OSJ OPEN SCIENCE JOURNAL

RESEARCH ARTICLE

Effects of Beet Juice Supplementation in Different Concentrations and the Importance of Nitric Oxide in Endurance Runners

Richard Danilo Serrano[1*], William Fioravante Victor[1], Nailza Maesta[1], Rodrigo Elias Diniz[1], Laurita Maluf Morelato[1], Isabela Souza Campos[2], Raphael dos Santos Canciglieri[1,3]

[1]Methodist University of Piracicaba, Brazil
[2]Technical nurse at the city of Piracicaba, Brazil
[3]State University of Campinas, Brazil

*Corresponding author: Richard Danilo Serrano: niloserrano@hotmail.com

OPEN ACCESS

Citation: Serrano R.D., Victor W.F., Maesta N., Diniz R.E., Morelato L.M., Campos I.S., Cancriglieri R.S..(2023) Effects of Beet Juice Supplementation in Different Concentrations and the Importance of Nitric Oxide in Endurance Runners. Open Science Journal 8(2)

Received: 8th March 2022

Accepted: 10th July 2022

Published: 23rd August 2023

Funding: The author(s) received no specific funding for this work

Competing Interests: The authors have declared that no competing interests exists.

Abstract:

More and more beetroot has been highlighted in sports, mainly in long-term aerobic modalities, due to its high concentration of nitrate (NO_3-), being an important precursor of nitric oxide (NO), providing an improvement in sports performance. The main objective of this study was to investigate the effects of acute ingestion of beetroot juice (BR), rich in NO_3- in different concentrations in endurance exercise, evaluating performance, glycemic, cardiorespiratory and urinary responses of amateur runners. We hypothesized that BR would increase glucose uptake, would minimize the maximum oxygen uptake (VO_{2max}), improve exercise economy and runners' performance. This is quantitative cross-sectional study with nutritional intervention, randomized Nineteen male amateur runners were exposed to two conditions, ingestion of BR and Placebo with carbohydrate isomaltulose Palatinose (PL), the individuals were divided into three groups (minimum, average and maximum concentration), being submitted to a 30-minute running test at maximum intensity, on a racetrack. Pre and posttest blood and urine samples were collected to determine glucose uptake, NO_2- (nitrite) excretion, urinary pH and urinary density. Cooper's test also performed in order to assess VO_{2max} (mL kg^{-1} min^{-1}). The main posttest results showed that through nitrite excretion there was a reduction of nitrate to nitric oxide (0 ± 0 vs 0.94 ± 0.23, $P < 0.05$ (pre vs post)), increase in glucose uptake (139.94 ± 35.02 vs 122.88 ± 37.69, $P < 0.05$ (PL vs BR)) and VO_{2max} improvement (54.96 ± 6.87 vs 55.99 ± 6.88, $P < 0.05$ (PL vs BR))], coinciding with the increase in physical performance (2972.63 ± 308.84 vs 3018.95 ± 309.29, P <0.05 (PL vs BR))]. The results found observed that beet supplementation in amateur runners increased glucose uptake, improved VO_{2max} and running performance.

Keywords: Beet juice, Nitrate, Nitrite, Nitric oxide, Runners.

Introduction

Physical resistance is a condition that is directly linked to the sports performance of the athlete, however, there are physiological factors of the exercise that can interfere, such as the maximum oxygen uptake (VO_{2max}) by the muscles, ventilatory thresholds and physical conditioning that provides the economy energy [1,2]. However, nutritional strategies before, during and after training and competitions will impact physical performance and, as supporting to adjusted nutrition, the use of supplements and ergogenic compounds has become present, in order to optimize performance for sporting events [3].
According to the Australian Institute of Sport (AIS), among the supplements with greater scientific evidence and practical considerations on sports performance are caffeine, sodium bicarbonate, creatine, beta-alanine and nitrate (NO_3-). Particularly Nitrate is an anion compound, highly soluble in water. It can be found in large amounts in vegetables such as spinach, arugula, celery and "beetroot" [5,6]. Despite the high adherence to the use of industrialized products such as nutritional supplements, currently beet juice (BR) has been standing out among athletes of long-term aerobic modalities, due to its high NO_{3-} present in the beet. After BR ingestion, NO_{3-} will be reduced to nitrite (NO_{2-}) through the action of the oral microbiota and subsequently to nitric oxide (NO) in the stomach, but a portion ends up going to the systemic circulation [7,8,9]. In the muscle, there is also a reduction of NO_{2-} to NO, due to the conditions of hypoxia and low pH present during physical exercise [10].
Endogenous NO synthesis occurs through NO synthases (NOS), which are expressed in 3 isoforms in skeletal muscle, neuronal (nNOS), inducible (iNOS) and endothelial (eNOS) [11,12]. The three isoforms of NOS have L-arginine as substrate, oxygen and nicotinamide adenine dinucleotide phosphate (NADPH) as co-substrates, flavin adenine dinucleotide (FAD), and flavin mononucleotide (FMN) as enzyme cofactors. NOS are homodimers, which in the presence of a heme group come together to form a functional dimer (functional NOS), which conducts electron transfer via FAD and FMN to the heme site to oxidize L-arginine to L-citrulline and NO [13]. NO synthesis takes place in two stages: first, NOS hydroxylates L-arginine into Nω-hydroxy-L-arginine (this remains bound to the enzyme), then NOS oxidizes Nω-hydroxy-L-arginine into L-citrulline and NO [14,15,16,17].
Through the guanylate cyclase receptor, NO aims to promote vasodilation of muscle fibers, increasing blood flow and thus improving the delivery of O_2 and nutrients to the muscles [18,19,20,2,21]. In trained and active individuals (VO_{2max} <60mL/min/kg) there is a better use of the effects of NO, as when exposed to

submaximal exercise sessions, it results in a reduction in VO_2 (oxygen uptake) levels and an increase in the time until exhaustion [1,22,23,24,25].

Is ingestion of beetroot juice at ideal concentrations to provide nitrate and promote vasodilation in athletes so positive for minimizing the use of doping substances, such as erythropoietin, for example?

However, highly trained elite athletes (VO_{2max} >70mL/min/kg) do not seem to respond to the effects of VO_2 reduction and exercise economy [10,26,7,27], or show little response [28,29], this can be explained due to the high endogenous concentration of NO from the diet and adaptations generated by physical exercise, contributing to greater activity of the NOS pathway [30,31].

Given these statements, the present study aims to investigate the effects of acute NO3- ingestion at different concentrations in endurance exercise, analyzing performance, glycemic, cardiorespiratory and urinary responses of amateur runners. We hypothesized that BR would increase glucose uptake, minimize VO_{2max}, thus improving exercise economy and runner's performance.

Materials and methods

Experimental study approach

This is a quantitative cross-sectional study with nutritional intervention, randomized, with its sample selected for convenience. The study included 19 male amateur runners aged between 29 and 69 (table 1), with a minimum of 12 months of training. The study was approved by the Research Ethics Committee of the Methodist University of Piracicaba (Unimep), under CAAE: 54051721.0.0000.5507, on 12/03/2021.

As inclusion criteria, those who practice long distance running, male, without intolerances to beets or any other food source of nitrate and without metabolic compromises. Those who met these criteria and agreed to participate signed an informed consent form.

Participants were randomly divided into three groups (G), low beet concentration group (G1), medium concentration group (G2) and high concentration group (G3), using a randomization tool as a criterion (https://www.random.org). Both groups were submitted to the same test protocol, which consisted of 30 minutes of running, on the track on the campus of the Methodist University of Piracicaba.

Table 1. Physical characteristics, age and training intensity in male runners

Features	we
Age (years)	33.8 ± 9.91
Height (m)	1.74 ± 0.05
Weight (kg)	74.70 ± 10.64
BMI (kg/m2)	24.5 ± 3.30
Fat Free Mass (kg)	64.81 ± 6.14
Fat (%)	12.72 ± 5.55
Muscle Mass (kg)	32.23 ± 3.48

MMI (kg/m^2)	10.56 ± 1.21
Pace (min/km)	05:17 ± 0.03

BMI: Body Mass Index; MMI: Muscle Mass Index

Procedures

Participants were submitted to an anamnesis about food, disease history, hydration, use of medication, supplementation and consumption of nitrate-rich foods. Used to characterize and fulfill the inclusion criterion. Subsequently, the runners were subjected to a urine collection, all carrying an individual collector, being instructed to discard the first jet and then collect the sample. For urine collection, 15mL centrifuge tubes (falcon type) were used, from supplier Corning ® 430790, RCF12500xg disposable graduations 1.5 - 14.5 mL self-standing: no.

Subsequently, blood samples, blood pressure (BP), oxygen saturation (SpO2) in the blood and blood glucose. Blood samples were collected with scalp 23 needles Scalp 23 MEDIX BRASIL SCALP (Stainless steel needle; Medical grade flexible and transparent PVC needle hub; ABS needle handle; Medical grade polyethylene protector; Flexible and transparent grade PVC tube), PVC tubes of approximately 30 cm, pvc tubes of approximately 30 centimeters and stored in vacuum tubes with EDTA K3 anticoagulant - DESCARPACK. For BP collection, a manual sphygmomanometer (Durashock DS44-BR Welch Allyn) was used, accurate from 0 to 300 mmHg, oxygen saturation was measured using a digital fingertip - Ox520 Incoterm, measurement range and pulse rate from 30 to 250 bpm, 1 bpm resolution, ± 2 bpm accuracy. Glycemia was measured using the AccuCheck – model Active glucometer, with 5 second analysis time, storage of 500 results and capacity of 1 µL of blood.

Then, to determine the physical characteristics of the sample (pre-test), measurements of body composition were performed, such as height, total body mass, fat-free mass and fat mass. Height was measured using a portable Alturexata® stadiometer, precision of 1 mm. Body mass was verified using a Welmy® mechanical scale, precision of 100 g. From these parameters, the body mass index (BMI) in kg/m^2 was calculated [32]. To determine the free fat mass and fat mass, a single experienced and previously trained evaluator used a Lange Skinfold Caliper adipometer, with a scale from 0 to 60 mm and a resolution of 1 mm. Based on the data obtained from skinfolds, the 3-fold protocol by Jackson and Pollock (1978) [33] was used to establish the density value and subsequent calculation of the percentage of body fat [34], and free fat mass by subtraction (table 1).

The amount of body water was quantified by the bioelectric impedance method (BIA - Biodinâmics ®, model 310e, USA), this method consists of a low-level electric current (50 kHz) (500 µA to 800 µA) that runs through the individual's body, considering absorption (reactance) and non-absorption (resistance), by body tissues, given in ohms. With this measurement, it was possible to obtain the amount of body water [35]. Water and body electrolytes, present in lean tissues, are excellent conductors of electric current, unlike fat tissue contains a low concentration of water, therefore, resistance to current flow is greater in individuals with a large amount of body fat. With four electrodes from supplier Sanny® SNYBIO100BRD, placed on the hand, wrist, foot and ankle, an electrical current of 500 to 800 μA is applied to the distal electrodes on the hand and foot and the voltage drop is detected by the proximal electrodes on the wrist and ankle [36].

To carry out the test, all participants were instructed to drink 2 liters of water the day before the body assessment, not to perform physical exercise 24 hours before, not to consume caffeine, chocolate, tea and alcoholic beverages 48 hours before, in Fasting for at least 4 hours and emptying the bladder.

To classify the percentage of fat obtained by the 3-fold protocol, 12 to 21% as a reference for males [37]. Used the equation to calculate muscle mass (MM(kg)) proposed by Lee et al (2000) [38]. From the result of muscle mass (kg), these individuals were classified by the muscle mass index (BMI), with the equation BMI ((kg/m^2) = MM (kg)/height^2 (table 1) [39].

Study design and experimental test

The runners were separated into three groups, both groups received carbohydrate isomaltulose Palatinose (PL) as control and beetroot juice (BR) as treatment. Between the two drinks, there was an interval of seven days. On the first day of the test, G1 (n=6), G2 (n=7) and G3 (n=6) ingested carbohydrate isomaltulose Palatinose at concentrations of 9g (G1), 22g (G2) and 40g (G3), diluted in 200 ml of water without flavor. On the second of the test (7 days after the first test), the runners ingested beetroot juice BR at concentrations: G1(140g (5.4 mmoL NO_{3-})), G2 (328g (12.8 mmoL NO_{3-})) and G3 (600g (23.3 mmol NO_{3-})) (Table 2), all concentrations were prepared and separated into individual bottles containing the same volume of liquid, being stored at room temperature. The amounts of beet were according to the concentration of carbohydrates, that is, 140g of beet offers 9g of CHO, 328g contains 22g of CHO and 600g with 40g of CHO, amounts similar to the isomaltulose Palatonose groups.

Table 2. Quantity and dilution of beet in the respective groups of runners

Group	Beetroot (g)	Juice (g)	Water (g)	Total (g)
G1	140	122	388	510
G2	330	299	211	510
G3	610	288	222	510

G1: 140g of beetroot with 9g of CHO; G2: 328g with 22g of CHO; G3: 600g with 40g of CHO

On the first day, both groups waited 20 minutes to start the test, on the second day, the groups started running 2 hours after BR ingestion, as scientific evidence demonstrates that the peak of NO_{2-} in the current blood pressure occurs 2 hours after BR ingestion (40). For the second day, the subjects were instructed not to use oral antiseptic, antibacterial toothpaste and chewing gum 24 hours before the study, due to the effects of oral antiseptics on oral bacteria, thus being able to attenuate the increases in NO_{2-} after ingestion of NO_{3-} [41].

On both days the individuals were submitted to a 30-minute running test on a 400-meter track with markings every 20 meters, the first 12 minutes all ran at the maximum of their physical capacity (to determine VO_{2max}) and 18 minutes at a moderate cadence. When the timer reached 12 minutes, a whistle sounded, so the inspector in charge would observe which marking the runner was on, this condition was made so that the maximum distance covered in that time was calculated and later the calculation of VO_{2max}, calculation performed by the test by Cooper (1968). After this short stop, the individuals completed the test with 18 minutes of running, in order to observe the total distance in 30 minutes of test. At the end of the test, the runners answered a questionnaire regarding the degree of perceived exertion (Borg scale) [43]. After the running test, the participants again collected urine, body weight, blood, BP, SpO2, blood glucose and bioimpedance analysis.

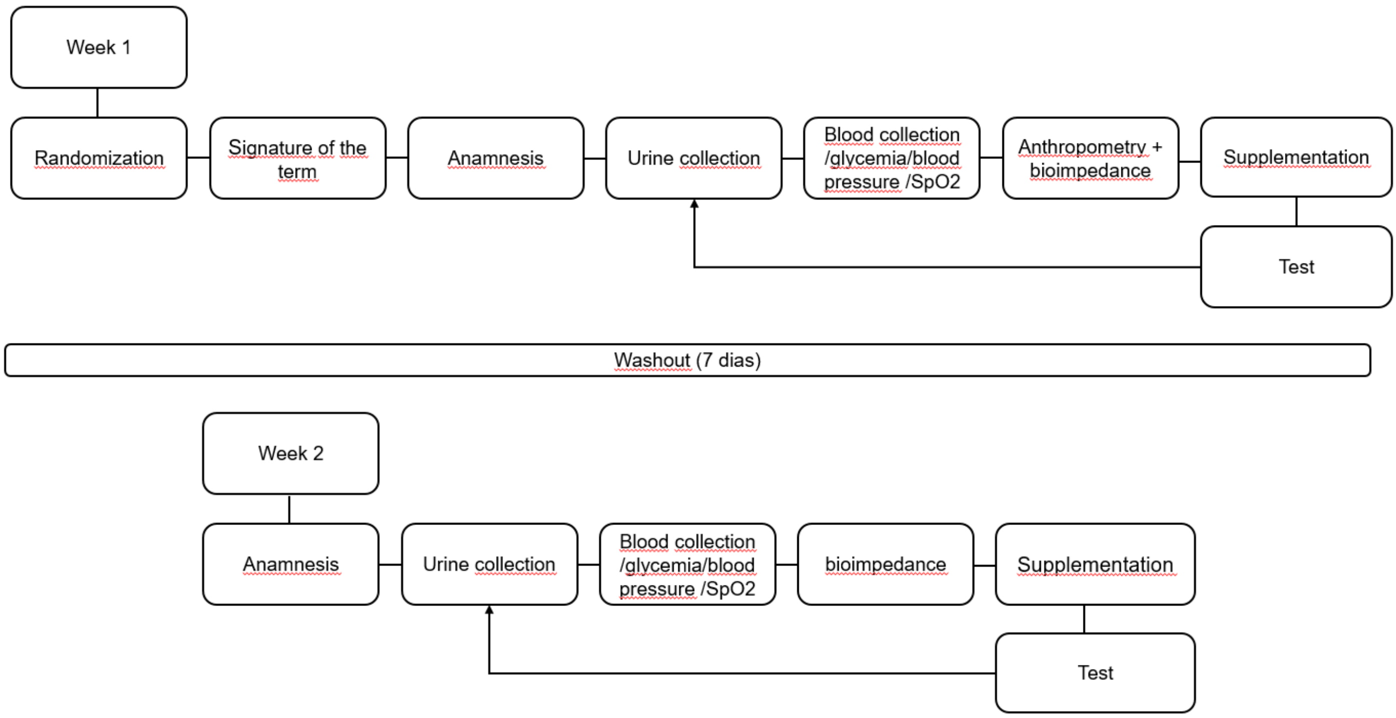

Figure 1. Timeline, referring to the processes that occurred in the first and second week.

Statistical analysis

GraphPad software version 8.0 used to calculate data normality values, using the Shapiro-Wilks test. To compare the variables of weight, fat, glycemia, hematocrit, BP, SpO2, subjective perception of exertion, urinary pH, urinary density, VO_{2max} and total distance covered, the paired t test (data with parametric distribution) and the test were performed. Anova One Way. The significance level used will be $p < 0.05$.

Results

Post-test Glycemia Analysis (mg/dl)

When comparing blood glucose analyzes (mg/dL) between groups (PL and BR), it is notable that there is an increase (Fig 2A) in glucose uptake after 30 minutes of test (139.94 ± 35.02 vs. 122.88 ± 37.69, $P < 0.05$ (PL vs BR)).

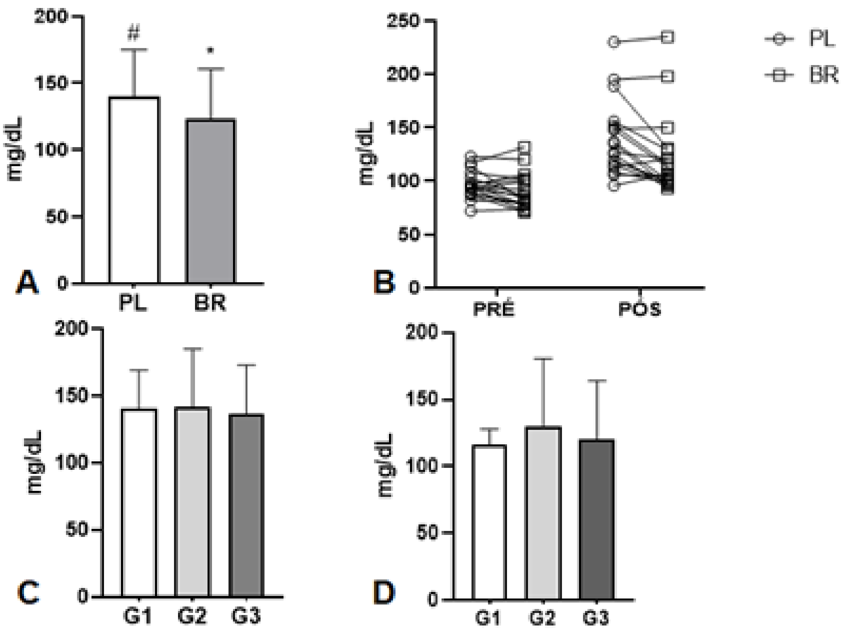

Figure 2. Post-test glycemic response (mg/dL) to isomaltulose Palatinose (PL) and beetroot juice (BR) intake in runners. Mean glycemic response between groups (A); Individual blood glucose responses (B); Glycemic response at minimum (G1), average (G2) and maximum (G3) concentrations of PL (C); Glycemic response in BR concentrations G1, G2 and G3 (D). * Indicates differences within the group ($p < 0.05$).

When the concentration (minimum, average and maximum) of BR or PL is considered, no significant differences observed, within groups, in glycemia. Fig 2C (140.66 ± 28.73, 141.85 ± 43.31 and 136.4 ± 36.64, $P > 0.05$ (G1, G2 and G3)); Fig 2D (116.5 ± 11.34, 130 ± 50.56 and 120.6 ± 43.44, $P > 0.05$ (G1, G2 and G3)).

Oxygen saturation (SpO2) and blood pressure (BP) results

As for the SpO2 (%) post-race analyzes (PL vs BR), no significant difference was observed (95.88 ± 1.40 and 96.77% ± 1.35, $P > 0.05$ (PL and BR)). It was not possible to find any significant difference in the values of BP (mmHg) systolic (115.55 ± 6.15 and 115.77 ± 8.24, $P > 0.05$ (PL and BR)) and diastolic (81.11 ± 7.58 and 80 ± 5.94 $P > 0.05$ (PL and BR)) post exercise (PL x BR).

Dehydration response

Hematocrit concentration (%) showed difference after PL or BR ingestions. It was possible to observe an increase in BR powders in relation to PL powders [Fig 3A (43.08 ± 1.78 vs 46.41 ± 3.82, P <0.05 (PL vs BR))]. However, when comparing the groups at different concentrations, no significant difference was found for PL [Fig 3B (44 ± 2.82, 42.66 ± 1.96 and 43.25 ± 1.25, P >0.05 (G1, G2 and G3))] and BR [Fig 2C (48 ± 1.41, 47 ± 4.93 and 44.75 ± 2.5, P >0.05 (G1, G2 and G3))].

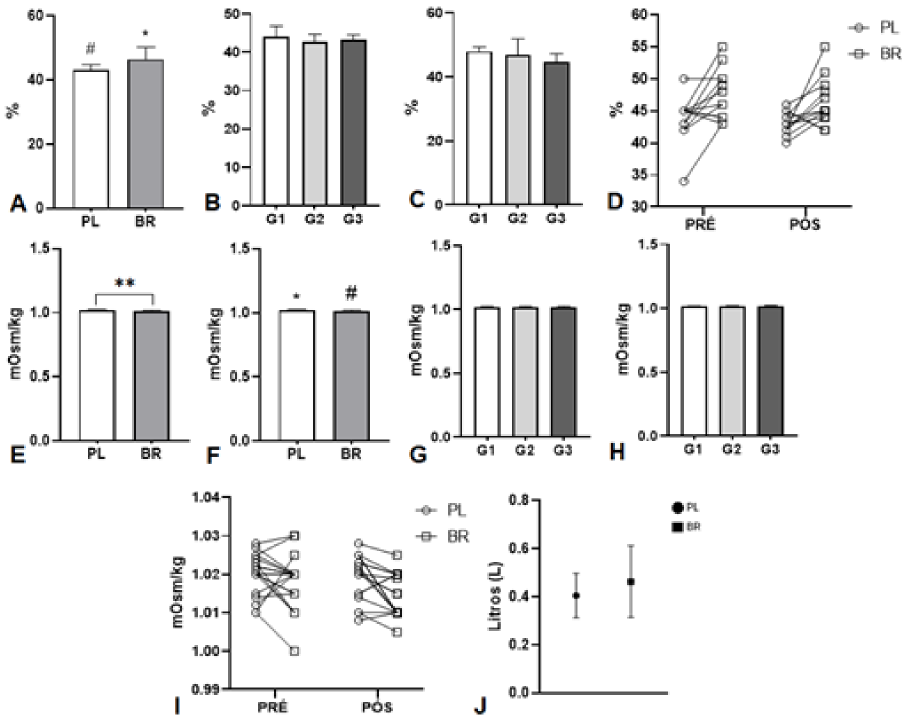

Figure 3. Post-test dehydration response to isomaltulose Palatinose (PL) and beetroot juice (BR) intake in runners. Total mean hematocrit (%) between groups (A); Hematocrit response (%) at the minimum (G1), average (G2) and maximum (G3) concentrations of PL (B); Hematocrit response (%) in BR concentrations G1, G2 and G3 (C); Individual hematocrit responses (%) between groups (D); Mean total urinary density (mOsm/kg) between groups (E); Post-test urinary density (mOsm/kg) referring to the maximum concentration (G3) in the ingestion of PL and BR (F); Urinary density response in G1, G2 and G3 concentrations of PL (F);); Urinary density response in BR concentrations G1, G2 and G3 (G); Individual responses of urinary density between groups (I) and difference in body water loss in liters (L) between groups (J). * Indicates differences within the group (p <0.05).

The post-test density analyzes demonstrate a significantly greater difference for PL [Fig 3E (1.0195 ± 0.0058 vs 1.0141 ± 0.0055, P <0.05 (PL vs BR))], however, the influence for this difference occurred in relation to the highest concentration (G3) [Fig 3F (1.019 ± 0.0064 vs 1.015 ± 0.0063, P <0.05 (PL vs BR))]. When comparing the results of density between concentrations, there was no difference between groups, both for PL [Fig 3G (1.02 ± 0.0061, 1.0192 ± 0.0060 and 1.0193 ± 0.0064, P >0.05 (G1, G2 and G3))], as for BR [Fig 3H (1.012 ± 0.0061, 1.014 ± 0.0048 and 1.015 ± 0.0063, P >0.05 (G1, G2 and G3))].

When comparing the reduction of body water (L), after intervention, it was not possible to show relevant difference, in relation to this loss of body water, in both supplements. Fig 2J (0.41 ± 0.08 vs 0.46 ± 0.14, P >0.05 (PL vs BR)).

pH and NO_2 analysis - in urine (µmol/L)

The post-intervention pH analyzes, it was possible to notice an increase in the mean pH between the groups. Fig 4 (5.05 ± 0.236 vs 5.94 ± 0.235, $P < 0.05$ (PL vs BR)).

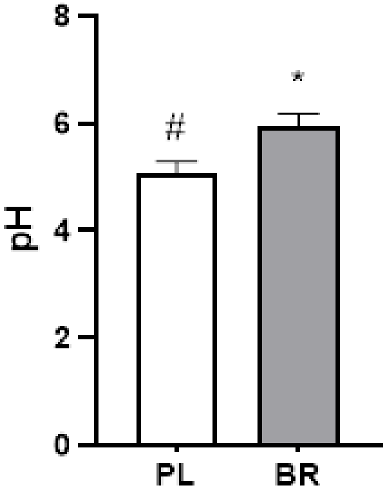

Figure 4. Post-test urinary pH after ingestion of isomaltulose Palatinose (PL) and beetroot juice (BR). * Indicates differences within the group (p <0.05).

Urine results, pre and posttest, indicate that there was a significant consideration of NO_{2}^{-} in the urine of the beetroot group, Fig 5 (0 ± 0 vs 0.94 ± 0.23, $P < 0.05$ (pre vs post)).

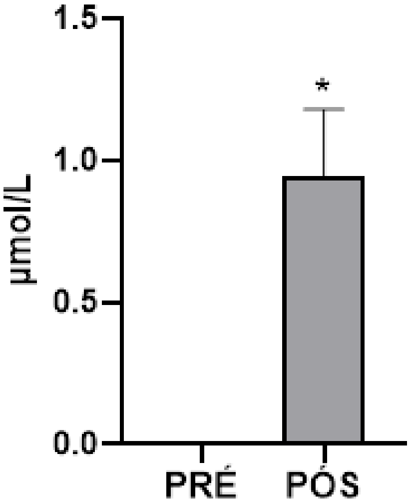

Image 5. Results of NO2- in the urine before and after beetroot ingestion. * Indicates differences within the group (p <0.05).

Athletic performance

Regardless of the concentrations, the total distance covered (meters (m)) was greater (p <0.05) in the BR group [Fig 6A (2972.63 ± 308.84 vs 3018.95 ± 309.29, P <0. 05 (PL vs BR))]. The concentrations that influenced this difference were those of the groups with minimum [Fig 6B (3020 ± 408.50 vs 3090 ± 417.37, P = 0.0147 (PL vs BR))] and mean [Fig 6C (2797, 14 ± 229.03 vs 2868.57 ± 249.76, P = 0.0168 (PL vs BR))] concentration. When comparing the three concentrations in the drinks, it was not possible to observe significant results between them, both in the PL [Fig 6D (3020 ± 408.50, 2797.14 ± 229.03 and 3130 ± 195.03, P >0.05 (G1, G2 and G3))] and BR [Fig 6E (3090 ± 417.37, 2868.57 ± 249.76 and 3123.33 ± 211.06, P >0.05 (G1, G2 and G3))].

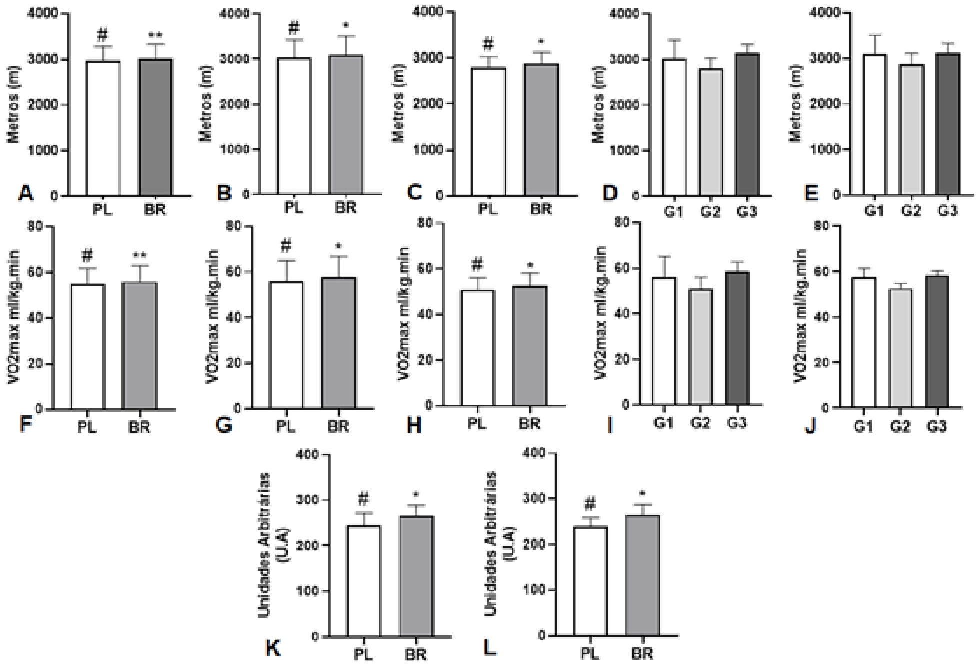

Figure 6. Athletic performance responses to isomaltulose Palatinose (PL) and beetroot juice (BR) intake of runners. Mean of the maximum distance covered in meters (m) between groups (A); Maximum distance traveled in meters at the minimum concentration (G1) (B); Maximum distance covered in meters at the average concentration (G2) (C); Total distance covered in meters at different concentrations of palatinosis (PL), minimum (G1), medium (G2) and maximum (G3) (D); Total distance covered in meters in the different concentrations G1, G2 and G3 of BR (E); Average Vo_{2max} between groups (F); Average Vo_{2max} in G1 (G); Mean of Vo2max in G2 (H); Vo_{2max} at different concentrations of PL, G1, G2 and G3 (I); Vo_{2max} at different concentrations of BR, G1, G2 and G3 (J); Arbitrary units (AU), indicating the subjective perception of effort in PL and BR intake (K); UA indicating the subjective perception of exertion in the intake of PL and BR, in G3 (L). * Indicates differences within the group (p <0.05).

VO_{2max} analyzes indicate an increase in the BR group [Fig 6F (54.96 ± 6.87 vs 55.99 ± 6.88, P <0.05 (PL vs BR))], having a greater relevance only in the minimal [Fig 6G (56.01 ± 9.09 vs 57.57 ± 9.29, P < 0.05 (PL vs BR))] and mean groups [Fig 6H (51.05 ± 5.10 vs 52 .64 ± 5.56, P < 0.05 (PL vs BR))] concentration. As for the different concentrations, no considerable differences were observed in the VO2max of PL [Fig 6I (56.01 ± 9.09, 51.05 ± 5.10 and 58.46 ± 4.34, P >0.05 (G1, G2 and G3))] and BR [Fig 6J (57.57 ± 9.29, 52.64 ± 5.56 and 58.31 ± 4.70, P >0.05 (G1, G2 and G3))].

After the test, the perception of exertion was greater in the beet group [Fig 6K (244.73 ± 26.95 vs 265.26 ± 22.94, P = 0.0149 (PL vs BR))], however, it is possible observe that the highest concentration of beet (G3) was the one that influenced the increase in PSE [Fig 6L (240 ± 18.97 vs 265 ± 22.58, P = 0.0422 (PL vs BR)), the other concentrations had no significant difference.

Discussion and conclusion

This is the first study to investigate the effects of BR supplementation at minimum, average and maximum concentrations during exercise, on the physiological responses generated during a 30-minute running test.

The main results found in this study were that, compared to PL, BR ingestion: 1) improved glucose uptake by 13.88% during running; 2) increased the hematocrit concentration by 7.72%; 3) increased the mean urinary pH by 17.62%; 4) showed a decrease in post-test urinary density, in the highest concentration (G3); 5) significantly evidenced the excretion of NO_{2-} in the urine, after the intervention; 6) it improved the runners' average performance by 1.55%, with G1 and G2 being the most induced concentrations; 7) it increased the mean VO_{2max} by 1.9%, so that such increases were more relevant in G1 and G2; 8) caused a higher RPE (8.4%), however, this perception was greater in G3.

The present study demonstrated that after ingestion of beetroot juice, there is a considerable increase in glucose capitation and VO_{2max} elevation, such responses coincide with the improvement in performance in amateur runners (1.55%), these results are similar to with a range of current evidence, which demonstrate that during physical activity there is an increase in glucose oxidation [44,45], coinciding with the high maximum oxygen consumption (VO_{2max}) and performance improvement in runners highly trained [46,47,48,49].

In turn, evidence shows that NO increases the expression of glucose transporter 4 (GLUT-4) (50), through stimulation of AMP-activated protein kinase (AMPK), when this protein is activated it inhibits the synthesis of glycogen, fatty acids and proteins, thus providing an increase in the uptake of glucose, fat and stimulating mitochondrial biogenesis, in several tissues, even the skeletal muscle [51,52,53].

The mean VO2max in the present study was shown to be higher during BR consumption (55.99 ± 6.88 mL kg^{-1} min^{-1}), these findings do not corroborate with previous trials, in which moderately and well-trained individuals, which were supplemented with NO and exposed to submaximal exercise sessions obtained a significant reduction in oxygen utilization (VO_{2max}) [23,24,54,55,56,57]. However, the study conducted by Lausch et al. (2019) (1) analyzed the effects of chronic BR supplementation (12.4 mmoL NO_{3-}) in well-trained cyclists, during 7 days, these cyclists performed a time trial (10km), being exposed to a gas that left them in a state of normoxia or hypoxia, the results showed that there was an increase in performance and VO_{2max}, but there was no significant difference between normoxia and hypoxia. These findings validate the results, since, after ingestion of 5.4 mmoL and 12.8 mmoL NO_{3-} the runners had an increase in performance and VO_{2max}, which was not observed in the highest concentration (23.3 mmoL NO_{3-}).

It is evident that the low oral administration with 2 mmoL of NO_3- promotes the reduction of NO_{3-} to NO in the organism [58], considering that approximately 75% of the total nitrate intake in the circulation is excreted in the urine and 25% is concentrated in the salivary glands, thus being reused by the enterosalivar cycle [59], these statements validate our results, confirming the increase in systemic NO bioavailability through the nitrate – nitrite – nitric oxide route [7,8], promoting vasodilation, consequently improving the performance of athletes and well-trained individuals [1,41,60], since G1 and G2 demonstrated to improve performance and increase VO_{2max}. On the other hand, there was a considerable excretion of NO_{2-} in the urine of the runners, which indicates the efficiency of the reduction of NO_{3-} to NO and also an excess of NO_{3-} consumption when administered in acute form.

A double-blind randomized placebo-controlled crossover study sought to analyze 27 hypertensive individuals who consumed a low-nitrate diet for 1 week and subsequently switched to a high-nitrate diet, supplemented twice a day with 70 ml of beet juice, therefore, it was evidenced that the consumption of the diet rich in NO3- obtained a 4 times greater elevation of NO_{3-} and NO_{2-} urinary than the diet low in nitrate, not presenting to reduce the arterial pressure of the studied population [61].

Francesconi et al. (1985) [62] showed that urinary analyzes would be more sensitive than hematocrit and plasma osmolarity values when indicating dehydration.

Through the values of urinary density it is possible to analyze how concentrated the urine is, since the closer the values are to 1,000 mOsm/kg, it is an indication that the urine contains less residues and is more diluted, given that the National Athletic Trainer's Association (2000) demonstrated in its study that, after comparing the values of urinary density before and after the competition of the participants, greater dehydration was observed after the competition [63].

In study, observed that individuals ingested BR had a density closer to the lower limit (1,000 mOsm/kg), indicating less dehydration compared to PL, however cannot say that individuals who ingested PL they were dehydrated. The bioimpedance data show that there was no significant loss in body water results [64], and the PL density values are not as close to the lower limit.

Post-exercise acid-base balance occurs through the buffering system of blood and tissues, so that diffusion of carbon dioxide from the bloodstream to the lungs through respiration and the excretion of hydrogen ions from the blood into the urine takes place. Through the kidneys. Therefore, metabolic acidosis occurs through muscle contraction during exercise, as there is an increase in the accumulation of hydrogen ions (H+), causing acidosis, thus impairing muscle contraction during high-intensity exercise and consequently performance [65].

Abián-Vicén et al. (2012)[65] highlighted that after intense exercise, the urine becomes acidic due to the increase in the elimination of ammonia, leading to a decrease in the pH and that the urinary pH data is an indication of the degree of pH alteration blood.

Through these statements and with the results achieved in study, it is possible to show that the 30 minutes of running were not enough for the individuals to exert maximum effort and enter into acidosis, since G1 and G2 did not show significance in relation to PSE, these findings corroborates are the data found by Arnold et al. (2015)[66], when investigating the effect of beetroot supplementation in well-trained athletes, submitted to a race at altitude, did not find significant results as the time to exhaustion, 10km time trial and the PSE data.

This study has some limitations. The number of runners was relatively low, but the inclusion and exclusion criterion restricted some participants, who did not have at least 12 months of training. Did not control a standardized diet for runners. Only fasting and post-test glycemia were collected, and we could have performed a pre-test collection, this collection could have better elucidated glucose uptake, when comparing PL x BR.

As reviewed, most studies with beets apply tests, in athletes, in laboratories or in the field, to analyze effects on physical performance, however, there is no evidence in the competition situation, the few studies that show a positive result, present many biases, such as diet, physical conditioning, gender, age, biochemical tests, nutritional and pharmacological supplements.

Despite the limitations of study, based on hypothesis, it was evident that beet supplementation in amateur runners increased glucose uptake when comparing PL x BR, but, there was no decrease in VO_{2max}, thus increasing VO_{2max} and improved running performance in G1 and G2. It can be concluded that there is a limit to nitrate supplementation, since no significant results were found in relation to the highest concentration of supplementation with beetroot juice.

References

1. Rokkedal-Lausch, Torben, et al. "Chronic high-dose beetroot juice supplementation improves time trial performance of well-trained cyclists in normoxia and hypoxia." Nitric Oxide 85 (2019): 44-52.
2. Domínguez, Raúl, et al. "Effects of beetroot juice supplementation on cardiorespiratory endurance in athletes. A systematic review." Nutrients 9.1 (2017): 43.
3. Burke, Louise M., et al. "International association of athletics federations consensus statement 2019: nutrition for athletics." International Journal of Sport Nutrition and Exercise Metabolism 29.2 (2019): 73-84.
4. The Australian Institute of Sport (AIS). <https://www.ais.gov.au/nutrition/supplements/group_a> Accessed March 28, 2022.
5. Hoon, Matthew W., et al. "The effect of variable doses of inorganic nitrate-rich beetroot juice on simulated 2000-m rowing performance in trained athletes." International journal of sports physiology and performance 9.4 (2014): 615-620.
6. Karwowska, Małgorzata, and Anna Kononiuk. "Nitrates/nitrites in food—Risk for nitrosative stress and benefits." Antioxidants 9.3 (2020): 241.
7. Garnacho-Castaño, Manuel Vicente, et al. "Effects of a single dose of beetroot juice on cycling time trial performance at ventilatory thresholds intensity in male triathletes." Journal of the International Society of Sports Nutrition 15.1 (2018): 1-12.
8. Kozłowska, Lucyna, et al. "Changes in oxidative stress, inflammation, and muscle damage markers following diet and beetroot juice supplementation in elite fencers." Antioxidants 9.7 (2020): 571.
9. Poole, David C., et al. "Role of nitric oxide in convective and diffusive skeletal muscle microvascular oxygen kinetics." Nitric Oxide 121 (2022): 34-44.
10. Boorsma, Robert K., Jamie Whitfield, and Lawrence L. Spriet. "Beetroot juice supplementation does not improve performance of elite 1500-m runners." Med Sci Sports Exerc 46.12 (2014): 2326-2334.
11. Lira, Vitor A., et al. "Nitric oxide and AMPK cooperatively regulate PGC - 1 α in skeletal muscle cells." The Journal of physiology 588.18 (2010): 3551-3566.
12. Wu, Guoyao, et al. "Role of L-Arginine in Nitric Oxide Synthesis and Health in Humans." Amino acids in Nutrition and Health . Springer, Cham, 2021. 167-187.
13. Session. "Nitric oxide synthases: regulation and function." European heart journal 33.7 (2012): 829-837.
14. de Betue, Carlijn TI, et al. "24-Hour protein, arginine and citrulline metabolism in fed critically ill children–A stable isotope tracer study." Clinical Nutrition 36.3 (2017): 876-887.
15. Tsuboi, Tomoe, Morihiko Maeda, and Toshio Hayashi. "Administration of L-arginine plus L-citrulline or L-citrulline alone successfully retarded endothelial senescence." PloS one 13.2 (2018): e0192252.
16. Gambardella, Jessica, et al. "Arginine and endothelial function." Biomedicines 8.8 (2020): 277.
17. Sibisi, NC, et al. "Evaluating the role of nitric oxide in myogenesis in vitro." Biochimie 196 (2022): 216-224.
18. Montfort, William R., Jessica A. Wales, and Andrzej Weichsel. "Structure and activation of soluble guanylyl cyclase, the nitric oxide sensor." Antioxidants & redox signaling 26.3 (2017): 107-121.
19. de Castro, Talitha F., et al. "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." European Journal of Applied Physiology 119.5 (2019): 1043-1053.
20. Domínguez, Raúl, et al. "Effects of beetroot juice supplementation on intermittent high-intensity exercise efforts." Journal of the International Society of Sports Nutrition 15.1 (2018): 1-12.

21. Peeling, Peter, et al. "Beetroot juice improves on-water 500 m time-trial performance, and laboratory-based paddling economy in national and international-level kayak athletes." International journal of sport nutrition and exercise metabolism 25.3 (2015): 278-284.
22. Eggebeen, Joel, et al. "One week of daily dosing with beetroot juice improves submaximal endurance and blood pressure in older patients with heart failure and preserved ejection fraction." JACC: Heart Failure 4.6 (2016): 428-437.
23. Carriker, Colin R., et al. "Nitrate-containing beetroot juice reduces oxygen consumption during submaximal exercise in low but not high aerobically fit male runners." Journal of Exercise Nutrition & Biochemistry 20.4 (2016): 27.
24. Lansley, Katherine E., et al. "Dietary nitrate supplementation reduces the O2 cost of walking and running: a placebo-controlled study." Journal of applied physiology 110.3 (2011): 591-600.
25. Tan, Rachel, et al. "Beetroot juice ingestion during prolonged moderate-intensity exercise attenuates progressive rise in O2 uptake." Journal of Applied Physiology 124.5 (2018): 1254-1263.
26. Christensen, Peter Moller, M. Nyberg, and Jens Bangsbo. "Influence of nitrate supplementation on VO2 kinetics and endurance of elite cyclists." Scandinavian journal of medicine & science in sports 23.1 (2013): e21-e31.
27. Burke, Louise M., et al. "Neither beetroot juice supplementation nor increased carbohydrate oxidation enhance economy of prolonged exercise in elite race walkers." Nutrients 13.8 (2021): 2767.
28. Balsalobre-Fernández, Carlos, et al. "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 13.7 (2018): e0200517.
29. Jonvik, Kristin L., et al. "Repeated-sprint performance and plasma responses following beetroot juice supplementation do not differ between recreational, competitive and elite sprint athletes." European journal of sport science 18.4 (2018): 524-533.
30. Sandbakk, Silvana Bucher, et al. "Effects of acute supplementation of L-arginine and nitrate on endurance and sprint performance in elite athletes." Nitric Oxide 48 (2015): 10-15.
31. Jonvik, Kristin L., et al. "Can elite athletes benefit from dietary nitrate supplementation?." Journal of applied physiology 119.6 (2015): 759-761.
32. World Health Organization – WHO. Physical status: use of World Health Organization criteria to classify nutritional status in children.
33. Jackson, Andrew S., and Michael L. Pollock. "Generalized equations for predicting body density of men." British journal of nutrition 40.3 (1978): 497-504.
34. Siri, WE Body composition from fluid spaces and obesity: analysis of methods. IN Brozek, J.; Henschel, A. Techniques for measuring body composition. National Academy of Sciences. 1961.
35. Matias, Catarina N., et al. "Estimation of total body water and extracellular water with bioimpedance in athletes: A need for athlete-specific prediction models." Clinical nutrition 35.2 (2016): 468-474.
36. Lukaski, Henry C., et al. "Assessment of fat-free mass using bioelectrical impedance measurements of the human body." The American journal of clinical nutrition 41.4 (1985): 810-817.
37. BRAY, GA Pathophysiology of obesity. Amer. J. clin. Nutr., 55(Suppl 2): 488-95,1992.
38. Lee, Robert C., et al. "Total-body skeletal muscle mass: development and cross-validation of anthropometric prediction models." The American journal of clinical nutrition 72.3 (2000): 796-803.
39. Janssen, Ian, et al. "Skeletal muscle cutpoints associated with elevated physical disability risk in older men and women." American journal of epidemiology 159.4 (2004): 413-421.
40. Garnacho-Castaño, Manuel Vicente, et al. "Understanding the effects of beetroot juice intake on CrossFit performance by assessing hormonal, metabolic and mechanical response: a randomized, double-blind, crossover design." Journal of the International Society of Sports Nutrition 17.1 (2020): 56.
41. Nyakayiru, Jean, et al. "Beetroot juice supplementation improves high-intensity intermittent type exercise performance in trained soccer players." Nutrients 9.3 (2017): 314.
42. Cooper, Kenneth H. "A means of assessing maximal oxygen intake: correlation between field and treadmill testing." Jama 203.3 (1968): 201-204.
43. Borg, Gunnar AV. "Psychophysical bases of perceived exertion." Medicine & science in sports & exercise (1982).
44. Roberts, Justin D., et al. "Assessing a commercially available sports drink on exogenous carbohydrate oxidation, fluid delivery and sustained exercise performance." Journal of the International Society of Sports Nutrition 11.1 (2014): 8.
45. Jentjens, Roy LPG, and Asker E. Jeukendrup. "High rates of exogenous carbohydrate oxidation from a mixture of glucose and fructose ingested during prolonged cycling exercise." British Journal of Nutrition 93.4 (2005): 485-492.
46. Maunder, ED, TIM Podlogar, and Gareth A. Wallis. "Postexercise Fructose-Maltodextrin Ingestion Enhances Subsequent Endurance Capacity." Medicine and science in sports and exercise 50.5 (2018): 1039-1045.

47. Wilson, PB, and SJ Ingraham. "Glucose - fructose likely improves gastrointestinal comfort and endurance running performance relative to glucose - only." Scandinavian journal of medicine & science in sports 25.6
48. Tsintzas, Orestis-Konstantinos, et al. "Carbohydrate intake and glycogen utilization in different muscle fiber types in man." The Journal of physiology 489.1 (1995): 243-250.
49. Wilber, Randall L., and Robert J. Moffatt. "Influence of carbohydrate ingestion on blood glucose and performance in runners." International Journal of Sport Nutrition and Exercise Metabolism 2.4 (1992): 317-327.
50. Lira, Vitor A., et al. "Nitric oxide increases GLUT4 expression and regulates AMPK signaling in skeletal muscle." American Journal of Physiology-Endocrinology and Metabolism 293.4 (2007): E1062-E1068.
51 . Nisoli, Enzo, et al. "Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS." Science 310.5746 (2005): 314-317.
52. Nisoli, Enzo, et al. "Mitochondrial biogenesis by NO yields functionally active mitochondria in mammals." Proceedings of the National Academy of Sciences 101.47 (2004): 16507-16512.
53. Nisoli, Enzo, et al. "Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide." Science 299,5608 (2003): 896-899.
54. Porcelli, Simone, et al. "Aerobic fitness affects the exercise performance responses to nitrate supplementation." (2015): 1643-1651.
55. Larsen, FJ, et al. "Effects of dietary nitrate on oxygen cost during exercise." Acta physiologica 191.1 (2007): 59-66.
56. Tan, Rachel, et al. "Beetroot juice ingestion during prolonged moderate-intensity exercise attenuates progressive rise in O2 uptake." Journal of Applied Physiology 124.5 (2018): 1254-1263.
57. Shannon, Oliver Michael, et al. "Dietary nitrate supplementation enhances high-intensity running performance in moderate normobaric hypoxia, independent of aerobic fitness." Nitric Oxide 59 (2016): 63-70.
58. McKnight, GM, et al. "Chemical synthesis of nitric oxide in the stomach from dietary nitrate in humans." Gut 40.2 (1997): 211-214.
59. Pannala, Ananth S., et al. "The effect of dietary nitrate on salivary, plasma, and urinary nitrate metabolism in humans." Free Radical Biology and Medicine 34.5 (2003): 576-584.
60. Santana, Jeferson, et al. "Nitrate supplementation combined with a running training program improved time-trial performance in recreationally trained runners." Sports 7.5 (2019): 120.
61. Bondonno, Catherine P., et al. "Absence of an effect of high nitrate intake from beetroot juice on blood pressure in treated hypertensive individuals: a randomized controlled trial." The American journal of clinical nutrition 102.2 (2015): 368-375.
62. Francesconi, RP, et al. "Urinary and hematologic indexes of hypohydration." Journal of Applied Physiology 62.3 (1987): 1271-1276.
63. House, Douglas J., et al. "National athletic trainers' association position statement: fluid replacement for athletes." Journal of athletic training 35.2 (2000): 212.
64. Mueller, Sandro Manuel, et al. "Changes in body composition in triathletes during an Ironman race." European Journal of Applied Physiology 113.9 (2013): 2343-2352.
65. Abián-Vicén, Javier, et al. "Analysis of dehydration and strength in elite badminton players." PloS one 7.5 (2012): e37821.
66. Arnold, Josh Timothy, et al. "Beetroot juice does not enhance altitude running performance in well-trained athletes." Applied Physiology, Nutrition, and Metabolism 40.6 (2015): 590-595.