

Research article

## A Single Dose of Beetroot Juice not Enhance Performance during Intervallic Swimming Efforts

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### Abstract

Despite the numerous scientific evidence on the topic, there is no clear and consistent answer that clarifies the true effects of beetroot juice (BJ) supplementation on different types of physical performance. This study examined whether an acute intake of BJ improves swimming performance, physiological variables of anaerobic metabolism, or subjective measures during high-intensity interval exercise with incomplete rest in competitive swimmers. Eighteen competitive swimmers (nine females and nine males) participated in this cross-over randomized, placebo-controlled, double-blind and counterbalanced study. In two trials, swimmers ingested BJ (70 mL, 6.4 mmol/400 mg NO<sub>3</sub><sup>-</sup>) or placebo (PLA) (70 mL, 0.04 mmol/3 mg NO<sub>3</sub><sup>-</sup>) three hours before a 2×6×100 m maximal effort with 40 seconds rest between repetitions and three minutes between blocks. The 100 m times showed no differences between groups ( $p > 0.05$ ), but there was an interaction between block×repetition×condition ( $F_5 = 3.10$ ;  $p = 0.046$ ;  $\eta^2 = 0.54$ ), indicating that the BJ group decreased the time of the sixth repetition of block2 compared to block1 ( $p = 0.01$ ). Lactate concentration showed no differences between conditions ( $p > 0.05$ ), but there was a main effect of block ( $\eta^2 = 0.60$ ) and a block×repetition interaction ( $\eta^2 = 0.70$ ), indicating higher values in block2 and increasing values between repetitions in block1. The subjective scales, perception of exertion (RPE) and Total Quality Recovery (TQR), showed no effects of condition ( $p > 0.05$ ), but BJ swimmers had a greater TQR in the last repetitions of each block. In conclusion, a single dose of BJ did not enhance intermittent swimming performance or modified the physiological (lactate and heart rate) or subjective (RPE and TQR) variables; although there was a possible positive effect on the exercise tolerance at the end of effort.

**Key words:** Nitrate, ergogenic aids, intermittent efforts, swimming performance, exercise tolerance.

### Introduction

In competitive swimming, 81% of the events on the Olympic schedule are 200m races or shorter, typically lasting no more than two and a half minutes. However, in contrast to these relatively brief times, the in-water training volume of young competitive swimmers (aged 13 - 17) can traditionally range from 4,000 m to 7,000 m per session (Amara et al., 2022) with a large proportion of the weekly training volume usually performed at low intensities (Mujika, 1998). In recent years, the effects of high-intensity interval training in competitive swimmers have been investigated and the results suggest similar performance responses

compared to traditional higher volume training (Nugent et al., 2017). In a study by Clemente-Suarez, et al. (2018), after ten weeks of traditional training based on high training volume and low intensity, there was no benefit compared to ten weeks of reverse training based on lower training volume and higher intensity (Clemente-Suárez et al., 2018).

In addition to high-intensity interval work, another strategy used by athletes to improve performance is the use of sports supplements (SS), with swimmers being no exception. In a recent study, 86.9% of the sample of national and international swimmers had used SS on regular basis, with no differences between men and women (Moreno et al., 2022). Although there is a wide variety of supplements on the market, the Australian Institute of Sport (AIS) classifies them into four groups (A-B-C-D) from highest to lowest level of scientific evidence, with group A having the most evidence and group D having the least (AIS, 2022). One of the SS that the AIS classifies as Group A is dietary nitrate. Nitrate is continuously produced in our bodies as a result of the oxidation of the amino acid arginine to nitric oxide (NO) (Silva et al., 2022).

There is reason to believe that improving NO bioavailability by increasing the nitrate-nitrite-NO pathway may influence muscle function and exercise performance (Jones et al., 2003). Nitrate and nitrite are the main substrates to produce NO via the NO synthase (NOS)-independent pathway. Inhibition of NOS, which would reduce endogenous NO production, could regulate blood flow and increase oxygen consumption (Bescós et al., 2012). In addition, it has been suggested that L-citrulline could be an alternative NO donor in the NOS-dependent pathway, as it can increase L-arginine levels, which is also a participant in the NOS-dependent pathway (Bescós et al., 2012) and could improve the respiratory response and the maximal power in relation to body mass (Koppo et al., 2009). Increasing dietary nitrate intake or using a nitrate supplement, such as beetroot juice (BJ), may increase NO bioavailability and have the potential to improve exercise performance in situations where NO production may be impaired (Lowings et al., 2017), like in high-intensity intervallic efforts with a predominant oxidative metabolism (Domínguez et al., 2018). In fact, the levels of nitrate intake reported in humans through diet are much lower than those ingested with a 70 mL nitrate shot, as was the case in our study (Jonvik et al., 2017). There is evidence that nitrate supplementation may be particularly effective in reducing

muscle fatigue associated with high-intensity exercise by a selective physiological efficacy of nitrates in type II (fast twitch) muscle (Jones et al., 2016). The nitrate supplementation may help to restore the phosphocreatine stores, prevent their depletion during repeated efforts and limit the accumulation of metabolites such as adenosine diphosphate (ADP) and inorganic phosphates, which are known to induce muscle fatigue (Domínguez et al., 2018). Consequently, the use of BJ could have ergogenic effects that may increase time to exhaustion and, therefore, exercise tolerance (Bailey et al., 2009).

At present, there is no clear and consistent answer that clarifies the true effects of BJ on physical performance, as many studies have been based on lesser-trained athletes and differ in experimental design in terms of type of physical exertion, recovery times, and intake protocol. A systematic review of nine articles on the effects of beet juice supplementation showed that both acute and chronic supplementation can improve performance during high-intensity, intermittent exercise with short rest periods (Domínguez et al., 2018). Wylie et al. (2016) also found that BJ could be ergogenic during repeated short duration sets of maximal intensity cycling sprints, interspersed with short recovery periods (Wylie et al., 2016). On the other hand, during a 15-second cycling bout at 170% of maximal aerobic power output interspersed with 30-second passive recovery periods, Pawlak-Chaouch et al. (2019) reported that BJ supplementation did not increase tolerance to supramaximal intensity intermittent exercise. Specifically in the sport of swimming, few studies have examined the effects of BJ intake despite the specific characteristics of the sport (with a partial hypoxia in nature and a more dominant use of upper-limbs for propulsion) that could enhance or at least modify the responses to BJ supplementation (Muggeridge et al., 2013; Patrician and Schagatay, 2017). With a similar intake protocol, Pinna et al. (2014) observed significant improvement in exercise tolerance during a swimming test to exhaustion with a chronic BJ intake, while Esen et al. (2018) and Lowings et al., (2017) did not obtain significant improvements in time trials over 100 and 200m front-crawl or 168m backstroke performance, respectively. During a repeated swimming effort of 6x100m with seven minutes rest, and following an acute BJ ingestion, Moreno et al. (Moreno et al., 2023) failed to show significant improvements in 100m times; although, no information is available for repeated efforts with reduced recovery times as typically employed by swimmers during training blocks.

Given the controversial results on the effects of BJ ingestion on performance, but considering the possible effect during high-intensity intermittent exercise with short rest periods in other sport disciplines than swimming (Domínguez et al., 2018), the aim of our study was to examine whether an acute intake of BJ improves swimming performance, physiological variables of anaerobic metabolism, or subjective measures during high-intensity interval exercise with incomplete rest in competitive swimmers. In this regard, we hypothesized that an acute BJ ingestion protocol could show an improvement in overall performance or, at least, in the exercise tolerance compared to the control group during a high-intensity intermittent swimming effort with incomplete rests.

## Methods

### Participants

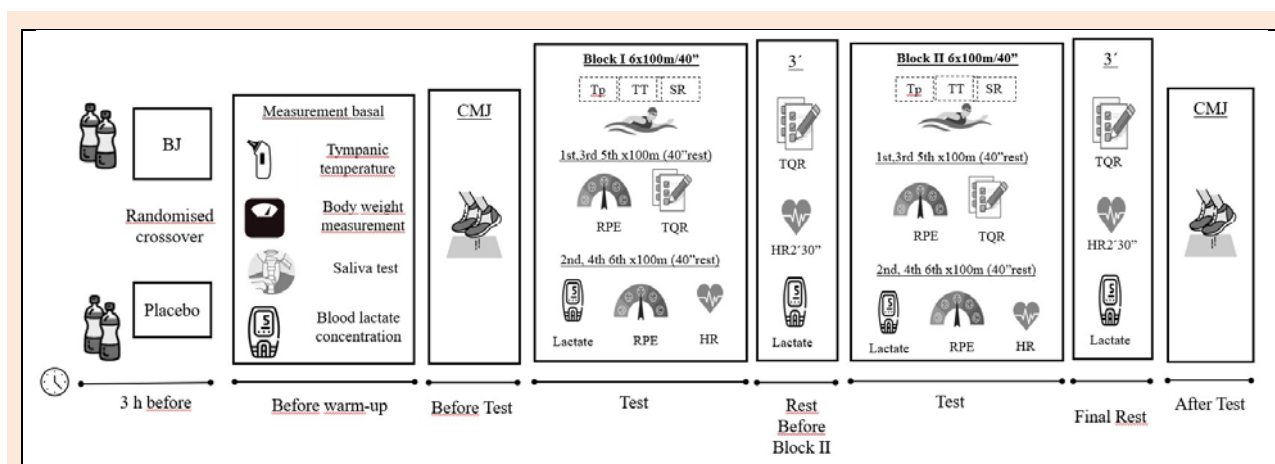
Eighteen competitive swimmers, nine females ( $17.11 \pm 1.05$  years old,  $1.71 \pm 1.41$  m and  $55 \pm 1.41$  kg) and nine males ( $17.00 \pm 1.32$  years old,  $1.78 \pm 8.29$  m and  $69.33 \pm 10.02$  kg) participated in this study. They were part of a training program of at least 18 hours per week in the water (approximately 40 - 50 km/week), 5 hours per week out of the water, and had at least 4 years of competitive experience. Their average personal best times corresponded to level 3 (national level) according to Ruiz-Navarro et al. (Ruiz-Navarro et al., 2023). Written informed consent was obtained from all participants and their legal guardians after they were informed of the method and purpose of the study. The experimental protocol was approved by the local University Ethics Committee (No. 79/2022) and complied with the recommendations of the Declaration of Helsinki.

### Experimental design

The experiment was a cross-over study with randomized, placebo-controlled, double-blind, and counterbalanced design. An external researcher randomized the drinks of all participants to eliminate any order effects (Research Randomizer, <https://www.randomizer.org>). The ClinicalTrials.gov identifier was NCT05657093. In the first session, ten swimmers consumed the BJ supplement and eight consumed a placebo (PLA). Each participant completed two sessions, separated by 7 days to allow for recovery and washout of the substance, in their usual training pool and under the same experimental conditions (i.e., same time of day). The pool was indoor, 50x25 meters, with a water temperature of 27°C on both days.

### Procedure

In both experimental conditions, the swimmers ingested the Beetroot Juice (BJ) in a pocket-sized delivers; BJ (70 mL, 6.4 mmol/400 mg NO<sub>3</sub><sup>-</sup>; Beet-It-Pro Elite Shot; James White Drinks Ltd., Ipswich, UK) or PLA (70 mL, 0.04 mmol/3 mg NO<sub>3</sub><sup>-</sup>), matched in flavour, dosage, appearance, and packaging as described elsewhere (Cuenca et al., 2018; López-Samanes et al., 2020). In line with previous studies (Cuenca et al., 2018; López-Samanes et al., 2020; Silva et al., 2022), the juice intake was done for all swimmers three hours before the swimming test (Figure 1). This ensured a peak of nitrate levels during the exercise test, as reported in Wylie et al. (2013). The day before the test, swimmers followed an intake protocol of approximately 60% carbohydrate, 30% fat and 10% protein, with a list of NO<sub>3</sub>-rich foods (e.g., beets, celery, spinach) to be eliminated from the diet (López-Samanes et al., 2020). In addition, swimmers avoided brushing their teeth, using mouthwash, chewing gum, and consuming sweets, stimulants (e.g., caffeine), or alcohol, all of which could alter the oral microbiota and interfere with NO<sub>3</sub><sup>-</sup> reduction, for 24 h prior to the experiments (López-Samanes et al., 2020). To determine whether participants had identified which day they had ingested the BJ and/or PLA they were asked "What do you think you ingested?" at the end of each condition (López-Samanes et al., 2020). Less than half of the participants correctly guessed which group they belonged to.



**Figure 1. Diagram of the intervention procedure.** BJ: Beetroot Juice; PLA: placebo; HR: Heart Rate; RPE: Rate of Perceived Exertion; TQR: Total Quality Recovery Scale; CMJ: Counter Movement Jump; Tp: Partial Time; TT: Total Time; SR: Stroke Rate.

Before starting the swimming test, the swimmers' resting lactate was measured (Lactate Pro2 lactate analyzer; Arkray, Shiga, Japan) to ensure similar baseline conditions before each test. A record sheet was used to record what the swimmers had for dinner the night before the experiment, so that they could be reminded of this before the second experiment.

After the resting values were measured, the swimmers performed 3 repetitions of the counter movement jump (CMJ) with hands on waist and 45 s of passive recovery using an infrared jump system (Optojump Next; Microgate, Bolzano, Italy). This CMJ measure was included as an indicator of general fatigue of athletes (Gathercole et al., 2015). The swimming test consisted of 2 blocks of  $6 \times 100$  m front crawl at maximal effort with push start (Trinidad et al., 2022). Recovery time between 100m repetitions was 40 seconds, while a rest period of 3 minutes was allowed between the first and second blocks. To ensure accurate timing, two experienced coaches recorded split and total times for each 100m repetition, which were then given to the swimmers during the recovery period. The Finnis Chronometer 3 $\times$ 100 digital stopwatch (Seiko SVAS003, Seiko Watch Corporation, Japan) was utilized to record and average the times. This training set represented a typical training exercise for middle-distance competitive swimmers when aiming for intensity zones 3 and 4 (above 4 mmol.L<sup>-1</sup>), as previously reported (Hellard et al., 2017).

Blood lactate concentrations were measured twice. During the even repetitions, when the swimmers completed the repetition, and during the three minutes rest period before the start of block 2. Heart rate was also measured with a Geonaute OxyLane-4 heart rate monitor at the end of each block and also after the rest period before the start of the next block.

The perceived exertion scale (RPE) with Borg's CR-10 category ratio scale was given to the swimmers on a laminated board so that they could identify their perceived exertion at the end of the repetition (Borg, 1982). The recovery quality scale (TQR) with Kenttä's scale was measured after each 100 m repetition, just before the start of the next repetition (Kenttä and Hassmén, 1998). Finally, the swimmers performed three CMJ jumps after the test, following the same procedure as before the test.

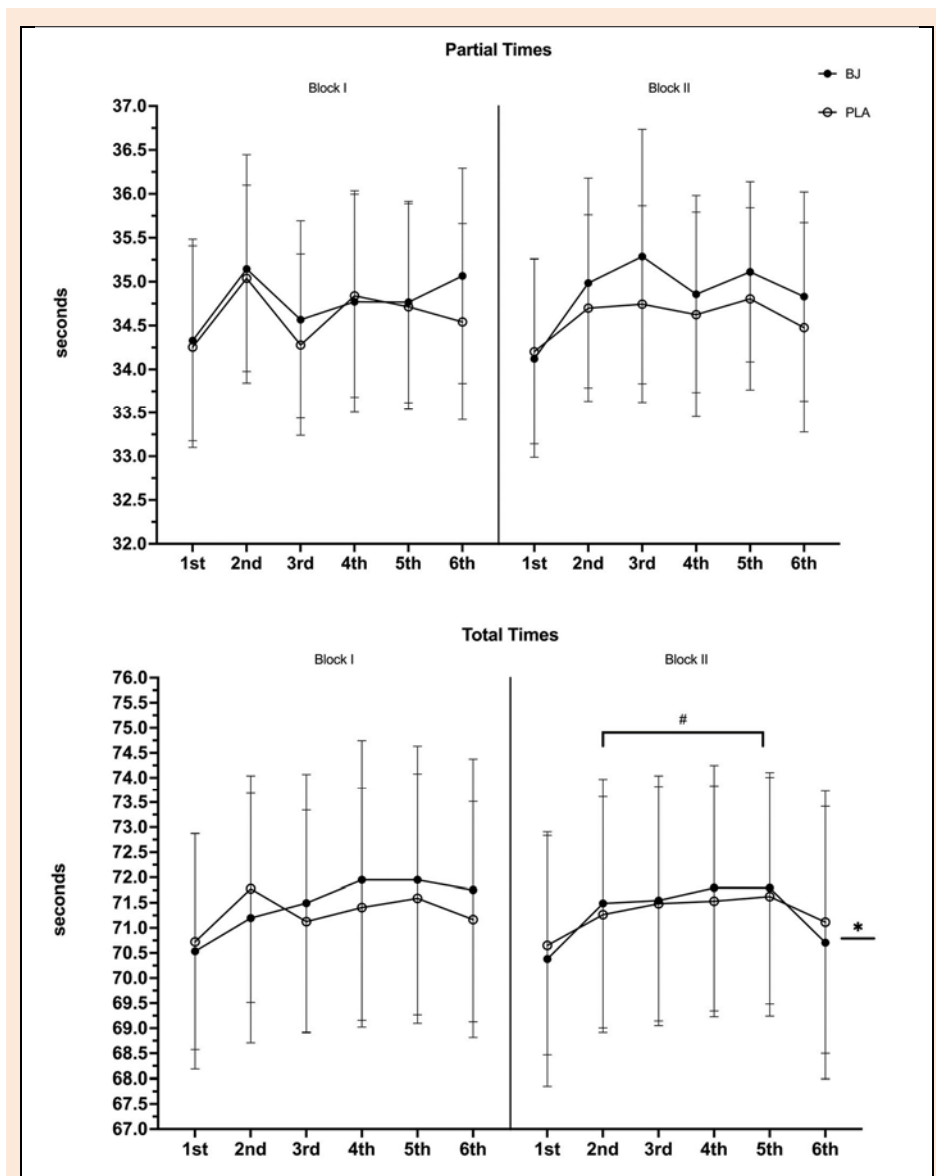
### Statistical Analysis

Statistical tests were performed using IBM SPSS Statistics for Macintosh (version 26.0; IBM Corp., Armonk, NY). Assuming an alpha level of 0.05 and  $\beta$  of 0.80, the sample size was estimated at 18 participants using G\*Power software (Faul et al., 2007). A Shapiro-Wilk test was performed to determine whether the distribution of the data met the assumption of normality. A three-way repeated measures analysis of variance (condition: BJ or PLA, block: first or second, repetition: first to sixth) was then used to identify effects on the dependent variables. Assumptions of variance and sphericity were tested using Levene's and Mauchly's tests, respectively. Partial eta squared ( $\eta^2$ ) was used as an effect size indicator, and it was classified as 0.01 - 0.1 small effect, 0.11 - 0.15 medium effect, and 0.16 - 1 large effect (Pierce et al., 2004). Bonferroni post hoc test was used to check pairwise comparisons. The significance level was set at  $p < 0.05$ .

### Results

Overall, the partial and total times during the  $2 \times 6 \times 100$  m test showed no differences ( $p > 0.05$ ) between the PLA and BJ conditions. However, for total times, there was a block $\times$ repetition $\times$ condition interaction ( $F_5 = 3.10$ ;  $p = 0.046$ ;  $\eta^2 = 0.54$ ), suggesting that swimmers in the BJ group reduced the time of the sixth repetition of block 2 compared to block 1 ( $p = 0.011$ ) (Figure 2). In addition, there was a main effect of repetitions on both partial ( $F_5 = 10.45$ ;  $p < 0.001$ ;  $\eta^2 = 0.80$ ) and total ( $F_5 = 11.71$ ;  $p < 0.001$ ;  $\eta^2 = 0.82$ ) times, pointing out that swimmers in the BJ group performed longer times in the second through fifth repetitions compared to the first (all  $p < 0.005$ ) in the total times of block 2.

There were no differences between conditions in blood lactate concentrations measured at the end of the 100 m paired repetitions ( $p > 0.05$ ) (Table 1). The main effects were between blocks ( $F_1 = 19.79$ ;  $p = 0.001$ ;  $\eta^2 = 0.60$ ), between repetitions ( $F_3 = 9.57$ ;  $p = 0.002$ ;  $\eta^2 = 0.72$ ) and between block $\times$ repetition interaction ( $F = 8.52$ ;  $p = 0.003$ ;  $\eta^2 = 0.70$ ), being lactate concentrations higher in all repetitions of block 2 compared to block 1 (except for the 6th repetition of the PLA condition) and increasing lactate



**Figure 2.** Total and partial times (mean  $\pm$  standard deviation) of competitive swimmers in BJ and PLA conditions during a 2 x 6 x 100m test. BJ: beetroot juice; PLA: placebo. \*Difference between blocks for the 6th repetition in condition BJ. #Significant differences with respect to the 1st repetition.

**Table 1.** Physiological variables (mean  $\pm$  standard deviation) in beetroot juice (BJ) and placebo (PLA) conditions in a 2 x 6 x 100m swim test.

		Block 1			
		2nd	4th	6th	Rest
Lactate	BJ	4.93 $\pm$ 1.95*	7.31 $\pm$ 2.60#*	8.99 $\pm$ 2.67#*	8.15 $\pm$ 2.97
	PLA	5.67 $\pm$ 2.20*	7.14 $\pm$ 2.70*	8.36 $\pm$ 2.21#	7.27 $\pm$ 2.48*#
Heart rate	BJ			157.29 $\pm$ 12.11	114.29 $\pm$ 10.61#
	PLA			154.71 $\pm$ 21.75	110.71 $\pm$ 9.13#
		Block 2			
Lactate	BJ	9.56 $\pm$ 3.32	10.16 $\pm$ 3.77	11.05 $\pm$ 3.81	9.77 $\pm$ 4.28
	PLA	9.11 $\pm$ 2.96	9.09 $\pm$ 2.67	9.91 $\pm$ 4.10	9.23 $\pm$ 3.64
Heart rate	BJ			160.06 $\pm$ 21.33	110.71 $\pm$ 9.13#
	PLA			157.24 $\pm$ 13.35	111 $\pm$ 9.9#

\* Different values ( $p < 0.05$ ) than block 2 in the same condition. # Different values ( $p < 0.05$ ) from previous repetition in the same block and condition.

values between repetitions were detected only in block 1. For heart rate and CMJ (Pre-Post  $\Delta_{PLA}$  1.64  $\pm$  2.77cm;  $\Delta_{BJ}$  0.84  $\pm$  2.31cm), no variations were found between conditions ( $p > 0.05$ ), although the resting values for HR were lower than the values at the end of the sixth repetition of

each block (Table 1).

In the cases of RPE and TQR variables, no main effects of condition were detected ( $p > 0.05$ ). However, there was a block ( $F_1 = 31.83$ ;  $p < 0.001$ ;  $\eta^2 = 0.68$  for RPE and  $F_1 = 22.40$ ;  $p < 0.001$ ;  $\eta^2 = 0.58$  for TQR), repetition ( $F_5 =$



31.26;  $p < 0.001$ ;  $\eta^2 = 0.93$  for RPE and  $F_1 = 10.72$ ;  $p < 0.001$ ;  $\eta^2 = 0.82$  for TQR), and block $\times$ repetition interaction ( $F_5 = 7.19$ ;  $p = 0.003$ ;  $\eta^2 = 0.77$  for RPE). RPE was lower in all repetitions of block 1 compared to block 2 (except for the first and second repetitions of the BJ condition) and no changes in RPE were detected between repetitions of block 1 for the BJ group and between repetitions of block 2 for the PLA group (Table 2). As far TQR scale is

concerned, post hoc comparisons revealed no changes in recovery perception between consecutive repetitions for BJ swimmers, but a lower recovery perception for PLA swimmers in the sixth repetition of block 1 and in the fifth and sixth repetitions of block 2. In addition, all swimmers showed a lower recovery perception before the third, fourth and fifth repetitions of block 2 compared to block 1 (Table 2).

**Table 2.** Descriptive data of the RPE and TQR subjective scales (mean  $\pm$  standard deviation) in the beetroot juice (BJ) and placebo (PLA) conditions in a 2 x 6 x 100m swimming test.

		Block 1					
		1st	2nd	3rd	4th	5th	6th/Rest
Lactate	BJ	13.22 $\pm$ 2.30	14.11 $\pm$ 2.90	14.39 $\pm$ 2.83*	15.11 $\pm$ 2.56*	15.61 $\pm$ 2.50*	16.28 $\pm$ 2.10*
	PLA	11.38 $\pm$ 2.72*	13.06 $\pm$ 2.46#*	14.22 $\pm$ 2.13*	14.83 $\pm$ 2.20*	15.33 $\pm$ 2.00*	16.06 $\pm$ 1.62*
Heart rate	BJ	13.47 $\pm$ 3.47	13.18 $\pm$ 3.41	12.53 $\pm$ 3.00*	11.88 $\pm$ 2.7*	12.94 $\pm$ 3.07*	13.65 $\pm$ 3.16
	PLA	14.12 $\pm$ 3.00	13.41 $\pm$ 2.58	12.35 $\pm$ 2.29*	12.12 $\pm$ 2.7*	11.47 $\pm$ 2.21*	14.47 $\pm$ 2.93#
		Block 2					
Lactate	BJ	14.33 $\pm$ 2.30	14.72 $\pm$ 2.08	16.83 $\pm$ 1.72#	17.00 $\pm$ 2.08	17.78 $\pm$ 1.48#	18.44 $\pm$ 1.38#
	PLA	15.22 $\pm$ 2.04	15.06 $\pm$ 2.38	17.00 $\pm$ 2.10	17.11 $\pm$ 1.78	17.56 $\pm$ 1.85	18.22 $\pm$ 1.73
Heart rate	BJ	13.06 $\pm$ 2.41	12.18 $\pm$ 2.98	10.76 $\pm$ 2.14	10.12 $\pm$ 2.18	9.71 $\pm$ 2.14	12.00 $\pm$ 3.52
	PLA	13.00 $\pm$ 2.83	12.06 $\pm$ 2.51	11.00 $\pm$ 2.94	9.88 $\pm$ 2.32	9.24 $\pm$ 2.11#	13.59 $\pm$ 2.57#

\*Different values ( $p < 0.05$ ) than block 2 in the same condition. #Different values ( $p < 0.05$ ) from previous repetition in the same block and condition.

## Discussion

The aim of the present study was to determine whether an acute intake of BJ could improve performance during a high-intensity intermittent effort in competitive swimmers. The results did not confirm any overall performance differences between the PLA and BJ groups. However, there was a potential ergogenic effect on exercise tolerance at the end of interval exercise.

Competitive swimmers in the present study performed 100m swim repetitions in an average time of 75 to 73 seconds for females and 68 to 66 seconds for males, with no differences regardless of BJ or PLA conditions. This is consistent with previous studies in swimming, which found no beneficial effects on swimmers' performance after BJ ingestion (Lowings et al., 2017; Esen et al., 2018; Moreno et al., 2023), even when the swim distance and thus the type of effort (Moreno et al., 2023), ingestion protocol (Lowings et al., 2017; Moreno et al., 2023), and rest conditions were matched (Esen et al., 2018; Moreno et al., 2023). Nevertheless, by consuming BJ in the present study, swimmers were able to reduce their times of the last repetition in block 2 compared to block 1. The average magnitude of these differences reached 0.20 units of the standard deviation of 100m times (calculated as effect size), which would correspond to a percentile gain of eight positions (Marzano et al., 2011) for a given sample of competitive swimmers. This improvement could be related to a ergogenic effect of BJ in the later phases of the effort, as others have shown in prolonging the time to exhaustion (Lowings et al., 2017; Behrens CE Jr. et al., 2020) or detecting a possible improvement in the last part of a 168m swimming effort (Lowings et al., 2017). An increased blood flow through vasodilation as well as a lower blood pressure following nitrates supplementation (Anderson et al., 2022) could reduce the cardiovascular stress and increase the efficiency of oxygen utilization, potentially speeding recovery (Bailey et al., 2009). One can argue, the

improved performance of swimmers after BJ ingestion in the last repetition of the intermittent bout could also be related to their effort regulation in the previous repetitions (Figure 2), as the pace profile over the six 100m repetitions showed a parabolic pattern typically observed in middle-distance swimming events (Mauger et al., 2012; Mytton et al., 2015). However, this hypothesis does not seem to be supported by the subjective variables between groups (Table 2).

The lack of overall differences in swimming performance was supported by no differences in blood lactate concentrations between conditions during the high-intensity efforts with incomplete recovery (Table 1). These findings support previous studies that described no effects of BJ supplementation on lactate concentration after high-intensity intermittent exercise in competitive swimmers over the same swimming distances (Moreno et al., 2023), nor in other sports like taekwondo during an anaerobic effort (Mirafrafi et al., 2021), team sports after an intense intermittent effort undergoing a yo-yo test (Wylie et al., 2013). Acute ingestion of nitrates has been shown to reduce the  $O_2$  cost during submaximal exercise, thereby reducing the physiological load by reducing the contribution of substrate-level phosphorylation and lactate production during exercise (Jones et al., 2014). Furthermore, BJ supplementation has been shown to increase microvascular  $PO_2$  in rat type II muscle during submaximal exercise, thereby enhancing capillary-myofibril oxygen exchange and allowing for better maintenance of intramuscular homeostasis (Ferguson et al., 2013; 2015). By improving oxidative function, this mechanism may be important in delaying lactate accumulation during high-intensity exercise (Krustrup et al., 2004). Our results do not support this argument, as there was no complete recovery between efforts following recommendations for high-intensity interval training in competitive swimmers (Kilen et al., 2014). Therefore, we could speculate that during longer intervals ( $>3$  min), which rely more on oxidative phosphorylation, acute BJ

ingestion could potentially have ergogenic effects on performance, as shown by other authors (Domínguez et al., 2018). However, this hypothesis does not hold in view of the results obtained in other competitive swimmers with similar training workloads and performance levels (Moreno et al., 2023).

Regarding the values of the subjective scales RPE and TQR, in line with CMJ results as an indicator of general fatigue of athletes, there were no overall differences between the conditions. However, the swimmers in the BJ group (in contrast to the PLA group) started block 2 with similar RPE values as in block 1 whereas the PLA group (unlike the BJ group) showed a lower perceived recovery (TQR) in the final repetitions of each block (Table 2). Possible improvements in performance during this type of effort have been linked to a faster resynthesis of phosphocreatine. It is speculated that the ingestion of beetroot juice may delay depletion during repetitive exercise efforts (Domínguez et al., 2018). Since BJ may have an ergogenic effect on efforts with an oxidative metabolic component, as shown in the study by Mosher et al (Mosher et al., 2016), it is possible that the supplement accelerates this recovery, thereby delaying the onset of fatigue. This may be one of the reasons why our swimmers in the BJ group had better RPE and TQR than those in the PLA group.

The present results provided valuable information about the swimmers' performance at the end of a  $2 \times 6 \times 100$  at maximal effort, suggesting that BJ supplementation may provide improvements in exercise tolerance or subjective sensation during the later phases of training. That said, physiological measures other than lactate or heart rate may help to understand how BJ affects swimmers. But, overall, considering the small differences between successful and unsuccessful performance at the competitive level (Born et al., 2022), a possible ergogenic effect of BJ on exercise tolerance could play a role on the competitive outcome of high-level swimmers. Future research in this area is encouraged to conduct tests with incomplete rest periods but with chronic BJ ingestion to determine if this might produce different results. For example, it is unknown whether higher doses of BJ (i.e.,  $\sim 12.5$  mmol) or multi-day supplementation (i.e., 3 or 7 days) could produce an ergogenic effect on performance in elite swimmers. In addition, data about the nitrate absorption through plasma and/or saliva levels could increase the understanding of the experimental results about BJ supplementation.

## Conclusion

The results of our study point out that acute ingestion of BJ three hours prior to a high-intensity  $2 \times 6 \times 100$  test with reduced recovery did not significantly affect the overall exercise performance, physiological variables of anaerobic metabolism, or subjective measures of fatigue/recovery of swimmers. However, an improvement in the perceived recovery at the end of the interval effort was observed that could be connected to a potential positive effect of BJ supplementation in the performance of the final effort repetition.

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## References

- AIS, Australian Institute of Sport (2022) Sports supplement framework 2019. Available at: <https://www.ais.gov.au/nutrition/supplements> (Accessed: 3 January 2023)
- Amara, S., Barbosa, T. M., Chortane, O. G., Hammami, R., Attia, A., Chortane, S. G., & van den Tillaar, R. (2022) Effect of Concurrent Resistance Training on Lower Body Strength, Leg Kick Swimming, and Sport-Specific Performance in Competitive Swimmers. *Biology* **11**(2), 299. <https://doi.org/10.3390/biology11020299>
- Anderson, O. K., Martinez-Ferran, M., Lorenzo-Calvo, J., Jiménez, S. L., & Pareja-Galeano, H. (2022) Effects of Nitrate Supplementation on Muscle Strength and Mass: A Systematic Review. *Journal of Strength and Conditioning Research* **36**(12), 3562-3570. <https://doi.org/10.1519/JSC.00000000000004101>
- Bailey, S. J., Winyard, P., Vanhatalo, A., Blackwell, J. R., Dimenna, F. J., Wilkerson, D. P., Tarr, J., Benjamin, N., & Jones, A. M. (2009) Dietary nitrate supplementation reduces the O<sub>2</sub> cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *Journal of Applied Physiology* **107**(4), 1144-1155. <https://doi.org/10.1152/japplphysiol.00722.2009>
- Behrens CE Jr, Ahmed, K., Ricart, K., Linder, B., Fernández, J., Bertrand, B., Patel, R. P., & Fisher, G. (2020) Acute beetroot juice supplementation improves exercise tolerance and cycling efficiency in adults with obesity. *Physiological Reports* **8**(19), e14574. <https://doi.org/10.14814/phy2.14574>
- Bescós, R., Sureda, A., Tur, J. A., & Pons, A. (2012) The effect of nitric-oxide-related supplements on human performance. *Sports Medicine* **42**(2), 99-117. <https://doi.org/10.2165/11596860-000000000-00000>
- Borg, Gunnar A.V. (1982) Psychophysical bases of perceived exertion. *Medicine and Science in Sports and Exercise* **14**(5), 377-381. <https://doi.org/10.1249/00005768-198205000-00012>
- Born, D.-P., Schönfelder, M., Logan, O., Olstad, B. H., & Romann, M. (2022) Performance Development of European Swimmers Across the Olympic Cycle. *Frontiers in Sports and Active Living* **4**, 894066. <https://doi.org/10.3389/fspor.2022.894066>
- Clemente-Suárez, V. J., Fernandes, R. J., de Jesus, K., Pelarigo, J. G., Arroyo-Toledo, J. J., & Vilas-Boas, J. P. (2018) Do traditional and reverse swimming training periodizations lead to similar aerobic performance improvements? *The Journal of Sports Medicine and Physical Fitness* **58**(6), 761-767. <https://doi.org/10.23736/S0022-4707.17.07465-5>
- Cuenca, E., Jodra, P., Pérez-López, A., González-Rodríguez, L. G., Fernandes da Silva, S., Veiga-Herreros, P., & Domínguez, R. (2018) Effects of Beetroot Juice Supplementation on Performance and Fatigue in a 30-s All-Out Sprint Exercise: A Randomized, Double-Blind Cross-Over Study. *Nutrients* **10**(9), 1222. <https://doi.org/10.3390/nu10091222>
- Domínguez, R., Maté-Muñoz, J. L., Cuenca, E., García-Fernández, P., Mata-Ordoñez, F., Lozano-Estevan, M. C., Veiga-Herreros, P., da Silva, S. F., & Garnacho-Castaño, M. V. (2018) Effects of beetroot juice supplementation on intermittent high-intensity exercise efforts. *Journal of the International Society of Sports Nutrition* **15**(1), 2. <https://doi.org/10.1186/s12970-017-0204-9>
- Esen, O., Nicholas, C., Morris, M., & Bailey, S. J. (2018) No Effect of Beetroot Juice Supplementation on 100-m and 200-m Swimming Performance in Moderately-Trained Swimmers. *International Journal of Sports Physiology and Performance* **14**(6), 1-19. <https://doi.org/10.1123/ijspp.2018-0654>

- Faul, F., Erdfelder, E., Lang, A. G., & Buchner, A. (2007). G\*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior research methods*, 39(2), 175–191. <https://doi.org/10.3758/bf03193146>
- Ferguson, S. K., Hirai, D. M., Copp, S. W., Holdsworth, C. T., Allen, J. D., Jones, A. M., Musch, T. I., & Poole, D. C. (2013) Impact of dietary nitrate supplementation via beetroot juice on exercising muscle vascular control in rats: Beetroot juice and exercising muscle blood flow. *The Journal of Physiology* 591(2), 547-557. <https://doi.org/10.1113/jphysiol.2012.243121>
- Ferguson, S. K., Holdsworth, C. T., Wright, J. L., Fees, A. J., Allen, J. D., Jones, A. M., Musch, T. I., & Poole, D. C. (2015) Microvascular oxygen pressures in muscles comprised of different fiber types: Impact of dietary nitrate supplementation. *Nitric Oxide* 48, 38-43. <https://doi.org/10.1016/j.niox.2014.09.157>
- Gathercole, R., Sporer, B., Stellingwerff, T., & Sleivert, G. (2015) Alternative Countermovement-Jump Analysis to Quantify Acute Neuromuscular Fatigue. *International Journal of Sports Physiology and Performance* 10(1), 84-92. <https://doi.org/10.1123/ijspp.2013-0413>
- Hellard, D. P., Scordia, C., Avalos, M., Mujika, I., & Pyne, D. B. (2017) Modelling of optimal training load patterns during the 11 weeks preceding major competition in elite swimmers. Applied physiology, nutrition, and metabolism. *Physiologie Appliquee, Nutrition et Metabolisme* 42(10), 1106-1117. <https://doi.org/10.1139/apnm-2017-0180>
- Jones, A. M., Wilkerson, D. P., Koppo, K., Wilmshurst, S., & Campbell, I. T. (2003) Inhibition of nitric oxide synthase by L-NAME speeds phase II pulmonary. VO<sub>2</sub> kinetics in the transition to moderate-intensity exercise in man. *The Journal of physiology* 552(1), 265-272. <https://doi.org/10.1113/jphysiol.2003.045799>
- Jones, A.M. (2014) Dietary Nitrate Supplementation and Exercise Performance. *Sports Medicine (Auckland, N.z.)* 44(Suppl 1), 35-45. <https://doi.org/10.1007/s40279-014-0149-y>
- Jones, A. M., Ferguson, S. K., Bailey, S. J., Vanhatalo, A., & Poole, D. C. (2016) Fiber Type-Specific Effects of Dietary Nitrate. *Exercise and Sport Sciences Reviews* 44(2), 53-60. <https://doi.org/10.1249/JES.00000000000000074>
- Jonvik, K. L., Nyakayiru, J., van Dijk, J. W., Wardenaar, F. C., van Loon, L. J., & Verdijk, L. B. (2017) Habitual Dietary Nitrate Intake in Highly Trained Athletes. *International Journal of Sport Nutrition and Exercise Metabolism* 27(2), 148-157. <https://doi.org/10.1123/ijsnem.2016-0239>
- Kenttä, G. and Hassmén, P. (1998) Overtraining and Recovery: A Conceptual Model. *Sports Medicine* 26(1), 1-16. <https://doi.org/10.2165/00007256-199826010-00001>
- Kilen, A., Larsson, T. H., Jørgensen, M., Johansen, L., Jørgensen, S., & Nordsborg, N. B. (2014) Effects of 12 Weeks High-Intensity & Reduced-Volume Training in Elite Athletes. *Plos One*. Edited by J.M. Haddad, 9(4), e95025. <https://doi.org/10.1371/journal.pone.0095025>
- Koppo, K., Taes, Y. E., Pottier, A., Boone, J., Bouckaert, J., & Derave, W. (2009) Dietary arginine supplementation speeds pulmonary VO<sub>2</sub> kinetics during cycle exercise. *Medicine and Science in Sports and Exercise* 41(8), 1626-1632. <https://doi.org/10.1249/MSS.0b013e31819d81b6>
- Krustrup, P., Söderlund, K., Mohr, M., & Bangsbo, J. (2004) The slow component of oxygen uptake during intense, sub-maximal exercise in man is associated with additional fibre recruitment. *Pflügers Archiv European Journal of Physiology* 447(6), 855-866. <https://doi.org/10.1007/s00424-003-1203-z>
- López-Samanes, Á., Pérez-López, A., Moreno-Pérez, V., Nakamura, F. Y., Acebes-Sánchez, J., Quintana-Milla, I., Sánchez-Oliver, A. J., Moreno-Pérez, D., Fernández-Elias, V. E., & Domínguez, R. (2020) Effects of Beetroot Juice Ingestion on Physical Performance in Highly Competitive Tennis Players. *Nutrients* 12(2), 584. <https://doi.org/10.3390/nu12020584>
- Lowings, S., Shanon, O. M., Deighton, K., Matu, J., & Barlow, M. J. (2017) Effect of Dietary Nitrate Supplementation on Swimming Performance in Trained Swimmers. *International Journal of Sport Nutrition and Exercise Metabolism* 27(4), 377-384. <https://doi.org/10.1123/ijsnem.2016-0251>
- Marzano, R.J., Pickering, D.J. and Heflebower, T. (2011) The Highly Engaged Classroom. United States os America: Marzano by Solution Tree.
- Mauger, A.R., Neuloh, J. and Castle, P.C. (2012) Analysis of Pacing Strategy Selection in Elite 400-m Freestyle Swimming. *Medicine & Science in Sports & Exercise* 44(11), 2205-2212. <https://doi.org/10.1249/MSS.0b013e3182604b84>
- Miraftebi, H., Avazpoor, Z., Berjisan, E., Sarshin, A., Rezaei, S., Domínguez, R., Reale, R., Franchini, E., Samanipour, M. H., Koozehchian, M. S., Willems, M. E. T., Rafiei, R., & Naderi, A. (2021) Effects of Beetroot Juice Supplementation on Cognitive Function, Aerobic and Anaerobic Performances of Trained Male Taekwondo Athletes: A Pilot Study. *International Journal of Environmental Research and Public Health* 18(19), 10202. <https://doi.org/10.3390/ijerph181910202>
- Moreno, B., Veiga, S., Sánchez-Oliver, A. J., Domínguez, R., & Morencos, E. (2022) Analysis of Sport Supplement Consumption by Competitive Swimmers According to Sex and Competitive Level. *Nutrients* 14(15), 3218. <https://doi.org/10.3390/nu14153218>
- Moreno, B., Morencos, E., Vicente-Campos, D., Muñoz, A., González-García, J., & Veiga, S. (2023) Effects of beetroot juice intake on repeated performance of competitive swimmers. *Frontiers in Physiology* 13, 1076295. <https://doi.org/10.3389/fphys.2022.1076295>
- Mosher, S. L., Sparks, S. A., Williams, E. L., Bentley, D. J., & Mc Naughton, L. R. (2016) Ingestion of a Nitric Oxide Enhancing Supplement Improves Resistance Exercise Performance. *Journal of Strength and Conditioning Research* 30(12), 3520-3524. <https://doi.org/10.1519/JSC.0000000000001437>
- Muggeridge, D. J., Howe, C. C., Spendiff, O., Pedlar, C., James, P. E., & Easton, C. (2013) The Effects of a Single Dose of Concentrated Beetroot Juice on Performance in Trained Flatwater Kayakers. *International Journal of Sport Nutrition and Exercise Metabolism* 23(5), 498-506. <https://doi.org/10.1123/ijsnem.23.5.498>
- Mujika, I. (1998) The Influence of Training Characteristics and Tapering on the Adaptation in Highly Trained Individuals: A Review. *International Journal of Sports Medicine* 19(07), 439-446. <https://doi.org/10.1055/s-2007-971942>
- Mytton, G. J., Archer, D. T., Turner, L., Skorski, S., Renfree, A., Thompson, K. G., & St Clair Gibson, A. (2015) Increased Variability of Lap Speeds: Differentiating Medalists and Nonmedalists in Middle-Distance Running and Swimming Events. *International Journal of Sports Physiology and Performance* 10(3), 369-373. <https://doi.org/10.1123/ijspp.2014-0207>
- Nugent, F. J., Comyns, T. M., Burrows, E., & Warrington, G. D. (2017) Effects of Low-Volume, High-Intensity Training on Performance in Competitive Swimmers: A Systematic Review. *Journal of Strength and Conditioning Research* 31(3), 837-847. <https://doi.org/10.1519/JSC.0000000000001583>
- Patrician, A. and Schagatay, E. (2017) Dietary nitrate enhances arterial oxygen saturation after dynamic apnea. *Scandinavian Journal of Medicine & Science in Sports* 27(6), 622-626. <https://doi.org/10.1111/sms.12684>
- Pawlak-Chaouch, M., Boissière, J., Munyaneza, D., Gamelin, F. X., Cuvelier, G., Berthoin, S., & Aucouturier, J. (2019) Beetroot Juice Does Not Enhance Supramaximal Intermittent Exercise Performance in Elite Endurance Athletes. *Journal of the American College of Nutrition* 38(8), 729-738. <https://doi.org/10.1080/07315724.2019.1601601>
- Pierce, C.A., Block, R.A. and Aguinis, H. (2004) Cautionary Note on Reporting Eta-Squared Values from Multifactor ANOVA Designs. *Educational and Psychological Measurement* 64(6), 916-924. <https://doi.org/10.1177/0013164404264848>
- Pinna, M., Roberto, S., Milia, R., Marongiu, E., Olla, S., Loi, A., Migliaccio, G. M., Padulo, J., Orlandi, C., Tocco, F., Concu, A., & Crisafulli, A. (2014) Effect of Beetroot Juice Supplementation on Aerobic Response during Swimming. *Nutrients* 6(2), 605-615. <https://doi.org/10.3390/nu6020605>
- Ruiz-Navarro, J J., López-Belmonte, Ó., Gay, A., Cuenca-Fernández, F., & Arellano, R. (2023) A new model of performance classification to standardize the research results in swimming. *European Journal of Sport Science* 23(4), 478-488. <https://doi.org/10.1080/17461391.2022.2046174>
- Silva, K.V. C., Costa, B. D., Gomes, A. C., Saunders, B., & Mota, J. F. (2022) Factors that Moderate the Effect of Nitrate Ingestion on Exercise Performance in Adults: A Systematic Review with Meta-Analyses and Meta-Regressions. *Advances in Nutrition* 13(5), 1866-1881. <https://doi.org/10.1093/advances/nmac054>



- Trinidad A, Navandar A, Navarro E, Veiga S. (2022) Kinematic Determinants of the Swimming Push Start in Competitive Swimmers. *Applied Sciences* **12**(18), 9278. <https://doi.org/10.3390/app12189278>
- Wylie, L. J., Mohr, M., Krstrup, P., Jackman, S. R., Ermdis, G., Kelly, J., Black, M. I., Bailey, S. J., Vanhatalo, A., & Jones, A. M. (2013) Dietary nitrate supplementation improves team sport-specific intense intermittent exercise performance. *European Journal of Applied Physiology* **113**(7), 1673-1684. <https://doi.org/10.1007/s00421-013-2589-8>
- Wylie, L. J., Bailey, S. J., Kelly, J., Blackwell, J. R., Vanhatalo, A., & Jones, A. M. (2016) Influence of beetroot juice supplementation on intermittent exercise performance. *European Journal of Applied Physiology* **116**(2), 415-425. <https://doi.org/10.1007/s00421-015-3296-4>

### Key points

- The effect of an acute dose of beet juice three hours before a high-intensity  $2 \times 6 \times 100$  test with reduced recovery, as typically used during training blocks, was tested in competitive swimmers.
- The BJ supplementation did not significantly affect the overall performance of competitive swimmers or their physiological variables.
- However, an improvement in perceived recovery (TQR) at the end of the interval effort was noted for the BJ group, suggesting an improvement in exercise tolerance that may play a role in the competitive outcome of competitive swimmers.

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