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The impact of beetroot juice supplementation on muscular endurance, maximal strength and countermovement jump performance.

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ABSTRACT

Purpose: Dietary nitrate has been shown to enhance muscle contractile function and has, therefore, been linked to increased muscle power and sprint exercise performance. However, the impact of dietary nitrate supplementation on maximal strength, performance and muscular endurance remains to be established.

Methods: Fifteen recreationally active males (25±4 y, BMI 24±3 kg/m^2) participated in a randomized double-blinded cross-over study comprising two 6-d supplementation periods; 140 mL/d nitrate-rich (BR; 985 mg/d) and nitrate-depleted (PLA; 0.37 mg/d) beetroot juice. Three hours following the last supplement, we assessed countermovement jump (CMJ) performance, maximal strength and power of the upper leg by voluntary isometric (30° and 60° angle) and isokinetic contractions (60, 120, 180 and 300°·s^{-1}), and muscular endurance (total workload) by 30 reciprocal isokinetic voluntary contractions at 180°·s^{-1}.

Results: Despite differences in plasma nitrate (BR: 879±239 vs PLA: 33±13 μmol/L, P<0.001) and nitrite (BR: 463±217 vs PLA: 176±50 nmol/L, P<0.001) concentrations prior to exercise testing, CMJ height (BR: 39.3±6.3 vs PLA: 39.6±6.3 cm; P=0.39) and muscular endurance (BR: 3.93±0.69 vs PLA: 3.90±0.66 kJ; P=0.74) were not different between treatments. In line, isometric strength (P>0.50 for both angles) and isokinetic knee extension power (P>0.33 for all velocities) did not differ between treatments. Isokinetic knee flexion power was significantly higher following BR compared with PLA ingestion at 60°·s^{-1} (P=0.001), but not at 120°·s^{-1} (P=0.24), 180°·s^{-1} (P=0.066), and 300°·s^{-1} (P=0.36).

Conclusion: Nitrate supplementation does not improve maximal strength, countermovement jump performance and muscular endurance in healthy, active males.

Keywords: Exercise; Fitness; Musculoskeletal; Nutrition

INTRODUCTION

Beetroot juice has become a popular supplement among athletes due to the recent research on the ergogenic properties of dietary nitrate. The effects of dietary nitrate ingestion have been attributed to its reduction to nitrite by oral bacteria, and the further reduction in the circulation to nitric oxide (NO), particularly in environments of low oxygen availability and low pH (Lundberg, Weitzberg, & Gladwin, 2008). NO is important for several physiological processes associated with exercise, including the regulation of blood flow and skeletal muscle contraction (Stamler & Meissner, 2001). NO has the ability to stimulate the conversion of cyclic guanosine monophosphate (cGMP), which causes relaxation of smooth muscle (i.e. vasodilation) (Katsuki, Arnold, Mittal, & Murad, 1977). Furthermore, an increase in cGMP increases the contraction velocity of skeletal muscle fibers and could, as such, increase peak power (Morrison, Miller, & Reid, 1996).

Due to its impact on blood flow and muscle contractile function, it has recently been suggested that nitrate supplementation can improve muscle strength and sprint performance (Jones, Thompson, Wylie, & Vanhatalo, 2018). Supplementation with beetroot juice has been shown to reduce the phosphocreatine cost of force production (Fulford et al., 2013). Furthermore, it has been suggested that nitrate supplementation can improve local perfusion, fatigue resistance, and contractility of low oxygenated type II (fast twitch) muscle fibers in particular (Jones, Ferguson, Bailey, Vanhatalo, & Poole, 2016). Therefore, dietary nitrate has the potential to improve maximal strength and sprint performance, during which mainly type II muscle fibers are recruited. A few studies reported that nitrate-rich beetroot juice improved maximal power output of inertial-load sprints in trained cyclists (Rimer, Peterson, Coggan, & Martin, 2016), maximal voluntary isokinetic knee extensor power and velocity in healthy subjects (Coggan et al., 2015) and time to reach peak power output during 30 s all out Wingate tests (Cuenca et al., 2018; Jonvik et al., 2018). Others reported no effect of nitrate supplementation on maximal isometric voluntary contractions, but enhancement of electrically evoked muscle force production (Haider & Folland, 2014; Whitfield et al., 2017).

Given the impact of dietary nitrate supplementation on repeated high-intensity exercise performance and maximal force production, it can be speculated that nitrate supplementation blunts the development of fatigue during repeated maximal contractions, and thus improves muscular endurance. Previous studies are conflicting regarding the effects of beetroot juice on repeated maximal contractions. Tillin, Moudy, Nourse, and Tyler (2018) reported that beetroot juice supplementation attenuated the decline in power during a series of maximal voluntary isometric knee extensions, while Coggan et al. (2015) reported no effect of beetroot juice supplementation on the decline in power during 50 reciprocal isokinetic contractions. A possible benefit of nitrate supplementation on maximal strength and repeated maximal efforts has been linked to direct performance effects of jumping exercises, as Clifford et al. (2016) showed that beetroot juice can reduce the decrement in countermovement jumps (CMJ) between bouts of repeated-sprint exercise.

Taken together, evidence regarding the impact of nitrate supplementation on strength outcomes is equivocal. This might, at least partly, be related to the heterogeneity in selected strength outcomes. To firmly establish the potential benefits of nitrate supplementation, we designed a comprehensive test battery with complementary strength outcomes, including maximal strength, muscular endurance, and strength performance. Hence, we aimed to assess the impact of beetroot juice supplementation on maximal isometric strength and isokinetic power, workload achieved during thirty reciprocal voluntary isokinetic contractions and CMJ performance in recreationally active males.

METHODS

Study design

In a randomized, double-blinded, placebo-controlled crossover design, participants underwent two 6-days supplementation periods of nitrate-rich (BR) and nitrate-depleted (PLA) beetroot juice, interspaced by a washout period of at least one week. The study included a screening and familiarization session (visit 1) and two experimental test days (visits 2 & 3). The two experimental test days were performed at day six of each supplementation period, at the same day of the week and same time of day (±1 h) for each individual. The experimental protocol and procedures were reviewed by the HAN University of Applied Sciences ethical advisory board, and conducted according to the principles of the Declaration of Helsinki (World Medical, 2013).

Participants

Fifteen young (18-40 y), non-obese (BMI<30 $kg \cdot m^{-2}$) recreationally active (exercising 0.5-8.0 h weekly), healthy males were included in the present study. Participants' characteristics are reported in Table 1. The participants were free from disease and injury, were non-smokers, and were currently not using dietary nitrate supplements. All participants were informed of the nature and possible risks of the experimental procedures before their written informed consent was obtained.

Supplementation

Over two 6-day periods, participants ingested 2x70 mL nitrate-rich (BR; 985 mg nitrate) or nitrate-depleted (PLA; 0.37 mg nitrate) beetroot juice per day divided over breakfast and dinner. The nitrate-rich and nitrate-depleted beetroot juice were produced by the same manufacturer (Beet it, James White Drinks Ltd., Ipswich, UK). The products could not be discerned by taste, smell, texture, or colour. All researchers and participants were blinded to treatment assignment for the duration of the study. To stimulate compliance with the supplementation protocol, participants received a daily reminder by text message from the researchers. On day 6 (test day) participants ingested the last two supplemental bottles exactly 3 h prior to the start of the performance tests. The order of supplementation was BR-PLA for 8 participants and PLA-BR for the other 7 participants. There was a washout period of at least one week between the supplementation periods.

Study procedures

During visit 1, baseline characteristics were obtained, including participants' height, body mass, and blood pressure (Omron HEM-907; Omron Healthcare Inc.). Subsequently, participants were familiarized with all exercise testing procedures as described below. On day 6 of each experimental period, participants arrived at the laboratory ~2h30min after ingesting the last 2 supplements. Fifteen min before exercise testing, a single venous blood sample (5 mL) was obtained. Following a 5-min warm-up on a cycle ergometer, participants performed five countermovement jumps (CMJ) on a ground force platform (AMTI BP400600-1K) to assess the ground reaction force and vertical jump height. Participants began the CMJ in standing position with the hands placed on the hips, dropped into the squat position, and then immediately jumped vertically as high as possible. One minute of rest was allowed between each attempt. Force platform data were collected using Nexus software (Vicon Motion Systems, Oxford, UK) with a sampling frequency of 100 Hz and a low-pass filter. Peak ground reaction force ($yGRF_{max}$) was determined and peak jump height (derived from time of flight (t) and gravitation constant (g): $½*g*(t/2)^2$) was calculated in MATLAB (Mathworks, Natick, MA). The coefficient of variation (CV) for CMJ height is ~2.5 to 5% (Cormack, Newton, McGuigan, & Doyle, 2008; Moir, Button, Glaister, & Stone, 2004).

Subsequently, participants performed isometric and isokinetic strength testing of the right upper leg on a dynamometer (Humac Norm Isokinetic Extremity System, CSMI, Stoughton

MA, USA). Participants were placed in an upright seated position with the back-chair seat set to an angle of 85° and fastened to the seat and lever arm of the dynamometer by torso, thigh, and shin straps to reduce contribution of any irrelevant body movement. The dynamometer was adjusted so that the femoral epicondyle was in line with the axis of rotation of the lever arm. Three maximal voluntary isometric knee extensions were performed for a duration of 4 s at a knee angle of 30° as well as at 60°, with 60 s rest intervals between successive attempts. Subsequently, maximally voluntary isokinetic knee extension and flexion were determined at angular velocities of 60, 120, 180 and 300°$\cdot$s^{-1} (1.05, 2.09, 3.14 and 5.24 rad$\cdot$s^{-1}). At all velocities, five reciprocal contractions were performed. For each isometric and isokinetic test, the single best contraction (isokinetic flexion and extension separately) was used for analyses, and using maximal strength (Nm) and velocity (s), the maximal power (W) was calculated at each angular velocity. The CVs of isometric (Morton et al., 2005) and isokinetic MVC (Impellizzeri, Bizzini, Rampinini, Cereda, & Maffiuletti, 2008) of the upper leg are generally <5%. Finally, isokinetic muscular endurance was determined by 30 reciprocal contractions at an angular velocity of 180°$\cdot$s^{-1} (3.14 rad$\cdot$s^{-1}). Total area under the curve (AUC) for the 30 reciprocal contractions was calculated to determine total work (kJ) as a measure of muscular endurance. We calculated total work for isokinetic knee extensions and flexions separately and combined (the sum of extension and flexion total work). The CV of the total work performed during 30 reciprocal contractions is <5% (Gleeson & Mercer, 1992). Fatigue index was calculated as the percentage change in work between the first 10 and the final 10 contractions. Participants were instructed and verbally encouraged to execute each contraction with maximal force and were given verbal feedback on the number of repetitions. Between each different isokinetic test, participants rested for 90 s. All isokinetic tests were performed over an 85º range of motion with the knee fully extended being 0º. Analyses were performed over a 10-75º range of motion to dismiss any end-range deceleration. Data were generated using the Humac Norm system's software package and MATLAB.

Blood sampling and plasma analyses

Blood samples were obtained in lithium-heparin containing tubes, and centrifuged immediately at 1,000 *g* for 5 min, at 20±1°C. Aliquots of the plasma were transferred to 2 mL Eppendorf tubes and immediately frozen in a –20°C freezer, before being stored in a –80°C freezer for subsequent analysis. Plasma nitrate and nitrite concentrations were determined after reduction to NO using the chemiluminescence technique (NOA; Sievers NOA 280i; Analytix, Durham, UK) as described previously (Jonvik et al., 2016).

Physical activity and dietary standardization

Participants recorded their dietary intake 48 h prior to the first test day (visit 2). They received a copy of this dietary record, and were subsequently instructed to replicate their dietary intake 48 h prior to the second test day (visit 3). Participants did not consume caffeine (12 h) and alcohol (24 h) prior to each test day. No restrictions were set for the intake of nitrate-rich foods during the intervention period, to allow for the determination of the additional effect of nitrate supplementation on plasma nitrate and nitrite concentrations and performance outcomes on top of the normal diet (Vanhatalo et al., 2010). However, to prevent any attenuation in the reduction of nitrate to nitrite in the oral cavity by commensal bacteria, participants were asked to refrain from using any antibacterial mouthwash/toothpaste, chewing gum and tongue-scraping during the supplementation period (Govoni, Jansson, Weitzberg, & Lundberg, 2008). In addition to the dietary standardization, participants were instructed to refrain from any heavy physical exercise 48 h prior to the test days.

Sample size and statistical analysis

Sample size was calculated using GPower version 3.1.9.2. Given the reported increments in maximal knee extensor power and velocity by Coggan et al. (2015), and the potential of acute dietary interventions to improve CMJ performance (Del Coso et al., 2014), we assumed a 5±6% greater CMJ height in BR compared with PLA. With 0.8 power to detect a significant difference ($P<0.05$, two-sided), a minimum of 14 participants was required for this cross-over study.

Statistical analyses were carried out using SPSS 22.0 (IBM Corp., Armonk, USA). Data were checked for normality using Kolmogorov–Smirnov tests. Data obtained during exercise testing in the PLA and BR condition were compared by paired t-tests or Wilcoxon signed-rank tests for normally and non-normally distributed variables, respectively. Statistical significance was set at $P<0.05$. In case of significant outcomes, the differences were further analyzed to assess whether individual changes were meaningful, taking the smallest worthwhile change (SWC) and typical error into account. For this purpose, we analyzed whether the individual '50% true change CI' exceeded the smallest worthwhile change (SWC), according to the approach recently described by Swinton and colleagues (Swinton et al., 2018).

Data are presented as means ± SD, or medians [IQR] where appropriate.

RESULTS

Plasma nitrate and nitrite concentrations

Plasma nitrate concentrations were ~26 fold higher following BR vs PLA (870±239 vs 33±13 µmol/L; *P*<0.001), while plasma nitrite concentrations were ~3 fold higher (463±217 vs 176±50 nmol/L; *P*<0.001).

Maximal isometric strength and isokinetic power

Maximal isometric knee extensor strength at 30° knee flexion (204±39 vs 200±37 Nm; *P*=0.50) and at 60° knee flexion (286±43 vs 285±47 Nm; *P*=0.90; Figure 1A) did not differ between BR and PLA, respectively. For the isokinetic tests, no differences in maximal power between BR and PLA were found for maximal knee extensions at any velocity (60°·s^{-1}: 220±45 vs 218±40 W; *P*=0.69; 120°·s^{-1}: 392±74 vs 387±62 W; *P*=0.49; 180°·s^{-1}: 500±86 vs 487±67 W; *P*=0.33; 300°·s^{-1}: 554±102 vs 544±81 W; *P*=0.50; Figure 1B). However, maximal knee flexion power improved marginally by BR at 60°·s^{-1} (151 [144-157] vs 148 [135-154] W; *P*=0.001), with a meaningful change being observed in 6 out of 15 participants. A comparable change was seen for maximal knee flexion power at 180°·s^{-1} (391±57 vs 380±58 W), although this finding did not reach statistical significance (*P*=0.066). Moreover, the magnitude of change for this outcome appeared to be meaningful for only 1 of the 15 participants. No significant differences in knee flexor power between BR and PLA were seen at 120°·s^{-1} (392±74 vs 387±62 W; *P*=0.24) and 300°·s^{-1} (493±73 vs 485±81 W; *P*=0.36; Figure 1C).

Countermovement jump performance

The maximal vertical jump height during a countermovement jump did not differ between BR and PLA (39.3±6.3 vs 39.6±6.3 cm; *P*=0.39; Figure 2). The peak ground reaction force also did not differ between BR and PLA (3,040 [3,035-3,087] vs 3,055 [3,035-3,086] N; *P*=0.57).

Muscular endurance

There was no difference between BR and PLA for total workload over the 30 reciprocal isokinetic repetitions (3.93±0.69 vs 3.90±0.66 KJ; *P*=0.74; Figure 3). Neither did the total workload differ between BR and PLA when analyzed for the extensions (2.18±0.41 vs 2.15±0.35 KJ; *P*=0.58) and flexions (1.75±0.35 vs 1.75±0.34 KJ; *P*=0.94), respectively. In line, the fatigue index (the decline in work performed for the 10 last compared to the 10 first contractions) did not differ between BR and PLA for either extensions (28±9 vs 31±14%; *P*=0.27) or flexors (34±11 vs 34±12%; *P*=0.73), respectively.

DISCUSSION

Six days of beetroot juice supplementation substantially increased plasma nitrate and nitrite concentrations, but did not improve maximal isometric knee extensor strength and isokinetic knee extension power, countermovement jump performance, and muscular endurance. A small, but significant effect of beetroot juice supplementation was seen for some indicators of maximal knee flexor power.

The increase in plasma nitrate and nitrite concentrations following beetroot juice was comparable to our previous studies in athletes (Jonvik et al., 2018; Jonvik, Van Dijk, Senden, Van Loon, & Verdijk, 2017; Nyakayiru et al., 2017). Despite a successful supplementation protocol, we observed no benefits of short-term nitrate-rich beetroot juice supplementation on maximal isometric knee extensor strength. This is in line with most literature on maximal isometric strength (Aucouturier, Boissiere, Pawlak-Chaouch, Cuvelier, & Gamelin, 2015; Fulford et al., 2013; Haider & Folland, 2014). Only Bender et al. (2018) reported an improvement in peak force of isometric mid-thigh pulls following beetroot juice supplementation, although this improvement did not translate into improved repeated-sprint performance.

We observed no effects of short-term nitrate-rich beetroot juice supplementation on isokinetic knee extensor power for any velocity (up to 300°·s^{-1}). This finding partly confirms the findings of (Coggan et al., 2015), who reported no improvements in isokinetic knee extensor power up to a velocity of 270°·s^{-1}. However, they did report improvements in isokinetic knee extensor power at the highest velocity tested (360°·s^{-1}), which was extrapolated to a greater estimated maximal power and velocity following beetroot juice supplementation. By increasing the intracellular release of calcium in the muscle (Stamler & Meissner, 2001), it has been speculated that dietary nitrate is most effective during the initial phase of contraction where the calcium saturation normally is incomplete (Haider & Folland, 2014). As such, the impact of nitrate supplementation may be greater during high velocity contractions where the acceleration phase comprises a great part of the contraction. We cannot rule out that even higher velocities of contraction than used in the current study (300°·s^{-1}) would have led to an improvement following beetroot juice. However, our positive findings on maximal flexor power at the lower velocities contrast with this view.

Despite the lack of effect on maximal knee extensor power, we observed a significant effect of beetroot juice on maximal knee flexor power at 60°·s^{-1}. A similar benefit from beetroot juice was seen for flexor power at 180°·s^{-1}, although this finding did not reach statistical significance (*P*=0.066). Even though these findings are encouraging, the magnitude of change induced by beetroot juice appeared to be meaningful for the smaller part of the participants. Hence, the practical relevance of these findings for athletic performance remain to be evaluated in exercises or sports that are highly dependent on hamstring contractions such as sprinting or certain combat sports.

In the current study, CMJ performance was included as an integrated measurement of strength performance. In line with the lack of effect on maximal power, beetroot juice did not improve jump height or ground reaction force during countermovement jumps (CMJ). This seems logical as functional performance of jumping is highly reliant on the maximal force production (Peterson, Alvar, & Rhea, 2006). In agreement with our findings, Cuenca et al. (2018) also found no effects of beetroot juice supplementation on CMJ performance in a non-fatigued state. In a fatigued state on the other hand, it has been reported that beetroot juice may reduce the decrement in CMJ performance between successive bouts of repeated-sprint exercise (Clifford et al., 2016). This could be explained by the theory that beetroot juice supplementation may reduce the phosphocreatine cost of force production (Fulford et al., 2013), thereby delaying fatigue development for repeated maximal efforts. However, three other studies have been unable to detect any effects of beetroot juice supplementation on the decrement in CMJ during recovery from strenuous exercise (Clifford, Allerton, et al., 2017; Clifford, Howatson, West, & Stevenson, 2017; Cuenca et al., 2018), which seem to invalidate the proposed theory.

To investigate the impact of short-term beetroot juice supplementation on fatigue development during repeated maximal isokinetic contractions, participants in our study were subjected to a muscular endurance protocol comprising 30 maximal reciprocal isokinetic contractions of the knee extensors and flexors. Neither the total work conducted during the 30 reciprocal contractions, nor the fatigue index (reduction in power from the first 10 to the final 10 contractions) were improved following nitrate-rich beetroot juice supplementation. These findings are in line with those of Coggan et al. (2015) who found no differences in muscle function during 50 reciprocal isokinetic contractions following beetroot juice supplementation in healthy men and women. In contrast, Tillin et al. (2018) found that beetroot juice supplementation attenuated the decline in power during 60 reciprocal isometric 3 s contractions (2 s rest in between). The fatigue index (reduction in power from the first 6 to the final 6 contractions) was above 50% in their study, which is more substantial than the fatigue index of ~30% in our study. Hence, beetroot juice supplementation might be beneficial during more extreme fatigue development than tested in the current study.

In the current study, we assessed muscular strength and endurance along with countermovement jump performance. Despite the extensive assessment of muscular strength and endurance, we should also consider some potential limitations. During an extensive exercise testing battery, accumulating fatigue may potentially interfere with consecutive exercise tests. However, it can be argued that the total volume of exercise testing was low, thereby minimizing the potential interference between consecutive exercise tests. Secondly, participants were tested in a non-fasted state with some participants being tested in the morning, whereas others were tested in the afternoon. Although this approach reflects real-life practice, there could be an interaction between food intake prior to exercise testing and the impact of nitrate supplementation. It should be noted, however, that food intake prior to exercise testing and time of exercise testing were standardized within participants, thereby minimizing noise of repeated exercise testing. The current study included healthy recreationally active individuals. Despite the fact that we did not observe different responses to beetroot juice between various training levels in our previous work (Jonvik et al. 2018), the current findings cannot be simply generalized to other populations such as females, well-trained and elite athletes, or patient groups.

CONCLUSION

Nitrate supplementation through daily beetroot juice ingestion did not improve maximal strength, countermovement jump performance and muscular endurance in healthy, active males.

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DISCLOSURE STATEMENT

KLJ, DH, HBP and MdN declare that they have no conflict of interest. Over the last 5 years, LBV, LJCvL and JWvD have received research grants, consulting fees, and/or speaking honoraria from FrieslandCampina, and. LBV and LJCvL have received research grants, consulting fees, and speaking honoraria from Pepsico.

REFERENCES

Aucouturier, J., Boissiere, J., Pawlak-Chaouch, M., Cuvelier, G., & Gamelin, F. X. (2015). Effect of dietary nitrate supplementation on tolerance to supramaximal intensity intermittent exercise. *Nitric Oxide, 49*, 16-25. doi: 10.1016/j.niox.2015.05.004

Bender, D., Townsend, J. R., Vantrease, W. C., Marshall, A. C., Henry, R. N., Heffington, S. H., & Johnson, K. D. (2018). Acute beetroot juice administration improves peak isometric force production in adolescent males. *Appl Physiol Nutr Metab, 43*(8), 816-821. doi: 10.1139/apnm-2018-0050

Clifford, T., Allerton, D. M., Brown, M. A., Harper, L., Horsburgh, S., Keane, K. M., . . . Howatson, G. (2017). Minimal muscle damage after a marathon and no influence of beetroot juice on inflammation and recovery. *Appl Physiol Nutr Metab, 42*(3), 263-270. doi: 10.1139/apnm-2016-0525

Clifford, T., Berntzen, B., Davison, G. W., West, D. J., Howatson, G., & Stevenson, E. J. (2016). Effects of Beetroot Juice on Recovery of Muscle Function and Performance between Bouts of Repeated Sprint Exercise. *Nutrients, 8*(8). doi: 10.3390/nu8080506

Clifford, T., Howatson, G., West, D. J., & Stevenson, E. J. (2017). Beetroot juice is more beneficial than sodium nitrate for attenuating muscle pain after strenuous eccentric-bias exercise. *Appl Physiol Nutr Metab*. doi: 10.1139/apnm-2017-0238

Coggan, A. R., Leibowitz, J. L., Kadkhodayan, A., Thomas, D. P., Ramamurthy, S., Spearie, C. A., . . . Peterson, L. R. (2015). Effect of acute dietary nitrate intake on maximal knee extensor speed and power in healthy men and women. *Nitric Oxide, 48*, 16-21. doi: 10.1016/j.niox.2014.08.014

Cormack, S. J., Newton, R. U., McGuigan, M. R., & Doyle, T. L. (2008). Reliability of measures obtained during single and repeated countermovement jumps. *Int J Sports Physiol Perform, 3*(2), 131-144. doi: 10.1123/ijspp.3.2.131

Cuenca, E., Jodra, P., Perez-Lopez, A., Gonzalez-Rodriguez, L. G., Fernandes da Silva, S., Veiga-Herreros, P., & Dominguez, R. (2018). Effects of Beetroot Juice Supplementation on Performance and Fatigue in a 30-s All-Out Sprint Exercise: A Randomized, Double-Blind Cross-Over Study. *Nutrients, 10*(9). doi: 10.3390/nu10091222

Del Coso, J., Perez-Lopez, A., Abian-Vicen, J., Salinero, J. J., Lara, B., & Valades, D. (2014). Enhancing physical performance in male volleyball players with a caffeine-containing energy drink. *Int J Sports Physiol Perform, 9*(6), 1013-1018. doi: 10.1123/ijspp.2013-0448

Fulford, J., Winyard, P. G., Vanhatalo, A., Bailey, S. J., Blackwell, J. R., & Jones, A. M. (2013). Influence of dietary nitrate supplementation on human skeletal muscle metabolism and force production during maximum voluntary contractions. *Pflugers Arch, 465*, 517-528. doi: 10.1007/s00424-013-1220-5. Epub 2013 Jan 26.; ID: 749

Gleeson, N. P., & Mercer, T. H. (1992). Reproducibility of isokinetic leg strength and endurance characteristics of adult men and women. *Eur J Appl Physiol Occup Physiol, 65*(3), 221-228. doi: 10.1007/bf00705085

Govoni, M., Jansson, E., Weitzberg, E., & Lundberg, J. (2008). The increase in plasma nitrite after a dietary nitrate load is markedly attenuated by an antibacterial mouthwash. *Nitric Oxide, 19*, 333-337.

Haider, G., & Folland, J. P. (2014). Nitrate supplementation enhances the contractile properties of human skeletal muscle. *Med Sci Sports Exerc, 46*(12), 2234-2243. doi: 10.1249/MSS.0000000000000351

Impellizzeri, F. M., Bizzini, M., Rampinini, E., Cereda, F., & Maffiuletti, N. A. (2008). Reliability of isokinetic strength imbalance ratios measured using the Cybex NORM dynamometer. *Clin Physiol Funct Imaging, 28*(2), 113-119. doi: 10.1111/j.1475-097X.2007.00786.x

Jones, A. M., Ferguson, S. K., Bailey, S. J., Vanhatalo, A., & Poole, D. C. (2016). Fiber Type-Specific Effects of Dietary Nitrate. *Exerc Sport Sci Rev, 44*(2), 53-60. doi: 10.1249/JES.0000000000000074

Jones, A. M., Thompson, C., Wylie, L. J., & Vanhatalo, A. (2018). Dietary Nitrate and Physical Performance. *Annu Rev Nutr, 38*, 303-328. doi: 10.1146/annurev-nutr-082117-051622

Jonvik, K. L., Nyakayiru, J., Pinckaers, P. J., Senden, J. M., van Loon, L. J., & Verdijk, L. B. (2016). Nitrate-Rich Vegetables Increase Plasma Nitrate and Nitrite Concentrations and Lower Blood Pressure in Healthy Adults. *J Nutr*. doi: 10.3945/jn.116.229807

Jonvik, K. L., Nyakayiru, J., van Dijk, J. W., Maase, K., Ballak, S. B., Senden, J. M. G., . . . Verdijk, L. B. (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*, 1-10. doi: 10.1080/17461391.2018.1433722

Jonvik, K. L., Van Dijk, J. W., Senden, J. M. G., Van Loon, L. J. C., & Verdijk, L. B. (2017). The Effect of Beetroot Juice Supplementation on Dynamic Apnea and Intermittent Sprint Performance in Elite Female Water Polo Players. *Int J Sport Nutr Exerc Metab*, 1-20. doi: 10.1123/ijsnem.2017-0293

Katsuki, S., Arnold, W., Mittal, C., & Murad, F. (1977). Stimulation of guanylate cyclase by sodium nitroprusside, nitroglycerin and nitric oxide in various tissue preparations and comparison to the effects of sodium azide and hydroxylamine. *J Cyclic Nucleotide Res, 3*(1), 23-35.

Lundberg, J. O., Weitzberg, E., & Gladwin, M. T. (2008). The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nature Review Drug Discovery, 7*, 156-167.

Moir, G., Button, C., Glaister, M., & Stone, M. H. (2004). Influence of familiarization on the reliability of vertical jump and acceleration sprinting performance in physically active men. *J Strength Cond Res, 18*(2), 276-280. doi: 10.1519/R-13093.1

Morrison, R. J., Miller, C. C., 3rd, & Reid, M. B. (1996). Nitric oxide effects on shortening velocity and power production in the rat diaphragm. *J Appl Physiol (1985), 80*(3), 1065-1069. doi: 10.1152/jappl.1996.80.3.1065

Morton, J. P., Atkinson, G., MacLaren, D. P., Cable, N. T., Gilbert, G., Broome, C., . . . Drust, B. (2005). Reliability of maximal muscle force and voluntary activation as markers of exercise-induced muscle damage. *Eur J Appl Physiol, 94*(5-6), 541-548. doi: 10.1007/s00421-005-1373-9

Nyakayiru, J., Jonvik, K. L., Trommelen, J., Pinckaers, P. J., Senden, J. M., van Loon, L. J., & Verdijk, L. B. (2017). Beetroot Juice Supplementation Improves High-Intensity Intermittent Type Exercise Performance in Trained Soccer Players. *Nutrients, 9*(3). doi: 10.3390/nu9030314

Peterson, M. D., Alvar, B. A., & Rhea, M. R. (2006). The contribution of maximal force production to explosive movement among young collegiate athletes. *J Strength Cond Res, 20*(4), 867-873. doi: 10.1519/R-18695.1

Rimer, E. G., Peterson, L. R., Coggan, A. R., & Martin, J. C. (2016). Increase in Maximal Cycling Power With Acute Dietary Nitrate Supplementation. *Int J Sports Physiol Perform, 11*(6), 715-720. doi: 10.1123/ijspp.2015-0533

Stamler, J. S., & Meissner, G. (2001). Physiology of nitric oxide in skeletal muscle. *Physiological Reviews, 81*(1), 209-237.

Swinton, P. A., Hemingway, B. S., Saunders, B., Gualano, B., & Dolan, E. (2018). A Statistical Framework to Interpret Individual Response to Intervention: Paving the Way for Personalized Nutrition and Exercise Prescription. *Front Nutr, 5*, 41. doi: 10.3389/fnut.2018.00041

Tillin, N. A., Moudy, S., Nourse, K. M., & Tyler, C. J. (2018). Nitrate Supplement Benefits Contractile Forces in Fatigued but Not Unfatigued Muscle. *Med Sci Sports Exerc, 50*(10), 2122-2131. doi: 10.1249/MSS.0000000000001655

Vanhatalo, A., Bailey, S., Blackwell, J., DiMenna, F., Pavey, T. G., Wilkerson, D. P., . . . Jones, A. M. (2010). Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate-intensity and incremental exercise. *American Journal of Physiology. Regulatory, integrative and comparative physiology, 299*, 1121-1131.

Whitfield, J., Gamu, D., Heigenhauser, G. J. F., LJC, V. A. N. L., Spriet, L. L., Tupling, A. R., & Holloway, G. P. (2017). Beetroot Juice Increases Human Muscle Force without Changing Ca2+-Handling Proteins. *Med Sci Sports Exerc, 49*(10), 2016-2024. doi: 10.1249/MSS.0000000000001321

World Medical, A. (2013). World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA, 310*(20), 2191-2194. doi: 10.1001/jama.2013.281053

Table 1 Participants' characteristics[1]

N	15
Age, y	25±4
Height, m	1.82±0.08
Weight, kg	81±10
BMI, kg·m^{-2}	24±3
Systolic BP, mmHg	129±11
Diastolic BP, mmHg	69±6
Resting HR, beats·min^{-1}	68±10

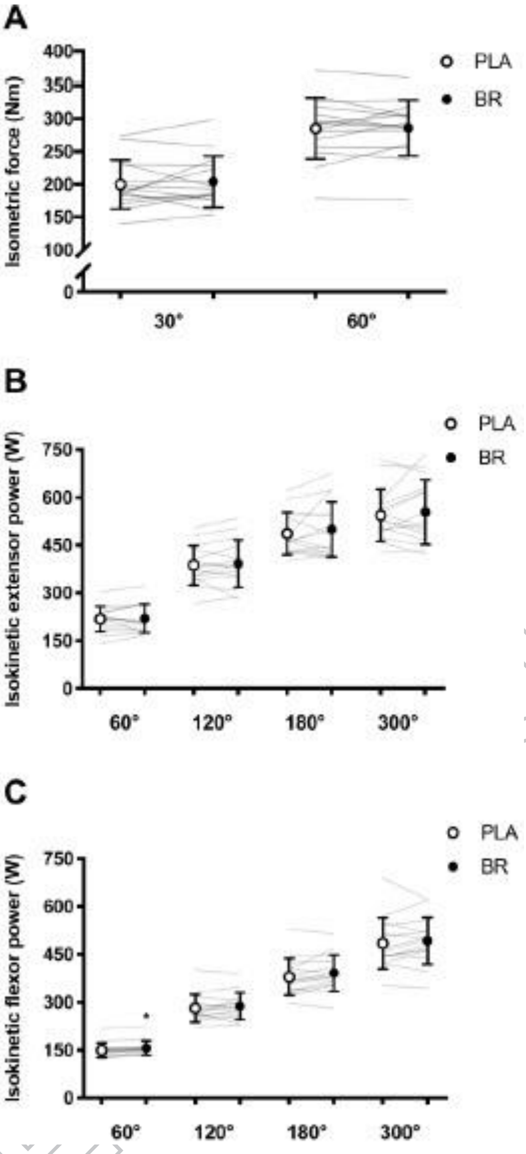
[1] Values are means±SDs. BP: blood pressure, HR: heart rate.

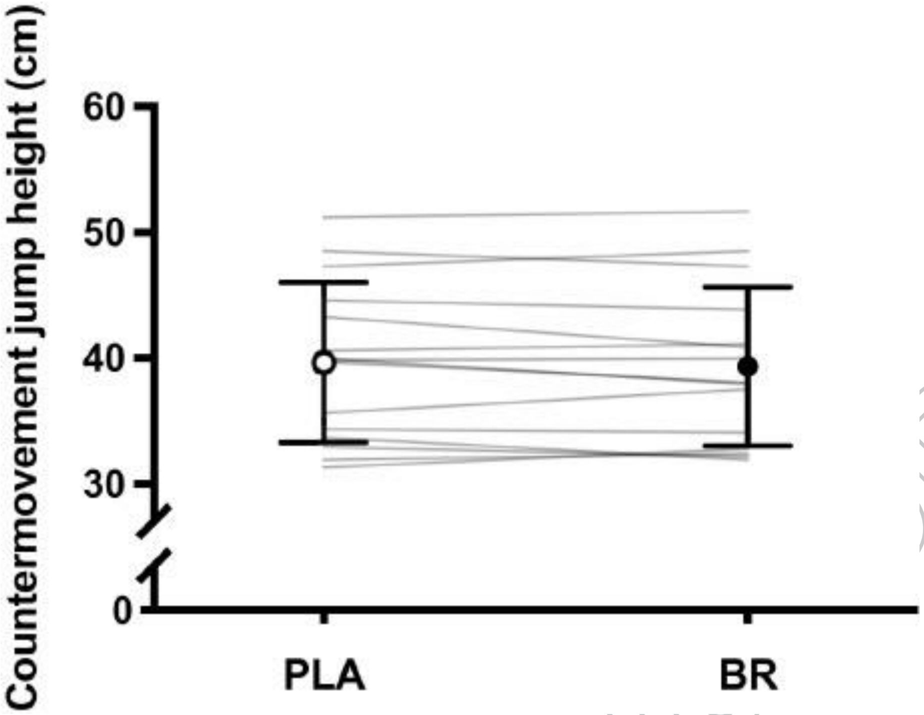
FIGURE CAPTIONS

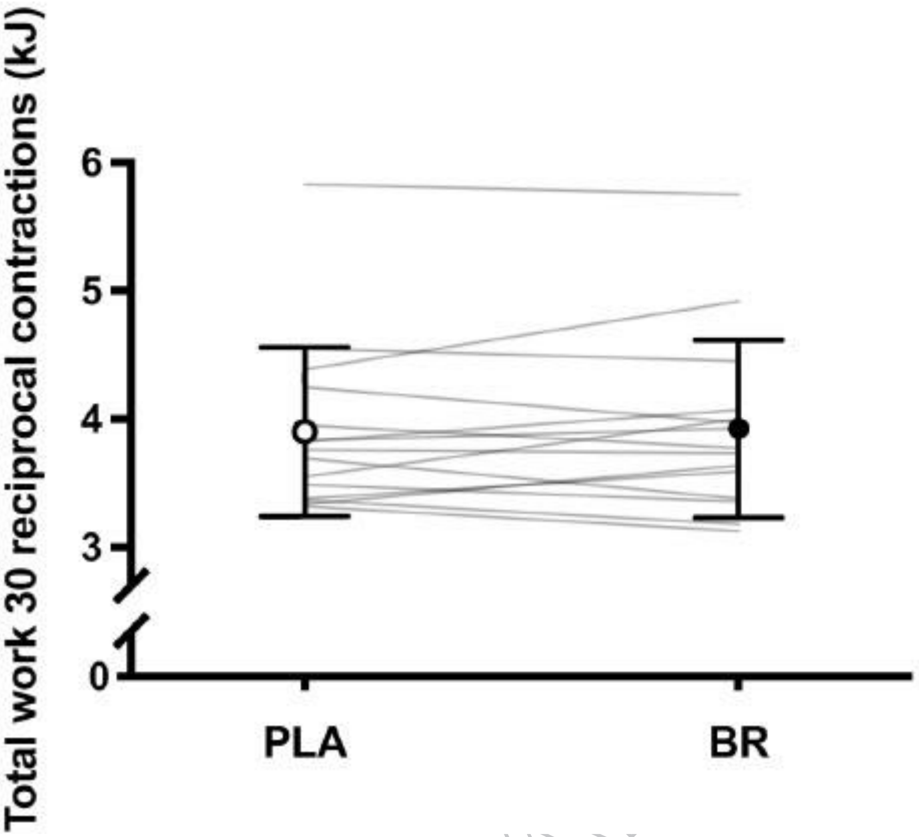
Figure 1: Maximal knee extensor strength measured by isometric force at 30° and 60° knee flexion angle (A), and maximal isokinetic knee extensor- (B) and knee flexor (C) power at velocities of 60, 120, 180 and 300°·s^{-1} (*n*=15). PLA: placebo (white symbol), BR: beetroot juice (black symbol). The grey lines represent individual cases, the black lines and symbols represent means±SD. *Significantly different from PLA.

Figure 2: Countermovement jump (CMJ) height (*n*=15). PLA: placebo (white symbol), BR: beetroot juice (black symbol). The grey lines represent individual cases, the black lines and symbols represent means±SD.

Figure 3: Muscular endurance measured as total work (kJ) for 30 reciprocal isokinetic contractions (*n*=15). PLA: placebo (white symbol), BR: beetroot juice (black symbol). The grey lines represent individual cases, the black lines and symbols represent means±SD.


A
Isometric force (Nm)
400
350
300
250
200
150
100
0
30°
60°
PLA
BR
B
Isokinetic extensor power (W)
750
600
450
300
150
0
60°
120°
180°
300°
PLA
BR
C
Isokinetic flexor power (W)
750
600
450
300
150
0
*
60°
120°
180°
300°
PLA
BR


Countermovement jump height (cm)
60
50
40
30
0
PLA
BR


Total work 30 reciprocal contractions (kJ)
6
5
4
3
0
PLA
BR