Beetroot juice ingestion does not improve neuromuscular performance and match-play demands in elite female hockey players: A randomized, double-blind, placebo-controlled study.

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ABSTRACT

Purpose: Beetroot juice is a dietary supplement that contains high levels of inorganic nitrate (NO_{3-}) and that its intake has proven effective at increasing blood nitric oxide (NO) concentrations improving endurance performance. However, the effect of this supplement in team sport performance, especially in female athletes, has been barely studied. This study aimed to compare the acute effects of beetroot juice supplementation on neuromuscular performance and match-play demands in elite female field hockey players. **Methods:** Eleven elite female hockey players (22.8 ±5.1 years) belonging to a bronze team medal in Eurohockey Club Champions Cup participated in this study. Participants were randomly divided into two groups undergoing a test battery with beetroot juice (70mL, 6.4 mmol NO_3^-) or placebo (70mL, 0.04 mmol NO_3^-) in two different days with one week between protocols. The neuromuscular test battery consisted of a countermovement jump, isometric handgrip strength (i.e., dominant hand), 20 meters-sprint and repeated sprint ability test (RSA). Afterward, a simulated hockey match play (2 x 12.5 minutes) was performed and recorded by Global Positioning System (GPS). **Results:** No statistically significant improvements were observed in any physical parameters analysed comparing beetroot juice compared to placebo ingestion, countermovement jump (p=0.776, ES=0.16), isometric handgrip strength (p=0.829; ES=-0.08), 20-meter sprint test (p=0.227; ES=-0.23), mean repeated sprint ability (p=0.955, ES=0.03) and in any physical match demands measured by GPS (p=0.243-1.000; ES=0.02-0.47). **Conclusion:** Acute beetroot juice supplementation did not produce any statistically significant improvement in neuromuscular performance or match-play demands in elite female field hockey players.

Trial Registration: The study was registered in ClinicalTrials.gov with the following ID: NCT05209139. The study was retrospectively registered by 26 January 2022**.**

Keywords: team-sports, female, dietary supplements, nitrates

INTRODUCTION

Field hockey is a team-sport characterized by high-intermittent efforts (i.e., continuous accelerations/decelerations) interspersed with moderate to low actions (i.e., walking) [1]. In this sport, competitive match-play consists of two teams of eleven players participating in 4 quarters of 15 minutes each [2] characterized by continuous sprints, acceleration, decelerations and changes of direction [3,4]. Although field hockey has similarities with other team sports (e.g., soccer), the sport (e.g., stick) and protective equipment required (e.g., mouth protection) [5] with the particular field-space for playing (i.e., 91.4 × 55 m) provoked that hockey players must possess an excellent combination of technical and tactical skills [6] and physical conditioning levels [7]. According to the physical-point-of view, high performance in field hockey requires high levels of strength and agility values [8] and good repeated sprint ability capacity [9].

Due to the importance of performing intermittent and explosive efforts in field hockey, the use of adequate nutritional strategies in field hockey based on current recommendations for team sports (i.e., 5–7 $g \cdot kg^{-1}$ body weight d^{-1}, 1.2-1.7 $g \cdot kg^{-1}$ body weight d^{-1} of protein and between from 20% to 35% of total energy from fat intake) [10] could promote ergogenic effects which may enhance repeated high-intensity actions during training/competitions that could be reflected in the overall performance . Only a few dietary supplements have reported good scientific evidence for improving team sport performance, among them, beetroot juice supplementation provided acutely (i.e., 2-3 hours before) at doses > 5 mmol of nitrate (NO_{3-}) [11] has reported potential ergogenic effects [12]. Beetroot juice is a source of high bioavailability nitrate (NO_{3-}) that is reduced to nitrite (NO_{2-}) and nitric oxide (NO), especially in hypoxia and acidosis conditions [13]. In fact, beetroot juice effect is mediated by its function as NO precursor [14] and may elicit performance enhancement due to different physiological mechanisms related to

vasodilatation [11], skeletal muscle contractility or delaying fatigue development [13]. Thus, previous studies have reported improvements in time to exhaustion [15], repeated sprints [16] and high-intensity intermittent performance (e.g. Yo-Yo test) [17,18]; however, other studies do not report ergogenic benefits of beetroot juice ingestion in high-intensity efforts (e.g., repeated sprint ability, distance covered at high velocities, number of accelerations/decelerations during simulated matches) [19-21]. Indeed, most studies have been conducted in male athletes, and hence female athletes are underrepresented [11,22].

To the best of our knowledge, only two previous studies have analysed the acute or chronic effects of beetroot juice ingestion (6.4-12.8 mmol NO_{3-}) in female team-sports athletes (i.e., water polo and soccer, basketball and netball) on high-intensity intermittent efforts (e.g., repeated -sprint ability) [23,24] while no studies have explored the effects of beetroot juice ingestion focused on elite female athletes during specific on-court scenarios (e.g. simulated match-play). Therefore, the aim of this study was to determine the effect of acute supplementation with beetroot juice (6.4 mmol of NO_{3-}) on neuromuscular performance and match-play demands in elite female hockey players.

METHODS

Participants

Seventeen females professional field hockey players from an elite field hockey club (i.e., bronze team medal in Eurohockey Club Champions 2018), therefore, six of them members of Spanish National Team (bronze medal Women's Hockey World Cup London, 2018), volunteered to take part in the study. Female field hockey players between 18 and 35 years old were eligible for inclusion. Exclusion criteria were; goalkeepers, intolerance to beetroot juice, suffering from any chronic pathology or an injury in the month prior to

77 the investigation and the use of medicines or dietary supplements during the study. As
78 players were tested as part of squad logistics it was not possible to ensure all players were
79 tested at the same phase of their menstrual cycle. However, using the mobile application
80 (Mycalendar; Period Tracker, Singapore[25]), it was determined that eleven participants
81 were tested during the follicular phase of their menstrual cycle, and six were tested during
82 the luteal phase. Two participants were goalkeepers and were excluded from the study,
83 one participant was unable to attend the second testing sessions due to anterior knee pain
84 and three participants did not attend to the second day testing for unspecified reasons,
85 thus a final sample of eleven female field hockey players participated in this study. After
86 being fully informed of the experimental protocols, all female players gave their informed
87 written consent to participate. The Bioethics Commission of the Francisco de Vitoria
88 University (code 46/2018) approved the study, which complied with the Declaration of
89 Helsinki.

90 **Experimental design**

91 The required sample size was determined by statistical power calculation based on
92 previous studies [26]. The minimum number of participants required to detect an 8 ± 6 %
93 difference in counter movement jump performance between two groups, with a power of
94 0.80 and two-tailed α level set at 0.05 was estimated as seven per group using G*Power
95 software (v. 3.1.9, Düsseldorf, Germany). A randomized, double-blind, placebo-
96 controlled crossover experimental design was used (NCT05209139) (in each trial, 50%
97 of participants ingested placebo and 50% ingested beetroot juice) with random
98 assignment to each supplement (Research Randomizer, www.randomizer.org). Three
99 hours after intake of beetroot or placebo, female hockey players underwent two identical
100 testing sessions on two different days separated by one week to allow a full recovery and
101 substance wash-out. Female hockey players were allocated to receive a 70 mL dose of

beetroot juice containing 6.4 mmol of NO_3^-(Beet-It-Pro Elite Shot, James White Drinks Ltd., Ipswich, UK) or 70 mL nitrate-depleted beetroot juice placebo (0.04 mmol of NO_3^-), matched in flavour, appearance, and packaging (Beet-It-Pro Elite Shot, James White Drinks Ltd., Ipswich, UK) 3 hours before each testing session. Prior to the assessment, participants were requested to attend a preliminary assessment of body composition and underwent a familiarization session during warm-up. Testing sessions included a neuromuscular test battery consisting of countermovement jump, isometric handgrip strength, 20-m sprint test, repeated sprint ability test and simulated match-play. Experimental procedures were performed at the same hour in the evening (19:00 h) coinciding with typical training time and for avoiding the influence of circadian rhythms on intermittent sports performance [27] or beetroot juice supplementation [28]. Environmental conditions were similar between trials (12 °C, 37% humidity), measured using a portable weather station (Metereological Station, Künken, Spain). All testing sessions started with a saliva test strip (Nitric Oxide Saliva Test Strips, Berkeley Life, Chicago, USA) to verify that the supplement was consumed, following manufacturer's guidelines [29], and a 15 min standardized dynamic warm-protocol, consisting in 5 min jogging, 5 min joint mobility, 2 x 15 m progressive accelerations with 1 min rest in between.

Dietary study protocol

Dietary NO_3^- intake was restricted by providing subjects with a list of NO_3^- rich foods (e.g., beetroot, celery, or spinach) that they should avoid in the 48h before each testing session [20]. Subjects were encouraged to avoid brushing their teeth or using any oral antiseptic rinse or chewing gum or ingesting sweets that could alter their oral microbiota and interfere with NO3- reduction during the 24 h leading up to each experimental trial [30]. Subjects were instructed to refrain from any type of exercise or the ingestion of

caffeine 24-hours before the experimental trials and to follow a diet sheet consisting of 60% carbohydrates, 30% fat, and 10% proteins that should be replicated during the two days of the study. Additionally, participants were provided with a survey to be filled out the following morning relating to potential symptoms (e.g. gastrointestinal upset, red urine, acid reflux, nausea and other perceived discomfort). This survey included several typical side effects associated with beetroot juice on a yes/no scale that has been used previously to assess the side effects derived from beetroot juice ingestion [31].

Exercise study protocol.

Countermovement jump and isometric handgrip strength

Countermovement jump test started with participants standing in the anatomical position, but with hands on the hips. On command, participants flexed their knees to 90º and jumped as high as possible while maintaining their hands on their hips. Jump height was measured with an optical timing system (Optojump Next, Microgate, Bolzano, Italy). Two CMJ attempts were performed interspersed with 45 seconds of passive recovery as previously described [32] and the highest attempt was used for subsequent statistical analysis.

Furthermore, an isometric handgrip strength test was performed. Two maximum isometric voluntary contractions were measured in the dominant hand using a calibrated handgrip dynamometer (Takei 5101, Tokyo, Japan). Volunteers sat with 0 degrees of shoulder flexion, 0 degrees of elbow flexion, and the forearm and hand in a neutral position [33]. The highest value out of two attempts was recorded as the maximum voluntary handgrip strength.

20-meters sprint and repeated sprint ability

In the 20-m sprint test, female field hockey players ran at maximal speed for 20-metres in a straight line, and the time needed to cover the distance was measured using two photocell gates placed 1 m above the ground (Polifemo Radio Light, Microgate, Bolzano, Italy) as previously described in other team-sports disciplines [34]. This test was selected based on normal distance covered by field hockey players during training/competitions. Two attempts were performed interspersed with 2 minutes of passive recovery between repetitions. The faster attempt was used for subsequent analysis.

Repeated sprint ability protocol consisted on six repetitions of 30 m maximal sprints interspersed with 30 s of active recovery periods, where players decelerated during 10 m and jogged 40 m to position themselves for a new start [35], measured using electronic photocells (Polifemo Radio Light, Microgate, Bolzano, Italy). Best sprint time, mean sprint time and percentage sprint decrement (RSAindex) were recorded for analyses. Mean sprint times and RSAindex were calculated according to the following equation: 100-(total time/ideal time *100); and the total time was the time spent to complete the 6 x 30-m shuttle sprints and ideal time is the product of 6 x best sprint time [36].

Simulated field hockey match-play

Players competed in a 7-on-7 field hockey match on an outdoor court, regulated by two referees and following the field hockey rules. The game consisted of two parts of 12.5 minutes with a break of 2 minutes between them. No substitutions were allowed to avoid data inconsistency. Players' match activity was monitored using a wearable GPS system (SPI Elite, GPSports Systems, Canberra, Australia) inserted in a purpose-built backpack. Speed and distance were tracked at 15Hz, and accelerations were recorded at 100Hz. Players wore the same GPS unit for both matches in order to reduce measurement error. Data collected were analyzed using specialized software (TeamAMS, GPSports,

Canberra, Australia). The following variables were recorded and used to assess the external load during match-play: total distance covered per match (walking: < 7.0 km h^{-1}, jogging: 7.0 to 14.9 km h^{-1}, running: 15.0 to 17.9 km h^{-1}, high-intensity running: 18.0 to 24.9 km h^{-1} and sprinting: >25.0 km h^{-1}), peak velocity (km/h), number of high intensity accelerations (> 3 m s^{-2}) and decelerations (< 3m s^{-2}) during the 20 minutes simulated match. Finally, session rating of perceived exertion (10-point scale) was obtained 30 minutes after of the end of the match such as previously reported in field hockey [1].

Statistical analysis

Data are presented as mean ± standard deviation (SD). The Shapiro-Wilks test revealed that data were normally distributed. Paired t-tests were performed to compare neuromuscular values and match-play running performance between beetroot juice and placebo trials and statistical significance was set at $p < 0.05$. Cohen's d (± 95% confidence intervals) for t-tests were calculated to estimate the effect size (ES) [37] considering trivial (< 0.19), small (0.20–0.49), medium (0.50–0.79), and large (> 0.80). Calculations were made using SPSS software (version 24, IBM, Armonk, NY, USA).

RESULTS

Eleven elite female hockey players (age: 28.8 ± 3.7 years; body mass: 67.0 ± 5.7 kg; height: 1.65 ± 0.04 m; hockey experience: 16.0 ± 5.2 years took part of this study. The study blinding was successful with 36% of the participants (4/11 participants) correctly identifying the supplement that they were receiving, and the nutritional strategy was well tolerated without severe adverse effects reporting only two participants showed nauseas and other participant gastric discomfort after the beetroot juice ingestion.

Countermovement jump and isometric handgrip strength

In comparison to the placebo, no statistical differences were reported for acute beetroot ingestion in countermovement jump height (2.1 ± 11.0%; p = 0.776; ES = 0.16 [-0.09; 0.42] (Figure 1a) or isometric handgrip strength (-1.3 ± 7.8%; p = 0.829; ES = -0.08 [-0.33; 0.17] (Figure 1b).

20-meters sprint and repeated sprint ability

No statistical differences were reported in 20-m sprint for beetroot versus placebo conditions (1.4 ± 3.5%; p = 0.227; ES = -0.23 [-0.02; 0.48] (Figure 1c) or in repeated sprint ability in the best (2.13 ± 4.38%; p = 0.313; ES = 0.25 [0.00; 0.50] and mean sprint (-0.04 ± 3.54%. p = 0.995; ES = 0.03 [-0.22; 0.28] (Figure 1d). Therefore, no differences were reported in RSAindex (1.3 ± 3.6 %; p = 0.246; ES = 0.55 [0.29; 0.81]).

Simulated field hockey match-play and rating of perception effort.

In comparison to placebo, beetroot juice ingestion did not show statistical differences in the GPS variables measured during simulated field hockey match (Table 1). In addition, trivial to small changes effect sizes were reported (i.e., 0.02-0.47) (Figure 2). Finally, no differences were reported either in rating of perception effort between beetroot juice and placebo conditions (6.00 ± 1.20 vs 6.5 ± 1.43 points; p = 0.246; ES = -0.38 [-0.63; -0.12]).

DISCUSSION

The aim of this study was to determine the acute effect of beetroot juice ingestion on field hockey neuromuscular demands and match-play running performance during a simulated field hockey game (2 x 12.5 minutes), due to the ingestion of 70 mL has been enough for eliciting improvements in high intensity efforts [38]. According to our results, no benefits were reported for beetroot juice intake (70mL, 6.4 mmol NO_3) on neuromuscular

221 performance (i.e., jump height or repeated sprint capacity) or match-play demands during
222 a simulated field hockey match (i.e., measured by GPS). Thus, our results suggest that
223 acute (single) dose of 6.4 mmol NO_3 of beetroot juice seem not to cause a meaningful
224 ergogenic effect on elite female field hockey performance. Therefore, longer
225 supplementation periods may be warranted.

226 In field hockey, higher values of strength and muscle power in the lower-limbs are
227 required for an enhancement of on-court performance due to the continuous
228 accelerations/decelerations and change-of-direction [39]. Thus, functional measurements
229 such a vertical jump test (e.g., countermovement jump test), could be an excellent tool
230 for quantifying explosive performance in field hockey [40]. When compared to the
231 placebo condition, beetroot juice did not induce any statistically significant difference in
232 this test (+2.1%). Although no previous studies have analysed the effect of beetroot juice
233 ingestion (70mL, 6.4 mmol NO_{3-}) in field hockey players, our data are in agreement with
234 previous studies in team-sports athletes (i.e., basketball or soccer) that reported no
235 additional benefits with acute beetroot juice ingestion [21,41]. In addition, isometric
236 handgrip strength is not a specific action of field hockey, however, an adequate handgrip
237 strength is required in field hockey to use the stick during specific actions (e.g. drag-flick).
238 Thus, this test is a simple method to test the effect of beetroot juice on force production
239 and might be indicative of a higher capacity to produce force during game actions [42].
240 Our data did not report any differences compared to placebo ingestion, that is also in
241 agreement with previous studies in tennis players [20] and basketball players [21].

242 During field hockey training/competitions, participants need to produce maximal or near
243 maximal sprints of short duration with moderate times of recovery [9], thus the ability to
244 cover short distances (20-meters) and the capacity of repeated sprints has been linked to
245 a better performance in elite field hockey. However, no benefits were shown in 20-m

246 sprint (+1.40%) as previously reported in young basketball players [21]. In addition, no
247 differences were reported in best (+4.71%), mean (-0.04%) and fatigue index (-1.34%)
248 obtained during repeated sprint ability test. Our findings do not concur with a recent
249 systematic review developed by Rojas-Valverde et al. (2021) which established that
250 beetroot juice ingestion improved fatigue resistance during repeated sprints (+1.2%-5.3%)
251 when consuming beetroot juice derived NO3-. However, it is worth mentioning that only
252 11.1% (2 of 18) of the all studies included in this systematic are composed exclusively of
253 females athletes, and beetroot juice intake was not shown to produce any benefits in
254 amateur [23] and elite female athletes [24] in repeated sprint ability.

255 To our knowledge this is the first study that has evaluated the effect of acute beetroot
256 juice supplementation on match-play demands in elite female field hockey players
257 athletes in specific on-court scenarios (i.e., simulated field hockey match). Global
258 position tracking systems are commonly used for quantifying the external load during
259 training/competitions recording different performance parameters such as peak speed,
260 distances covered at different velocities or number of accelerations/decelerations being
261 commonly used in field hockey [4]. Our data are in agreement with previous studies in
262 young amateur basketball players during a simulated basketball match [21] that reported
263 no benefits in any of the variables (e.g. total distance covered, peak speed, number the
264 accelerations/decelerations) when comparing beetroot juice to placebo supplementation.
265 Finally, possible beneficial effects of beetroot juice ingestion not observed in this study
266 could be attributed that elite athletes may benefit less from beetroot juice supplementation
267 [43] due to have high baseline blood nitrate and nitrite concentrations associated to
268 training-induced nitric oxide synthase enzyme upregulation [44] comparing to
269 recreational athletes [45] . In addition, acute ingestion protocol used in this study could

explain the different findings obtained when comparing with chronic protocols (i.e., 3-28 days) in other team-sports athletes [46,47].

LIMITATIONS OF THE STUDY

The current investigation has several limitations that need to be addressed. First, it was selected only a lower dose of beetroot juice (70mL, 6.4 mmol NO_{3-}) reporting no statistical differences in performance. Nonetheless, it is unknown if higher doses (i.e., ~12.5 mmol) or a multiday supplementation (i.e., 3 or 7 days) of this supplement could produce an ergogenic effect in female elite hockey performance or match play demands. Second, the simulated field hockey match (2 x 12.5 minutes) was shorter in duration that an official match (4 x 15 minutes), thus the fatigue that this simulated match produced in female athletes predictably was lower compared to an official field hockey match, and this could potentially reduce the benefits of beetroot juice in this study. Third, the number of participants, initially, 17 female field hockey athletes were recruited, but after eligibility and drop-outs, finally 11 participants took part in the study. Although, this number of participants may compromise statistical power (despite *a priori* power calculation), the exclusive type and the difficulties of recruiting this calibre of athlete (Bronze European Club finalist) preserve the relevance of the data shown in this study. Additionally, whilst squad logistics determined testing convenience, and although menstrual phase was tracked for all participants, specific control of menstrual phase was recognised as a limitation of the study. Future research should assess whether the impact of beetroot supplementation varies according to menstrual phase. Future investigations should also be conducted to determine the effect of beetroot juice ingestion in a real competitive field hockey context including a multiday supplementation protocol and a higher dosage than used in the present study.

CONCLUSIONS

Acute ingestion of 70 mL of beetroot juice (6.4 mmol NO_3^-) does not improve jump height, dominant isometric handgrip strength, sprint in short distance (20-meters), repeated sprint capacity and match play demands measured by GPS during a simulated field hockey match in elite female athletes.

STATEMENTS AND DECLARATIONS

CONFLICT OF INTEREST

None of the authors declare a conflict of interest related to the study.

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FIGURES AND TABLES

Table 1. Running performance during a simulated field hockey match (2 halves of 12.5 min) after the ingestion of 70 mL of beetroot juice or a placebo in female players.

Figure 1. Height during a countermovement jump (A), Dominant isometric handgrip strength (B), Time during a 20-m sprint test (C) and Mean repeated sprint ability (D) after the beetroot juice ingestion or the placebo in female field hockey players. Data are mean ± SD for 11 female field hockey players.

Figure 2. Effect sizes (±95% confidence intervals) for movement patterns during simulated field hockey match with the ingestion of 70 mL of beetroot juice (6.4 mmol NO3-) or a placebo in hockey players.