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**Article Title:** No Effect of Beetroot Juice Supplementation on 100-m and 200-m Swimming Performance in Moderately-Trained Swimmers

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## Abstract

**Purpose:** Dietary nitrate supplementation has been reported to improve performance in kayaking and rowing exercise which mandate significant recruitment of the upper body musculature. Since the effect of dietary nitrate supplementation on swimming performance is unclear, the purpose of this study was to assess the effect of dietary nitrate supplementation on 100-m and 200-m swimming freestyle time-trial (TT) performance. **Methods:** In a double blind, randomized crossover design, ten moderately-trained swimmers underwent two separate 3-day supplementation periods, with a daily dose of either 140 mL nitrate-rich (BRJ; ~800 mg/d nitrate) or nitrate-depleted (PLA) BRJ. Following blood sampling on day 3, the swimmers performed both 200-m and 100-m freestyle swimming TTs, with 30 min recovery between trials. **Results:** Plasma nitrite concentrations was greater after BRJ relative to PLA consumption ( $432 \pm 203$  nmol/L,  $111 \pm 56$  nmol/L, respectively,  $p = 0.001$ ). Systolic BP was lowered after BRJ compared to PLA supplementation ( $114 \pm 10$ ,  $120 \pm 10$  mmHg, respectively  $p = 0.001$ ), but time to complete the 200-m (BRJ:  $152.6 \pm 14.1$  s, PLA:  $152.5 \pm 14.1$  s) and 100-m (BRJ:  $69.5 \pm 7.2$  s, PLA:  $69.4 \pm 7.4$  s) freestyle swimming TTs were not different between BRJ and PLA ( $p > 0.05$ ). **Conclusion:** While 3 days of BRJ supplementation increased plasma nitrite concentration and lowered blood pressure, it did not improve 100-m and 200-m swimming TT performance. These results do not support an ergogenic effect of nitrate supplementation in moderately-trained swimmers, at least for 100-m and 200-m freestyle swimming performance.

**Key words:** Nitrite, exercise performance, ergogenic aid

## Introduction

Dietary supplementation with inorganic nitrate ( $\text{NO}_3^-$ ) has emerged as a popular nutritional intervention to enhance exercise performance. After ingestion,  $\text{NO}_3^-$  is chemically reduced to nitrite ( $\text{NO}_2^-$ ), via anaerobic bacteria that populate the oral cavity, and subsequently to nitric oxide (NO) through a variety of ubiquitously expressed  $\text{NO}_2^-$  reductases<sup>1</sup>. NO, and associated reactive nitrogen intermediates, can exert a positive influence on numerous physiological processes that could conflate to improve exercise performance, including skeletal muscle perfusion and oxygenation, metabolism and contractility<sup>2</sup>. Therefore, short-term  $\text{NO}_3^-$  supplementation has been shown to improve high-intensity continuous<sup>3,4</sup> and intermittent<sup>5</sup> exercise performance during running and cycling exercise, at least in recreationally-active participants.

Although  $\text{NO}_3^-$  supplementation appears to confer ergogenic potential during running and cycling exercise in recreationally-active participants, it has been suggested that an ergogenic effect of  $\text{NO}_3^-$  supplementation in these exercise modalities is less likely in well trained individuals<sup>2,3</sup>. However, there is evidence that  $\text{NO}_3^-$  supplementation can improve performance in highly trained athletes in events where a large muscle mass is recruited and the upper body musculature is heavily engaged, such as kayaking<sup>6</sup> and rowing<sup>7</sup>. Compared to leg exercise, exercise efficiency, vascular conductance and muscle  $\text{O}_2$  extraction are compromised, and acidosis and muscle sympathetic nerve activity are increased, at the same relative intensity during arm exercise<sup>8,9</sup>. The more acidic environment in the arm muscles during exercise might facilitate the reduction of  $\text{NO}_2^-$  to NO<sup>10</sup> and it has been reported that  $\text{NO}_3^-$  supplementation can improve efficiency, muscle  $\text{O}_2$  extraction and muscle blood flow, and can lower muscle sympathetic nerve activity during arm exercise<sup>11-13</sup>. Collectively, these physiological enhancements in the upper body musculature might account for improved performance in trained rowers<sup>7</sup> and kayakers<sup>6</sup> after  $\text{NO}_3^-$  supplementation.

Swimming is an exercise modality that mandates significant recruitment of the upper body musculature, with the arms making a greater contribution to propulsive force than the legs<sup>14</sup>. Therefore, the physiological enhancements in the arm muscles that have been reported after  $\text{NO}_3^-$  supplementation<sup>11-13</sup> might be expected to enhance swimming performance. Moreover, swimming exercise provokes exercise-induced arterial hypoxemia<sup>15</sup> and since the ergogenic effect of  $\text{NO}_3^-$  supplementation appears to be more pronounced in hypoxia compared to normoxia<sup>16</sup>, likely as a function of enhanced reduction of  $\text{NO}_2^-$  to  $\text{NO}$ <sup>17</sup>,  $\text{NO}_3^-$  supplementation might represent an effective ergogenic aid for swimmers. In addition, swimming is accompanied by periods of dynamic apnea<sup>18-20</sup>, with apnea duration linked to swimming performance<sup>21</sup>. Since there is some evidence that  $\text{NO}_3^-$  supplementation might enhance dynamic apnea performance<sup>18-20</sup>, this might also contribute to a potential improvement in swimming performance following  $\text{NO}_3^-$  supplementation. Accordingly, swimming might produce physiological conditions that enhance the potential for an ergogenic effect following  $\text{NO}_3^-$  supplementation.

In spite of an apparent synergy between the physiological demands of swimming and the conditions to optimize the effectiveness of  $\text{NO}_3^-$  supplementation, the influence of  $\text{NO}_3^-$  supplementation on physiological and performance responses during swimming exercise is unclear<sup>19,22,23</sup>. Indeed, 6 days of  $\text{NO}_3^-$  supplementation has been reported to enhance the ‘anaerobic threshold’ and swimming economy in trained masters swimmers<sup>23</sup>, who trained 3-4 times/week (6-7 h/week)<sup>23</sup>, and acute  $\text{NO}_3^-$  supplementation enhanced performance in the second half of a trial comprising  $8 \times 21$  m lengths in trained swimmers<sup>22</sup>, who completed 3 weekly swimming training sessions<sup>22</sup>. However, 6 days of  $\text{NO}_3^-$  supplementation did not improve performance during repeated 15 m sprints in elite female water polo athletes<sup>19</sup> who were preparing for the 2016 Olympic Games qualification and training 7 to 8 sessions a week<sup>19</sup>. Therefore, the existing studies suggest that  $\text{NO}_3^-$  supplementation is less likely to be ergogenic

for swimming performance as competitive standard and fitness status is increased, consistent with other exercise modalities<sup>2,3</sup>. However, since these studies did not assess the effect of  $\text{NO}_3^-$  supplementation on swimming performance over distances competed at major championships, further research is required to evaluate its potential as an ergogenic aid for moderately-trained swimmers.

The majority of studies assessing the effect of  $\text{NO}_3^-$  supplementation on time-trial performance in trained subjects have been  $> 6$  min and have mostly revealed no effect on performance<sup>2,3</sup>. In contrast,  $\text{NO}_3^-$  supplementation has been reported to enhance 500 m kayaking time-trial performance, which was  $\sim 2$  min<sup>6</sup>. Therefore,  $\text{NO}_3^-$  supplementation might have greater ergogenic potential during shorter duration time-trial performance tests in trained athletes, such as the 100 m and 200 m distances in swimming. Moreover, given the pronounced glycolytic energy turnover during such events, as reflected by a high post-competition  $\text{BLa}^{24}$ , and since the reduction of  $\text{NO}_2^-$  to NO is potentiated with acidosis<sup>10</sup>,  $\text{NO}_3^-$  supplementation has the potential to be ergogenic for trained swimmers competing over the 100 m and 200 m distances. However, this has yet to be investigated.

The purpose of this study was to test the hypothesis that short-term supplementation with  $\text{NO}_3^-$  would improve 200-m and 100-m freestyle swimming performance in moderately-trained swimmers.

## **Methods**

### **Participants**

Ten moderately-trained university swimmers (5 males) (mean  $\pm$  SD: age  $22 \pm 6$  years, body mass  $80.2 \pm 14.9$  kg, height  $1.75 \pm 0.06$  m) participated in this study. All participants had at least 10 years competitive swimming experience at club standard and at least 5 years experience competing in regional and university-level competitions. Participants completed at

least 3 to 4 weekly swimming training sessions (6-8 h a week). Ethical approval for this study was received from the Medicine, Dentistry and Clinical Sciences Research Ethics Committee at the University of Chester (reference no: 1256/17/OE/CSN). All participants provided written informed consent and completed health screening forms prior to participation in the study. Participants were required to record their dietary intake in the 24 h before the control trial and to repeat the same diet in the 24 h before subsequent trials. For 24 h prior to and for each of the testing days, participants were asked to refrain from high-intensity exercise, and the consumption of alcohol, caffeine, nutritional supplements and any anti-inflammatory drugs. Participants avoided antibacterial mouthwash throughout the testing period, given that it eradicates oral nitrate reducing bacteria<sup>25</sup>. The swimmers who participated in this study were in the middle stage of the general preparation training phase and that their training was standardized with 3 times/week, ~4000 m of swimming completed each time during the BRJ and PLA supplementation periods.

### **Experimental Design**

Participants completed three separate visits over ten days. On the first familiarization visit, all subjects performed a 200-m front-crawl time-trial (TT) following blood sample collection and blood pressure measurement. After 30 min of passive recovery, participants completed a 100-m front-crawl TT. The data during this familiarization visit were not used for further analyses.

Following completion of this initial familiarization, participants were assigned to consume either nitrate-rich beetroot juice (BRJ) or nitrate-depleted beetroot juice (PLA) for 3-days, in a randomized, double-blind, cross-over design. A minimum washout period of 72-h separated the BRJ and PLA supplementation periods to ensure plasma  $\text{NO}_2^-$  concentration had returned to baseline<sup>4</sup>.



## **Supplementation**

During the two 3-day supplementation periods, participants consumed  $2 \times 70$  mL/day of concentrated nitrate-rich (~8 mmol/day nitrite) or nitrate-depleted beetroot juice (Beet It, James White Drinks Ltd., Ipswich, UK). Participants ingested a 70 mL shot in the morning (~9 am) and evening (~9 pm) over the first 2 days of supplementation. On the final day of supplementation,  $2 \times 70$  mL shots were ingested together 3 h prior to the 200-m TT.

## **Simulated swimming time trials**

All trials were completed in the same swimming pool with a depth, length, width and water temperature of 1-3 m, 25-m, 12.5-m, and 28 °C respectively, with trials performed at the same time of day for each condition (~12 pm). The swimming performance tests consisted of 200-m and 100-m front crawl swimming distances using a protocol adopted from Lindh et al.<sup>26</sup> to provide a closer simulation of a real swimming competition situation. Each subject completed a standardized low-to-moderate intensity warm-up (~25 min) before each trial. Ten minutes following the warm-up, a 200-m freestyle TT was performed. After completing the 200-m TT, the subjects recovered in a seated position for 30 min and were only allowed to drink water, which was recorded and precisely replicated on the subsequent trial. Following the recovery, a 100-m freestyle TT was performed. Participants completed the TTs individually (with no other competitors present). All TTs were commenced with a diving start from diving blocks and were timed with a stopwatch.

## **Measurements**

Upon arrival at the laboratory and following 10 min of rest, supine blood pressure (BP) of the brachial artery was measured four times using an automated sphygmomanometer (Dinamap Pro, GE Medical Systems, Tampa, FL). The mean of the measurements was calculated and used for analysis. Subsequently, a venous blood sample (~4 mL) was collected

into a lithium-heparin tube. Samples were then centrifuged at 1160 g and 4°C for 10 min (hettich® 320 centrifuge, Canada). Plasma was subsequently aliquoted and stored in labelled tubes at -80°C for later analysis of the nitrite concentration using a modification of the chemiluminescence technique as previously described <sup>4</sup>. Capillary blood lactate concentration (BLa) was also measured using a lactate analyzer (Lactatepro®, Japan) from finger pinprick samples. BLa was measured prior to the warm up, and immediately before and after the 200-m and 100-m freestyle swimming performance trials.

### **Statistical analysis**

Paired samples *t*-tests were employed to test for differences between the BRJ and PLA supplements in 100-m and 200-m swimming performance, plasma nitrite concentration and blood pressure. A two-way (supplement × time) repeated-measures ANOVA was employed to assess blood lactate responses following PLA and BRJ supplementation. Where the ANOVA revealed a significant effect, paired samples *t*-tests were utilised using Fisher LSD to define the origin of any potential effect. Statistical significance was set at  $p < 0.05$ , and all data were analyzed using SPSS 23.0 (IBM Corp., Armonk, NY), and are presented as mean ± SD.

## **Results**

### **Plasma nitrite and blood pressure**

The plasma nitrite concentration was increased after BRJ ( $432 \pm 203 \text{ nmol}\cdot\text{L}^{-1}$ ) compared to PLA ( $111 \pm 56 \text{ nmol}\cdot\text{L}^{-1}$ ) supplementation ( $p = 0.001$ ; figure 1).

Systolic BP was lowered by 5 % after BRJ supplementation compared to PLA ( $114 \pm 10$ ,  $120 \pm 10 \text{ mmHg}$ , respectively  $p = 0.001$ ). However, diastolic BP was not different after BRJ ( $65 \pm 7 \text{ mmHg}$ ) compared to PLA ( $66 \pm 7 \text{ mmHg}$ ) supplementation ( $p > 0.05$ ; figure 2).

## **200-m and 100-m swimming time-trials**

There was no difference in 200-m freestyle swimming TT performance following BRJ ( $152.6 \pm 14.1$  s) and PLA ( $152.5 \pm 14.1$  s) supplementation ( $p > 0.05$ ; figure 3). There was also no difference in 100-m freestyle swimming TT performance following BRJ ( $69.5 \pm 7.1$  s) and PLA ( $69.4 \pm 7.3$  s) supplementation ( $p > 0.05$ ; figure 3).

## **Blood lactate concentration**

The two-way ANOVA demonstrated no significant main effect for supplement ( $p > 0.05$ ) or no supplement  $\times$  time interaction effect ( $p > 0.05$ ) for BLa.

## **Discussion**

The principal original findings of this study were that 3-days of BRJ juice supplementation, which increased plasma nitrite concentration and lowered systolic blood pressure, did not enhance 200-m or 100-m swimming TT performance in moderately-trained swimmers. These findings contrast with our experimental hypothesis and do not support short-term BRJ supplementation as an ergogenic intervention for moderately-trained freestyle swimmers over 100-m and 200-m.

Compared to the PLA condition, plasma nitrite concentration was 289% higher in the BRJ condition. This result is consistent with numerous previous studies<sup>16</sup>, including the study by Jonvik et al.<sup>4,19</sup> in elite female water polo athletes. Therefore, the BRJ intervention was successful at increasing the circulating reservoir for O<sub>2</sub>-independent NO generation by a magnitude that has previously been shown to enhance performance<sup>4</sup>.

In addition to an increase in plasma nitrite concentration, short-term BRJ supplementation lowered systolic BP (6 mmHg) in young moderately-trained swimmers. However, there was no reduction in diastolic BP after BRJ supplementation compared to PLA supplementation. A lowering of blood pressure after BRJ ingestion is consistent with previous

reports<sup>4,27</sup>, with the mechanisms that underpin this effect likely to be multifaceted, but are largely believed to be NO-mediated<sup>28</sup>. The magnitude of BP reduction in the current study is likely to be of clinical relevance<sup>29,30</sup>.

Despite increasing the circulating plasma nitrite concentration and by extension the potential for O<sub>2</sub>-independent NO generation, BRJ supplementation did not improve 200-m or 100-m swimming performance in moderately-trained swimmers in the current study. These findings are consistent with recent reports that BRJ supplementation did not improve overall performance in a 168 m trial in trained swimmers<sup>22</sup> or repeated 15 m sprints in elite female water polo athletes<sup>19</sup>. However, since the 168 m TT was performed in a 21 m swimming pool rather than a traditional 25 m or 50 m pool, and since 168 m is not an appropriate competition distance, the physiological demands of the trial assessed by Pinna et al.<sup>22</sup> would have differed compared to 100-m and 200-m swimming races. To overcome this limitation, a simulation test which was adapted from a previous protocol<sup>26</sup>, was applied in the present study to more closely reflect a real competition situation. Therefore, our findings significantly extend previous observations by testing the ergogenic potential of BRJ supplementation over competition-specific race distances. Since no effect of BRJ supplementation was observed for either short- or middle-distance swimming performance, our results imply that BRJ supplementation does not appear to provide an ergogenic effect for trained swimmers, at least over these distances. However, since the performance tests in the current study, and the previous study by Lowings et al.<sup>22</sup>, were in the region of 1-3 min, we cannot exclude the possibility that BRJ supplementation could be ergogenic for swimming events where the completion time is less than or greater than 1-3 min.

## Practical Applications

Collectively, the findings of the present study and other recent publications<sup>19,22</sup> do not support BRJ supplementation as a nutritional ergogenic aid for trained swimmers, at least up to distances of 200-m, but are in accord with the notion that BRJ supplementation is less likely to be ergogenic in well trained athletes<sup>2</sup>. However, since we did not measure plasma nitrite concentration prior to the 100-m bout, which occurred 30 min following completion of the 200-m bout, and since plasma nitrite concentration declines during intense exercise<sup>5</sup>, we cannot exclude the possibility that the plasma nitrite declined to a concentration that was too low to elicit an ergogenic effect in the 100-m TT and that BRJ supplementation could have been ergogenic in the 100-m TT if this had been completed without a prior maximal 200-m bout. Similarly, since aspects of endurance performance might be improved to a greater extent after prolonged compared to short-term BRJ supplementation<sup>27</sup>, improved swimming performance could have been observed if we had extended the supplementation window. Finally, since the TTs were hand timed in the current study and the error of such a method is likely to be higher than electronic timing methods, small performance changes may have been missed, especially during exercise over a short duration. Use of electronic timing pads is recommended to overcome this limitation. Therefore, further research is required to assess the potential ergogenic effects of BRJ supplementation in swimmers.

## Conclusions

In conclusion, 3-days of dietary supplementation with nitrate-rich beetroot juice increased plasma nitrite concentration and lowered BP but did not benefit middle (200-m) and short-distance (100-m) freestyle swimming performance in moderately-trained swimmers. These findings do not support short-term supplementation with nitrate-rich beetroot juice as a

nutritional ergogenic aid for trained swimmers, at least for the 100-m and 200-m freestyle events.

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### **Authorship**

The study was designed by OZ, CN, MM and SB. Data were collected, analysed, and subsequently interpreted by OZ, CN, MM and SB. Manuscript preparation, including drafting of the article and manuscript revisions, was undertaken by OZ, CN, MM and SB. All authors approved the final version of the article.

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This research did not receive any external funding. The authors declare no conflict of interest.

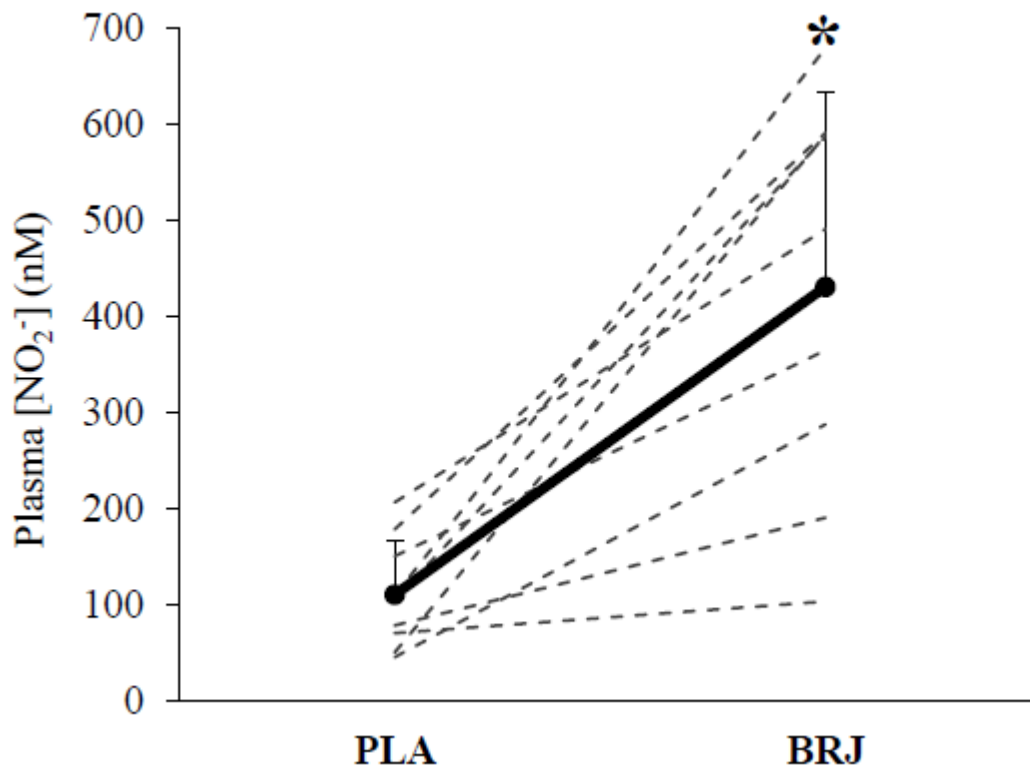
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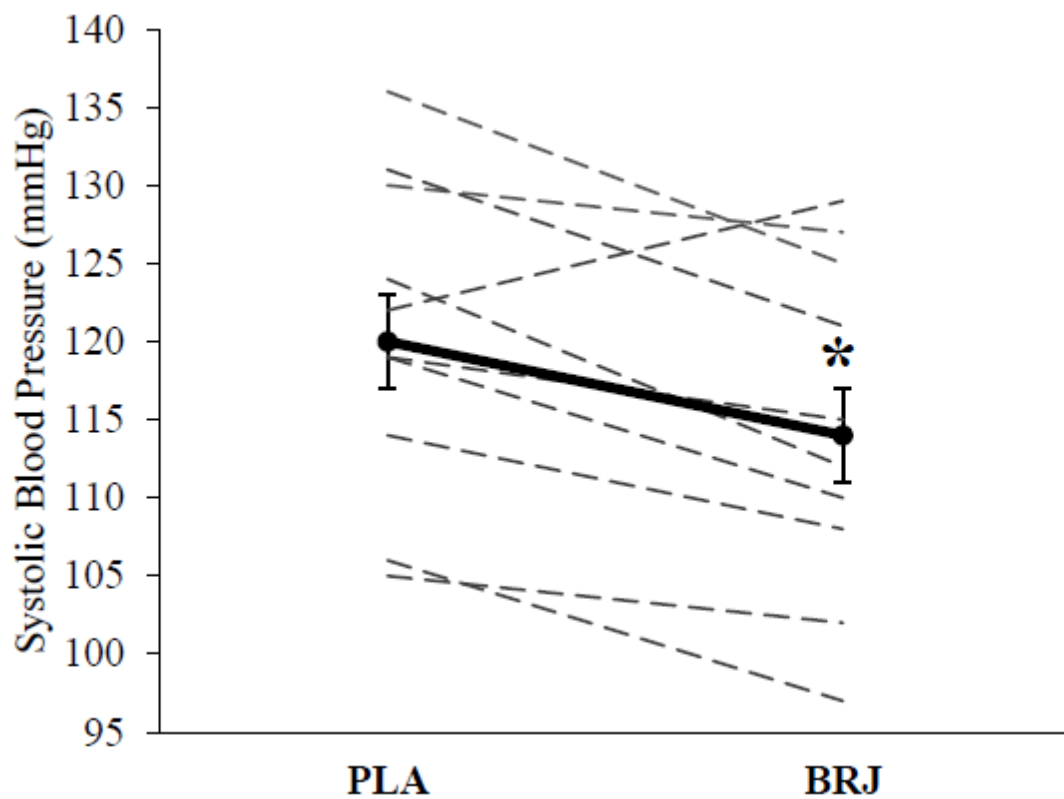
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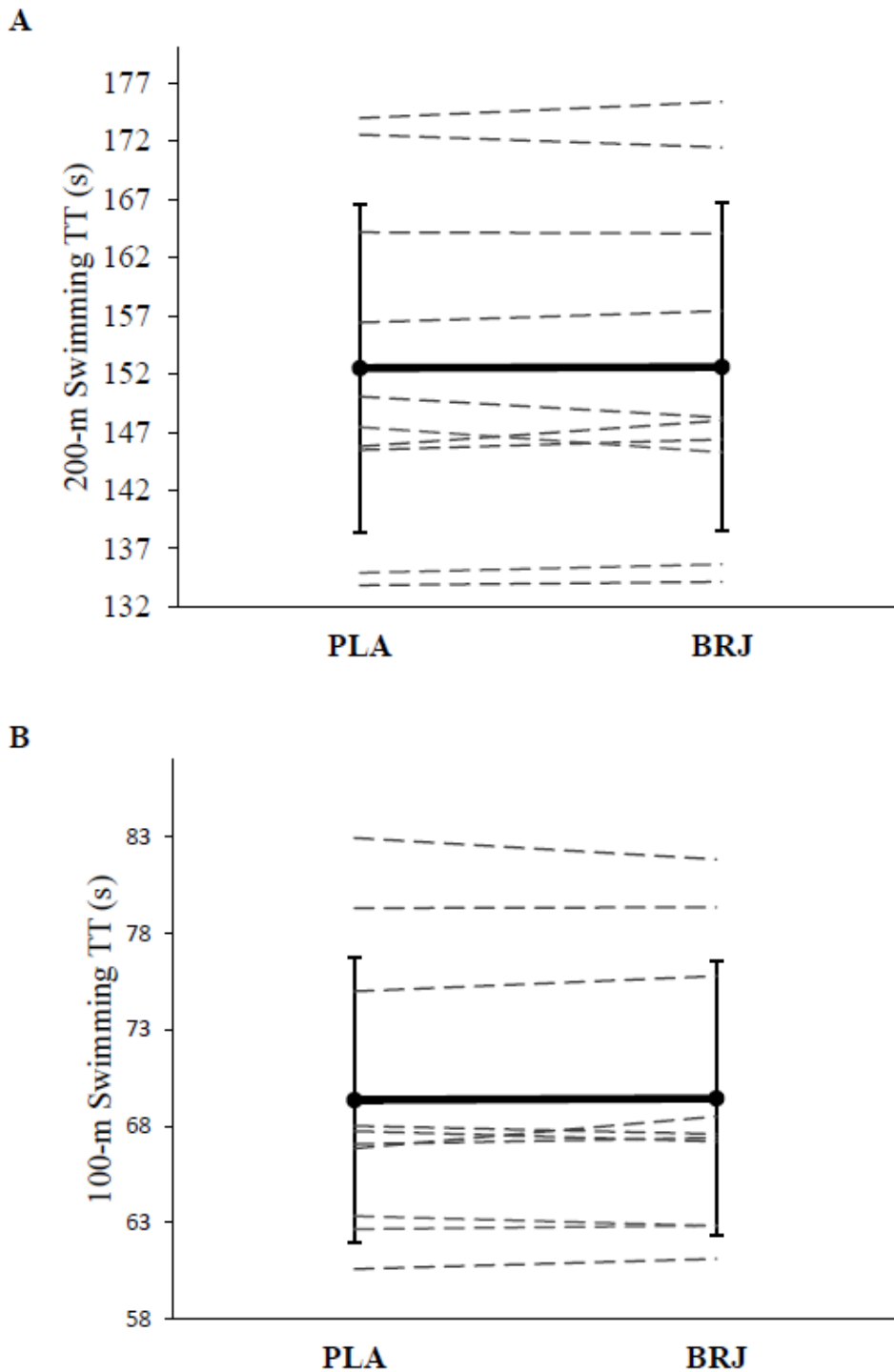
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**Figure 1:** Group mean (SD) and individual plasma nitrite concentration (NO<sub>2</sub><sup>-</sup>) responses after 3-days dietary nitrate or placebo supplementation are shown in the black and dashed lines, respectively. Plasma NO<sub>2</sub><sup>-</sup> was elevated following nitrate supplementation compared to placebo supplementation ( $p < 0.05$ )



**Figure 2:** Group mean (SD) and individual systolic blood pressure responses after 3-days dietary nitrate or placebo supplementation are shown in the black and dashed lines, respectively. Systolic blood pressure was lowered following nitrate supplementation compared to placebo supplementation ( $p < 0.05$ )



**Figure 3:** Group mean (SD) and individual 200-m swimming time trial (A) and 100-m swimming time trial (B) responses after 3-days dietary nitrate or placebo supplementation are shown in the black and dashed lines, respectively. There was no difference in 200-m and 100-m swimming time-trial performances between nitrate supplementation and placebo supplementation ( $p > 0.05$ ).