**Effect of beetroot juice supplementation on 10-km performance in recreational runners**

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Effect of beetroot juice supplementation on 10-km performance in recreational runners

Authors: Talitha Fernandes de Castro¹, Francisco de Assis Manoel², Diogo Hilgemberg Figueiredo², Diego Hilgemberg Figueiredo², Fabiana Andrade Machado¹²

¹ Post-graduate Program of Physiological Sciences, Department of Physiological Sciences, State University of Maringá-PR, Brazil.
² Associate Post-graduate Program in Physical Education UEM/UEL, Department of Physical Education, State University of Maringá-PR, Brazil.

Fabiana Andrade Machado (Corresponding Author)

Post-graduate Program of Physiological Sciences, Department of Physiological Sciences, State University of Maringá-PR, Brazil.
Associate Post-Graduate Program in Physical Education UEM/UEL, Department of Physical Education, State University of Maringá

Av. Colombo, 5790, Postal Code: 87.020-900, Maringá-PR, Brazil. Tel: (44) 3011-5861
E-mail: famachado_uem@hotmail.com, famachado@uem.br

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Abstract

The purpose of this study was to investigate the effects of chronic beetroot juice (BRJ) supplementation on 10-km running performance in recreational runners. In a double-blind, placebo-controlled, crossover-designed study, fourteen male recreational runners (age: 27.8 ± 3.4 y) performed three 10-km running tests at baseline, under the conditions of BRJ supplementation and placebo (PLA). Supplementation was administered for three days, and on the day of the assessments, the ingestion occurred two hours before each test and consisted of a dose of 420 mL of BRJ in natura (8.4 mmol NO\textsubscript{3}/day) or PLA with depleted NO\textsubscript{3} (0.01 mmol NO\textsubscript{3}/day). The mean velocity (MV) was calculated and the following variables were determined: maximum heart rate (HR\textsubscript{max}), maximal rating of perceived exertion (RPE\textsubscript{max}), and determined at pre and post test glucose concentrations (Glyc\textsubscript{pre}, Glyc\textsubscript{post}), and lactate peak. There was no main effect between conditions regarding to 10-km running time performance (BRJ: 50.1 ± 5.3; PLA: 51.0 ± 5.1 min, p = 0.391) and total MV (BRJ: 12.1 ± 1.3; PLA: 11.9 ± 1.2 km·h\textsuperscript{-1}, p = 0.321), as well as in the other analyzed variables. The time to complete the first half of the test (5 km) was statistically lower in the BRJ compared to that in the PLA (P = 0.027). In conclusion, chronic supplementation with BRJ increasing MV in the first half of the test and improves the final test time of ten of the fourteen runners, although we did not find a statistically significant difference in the performance of 10-km.

Keywords: nitrate supplementation; nitric oxide; running; exercise nutritional science; physical endurance.
Introduction

Dietary supplements are used to optimize performance, and therefore, help athletes gain an advantage in competition (Vernec et al., 2013). In recent years, a strong body of evidence indicates that the ergogenic effects of beetroot juice (BRJ), rich in inorganic nitrate (NO$_3^-$), can enhance performance in sports, and is becoming progressively more popular among competitive and recreational athletes (Dominguéz et al., 2017; McMahon et al., 2016; Cermak et al., 2012a). Once ingested, dietary inorganic NO$_3^-$ is reduced to nitrite (NO$_2^-$) and could favor nitric oxide (NO) synthesis by an independent pathway of the conventional L-arginine pathway (Lundberg et al., 2004).

NO is an important signaling molecule that plays a key role in many physiological processes that may impact sport performance, including the regulation of tissue blood flow, muscle contractility, mitochondrial respiration and biogenesis, favoring oxidative metabolism (Jones, 2014a; Dejam et al., 2004). It has already been demonstrated that BRJ can modify physiological variables associated with endurance performance (Jones, 2014b) as well as directly impacting parameters of cardiorespiratory endurance, such as maximal oxygen uptake (VO$_{2\text{max}}$), exercise efficiency or economy and the kinetics of pulmonary oxygen uptake (VO$_2$) (Dominguéz et al., 2017). However, the direct effects of supplementation on time trial performance tests that are closer to a competitive reality, especially in running, need to be further investigated.

Most of the studies reporting positive effects of acute or chronic NO$_3^-$ supplementation on time trial performances included tests with a duration of less than 30 minutes of moderate or high intensity (Peeling et al., 2014; Cermak et al., 2012a; Lansley et al., 2011a) when compared with longer duration exercise (Shannon et al., 2017; Cermak et al., 2012b; Wilkerson et al., 2012). It was shown that a single dose of BRJ was not able to
improve the 10-km time trial performance in trained runners (Shannon et al., 2017) as well as in the 50-mile test performance (Wilkerson et al., 2012) or even in the 1-hour time trial performance in well-trained cyclists (Cermak et al., 2012b).

Specifically with regard to running, few studies and with controversial results analyzed the effects of SB at different distances. Boorsma et al. (2014) did not find improvement in the completion time of 1500 m after the acute or chronic BRJ supplementation in trained runners despite the fact that two individuals were identified as high responders. On the contrary, in another study using the same distance and a similar sample, acute supplementation with BRJ was able to reduce the test time in supplemented individuals (Shannon et al. 2017). At longer distances (5-km), with moderately trained individuals, acute supplementation with 500 mg NO$_3^-$, despite having a tendency to increase the mean final velocity of the test and a statistically significant increase of 5% of velocity in the last third of the test with reduction of RPE in the supplemented group, did not find statistical differences in the time of conclusion of the test (Murphy et al., 2012).

In addition to the duration and intensity of the exercise, it is speculated that the level of training of athletes influences the effects of supplementation with NO$_3^-$ (Campos et al., 2018; Carriker et al., 2016; Porcelli et al., 2015). Although there is no agreement (Campos et al., 2018; McMahon et al., 2016; Hoon et al., 2014), it is believed that the effects of BRJ supplementation are less expressive in well-trained athletes when compared to active or moderately trained individuals (Shannon et al., 2017; Boorsma et al., 2014; Wilkerson et al., 2012; Cermak et al., 2012b), although there are athletes classified as “responders” (Jonvik et al., 2015).

Thus, considering the different BRJ supplementation responses in performances with different durations and intensities as well as in the direct impact of supplementation on performance in individuals with varying training levels, the purpose of this study was to
investigate the effects of chronic BRJ supplementation on 10-km running performance in recreational runners. In addition, we verified the effects of supplementation on the pacing strategy and other associated physiological variables. We hypothesized that BRJ NO$_3^-$ rich, compared to NO$_3^-$ depleted, can shorten the test time or increase the average speed of the race and improve the running performance in recreational runners.

**Materials and Methods**

**Participants**

Fourteen male, recreational runners (age: 27.8 ± 3.4 years, height: 176.9 ± 5.9 cm, body mass: 74.4 ± 9.2 kg, VO$_{2\text{max}}$: 45.4 ± 5.9 mL·kg$^{-1}$·min$^{-1}$) with 10-km performance time between 40-60 minutes (between 1.5 and 2.3 slower than the VM of the world record for the distance of 10-km (27.3min / 21.98 km·h$^{-1}$)) volunteered to participate in this study. All participants were physically active with at least one year of experience in running, presented medical clearance to perform exhaustive physical tests and reported no use of medication or nutritional supplements ergogenic during the study duration. Prior to testing, written informed consent was obtained from all participants. The experimental protocol was approved by the Local Human Research Ethics Committee (#1.262.502).

**Experimental overview**

After familiarization with the protocol, nutritional orientation for the study duration and anthropometrical assessment, participants visited the track for three 10-km running performances separated by a 1-week. On the first test participants performed a 10-km running performance at baseline moment. Next, in a randomized, double-blind, crossover-design, participants performed two 10-km running performances after consuming 3 doses of 420 mL of BRJ NO$_3^-$-rich (≅ 8.4 mmol NO$_3^-$)/day or placebo (PLA) NO$_3^-$ depleted (≅ 0.01 mmol NO$_3^-$).
/day) condition. All tests were performed a 400 m official outdoor track at the same time of the day under similar climatic conditions (temperature = 25 – 29 °C and relative humidity = 60–75%) to minimize the influence of circadian variance.

10-km running performance

The participants performed of 10-km running performance on a track of athletics on 400 m outdoor without the presence of opponents or another competitor in the track without even test moment track and preceded by a self-determined warm-up of 10 min. All of the participants were encouraged to give their best performance. Participants freely choose their pacing strategy during the performance and the time was recorded every 400 m. The overall mean velocity (MV) for each trial was calculated by dividing the total distance covered by the trial duration. Additionally, partial MVs were calculated in three phases: (1) start (first 400 m), (2) middle (400-9600 m) and (3) end (last 400 m), as previously reported (Bertuzi et al., 2014; Lima-Silva et al., 2010). Mineral water was provided ad libitum in cups throughout trials, so that runners could hydrate themselves as they were used to do in long-distance races. The time required to complete each test kilometer and 5-km was also recorded.

Earlobe capillary blood samples (25 µl) were collected into a capillary tube before, at the end of the tests (time zero of recovery) and at the third, fifth, and seventh minutes of passive recovery with participants seated in a comfortable chair. From these samples, [LA] was subsequently determined by electroenzymatic methods using an automated analyzer (YSI 2300 STAT, Yellow Springs, Ohio, USA). Peak [LA] (LApeak) was defined for each participant as the highest post-exercise [LA] value. The blood glucose concentration was analyzed at the pre and post-test time (Glycpre and Glycpost) from a blood sample of the index finger (0.6 µL) (OptiumXceed, Abbot, Brazil) and the result are reported in mg·dL⁻¹. Rating
of perceived exertion (RPE, Borg scale of 6-20 points) and heart rate (HR, Polar RS 800, Kempele, Helsinki) were checked at each turn (400m) and at the end of the test.

Participants were advised to attend the well-hydrated test local, performed a standardized meal three hours before the start of testing and instructed not to use any type of mouthwash during the testing period because of their potential inhibitory effect on the conversion of NO$_3^-$-NO$_2^-$ (Govoni et al., 2008). They were also instructed to abstain from caffeinated or alcoholic beverages and strenuous physical exercise within 24 hours prior to testing and to consume the same diet and to maintain the same physical exercise regimen 48 hours prior to testing.

**Supplementation Protocol**

BRJ was produced from *in natura* beets that were purchased from the same producer, without addition of any other food component. The juices were produced on the same day of consumption and delivered to the participants by the researcher in charge. The offered BRJ dose was 420 mL ($\cong$ 8.4 mmol NO$_3^-$/day). To produce the placebo substance (PLA), the previously produced BRJ was filtered by an ion exchange resin (PA101 OH-, Permution®) capable of removing selectively NO$_3^-$ (Lansley et al., 2011). The net amount of juice in the PLA condition was the same as the BRJ without filtration (420 mL $\cong$ 0.01 mmol NO$_3^-$/day).

The two substances (BRJ or PLA) did not show any visual or organoleptic differences that could distinguish them, and both were sent to a specialized laboratory to analyze the final amount of NO$_3^-$, attesting that the PLA substance contained an amount of NO$_3^-$ that would bring no ergogenic effects. BRJ supplementation was initiated three days prior to the tests and the last dose was administered two hours before each test. Participants were instructed to consume the dose within a maximum of 15 minutes. The order of consumption of the
substances was previously drawn and an external collaborator was responsible for the blindness of the condition of the participants.

Statistical analysis

Data are presented as means ± standard deviations (SD) and were analyzed using the Statistical Package for the Social Sciences 20.0 software (SPSS Inc., USA). The normality of data was verified using the Shapiro-Wilk test and sphericity was verified by the Mauchly test and when violated the Greenhouse-Geisser correction was used. One-way ANOVA for repeated measures followed by the Bonferroni post hoc test was used to evaluate differences between the baseline moment, BRJ and PLA conditions for the 10-km running performance, mean velocity (MV), pacing strategy, maximum heart rate (HR$_{max}$), maximal rating of perceived exertion (RPE$_{max}$), pre-test glucose concentration (Glyc$_{pre}$), post-test glucose concentration (Glyc$_{post}$) and peak lactate concentration (La$_{peak}$). Student’s paired t-test was used to determine any significant difference between the BRJ and PLA conditions for MV at each kilometer for the 10-km running performance and the first and the last 5 km running performance. In order to calculate the effect size (ES) and percentage difference (Dif.%), a comparison was made between the means of the BRJ and PLA conditions. The ES was used to estimate the (standardized) magnitude of the difference, and the values were classified according to Cohen (1988) in: ≤ 0.20 (trivial), 0.21-0.50 (small), 0.51-0.80 (moderate) and > 0.80 (large). For all analyses, a significance level of $P < 0.05$ was adopted.

Results

Table 1 shows the results of the 10-km running performance test at baseline, under BRJ, and the PLA conditions. There was no main effect between conditions regarding to test
time ($F_{2,26} = 2.38; P = 0.112$), total MV ($F_{2,26} = 3.32; P = 0.052$), and the values relating to
HR$_{\text{max}}$ ($F_{1,3;17.1} = 0.08; P = 0.92$), RPE$_{\text{max}}$ ($F_{1,2;15.9} = 0.19; P = 0.821$), La$_{\text{peak}}$ ($F_{2,26} = 0.841; P =
0.443$), Gly$_{\text{pre}}$ ($F_{1,4;18.7} = 0.07; P = 0.925$) and Gly$_{\text{post}}$ ($F_{2,26} = 1.46; P = 0.249$). The value of the
percent difference ($\%$Diff) for comparison between the total test time (min) of BRJ and PLA
conditions was $-1.9 \pm 4.2\%$. Considering the baseline values the $\%$Diff of the BRJ condition
was $-2.5 \pm 4.7\%$ and PLA condition was $-0.7 \pm 3.8\%$.

*****Table 1 here*****

A significant main effect in the MV was observed for each phase in BRJ ($F_{2,26} = 5.15,
P = 0.013$). The MV in the start phase was higher than that in the middle phase ($P = 0.008$),
but there was no difference in relation to the end phase ($P = 0.369$), and MV in the middle
phase also reported no difference than that in the end phase ($P = 0.602$). For PLA condition, a
significant main effect in the MV was observed for each phase ($F_{2,26} = 3.84, P = 0.034$). The
MV in the start phase was higher that in the middle phase ($P = 0.002$), but not different from
that in the end phase ($P = 1.000$), and the MV in the middle phase was no different from that
in the end phase ($P = 0.136$).

Figure 1 presents the comparisons between the MV for the 10-km running
performance for the BRJ and PLA conditions at each kilometer. There were statistically
significant differences in favor of BRJ between the fourth and seventh kilometer ($P < 0.05$),
but not in the other parts of the test. When the performance was divided considering the first
and the last 5 km, both the MV (BRJ: 12.48 ± 1.2 vs PLA: 12.14 ± 1.2 km·h$^{-1}$, $P = 0.019$) and
the time to complete the first half of the test presented a statistically significant difference
between the BRJ and PLA conditions (BRJ: 24.25 ± 2.36 vs PLA: 24.94 ± 2.44 min, $P =
0.027$). There was no statistically significant difference in the reported values of RPE in the
first 5 km (BRJ: 13.5 ± 2.4 vs PLA: 13.8 ± 2.1 km·h$^{-1}$, $P = 0.272$) or any other time of the
test. Analysis based on magnitude of effect indicated a "small" effect size in favor of supplementation with BRJ in the MV and the total time of the 5-km during the test. There was no statistically significant difference in the MV or in the time to complete the second half of the test.

The individual results of the 10-km running performance in the BRJ and PLA conditions are presented in Figure 2. Of the 14 participants who completed the study, 10 presented a shorter test time in the BRJ test when compared to the PLA condition, and the other participants were faster in the PLA condition.

Discussion

The aim of the present study was to investigate the effects of chronic BRJ supplementation on the 10-km running performance in recreational runners. The main finding was that supplementation for three days with BRJ (8.4 mmol NO\(_3^-\)/day) increasing MV in the first half of the test and improves the final test time of ten of the fourteen runners, although we did not find a statistically significant difference in the performance of 10-km, contrary to our initial hypothesis.

The variation at the final time of the 10-km running performance test between the BRJ and PLA conditions was 0.9 ± 2.1 minutes (\(P = 0.130\)), indicating an improvement of 1.9 ± 4.2% on running performance with a “trivial” effect size. Our results are in agreement with
previous studies that evaluated the effects of BRJ on long duration endurance performance (Shannon et al., 2017; Wilkerson et al., 2012) and with a literature review and meta-analysis that found a "trivial" but not significant effect in favor of dietary supplementation of NO$_3^-$, which can be translated into improvement of 0.8% in the final performance (McMahon et al., 2016). Although the result of the present study may seem small in the competitive environment in which athletes have very similar levels of performances, the improvement of 0.5 to 1.5% is considered sufficient to make a difference between the competitors (Dominguéz et al., 2018; Paton and Hopkins, 2006) as well as the final result of the performance.

The pacing strategy adopted during the 10-km running performance in the BRJ and PLA conditions was similar. In both conditions, the progressive decrease of MV throughout the test was observed, which characterizes a negative strategy (do Carmo et al., 2012). However, a statistically significant difference was observed, with a greater MV from the fourth to the seventh kilometer, in the BRJ condition compared to the PLA. The improvement in MV in this part of the test may be associated to the effects of NO$_3^-$ supplementation on the exercise economy (Dominguéz et al., 2017; Jones, 2014b), a parameter that expresses the relationship between VO$_2$ or RPE and the distance traveled by an athlete (Peeling et al., 2015). The improvement in the exercise economy will allow an athlete to exercise at a higher power output or running speed for the same VO$_2$ and reduced time required to complete the 10-km performance (Jones, 2014b). Another mechanism that could explain these results was the beneficial effects of BRJ in VO$_{2max}$, a parameter that, along with two other variables (treadmill peak velocity and 1 repetition maximal), are considered to best explain this performance in the middle part of the test (Bertuzzi et al., 2014). It has already been shown that BRJ can increase VO$_{2max}$ in physically active men and it is believed that both its vasodilatory properties, as well as its additional action on mitochondrial respiration and
improved muscle contraction efficiency, may be involved in this process (Dominguéz et al., 2018; Fergunson et al., 2015; Vanhatalo et al., 2010).

Although in the present study BRJ changed the MV in the intermediate phase of the performance, no difference was found in the final phase. Our results are in agreement with the data reported in the literature, which show that NO$_3^-$ supplementation seems to have a positive effect on time trial performances lasting less than 30 minutes (Porcelli et al., 2015; Peeling et al., 2014; Cermak et al., 2012b) when compared to longer tests of lower intensities (Shannon et al., 2017; Glaister et al., 2015; Wilkerson et al., 2012). The exact reasons for this difference between the results in performances with different durations are still unclear; however, one of the hypotheses supported is the difference in the predominance of recruitment of muscle fibers in these two types of activity (Dominguéz et al., 2018; Shannon et al., 2017). It is known that the effects of NO$_3^-$ can enhance type II muscle fibre contractility and blood flow (Ferguson et al. 2013a, 2013b) suggesting the possibility that BRJ supplementation might benefit human performance during high-intensity exercise and which could mean a physiological advantage for intermittent and/or high intensity exercises (Dominguéz et al., 2018; Coggan et al., 2015; Jones, 2014b). Additionally, when compared to other sport modalities, endurance athletes tend to present a greater predominance of type I skeletal muscle fibers (Tesch and Karlsson, 1985). Another hypothesis that could justify the difference between performance results with different durations is that the low intensity endurance exercise, in which the skeletal muscle remains well oxygenated, would not lead to a significant decrease in the local pH and to the stimulus of NO$_2^-$ conversion in NO, since this conversion is stimulated by hypoxia (Jones, 2014a; Jones, 2014b).

Considering the results in which the effects of NO$_3^-$ supplementation are more expressive in active and moderately trained individuals with VO$_{2\text{max}}$ < 60 mL·kg$^{-1}$·min$^{-1}$ (Carriker et al., 2016; Hoon et al., 2014), another hypothesis tested in this study is that BRJ...
could improve the 10-km running performance in recreational runners (VO2max = 45.4 ± 5.9 mL·kg⁻¹·min⁻¹). Although the mechanisms that explain the effects of performance supplementation in individuals with different levels of training are not fully elucidated, and the studies still present controversial results (Campos et al., 2018; McMahon et al., 2016; Jones, 2014a, 2015b), it is believed that well-trained athletes have higher NO3⁻ and NO2⁻ plasma levels than most individuals with a lower level of training, so that the response of a standard dose (4 - 8 mmol NO3⁻) of BRJ can be decreased in this population (Boorsma et al., 2014; Jones, 2014b). In addition, they appear to have a higher activity of the NOS enzyme, responsible for endogenous NO generation, which could decrease NO production from NO3⁻ and attenuate the benefits of BRJ supplementation (Lowings et al., 2017). However, contrary to our hypothesis and other studies with a similar population (Bailey et al., 2015; Wylie et al., 2013; Breese et al., 2013), we found no statistical difference in favor of supplementation, which suggests that other mechanisms, besides level of physical fitness, are involved in the physiological responses to NO3⁻.

In addition to the duration and intensity of the test, and the level of training or physical fitness of the participants, an important aspect that has been considered in research involving supplementation with BRJ or other sources of NO3⁻ is the existence of individual variability in responses to supplementation, leading to the classification of responders and non-responders according to plasma NO2⁻ elevation and individual performance results (Jonvik et al., 2015). It has already been shown that there is a positive correlation between NO2⁻ plasma levels and improvement in performance (Carriker et al., 2016) and that, despite following the same recommendations and ingesting the same BRJ doses, some individuals do not have this response (Wilkerson et al., 2012). However, even with similar responses of plasma concentrations of NO2⁻ and NO3⁻ after supplementation, not all studies have found results in favor of BRJ supplementation (Shannon et al., 2017; Cermak et al., 2012b).
Although we did not perform plasma NO$_2^-$ and NO$_3^-$ dosing in our study, which prevents us from contributing to this classification, 10 of the 14 participants in this study finished the 10-km run faster in the BRJ supplementation condition than in the PLA condition, which is in agreement with the literature and may indicate an interpersonal variation in relation to the beneficial effects of NO$_3^-$ on performance. Therefore, analysis of the individual performance results after BRJ supplementation should be considered.

In view of this, we can conclude that chronic supplementation (three days) with 420 mL of BRJ (8.4 mmol NO$_3^-$/day) increasing MV in the first half of the test and improves the final test time of ten of the fourteen runners, although we did not find a statistically significant difference in the performance of 10-km. These findings provide important insight into the specific conditions where BRJ supplementation may be ergogenic for a 5-km distance or for some individuals, and can be an important tactical advantage in 10-km running performance.

The authors have no conflicts of interest to report.
References


Table 1 - Mean values ± standard deviation (SD) of the variables obtained during 10-km running performance at baseline moment, BRJ and PLA conditions (n = 14)

<table>
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<th>Baseline</th>
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<th>PLA</th>
<th>ES (CI 90%)</th>
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<tr>
<td>Performance time (min)</td>
<td>51.3 ± 4.5</td>
<td>50.1 ± 5.3</td>
<td>51.0 ± 5.1</td>
<td>-0.17 “trivial”</td>
</tr>
<tr>
<td>MV 10 km (km·h⁻¹)</td>
<td>11.8 ± 1.1</td>
<td>12.1 ± 1.3</td>
<td>11.9 ± 1.2</td>
<td>0.16 “trivial”</td>
</tr>
<tr>
<td>HR_{max} (bpm)</td>
<td>185 ± 13.0</td>
<td>185 ± 10.9</td>
<td>185 ± 11.2</td>
<td>0.00 “trivial”</td>
</tr>
<tr>
<td>RPE_{max} (AU)</td>
<td>17.9 ± 1.7</td>
<td>18.2 ± 2.0</td>
<td>17.5 ± 2.8</td>
<td>0.28 “small”</td>
</tr>
<tr>
<td>Glyc_{pre} (mg·dL⁻¹)</td>
<td>96.8 ± 15.4</td>
<td>94.5 ± 12.4</td>
<td>95.8 ± 16.1</td>
<td>-0.09 “trivial”</td>
</tr>
<tr>
<td>Glyc_{post} (mg·dL⁻¹)</td>
<td>96.7 ± 15.1</td>
<td>95.5 ± 10.7</td>
<td>89.5 ± 14.1</td>
<td>0.47 “small”</td>
</tr>
<tr>
<td>Lac_{peak} (mmol·L⁻¹)</td>
<td>8.6 ± 0.9</td>
<td>8.8 ± 0.6</td>
<td>8.9 ± 0.6</td>
<td>-0.16 “trivial”</td>
</tr>
</tbody>
</table>

BRJ, beetroot juice condition; PLA, placebo condition; ES, effect size between BRJ and PLA conditions; Total performance time (min); MV, mean velocity; HR_{max}: maximum heart rate; Lac_{peak}: peak lactate concentration; RPE_{max}: maximal rating of perceived exertion; AU: arbitrary units; Glyc_{pre}: pre-test glucose concentration; Glyc_{post}: post-test glucose concentration.

Fig 1 Mean velocity (MV) for each km test in the BRJ and PLA conditions.

Fig 2. Individual performance on 10-km running performance in BRJ and PLA conditions.
Mean velocity (MV) for each km test in the BRJ and PLA conditions.
Individual performance on 10-km running performance in BRJ and PLA conditions.