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Original Article

The effect of beetroot juice on repeated sprint performance and muscle force production

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Abstract
Nitrate can impact a range of physiological functions through its conversion to nitric oxide (NO) and as such, it has received attention as a dietary supplement. Beetroot juice (BR) is a nitrate-rich dietary source which has been used in the athletic setting for evaluating its performance through time-to-exhaustion protocols, which test exercise capacity but not athletic performance using in most research cyclists as subjects. The aim of this study was to examine the effects of beetroot juice on muscle force production and repeated sprint performance through measurements on an isokinetic dynamometer through extension and flexion knee movements at 60 and 240 degrees and a non-motorized treadmill through a high intensity sprint (5x6 seconds) protocol after a single dose intake of BR (70ml with 0.4g nitrate), placebo (blackcurrant juice) or control (no juice) in young and healthy adults. The results did not show any differences between the three trials for the muscle strength measurements but a small difference in peak power production performing the sprint protocol, suggesting that either higher doses or longer intake period may be required prior to observation of an impact on either muscle strength or sprint performance.
Keywords: Sprint performance, Muscle force production, nitrate, beetroot juice.

Introduction

The importance of nitric oxide (NO) for the physiology of mammals, including humans, has now been extensively studied and established, with a large number of studies supporting its role in signal transduction (Murad, 1996; Martin et al., 2000; Hanafy et al., 2001; Murad, 2004) and a range of body processes like vasodilation (Higashi & Yoshizumi, 2003) and vessel homeostasis (Palmer, 2003), liver regeneration (Carnovale & Ronco, 2012), immune response by the macrophages (Bogdan, 2001; Wink et al., 2011), neurotransmission (Oh, 1995; Kiss, 2000). Nitric oxide becomes available in the body through oxidation of L-arginine by the nitric oxide synthetases (NOS) (Alderton et al., 2001), but an additional pathway was recently unveiled, where NO becomes available through the sequential reduction of nitrate to nitrite and of nitrite to NO (Lundberg et al., 2008). As a result, inorganic nitrate from dietary sources emerges as a substrate for the in vivo generation of NO; indeed, dietary intake of nitrate produces the same effect as NO, namely vasoprotective activity, blood pressure reduction and additionally, decreases the whole body oxygen cost during exercise or enhanced maximal performance (Ferreira &Behnke, 2011; Lansley et al., 2011). The effects of nitrate can be achieved through consumption of large amount of vegetables which are nitrate-rich.
Beetroot juice (BR) is a nitrate-rich dietary source, containing approximately 5-8 mmol and has therefore been used as a dietary supplement of inorganic nitrate (NO_3^-) in order to provide benefits to the body through the mechanisms as described above. Given the impact of a range of physiological functions by dietary nitrate supplementation, a number of studies addressed the question of the impact of beetroot juice (as a nitrate-rich food) on physiological responses to exercise, with the prospect of using it as a supplement to enhance performance. One study showed that three days of sodium nitrate supplementation at 0.1 mmol/kg/day resulted in reduction of resting blood pressure and the O_2 cost of sub-maximal cycle exercise (Larsen et al. 2007), while reduction of O_2 cost of low intensity exercise with an enhanced tolerance to high intensity exercise in humans was observed in a later study, after a six-consecutive day nitrate supplementation (Bailey et al. 2009). The same research group concluded that the same supplements enhance muscle contractile efficiency during knee-extensor exercise (Bailey et al., 2010), while a recent study showed that six days of nitrate supplementation in the form of beetroot juice (~0.5 L/d with~8 mmol/d nitrate) reduced VO_2 during submaximal exercise, improved time-trial performance in trained cyclists, thus increasing tolerance of high-intensity work rates and presenting nitrate as a potent ergogenic aid (Cermak et al., 2012a). A follow-up study testing the effect of a single dose of beetroot

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juice (500 ml, approx. 6.2 mmol NO_3) indicated that this dose improved subsequent time-trial performance. Recently, one study showed that dietary nitrate supplementation improved team sport-specific intense intermittent exercise performance after intake of 490 mL of concentrated, nitrate-rich BR by 4.2% when compared to performance after intake of placebo juice (Wylie et al., 2013). In this study 14 male team-sport players were assigned in a double blind, randomized, crossover design performing 30 hours before the completion of a Yo-yo intermittent recovery level 1 test. The most significant improvement was the 4.2% greater performance of the Yo-yo test consuming Beetroot Juice compared to placebo as well as the 400% greater resting plasma nitrite concentration.
A number of studies have also tested the effect of BR on force production using the isokinetic dynamometer by completing a series of high intensity exercises and demonstrating that it can improve time-to-exhaustion. In one of these studies 8 men (19-38 years old) consumed a single dose of beetroot juice or placebo for 6 consecutive days and completed series of step moderate-intensity and severe-intensity exercise tests on the last three days resulting a reduction to the O2 uptake and extension to the time-to-exhaustion (Bailey et al., 2009;2010). There are two possible mechanisms through which NO could be involved in the O_2 usage and impact both high intensity exercise and force production. One of the mechanisms proposes that hypoxia can facilitate the NO_2 reduction, cause the production of more NO in parts of muscle that are receiving less or using more O_2 and as a result local blood flow is matched to O_2 requirement, providing a more homogenous distribution of O_2 within skeletal muscle. This mechanism would not justify a reduction on O_2 expenditure during exercise. The second mechanism supports that NO/NO_2 may act as regulators of O_2 exertion and NO might enhance the efficiency of oxidative phosphorylation by reducing "slippage" of the mitochondrial proton pumps. Given that NO_3 is relatively inert, its effect must be mediated through action of its bioactive nitrogen derivative, nitrite (NO_2), or the subsequent bioconversion of NO_2 to nitric oxide (NO). Perkins et al. (1997) have described the relation between force production and nitric oxide and concluded that exposure to the NO donor SNP (Sodium Nitroprusside) inhibited isometric force, Ca^2 sensitivity and actomyosin ATPase activity.
As regards physiological responses to exercise, BR is deemed salutary in most kinds of endurance, but little is known on its role on strength and power exercise. Most studies evaluating its performance have used time-to-exhaustion protocols and as a result were deemed of limited validity in the athletic setting since such protocols test exercise capacity but not athletic performance.
The aim of the current research was to examine the effects of beetroot juice on muscle force production (strength) and repeated sprint performance. Our hypothesis was that intake of beetroot juice could positively impact both sprint performance and muscle force production in comparison to placebo.

Materials and methods

Participants
Seven healthy men participated in this study. They had an average age of 25,2±3,3 years, height of 181,5±7,5 cm and body mass of 81,1±9,1 kg. Before taking part in the study, all subjects completed a PAR-Q in order to assess their suitability for the study. All participants received a participant information sheet and provided written informed consent prior to the initiation of the study. Subjects had no previous history of metabolic or cardiovascular problems, refrained from exercise, alcohol, tobacco and caffeine 24 hours before testing and maintained their normal diet prior to each visit. All participants verbally reported compliance with the instructions in the pre-testing period (refraining from tobacco, alcohol and exercise and maintenance of normal diet). All procedures were approved by the Ethics committee of the University.

Study design

Experimental design: The subjects participated in two repeated measures familiarisation sessions using a cross-over design in order to become accustomed to the equipment through testing of the movements of the isokinetic dynamometer and use of the treadmill. The sessions happened once per week for two weeks before testing and all at the same time of the day and the same day of the week. Every subject participated in three trials (beetroot, placebo, control) randomly and under double blind conditions. Each testing day, the participants consumed 3 hours prior the testing protocols either beetroot juice or placebo or nothing depending on the group that they are assigned to, using a face mask and a nose clip to limitate the smell and the taste of each drink. The beetroot juice was offered in a single dose of 70 ml containing 0,4 g of nitrate (James White Drinks, Beet It Sport) , while the placebo was blackcurrant cordial with negligible nitrate content. Extension and flexion movements were performed using the isokinetic dynamometer.
To limit possible threats to experimental internal validity, participants were advised to follow a balanced diet during the lab testing period and avoid intense exercise during this time. To control this, the normal diet that participants followed was recorded on a sheet and replicated, allowing observation of differences related to the intervention.
Participants had an equal chance of being in the beetroot, the placebo or the control group and so, we can assume that groups do not differ at the start of the experiment. This can be achieved performing a counterbalancing

method using a Latin square. Additionally, the placebo (blackcurrant cordial) is another means for the internal validity of the experiment.
To maximise the external validity, we performed the isokinetic tests in fast speed which is more functional and is related to real life.

Protocol: On the first day of testing, subjects entered the muscle strength laboratory and their body mass (kg) and height (cm) were measured using calibrated scales and equipment (Charder HM200PW Wall Mount Height Rod Stadiometer). Heart Rate (HR) using Polar Heart Rate Monitor FT1 (Polar Electro Oy, Finland). Rating of Perceived Exertion (RPE) was measured using Borg Scale (Borg, 1970). Blood Lactate (BL) concentrations from capillary blood were assessed using the LactatePro for each of the participants before the test began. Hamstrings and quadriceps muscle force production was assessed using an isokinetic dynamometer (Biodex) at speeds of 60 and 240 degrees per second. The subjects sat upright in the testing chair, with the trunk vertical and knee flexion at 90^{O}, while being tightly secured to the seat with straps across the trunk and thigh to isolate the muscles being assessed. Following the extension and flexion movements using the isokinetic dynamometer, participants were measured for their Heart Rate, Rating of Perceived Exertion and Blood Lactate and prior to the performance of repeated sprints on treadmill with recovery time between each sprint.

The participants performed repeated high intensity sprints (5 x 6 seconds) on a non-motorised treadmill (Woodway, Weil am Rhein, Germany) with 30 seconds of recovery between each exercise bout. Power output was assessed during each sprint and the decrement in performance and fatigue index was calculated . HR and RPE were measured
exactly before and after every bout and Blood Lactate was measured once before and after the sprint protocol.

Statistical analysis
To describe the data obtained we used the mean and the standard deviation. Two-way repeated measures of ANOVA was used to assess repeated sprint protocols. One-way ANOVA was used for all other measurements. The statistical software SPSS (version16.0.2) was used for the analysis of variance (http://www-01.ibm.com/software/analytics/spss/).

Results
Muscle strength
Data was obtained on muscle strength using an isokinetic dynamometer. The data on knee extension and flexion peak torque for the subjects are presented in Table 1.

Table 1: Isokinetic peak torque (Nm) (Mean±Standard Deviation) and Flexion/ /extension (F/E) ratio (Mean±Standard Deviation) data

Measurement	**A (beetroot juice)**	**B (blackcurrant juice-placebo)**	**C (control-no juice)**	**F**	**P value (95% confidence interval)**
Extension at 60°/s	200,2±25,8	207,4±37,5	189,4±30,3	0.5813	0.57
Extension at 240°/s	124,1±9,2	131,4±17,1	118,5±26	0.8448	0.45
Flexion at 60°/s	103,3±27,7	110,9±29,9	101,8±26,3	0.2105	0.81
Flexion at 240°/s	59,8±29,5	69,4±21,5	65,7±25,4	0.2506	0.78
F/E ratio (%) at 60°/s	51±11,9	54,1±14,5	54,4±14,6	0.7356	0.49
F/E ratio (%) at 240°/s	49,1±25,6	54,7±21,5	56,7±20,5	0.2847	0.75

Knee flexion and extension measures are different in most cases between the three trials, A, B and C, where the subjects received different supplements. At the high velocity of 240°/s, mean flexion and extension peak torque are lower when compared to the corresponding ones at the slow velocity of 60°/s. Comparison of the measurements between extension and flexion peak torque at either 60°/s or 240°/s shows that in both cases, flexion values are almost half of the corresponding extension values. Interestingly, the strength measures are larger in the placebo trial than the measures in the beetroot trial and this finding is consistent across all different measurements, in both extension and flexion mean peak torque.
The strength measures are lower in the control trial where the subjects received no juice, than the measures in the beetroot trial. One exception to this result is the case of flexion measurements at 240°/s, where peak torque after intake of beetroot juice is lower when compared to measurements without any intake of juice (by around 9%).

Analysis of the variance of the means of the measurements using one-way ANOVA showed that the probability that the null hypothesis, i.e. that the means of the different groups of the subjects are all equal. Given that the F value obtained is much lower than the F critical value for 2 degrees of freedom of our case (19.000, 5% confidence interval), the results of the analysis of variance suggests that our null hypothesis is accepted.

Repeated sprint performance

Data obtained using a non-motorised treadmill was collected and the mean values (±standard deviation) for the peak power of the subjects are shown in Table 2 and shown as a plot in Figure 1.

Table 2: Peak power (W) by subjects on the non-motorised treadmill

	***A* (beetroot juice)**	***B* (blackcurrant juice-placebo)**	***C* (no juice- control)**	***F***	***P value* (95% confidence interval)**
Mean Peak power (W) ± Standard deviation	4133,5±674,4	3938,3±603,1	4013,7±572,9	0.238	0.79

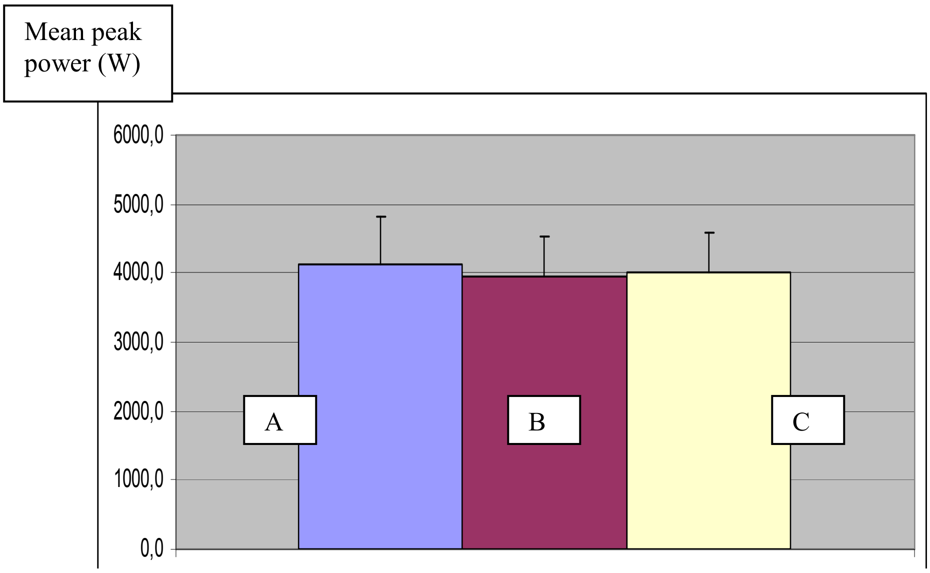

Figure 1: Mean peak power (±standard deviation) of subjects following consumption of beetroot juice (A), blackcurrant juice (B) and no juice(C). Standard deviations are shown per column.

The results show a small difference (~100W) between the mean calculated value of the peak average power produced by the subjects on the non-motorised treadmill after the intake of the BR supplement when compared to the corresponding one after intake of the placebo or the control. Both the placebo and the control groups give nearly similar values and unlike the experiments of the isokinetic dynamometer, the placebo does not appear to cause any positive effect to the repeated sprint performance. It should be mentioned, however, that statistically, the evidence was not significant.

Table 3: HR and RPE values during bouts

	HR pre	HR post	RPE
Trial A –beetroot	143,7±8,2	157,4±6,5	13,9±1,6
Trial B- placebo	141,7±11,2	155,9±8,2	14,3±1,7
Trial C-control	140,8±8,7	152,3±9,8	14,1±1,3

Across subjects, the mean sprint time was the same(6 seconds). HR and RPE increased during the bouts and a small effect is seen on HR (pre or post the bouts) from beetroot intake, when compared to either placebo or control. Accordingly, the RPE index is marginally lower after intake of beetroot when compared to placebo and control. (Table 3)

Discussion

The obtained results from the isokinetic dynamometer (Table 1) partly supported the hypothesis that BR positively effects muscle strength as some difference was indeed observed between the three trials. The intake of the beetroot juice gave a higher peak force performance to the subjects when compared to either the result of the placebo juice or the control. The interesting finding in this set of experiments was that the placebo cordial also affected positively the muscle strength performance of the subjects, suggesting a placebo effect on the subjects.

The data from the repeated sprint performance testing also showed a small difference between beetroot intake and either placebo or control. In these measurements, no placebo effect was observed and placebo and control produced similar values on the mean peak power. This may suggest that the beetroot intake enables a fuller recovery between bouts, thus allowing for higher power output compared to placebo or control, which both gave very similar values. A number of delimitations were present in our study which could be addressed in a follow-up experimental design.

First, we applied only a single BR dose to the subjects prior to the experiment. In a study where administration of a single dose of BR was used on trained cyclists, there was no improvement on the subsequent 1-hr time-trial performance in well-trained cyclists (Cermak et al., 2012b). In a different study where a six-day supplementation with BR (0,5L/d) was followed, reduced VO□ during submaximal exercise and improved time-trial performance in trained cyclists (Cermak et al., 2012a). It is therefore possible that we may have needed a much higher dose of nitrate within the BR supplement and/or a prolonged administration of the supplement (as an example for six consecutive days) but following the literature sources we reached to the single dose as it was suggested. Another limitation was the short testing period, which may have not sufficed to test the effect of the supplement could not be measured. Indeed, it has been shown that 5-6 weeks are required for safe conclusions in endurance-performance studies using beetroot juice, time which was not used in this study. In this design, there was no harmonization of the administration of the supplement. The participants consumed the juices and were asked to take part in the exercises. There exists a chance that they disliked the taste and in this case they should be replaced. Another limitation is that the isokinetic dynamometer doesn't reflect to real life and has limited applicability, being used to measure force production for isolated muscle movements and not for whole body muscle groups.

Conclusion

Our results indicated that there was some measurable effect of a single dose of BR intake on either muscle strength or sprint performance in a set of seven healthy male subjects. While our obtained results were not statistically significant, a future experiment addressing the current delimitations mainly regarding the duration for supplement intake (for instance over six consecutive days like in a number of studies) may be necessary before any statistically significant data is obtained.

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