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ORIGINAL ARTICLE

Effects of chronic beetroot juice supplementation on maximum oxygen uptake, velocity associated with maximum oxygen uptake, and peak velocity in recreational runners: a double-blinded, randomized and crossover study

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Abstract

Purpose This study investigated the effects of chronic 3-day beetroot juice (BRJ) supplementation on maximum oxygen uptake (VO_{2max}), velocity associated with VO_{2max} (vVO_{2max}), and peak velocity (V_{peak}) in recreational runners.

Methods Thirteen male recreational runners (age 28.2 ± 3.0 years, height 176.8 ± 0.1 cm, body mass 74.4 ± 9.5 kg) performed four tests on a treadmill in a randomized, double-blind, crossover design: two maximum incremental tests to determine VO_{2max} and $vVO_{2max,}$ and two tests to determine V_{peak}. Trials were performed following 3 days of supplementation of NO_3^--rich BRJ in natura (8.4 mmol NO_3^- day^{-1}) or BRJ NO_3^--depleted placebo (0.01 mmol NO_3^- day^{-1}), with the last dose being ingested 2 h before each test. During the tests, maximum heart rate (HR_{max}), maximal rating of perceived exertion (RPE_{max}), pre- and post-test glucose concentrations ($Gluc_{pre,}$ $Gluc_{post}$), and peak blood lactate concentration were determined.

Results VO_{2max} was higher following BRJ vs PLA (46.6 ± 6.4 vs 45.1 ± 5.8 mL kg^{-1} min^{-1}; $P = 0.022$), as well as vVO_{2max} (14.5 ± 0.8 vs 13.9 ± 1.0 km h^{-1} $P = 0.024$) and V_{peak} (15.5 ± 1.1 vs 15.2 ± 1.2 km h^{-1} $P = 0.038$), with no differences in the other variables.

Conclusion Consumption of NO_3^--rich BRJ in natura (8.4 mmol NO_3^- day^{-1}) once per day for 3 days improved VO_{2max}, vVO_{2max} and V_{peak} in recreational runners without changing the other analyzed variables.

Keywords Nitrate supplementation · Nitric oxide · Running · Exercise nutritional science · Physical endurance

Abbreviations

LA	Blood lactate concentration
LA_{peak}	Peak blood lactate concentration
ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
BRJ	Beetroot juice supplementation
ES	Effect size
$Gluc_{pre}$	Pre-glucose concentrations
$Gluc_{post}$	Post-glucose concentrations
HR	Heart rate
HR_{max}	Maximum heart rate
Inc	Speed increment
$NaNO_3^-$	Sodium nitrate
NO	Nitric oxide
NOS	Nitric oxide synthase
NO_2^-	Nitrite
NO_3^-	Inorganic nitrate
O_2	Oxygen
PCr	Phosphocreatine
P_i	Inorganic phosphate
PLA	Placebo
P/O	Phosphate/oxygen ratio
RE	Running economy
RPE	Rating of perceived exertion

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RPE_{max}	Maximal rating of perceived exertion
SIRT1	Regulatory protein sirtuin
T	Number of seconds required to complete a stage
t	Number of seconds sustained during the incomplete stage
$V_{complete}$	Running velocity of the last complete stage
VO_2	Oxygen uptake
VO_{2max}	Maximum oxygen uptake
V_{peak}	Peak velocity
vVO_{2max}	Velocity associated with VO_{2max}

Introduction

Beetroot juice (BRJ) supplementation has been widely studied after evidence showed that inorganic nitrate (NO_3^-) consumption increased plasma concentrations of NO_3^- and nitrite (NO_2^-) in a dose-dependent manner (Nyakayiru et al. 2017; Wylie et al. 2013). This supplementation could also induce the production of nitric oxide (NO) independent of the conventional L-arginine pathway. This occurs mainly under conditions of hypoxia and low pH, when the activity of the nitric oxide synthase (NOS) enzyme family, dependent on oxygen (O_2), is impaired (Modin et al. 2001; Moncada and Higgs 1993). NO is an important signaling molecule that promotes relaxation of vascular smooth muscle and increases tissue blood flow mediated by guanylyl cyclase (Francis et al. 2010; Vanni et al. 2007). In addition to its direct effect on vasodilation and hemodynamics (Govoni et al. 2008), NO plays a key role in many physiological processes that may impact endurance exercise performance and even facilitates adaptation to exercise training (de Castro et al. 2018; Thompson et al. 2017; Bailey et al. 2009). In addition, chronic and acute dietary NO_3^- supplementation reduces the O_2 cost of exercise and increases exercise efficiency, as is reported in many (Whitfield et al. 2016; Lansley et al. 2011; Larsen et al. 2007, 2011; Bailey et al. 2009, 2010; Vanhatalo et al. 2010), although not in all studies (Balsalobre-Fernández et al. 2018; Betteridge et al. 2016; Thompson et al. 2015).

Some mechanisms are proposed to explain this ergogenic effect on endurance performance and on the cardiorespiratory components of aerobic exercise: reduction of the cost of ATP production of contractile force and attenuation of the stimulus for oxidative phosphorylation and oxygen uptake (VO_2) (Vanhatalo et al. 2011; Bailey et al. 2010), an increase in the mitochondrial phosphorylation efficiency, evaluated by the phosphate/oxygen ratio (P/O) ratio (Bailey et al. 2009). In addition, there is evidence that NO_2^- can act as an alternative acceptor of electrons, thus replacing the role of O_2 in respiration (Bailey et al. 2009; Basu et al. 2008; Brown 2001) and inhibits O_2 binding at cytochrome C oxidase (Brown and Cooper 1994; Cleeter et al. 1994), resulting in "local hypoxia" (Larsen et al. 2011) and consequent increase in tissue availability (Tong et al. 2011). Regarding the improvement of muscular function, besides its role in the modulation of the contractile function, especially in type II muscle fibers (Ferguson et al. 2013; Hernandés et al. 2012), it is believed that NO can match blood flow with local demand for O_2 providing the best distribution (Hord et al. 2009).

It is believed that in addition to directly impacting O_2 cost of endurance running at a given submaximal speed, NO_3^- supplementation may improve other physiological determinants of endurance exercise such as maximal oxygen consumption (VO_{2max}) (Joyner and Coyle 2008; Dominguez et al. 2017; Vanhatalo et al. 2011). This variable is frequently used to indicate the cardiorespiratory fitness of an individual because it is quantitatively and qualitatively related to metabolic and cardiovascular changes (Edvardsen et al. 2014; Bassett and Howley 2000). An individual's VO_{2max} is determined by genetic factors, age, gender, and level of training (Rowland 1996). Among its limiting factors are pulmonary ventilation, alveolar–capillary O_2 diffusion, cardiovascular system, and the arteriovenous O_2 difference (Saltin and Strange 1992). It is directly affected by vasodilation and muscular vasoconstriction, and also the capacity of energy generation by oxidative phosphorylation (Amann 2012; Denadai 1999; Holloszy and Coyle 1984). In addition, VO_{2max} is considered a useful variable to predict endurance performance (Midgley et al. 2007; Billat et al. 1999).

From the determination of VO_{2max} it is possible to identify other variables such as the velocity of VO_{2max} occurrence (vVO_{2max}), which in physiological terms reflects the interaction between endurance running at a given submaximal speed and VO_{2max} (Buchheit al. 2010; Almarwaey et al. 2004; Billat and Koralsztein 1996). Moreover, vVO_{2max} can explain variations in performance that endurance running at a given submaximal speed and VO_{2max} alone would not be able to do (Hill and Rowell 1996).

Another variable that has gained attention among researchers, trainers, and endurance runners is the maximal velocity attained during an incremental test (V_{peak}) (McLaughlin et al. 2010; Saunders et al. 2010). Previous studies have shown that V_{peak} reflects the intensity of VO_{2max} and vVO_{2max}; however, it is determinate and not estimated, thus associating itself directly with aerobic power (da Silva et al. 2015). In addition, V_{peak} is a great predictor of endurance performance over 3–90 km (Machado et al. 2013; Midgley et al. 2007; Mclaughlin et al. 2010), and like $vVO_{2max,}$ is considered a good variable to monitor and prescribe endurance training (Manoel et al. 2017, 2018; Da Silva et al. 2017). Since determination of V_{peak} does not require ergospirometry, it is considered a very practical and attractive variable compared to vVO_{2max}. In

addition, different from the determination of $vVO_{2max,}$ which considers the minimum velocity that VO_{2max} reached during an incremental test to exhaustion, independent of the time remained in the stage that it occurs (Billat et al. 1994, 1996), the determination of V_{peak} considers every second remained during the test, even if the last stage is not completed adjusted with Kuipers et al. (2003) equation, which in turn may be more sensitive to detect the effects of training. For a proper training prescription, it is necessary to use variables that control and monitor the intensity of effort and possible physiological adaptations resulting from this practice and, most importantly, that show a correlation with performance. Thus, vVO_{2max} and V_{peak} are the preferred variables to monitor and to prescribe training among endurance athletes. More recently, Manoel et al. (2017) demonstrated that 4 weeks of endurance training prescribed by V_{peak} and its respective time limit promoted similar improvements in 10-km time trial and V_{peak}, compared to 4 weeks of endurance training prescribed by vVO_{2max} and its respective time limit in moderately trained endurance runners. However, no difference was found for the VO_{2max} after the training prescribed by the V_{peak} and vVO_{2max}, suggesting that for a given training intervention, VO_{2max}, V_{peak}, and vVO_{2max} could change differently.

Nonetheless, recently evidences indicate that acute or chronic NO_3^- supplementation can improve running endurance performance from different distances (i.e., 5-km and 10-km time trial) (Shannon et al. 2017; de Castro et al. 2018). For instance, Shannon et al. (2017) reported a significant improvement in 5-km time trial (performance improvement: ≅ 1.9%) after acute BRJ supplementation compared to PLA. Similarly, de Castro et al. (2018) showed improvements in 10-km time trial after BRJ supplementation in recreational endurance runners compared to PLA condition (performance improvement: ≅ 1.9%). These improvements in time trial performance after acute or chronic BRJ supplementation were comparable and greater than those reported in studies with endurance running training protocols prescribed with vVO_{2max} and V_{peak} (performance improvement in 3-km and 10-km time trial: ≅ 1% and 1.4%, respectively) (Smith et al. 2003; Manoel et al. 2017). Furthermore, most studies that have examined these effects have used time-to-exhaustion protocols with exercise being performed at a constant work rate (Bailey et al. 2009, 2010; Lansley et al. 2011), which in turn do not simulate normal athletic competition. Therefore, since there is a correlation between monitoring and training prescription variables, such as V_{peak}, vVO_{2max} and endurance performance (i.e., 10 km time trial), it would be interesting to verify the effect of chronic NO_3^- supplementation on these variables.

Thus, the aim of the present study was to investigate the effects of chronic 3-day BRJ supplementation on VO_{2max}, vVO_{2max} and V_{peak} in recreational runners. Due to the vasodilatory properties of NO_3^- and their influence on O_2 use during skeletal muscle contraction, and the mitochondrial benefits and their relationship with the performance variables, we hypothesized that after chronic BRJ supplementation VO_{2max}, vVO_{2max} and V_{peak} would be increased compared to PLA condition.

Methods

Participants and design

Thirteen male recreational runners (age 28.2 ± 3.0 years, height 176.8 ± 0.1 cm, body mass 74.4 ± 9.5 kg) with 10-km performance time between 40 and 60 min (≅ 45 to 70% of the World record established in 2005) volunteered to participate in this study. All participants had at least 1 year of experience in running, presented medical clearance to perform exhaustive physical tests and reported no use of medication or nutritional supplements during the study. Prior to testing, written informed consent was obtained from all participants. The experimental protocol was approved by the Local Human Research Ethics Committee (#1.262.502/2016).

After familiarization with the protocol, nutritional orientation (i.e., dietary procedures to be adopted throughout the testing period) and anthropometrical assessment (i.e., height and body mass), participants visited the laboratory (temperature = 21 ± 1 °C and relative humidity = 55 ± 5–60%) four times separated by 1 week between each test. In a randomized, double-blind, placebo-controlled cross-over design, participants performed two maximum incremental tests to determine the VO_{2max} and vVO_{2max}, and two tests to determine the V_{peak}, after the consumption of one dose per day of 420 mL NO_3^--rich BRJ (8.4 mmol NO_3^- day^{-1}) or NO_3^--depleted placebo (PLA) (0.01 mmol NO_3^- day^{-1}) condition over 3 days (de Castro et al. 2018). The supplementation periods were interspaced by 4 days of washout (Wylie et al. 2013). The experimental protocol is illustrated in Fig. 1.

Experimental protocol

Determination of VO_{2max} and vVO_{2max}

The incremental exercise tests were performed on a motorized treadmill (Super ATL, Inbrasport, Porto Alegre, Brazil) with a gradient set at 1% (Jones and Doust 1996). After a warm-up that consisted of walking at 6 km h^{-1} for 3 min, the protocol started with an initial speed of 8 km h^{-1}, followed by an increase of 1 km h^{-1} every 3 min between each successive stage until volitional exhaustion (i.e., the participant was unable to continue running) (Machado et al. 2013).

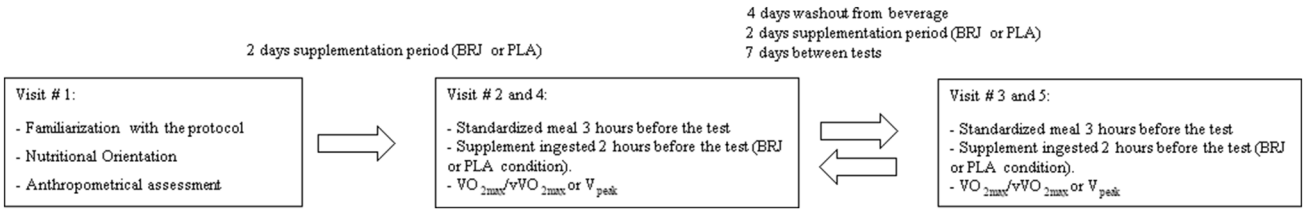

Fig. 1 Overview of the experimental protocol. *BRJ* beetroot juice supplementation, *PLA* placebo, VO_{2max} maximum oxygen uptake, vVO_{2max} velocity associated with VO_{2max}, V_{peak} peak velocity

Consistently across each trial, participants were strongly encouraged, verbally, to perform the maximum effort. Gas exchange was collected to determine the VO_{2max} using a portable gas analyzer (k4b^2 Cosmed®, Rome, Italy), and the VO_{2max} was regarded as the maximum value obtained during the test, measured at an average of 15-s intervals. In addition to the participants having to perform the test until voluntary exhaustion (i.e., the participant was unable to continue running), they should have met at least two of the following criteria to have been considered a successful test: (1) peak blood lactate concentration ($[LA_{peak}] \geq 8$ mmol L^{-1}, (2) maximum heart rate (HR_{max}) $\geq 100\%$ of endurance-trained age-predicted HR_{max} using the age-based "206–0.7 × age" equation (Tanaka et al. 2001) and (3) maximal rating of perceived exertion (RPE_{max}) ≥ 18 in the 6–20 Borg scale (Borg 1982). The vVO_{2max} was the minimum velocity at which the participants were running when VO_{2max} occurred (Billat et al. 1996, 1999).

Determination of V_{peak}

The protocol used to determine the V_{peak} was the same as that used to determine of VO_{2max}, however, without the use of a portable gas analyzer. The V_{peak} was considered the maximum running speed reached during the incremental test and if the last stage was not completed, was calculated on the part time remained in the last stage achieved from the equation proposed by Kuipers et al. (2003):

$$V_{peak} = V_{complete} + (Inc \times t/T),$$

where $V_{complete}$ is the running velocity of the last complete stage, Inc the speed increment (i.e., 1 km h^{-1}), t the number of seconds sustained during the incomplete stage and T is the number of seconds required to complete a stage (i.e., 180 s).

Dietary standardization and physical activity

The nutritional evaluation was carried out by a Nutritional Professional with the purpose of estimating the consumption of foods rich in NO_3^-, betaine and other compounds present in BRJ that could interfere with the results of the tests, besides discarding interferences of inadequate eating habits during the protocol. Participants received written guidelines on dietary procedures to be adopted throughout the testing period, which involved no consumption of foods rich in NO_3^-. Three hours before each test procedure, the participants were instructed to consume a standardized meal with the minimum amount of carbohydrate and protein calculated individually, respecting each participant eating habits. It was advised to replicate the meals and to maintain the choice of the same foods before the tests to avoid interference of any food factor. Before the beginning of each test, they wrote down the foods consumed and their quantities (Gibson 1990; Buzzard 1998). They were also instructed to abstain from strenuous physical exercise within 24 h prior to testing and to maintain the same physical exercise regimen 48 h prior to testing (de Castro et al. 2018; Machado et al. 2013).

Supplementation protocol

BRJ was produced by a Nutritional Professional at a controlled environment from natura beets that were purchased from the same producer, without the 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 (8.4 mmol NO_3^- day^{-1}). To produce the PLA substance, the previously produced BRJ was filtered by an ion exchange resin (PA101 OH^-, Permution®, Curitiba—PR, Brazil) capable of filtering NO_3^- (Lansley et al. 2011). The PLA substance was offered in the same quantity (420 mL) but without NO_3^- (0.01 mmol NO_3^- day^{-1}). 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 3 days prior to the tests and the last dose was administered 2 h before each test. Participants were instructed to consume the dose within a maximum of 15 min (de Castro et al. 2018). The order of consumption of the substances was previously drawn and an external collaborator was responsible for the blinding the condition to the participants. Participants consumed a standardized meal

3 h before the start of testing and were instructed not to use any type of mouthwash during the testing period because of their potential inhibitory effect on the conversion of NO_3^- to NO_2^- (Govoni et al. 2008).

Determination of blood lactate and glucose concentration, heart rate (HR), and rating of perceived exertion (RPE)

Earlobe capillary blood samples (25 µl) were collected into a capillary tube at the end of the tests (time zero of recovery) and at the 3rd, 5th, and 7th min of passive recovery with participants seated in a comfortable chair. From these samples, blood lactate concentration [LA] was subsequently determined by electroenzymatic methods using an automated analyzer (YSI 2300 STAT, Yellow Springs, Ohio, USA). [LA_{peak}] 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 ($Gluc_{pre}$ and $Gluc_{post}$) from a blood sample of the index finger (0.6 µL) (OptiumXceed, Abbot, Brazil) and the results are reported in mg dL^{-1}. Before testing, participants were familiarized with the 6–20 Borg scale (Borg 1982), which was used to measure the rating of perceived exertion (RPE) during the last 10 s of each stage and at exhaustion. The highest RPE value was adopted as the RPE_{max}. HR was monitored during all tests (Polar RS800sd; Kempele, Finland) and HR_{max} was defined as the highest HR value recorded during the test.

Statistical analyses

All statistical analyses were performed using the Statistical Package for the Social Sciences software (v.20.0, SPSS Inc., Chicago, IL, USA). Data normality was verified by Shapiro–Wilk test and the variables are presented as mean ± standard deviation (SD). The results of the incremental tests to determine VO_{2max}, vVO_{2max}, V_{peak}, HR_{max}, RPE_{max}, $Gluc_{pre}$, $Gluc_{post}$ and [La_{peak}] of the BRJ and PLA conditions were compared by Student's paired *t* test. To calculate the effect size (ES) and percentage difference (Dif. %), a comparison was made between the means. 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 comparisons between the variables obtained in the incremental tests to determine VO_{2max} between BRJ and PLA conditions, as well as Dif. % and ES among the conditions. Statistically significant differences were found in VO_{2max} (absolute and relative), in vVO_{2max} and in the duration of the incremental

Table 1 Mean values ± standard deviation (SD) of the variables obtained during the incremental test for VO_{2max} determination ($n = 13$)

Variables	PLA	BRJ	*P*	Dif. %	ES (CI 90%)
VO_{2max} (mL kg^{-1} min^{-1})	45.1 ± 5.8	46.6 ± 6.4*	0.022	3.7 ± 5.8	0.22 Small
VO_{2max} (L min^{-1})	3.4 ± 0.3	3.6 ± 0.4*	0.024	3.0 ± 5.0	0.55 Moderate
vVO_{2max} (km h^{-1})	13.9 ± 1.0	14.5 ± 0.8*	0.018	3.8 ± 4.9	0.54 Moderate
Duration (min)	25.6 ± 2.8	26.8 ± 3.4*	0.007	4.1 ± 4.9	0.32 Small
HR_{max} (bpm)	184 ± 6.3	184 ± 7.3	0.673	0.3 ± 2.7	0.04 Trivial
RPE_{max} (AU)	19.4 ± 1.0	19.9 ± 0.3	0.089	2.7 ± 5.3	0.70 Moderate
$Gluc_{pre}$ (mg dL^{-1})	98.5 ± 11.3	93.2 ± 9.5	0.169	-6.5 ± 14.4	−0.44 Small
$Gluc_{post}$ (mg dL^{-1})	104.0 ± 28.8	98.6 ± 23.3	0.502	-7.7 ± 33.5	−0.17 Trivial
[Lac_{peak}] (mmol L^{-1})	9.2 ± 0.6	9.1 ± 0.7	0.299	-1.4 ± 4.1	−0.15 Trivial

BRJ beetroot juice condition, *PLA* placebo condition, *ES* effect size, VO_{2max} maximal oxygen uptake, vVO_{2max} velocity associated with the occurrence of maximum oxygen uptake, HR_{max} maximum heart rate, RPE_{max} maximal rating of perceived exertion, *AU* arbitrary units, $Gluc_{pre}$ pre-test glucose concentration, $Gluc_{post}$ post-test glucose concentration, [La_{peak}] peak lactate concentration

*$P < 0.05$ in relation placebo condition

Table 2 Mean values ± standard deviation (SD) of the variables obtained during the incremental test for V_{peak} determination ($n = 13$)

Variables	PLA	BRJ	*P*	Dif. %	ES (CI 90%)
V_{peak} (km h^{-1})	15.2 ± 1.2	15.5 ± 1.1*	0.038	2.0 ± 3.1	0.17 Trivial
Duration (min)	27.6 ± 3.6	28.5 ± 3.2*	0.038	3.2 ± 5.1	0.21 Small
HR_{max} (bpm)	185 ± 8.2	185 ± 8.1	0.883	0.1 ± 3.0	0.06 Trivial
RPE_{max} (AU)	18.8 ± 1.4	19.0 ± 1.4	0.427	1.1 ± 5.2	0.14 Trivial
$Gluc_{pre}$ (mg dL^{-1})	93.1 ± 14.7	91.2 ± 7.5	0.658	-2.3 ± 15.7	−0.12 Trivial
$Gluc_{post}$ (mg dL^{-1})	104.0 ± 28.0	114.6 ± 23.3	0.111	8.6 ± 20.0	0.34 Small
[Lac_{peak}] (mmol L^{-1})	10.1 ± 1.0	10.1 ± 0.6	0.947	-0.2 ± 8.9	0.00 Trivial

BRJ beetroot juice condition, *PLA* placebo condition, *ES* effect size, V_{peak} peak velocity, HR_{max} maximum heart rate, RPE_{max} maximal rating of perceived exertion, *AU* arbitrary units, $Gluc_{pre}$ pre-test glucose concentration, $Gluc_{post}$ post-test glucose concentration, [La_{peak}] peak lactate concentration

*$P < 0.05$ in relation placebo condition

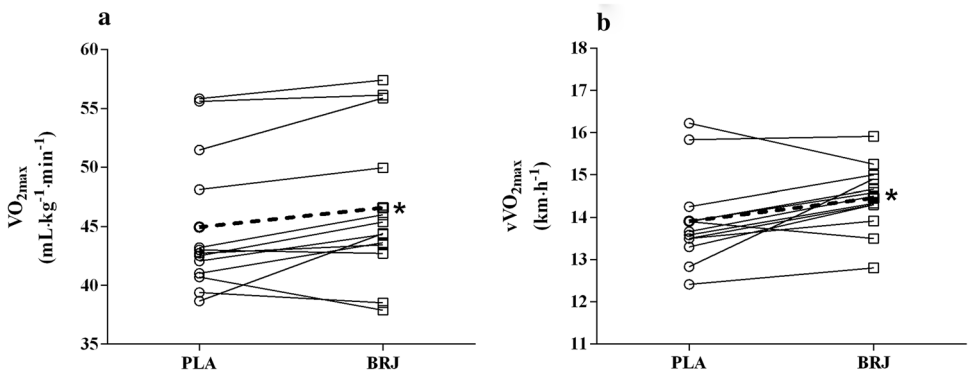

Fig. 2 Mean and individual values: **a** maximum oxygen uptake (VO_{2max}), **b** velocity associated with VO_{2max} (vVO_{2max}) in BRJ and PLA conditions. Dash line represents the mean values. *$P < 0.05$ in relation placebo condition

test. No differences were found in the values relating to HR_{max}, RPE_{max}, [La_{peak}], $Gluc_{pre}$ and $Gluc_{post}$ between the conditions.

Table 2 shows the results of the comparisons between the variables obtained in the incremental tests to determine V_{peak} between BRJ and PLA conditions, as well as Dif. % and ES among the conditions. Statistically significant differences were found in V_{peak} and in the duration of the incremental test. No differences were found in the values relating to HR_{max}, RPE_{max}, [La_{peak}], $Gluc_{pre}$, and $Gluc_{post}$ between the conditions.

The mean and individual results of the values obtained in the incremental tests to determine VO_{2max}, $vVO_{2max,}$ in the BRJ and PLA conditions are presented in Fig. 2. Eleven of the 13 participants improved VO_{2max} (absolute and relative) as well as vVO_{2max} in the BRJ condition compared to PLA condition.

The mean and individual results of the values obtained in the incremental tests to determine V_{peak} in the BRJ and PLA conditions are presented in Fig. 3. Nine of the 13

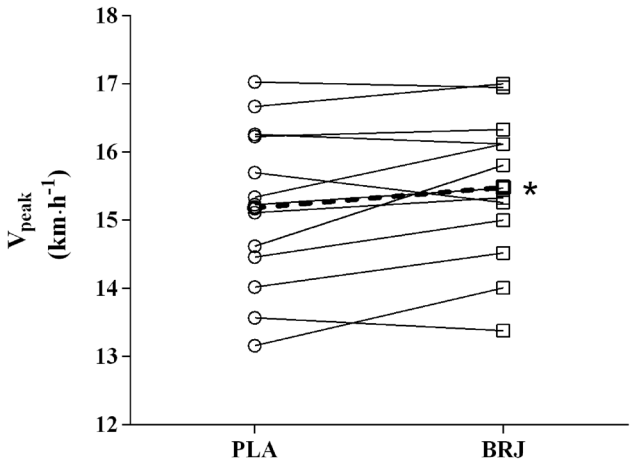

Fig. 3 Mean and individual values of peak velocity (V_{peak}) in BRJ and PLA conditions. Dash line represents the mean values. *$P < 0.05$ in relation placebo condition

participants presented higher values in the V_{peak} in the BRJ condition compared to PLA condition.

Discussion

The aim of the present study was to investigate the effects of chronic BRJ supplementation on VO_{2max}, vVO_{2max} and V_{peak} in recreational runners. The main finding was that 3-day supplementation with BRJ (8.4 mmol NO_3^- day^{-1}) increased VO_{2max}, vVO_{2max}, and V_{peak}. To our knowledge, this was the first study to evaluate the effects of BRJ supplementation on vVO_{2max} and V_{peak} variables in recreational runners.

It has already been shown that NO_3^- supplementation can improve cardiorespiratory endurance in athletes by increasing efficiency and by improving performance in time to exhaustion tests at submaximal intensities and graded exercise tests (Dominguéz et al. 2017; McMahon et al. 2016). These results are more remarkable in active and moderately trained individuals with a $VO_{2max} < 60$ mL kg^{-1} min^{-1} (Carriker et al. 2016; Hoon et al. 2014). However, few studies, reporting controversial results, have investigated the effects of both $NaNO_3^-$ (Bescós et al. 2011; Larsen et al. 2010) and BRJ supplementation (Lansley et al. 2011a, b; Vanhatalo et al. 2010; Bailey et al. 2009) on the VO_{2max} response during exercise. Contrary to our study, both Bescós et al. (2011) and Larsen et al. (2010) observed a reduction in VO_{2max} followed by maintenance or a small increase in time to exhaustion, after a single dose (10 mg kg^{-1} of body mass) 3 h before exercise and chronic dose (0.1 mg kg^{-1} day^{-1}) for 2 days before the test, respectively. In these two studies, authors suggest that there may be two distinct mechanisms involved: one supplement is able to reduce VO_{2max}, and the other is able to improve muscle efficiency and energetic function in the muscles involved (Bescós et al. 2011; Larsen et al. 2010). Similar to our study, Waldron et al. (2018) observed improvements in energy cost of exercise, recovery of VO_2, resting mean arterial pressure, and blood markers after 350 mL of NO_3^--rich supplementation (≅ 20.5 mmol). Vanhatalo et al. (2010) analyzing the effect of acute and chronic effects of BRJ (5.2 mmol NO_3^- day^{-1}) in physically active men found a statistically significant increase in VO_{2max} (≅ 140 mL min^{-1}) after 15 days of chronic supplementation but not after 2.5 h and 5 days.

During exercise, the O_2 and blood flow requirement for active muscles increases significantly in relation to rest (Denadai 1995; Noakes et al. 1990). The increase in blood flow can be attributed to increases in cardiac output, muscle contraction, and vasodilation stimulated by hypoxia and metabolic acidosis, with NO being mainly responsible for this last process (Casey et al. 2015; Amann 2012). The chronic exposure of cells to NO results in cGMP-mediated activation of regulatory protein sirtuin (SIRT1), which upregulates the transcriptional factors and nuclear respiratory factors involved in the coordination of mitochondrial fusion and fission events (Kelly and Scarpulla 2004). It has also recently been shown that BRJ increases the basal oxidative metabolism and is an inducer of metabolic gene expression and mitochondrial biogenesis by elevating metabolic gene expression of peroxisome proliferator-activated receptor gamma coactivator-1 alpha, nuclear respiratory factor 1, mitochondrial transcription factor A, and glucose transporter 4 (Vaughan et al. 2016) to name a few. In addition, two polyphenols found in beetroot, quercetin and resveratrol may increase aerobic capacity through stimulation of mitochondrial biogenesis and antioxidant function (Davis et al. 2009; Lagouge et al. 2006).

Although acute supplementation with BRJ may have an ergogenic effect on reducing VO_2 at less than or equal to VO_{2max} intensity (Dominguéz et al. 2017; Muggeridge et al. 2013), a single dose is not sufficient to induce mitochondrial biogenesis, suggesting that mitochondrial adaptations could only occur after longer supplementation protocols (Dominguéz et al. 2017; Vanhatalo et al. 2010). BRJ may be more beneficial compared to $NaNO_3^-$ and KNO_3^-, with respect to performance of physical exercise or VO_2 (McQuillan et al. 2017; McMahon et al. 2016) since the additional beetroot compounds play an important role in the metabolism of NO_3^- and may facilitate the reduction of NO_2^- to NO in the gut (Wootton-Beard and Ryan 2011; Peri et al. 2005).

In our study, in addition to the improvements observed in VO_{2max}, we also observed a statistically significant improvement in vVO_{2max} values in the BRJ condition. These changes presented the largest effect (ES of 0.55 for VO_{2max} and 0.54 for vVO_{2max}) compared with V_{peak} changes after BRJ condition (ES of 0.17). It has already been showed that NO_3^- can increase VO_{2max} probably by its vasodilatory properties, as well as by its additional action on mitochondrial respiration improving muscle contraction efficiency (Dominguéz et al. 2018; Ferguson et al. 2015). This is a very important result, because vVO_{2max} is a performance variable with good sensitivity for predicting the aerobic performance of middle- and long-distance runners (Buchheit et al. 2010; McLaughlin et al. 2010; Billat and Koralsztein 1996), and is widely used for prescribing and monitoring endurance training with the objective of improving VO_{2max} (Buchheit et al. 2010; Midgley et al. 2007; Laursen and Jenkins 2002). The moderate vVO_{2max} improvement of 0.5 km h^{-1} found in the current study represents an increase of 3.8% (Table 1) after supplementation. This is very important as it is similar to results found in studies with a training protocol. For instance, Manoel et al. (2018) also observed a 0.5-km h^{-1} improvement in vVO_{2max} after 4 weeks of endurance training in moderately trained endurance runners.

In relation, improvements in V_{peak} were found in the BRJ conditions. Since it occurs at intensities higher than

anaerobic threshold (Hill and Rowell 1996), V_{peak} is a variable that jointly represents the capacity of the aerobic and anaerobic systems for energy supply (Noakes et al. 1990). It has already been shown that BRJ, besides positively impacting exhaustion (Dominguéz et al. 2017; McMahon et al. 2016; Hoon et al. 2013), may have a more expressive effect in situations of hypoxia, low pH (Modin et al. 2001), and in activities that have a greater recruitment of type II muscle fibers, improving muscular contraction efficiency (Jones et al. 2016; Ferguson et al. 2013; Hernandéz et al. 2012). Since V_{peak} is a variable that correlates with endurance performance, small changes in V_{peak} could be reflected in the performance (Manoel et al. 2017; Machado et al. 2013).

Although the change in V_{peak} observed in the present study may seem small in a competitive environment in which athletes have very similar levels of performance, this small improvement is considered sufficient to make a difference between competitors (Dominguez et al. 2018; Paton and Hopkins 2006), and in the final result of the performance. This improvement is similar to previous studies that have verified the effect of BRJ on endurance performance (de Castro et al. 2018; Shannon et al. 2017; Wilkerson et al. 2012). Recently, after 15 days of BRJ supplementation in elite middle- and long-distance runners, Balsalobre-Fernández et al. (2018) demonstrated improvements in time to exhaustion. The protocol used consisted of a 10-min warm-up at 10 km h^{-1} followed by three steady-state steps of 3 min each, at 15, 17.1 and 20 km h^{-1}, with an increase of 0.2 km h^{-1} every 12 s until exhaustion after the last steady state step. The athletes who consumed NO_3^--rich BRJ endured more time before voluntarily stopping the incremental treadmill test (Beetroot: 1269 ± 53.6 vs Placebo: 1230 ± 73.5 s). The current study presents similar results, since after 3 days of supplementation the duration of vVO_{2max} and V_{peak} incremental tests to exhaustion were increased. Balsalobre-Fernández et al. (2018) stated that a possible mechanism explaining the increase in time to exhaustion after BRJ supplementation is a moderate increase in O_2 saturation. Thus, the increase in O_2 saturation could have limited the accumulation of fatigue-related metabolites and reduced phosphocreatine (PCr) depletion; thereby increasing time-to-exhaustion.

No differences in the values of HR_{max}, RPE_{max} or $[La_{peak}]$ were found between the supplementation conditions. The absence of change in these variables was expected, as they are routinely used for the identification of physiological responses generated by effort (Hill and Rowell 1996), and they serve as a parameter to identify maximum effort during the incremental test (Fernandes et al. 2006). Additionally, no change was observed in $Gluc_{pre}$ or $Gluc_{post}$ between conditions.

Limitations of the current study include the lack of a measurement of circulating plasma NO_3^- or NO_2^- concentrations, which could augment the interpretation of the effects observed when nitrate is supplemented. Since increases in performance variables after chronic BRJ supplementation were observed, it may be worthwhile for individuals to test these nutritional ergogenic aids to improve performance, which in certain competitive situations could be meaningful.

Conclusion

In conclusion, the once daily consumption of BRJ rich in natura NO_3^- (8.4 mmol NO_3^- day^{-1}) for 3 days improved VO_{2max}, vVO_{2max}, and V_{peak} in recreational runners without changing the other analyzed variables. Since these are important variables for the prediction, monitoring, and prescription of endurance training, recreational runners may benefit from the effects of BRJ if they add this supplement to their dietary routine.

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Compliance with ethical standards

Conflict of interest The author(s) declare that they have no conflict of interest.

References

Almarwaey OA, Jones AM, Tolfrey K (2004) Maximal lactate steady state in trained adolescent runners. J Sports Sci 22:215–225. https://doi.org/10.1080/02640410310001641520

Amann M (2012) Pulmonary system limitations to endurance exercise performance in humans. Exp Physiol 97:311–318. https://doi.org/10.1113/expphysiol.2011.058800

Bailey SJ, Winyard P, Vanhatalo A, Blackwell JR, DiMenna FJ, Wilkerson DP, Tarr J, Benjamin N, Jones AM (2009) Dietary nitrate supplementation reduces the O_2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. J Appl Physiol 107:1144–1155. https://doi.org/10.1152/japplphysiol.00722.2009

Bailey SJ, Fulford J, Vanhatalo A, Winyard PG, Blackwell JR, DiMenna FJ, Wilkerson DP, Benjamin N, Jones AM (2010) Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans. J Appl Physiol 109:135–148. https://doi.org/10.1152/japplphysiol.00046.2010

Balsalobre-Fernández C, Romero-Moraleda B, Cupeiro R, Peinado AB, Butragueño J, Benito PJ (2018) The effects of beetroot juice supplementation on exercise economy, rating of perceived exertion and running mechanics in elite distance runners: a double-blinded, randomized study. PloS one 13:e0200517. https://doi.org/10.1371/journal.pone.0200517

Bassett DR, Howley ET (2000) Limiting factors for maximum oxygen uptake and determinants of endurance performance. Med Sci Sports Exerc 32:70–84

Basu S, Azarova NA, Font MD, King SB, Hogg N, Gladwin MT, Shiva S, Kim-Shapiro DB (2008) Nitrite reductase activity of cytochrome c. J Biol Chem 47:32590–32597. https://doi.org/10.1074/jbc.M806934200

Bescós R, Rodríguez FA, Iglesias X, Ferrer MD, Iborra E, Pons A (2011) Acute administration of inorganic nitrate reduces VO_2(peak) in endurance athletes. Med Sci Sports Exerc 43:1979–1986. https://doi.org/10.1249/MSS.0b013e318217d439

Betteridge S, Bescós R, Martorell M, Pons A, Garnham AP, Stathis CC, McConell GK (2016) No effect of acute beetroot juice ingestion on oxygen consumption, glucose kinetics, or skeletal muscle metabolism during submaximal exercise in males. J Appl Physiol 120:391–398. https://doi.org/10.1152/japplphysiol.00658.2015

Billat LV, Koralsztein JP (1996) Significance of the velocity at VO_{2max} and time to exhaustion at this velocity. Sports Med 22:90–108

Billat V, Renoux JC, Pinoteau J, Petit B, Koralsztein JP (1994) Reproducibility of running time to exhaustion at VO_{2max} in subelite runners. Med Sci Sports Exerc 26:254–257

Billat VL, Hill DW, Pinoteau J, Petit B, Koralsztein JP (1996) Effect of protocol on determination of velocity at VO_{2max} and on its time to exhaustion. Arch Physiol Biochem 104:313–321. https://doi.org/10.1076/apab.104.3.313.12908

Billat VL, Flechet B, Petit B, Muriaux G, Koralsztein JP (1999) Interval training at VO_{2max}: effects on aerobic performance and overtraining markers. Med Sci Sports Exerc 31:156–163. https://doi.org/10.1097/00005768-199901000-00024

Borg GA (1982) Psychophysical bases of perceived exertion. Med Sci Sports Exerc 14:377–381

Brown GC (2001) Regulation of mitochondrial respiration by nitric oxide inhibition of cytochrome c oxidase. Biochim Biophys Acta 1504:46–57. https://doi.org/10.1016/S0005-2728(00)00238-3

Brown GC, Cooper CE (1994) Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase. FEBS Lett 356:295–298. https://doi.org/10.1016/0014-5793(94)01290-3

Buchheit M, Chivot A, Parouty J, Mercier D, Al Haddad H, Laursen PB, Ahmaidi S (2010) Monitoring endurance running performance using cardiac parasympathetic function. Eur J Appl Physiol 108:1153–1167. https://doi.org/10.1007/s00421-009-1317-x

Buzzard M (1998) 24-hours dietary recall and food record methods. In: Willett WC Nutritional epidemiology, 2 edn. Oxford University Press, Oxford, pp 50–73

Carriker CR, Vaughan RA, VanDusseldorp TA, Johnson KE, Beltz NM, McCormick JJ, Cole NH, Gibson AL (2016) Nitrate-containining beetroot juice reduces oxygen consumption during submaximal exercise in low but not high aerobically fit male runners. J Exerc Nutrition Biochem 20:27–34

Casey DP, Treichler DP, Ganger CT, Schneider AC, Ueda K (2015) Acute dietary nitrate supplementation enhances compensatory vasodilation during hypoxic exercise in older adults. J Appl Physiol 118:178–186. https://doi.org/10.1152/japplphysiol.00662.2014

Cleeter MWJ, Cooper JM, Darley-Usmar VM, Moncada SA, Schapira AHV (1994) Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. FEBS Lett 345:50–54. https://doi.org/10.1016/0014-5793(94)00424-2

Cohen J (1988) Statistical power analysis for the behavioral sciences. Lawrence Erlbaum, Hillsdale

da Silva DF, Simões HG, Machado FA (2015) vVO_{2max} versus V_{peak}, what is the best predictor of running performances in middle-aged recreationally-trained runners? Sci Sports 30:e85–e92. https://doi.org/10.1016/j.scispo.2014.10.006

Davis JM, Murphy EA, Carmichael MD, Davis B (2009) Quercetin increases brain and muscle mitochondrial biogenesis and exercise tolerance. Am J Physiol Regul Integr Comp Physiol 296:R1071–R1077. https://doi.org/10.1152/ajpregu.90925.2008

Da Silva DF, Ferraro ZM, Adamo KB, Machado FA (2017) Endurance training individually guided by HRV in untrained women. J Strength Con Res. https://doi.org/10.1519/JSC.0000000000002001

de Castro TF, Manoel FA, Figueiredo DH, Figueiredo DH, Machado FA (2018) Effect of beetroot juice supplementation on 10-km performance in recreational runners. Appl Physiol Nutr Metab. https://doi.org/10.1139/apnm-2018-0277

Denadai BS (1995) Consumo máximo de oxigênio: fatores determinantes e limitantes. Revista Brasileira de Atividade Física Saúde 1:85–94. https://doi.org/10.12820/rbafs.v.1n1p85-94

Denadai BS (1999) Índices fisiológicos de avaliação aeróbia: conceitos e aplicações, 4. BSD, Ribeirão Preto

Domínguez R, Cuenca E, Maté-Muñoz JL, García-Fernández P, Serra-Paya N, Estevan MCL, Garnacho-Castaño MV (2017) Effects of beetroot juice supplementation on cardiorespiratory endurance in athletes. A systematic review. Nutrients 9:43. https://doi.org/10.3390/nu9010043

Domínguez R, Maté-Muñoz JL, Cuenca E, García-Fernández P, Mata-Ordoñez F, Lozano-Estevan MC, Veiga-Herreros P, da Silva SF, Garnacho-Castaño MV (2018) Effects of beetroot juice supplementation on intermittent high-intensity exercise efforts. J Int Soc Sports Nutr. https://doi.org/10.1186/s12970-017-0204-9

Edvardsen E, Hem E, Anderssen SA (2014) End criteria for reaching maximal oxygen uptake must be strict and adjusted to sex and age: a cross-sectional study. PloS one 9:e85276. https://doi.org/10.1371/journal.pone.0085276

Ferguson SK, Hirai DM, Copp SW, Holdsworth CT, Allen JD, Jones AM, Musch TI, Poole DC (2013) Impact of dietary nitrate supplementation via beetroot juice on exercising muscle vascular control in rats. J Physiol 591:547–557. https://doi.org/10.1113/jphysiol.2012.243121

Ferguson SK, Holdsworth CT, Wright JL, Fees AJ, Allen JD, Jones AM, Musch TI, Poole DC (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

Fernandes RJ, Billat VL, Cruz AC, Colaço PJ, Cardoso CS, Vilas-Boas JP (2006) Does net energy cost of swimming affect time to exhaustion at the individual's maximal oxygen consumption velocity? J Sports Med Phys Fitness 46:373–380

Francis SH, Busch JL, Corbin JD (2010) cGMP-dependent protein kinases and cGMP phosphodiesterases in nitric oxide and cGMP action. Pharmacol Rev 62:525–563. https://doi.org/10.1124/pr.110.002907

Gibson RS (1990) Principles of nutritional assessment. Oxford University Press. Food consumption of individuals, Oxford, pp 37–54

Giulivi C (2003) Characterization and function of mitochondrial nitric-oxide synthase. Free Radic Biol Med 34:397–408. https://doi.org/10.1016/S0891-5849(02)01298-4

Govoni M, Jansson EA, Weitzberg E, Lundberg JO (2008) The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash. Nitric Oxide Biol Chem Off J Nitric Oxide Soc 19:333–337. https://doi.org/10.1016/j.niox.2008.08.003

Hernandez A, Schiffer TA, Ivarsson N, Cheng AJ, Bruton JD, Lundberg JO, Weitzberg E, Westerblad H (2012) Dietary nitrate increases tetanic [Ca2+]i and contractile force in mouse fast-twitch muscle. J Physiol 590:3575–3583. https://doi.org/10.1113/jphysiol.2012.232777

Heunks LM, Cody MJ, Geiger PC, Dekhuijzen PR, Sieck GC (2001) Nitric oxide impairs Ca_2^+ activation and slows cross-bridge

cycling kinetics in skeletal muscle. J Appl Physiol (1985) 91:2233–2239. https://doi.org/10.1152/jappl.2001.91.5.2233
Hill DW, Rowell AL (1996) Running velocity at VO_{2max}. Med Sci Sport Exerc 28:114–119. https://doi.org/10.1097/00005768-199601000-00022
Holloszy JO, Coyle EF (1984) Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. J Appl Physiol Respir Environ Exerc Physio 56:831–838. https://doi.org/10.1152/jappl.1984.56.4.831
Hoon MW, Johnson NA, Chapman PG, Burke LM (2013) The effect of nitrate supplementation on exercise performance in healthy individuals: a systematic review and meta-analysis. Int J Sport Nutr Exerc Metab 23:522–532. https://doi.org/10.1123/IJSPP.2013-0207
Hoon MW, Hopkins WG, Jones AM, Martin DT, Halson SL, West NP, Johnson NA, Burke LM (2014) Nitrate supplementation and high-intensity performance in competitive cyclists. Appl Physiol Nutr Metab 39:1043–1049
Hord NG, Tang Y, Bryan NS (2009) Food sources of nitrates and nitrites: the physiologic context for potential health benefits. Am J Clin Nutr 90:1–10. https://doi.org/10.3945/ajcn.2008.27131
Jones AM, Doust JH (1996) A 1% treadmill grade most accurately reflects the energetic cost of outdoor running. J Sports Sci 14:321–327. https://doi.org/10.1080/02640419608727717
Jones AM, Ferguson SK, Bailey SJ, Vanhatalo A, Poole DC (2016) Fiber-type specific effects of dietary nitrate. Exerc Sport Sci Rev. https://doi.org/10.1249/JES.0000000000000074
Jonvik KL, Nyakayiru J, Van Dijk JW, Maase K, Ballak SB, Senden JMG, Verdijk LB (2018) Repeated-sprint performance and plasma responses following beetroot juice supplementation do not differ between recreational, competitive and elite sprint athletes. Eur J Sport Sci 18:524–533. https://doi.org/10.1080/17461391.2018.1433722
Joyner MJ, Coyle EF (2008) Endurance exercise performance: the physiology of champions. J Physiol 586:35–44. https://doi.org/10.1113/jphysiol.2007.143834
Kelly DP, Scarpulla RC (2004) Transcriptional regulatory circuits controlling mitochondrial biogenesis and function. Genes Dev 18:357–368. https://doi.org/10.1101/gad.1177604
Kuipers H, Rietjens GJWM, Verstappen F, Schoenmakers H, Hofman G (2003) Effects of stage duration in incremental running tests on physiological variables. Int J Sports Med 24:486–491. https://doi.org/10.1055/s-2003-42020
Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Geny B (2006) Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1α. Cell 127:109–1122. https://doi.org/10.1016/j.cell.2006.11.013
Lansley KE, Winyard PG, Fulford J, Vanhatalo A, Bailey SJ, Blackwell JR, DiMenna FJ, Gilchrist M, Benjamin N, Jones AM (2011a) Dietary nitrate supplementation reduces the O_2 cost of walking and running: a placebo-controlled study. J Appl Physiol 110:591–600. https://doi.org/10.1152/japplphysiol.01070.2010
Lansley KE, Winyard PG, Bailey SJ, Vanhatalo A, Wilkerson DP, Blackwell JR, Gilchrist M, Benjamin N, Jones AM (2011b) Acute dietary nitrate supplementation improves cycling time trial performance. Med Sci Sports Exerc 43:1125–1131. https://doi.org/10.1249/MSS.0b013e31821597b4
Larsen FJ, Weitzberg E, Lundberg JO, Ekblom B (2007) Effects of dietary nitrate on oxygen cost during exercise. Acta Physiol 191:59–66. https://doi.org/10.1111/j.1748-1716.2007.01713.x
Larsen FJ, Weitzberg E, Lundberg JO, Ekblom B (2010) Dietary nitrate reduces maximal oxygen consumption while maintaining work performance in maximal exercise. Free Radic Biol Med 48:342–347. https://doi.org/10.1016/j.freeradbiomed.2009.11.006
Larsen FJ, Schiffer TA, Borniquel S, Sahlin K, Ekblom B, Lundberg JO, Weitzberg E (2011) Dietary inorganic nitrate improves mitochondrial efficiency in humans. Cell Metab 13:149–159. https://doi.org/10.1016/j.cmet.2011.01.004
Laursen PB, Jenkins DG (2002) The scientific basis for high-intensity interval training. Sports Med 32:53–73. https://doi.org/10.2165/00007256-200232010-00003
Machado FA, Kravchychyn ACP, Peserico CS, da Silva DF, Mezzaroba PV (2013) Incremental test design, peak 'aerobic'running speed and endurance performance in runners. J Sci Med Sport 16:577–582. https://doi.org/10.1016/j.jsams.2012.12.009
Manoel FA, da Silva DF, de Lima JRP, Machado FA (2017) Peak velocity and its time limit are as good as the velocity associated with VO_{2max} for training prescription in runners. Sports Med Int Open 1:E8–E15. https://doi.org/10.1055/s-0042-119951
Manoel FA, Figueiredo DH, Machado FA (2018) Can the endurance training change the pacing strategy during 10 km running performance? Int J Perform Anal Sport 18:127–136. https://doi.org/10.1080/24748668.2018.1454744
McLaughlin JE, Howley ET, Bassett JD, Thompson DL, Fitzhugh EC (2010) Test of the classic model for predicting endurance running performance. Med Sci Sports Exerc 42:991–997. https://doi.org/10.1249/MSS.0b013e3181c0669d
McMahon NF, Leveritt MD, Pavey TG (2016) The effect of dietary nitrate supplementation on endurance exercise performance in healthy adults: a systematic review and meta-analysis. Sports Med. https://doi.org/10.1007/s40279-016-0617-7
McQuillan JA, Dulson DK, Laursen PB, Kilding AE (2017) Dietary nitrate fails to improve 1 and 4 km cycling performance in highly trained cyclists. Int J Sport Nutr Exerc Metab 3:255–263
Midgley AW, McNaughton LR, Jones AM (2007) Training to enhance the physiological determinants of long-distance running performance. Sports Med 37:857–880. https://doi.org/10.2165/00007256-200737100-00003
Modin A, Björne H, Herulf M, Alving K, Weitzberg E, Lundberg JO (2001) Nitrite-derived nitric oxide: a possible mediator of "acidic-metabolic" vasodilation. Acta Physiol Scand 171:9–16. https://doi.org/10.1046/j.1365-201X.2001.00771.x
Moncada S, Higgs A (1993) The L-arginine-nitric oxide pathway. N Engl J Med 329:2002–2012. https://doi.org/10.1056/NEJM199312303292706
Muggeridge DJ, Christopher CFH, Spendiff O, Pedlar C, James PE, Easton C (2013) The effects of a single dose of concentrated beetroot juice on performance in trained flatwater kayakers. Int J Sport Nutri Exerc Metab 23:498–506
Noakes TD, Myburgh KH, Schall R (1990) Peak treadmill running velocity during the VO_{2max} test predicts running performance. J Sports Sci 8:35–45. https://doi.org/10.1080/02640419008732129
Nyakayiru JM, Jonvik KL, Pinckaers PJ, Senden J, van Loon LJ, Verdijk LB (2017) No effect of acute and 6-day nitrate supplementation on Vo2 and time-trial performance in highly trained cyclists. Int J Sport Nutr Exerc Metab 27:11–17. https://doi.org/10.1123/ijsnem.2016-0034
Paton CD, Hopkins WG (2006) Variation in performance of elite cyclists from race to race. Eur J Sport Sci 6:25–31. https://doi.org/10.1080/17461390500422796
Peri L, Pietraforte D, Scorza G, Napolitano A, Fogliano V, Minetti M (2005) Apples increase nitric oxide production by human saliva at the acidic pH of the stomach: a new biological function for polyphenols with a catechol group? Free Radic Biol Med 39:668–681
Rowland TW (1996) Developmental exercise physiology. Human Kinetics Books, Champaign
Saltin B, Strange S (1992) Maximal oxygen uptake: "old" and "new" arguments for a cardiovascular limitation. Med Sci Sports Exerc 24:30–37

Saunders PU, Cox AJ, Hopkins WG, Pyne DB (2010) Physiological measures tracking seasonal changes in peak running speed. Int J Sports Physiol Perform 5:230–238. https://doi.org/10.1123/ijspp.5.2.230

Shannon OM, Duckworth L, Barlow MJ et al (2017) Effects of dietary nitrate supplementation on physiological responses, cognitive function, and exercise performance at moderate and very-high simulated altitude. Front Physiol 8:401. https://doi.org/10.3389/fphys.2017.00401

Shanon OM, Barlow MJ, Duckworth L, Willians E, Wort D, Woods D, Seirvo M, O'Hara JP (2017) Dietary nitrate supplementation enhances short but not longer duration running time-trial performance. Eur J Appl Physiol 117:775–785. https://doi.org/10.1007/s00421-017-3580-6

Smith TP, Coombes JS, Geraghty DP (2003) Optimising high-intensity treadmill training using the running speed at maximal O_2 uptake and the time for which this can be maintained. Eur J Appl Physiol 89:337–343. https://doi.org/10.1007/s00421-003-0806-6

Tanaka H, Monahan KD, Seals DR (2001) Age-predicted maximal heart rate revisited. J Am Coll Cardiol 37:153–156. https://doi.org/10.1016/S0735-1097(00)01054-8

Tengan CH, Moraes CT (2017) NO control of mitochondrial function in normal and transformed cells. Biochim Biophys Acta 1858:573–581. https://doi.org/10.1016/j.bbabio.2017.02.009

Thompson C, Wylie LJ, Fulford J, Kelly J, Black MI, McDonagh ST, Jeukendrup AE, Vanhatalo A, Jones AM (2015) Dietary nitrate improves sprint performance and cognitive function during prolonged intermittent exercise. Eur J Appl Physio 115:1825–1834. https://doi.org/10.1007/s00421-015-3166-0

Thompson C, Wylie LJ, Blackwell JR, Fulford J, Black MI, Kelly J, McDonagh ST, Carter J, Bailey SJ, Vanhatalo A, Jones AM (2017) Influence of dietary nitrate supplementation on physiological and muscle metabolic adaptations to sprint interval training. J Appl Physiol 122:642–652. https://doi.org/10.1152/japplphysiol.00909.2016

Tong L, Heim RA, Wu S (2011) Nitric oxide: a regulator of eukaryotic initiation factor 2 kinases. Free Radic Biol Med 50:1717–1725. https://doi.org/10.1016/j.freeradbiomed.2011.03.032

Vanhatalo A, Bailey SJ, Blackwell JR, DiMenna FJ, Pavey TG, Wilkerson DP, Benjamin N, Winyard PG, Jones AM (2010) Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate-intensity and incremental exercise. AJP Regul Integr Comp Physiol 299:R1121–R1131. https://doi.org/10.1152/ajpregu.00206.2010

Vanhatalo A, Fulford J, Bailey SJ, Blackwell JR, Winyard PG, Jones AM (2011) Dietary nitrate reduces muscle metabolic perturbation and improves exercise tolerance in hypoxia. J Physiol 589:5517–5528. https://doi.org/10.1113/jphysiol.2011.216341

Vanni DS, Horstmann B, Benjo AM, Daher JPL, Kanaan S, Sleiman M (2007) Óxido nitrico: inibição das plaquetas e participação na formação do trombo. J Bras Patol Med Lab 43:181–189

Vaughan RA, Gannon NP, Carriker CR (2016) Nitrate-containing beetroot enhances myocyte metabolism and mitochondrial content. J Tradit Complement Med 6:17–22. https://doi.org/10.1016/j.jtcme.2014.11.033

Waldron M, Waldron L, Lawlor C, Gray A, Highton J (2018) Beetroot supplementation improves the physiological responses to incline walking. Eur J Appl Physiol 118:1131–1141. https://doi.org/10.1007/s00421-018-3843-x

Whitfield J, Ludzki A, Heigenhauser GJF, Senden JM, Verdijk LB, van Loon LJ, Spriet LL, Holloway GP (2016) Beetroot juice supplementation reduces whole body oxygen consumption but does not improve indices of mitochondrial efficiency in human skeletal muscle. J Physiol 594:421–435. https://doi.org/10.1113/JP270844

Wilkerson DP, Hayward GM, Bailey SJ, Vanhatalo A, Blackwell JR, Jones AM (2012) Influence of acute dietary nitrate supplementation on 50 mile time trial performance in well-trained cyclists. Eur J Appl Physiol 112:4127–4134. https://doi.org/10.1007/s00421-012-2397-6

Wootton-Beard PC, Ryan L (2011) A beetroot juice shot is a significant and convenient source of bioaccessible antioxidants. J Funct Foods 3:329–334

Wylie LJ, Kelly J, Bailey SJ, Blackwell JR, Skiba PF, Winyard PG, Jeukendrup AE, Vanhatalo A, Jones AM (2013) Beetroot juice and exercise: Pharmacodynamic and dose-response relationships. J Appl Physiol 115:325–336. https://doi.org/10.1152/japplphysiol.00372.2013