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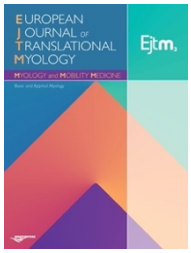

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Acute beetroot juice supplementation improves selected physical performance parameters, but not cardiovascular responses in healthy older women: a randomized clinical trial

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Abstract

Aging is associated with declines in muscle strength and functional capacity, driven by both neuromuscular and cardiovascular changes. In parallel, ageing is accompanied by reduced nitric oxide (NO) bioavailability and potentially a reduced efficacy of the nitrate (NO_3^-)–nitrite (NO_2^-)–NO pathway, a route for NO generation that supports vascular and skeletal-muscle function. Thus, the aim of the present study was to examine the effects of acute ingestion of 70 mL of nitrate-rich beetroot juice (6.4 mmol NO_3^-) versus 70 mL placebo (0.04 mmol NO_3^-) on strength and functional performance in healthy older women aged between 70-80 years. Nine healthy women (75.4 ± 4.0 years) participated in this randomized, double-blind, placebo-controlled crossover experiment. Participants completed a physical performance test consisting of 6-m walk test, isometric handgrip strength (i.e., dominant and non-dominant hand), Timed Up-and-Go test (TUG), and sit-to-stand (STS) muscle power test along with cardiovascular assessments (systolic/diastolic blood pressure, heart rate response and 6-minutes' walk performance) after ingesting either 70 mL of beetroot juice (6.4 mmol NO_3^-) or 70 mL of placebo (0.04 mmol NO_3^-) in a randomized order. Beetroot juice ingestion resulted in significant improvement vs. placebo in isometric dominant handgrip strength (9.6%, p = 0.046, ES = 0.78), with no changes observed in the non-dominant hand (1.2%, p = 0.553, ES = 0.11). No differences were found in beetroot juice vs placebo for 6-m walk

performance (−3.3%, p = 0.306, ES = −0.19), TUG (−5.0%, p = 0.225, ES = −0.17), STS leg extensor muscle power (5.0%, p = 0.121, ES = −0.25), or 6-minute walk performance (−7.7%, p = 0.110, ES = −0.48). Acute ingestion of nitrate-rich beetroot juice led to increased isometric handgrip strength in healthy older women, with no significant effects observed in functional performance (TUG), lower limb STS power, cardiovascular responses, or short-term (6-m) as well as long-term (6-min) walking performance compared with placebo conditions.

Key words: elderly, nitric oxide, dietary supplements, cardiovascular.

Introduction

Ageing is a complex biological process characterized by progressive physiological and behavioral changes that contribute to declines in physical function, functional independence, and overall health.[1] Among these changes, increased physical inactivity plays a central role, accelerating neuromuscular deterioration[2] and impairing the ability to perform activities of daily living in older adults.[3] For this reason, physical function at old age has become a growing public health priority in modern-day societies.[2] One of the main strategies proposed to prevent this decline in physical activity is the regular practice of resistance exercise, which has demonstrated robust benefits in maintaining health, improving quality of life, and attenuating neuromuscular impairments in older populations.[4,5]

Specifically, resistance training interventions appear to be highly effective in enhancing neuromuscular function and increasing muscle strength and mass in older adults, even when approaching advanced ages.[6] Consequently, resistance training represents a powerful countermeasure against age-related neuromuscular decline in muscle strength and muscle mass quantity and quality (*i.e.*, hallmarks of sarcopenia).[7] When combined with adequate nutritional support, resistance training may offer a particularly effective strategy to counteract muscle mass loss and neuromuscular deterioration associated with ageing.[8,9] From a nutritional perspective, ageing is often accompanied by a reduction in total food intake, which may result in an insufficient availability of essential nutrients required to sustain muscle protein synthesis and preserve neuromuscular integrity.[10] Thus, dietary supplements capable of enhancing exercise-induced neuromuscular and muscle mass adaptations have gained increasing attention. Among them, nitric oxide (NO) precursors, particularly dietary nitrate (NO_3^-) in beetroot juice form, have emerged as promising agents to improve neuromuscular performance and muscle function.[11]

Beetroot extract juice is a rich source of dietary nitrate and has been shown to enhance nitric oxide (NO) bioavailability via the NO_3^-–nitrite (NO_2^-)–NO pathway.[12] Nitric oxide plays a key role in vascular and muscular physiology by promoting vasodilation, stimulating myogenic stem cell (satellite cell) proliferation,[13] and increasing neurotransmitter release and enhancing motor unit recruitment.[14] Together, these mechanisms may contribute to improvements in mechanical muscle function and neuromuscular performance, respectively, and may be particularly relevant in older populations, in whom ageing-related impairments in the NO_3^-–NO_2^-–NO pathway have been suggested to result in reduced endogenous NO bioavailability[15] especially in older adults with low physical fitness levels or compromised vascular and metabolic function.[16] Although previous studies have shown that acute NO_3^- supplementation can improve mechanical muscle function (maximal knee extensor strength) assessed by means of isokinetic dynamometry,[17,18] these studies included mixed cohorts of older men and women. To our best of our knowledge, however, no previous investigations have exclusively examined the acute effects of beetroot juice ingestion in women aged over 65 years while concurrently evaluating the impact on physical performance (muscle strength and power output) and cardiovascular capacity (*i.e.*, blood pressure, submaximal cardiovascular exercise responses). Therefore, the aim of the present study was to examine the acute effects of ingesting 70 mL of beetroot juice on physical performance, mechanical muscle function, and cardiovascular exercise responses in healthy older women.

Material and Methods

Participants

Initially, a total of twenty-one potential participants (n = 21) were enrolled and visited the Lab facilities. Exclusion criteria were: i) age outside the range of 65-80 years; ii) orthopedic limitations or other contraindications to strenuous exercise; iii) inability to provide informed consent; iv) currently smokers; v) were taking proton pump inhibitors, antacids, or xanthine oxidoreductase inhibitors; vi) history of major metabolic (thyroid disorders, type I or II diabetes), cardiovascular (e.g., moderate or severe valvular disease, myocardial/pericardial disease, stage II or greater hypertension, heart failure, myocardial infarction/ischemia), renal (eGFR <60 mL/min/1.73 m^2, or 61– 90 mL/ min/1.73 m^2 and albumin: creatine ratio > 30), affected by neuromuscular diseases (e.g., cervical spondylosis radiculomyelopathy, lumbar spondylosis, amyotrophic lateral sclerosis, Guillain- Barre syndrome, acquired demyelinating polyneuropathies), or liver (e.g., SGOT/SGPT >2× normal) diseases, anemic state (hematocrit <30%).[19] During their initial visit, participants underwent a health history assessment and a physical examination and were familiarized (practiced) with all physical and cardiovascular tests performed in the study. Ten participants (n = 10) were

excluded after the initial visit due to the following reasons: age outside the inclusion range (n = 6; younger or older than 65–79 years), current smoking status (n = 3), and diagnosed hypertension (n = 1). Consequently, eleven participants were recruited for the study. Subsequently, two participants were excluded from the analysis. Two participants were excluded due to failure to attend one experimental session (n = 2) (Figure 1). As a result, the final study sample consisted of nine healthy older women (Table 1). The study protocol was approved by the Bioethics Commission of Francisco de Vitoria University (approval no. 05/2022) and was conducted in accordance with the Declaration of Helsinki and register in ClinicalTrials.gov (U.S. National Institutes of Health; Identifier: NCT05337527).

Experimental design

A randomized, double-blind, placebo-controlled crossover design was employed in the present study. Each participant completed two identical experimental trials separated by one week to allow for physical recovery and a sufficiently long washout period. The experimental trials were identical in all aspects except for the beverage ingested prior to exercise. On one occasion, participants consumed 70 mL of beetroot juice (6.4 mmol NO_3^-; Beet It Pro Elite Shot, James White Drinks Ltd., Ipswich, UK), produced from beetroot juice concentrate (98%) and lemon juice (2%). The nutritional composition per 100 mL was: energy, 373 kJ; fat, 0 g; carbohydrates, 18 g (of which sugars: 17 g); protein, 3.7 g; salt, 0.48 g. On a separate occasion, participants consumed 70 mL of a taste-matched placebo beverage containing negligible nitrate content (0.04 mmol NO_3^-; Beet It Pro Elite Shot, James White Drinks Ltd., Ipswich, UK). All beverages were administered at the same time of day (8:30 AM), provided in opaque bottles to maintain blinding, and ingested 150 min prior to the initiation of performance assessments, in accordance with previously published protocols.[20] This timing was chosen to align with previous studies that established the peak plasma response of oral nitrate/nitrite (NO_3^- and NO_2^-) ingestion occurs 2–3 hours after ingestion.[21] The order of the test days (beetroot vs placebo) was randomized for each participant using an online randomization tool (randomizer.org). An independent researcher assigned alphanumeric codes to each trial to ensure blinding of both participants and investigators during data collection and analysis. These codes were revealed only after completion of the statistical analyses. All tests were administered in the same sequence across sessions and conducted at the Lab facilities at the same time of day (between 11:00 and 12:00 h) to minimize the influence of circadian variations on performance. During the testing sessions, the mean ± Standard Deviation (SD) air temperature was 19.5 ± 2.3°C, and relative humidity was 35 ± 6%, as measured using a portable weather station (Meteorological Station, Küken, Spain).

Experimental protocol

Two days before each experimental trial, dietary nitrate (NO_3^-) intake was restricted by instructing participants to avoid nitrate-rich foods, including beetroot, celery, lettuce, arugula, spinach, and kale, in accordance with written guidelines reported in previous studies.[22] In addition, participants were asked to refrain from brushing their teeth, using oral antiseptic mouthwashes, chewing gum, or consuming sweet foods during the 24 h preceding each experimental session, as these behaviors may alter the oral microbiota and interfere with nitrate metabolism.[23] During the experimental period, the participants were also instructed to avoid strenuous physical activity for 24 h prior to testing and to adhere to a standardized daily macronutrient distribution consisting of 60% carbohydrates, 30% fat, and 10% protein (all participants completed 3-day food diaries prior to testing to confirm they followed the macronutrient distribution). On both experimental days, participants arrived at the Lab and consumed the assigned beverage (beetroot juice or placebo) under the supervision of an investigator to ensure complete ingestion. Body mass and body composition were subsequently assessed using a Tanita B-601 analyzer (Tanita Corp., Tokyo, Japan), while body height was measured with a stadiometer (Seca, Germany). Subsequently, 150 minutes after beverage ingestion, resting blood pressure (systolic and diastolic) and heart rate were measured using a validated oscillometric blood pressure monitor (OMRON MX3 Plus, Osaka, Japan)[24] to verify comparable trial conditions across sessions. Immediately after blood pressure assessment, participants completed a neuromuscular test battery comprising several physical performance tests, all of which have previously demonstrated acceptable test–retest reliability (ICC = 0.84–0.98). This battery included consisting of the 6-m walk test to assess maximal gait speed;[25] maximal isometric handgrip strength (*i.e.*, dominant and non-dominant hand)[26] the timed up-and-go (TUG) test to determine functional capacity,[27] the sit-to-stand (STS) test to assess functional capacity including lower limb muscular power and short-term endurance;[28] and the 6-min walk test to evaluate cardiorespiratory fitness (Figure 2).[29] Upon completion of the testing battery, ratings of perceived exertion (RPE; 0–10 scale) were recorded, and participants were asked to report any perceived side effects related to beetroot juice or placebo intake.[20]

Gait speed and isometric handgrip strength testing

Maximal horizontal gait speed was assessed by means of a 6-m walk test in which participants were instructed to walk as fast as possible along a 6-m walkway marked with cones, preceded by a 1-m acceleration zone before the first cone using photoelectric timing gates (Witty Microgate, Bolzano, Italy). Participants completed two trials separated by 1 min of passive seated rest, and the fastest

trial was selected for subsequent analysis.[30] The ICC of this test 0.88 was Upper-limb isometric muscle strength was evaluated through an isometric handgrip strength test (TKK 5101 Grip-D, Takei, Tokyo, Japan). Participants were seated with their shoulders in a neutral position and their elbows fully extended during the test.[26] Two maximal 5-second trials were performed for each hand, with a 1-minute passive seated rest between trials, and the highest value in Newtons (N) obtained for each hand was selected for further analysis.

Timed Up & Go (TUG) and Sit to Stand (STS) testing

Functional mobility was assessed using the TUG test using a standard chair with a backrest, positioned at one end of a hallway. A marker was placed on the floor at 3 m from the front edge of the chair. Participants began the test seated, with their back supported by the chair, arms resting on the armrests, and feet flat on the floor. Upon the verbal command "go," participants were instructed to stand up, walk at a comfortable and safe pace to the 3-m marker, turn around, return to the chair, and sit down again.[27] Timing commenced at the verbal cue and stopped once the participant was fully seated, and time was measured with a handheld stopwatch (Zastor 2, Alicante, Spain). Participants completed two trials separated by 1 min of passive seated rest, and the fastest trial was selected for subsequent analysis. Functional lower-limb performance was assessed using the five-reps STS test. The test was performed on a standardized armless chair (0.50 m height). Participants started from a seated position with their buttocks in contact with the chair, arms crossed over the chest and were instructed to stand up and sit down five times as fast as possible following the verbal command "ready, set, go!". Test execution and timing were recorded using a validated mobile application (PowerFrail, Toledo, Spain), as previously described.[28] The test ended when the participant sat down after completing the fifth repetition. Participants were allowed one to two familiarization trials with 30–60 s of rest before the recorded trial. Verbal encouragement was provided during the test.

6-minutes' walk testing (6MWT)

Functional aerobic capacity was assessed using the six-minute walk test (6MWT) following standardized guidelines.[29] The test was performed along a straight 30-m indoor walkway marked with cones every 3 m. Participants were instructed to walk back and forth along the walkway for six minutes at a self-selected, comfortable pace, aiming to cover as far as possible.[29] The evaluator recorded the start and end of the test using a stopwatch (Zastor 2, Alicante, Spain), and the total distance was calculated by summing the number of completed laps and the remaining distance from the last turn to the nearest cone.[31] Participants were allowed to stop and rest if necessary and resume

walking when able and the test leader was responsible for determining the start and end of the test using a stopwatch.

Rating of perceived exertion and side effects questionnaire

Participants reported their subjective perception of effort (RPE) using a numerical scale ranging from 0 to 10, which was recorded 30 minutes after cessation of the physical performance and cardiovascular tests, respectively.[32] In addition, on the morning following each experimental condition, participants completed a questionnaire to assess potential side effects (e.g., gastrointestinal discomfort, gastric reflux, nausea, muscle soreness, headache, increased fatigue, or nervousness) after beetroot juice ingestion in the hours post-test time interval following completion of the neuromuscular test battery.[20]

Statistical analysis

Normality of the data distribution was assessed using the Shapiro–Wilk test, and homoscedasticity was verified using Levene's test. Students' t-tests were used to compare intra-individual differences in dependent variables between the two conditions (beetroot juice vs. placebo juice). The McNemar test was applied to analyze side effects outcomes related to beetroot juice or placebo ingestion. Effect size was calculated using Cohen's d and interpreted as trivial (<0.15), small (0.15–0.39), moderate (0.40–0.75), or large (≥0.75), according to the Brydges classification for effect sizes in gerontology [33]. Statistical significance was set at $p \leq 0.05$. Statistical analyses were performed using JASP (version 0.95.4, University of Amsterdam) while figures were produced using GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA).

Results

Blood pressure and resting heart rate

No significant differences between conditions (beetroot juice vs. placebo) were observed for resting systolic blood pressure (136.22 ± 22.63 vs. 145.22 ± 21.14 mmHg, p = 0.135; ES = 0.55 [−1.25, 0.17], *moderate*), diastolic blood pressure (83.00 ± 12.08 vs. 87.89 ± 15.46 mmHg, p = 0.225; ES = 0.43 [−1.13, 0.26], *moderate*), or heart rate (75.3 ± 16.9 vs. 76.6 ± 10.1 beats·min^{-1}, p = 0.700; ES= -0.13 [−0.78, 0.52]).

Gait speed and isometric handgrip strength

No statistically significant differences were observed following acute beetroot juice ingestion compared with placebo ingestion in 6-m walk gait speed test (−3.29 ± 10.4%; p = 0.312; ES = -0.36

[−1.02, 0.32]); Figure 3a). In contrast, statistically significant differences were found in favor of beetroot ingestion for maximal handgrip strength of the dominant hand (9.6 ± 10.8%; p = 0.046; ES = 0.78 [0.01, 1.56]; Figure 3b), whereas no differences were observed in the non-dominant hand (1.1 ± 11.0%; p = 0.556; ES = 0.11 [−0.86, 0.46]; Figure 3c).

TUG, STS Muscle Power and 6MWT

Compared with placebo conditions, no statistically significant differences were observed following acute beetroot juice ingestion in the TUG test (4.9 ± 9.6%; p = 0.225; ES = −0.17 [−0.48, 0.14] Figure 3d), STS performance (−4.9 ± 10.0%; p = 0.121; ES = −0.25 [−0.56, 0.06]; Figure 3e), or the six-minute walking distance (−7.7 ± 12.6%; p = 0.110; ES = −0.48 [0.18, 0.78]; Figure 3f). Notably, however, STS performance and 6-minute walking distance tended (p = 0.121 and p = 0.110) to be superior with beetroot vs. placebo ingestion without reaching statistical significance.

Perceptual responses and side effects

Regarding rating of perceived exertion (RPE), statistically significant differences were observed between placebo trials vs beetroot juice (2.9 ± 1.7 vs. 2.0 ± 1.5 points, respectively; (p = 0.041; ES = −0.55 [−0.87, −0.24]). No statistically significant differences were observed between placebo and beetroot juice conditions for any of the reported adverse effects (p > 0.05). Urine discoloration (reddish) was reported by four participants (44.4%) following placebo ingestion and by five participants (55.5%) following beetroot juice ingestion, and an increase in urine output was reported by a single participant (11.1%) in both conditions (p = 1.000). No participants reported gastrointestinal discomfort, gastric reflux, nausea, muscle soreness, headache, increased fatigue, or nervousness in either condition. Finally, 33% of the participants (3 out of 9) were able to correctly identify their supplementation conditions, indicating successful blinding.

Discussion

The aim of the present study was to evaluate the acute effects of beetroot juice ingestion (70 mL providing a total of 6.4 mmol of NO_3^-) versus placebo intake (70 mL providing a total of 0.04 mmol of NO_3^-) on physical performance and cardiovascular demands in healthy older women. To our best knowledge, this is the first study to examine the acute effects of beetroot juice intake on strength and power-related physical performance parameters in an older female cohort. The main findings revealed significant improvements in dominant isometric handgrip strength compared with placebo, while no differences were reported in the other physical performance or cardiovascular variables.

The six-meter walk test is often used to evaluate maximal horizontal gait speed in elderly and older adults to predict aging-related functional declines and fall risk.[34] In addition, performance in short-distance gait speed tests (e.g., 6-m walk test) has been shown to be strongly positively associated with functional fitness and indicators of health status in older adults which makes gait speed represent a feasible biomarker of functional capacity and risk of adverse outcomes in aging populations.[35] According to the present data, acute beetroot juice ingestion did not result in an improvement in 6-m maximal gait speed in the present cohort of older women. These findings are consistent with previous reports by Siervo *et al.* (2016), who observed no significant changes in 10-m maximal gait speed following seven days of beetroot juice ingestion in a cohort of male and female healthy individuals.(≈65 years).[25] As a possible explanation, horizontal gait speed is a highly automated motor task that relies strongly on motor control and balance, particularly in habitual walkers, and may therefore be less sensitive to peripheral physiological enhancements induced by acute beetroot juice supplementation.

Maximal isometric handgrip strength is a key metric for identifying probable sarcopenia[7] and is also associated with other relevant health outcomes, such as blood pressure regulation,[36] highlighting its clinical relevance in older adults. A novel and clinically meaningful finding in the present study was the selective improvement in dominant handgrip isometric strength following acute beetroot juice ingestion. We hypothesized that this asymmetric response (no changes were observed for the non-dominant hand) may be explained by differences in habitual use and neuromuscular efficiency between dominant and non-dominant limbs.[37] Specifically, the dominant hand is typically more heavily engaged in daily activities, which may enhance neural drive and intermuscular coordination,[38] allowing acute physiological enhancements to be manifested as measurable performance gains. As a potential adaptive mechanism, beetroot ingestion may have led to increase NO bioavailability via the NO_3^-–NO_2^-–NO pathway, which plays an important role in skeletal muscle function by improving excitation–contraction coupling, calcium handling, enhancing myofibrillar function, and increasing motor unit recruitment.[12,14,17] These mechanisms appear to preferentially benefit short-duration, high neural-demand tasks such as maximal isometric contractions, particularly in muscles with higher baseline neuromuscular efficiency, while the absence of improvements in the non-dominant hand could reflect a reduced capacity to exploit these acute NO-mediated effects. Supporting this interpretation, previous experiments in older adults have shown that the ergogenic responses to acute nitrate supplementation may be task- and muscle-specific rather than global.[17] However, more research is needed to confirm this preliminary outcome.

The TUG test is a reliable and valid assessment for evaluating functional mobility, balance, and fall risk in older adults[27] and has been shown to be closely associated with maximal gait speed.[39] According to the present data, acute beetroot juice ingestion did not result in an improvement in TUG performance as also failed to be observed in previous investigations.[25] Although beetroot juice supplementation has been shown to increase nitric oxide bioavailability,[40] these physiological adaptations may not directly translate into improvements in short-duration, multi-component functional tasks such as the TUG, as discussed in detail above. Furthermore, age-related declines in muscle power, neuromuscular activation, and postural control may attenuate the potential ergogenic effects of beetroot juice administration in complex mobility tasks requiring rapid transitions and precise balance control.[41] These results suggest that acute beetroot juice ingestion is unlikely to meaningfully enhance functional mobility in healthy older women during complex (multi-directional) locomotion's tasks, highlighting the potential need for longer-term supplementation strategies and/or combined exercise interventions to elicit measurable improvements in TUG performance.

STS muscle power testing has emerged as an easy, portable and inexpensive procedure to assess lower limb mechanical muscle power in older adults, including various clinical settings.[42] Although acute NO_3^- supplementation has previously been associated with improvements in muscle contractile properties and maximal muscle power output in older adults,[19] respectively, according to the present data these effects may not necessarily translate into improvements in complex functional mobility tasks such as the STS maneuver.[25] Thus, in conjunction, the absence of improvements in TUG and STS performance suggests that acute nitrate supplementation may not be effective to overcome the multifactorial limitations governing complex functional movement enhancements in healthy older women.

The 6MWT is a submaximal, self-paced test designed to assess functional aerobic capacity and overall mobility in older adults.[29] While beetroot juice supplementation has been shown to reduce the oxygen cost of exercise and improve vascular function in certain populations,[43] its effects on functional walking performance in healthy older adults appear to be limited. A possible explanation for the lack of improvement in 6-min walking distance may be that the present participants all were free from overt cardiovascular or metabolic diseases, which may have constrained the potential for acute nitrate-induced enhancements in oxygen delivery or utilization compared with populations exhibiting impairments in the NO_3^-–NO_2^-–NO pathway (e.g. cardiometabolic diseases).[44] Moreover, the self-regulated nature of the 6MWT (*i.e.* the habitually chosen gait speed) may have reduced its sensitivity to detect subtle physiological changes, as participants may subconsciously have adjusted their walking pace to maintain comfort and ensure high perceived safety. Consistent

with this interpretation, previous studies investigating beetroot juice supplementation in older adults have reported null or only modest effects on a range of functional endurance outcomes[18, 19] particularly following acute single-session and chronic supplementation protocols.[25] Thus, future studies examining the effects of beetroot juice supplementation in older populations should include cohorts with different pathologies and compare acute versus more chronic supplementation protocols.

Limitations

A number of limitations with the present study should be acknowledged. Firstly, the small sample size is likely to have caused limited levels of statistical power and generalizability, although the randomized crossover design has helped to reduce inter-individual variability. Secondly, plasma or salivary $NO_3^-$and NO_2^-concentrations were not measured, preventing direct confirmation of the efficacy of beetroot supplementation on systemic NO bioavailability thereby hampering the characterization of responders versus non-responders, which otherwise would seem relevant given the relatively high degrees of inter-individual variability. Thirdly, the study evaluated only the acute effects of beetroot juice ingestion, and therefore the findings cannot be extrapolated to multi-day or more chronic supplementation protocols. Fourth, although the present test battery included several clinically relevant assessments of functional capacity, the absence of more extensive direct neuromuscular or biomechanical measurements (e.g., electromyography or kinetic analyses) is likely to have limited any mechanistic interpretations of the task-specific responses observed in the present study.

Conclusions

Acute supplementation with beetroot juice led to significantly greater increases compared to placebo in maximal isometric handgrip strength of the dominant hand. Conversely, no corresponding effects were observed for a number of other physical performance parameters (i.e., 6-m walking, non-dominant isometric handgrip strength, TUG performance and lower limb STS muscle power) no measurable effects of acute beetroot supplementation were observed on selected cardiovascular outcomes (6-min walking distance, systolic/diastolic blood pressure, heart rate responses) in healthy older women.

List of Abbreviations

NO, Nitric Oxide

NO_3^-, Nitrate

NO_2^-, Nitrite
TUG, Timed Up-and-Go test
STS, Sit-to-Stand Muscle Power Test
SD, Standard Deviation
ICC, Intraclass Correlation Coefficient
TUG, Timed Up-and-Go
RPE, Rating of Perceived Exertion
N, Newton

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Contributions

Sofía Gaos, Michelle Matos-Duarte, Sandra Ortiz, Daniel Ramasco and Álvaro López-Samanes conceived the idea presented and prepared the manuscript. Sofía Gaos, Michelle Matos-Duarte, Per Aagaard and Álvaro López-Samanes wrote the article. Sandra Ortiz, Daniel Ramasco, Alejandro Muñoz, Millán Aguilar-Navarro, Miguel López-Moreno, Julio Martín-López, Marta González-Ramos, Luis Berlanga,, Per Aagaard and Álvaro López-Samanes critically reviewed the manuscript for intellectual content. All authors contributed substantially to the conception of the article, read and approved the final edited manuscript.

Conflict of interest

The authors declare no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous three years; and no other relationships or activities that could appear to have influenced the submitted work.

Ethics approval

The Ethics Committee of Francisco de Vitoria University approved this study (EC 05/2022). The study is conformed with the Helsinki Declaration of 1964, as revised in 2013, concerning human and animal rights.

Informed consent

All patients participating in this study signed a written informed consent form for participating in this study.

Patient consent for publication

Written informed consent was obtained from a legally authorized representatives for anonymized patient information to be published in this article.

Availability of data and materials:

All data generated or analyzed during this study are included in this published article.

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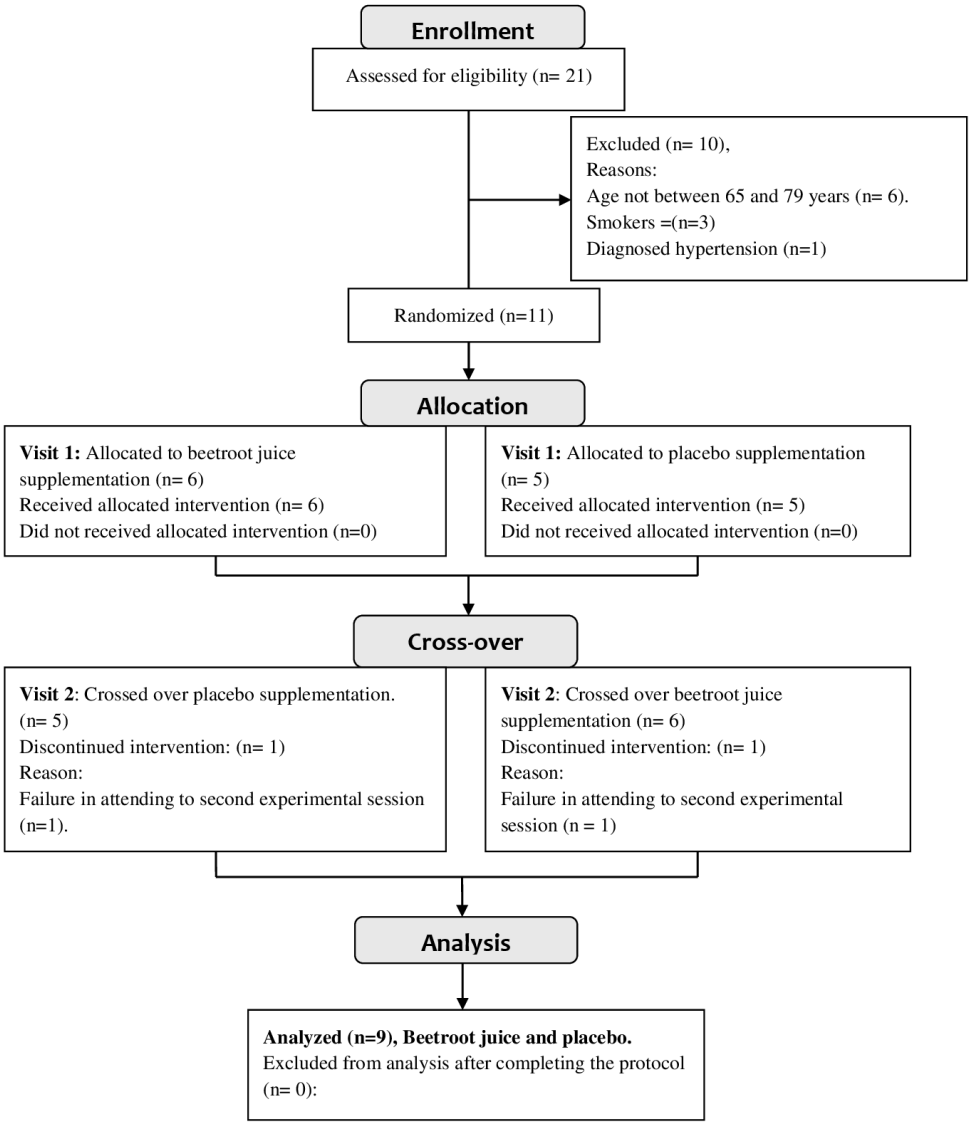

Figure 1. CONSORT flowchart of randomized, double-blind, crossover experiments.

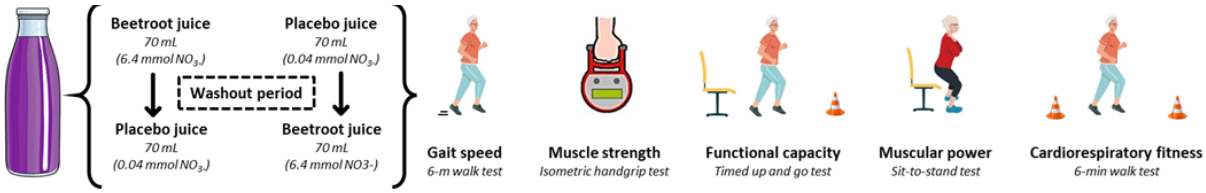

Figure 2. A schematic diagram outlining the sequence of experimental procedures at each testing session.

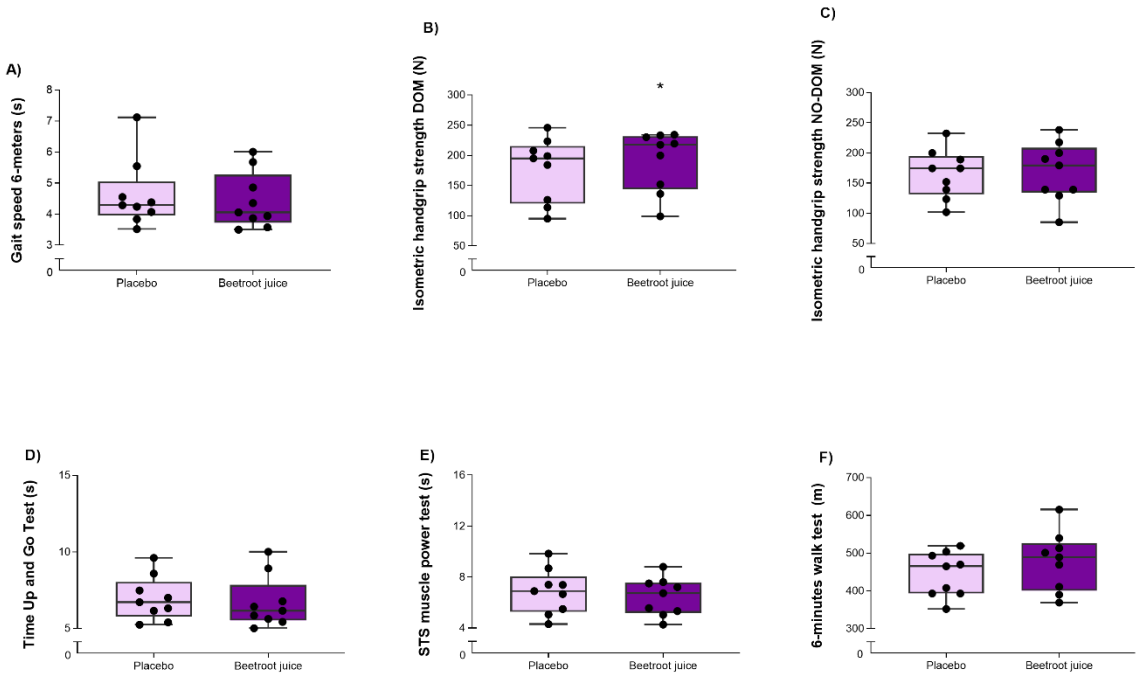

Figure 3. Gait speed 6-meters (A), Dominant isometric handgrip strength (B), Non-dominant isometric handgrip strength (C), Time Up and Go Test (s) (D), STS muscle power test (E) and 6-minutes' walk test (s) (F). Data are presented as box-and-whisker plots, with boxes representing mean and standard deviation, while individual data points are shown as dots. *Statistically significant differences occurred at $p < 0.05$. Abbreviations: DOM =dominant, NO-DOM =non-dominant

Table 1. Characteristics of the study participants (mean ± SD).

Variable	**Mean ± SD**
Age (years)	75.4 ± 4.0
Height (m)	1.53 ± 0.09
Body mass (kg)	67.96 ± 9.04
BMI (kg/m²)	29.16 ± 4.01

SD, standard deviation; BMI, body mass index.