST: 1.76 ± 0.15 μ M; p = 0.21; Table 1). Based on plasma NO₂⁻ response, 4 (3 males and 1 female) of the 8 athletes were 'responders' (classified as subjects with an increase in plasma [NO₂⁻] greater than the group mean of 8.7%) to BRJ supplementation. The mean plasma [NO₂⁻] increase of the 'responders' was significant (PRE: 1.35 ± 0.21, POST: 1.84 ± 0.19, p=0.03) from baseline values and increased by 36.5%.

Part B: Time trial performance

There was no significant difference in mean PO during the 30 min TT between the PL (288 ± 23 W) and the BRJ (285 ± 22 W) treatments (Table 1, p > 0.05). Similarly, there was not a significant difference for the total distance covered (PL: 16.3 ± 0.7 km; BRJ: 16.1 ± 0.6 km; Table 1, p > 0.05) or for the TT speed (PL: 32.4 ± 1.4; BRJ: 32.6 ± 1.3 kph; Table 1, p > 0.05). Further, there was no significant difference in lactate, heart rate, and RPE between BRJ vs. PL at all-time points during the TT (Table 1, p > 0.05). There was, however, a significant positive relationship of time on blood lactate response and heart rate in both trials (Table 2, p < 0.05).

When the subjects were separated into 'responders' and 'nonresponders' there were no significant changes in any of the blood or performance measures within the 'responder' group between the PL and BRJ treatments (Table 3). Further, we were unable to compare between the 'responder' and 'non-responder' groups since the sexes were not equally representative in each group, with 3 males and 1 female representing the 'responder' group while 3 females and 1 male represented the 'non-responder' group. Thus, if 'responder' and 'nonresponder' groups were compared, our performance measure results would likely have represented a sex effect rather than an effect of plasma [NO,].

Discussion

To our knowledge, this is the first study in competitive elite triathletes to report that an acute high dose of exogenous NO_3^- -rich BRJ (210 mL; 19.4 mmol) does not result in performance benefits during a high intensity, middle distance cycling performance. The results of this study are similar to the majority of studies reporting that BRJ supplementation in athletic cohorts does not point to performance benefits when evaluating the group as a whole [4,12,13,18,23]. However, research has reported variability in populations of athletes, whereby some athletes are 'responders' to BRJ supplementation (elevate their plasma $[NO_2^-]$ above the group mean) while others are 'non-responders'. When the subjects of the current study were separated into BRJ 'responders' and 'non-responders' we did not observe statistically significant changes in any of the performance measures between the PL and BRJ supplementation.

Variable	Placebo	Beetroot
Baseline plasma [NO ₃] (uM)		45.6 ± 7.4
Post-beverage plasma [NO ₃] (uM)		461.2 ± 97.3
Baseline plasma [NO ₂] (uM)		1.62 ± 0.17
Post-beverage plasma [NO ₂] (uM)		1.76 ± 0.15
Distance (km)	16.3 ± 0.7	16.1 ± 0.6
PO (W)	288 ± 23	285 ± 22
Blood [lactate] (mM)	8.3 ± 0.1	9.0 ± 0.3
HR (bpm)	174 ± 5	171 ± 7
RPE	16.8 ± 0.3	16.7 ± 0.4

Table 1: Blood and performance response to a 30 minute time trial after supplementation with nitrate-rich beetroot or placebo (n = 7).

Time (min)	0		1	0	2	0	30		
	PL	BRJ	PL	BRJ	PL	BRJ	PL	BRJ	
Lactate (mmol)	1.5 ± 0.2	1.6 ± 0.2	5.3 ± 0.4	6.1 ± 0.3	8.3 ± 0.7	7.9 ± 0.7	10.3 ± 0.8	10.9 ± 1.2	
HR (bpm)	74 ± 5	72 ± 4	173 ± 6	175 ± 4	179 ± 4	179 ± 4	189 ± 3	185 ± 4	
RPE			17 ± 1	16 ± 1	19 ± 1	19 ± 1	19 ± 1	19 ± 1	

Values are means ± SD. PL, placebo; BRJ, beetroot juice; HR, heart rate; RPE, rating of perceived exertion. Significant effect of time for all time points in both the PL and BRJ trials for lactate and HR (*p* < 0.05).

Table 2: Blood lactate, heart rate, and rate of perceived exertion during a 30 minute time trial in response to supplementation with nitrate-rich beetroot juice or placebo (n = 7).

Subject	2	BRJ	4 PL	BRJ	5 PL	BRJ	7 PL	BRJ	MEAN		MEAN		%
	PL								PL	SD	BRJ	SD	Difference
Distance (km)	14.58	14.27	18.17	18.32	16.24	16.71	18.3	17.85	16.82	0.9	16.79	0.9	0.2
PO (W)	221	210	366	377	330	334	331	315	312	31.5	309	35	1.0
Speed (km/hr)	29.2	28.6	36.3	36.6	32.6	33.4	36.6	35.7	33.7	1.8	33.6	1.8	0.2
HR (bpm)	189	154	176	172	166	166	183	180	179	4.9	168	5.5	5.9
Lactate (mmol)	7.9	9.1	9.3	10.4	5.8	7.1	6.1	6.5	7.3	0.8	8.3	0.9	-13.7
RPE	16	15.5	18	18	15.5	16.5	18	16.5	16.9	0.7	16.6	0.5	1.5

Values are means ± SD. PL, placebo; BRJ, beetroot juice; HR, heart rate; RPE, rating of perceived exertion; PO, power output.

Table 3: Performance response of the 'responders' to a 30 minute time trial after supplementation with nitrate-rich beetroot or placebo (n = 4).

Plasma [NO₂⁻]

The baseline plasma $[NO_2^{-1}]$ of the current study was 1.62 ± 0.1 μ M and, although not significant, was elevated by 8.7% in response to BRJ supplementation. Our plasma [NO₂⁻] values are most similar to, although lower than, those of Bescos et al. [18] who reported a baseline $[NO_{2}]$ of 2.31 ± 0.16 μ M and a mean increase of 16% following sodium nitrate intake (10 mg/kg) in trained endurance cyclists (male; n=11; 34.3 ± 4.8 yr). Interestingly, our basal levels of plasma NO₂⁻ were much higher than those reported in other populations of healthy adults [3,6,25]. The elevated baseline $[NO_2^{-1}]$ values of the current study may in part be due to the habitual dietary intake of foods containing NO₃. An analysis of the 24 hour food/drink log indicated that on average, subjects consumed 100-150 mg of NO3⁻/day. Females consumed greater amounts per kg body mass (BM) than males (females 2.4 mg/ kgBM vs. males 1.3 mg/kgBM). This may have been a contributing factor as to why more males (75%) were 'responders' while the majority of females (75%) were 'non-responders' to BRJ. In addition, the athletes consumed on average greater daily intakes of NO₃⁻/day in comparison to that of the general populous (62-124mg of NO_3^{-1}/day) [26,27].

As a group, the mean post-BRJ beverage plasma [NO,⁻] did not significantly change. Similar to other studies of trained subjects [13,24], some of our subjects were 'responders' while others were 'nonresponders' to NO3⁻ supplementation. In fact, 4 out of the 8 subjects (50%) experienced a significant increase in plasma [NO,] by 491 nM (37%) in response to BRJ intake. Among the 'non-responders' 3 subjects had a mean decrease in plasma [NO₂⁻] by 161 nM (61%), and 1 subject had a mean increase in plasma [NO₂] by 182 nM (0.9%). Interestingly, some previous studies have separated subjects based on plasma [NO₃⁻] rather than [NO₃⁻]. If we had done this, subject 1 would have been grouped as a 'responder' while subject 2 would have been a 'non-responder'. Subject 2 demonstrated no performance benefit to supplementation. In addition to elevated plasma [NO3-], subject 1 demonstrated performance improvements and thus would have potentially been miss-grouped as a true 'responder' to BRJ supplementation, while in fact, the idea of 'responders' and 'nonresponder' classification is based on the plasma $[\mathrm{NO}_3^{-1}]$ and not $[\mathrm{NO}_3^{-1}],$ since NO_{2}^{-1} is the bioactive source of NO [24].

Performance measures

The lack of performance improvement post-NO₃ supplementation in the current study is consistent with research conducted in trained cyclists and other trained athletes [4,13,14,15,18], although contrary to research in recreationally active/moderately trained individuals demonstrating improved exercise economy and performance [5,7,8,13,28]. When examining the group as a whole we did, however, observe that the distance and PO improved by 1.9% and 2.1% respectively, after consuming BRJ. When the subjects were separated into 'responders' and 'non-responders' there were no significant changes in any of the performance measures within the 'responder' group between the PL and BRJ treatments (Table 3). Two (male; subjects 4 and 5) of the four 'responders' did have higher mean PO (3% and 1.2%; respectively) and distance covered (0.8% and 2.9%; respectively) in the BRJ vs. PL trial (Table 3), although these changes were not significant. However, the mean percent change of the 'responders' for PO and distance was very similar to the mean percent change of all the subjects for PO and distance covered (1.2% and 1.0%; respectively). Further, when evaluating the 'non-responders', one subject (female) improved her performance with BRJ (1.2% PO; 2.6% distance; 1.2% speed) (Table 4). Even though the performance results (PO, distance) of all groups were insignificant with regard to BRJ supplementation, the coefficient of variation (CV) for-performance in sprint and endurance sports is 0.6-1.4% and our mean values fit within or are greater than this range [29]. However, since all the groups (whole, 'responders', and 'nonresponders') had values within this range our results as to whether BRJ supplementation is of benefit to performance is inconclusive. It is therefore probable that the percent change in the performance measures is due to small sample size and few performance trials daily variation rather than an effect of supplementation.

It has also been suggested that resting plasma [NO₃⁻] and/or [NO₃⁻] is directly proportional to exercise capacity; with athletes exhibiting increased training-related NOS activity in comparison to their untrained counterparts [20,24]. Thus, the likelihood of significantly improving exercise performance in elite populations may be reduced due to consistently elevated levels of plasma [NO,] in comparison to a population of untrained/moderately trained subjects. The lack of improvement in performance, even among the 'responders', supports the idea that in well-trained and in elite athletic subjects a threshold may exist; whereby training adaptations of endurance exercise may provide the subject with sufficient amounts of endogenous NO₂⁻ and the athlete may not require any additional NO3⁻ from exogenous sources to meet their needs [18,24]. In fact, the reliance on the nitrate-nitrite-NO pathway during endurance exercise may be reduced in trained athletes due to the reduction of hypoxic loci within the skeletal muscle [24]. Endurance training also increases the muscle capillary density and thus improvements in muscle perfusion and O₂ distribution may not be seen, since these individuals are already operating with optimal blood flow and O₂ distribution levels [11,19].

Limitations

A limitation in the current study was the time period between supplementing the subjects with BRJ and the measuring plasma $[NO_2^{-1}]$. Research has reported that individual variability exists in the time to peak plasma $[NO_2^{-1}]$. In fact, Wylie et al. [28] reported that the time to peak plasma $[NO_2^{-1}]$ can range from 2-6 hours. In the current study, plasma $[NO_2^{-1}]$ values were measured at the low end of the range (2.5 hours post intake). This may account for the lack of elevation in plasma

Subject	1		3 PL	BRJ	6 PL	BRJ	MEAN		MEAN		%
	PL	BRJ					PL	SD	BRJ	SD	Difference
Distance (km)	16.58	16.75	13.82	13.6	18.41	16.2	16.3	1.3	15.5	0.9	4.6
PO (W)	261	268	226	219	359	324	282	40	270	30	4.1
Speed (km/hr)	33.2	33.6	27.7	27.3	36.8	32.4	29.2	2.0	31.1	1.9	-6.4
HR (bpm)	176	179	195	194	177	179	183	6.2	184	5.0	-0.7
Lactate (mmol)	9.6	10.2	8.7	10.4	8.6	7.5	9.0	0.3	9.4	0.9	-4.5
RPE	19	19	18.5	18	18.5	18	18.7	0.2	18.3	0.3	1.8

Values are means ± SD. PL, placebo; BRJ, beetroot juice; HR, heart rate; RPE, rating of perceived exertion; PO, power output.

Table 4: Performance response of the 'non-responders' to a 30 minute time trial after supplementation with nitrate-rich beetroot or placebo (n = 3).

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 $[NO_2^{-1}]$ for some of the subjects ('non-responders') and potentially the lack of improvement in performance measures. Another limitation in the study was that we failed to measure VO₂. This would have provided information regarding the O₂ cost of exercise and the interaction between PO and VO₂ in response to BRJ supplementation. Lastly, this study would have been strengthened by testing a greater number of subjects and increasing the number of performance trials, which would also have eliminated/reduced the daily variation in exercise performance.

In conclusion, we have shown in competitive elite triathletes that an acute high dose of NO_3^- rich BRJ does not elevate plasma $[NO_2^-]$ and does not result in performance benefits in response to a high intensity, middle distance 30 min TT. Further, although our data indicated plasma $[NO_3^-]$ 'responders' and 'non-responders' this did not translate into improvements in performance.

Practical Applications

There is no ergogenic benefit to supplementing this caliber of athlete with an acute dose (19.4 mmol) of BRJ 2.5 hr prior to an intense cycling competition.

Competing interests

The authors declare that they have no competing interests.

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