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Beetroot Juice and Vitamin C Co-Supplementation Enhances Anaerobic Performance and Reduces Post-Exercise Glycemia in Wrestlers: A Randomized, Double-Blind, Placebo-Controlled Crossover Trial

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Abstract

Background: Wrestling, characterized by high-intensity intermittent efforts, demands exceptional anaerobic power and recovery capacity. Nitrate-rich beetroot juice (BRJ), supplemented with vitamin C, has emerged as a potential ergogenic aid through increased nitric oxide bioavailability. However, limited data exists regarding its acute effects on anaerobic performance in combat sport athletes. This study investigated the acute effects of BRJ supplemented with vitamin C on upper- and lower-body anaerobic test performance and selected biochemical markers in collegiate wrestlers.

Methods: In a randomized, double-blind, placebo-controlled crossover trial, 28 collegiate male wrestlers (18–24 years) consumed a single 250-ml BRJ drink (8.4 mmol nitrate + 90 mg vitamin C) or an isocaloric nitrate-free placebo three hours prior to testing, with a 14-day washout between conditions. Participants performed sequential 30-second upper- and lower-body Wingate anaerobic test (WAnTs) separated by 90 seconds. Venous blood was collected pre-test, immediately post-test, and 24 hours post-test and was analyzed for blood glucose, creatine kinase (CK), and lactate dehydrogenase (LDH).

Results: 24 completed the study. BRJ+vitamin C significantly increased upper-body WAnT peak power (419 ± 98 W vs. 403 ± 103 W, $P<0.001$) and lower-body WAnT mean power (390 ± 89 W vs. 376 ± 89 W, $P=0.002$) compared with placebo. Post-exercise blood glucose was significantly lower in the BRJ + vitamin C group (96.3 ± 19.2 mg/dl vs. 112.5 ± 20.4 mg/dl, $P<0.001$). No significant differences were observed for CK ($P = 0.59$) or LDH ($P = 0.27$) at any measured time point.

Conclusion: Acute BRJ + vitamin C supplementation improved anaerobic capacity and lowered post-exercise blood glucose in collegiate wrestlers, offering a potential ergogenic strategy for combat sports. Further studies with larger, diverse populations and chronic supplementation are needed to confirm and extend these findings.

Trial registration and date: Iranian Registry of Clinical Trials (IRCT20240407061440N1); registration date: June 7, 2024.

Keywords: Beetroot Juice, Vitamin C, Wingate Anaerobic Test, Anaerobic Capacity, Glycemic Control, Wrestling

1. Introduction

Wrestling is an Olympic sport characterized by high-intensity, intermittent efforts that demands exceptional anaerobic power, muscular strength, and endurance across upper and lower body musculature (1, 2). With up to 90% of energy derived from anaerobic glycolysis during matches, wrestlers rely heavily on strategies to optimize power output and recovery (3, 4). Athletes in competitive sports like wrestling increasingly utilize dietary supplements to enhance physical and psychological capabilities (5, 6).

Dietary supplements, as defined by the International Olympic Committee, are targeted nutritional compounds consumed to achieve specific health or performance benefits (6). Among these, beetroot (*Beta vulgaris rubra*), rich in nitrate NO_3^- and bioactive phytochemicals such as betalains, flavonoids, and polyphenols (7), has recently been recognized as a potent ergogenic aid capable of enhancing exercise performance and alleviating exercise-related tissue stress (8-12). Beetroot naturally contains high levels of inorganic NO_3^- , typically 1-10 mmol/kg fresh weight in raw beets (varying by cultivar and conditions), while concentrated beetroot juice products commonly deliver 5-8 mmol nitrate per acute dose for ergogenic purposes (13, 14).

NO_3^- from beetroot juice (BRJ) enhances nitric oxide (NO) bioavailability through its reduction to nitrite (NO_2^-) and subsequently NO, particularly under hypoxic and acidic conditions, which are prevalent during high-intensity exercise (15-18). This process promotes vasodilation, improves blood flow, enhances muscle efficiency, and reduces the oxygen cost of exercise, potentially benefiting anaerobic performance in sports like wrestling (17-21). Furthermore, emerging evidence suggests that increased

NO bioavailability may also improve glucose uptake in skeletal muscle, independent of insulin and muscle contraction pathways, by stimulating GLUT4 translocation and glucose transport (22, 23). This mechanism could help attenuate exercise-induced hyperglycemia, which commonly occurs during high-intensity anaerobic efforts due to catecholamine-mediated hepatic glucose output and reduced peripheral glucose disposal. Additionally, BRJ's antioxidant properties, driven by betalains, may reduce exercise-induced muscle damage and inflammation, as evidenced by decreased serum levels of creatine kinase (CK) and lactate dehydrogenase (LDH) (12, 24).

Vitamin C, a potent water-soluble antioxidant, enhances NO production by neutralizing reactive oxygen species (ROS) and facilitating NO_3^- reduction to NO under hypoxic and acidic conditions (25-27). Vitamin C acts as a cofactor in deoxyhemoglobin-mediated reactions and acidified NO_3^- conversion (25). By scavenging exercise-induced ROS, vitamin C may preserve NO bioavailability and further support NO-mediated glucose uptake in muscle, potentially providing a synergistic effect with BRJ in mitigating post-exercise hyperglycemia (28).

Therefore, it can be hypothesized that vitamin C may amplify the ergogenic benefits of nitrate-rich BRJ not only in improving anaerobic performance and recovery, but also in attenuating exercise-induced hyperglycemia, which may otherwise contribute to fatigue, osmotic stress, and delayed recovery in wrestlers. However, the combined effects of dietary nitrate and vitamin C supplementation, particularly in combat sports, remain underexplored, highlighting a critical gap in the literature (25, 29, 30). The rationale underlying the selection of this supplement, along with its design as a performance-enhancing strategy for wrestlers, is illustrated in Figure 1.

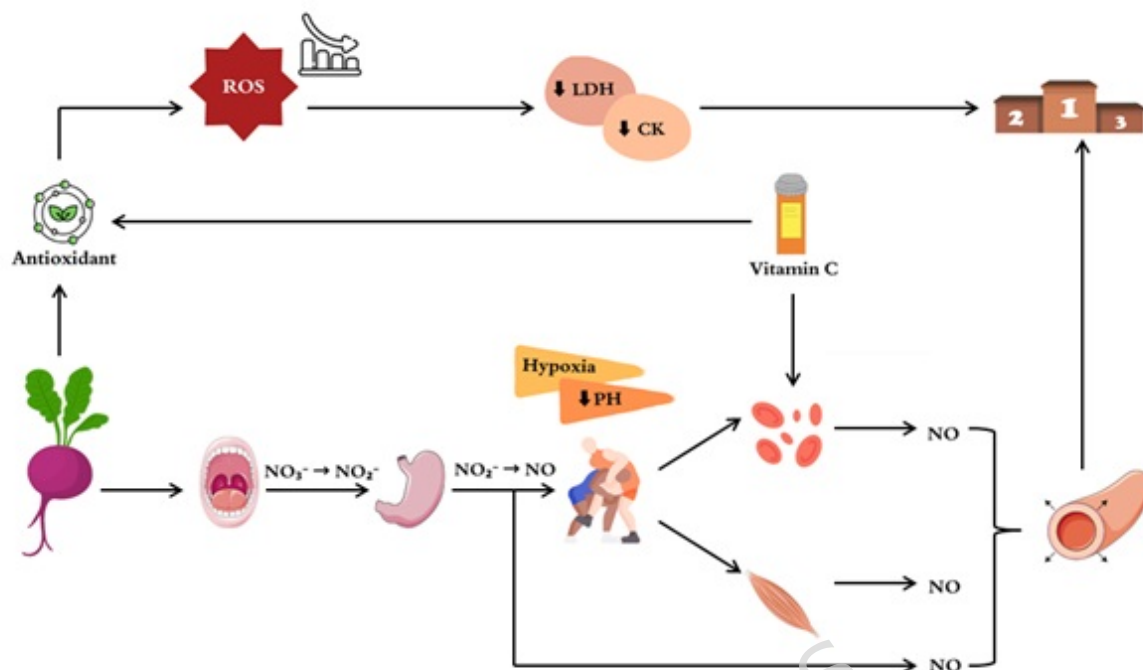


Figure 1. Hypothesized Mechanism of Anaerobic Performance Enhancement from Combined Beetroot Juice and Vitamin C Supplementation

Wrestling stands out as a discipline heavily dependent on anaerobic energy systems, characterized by intense exertions spanning 6 to 60 seconds, predominantly fueled by glycolytic processes (31). Although the acute administration of beetroot supplements has been thoroughly investigated for its influence on upper and lower body performance in the Wingate Anaerobic Test (WAnT), with separate evaluations conducted for upper-body and lower-body protocols (32-35), a combined alternating protocol has not yet been implemented, leaving significant gaps in this area (36). Previous studies have predominantly concentrated on successive trials within a single body region, neglecting the dynamic, sequential activation of both upper and lower extremities that is emblematic of combat sports (37-39).

This study sought to address a key gap in the literature by investigating the acute effects of a single dose of beetroot juice combined with vitamin C on

upper and lower body anaerobic performance and selected biochemical markers in collegiate wrestlers. To enhance ecological validity, a novel sequential WAnT protocol involving consecutive upper and lower body efforts was employed to closely replicate the multifaceted physiological demands characteristic of competitive wrestling. We hypothesized that this combination would improve anaerobic performance in wrestlers, attenuate exercise-induced hyperglycemia, and reduce muscle damage markers (6, 26, 27, 40).

2. Materials and Methods

2.1. Study Design

A randomized, double-blind, placebo-controlled, crossover trial was conducted from June to July 2024 at Ferdowsi University's Faculty of Sport Sciences, Iran. The study was approved by the Ethics Committee of Mashhad University of Medical Sciences (IR.MUMS.MEDICAL.REC.1403.011) and registered with the Iranian Registry of Clinical Trials (IRCT20240407061440N1). All procedures adhered to the Declaration of Helsinki.

2.2. Participants

Twenty-eight collegiate male wrestlers were recruited via coaches at Ferdowsi University of Mashhad, Iran. Inclusion criteria included age 18-24 years, non-elite status, at least 2 years of wrestling experience, prior WAnT experience, and adherence to the study protocol. Exclusion criteria encompassed caffeine consumption within 24 hours prior to testing, use of nitrate-containing medications or supplements within the past six months, herbal medication use, presence of any cardiovascular, pulmonary, metabolic, neurological, or orthopedic conditions limiting cycle ergometry,

recent acute cardiac events, beetroot allergies, use of oral antiseptics within the past four weeks, addiction, alcoholism, or intense exercise within 72 hours prior to testing. Eligibility was assessed through medical history questionnaires and physical examinations conducted by a study physician. Participants were withdrawn for acute illness, adverse effects impacting quality of life, or unwillingness to continue.

2.3. Randomization and Blinding

Participants were randomized to receive BRJ drink (8.4 mmol NO_3^- plus 90 mg vitamin C) or an isocaloric placebo in random order, with a 14-day washout period to eliminate carryover effects (41). Randomization was performed using a web-based tool (www.sealedenvelope.com), with allocation concealed in numbered, opaque, sealed envelopes managed by an independent staff member. Blinding was maintained for participants, assessors (conducting WAnT, blood sampling, and RPE questionnaires), and analysts. Intervention and placebo drinks, matched for appearance and taste, were prepared and labeled by an independent staff member. Allocation details were inaccessible until data collection was complete.

2.4. Intervention and Placebo

The intervention was a 250-ml BRJ drink containing 8.4 mmol NO_3^- (from 56 g freeze-dried beetroot powder) and 90 mg vitamin C, with 0.25 g/ml added sugar. The nitrate dose was based on evidence indicating performance benefits with 5–14.9 mmol NO_3^- consumed 2–3 hours pre-exercise (18, 42). The placebo drink was isocaloric to the intervention drink and contained 0.4 mg/ml citric acid, 0.2 mg/ml food coloring, with 0.25 g/ml added sugar. Both the intervention and placebo drinks were administered three hours prior to Wingate tests to maximize plasma levels of NO_3^- (41), vitamin C (43), and polyphenols (44), corresponding to peak bioavailability.

Beetroot was juiced and freeze-dried to preserve nutrients and minimize contamination (45-47). The powder was tested for heavy metals and NO_3^- content, confirming safety per FAO/WHO standards (48, 49). High-nitrate foods were avoided during the washout period in both conditions and compliance to this was confirmed throughout the study protocol.

2.5. Procedures

The study involved three visits (second and third visits separated by two weeks). At the first visit, participants completed medical history questionnaires, underwent eligibility screening, and were familiarized with Monark cycle ergometers (Ergomedic 891E for upper body, 894E for lower body; Vansbro, Sweden). Anthropometric (height via stadiometer, body composition via InBody 720 Bioelectrical Impedance Analyzer) and baseline assessments (72-hour food record, International Physical Activity Questionnaire) were performed (50, 51).

On testing days, participants consumed either the supplement drink or a placebo within 10 minutes and rested quietly in the laboratory for three hours before beginning a warm-up. Warm-up included 5 min of cycling at 50 W with three short sprints, 5 min of rest, and ~3 min of dynamic stretching immediately before the Wingate tests. From there, each participant performed a 30-second upper-body WAnT and a 30-second lower-body WAnT in a sequential fashion with a 60-second break between the tests. This specific order was followed for all participants for all testing sessions. Prior research indicates that a 30-second lower-body WAnT is unaffected by a preceding 30-second upper-body WAnT, whereas upper-body performance significantly declines following a lower-body WAnT (38). Despite elevated blood lactate levels, subsequent lower-body peak and mean power remain unchanged (38). This supports the use of sequential WAnT protocols targeting different body regions to assess integrated anaerobic performance. Further, this approach strengthens the face validity of our

protocol due to dynamic demands of wrestling involving both the upper-body and lower musculature. Upper-body WAnT was assessed using a modified Monark ergometer (fixed table, seated position, elbows near full extension). Lower-body WAnT was assessed using a standard ergometer (seat adjusted for $\sim 5^\circ$ knee flexion). Resistance was set to 5% and 7.5% of body weight for upper body and lower body WAnT tests, respectively (52, 53). Power output (peak power, mean power, minimum power, time to peak power, fatigue index) was recorded using Wingate (version 1.0.7) software. Blood samples (10 ml, brachial vein) were collected pre-test (3 hours post-supplementation), immediately post-WAnT, and 24 hours post-WAnT. Blood samples (10 ml, brachial vein) were collected pre-test (3 hours post-supplementation), immediately post-test, and 24 hours post-test. Samples were centrifuged at 3000 rpm for 10 minutes at 4°C to separate serum. Serum glucose, CK, and lactate LDH were analysed as follows:

Serum glucose was measured in triplicate using a colorimetric enzymatic assay (GOD-PAP) with commercial reagents from the Glucose Enzymatic Kit (BioRexFars Innovative Diagnostics, Iran). The assay was conducted at 546 nm, 37°C , with a 1 cm cuvette against a reagent blank.

Serum CK was measured in triplicate using an enzymatic assay (IFCC method) with commercial reagents from the CK-NAC Kit (BioRexFars Innovative Diagnostics, Iran), including reagent R1 (Imidazole buffer pH 6.7, Glucose 20 mmol/l, Mg-acetate 10 mmol/l, EDTA 2 mmol/l) and reagent R2 (ADP 2 mmol/l, NADP 2 mmol/l, AMP 3 mmol/l, Hexokinase ≤ 2.5 U/ml, Diadenosine pentaphosphate 5 $\mu\text{mol/l}$, G6PDH ≤ 1.5 U/ml, N-Acetyl Cysteine 20 mmol/l, Creatine phosphate 30 mmol/l). The assay was conducted at 340 nm, 37°C , with a 1 cm cuvette against a reagent blank.

Serum LDH was measured in triplicate using an enzymatic assay (IFCC method) with commercial reagents from the LDH Kit (BioRexFars Innovative Diagnostics, Iran), including R1 (Tris buffer pH 7.5 50 mmol/l,

Pyruvate 0.6 mmol/l) and R2 (NADH 0.18 mmol/l). The assay was conducted at 340 nm, 37°C, with a 1 cm cuvette against a reagent blank. Adverse effects were monitored daily.

Considering the nitrate half-life of roughly 5 to 8 hours, a 14-day washout interval was deemed adequate to substantially diminish any lingering influences from prior nitrate intake (54). By employing a crossover design where participants acted as their own controls, testing sessions were scheduled at identical times of day across both conditions to minimize circadian variability.

2.6. Control of confounders

To minimize the influence of confounding factors, diet, physical activity, and circadian rhythms were rigorously controlled. Participants were provided with standardized dietary guidelines and instructed to follow a consistent low-nitrate diet for 72 hours prior to each testing session, avoiding foods high in NO_3^- (e.g., leafy greens, beets, and processed meats). Intervention compliance was monitored through 72-hour food records collected at the first visit and reviewed by a study nutritionist before each session. Participants were also instructed to refrain from intense physical activity for 72 hours prior to testing, and their baseline physical activity levels were assessed using the International Physical Activity Questionnaire (IPAQ) to ensure consistency across sessions (50). To minimize potential influences of daily biological fluctuations, participants completed their assessments at a consistent time window (11:00–14:00) across all visits. Additionally, a washout interval of at least 14 days was implemented to reduce any residual or carryover effects between testing sessions.

2.7. Sample Size

Sample size was calculated based on mean power data from a previous study by Williams et al. (9) (placebo: 508.14 ± 117.55 W; BRJ: 607.36 ± 112.28 W). The required sample size was calculated to be 22 participants for a crossover design considering 95% confidence and 80% power using the following equation:

$$n = \frac{(k \times \delta_1^2 + \delta_2^2)(Z_{1-\alpha/2} + Z_{1-\beta})^2}{\Delta^2}$$

In the above equation n is the sample size in each group, k is the ratio of the groups, which is one in this study, δ is the standard deviation in each group, Δ is the difference of group means, $Z_{1-\alpha/2}$ is considered 1.96 for 95% confidence interval, and $Z_{1-\beta}$ is the power of the study. To mitigate the risk of attrition and to secure completion of all planned study visits, the study initially recruited 28 participants.

Statistical Analysis

Data were analyzed using SPSS v26. Normality was assessed via Shapiro-Wilk test. Independent t-test was used for the comparison of normally distributed variables and Mann-Whitney test was used for the comparison of non-normally distributed variables. In order to assess the carryover effect, univariate General Linear Model (GLM) was used independently for each variable with each outcome variable as dependent variable and treatment and sequence of interventions as fixed factors. The carryover effect was defined as the treatment-sequence (carryover) effects in the model. All tests were performed two-sided and the statistical significance level was set at $p < 0.05$.

3. Results

3.1. Participant Characteristics

Of 28 enrolled collegiate male wrestlers, 24 (85.7%) completed the study; four withdrew due to unwillingness to continue after the first session. The flow of participants is presented in Figure 2, following CONSORT guidelines.

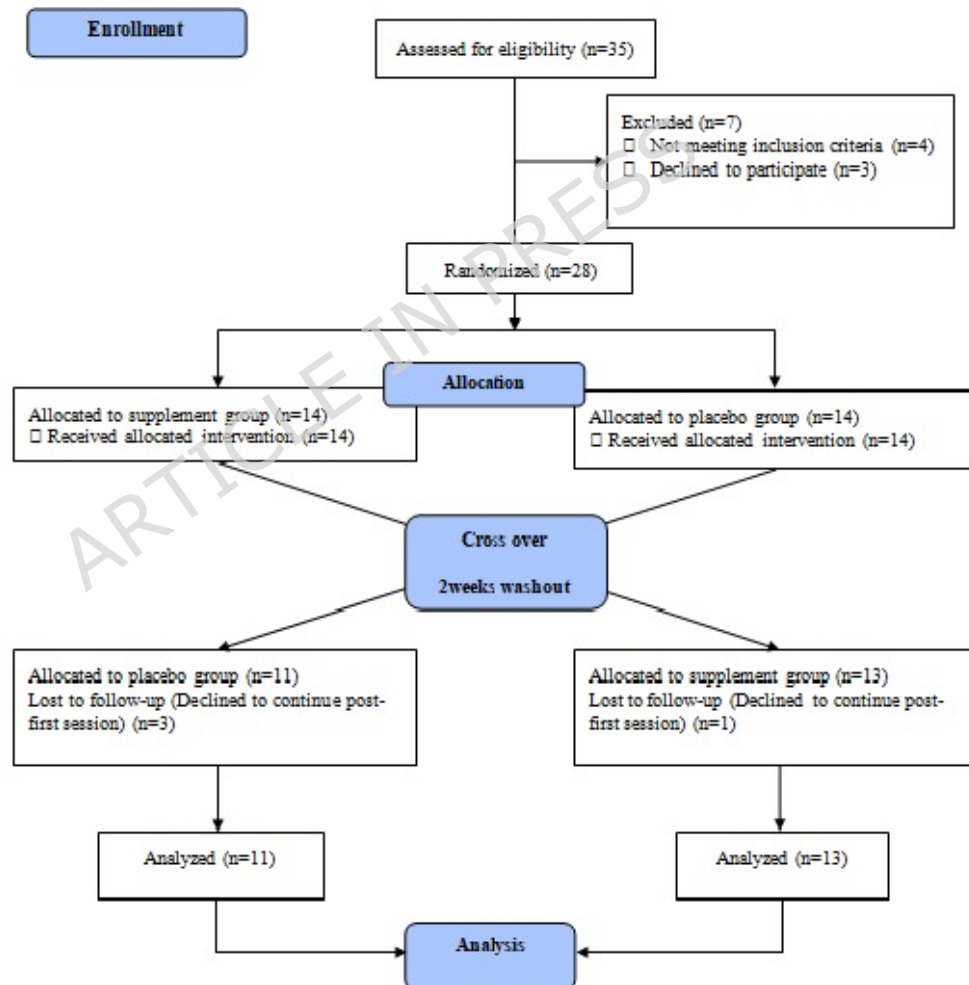


Figure 2. CONSORT 2010 Flow Diagram for a Crossover Trial

Baseline characteristics are summarized in Table 1.

Table 1 | Demographic and Anthropometric Characteristics

Variable	Value
Age (years)	21.0 ± 3.2
Weight (kg)	67.9 ± 11.8
Height (cm)	172.2 ± 6.0
BMI (kg/m ²)	22.7 ± 3.0

Data are means ± SD. SD = standard deviation; BMI = body mass index.

3.2. Upper-Body Wingate Anaerobic Test (WAnT) Outcomes

The BRJ+vitamin C group exhibited significantly higher peak power (419 ± 98 W vs. 403 ± 103 W, $P < 0.001$) and peak power per body weight (6.2 ± 0.8 W/kg vs. 5.9 ± 0.8 W/kg, $P < 0.001$) compared to the placebo group in the upper-body WAnT (Table 2). Although mean power was higher in the BRJ+vitamin C group (260 ± 72 W vs. 246 ± 75 W, $P = 0.099$), this difference did not reach statistical significance. No significant differences were observed for minimum power, minimum power per body weight, mean power per body weight, time to peak power, or fatigue index (all $P \geq 0.05$). No significant intervention-by-time interactions or carryover effects were detected ($P > 0.05$).

Table 2 | Upper-Body Wingate Anaerobic Test Outcomes (3 Hours Post-Supplementation)

Variable	BRJ+VitC	Placebo	P-value
Peak Power (W)*	419 ± 98	403 ± 103	<0.001
Peak Power (W/kg)*	6.2 ± 0.8	5.9 ± 0.8	<0.001
Mean Power (W)	260 ± 72	246 ± 75	0.099
Mean Power (W/kg)	3.7 ± 0.8	3.6 ± 0.8	0.178

Minimum Power (W)	42.7 ± 118.2	-81.2 ± 121.2	0.182
Minimum Power (W/kg)	-0.68 ± 1.77	-1.28 ± 1.73	0.165
Time to Peak Power (s)	10.3 ± 3.3	9.8 ± 3.5	0.369
Fatigue Index	113 ± 31	124 ± 30	0.158

*BRJ: beetroot juice; *: significant difference for placebo vs. BRJ+Vit C ($p < 0.05$); Data are means ± SD.*

3.3. Lower-Body Wingate Anaerobic Test (WAnT) Outcomes

In the lower-body WAnT, the BRJ+vitamin C group showed significantly higher mean power (390 ± 89 W vs. 376 ± 89 W, $P = 0.002$) and mean power per body weight (5.8 ± 0.9 W/kg vs. 5.3 ± 0.8 W/kg, $P = 0.001$) compared to placebo (Table 3). No significant differences were observed for peak power, peak power per body weight, minimum power, minimum power per body weight, time to peak power, or fatigue index (all $P \geq 0.05$). No significant intervention-by-time interactions or carryover effects were detected ($P > 0.05$).

Table 3 | Lower-Body Wingate Anaerobic Test Outcomes (3 Hours Post-Supplementation)

Variable	BRJ+VitC	Placebo	<i>P</i> -value
Peak Power (W)	516 ± 132	513 ± 126	0.752
Peak Power (W/kg)	7.4 ± 0.9	7.6 ± 1.0	0.320
Mean Power (W)*	390 ± 89	376 ± 89	0.002
Mean Power (W/kg)*	5.8 ± 0.9	5.3 ± 0.8	0.001
Minimum Power (W)	199 ± 67	205 ± 49	0.625
Minimum Power (W/kg)	2.9 ± 1.0	3.1 ± 0.7	0.493

Time to Peak Power (s)	5.3 ± 2.6	5.6 ± 2.2	0.690
Fatigue Index	60.4 ± 13.2	58.8 ± 9.1	0.494

*BRJ: beetroot juice; *: significant difference for placebo vs. BRJ+Vit C (p < 0.05); Data are means ± SD.*

3.4. Biochemical Markers

Serum glucose levels were significantly lower in the BRJ+vitamin C group compared to placebo both pre-test (72.0 ± 6.7 mg/dl vs. 82.6 ± 9.0 mg/dl, P < 0.001) and post-test (96.3 ± 19.2 mg/dl vs. 112.5 ± 20.4 mg/dl, P < 0.001) (Table 4). No significant differences were observed for CK or LDH levels in pre-test, post-test, or 24 hours post-test (P ≥ 0.05). No intervention-by-time interactions or carryover effects were detected (P > 0.05).

Table 4 | Biochemical Marker Outcomes

Variable	Time Point	BRJ+VitC	Placebo	P-value
CK (U/L)	Pre-Test	210 ± 122	219 ± 168	0.798
	Post-Test	290 ± 199	271 ± 192	0.729
	Difference (post-pre)	37.5 (9.8-124.8)*	21 (11.5-54.3)*	0.392
	24 h Post-Test	351 ± 222	321 ± 211	0.596
LDH (U/L)	Pre-Test	335 ± 73	354 ± 98	0.278
	Post-Test	405 ± 75	416 ± 90	0.535
	Difference (post-pre)	69.8 ± 51.5	48 (23.75-72.75)*	0.370
	24 h Post-Test	454 ± 99	467 ± 98	0.536
BG (mg/dl)*	Pre-Test	72.1 ± 6.7	82.6 ± 9.0	<0.001

Post-Test	96.3 ± 19.2	112.5 ± 20.4	<0.001
Difference (post-pre)	24.2 ± 18.2	29.8 ± 18.4	0.292

*BRJ: Beetroot Juice; CK: Creatine Kinase; LDH: Lactate dehydrogenase; BG: Blood Glucose; BG not measured at 24 h post-test; *: significant difference for placebo vs. BRJ+Vit C (p < 0.05);*

** Median and interquartile range was used due to non-normal distribution of data*

All tests were independent t-test except for comparison of the difference in CK and LDH, for which the Mann-Whitney test was used.

3.5. Carryover effect

The effect of treatment-sequence (carryover) is shown in Table 5. Significant carryover effect was seen only in upper body time to peak (p=0.042) and lower body minimum power (p=0.018).

Table 5 | Effect of treatment, sequence, and treatment-sequence (carryover)

	Variable	Treatment-sequence effect (carryover)
Upper body	Peak Power (W)	0.071
	Peak Power (W/kg)	0.113
	Mean Power (W)	0.123
	Mean Power (W/kg)	0.518
	Minimum Power (W)	0.153
	Minimum Power (W/kg)	0.146
	Time to Peak Power (s)	0.042*

Lower body	Peak Power (W)	0.187
	Peak Power (W/kg)	0.484
	Mean Power (W)	0.308
	Mean Power (W/kg)	0.910
	Minimum Power (W)	0.018*
	Minimum Power (W/kg)	0.070
	Time to Peak Power (s)	0.404
CK	Pre-Test	0.598
	Post-Test	0.446
	24 h Post-Test	0.591
LDH	Pre-Test	0.750
	Post-Test	0.467
	24 h Post-Test	0.586
BG	Pre-Test	0.593
	Post-Test	0.172

CK: Creatine Kinase; LDH: Lactate dehydrogenase; BG: Blood Glucose; BG not measured at 24 h post-test. Univariate general linear model was used to assess the effects.

4. Discussion

In this randomized, double-blind, placebo-controlled crossover trial, an acute dose of BRJ enriched with vitamin C (BRJ+VitC, 8.4 mmol nitrate, 90 mg vitamin C) produced modest but statistically significant improvements in selected measures of anaerobic performance, specifically upper-body WAnT peak power and lower-body WAnT mean power in collegiate male wrestlers. Additionally, BRJ+VitC attenuated the rise in post-exercise serum glucose. No treatment effect was observed for the muscle-damage biomarkers CK or lactate LDH. Collectively, these findings highlight the

ergogenic potential of combined nitrate and vitamin C supplementation in combat sports, particularly wrestling, where sequential high-intensity efforts are critical.

The improvements in upper-body peak power and lower-body mean power align with prior studies demonstrating that dietary nitrate can increase peak and mean power during short, high-intensity efforts (20, 55-57). For example, Domínguez et al. (55) reported increased peak and mean power in the WAnT with 5.6 mmol NO_3^- from BRJ and Hashemi Fard et al. (57) observed enhanced anaerobic power in karate athletes. Our results extend this literature by showing benefits in a repeated, sequential upper-then-lower body WAnT protocol designed to better mimic the mixed demands of wrestling.

In this crossover trial, BRJ significantly increased peak anaerobic power in the upper-body Wingate test but not in the lower-body test. Mechanistically, NO_3^- from BRJ is reduced to NO_2^- and NO via enterosalivary and tissue pathways, particularly under hypoxic and acidic conditions typical of maximal anaerobic bouts. NO can acutely improve muscle contractile function, calcium handling in type II fibers, and local blood flow effects that plausibly contribute to improved peak and mean power (58-60). Vitamin C may possibly augment these effects by (1) attenuating oxidative inactivation of NO and (2) supporting reductive pathways that favor $\text{NO}_2^- \rightarrow \text{NO}$ conversion, potentially enhancing NO bioavailability. BRJ also reduces ATP demand and phosphocreatine depletion, supporting sustained high-intensity performance (61, 62). The clearer response in upper-body peak power compared to lower-body peak power is consistent with heterogeneous responses across muscle groups reported in the literature, potentially reflecting differences in muscle mass, motor unit recruitment patterns, or local perfusion (63-65). Also a lower NO_3^- dose compared to studies reporting ergogenic effects (11.2 mmol) (66, 67). These findings highlight

BRJ's potential to enhance upper-body performance in wrestling. The lack of effect on minimum power, time to peak power, and fatigue index may reflect the specific muscle groups involved or the acute dosing strategy, as chronic supplementation often yields broader benefits (10, 42).

Although some studies suggest BRJ supplementation may reduce exercise-induced CK elevations (24, 68), most studies, including ours, indicate BRJ fails to mitigate CK and LDH levels (69-72). Similarly, cherry and pomegranate juices show limited efficacy in altering CK and LDH levels (73, 74), suggesting antioxidant-rich foods may not reduce muscle damage. CK in particular is highly variable between individuals (75) and influenced by exercise modality, muscle mass, and sampling time; a single acute dose is unlikely to meaningfully alter these markers. Chronic supplementation or different timing may be necessary to observe consistent effects on biochemical indices of muscle damage

The significant reduction in serum glucose post-exercise suggests that BRJ+VitC improves glycemic control during high-intensity exercise. High-intensity anaerobic exercise, such as the WAnT, typically elevates plasma glucose due to catecholamine-induced hepatic glucose output, followed by post-exercise hypoglycemia as counter-regulatory hormones decline (76-85). BRJ, rich in nitrate, and vitamin C, a potent antioxidant, are studied for their potential to influence glucose regulation, especially after high-intensity exercise like the WAnT (28, 86, 87). The reduction in post-exercise serum glucose observed in our trial could reflect increased insulin-independent glucose uptake via NO-mediated stimulation of GLUT4 translocation in skeletal muscle, a mechanism independent of insulin and contraction pathways (22, 23). Vitamin C likely contributes synergistically by neutralizing exercise-induced reactive oxygen species (ROS), thereby preserving NO bioavailability and supporting glucose disposal amid oxidative stress (28). These complementary actions, NO promotion of

GLUT4 translocation and ROS scavenging, may collectively attenuate the glycemic excursion, although direct measures of insulin, lactate, $\text{NO}_3^-/\text{NO}_2^-$, or muscle GLUT4 were not collected here and therefore mechanistic inferences remain speculative (29, 88-90).

The effects of BRJ on post-exercise glucose levels vary across studies. In contrast to Vasconcellos et al. (91), who found no significant reduction in post-exercise glucose with BRJ alone and greater reductions in the placebo group, our study observed improved glycemic control with BRJ supplemented with vitamin C, likely due to the synergistic effects of vitamin C. These effects may be attributed to vitamin C's antioxidant properties, which reduce oxidative stress and enhance insulin-independent glucose uptake, as well as its role in promoting NO production, which improves muscle perfusion and glucose metabolism (92-94). Furthermore, mitigating post-exercise hyperglycemia may positively influence anaerobic performance in wrestlers (95). Elevated blood glucose excursions can lead to osmotic stress, potential dehydration (96), exacerbated metabolic acidosis (97), and increased perceived fatigue, which may impair recovery between intermittent high-intensity efforts and reduce power output in subsequent bouts. By reducing these glycemic spikes, BRJ + VitC may decrease fatigue accumulation, enhance recovery kinetics, and support sustained explosive performance, particularly relevant in combat sports with repeated anaerobic demands (98). Variability in glucose responses may also arise from differences in exercise intensity and participant fitness levels (40).

Several studies have investigated the ergogenic effects of BRJ monotherapy (without vitamin C) on anaerobic performance, predominantly using Wingate anaerobic tests or high-intensity protocols in trained or recreationally active individuals. Acute BRJ supplementation has been shown to modestly improve peak power by approximately 3-5% and mean

power by 3–6%, with reductions in time to peak power in some cases (99–101). In combat sports athletes, including wrestlers, BRJ alone has enhanced isokinetic strength and explosive power, though effects on overall anaerobic power output vary (102–104).

In the present study, BRJ + vitamin C co-supplementation significantly improved upper-body peak power by approximately 4.0–5.1% and lower-body mean power by 3.7–9.4%, alongside a marked attenuation of post-exercise blood glucose elevation. These improvements in peak and mean power align with or modestly exceed the typical range reported in BRJ-only studies, particularly in mean power for lower-body efforts and in the context of wrestlers' high anaerobic demands. Notably, the substantial reduction in post-exercise glycemia is less commonly emphasized in BRJ monotherapy trials and may reflect an additive antioxidant contribution from vitamin C, potentially mitigating oxidative stress and supporting glucose uptake via preserved NO bioavailability.

However, direct head-to-head comparisons are limited, and co-supplementation studies remain scarce. A recent systematic review on BRJ co-supplementation highlighted that additive benefits often depend on chronic duration rather than acute dosing, though evidence for vitamin C specifically is sparse (104).

Compared to other supplements commonly used in wrestlers (Prieto Martínez et al., 2025) (105), our acute BRJ + VitC co-supplementation produced modest but significant improvements in anaerobic performance. Creatine monohydrate generally shows stronger effects on anaerobic power, strength and recovery in wrestlers, while sodium citrate aids body mass regain after weight cutting, and spirulina or BCAA yield mixed or weaker results on anaerobic metrics. Our protocol uniquely attenuated post-exercise hyperglycemia, a benefit rarely reported for other supplements, which may help reduce fatigue in repeated high-intensity bouts. Overall,

BRJ + VitC offers a practical acute option with additive metabolic advantages, though direct comparisons and larger studies are needed.

Strengths and limitations

This study was the first to examine the combined effects of BRJ+VitC on sequential upper- and lower-body WAnT performance in collegiate wrestlers, addressing a critical gap in combat sport research (10). Strengths include the crossover design, which minimized inter-subject variability, the use of a validated WAnT protocol on friction-braked Monark ergometers (106, 107), a familiarization session to reduce learning effects (108), and rigorous control of confounders (diet, physical activity, circadian rhythms). The double-blind methodology and use of freeze-dried beetroot powder helped to ensure all bioactive compounds were preserved, which both worked to support the internal validity of our design. Comprehensive assessments of anaerobic power, biochemical markers and anthropometrics further strengthen the findings.

Limitations include the small sample size (n=24 completers), which may have limited the detection of smaller effects, particularly for CK and LDH. The exclusion of female and elite wrestlers restricts generalizability to these populations. The acute supplementation protocol may not reflect the potential benefits of chronic dosing, which could yield broader ergogenic effects (10, 35). Additionally, a significant carryover effect was observed for upper body time to peak power ($P=0.042$) and lower body minimum power ($P=0.018$), though this was limited to only two of the 14 performance variables assessed. The 14-day washout period, while typically sufficient, may not have fully eliminated residual effects in these parameters. A major limitation of this study is the lack of a BRJ-only control group, which precludes definitive attribution of benefits to vitamin C's independent or synergistic role. Thus, while the observed effects suggest potential additive

ergogenic advantages of co-supplementation, particularly for anaerobic power and glycemic regulation in wrestlers, these findings remain speculative without appropriate controls. Future trials should include BRJ-only, vitamin C-only, and combined arms to elucidate mechanisms and synergies in combat sport athletes. These findings underscore the need for further research with extended washout periods or parallel designs to clarify the persistence of such effects. Also, the lack of serum NO_3^- , NO_2^- , or lactate measurements limits mechanistic insights into NO bioavailability and metabolic responses.

Future directions

Future studies should investigate chronic BRJ+VitC supplementation to assess cumulative effects on performance and recovery. Including female and elite wrestlers would broaden applicability. Measuring serum nitrate, nitrite, and lactate levels could clarify mechanistic pathways. Additionally, assessing inflammatory markers (e.g., cytokines) could provide deeper insights into recovery dynamics. Given the absence of significant adverse effects beyond mild gastrointestinal discomfort, longer-term safety studies are warranted.

5. Conclusion

In conclusion, acute BRJ+VitC supplementation improved selected measures of anaerobic power and reduced post-exercise serum glucose in collegiate male wrestlers. However, baseline imbalances in glucose and evidence of carryover for specific outcomes temper inference. Future trials should include serum $\text{NO}_3^-/\text{NO}_2^-$ and insulin measures, pre-specified primary outcomes with adequate power, and design features (longer washout or parallel groups) to eliminate carryover. If these issues are

addressed, the combined nitrate + vitamin C strategy is a promising nutritional approach for combat sports.

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Data Availability Statement

The data supporting this study's findings are available upon request from the corresponding author.

Ethics Statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Mashhad University of Medical Sciences (IR.MUMS.MEDICAL.REC.1403.011). The participants provided their written informed consent to participate in this study. The trial was registered at the Iranian Registry of Clinical Trials (IRCT20240407061440N1).

Author Contributions

Conceptualization and Supervision, R.R.; formal analysis, A.J.E.; investigation, M.N. and A.H.Kh.; Writing original draft, M.N. and A.J.; Writing review, H.R., C.K and O.O.; writing review, and editing, M.N. and R.R. . All authors have read and agreed to the published version of the manuscript.

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Statement on the Use of Artificial Intelligence (AI)

We acknowledge that this essay was edited and its writing style refined with the help of ChatGPT (GPT-5, OpenAI's large-scale language generation model, used via the editGPT extension). The AI was not used to create the manuscript's content or references; it was employed solely to enhance the language. All AI-assisted text was carefully reviewed, edited, and rewritten by the authors, who assume full responsibility for the article's accuracy and substance.

Conflict of Interest

The authors confirm no conflicts of interest.

Abbreviations

BRJ: Beetroot juice

VitC: Vitamin C

WAnT: Wingate anaerobic test

CK: Creatine kinase

LDH: Lactate dehydrogenase

NO: Nitric oxide

NO₃⁻: Nitrate

NO₂⁻: Nitrite

ROS: Reactive oxygen species

CONSORT: Consolidated Standards of Reporting Trials

IPAQ: International Physical Activity Questionnaire

GLM: General linear model

IRCT: Iranian Registry of Clinical Trials

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