The Effect of Beetroot Juice Supplementation on Dynamic Apnea and Intermittent Sprint Performance in Elite Female Water Polo Players

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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-blinded, randomized, crossover manner, the Dutch National female water polo team (N=14) was subjected to two 6-day supplementation periods (1 and 2), with either 140 ml/day of nitrate-rich (BR; ∼800 mg/day nitrate) or nitrate-depleted (PLA) beetroot juice. Following blood sampling on Day 6, 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 4 × 4 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 = .178). However, when correcting for test order, the distance covered was significantly larger in BR versus PLA when BR was ingested in Period 2 (50.1 ± 8.5 vs. 42.8 ± 5.7 m, p = .002), whereas no difference was observed when BR was ingested in Period 1 (48.8 ± 7.4 vs. 52.3 ± 10.4 m, p = .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 = .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.

Keywords: ergogenic, nitrate, nitrite

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 (Lundberg et al., 2008). Nitric oxide plays a key role in skeletal muscle function—for example, by regulating blood flow and muscle contractility (Stamler & Meissner, 2001). Hypoxic conditions with low oxygen availability and a low pH environment can stimulate the nitrate–nitrite–nitric oxide 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 underwater exercise (Schagatay, 2010), the term “dynamic apnea” more appropriately reflects the different physiological characteristics associated with these breath-holding 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 underwater (breath-holding) 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 versus untrained individuals (Porcelli et al., 2015), the effect of nitrate supplementation has not been investigated in elite team sport athletes. Therefore, the aim of this 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 team (N = 18), of which two athletes could not participate due to injuries. Out of 16 athletes, one was excluded due to illness, and one was excluded 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, 3 months after winning World Championship silver, training 7 ± 1 sessions (2016). Following a standardized warm-up protocol, the dynamic apnea test started 3 hr postingestion. Each athlete had 30-s active rest (arms stretched above the head) between each sprint and 30-s semiactive rest (wrists above water) between each block of four 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 a 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 with a “go” signal by lowering the arm.

Supplements were ingested for 5 days at home, and the sixth dose was provided on the test day at the sports facility. Subjects underwent baseline and 2.5 hr postingestion measurements of plasma, saliva, and gastrointestinal tolerance questionnaires. Plasma and saliva were collected and analyzed for nitrate and nitrite using chemiluminescence (Sievers NOA 280i; Analytix Ltd., Boldon, UK), as previously described (Jonvik et al., 2016). Following a standardized warm-up protocol, the dynamic apnea test started 3 hr postingestion. Each athlete had 30–35 min of rest between the dynamic apnea and the intermittent test. The rate of perceived exertion was obtained twice, immediately after termination of both exercise tests.

Physical Activity and Dietary Standardization
Subjects were instructed to record their dietary intake 30 hr prior to Test Day 1 and to replicate their intake prior to Test Day 2, which was checked by a dietitian; to avoid caffeine and alcohol for 12 and 24 hr, respectively, prior to each test day; and to 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 analysis of variance, where appropriate, with treatment (BR vs. PLA) as within-subjects factor. After these preplanned initial analyses, test order was added as a between-subjects factor (in view of potential period effect). In case of a significant Treatment × Test Order interaction, separate paired t tests were performed. Statistical significance was set at p < .05. All data were analyzed using SPSS version 22.0 (IBM Corp., Armonk, NY) and are presented as means ± SD.

Table 1 Participants’ Characteristics

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<tr>
<td>N</td>
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<tr>
<td>Age (years)</td>
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<td>Height (cm)</td>
<td>178 ± 5</td>
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<tr>
<td>Weight (kg)</td>
<td>74 ± 9</td>
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<tr>
<td>BMI (kg/m²)</td>
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Note. Values are presented as means ± SD. BMI = body mass index.
Results

Plasma and Saliva

Baseline plasma nitrate concentrations on the test day were significantly higher following 5 days of BR versus PLA (127 ± 98 vs. 56 ± 22 μmol/L, p = .016; Figure 1a) and further increased at 2.5 hr postingestion for BR on the test day (751 ± 118 μmol/L), remaining significantly higher than PLA (p < .001; Figure 1a). In line, plasma nitrite concentrations for BR increased from baseline to 2.5 hr postingestion compared with PLA (227 ± 246 vs. 516 ± 268 nmol/L, p < .001; Figure 1b). Salivary nitrate and nitrite concentrations were significantly higher following 5 days of BR versus PLA (1,078 ± 1,202 vs. 260 ± 241 μmol/L and 746 ± 1,221 vs. 286 ± 148 μmol/L, respectively) and further increased at 2.5 hr postingestion for BR (13,795 ± 5,365 and 3,852 ± 2,541 μmol/L, respectively, all ps < .001).

Performance

With the preplanned 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 = .18; Figure 2a). However, when test order was added as a between-subjects factor, a significant Treatment × Test Order interaction was observed (p = .001). Separate analyses showed no difference in the distance covered between BR and PLA (and no difference between Tests 1 and 2) when BR was ingested in Period 1 (48.8 ± 7.4 vs. 52.3 ± 10.4 m, respectively, p = .10; Figure 2b). By contrast, the distance covered was significantly larger in BR versus PLA (and thus in Test 2 vs. Test 1) when BR was ingested in Period 2 (50.1 ± 8.5 vs. 42.8 ± 5.7 m, respectively, p = .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 = .14), but a significant Treatment × Test Order interaction was observed (p = .01). Separate analyses revealed no difference when BR was ingested in Period 1 (p = .14), but an increased time to exhaustion when BR was ingested in Period 2 (p = .023). The average swim speed was not different between BR and PLA (1.63 ± 0.11 vs. 1.67 ± 0.069 m/s, p = .28), and no Treatment × Test Order interaction was observed (p = .40).

To complete the intermittent test was not different between BR and PLA (316.0 ± 7.9 s vs. 316.3 ± 6.9 s, p = .73,
Figure 3). Time to complete separate blocks (4 × 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 1 to Test Day 2 (−1.3 ± 2.1 s, p = .034).

Secondary Parameters

No serious adverse effects were reported. Four athletes (two following BR and two following PLA) reported mild gastrointestinal complaints (belching, bloating, or flatulence) 2.5 hr post-ingestion. The rate of perceived exertion was not different between BR and PLA for the dynamic apnea (15.3 ± 2.1 vs. 16.1 ± 2.0, p = .16) or the intermittent test (19.1 ± 0.9 vs. 18.9 ± 1.1, p = .58), with no interaction of test order (both ps > .70). However, the fatigue scores of training were 28% ± 19% higher in Period 1 than Period 2 (p < .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 2.

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 with 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 versus 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 increased distance covered in plasma nitrate/nitrite, we observed no benefit in intermittent performance.

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% confidence interval [−0.5, 13.3]) in dynamic apnea performance in BR versus 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 versus 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 submaximal 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-holding 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 underwater 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 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.

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References


