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# Acute doses of beetroot juice supplementation on aerobic and anaerobic performance of trained male taekwondo athletes

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## ABSTRACT

Studies have shown that Nitrate ( $\text{NO}_3^-$ ) rich beetroot juice (BJ) supplementation improves endurance and high-intensity intermittent exercise. The dose-response effect on taekwondo of BJ supplementation are yet to be determined. This study aimed to investigate two acute doses of 400 mg of  $\text{NO}_3^-$  (BJ-400) and (800 mg  $\text{NO}_3^-$  (BJ-800) on taekwondo-specific performance tests compared with a placebo (PL) and control (CON) conditions. Eight trained male taekwondo athletes (age:  $20 \pm 4$  years, height:  $180 \pm 2$  cm, weight:  $64.8 \pm 4.0$  kg) completed four experimental trials using a randomized, double-blind placebo-controlled design: BJ-400, BJ-800, PL, and CON. Countermovement jump (CMJ) was performed before the Multiple Frequency Speed of Kick Test (FSKT) and Progressive Specific Taekwondo Test (PSTT), respectively, whereas cognitive function was assessed (via the Stroop test) before and after supplementation and 10 minutes following PSTT. Blood lactate was collected before the CMJ tests, immediately, and 3 minutes after the FSKT and PSST, with rating of perceived exertion (RPE) was recorded during and after specific taekwondo tests. No significant difference between conditions were observed ( $p > 0.05$ ) for PSTT and FSKT performances. In addition, blood lactate, RPE, heart rate, and CMJ height and performance during the specific taekwondo tests were not significantly different among conditions ( $p > 0.05$ ). However, after the PSTT test, cognitive function was higher in BJ-400 compared to other treatments ( $p < 0.05$ ). In conclusion, acute intake of 400 and 800 mg of  $\text{NO}_3^-$  rich BJ did not improve taekwondo-specific performance tests in trained male taekwondo athletes.

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## KEYWORDS

combat sport, martial arts, high-intensity intermittent performance, ergogenic aid

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## MATERIALS TEXT

### Participant

The study starts with 12 black belt athletes from the Iranian National Taekwondo League, but four could not complete our study due to injury during the period of the investigation. All athletes will be from the same club to prevent potential interference/variation caused by different training programs implemented among taekwondo clubs.

Athletes need to have all of the following inclusion criteria: 1) more than five years of experience in taekwondo; 2) not have consumed any ergogenic aids three months before the study; 3) to be training at least five times a week; 4) be older than 18 years old; 5) not currently have any musculoskeletal injuries. This study will be conducted during the 4-week pre-competition preparation phase. During this time, participants train six sessions per week, including three taekwondo-specific training and three condition sessions, including strength training and taekwondo-specific fitness. The ethical committee approved the study of science ministry, research technology sport science research institute of Iran (IR.SSRC.REC.1399.062).

### Experimental design

This study has got a randomized, double-blind placebo-controlled crossover design with five testing sessions at the laboratory. In the first session, participants will familiarized with the Multiple Frequency Speed of Kick Test (FSKT), countermovement jump (CMJ), and a Progressive Specific Taekwondo Test (PSTT). Subsequently, in a randomization order, participants will be set to 4 conditions: 1) 60 mlBJ (400 mg NO<sub>3</sub><sup>-</sup>) + 60 mlPlacebo (PL) (BJ-400), 2) 60 ml BJ + 60 mlBJ (BJ-800), 3) 60 ml PL + 60 ml PL (PL), and 4) control (CON). The washout between sessions will be seven days, and all evaluations will be done between 10:00 a.m. and 4:30 p.m., and at the same day of each week to prevent the circadian rhythm effect. To prevent overreaching, coaches will request to control training volume and intensity throughout the study. The Hooper Index questionnaire will be used before each test to monitor and evaluate the recovery and accumulated fatigue [1]. The experimental design is summarized in the Figure 1.

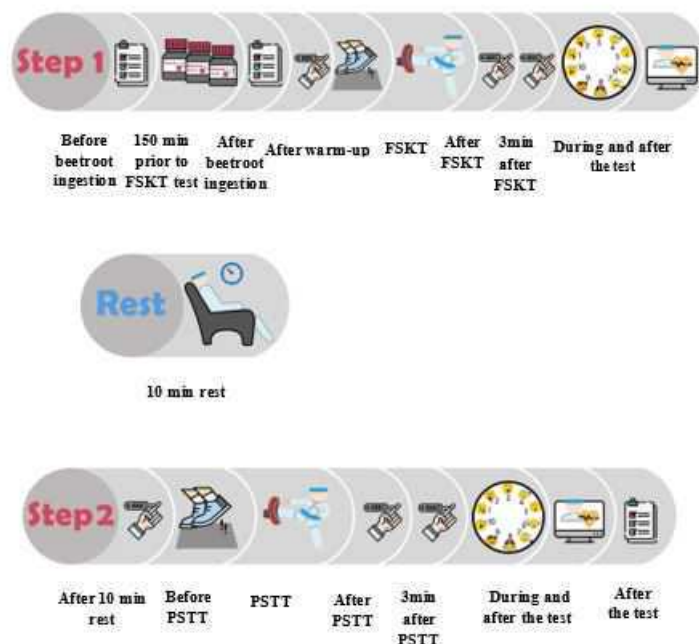


Figure 1. Schematic representation of the experimental design. FSKT = Frequency Speed of Kick Test; PSTT = Progressive Specific Taekwondo Test

#### Supplementation Protocol and Standardization of Physical Activity and Diet

Participants in the four trials (BJ-800, BJ-400, PL, CON) will arrive at the laboratory 3 hours prior to the tests. One-hundred-fifty min before starting the tests, each participant will ingest in a dose-response manner either one bottle of 60 ml BJ + 60 ml of PL or two bottles of BJ (120 ml) (Red Beet Vinitrox Shot; Sponsor Ltd, Germany) containing 400 mg  $\text{NO}_3^-$ , per serving or depleted dried powder  $\text{NO}_3^-$  for PL [2]. Based on previous instructions, PL will be prepared by dissolving 1 g of dried powder of BJ in one liter of water and adding lemon juice to mirror the taste of the commercial supplement [2]. Both, BJ and PL will be provided in identical bottles. The timing of BJ ingestion is based on previous published recommendations, i.e., ingesting 2.5-3 hours before exercise to synchronize with the peak of plasma  $\text{NO}_2^-$  [3]. In consideration of the anti-bacterial effect in the mouth by oral antiseptics which may prevent the rise in blood  $\text{NO}_2^-$  after the intake of  $\text{NO}_3^-$ , participants will be instructed to abstain from teeth brushing, using mouthwash, chewing gum, or sweets that could contain anti-bacterial substance during 24 hours prior to the test session [2].

Furthermore, a list of dietary sources rich in  $\text{NO}_3^-$  will be given to participants, with instructions to avoid eating high  $\text{NO}_3^-$  content (beetroot, celery, arugula, lettuce, spinach, turnip, endives, leek, parsley, cabbage etc.) in the 24 h prior to arriving at the laboratory. Participants will be instructed to avoid drinks containing caffeine and polyphenol-rich fruits 72 hours before the tests due to its potential ergogenic effect. Each participant replicated their intake 24 h prior to the first condition in subsequent sessions. A standardized snack will be ingested as a pre-workout meal and consisted of 1.5 g/kg carbohydrate and a 20-gram protein, and will be administered 4 hours before each test for participants.

#### Frequency Speed of Kick Test multiple

The FSKT included 5 sets of FSKT, and each set's period is 10 s interspersed with 10 s passive rest. Each athlete have to stay in front of the punching mitt to perform the test. After the sound signal, athletes will perform the maximal number of kicks, alternating right and left legs. Performance will be measured via the number of kicks in each set, the total number of kicks, and the kick decrement index (KDI) during the test. KDI indicates the degree that performance decreases during the test and will be calculated based on the number of kicks performed during five sets of the FSKT [4].

#### Progressive Specific Taekwondo Test

The PSTT uses a kick pad and will be performed in a 2 m × 2 m area. Kicks has to be between the navel and nipples height. Participants will start the PSTT from the first stage with six kicks, performed by the right leg alternating between right and left legs, then progressively increasing four kicks in each new stage. Athletes will be pending the test in step (fighting stance

hopping). Sound signals sit the pace performance with the, distance interval between each kick will be fixed for each new stage, intervals decrease for every new stage, increasing the kicking frequency (more details are provided in the original reference) [5]. Each athlete will continue to pace until exhaustion and will receive verbal encouragement so that participants will perform the kicks with maximum power and maintain the same power and technical quality of the kicks for the whole duration of the test. Heart rate (HR) will be recorded (Polar H10- Kempele, Finland) every 5 s during the protocol. The HR at the end of the test will be taken as HRpeak. During the PSTT, the highest frequency of kicks will be defined as the peak frequency kick that athletes reach in the last stage. Athletes will be instructed to perform the bandal-tchagui kick on the pad throughout the test. The test will be stopped upon the athlete failing to maintain more than two standard technical kicks or when the athlete stops the test [5].

### Counter Movement Jump

Subjects have to perform a maximal counter movement jump (CMJ) via a validated cellphone app, the My jump 2 app [6]. Athletes will perform the jump from a start position of extended knees, before squatting down to 90° knee flexion, with hands in mild pronation and without any subsequent arm swing, followed by an immediate jump for maximal height. Knees must be extended during the flight stage, as arms moved down with the elbows extended, and contact with the ground will be made with the toes. This test will perform prior to the conduct of each specific taekwondo tests. Three CMJ will be executed with a period of 30 seconds rest between jumps, and the highest jump will be recorded.

### Lactate Measurement and Rating of Perceived Exertion Recording

Finger-prick blood samples for lactate will be collected before the CMJ tests, immediately and 3 minutes after the PSST and FSKT (Lactate h/p/Cosmos, Germany). The rating of perceived exertion (RPE, 6-20 Borg scale) will be recorded after the FSKT and PSST, during FSKT, and PSST.

### Cognitive function assessment

Cognitive function will be assessed via the Stroop word-color test before and after supplementation and 10 minutes following the progressive specific taekwondo test [7]. The test has three pages including a word page printed with black words (W), a page containing 'XXXX' printed in different colors (C), a final page combines "RED", "GREEN" and "BLUE" words from the first page printed with colors from the second page, where the ink color do not match with text of the color word (CW). All subjects should respond as quickly as possible to each page for 45 seconds. The tests of the first two pages evaluate congruence, whereas the last test measures incongruence or interference to identify the true word without color effect on their choice to correct answer. The correct answers will be scored for percentage accuracy and reaction time will be considered for assessing cognitive function during period.

### Gastrointestinal symptoms assessment

Symptoms of gastrointestinal complaints (GI) after supplement ingestion will be reported by a gastrointestinal questionnaire [8]. Participants will select values ranging from 0 to 9, where 0 indicated "no problem at all" and 9 indicated "the worst it has ever been". The symptoms will be considered severe when the score will be equal to or higher than 5.

### Statistical analysis

All results will be reported as mean and standard deviation. Normality will be checked by using the Shapiro-Wilk test. A one-way repeated measure ANOVA will be used to compare aerobic performance (TTE), anaerobic performance (total kicks and KDI), and height reached on CMJ between the four treatments. A two-way repeated measure (treatment-by-time) ANOVA will be used for each variable of anaerobic performance, Stroop test, lactate, and rating of perceived exertion (RPE). Bonferroni test will be used as post hoc when a significant difference will be found in the ANOVA. ANOVA-partial eta squared denoted as is the effect size (ES) for all type of ANOVA which classified as small (0.01), medium (0.06), and large effect (0.14 and higher) (<https://www.spss-tutorials.com/effect-size/>). Significance will be set a priori at an alpha level of  $p \leq 0.05$ . Statistical analyses will be performed using SPSS 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp).

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