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Section: Rapid Communication

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Running Head: Beetroot and water polo performance

Journal: *International Journal of Sport Nutrition and Exercise*

Acceptance Date: November 26, 2017

DOI: https://doi.org/10.1123/ijsnem.2017-0293

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The effect of beetroot juice supplementation on dynamic apnea and intermittent sprint performance in elite female water polo players

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Running title: Beetroot and water polo performance

Word count main text: 2346 (introduction through conclusion, exclusive references)

Tables: 1 **Figures: 3**

Trial number: NCT02271633, www.trialregister.nl

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ABSTRACT

Nitrate-rich beetroot juice is thought to have ergogenic effects, particularly in conditions where oxygen availability is limited. Whether these effects also apply to elite athletes is currently unknown. The aim of this study was to assess the effects of beetroot juice supplementation on dynamic apnea and intermittent sprint performance in elite female water polo players. In a double-blind, randomized crossover manner the Dutch National female water polo team (*n*=14) was subjected to two 6-day supplementation periods (I and II), with either 140 mL/d nitrate-rich (BR; ~800 mg/d nitrate) or nitrate-depleted (PLA) beetroot juice. Following blood sampling on day six the athletes performed a maximal distance front crawl swimming-test without breathing (dynamic apnea test). In addition, intermittent sprint performance was assessed by performing 16 swim sprints of 15 m, in a 4x4 block with 30-s recovery between blocks (intermittent test). Distance covered during the dynamic apnea test did not differ between BR (49.5±7.8 m) and PLA (46.9±9.1 m, *P*=0.178). However, when correcting for test-order, distance covered was significantly larger in BR vs PLA when BR was ingested in period II (50.1±8.5 vs 42.8±5.7 m; *P*=0.002) whereas no difference was observed when BR was ingested in period I (48.8±7.4 vs 52.3±10.4 m; *P*=0.10). The time to complete the intermittent test was not different between BR and PLA (316.0±7.9 vs 316.3±6.9 s, *P*=0.73). In conclusion, beetroot juice supplementation does not improve intermittent performance in elite female water polo players, but there may be a potential for ergogenic effects during dynamic apnea.

Key words: nitrate; nitrite; ergogenic

INTRODUCTION

Over the past decade, the use of dietary nitrate to enhance performance has received increased attention, with possible ergogenic effects being caused by the reduction of dietary nitrate into nitrite and nitric oxide (NO) (Lundberg et al., 2008). NO plays a key role in skeletal muscle function, e.g. by regulating blood flow and muscle contractility (Stamler & Meissner, 2001). Hypoxic conditions with low oxygen availability and low pH environment can stimulate the nitrate-nitrite-NO pathway (Jones, 2014).

Several studies have found ergogenic effects of nitrate supplementation when exercising under hypoxic conditions. This could be 'local' tissue hypoxia such as during anaerobic, high-intensity intermittent exercise (Nyakayiru et al., 2017; Thompson et al., 2016; Wylie et al., 2013), or 'systemic' normobaric/hypobaric hypoxia (Carriker et al., 2016; Vanhatalo et al., 2010). Although 'systemic hypoxia' has also been used with respect to maximal under-water exercise (Schagatay, 2010), the term 'dynamic apnea' more appropriately reflects the different physiological characteristics associated with these breath-hold activities. Interestingly, recent work suggests that dietary nitrate may also prove beneficial under conditions of dynamic apnea (Patrician & Schagatay, 2017).

Water polo represents a high-intensity intermittent-type sport performed in and under water. Similar to the intermittent-type activity in soccer players (Krustrup et al., 2006), recruitment of type II muscle fibers is likely of key importance for water polo. Considering their relatively low oxygen tension, nitrate supplementation may be particularly effective in these type II muscle fibers (Bailey et al., 2015). Additionally, the sequence of short periods of under-water (breath-hold) activities in water polo could represent a condition in which dietary nitrate may prove beneficial (Patrician & Schagatay, 2017). Furthermore, although it has been postulated that the ergogenic properties of nitrate may be reduced in well-trained endurance athletes vs untrained individuals (Porcelli et al., 2015), the effect of nitrate supplementation has not been investigated in *elite* team-sport athletes. Therefore, the aim of the current study was to assess the effects of dietary nitrate supplementation on dynamic apnea and intermittent-type sprint performance in elite female water polo players.

METHODS

Subjects

We recruited the female Dutch National water polo selection (*n*=18), whereof two athletes could not participate due to injuries. Out of 16 athletes, one was excluded due to illness, and one due to lack of compliance with study protocol; 14 athletes completed the study (**Table 1**). The team was in preparation for the 2016 Olympic Games qualification, three months after winning World Championship silver, training 7±1 sessions (~12 h) per week. All athletes provided written informed consent to participate in the study that was approved by the local ethical committee CMO, Arnhem and Nijmegen, The Netherlands and was conducted in accordance with the Declaration of Helsinki (2013).

Study design

In a randomized, double-blinded crossover design, subjects underwent two 6-d supplementation periods (I and II), with either 140 mL/d nitrate-rich (BR; ~800 mg/d nitrate) or nitrate-depleted (PLA; placebo) beetroot juice (Beet It, James White Drinks Ltd., Ipswich, UK). Over a 4-week period, subjects underwent a screening and familiarization session (visit 1) and two experimental test days identical to visit 1, except for the 6-d supplementation periods (test days I and II). Intervention periods were interspaced by a 1-week washout. The exercise tests were performed at the same time of day (±10 min).

Experimental protocol

The athletes had practiced both the dynamic apnea and the intermittent test several times in training settings. On visit 1, they performed a standardized familiarization of both exercise tests. Testing was performed in a 25x30 m swimming pool at 28°C. The dynamic apnea test was adapted from apnea diving, which includes maximal distance swimming in shallow water (Schagatay, 2010). To create a setting more relevant to water polo, continuous front-crawl swimming was performed with the head kept under water, until exhaustion. Hyperventilation preceding the test was not allowed as this can affect performance and is difficult to standardize (Patrician & Schagatay, 2017). The test was performed individually and the pace, floating distance, start and turning technique were standardized. The main outcome was the completed distance when the athlete came to the surface to breath, measured to the closest 0.5 m using video recording. Secondary outcomes were apnea time and speed. The intermittent test consisted of 4x4 15-m swim sprints in the middle of the pool, interspersed with 5 s active rest (arms stretched above the head) between each sprint and 30 s semi-active rest (wrists above water) between each block of 4 sprints. This time-trial is adapted from water polo-specific performance tests previously validated in elite female water polo players (Tan et al., 2009), and was frequently used as performance test during regular training sessions. The intermittent test was performed in heats of 5-6 athletes, using the same heats and starting lanes during both test days. Each athlete had an individual supervisor clocking her in and out of the rest periods, and providing her a 'go' signal by lowering the arm.

Supplements were ingested for five days at home and the sixth dose was provided on the test day at the sports facility. Subjects underwent baseline and 2.5-h post-ingestion measurements of plasma, saliva and GI tolerance questionnaires. Plasma and saliva were collected and analyzed for nitrate and nitrite using chemiluminescence (Sievers NOA 280i; Analytix) as previously described (Jonvik et al., 2016). Following a standardized warm-up protocol, the dynamic apnea test started 3 h post-ingestion. Each athlete had 30-35 min rest between the dynamic apnea and the intermittent test. The rate of perceived exertion (RPE) was obtained twice; immediately after termination of both exercise tests.

Physical activity and dietary standardization

Subjects were instructed to: record their dietary intake 30 h prior to test day I, and replicate their intake prior to test day II which was checked by a dietitian; avoid caffeine and alcohol for 12 and 24 h prior to each test day, respectively; refrain from using any antibacterial mouthwash/toothpaste and tongue-scraping during each supplementation day (Govoni et al., 2008). Supplement logs and training diaries were kept for both intervention periods.

Statistical analysis

Dynamic apnea and intermittent test results, and plasma and salivary nitrate and nitrite concentrations were analyzed using paired samples t-tests or one-way repeated measures ANOVA where appropriate, with treatment (BR vs PLA) as within-subjects factor. After these pre-planned initial analyses, test-order was added as a between-subjects factor (in view of a potential period effect). In case of a significant treatment x test-order interaction, separate paired t-tests were performed. Statistical significance was set at $P<0.05$. All data were analyzed using SPSS 22.0 (IBM Corp., Armonk, NY), and are presented as means±SD.

RESULTS

Plasma and saliva

Baseline plasma nitrate concentrations on the test day were significantly higher following five days of BR vs PLA (127±98 vs 56±22 µmol/L; *P*=0.016; Figure 1A), and further increased at 2.5-h post-ingestion for BR on the test day (751±118 µmol/L), remaining significantly higher than PLA (*P*<0.001; Figure 1A). In line, plasma nitrite concentrations for BR increased from baseline to 2.5-h post-ingestion (227±246 vs 516±268 nmol/L; *P*<0.001 vs PLA; Figure 1B). Salivary nitrate and nitrite concentrations were significantly higher following five days of BR vs PLA (1078±1202 vs 260±241 µmol/L, and 746±1221 vs 286±148 µmol/L, respectively) and further increased at 2.5-h post-ingestion for BR (13795±5365 and 3852±2541 µmol/L, respectively, all *P*<0.001).

Performance

With the pre-planned primary analyses, distance covered during the dynamic apnea test did not significantly differ between BR and PLA (49.5±7.8 vs 46.9±9.1 m, *P*=0.18, Figure 2A). However, when test-order was added as a between-subjects factor, a significant treatment x test-order interaction was observed (*P*=0.001). Separate analyses showed no difference in the distance covered between BR and PLA (and no difference between test I and II) when BR was ingested in period I (48.8±7.4 vs 52.3±10.4 m, respectively, *P*=0.10, Figure 2B). In contrast, distance covered was significantly larger in BR vs PLA (and thus in test II vs I) when BR was ingested in period II (50.1±8.5 vs 42.8±5.7 m, respectively, *P*=0.002, Figure 2C). Likewise, time-to-exhaustion during dynamic apnea did not differ between BR and PLA (30.6±6.3 vs 28.1±5.9 s, *P*=0.14), but a significant treatment x test-order interaction was observed (*P*=0.01). Separate analyses revealed no difference when BR was ingested in period I (*P*=0.14), but an increased time-to-exhaustion when BR was ingested in period II (*P*=0.023). The average swim speed was not different between BR and PLA (1.63±0.11 vs 1.67±0.069 m/s, *P*=0.28), and no treatment x test-order interaction was observed (*P*=0.40).

Time to complete the intermittent test was not different between BR and PLA (316.0±7.9 s vs 316.3±6.9 s, *P*=0.73, Figure 3). Time to complete separate blocks (4x 15 m) also did not differ between BR and PLA (data not shown). Adding test-order as a between-subjects factor did not alter these findings. However, a small improvement (independent of test-order) was observed from test-day I to test-day II (-1.3±2.1 s, *P*=0.034).

Secondary parameters

No serious adverse effects were reported. Four athletes (two following BR and two following PLA) reported mild GI complaints (belching, bloating or windiness) 2.5-h post-ingestion. RPE was not different between BR and PLA for the dynamic apnea (15.3±2.1 vs 16.1±2.0, *P*=0.16) or the intermittent test (19.1±0.9 vs 18.9±1.1, *P*=0.58), with no interaction of test-order (both *P*>0.70). However, the fatigue scores of training were 28±19% higher in period I than period II (*P*<0.0001).

DISCUSSION

Six days of beetroot juice supplementation substantially increased plasma and salivary nitrate and nitrite concentrations, but did not improve intermittent sprint performance in elite female water polo players. Overall, no difference in dynamic apnea performance could be detected between BR and PLA. However, a significant interaction with test-order showed that a performance benefit was attained when BR was ingested during period II.

We specifically aimed to assess the effects of dietary nitrate supplementation in *elite* athletes. Baseline values and increases in plasma nitrate and nitrite concentrations of our elite athletes were comparable to those observed in female recreational and well-trained athletes (Buck et al., 2015; Glaister et al., 2015). This argues against the proposed attenuated plasma response in elite vs recreational athletes (Porcelli et al., 2015). Previous studies reported benefits of nitrate for intermittent team-sport performance in recreational (Thompson et al., 2016; Wylie et al., 2013) and trained athletes (Nyakayiru et al., 2017). Despite effective increases in plasma nitrate/nitrite however, we observed no beneficial effects on intermittent sprint performance in the water. Although high-intensity exercise may lower plasma nitrite concentrations (Bescos et al., 2011), it seems unlikely that the ~30 s apnea test would render the remaining BR-derived nitrite insufficient to also affect intermittent performance. Furthermore, distance covered in the dynamic apnea test was similar for BR vs PLA. This suggests that nitrate does not improve exercise performance in elite water polo players, in line with most studies in elite athletes of high-intensity sports (Boorsma et al., 2014; Peacock et al., 2012; Sandbakk et al., 2015). Advanced physiological adaptations through extensive training, such as higher muscular capillarization and optimized oxygen transport, may render nitrate supplementation less effective in elite athletes (Jones, 2014). Moreover, since by definition there are not many elite athletes, and there are likely only small benefits to be attained, it is difficult to establish potential ergogenic effects. On the other hand, by recruiting a homogenous group of Olympic level athletes, and testing them in a sport-specific environment, we minimized day-to-day variations and optimized the power for the small sample size. This particularly applies to the intermittent sprint-test, as it was adapted from a previously validated test (Tan et al., 2009), subjects were highly familiarized, and it closely resembles physical performance during water polo. Since absolutely no effect of BR was observed for intermittent performance, nitrate does not seem to be ergogenic for elite water polo players. However, despite great test-familiarization, a significant improvement was observed from the first to the second test day, independent of BR supplementation. Though we aimed to set up two identical test periods, standardizing training programs between test periods is challenging in elite athletes (Tanner, 2013). Higher fatigue scores of training were reported in period I, which likely caused the 'improvement' in intermittent performance.

The differences in training intensity/fatigue also appeared to have an effect on dynamic apnea performance. For this test however, there was an interaction of this 'period effect' with the effect of BR supplementation. Separate analyses showed a significant improvement in distance covered when BR was ingested during period II, whereas no difference in distance covered was found when BR was administered during period I. As such, the effect of BR was likely confounded by training periodization. In contrast to the lack of ergogenic effects for intermittent performance, our findings suggest minor ergogenic effects of BR for front crawl swimming without breathing. This is further supported when using the alternative analytical approach of magnitude-based inferences, which has been suggested to be of added value in (small scale) exercise performance studies (Batterham & Hopkins, 2006). Using this approach, here was a 'possibly small increase' (6.2% [90%CI: -0.5 to 13.3%]) in dynamic apnea performance in BR vs PLA, which even turned into a 'likely small increase' when including treatment order as a covariate. For the intermittent test, there was a '(very) likely trivial effect' of BR vs PLA, thus supporting a potential benefit of BR only for the dynamic apnea test.

Exhaustion testing like the dynamic apnea test is much more sensitive to changes than sport-specific performance testing like the intermittent test (Hopkins et al., 1999). Furthermore, despite rehearsal and standardization, subjects were less 'trained' in performing dynamic apnea exercise, which may have further increased the window of opportunity to detect beneficial effects of BR. Previously, Patrician and Schagatay (2017) found improved arterial oxygen saturation following beetroot juice supplementation for sub-maximal 75 m underwater swimming. They suggested that total distance would likely be increased during a maximal attempt, due to increased remaining oxygen stores. This is in line with our observation of increased distance covered during the dynamic apnea test. The test was adapted from maximal distance swimming in shallow water in apnea divers (Schagatay, 2010), using front crawl high-intensity swimming to increase the relevance to water polo. However, the dynamic apnea test simulates an extreme breath-hold situation of much longer duration than the intermittent underwater phases of a water polo game, and can therefore not be considered a water polo-specific test. It has been suggested that nitrate supplementation could be applied to sports with limited oxygen availability such as underwater rugby and hockey (Patrician & Schagatay, 2017), in which longer under-water phases are prevalent. Clearly though, we can only speculate whether an improved maximal distance of the dynamic apnea test could also translate to actual performance enhancement for water polo. Since there was absolutely no improvement of the intermittent water polo-specific performance test, a substantial performance enhancement of nitrate supplementation in elite water polo players is unlikely. Yet, it is obvious that even minor benefits could be very relevant in highly trained individuals, and future work should further examine which specific athlete populations may or may not benefit from nitrate supplementation.

CONCLUSIONS

Six days of beetroot juice supplementation does not improve intermittent sprint performance in elite female water polo players, but there may be a potential for beneficial effects during dynamic apnea.

AKNOWLEDGEMENTS

KLJ, JWvD, LJCvL, and LBV designed the research study; KLJ and JWvD conducted the research trials; KLJ, JMGS and LBV analyzed the data; all authors contributed to the writing of the paper; and KLJ had primary responsibility for the final content. All authors read and approved the final manuscript. The authors thank the Dutch Technology Foundation STW (grant 12877) for financial support. We also thank the Dutch Olympic Federation for the support in subject recruitment.

REFERENCES

Bailey, S. J., Varnham, R. L., DiMenna, F. J., Breese, B. C., Wylie, L. J., & Jones, A. M. (2015). Inorganic nitrate supplementation improves muscle oxygenation, O(2) uptake kinetics, and exercise tolerance at high but not low pedal rates. *J Appl Physiol (1985), 118*(11), 1396-1405. doi:10.1152/japplphysiol.01141.2014

Batterham, A. M., & Hopkins, W. G. (2006). Making meaningful inferences about magnitudes. *Int J Sports Physiol Perform, 1*(1), 50-57.

Bescos, R., Rodriguez, F. A., Iglesias, X., Ferrer, M. D., Iborra, E., & Pons, A. (2011). Acute administration of inorganic nitrate reduces VO(2peak) in endurance athletes. *Med Sci Sports Exerc, 43*, 1979-1986.

Boorsma, R. K., Whitfield, J., & Spriet, L. L. (2014). Beetroot Juice Supplementation Does Not Improve Performance in Elite 1500-m Runners. *Med Sci Sports Exerc, 46*(12), 2326-34.

Buck, C. L., Henry, T., Guelfi, K., Dawson, B., McNaughton, L. R., & Wallman, K. (2015). Effects of sodium phosphate and beetroot juice supplementation on repeated-sprint ability in females. *Eur J Appl Physiol, 115*(10), 2205-2213. doi:10.1007/s00421-015-3201-1

Carriker, C. R., Mermier, C. M., Van Dusseldorp, T. A., Johnson, K. E., Beltz, N. M., Vaughan, R. A., . . . Gibson, A. L. (2016). Effect of Acute Dietary Nitrate Consumption on Oxygen Consumption During Submaximal Exercise in Hypobaric Hypoxia. *Int J Sport Nutr Exerc Metab, 26*(4), 315-322. doi:10.1123/ijsnem.2015-0144

Glaister, M., Pattison, J. R., Muniz-Pumares, D., Patterson, S. D., & Foley, P. (2015). Effects of dietary nitrate, caffeine, and their combination on 20-km cycling time trial performance. *J Strength Cond Res, 29*(1), 165-174. doi:10.1519/JSC.0000000000000596

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.

Hopkins, W. G., Hawley, J. A., & Burke, L. M. (1999). Design and analysis of research on sport performance enhancement. *Med Sci Sports Exerc, 31*(3), 472-485.

Jones, A. (2014). Dietary Nitrate Supplementation and Exercise Performance. *Sports Med, 44*, 35-45. doi:10.1007/s40279-014-0149-y

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, 146*(5), 986-93. doi:10.3945/jn.116.229807

Krustrup, P., Mohr, M., Steensberg, A., Bencke, J., Kjaer, M., & Bangsbo, J. (2006). Muscle and blood metabolites during a soccer game: implications for sprint performance. *Med Sci Sports Exerc, 38*(6), 1165-1174. doi:10.1249/01.mss.0000222845.89262.cd

Lundberg, J. O., Weitzberg, E., & Gladwin, M. T. (2008). The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. *Nat Rev Drug Discov, 7*(2), 156-167.

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

Patrician, A., & Schagatay, E. (2017). Dietary nitrate enhances arterial oxygen saturation after dynamic apnea. *Scand J Med Sci Sports, 27*(6), 622-26. doi:10.1111/sms.12684

Peacock, O., Tjonna, A. E., James, P., Wisloff, U., Welde, B., Bohlke, N., . . . Sandbakk, O. (2012). Dietary nitrate does not enhance running performance in elite cross-country skiers. *Med Sci Sports Exerc, 44*, 2213-2219. doi:10.1249/MSS.0b013e3182640f48.; ID: 723

Porcelli, S., Ramaglia, M., Bellistri, G., Pavei, G., Pugliese, L., Montorsi, M., . . . Marzorati, M. (2015). Aerobic Fitness Affects the Exercise Performance Responses to Nitrate Supplementation. *Med Sci Sports Exerc, 47*(8), 1643-1651. doi: 10.1249/MSS.0000000000000577

Sandbakk, S. B., Sandbakk, O., Peacock, O., James, P., Welde, B., Stokes, K., . . . Tjonna, A. E. (2015). Effects of acute supplementation of L-arginine and nitrate on endurance and sprint performance in elite athletes. *Nitric Oxide, 48*, 10-15. doi:10.1016/j.niox.2014.10.006

Schagatay, E. (2010). Predicting performance in competitive apnea diving, part II: dynamic apnoea. *Diving Hyperb Med, 40*(1), 11-22.

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

Tan, F. H., Polglaze, T., & Dawson, B. (2009). Comparison of progressive maximal swimming tests in elite female water polo players. *Int J Sports Physiol Perform, 4*(2), 206-217.

Tanner, R. K., Gore, C.J. (2013). *Physiological Tests for Elite Athletes* (2nd ed.), Australian Institute of Sports, AUS: Human Kinetics.

Thompson, C., Vanhatalo, A., Jell, H., Fulford, J., Carter, J., Nyman, L., . . . Jones, A. M. (2016). Dietary nitrate supplementation improves sprint and high-intensity intermittent running performance. *Nitric Oxide, 61*, 55-61. doi:10.1016/j.niox.2016.10.006

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. *Am J Physiol Regul Integr Comp Physiol, 299(4)*, 1121-1131.

Wylie, L. J., Mohr, M., Krustrup, P., Jackman, S. R., Ermiotadis, G., Kelly, J., . . . Jones, A. M. (2013). Dietary nitrate supplementation improves team sport-specific intense intermittent exercise performance. *Eur J Appl Physiol*, *113*, 1673-1684.

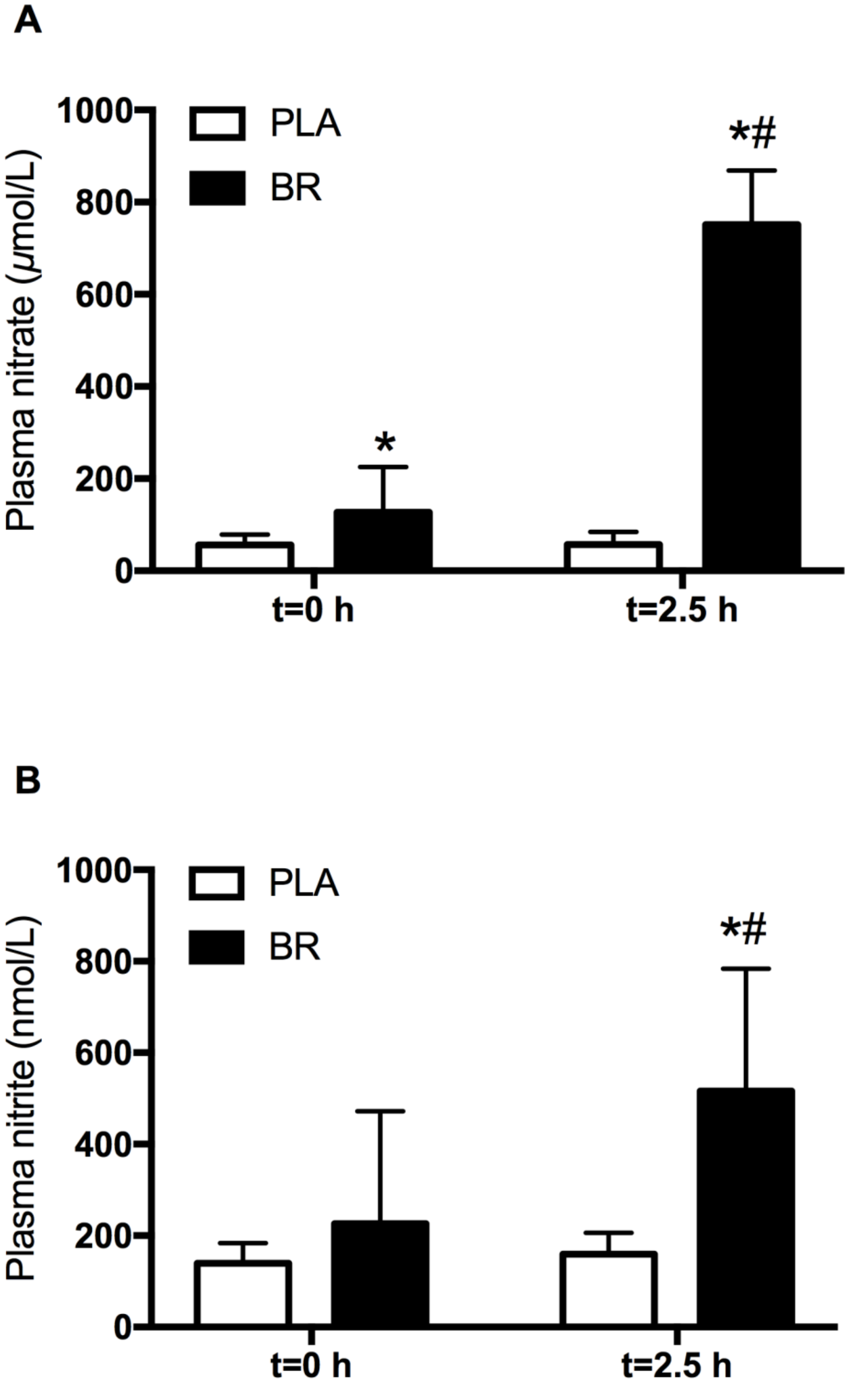

Figure 1: Plasma nitrate (A) and nitrite (B) concentrations at baseline (0 h) and 2.5-h post-ingestion on the test day (*n*=14). PLA: placebo, BR: beetroot juice, t: time point in hours. Values are means±SDs. *Significantly different from PLA, #significantly different from BR baseline P<0.001.

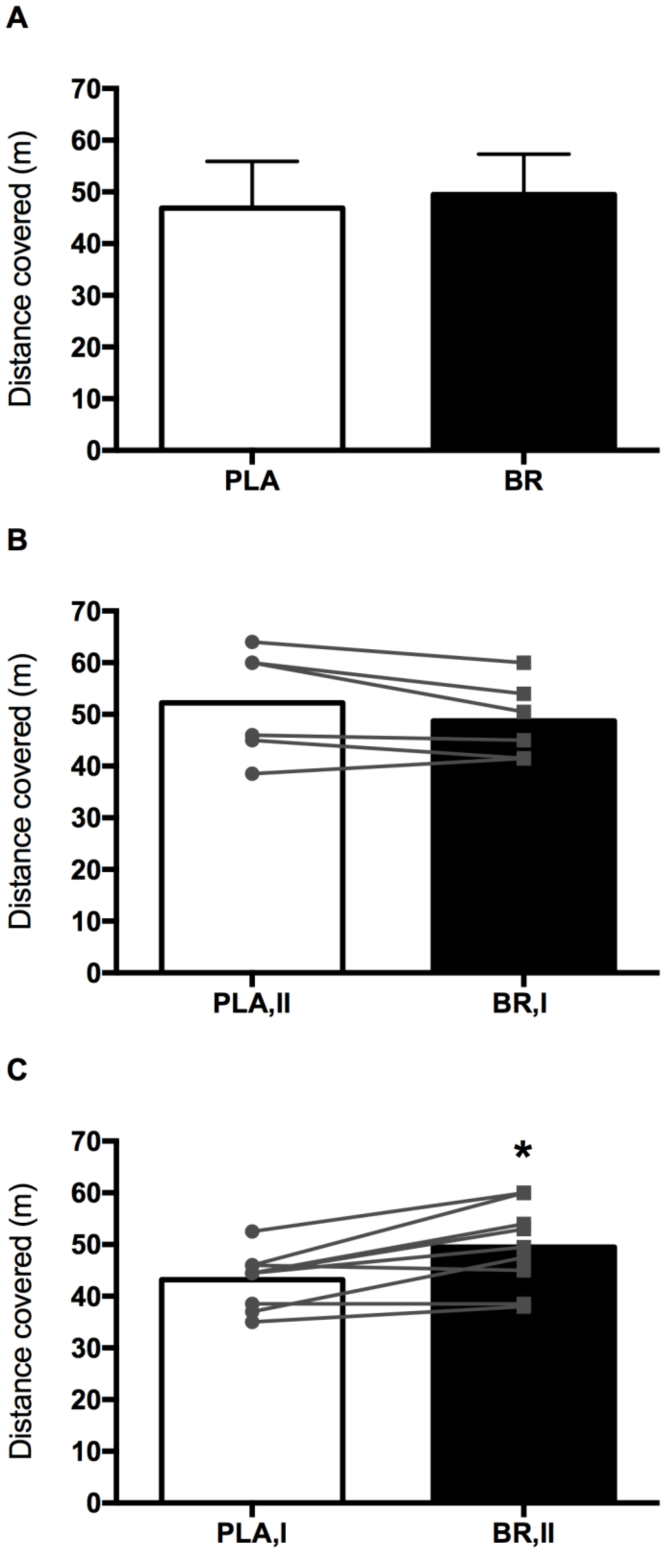

Figure 2: Dynamic apnea test performance for the total group (A; n=14), and separated by test order; BR-PLA (B; n=6) and PLA-BR (C; n=8). PLA: placebo, BR: beetroot juice. I: test I, II: test II. Values are means±SD. B: *Significantly different from PLA, P=0.002.

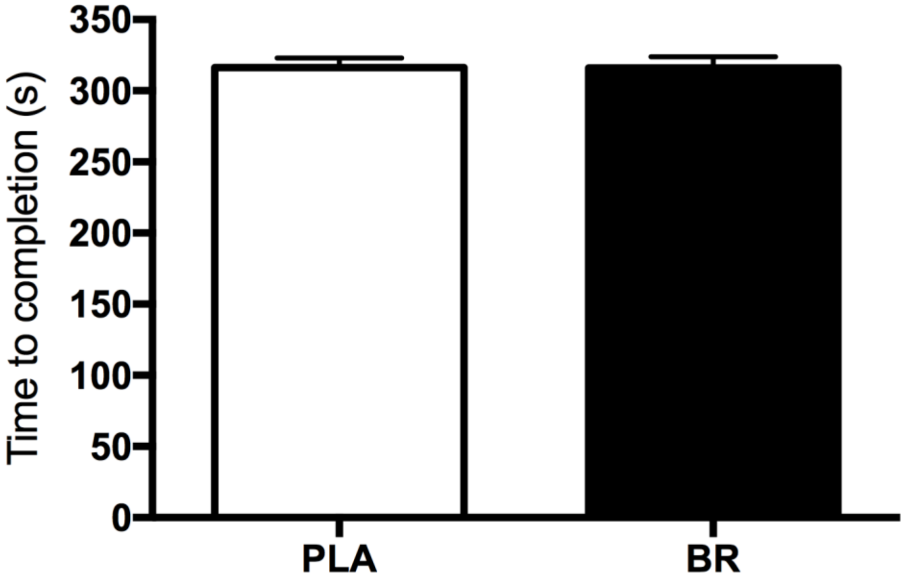

Figure 3: Intermittent test performance for the total group (n=14). Values are means±SD.

Table 1 Participants' characteristics[1]

n	14
Age, y	22±4
Height, cm	178±5
Weight, kg	74±9
BMI, kg/m^2	23±2

[1] Values are means±SDs.