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Brief Report

Effects of Acute Beetroot Juice Ingestion on Reactive Agility Performance

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Abstract: Beetroot juice (BRJ) is high in inorganic nitrate (NO_3) which has been shown to enhance vascular function, cognition, and physical performance. Acute BRJ supplementation has been reported to enhance explosive resistance exercise performance and simple reaction time in diseased populations. However, it remains unknown if acute BRJ supplementation can enhance agility and reactive drills in healthy individuals, which are relevant to many sports. The purpose of this study was to investigate the effects of acute BRJ supplementation on simple reaction time and reactive agility performance. In a counterbalanced double-blinded manner, physically active males completed two trials each with a differing single-dose treatment: (1) Placebo (PL), (2) BRJ. Each treatment was consumed 2-hrs before experimental testing. Plasma Nitrate (NO_3) and Nitrite (NO_2) were measured via capillary blood sampling and colorimetric assay. Reaction time was assessed using a simple reaction time light test. Reactive agility was measured via a semi-circle drill and a get-up-and-go drill. All tests used FITLIGHT LED sensors to record response time. Each visit was separated by a 72-h washout period. Acute BRJ ingestion resulted in significantly greater plasma NO_3 ($p < 0.001$) and NO_2 ($p = 0.008$) compared to PL. BRJ significantly improved response time during the semi-circle drill ($p = 0.011$) and get-up and go drill ($p = 0.027$) compared to PL. No differences between treatments were observed for simple reaction time ($p = 0.279$). Collectively, these findings suggest that acute BRJ ingestion may improve reactive agility performance likely mediated by systemic increases in NO_3/NO_2. Future research is needed to investigate how these findings translate to game-play and sports competition.

Keywords: nitrate; nitrite; reaction time; FITLIGHT

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1. Introduction

Beetroot juice (BRJ) contains high concentrations of inorganic nitrate (NO3) which has been repeatedly shown to result in the synthesis and accumulation of plasma nitrite (NO_2) [1]. Reduction of NO_3 to NO_2 is largely mediated by the oral microbiome and the elimination of anaerobic bacteria can attenuate NO3 conversion [2]. Once introduced into circulation, NO_2 is converted to nitric oxide (NO-) which has been shown to result in relaxation of vascular smooth muscle [3], promote local blood flow [4], increase vascular conductance [5], and improve metabolic efficiency [6]. Because of these effects, BRJ usage has been recently trending in sports and athletic populations with multiple investigations suggesting that acute BRJ ingestion may improve both aerobic and anaerobic exercise performance [7–9]. However, little to no evidence exists as to whether ergogenic benefits might translate to agility or visuomotor performance.

Evidence of how BRJ influences fast and explosive movements are primarily limited to the context of resistance exercise and sprinting [8–11]. For example, Williams et al., recently showed that an acute dose of concentrated BRJ resulted in faster movement velocity during bench press [8]. At the muscle level, BRJ ingestion has also been implicated in increasing

skeletal muscle shortening velocity [12]. Dumar et al., reported that BRJ abolished time-of-day decrements in power output during repeated sprints [9]. Other evidence supports these findings whereby acute BRJ ingestion increased peak and mean power output during a 30-s Wingate Anaerobic test (WAnT) [10]. Physiological underpinnings of performance enhancement are still being elucidated. However, improvements in power output may be mediated by increased calcium sensitivity and altered recruitment of fast twitch fibers. Hernandez et al., showed increased force output of fast twitch fibers with dietary NO_3 which concomitantly occurred with increases in sarcoplasmic reticulum calcium release [13]. Bolstering this, Ferguson et al., showed BRJ induced beneficial hemodynamic changes preferentially to fast-twitch fibers [4]. While findings are propitious, much less is known about the possible effects of BRJ ingestion on agility performance and reactive ability.

Although the literature is sparse, BRJ has been shown to improve cognitive function and reaction time [14,15]. Thompson et al., showed that 7 days of BRJ supplementation improved decision-response time during cognitive tasks [14]. Additionally, Gilchrist et al., observed improved simple reaction time with 14 days of dietary BRJ albeit participants were all T2-diabetic [15]. Reactive ability is important to optimize sports and exercise performance and may be dependent on the contractile function of fast twitch fibers [16]. Since BRJ exerts inotropic effects preferentially in fast-twitch fibers and may improve cognitive response time, it is plausible that BRJ may improve reactive agility performance. Thus, the purpose of this brief report was to investigate the acute effects of BRJ on reactive agility performance and simple reaction time. We hypothesized that BRJ would hasten response time during agility drills and simple reaction time.

2. Materials and Methods

2.1. Participants

To determine the appropriate sample size, an a priori power analysis was conducted using statistical software (G*power V 3.1.9.4). A previous investigation from our lab showed BRJ increased explosive power output versus PL during sprinting with an estimated effect size of d = 0.81 [9]. To calculate the minimal sample size needed, the following parameters were used: test = *t*-test (matched pairs), d = 0.81, $\alpha = 0.05$, 1-$\beta = 0.8$. This was calculated to a minimum sample size range of $n = 7$ for adequate power. To match sample sizes of previous investigations [8,9,17], 12 healthy physically active males (21.3 $\pm$ 1.2 yrs, 92.1 $\pm$ 12.8 kg, 184.7 $\pm$ 7.1 cm) were recruited. Physically active was defined as accruing 150 min/wk of moderate to vigorous physical activity including planned exercise and amateur participation in sports [18]. Further inclusion criteria included being free from orthopedic injury in the past six months and not currently supplementing with BRJ or nitrite precursors. Screening for exercise safety was completed using a physical activity readiness questionnaire (PAR-Q) and was required before participation. Participants were asked to refrain from alcohol, caffeine, and nicotine for a minimum of 6 h before testing [9,19]. Furthermore, participants were asked to not use any oral antiseptic or mouthwash 12 h before each visit [8]. Trial times were standardized ensuring all trials were completed within $\pm$ 1 h. Written and informed consent was obtained from each participant before data collection. All experimental procedures were conducted in accordance with the Declaration of Helsinki and approved by the Samford University Institutional Review Board (June 2021).

2.2. Procedures

2.2.1. Study Design

Using a double-blind, counterbalanced, crossover design approach, healthy adult males completed 2 visits each with a different experimental treatment: BRJ or PL. Following blood collection for plasma NO_3/NO_2, participants completed a simple reaction time test and 2 functional agility drills which occurred 2-hrs after the ingestion of treatment. Comparisons were drawn between BRJ and PL for average simple reaction time (SRT) and response time during agility drills. Each trial was separated by a 72 h washout period [8,9].

2.2.2. Supplementation and Plasma NO_3/NO_2

For BRJ supplementation, participants ingested a single concentrated dose (70 mL) of BRJ (Beetit Sports Pro-elite Shot; James White, UK) [8,9]. The BRJ provided approximately 400 mg of inorganic NO3. For PL treatment, concentrated black-currant juice (Suntory, Tokyo, Japan) was given in an identical volume of 70 mL [8,9]. Treatments were ingested 2-hrs prior to testing [8,9]. Participants were not aware of any experimental hypotheses. Supplements were distributed by an independent researcher not involved in data collection, and the distribution order was only divulged to researchers at the completion of all data analyses.

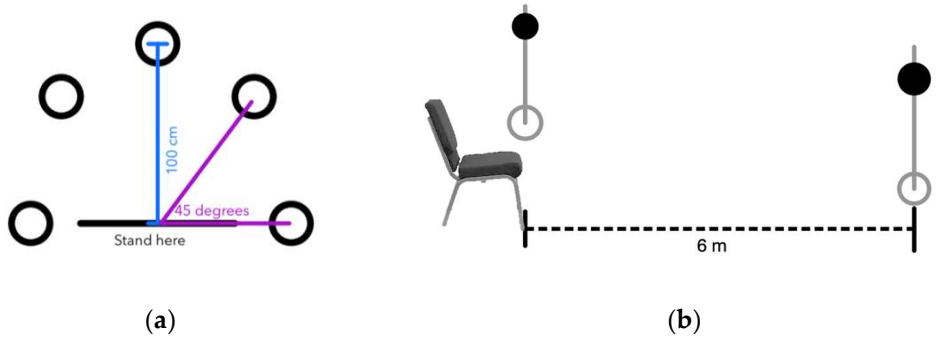
To measure plasma [NO_3] and [NO_2], ~500 µL of capillary blood was collected 2-hrs after treatment ingestion via finger prick as previously described by our lab [20,21]. A 17-gauge 2.0 mm depth disposable lancet was used to generate bleeding on either the third or fourth finger. A massage technique was used to promote steady blood flow and whole blood was collected via capillary action into potassium-EDTA coated microvette® tubes (SARSTEDT, Newton, NC, USA). Whole blood was then centrifuged at 10,000 rpm for 10 min, plasma was decanted, and subsequently stored at −80 °C until biochemical analysis which was completed following the conclusion data collection. Plasma concentrations of [NO_3] and [NO_2] were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Cayman Chemical, Ann Abor, MI, USA) [22,23]. All samples were analyzed in duplicate and according to the manufacturer's instructions.

2.2.3. Simple Reaction Time (SRT) and Functional Agility Testing

For our testing battery, participants completed a simple reaction time test (SRT) and two agility drills: semi-circle and get-up-and-go drill. Response time during all tests/drills was completed using a FITLIGHT trainer system (FITLIGHT USA Inc., Miami, FL, USA). The FITLIGHT trainer is a Bluetooth-based LED system whereby sensors can be placed in varying locations and used to detect reaction time in response to the illumination of the sensor. To prevent fatigue, participants always completed the SRT test first as adapted by Gilchrest et al. [15]. Briefly, one FITLIGHT sensor was placed flat on the table with the participant seated directly in front of it having hands placed on either side of the light with elbows at an approximate 90-degree angle. Participants were instructed to hit the sensor as quickly as possible once it illuminated. Participants completed 32 attempts and all attempts were averaged together for analysis [15]. The time at which it took for the sensor to illuminate was randomized as to prevent anticipatory behavior. In the event of a false start or miss, that attempt was excluded, and participants were given a repeat attempt.

Functional agility was assessed through a semi-circle and get-up-and-go drill which is diagramed in Figure 1. The semi-circle drill was adapted from Mackala et al., and assessed reactive agility and change of direction speed [24]. Five sensors were arranged in a uniform semicircular design on the floor. A centered marker was also put on the floor to indicate to participants where to start and return throughout the drill. From the marker, each light was spaced 100 cm away. The angle at which sensors were placed from the center marker were 2 sensors at an angle of 0°, 2 sensors at an angle of 45°, and 1 sensor at an angle of 90°. Upon commencement of the drill, sensors illuminated individually in a randomized fashion for a total of 15 times [24]. Participants were instructed to move to the illuminated sensor as quickly as possible, step on it, and return to the center marker before the next light flashed. There was a 1-s delay between the deactivation of a sensor and the activation of the next light to allow participants to reset to the center marker before the next attempt. The response time over the 15 attempts was averaged for analysis. The get-up-and-go drill was adapted from Donoghue et al., and assessed response time via processing speed [25]. This was accomplished by first having the participants complete a walkthrough of the drill for familiarization with the drill. Specifically, participants began in a chair in an upright, sitting position. A pole with one sensor was stationed 6 m in front of the chair and another immediately next to the chair. Once the sensor illuminated, participants stood up as quickly as possible and sprinted past the other sensor position 6 m away. The time it took to react

and complete the 6 m was recorded for a total of 2 attempts [25]. The fastest of the attempts was used for analysis.



(**a**) (**b**)

Figure 1. Diagrams of Reactive agility tests: (**a**) Semi-circle drill (**b**) Get-up-and-go drill.

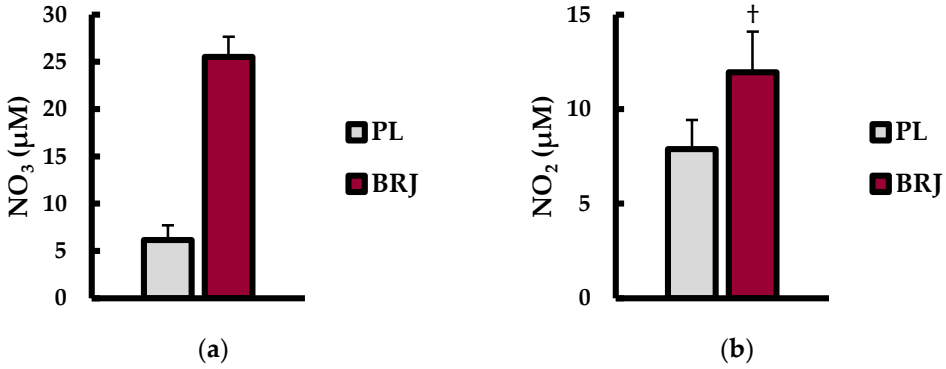
2.3. Data Analysis

Data analysis was completed using Jamovi software (Version 0.9; Sydney, Australia). Confirmation of data normality was conducted using a Shapiro–Wilk test. Means were compared and analyzed using a pairwise *t*-test. Effect sizes between means were calculated via Cohen's d (d) and interpreted as: 0.2—small; 0.5—moderate; 0.8—large [26,27]. All data are presented at mean ± standard deviation (SD). Significance was set at $p \leq 0.05$ a priori.

3. Results

3.1. Plasma Nitrate/Nitrite (NO_3/NO_2)

Plasma NO_3/NO_2 measurements are shown in Figure 2. For plasma NO_3 concentrations (µm; Figure 2a), BRJ resulted in significantly higher plasma NO_3 compared to PL ($p < 0.001$; d = 4.5). Furthermore, plasma NO_2 concentrations (µm; Figure 2b) were significantly higher with BRJ versus PL ($p = 0.008$; d = 2.1).



(**a**) (**b**)

Figure 2. Changes in (**a**) Plasma Nitrate (NO_3; µM) and (**b**) Plasma (NO_2; µM) between placebo (PL; grey bars) and beetroot juice (BRJ; dark red bars). Data are presented as mean ± SD. † indicates significantly different from PL ($p < 0.05$).

3.2. Simple Reaction Time (SRT) and Functional Agility Performance

SRT and agility performance are shown in Figure 3. For SRT (s; Figure 3a), BRJ did not alter reaction time compared to PL ($p = 0.279$; d = 0.32). For the get-up and go drill (s; Figure 3b), BRJ resulted in faster response times compared to PL ($p = 0.027$; d = 0.87). Lastly, response time for the semi-circle drill (s; Figure 3c) was significantly faster with BRJ ingestion versus PL ($p = 0.011$; d = 0.92).

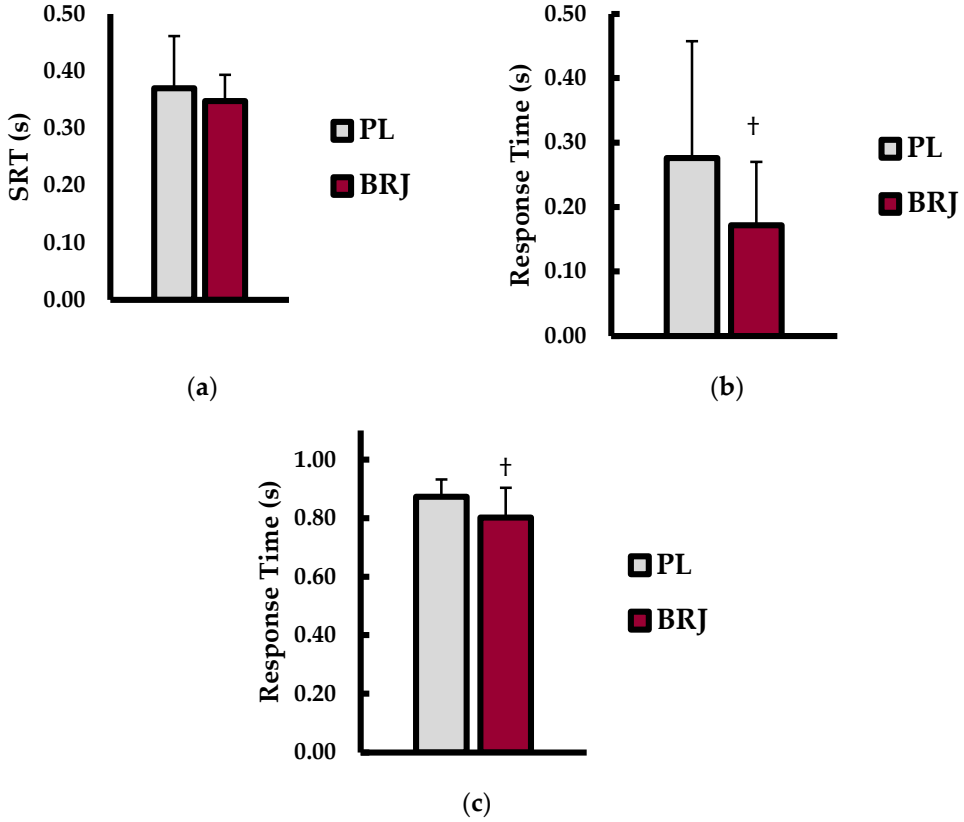


Figure 3. Differences in (**a**) Simple reaction time (s; SRT) (**b**) Get-up and go drill response time (s) and (**c**) Semi-circle drill response time (s) between placebo (PL; grey bars) and beetroot juice (BRJ; dark red bars). Data are presented as mean ± SD. † indicates significantly different from PL ($p < 0.05$).

4. Discussion

Acute BRJ ingestion has been previously shown to impart ergogenic benefits during sprint and explosive resistance exercise [9,21]. BRJ ingestion has also been shown to improve reactive and cognitive ability in diseased populations [15]. However, no studies to date have investigated the effects of acute BRJ ingestion on SRT and functional agility performance in healthy populations. Current results from this brief report indicate that an acute dose of nitrate-rich BRJ results in elevated plasma NO_3/NO_2 but did not alter SRT. However, BRJ ingestion resulted in faster response times during functional agility tests. While precise physiological underpinnings for alterations in agility performance with BRJ treatment are not fully clear, these findings support the use of BRJ to improve agility performance.

Dietary BRJ supplementation has been repeatedly shown to increase plasma NO_3/NO_2 [1,28]. Indeed, peak plasma NO_3 with BRJ has been shown to occur ~2 h post-ingestion [1]. Since increases in plasma NO_3/NO_2 are critical for the physiological benefits from BRJ, current data suggest that our dosing regimen was able to initiate the underlying benefit of nitrate accumulation and reduction of NO_3 to NO_2.

Although research is limited, Gilchrist et al., showed that elderly T2-diabetic populations supplementing with BRJ for 2-weeks improved SRT [15]. The authors suggested that this may be due to increased cerebral blood flow with BRJ ingestion. In contrast, the present findings did not see any improvements in SRT while at rest with acute BRJ ingestion. While reasons for disparities cannot be fully explained, it is plausible that differences in findings are due to the contrasting age of participants and dosing regimen. Older adults, especially those with insulin insensitivity, are at higher risk for vascular and endothelial dysfunction which may be partially explained by reduced NO production [28–30]. Furthermore, a previous report has shown that plasma NO_2 increases more in aged populations with BRJ supplementation [28]. Thus, poorer NO production/endothelial function paired

with heightened plasma NO_2 following BRJ ingestion in elderly and T2-diabetic populations may allow for a greater potential for adaptive responses to BRJ such as the previously suggested mechanism of increased cerebral blood flow [28]. Since young and healthy physically active subjects were studied currently, improvements in SRT with BRJ ingestion may not have translated fully due to already optimal endothelial function and blood flow at rest. Furthermore, the long-term nature of the BRJ dosing regimen utilized by Gilchrist et al. may have influenced SRT to a greater degree than the present acute ingestion period. Recent evidence has shown that many tissues (i.e., skeletal muscle, liver, etc.) possess the ability to store NO_3 in high concentrations which can be further elevated following BRJ ingestion [31]. Although uncertainty remains, longer BRJ supplementation periods may elicit greater NO_3 stores and availability which may allow for greater benefits including improvements in SRT. However, it should be cautioned that the paucity of research in BRJ dosing and NO_3 stores does not allow for full translation of these mechanisms to current findings.

BRJ resulted in improved reactive agility performance whereby BRJ resulted in faster response times compared to PL. These findings bolster previous studies showing improved performance in high-velocity movements with BRJ ingestion, albeit some disparities exist [8,32–34]. It is not fully clear what mediated these improvements, previous mechanistic studies may provide rationale. BRJ has been previously shown to increase sarcoplasmic reticulum calcium release with ensuing increases in muscle force output [35]. Beneficial effects like these have also been implicated to preferentially influence fast-twitch muscle fibers [3,35]. Since fast-twitch fiber recruitment is linked to great explosive ability, BRJ ingestion may have increased calcium release and fast-twitch fiber recruitment leading to improved agility performance. Although, the reader is cautioned that these mechanisms remain largely speculative until further research is done. Interestingly, performance during both agility drills was enhanced with BRJ versus PL despite SRT being unaffected. This may be due to the differences in task difficulty. Since our SRT test may have imposed only a small physical/cognitive demand during the task, BRJ may not have been able to enhance the completion of such a simple task. However, the reactive agility drills required more muscle mass recruitment, and the increase in the number of target choices imposed greater difficulty for decision-making. It could be feasible that more complex movements and tasks benefit the greatest from BRJ supplementation, but more research is needed for full realization.

Although this brief report presents novel information regarding BRJ supplementation and reactive agility performance, there were limitations. First, it remains largely speculative as to what the precise physiological mechanisms for agility performance enhancement are and the current study design did not allow for mechanistic exploration. However, future studies should use more powerful measurements to aid in optimizing training. Although the current sample size is supported statistically, it may not be representative of the general athletic population due to the low number of participants. However, the effect sizes of changes were large indicating that these data still have tremendous value. The current study only used one dosing regimen and NO_3 source which may not apply to other variations in these factors. Thus, future studies will be needed to optimize dosing for improved health and performance. Finally, although the FITLIGHT training system has been tested and validated in multiple contexts, the current brief report did not directly complete the test-retest or validation of FITLIGHT equipment. Therefore, we cannot eliminate possible sources of error from this although the implementation of counterbalancing the agility tests may have abrogated this as a possibility.

5. Conclusions

In conclusion, acute BRJ ingestion significantly increased plasma NO_3/NO_2 which was concurrently observed with improved reactive agility performance but not SRT. From a practical standpoint, BRJ may be used by athletes and recreational exercisers to improve reactive ability which may be important in sports that rely heavily on reactive ability (i.e., track-and-field/sprinting, football, basketball). BRJ is a natural and safe source of inorganic nitrate with well-supported claims of improved exercise performance. Furthermore, BRJ is also

relatively inexpensive and readily available for consumer use. Thus, coaches and athletes may use BRJ to optimize performance and training although more research with larger sample sizes and more mechanistic study designs is required to confirm the current findings.

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Informed Consent Statement: Prior to any data collection, verbal and written informed consent was obtained from each participant.

Data Availability Statement: All data are freely available within this manuscript.

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