

## Effect of Nitrate Supplementation on Delayed Onset Muscle Soreness Indices: A Double-Blind, Randomized, Cross-Over Clinical Trial

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### Abstract

**Background:** Delayed onset muscle soreness (DOMS) results from unaccustomed exercise, leading to pain, decreased muscle force, and limited joint range of motion (ROM). Nitrate supplementation may alleviate these symptoms. The aim of this study was to evaluate the impact of potassium nitrate on muscle tenderness, isometric force, and elbow ROM following eccentric exercise.

**Methods:** This double-blind, randomized, crossover clinical trial was conducted at Imam Khomeini Hospital, Tehran, Iran, during 2023. Participants (sedentary adults aged 18–40) ingested 1000 mg of potassium nitrate or a placebo (Stevia) 3 hours prior to performing eccentric biceps curls. Assessments included tenderness (Visual Analogue Scale), force (kilogram force), and ROM (degrees) at baseline, 48 hours, and 96 hours post-exercise. A minimum one-week washout period separated the interventions. Data were analyzed using non-parametric repeated-measures models in R software version 4.5.0 (significance set at  $P < 0.05$ ).

**Results:** Sixteen participants completed both arms of the crossover study. Median (IQR) tenderness scores for potassium nitrate were 20 (39) at baseline, 26.5 (45.5) at 48 hours, and 22 (36.75) at 96 hours, compared to 21 (36.75), 49.5 (26.25), and 32 (42.75) for the placebo. Nitrate reduced tenderness over time compared to the placebo ( $P < 0.001$ ). The p-values for the time-treatment interaction were  $P = 0.05$  for Wald-type statistics and  $P = 0.06$  for ANOVA-type statistics. Post-hoc testing confirmed lower pain in the nitrate group at 96 hours post-exercise ( $P = 0.03$ ). No significant between-group differences were found for isometric force or ROM ( $P > 0.05$ ).

**Conclusion:** A single dose of potassium nitrate supplementation before eccentric exercise significantly reduced muscle tenderness but had no effect on muscle force or joint ROM.

**Keywords:** Nitrates, Musculoskeletal Pain, Muscle Force, Joint Range Of Motion, Sports Medicine

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### Introduction

Delayed onset muscle soreness (DOMS), typically peaking 24 to 72 hours after unaccustomed exercise, is caused by microtrauma and inflammation in muscle and its respective connective tissue. This condition leads to pain, stiff-

ness, reduced muscle force, and a decreased range of motion that may last up to 10 days (1). Studies have demonstrated the complex mechanisms underlying DOMS, which is initiated by microtrauma to the Z-lines of type 2 skeletal

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#### ↑What is “already known” in this topic:

Delayed onset muscle soreness (DOMS) is primarily induced by unaccustomed eccentric exercise, resulting in muscle pain, decreased strength, and restricted range of motion. Various treatments, including dietary supplements such as nitrate, have been investigated to alleviate these symptoms, with some studies indicating potential benefits from nitrate-rich foods like beetroot juice.

#### →What this article adds:

This study provides new evidence regarding the acute effects of potassium nitrate supplementation prior to exercise. It demonstrates that potassium nitrate significantly reduces muscle tenderness following eccentric exercise, while having no effect on muscle strength or joint range of motion. These findings offer insights into the potential role of nitrates in managing delayed onset muscle soreness.

muscle fibers following eccentric contraction (2). This process eventually triggers inflammatory reactions and disturbances in cell homeostasis and neuromuscular processes (3-5). These aforementioned processes also result in an increase in certain blood markers, namely creatine kinase (CK), myoglobin, troponin I, lactate dehydrogenase, and alpha actin (6-8).

Numerous strategies for the prevention and treatment of complications associated with DOMS have been the focus of extensive research. These strategies include heat and cold therapy (9), physiotherapy modalities (10-12), massage (13), kinesiotaping (14), acupuncture (15, 16), blood flow restriction techniques (17), nutritional strategies (18-20), and the use of non-steroidal anti-inflammatory drugs (NSAIDs) (21).

Nitrate ( $\text{NO}_3^-$ ) is an anion found in various food sources, particularly in vegetables such as beetroot and leafy greens (e.g., spinach, celery) (22). After entering the nitrate cycle in the body, consumed nitrate is converted to nitrite ( $\text{NO}_2^-$ ) and ultimately to nitric oxide (NO), which serves multiple functions within the body (23). Nitric oxide exerts several effects on skeletal and vascular tissues, including exercise-associated vasodilation (24), increased muscle microcirculation (25), enhanced release of growth factors and Follistatin, inhibition of myostatin release, activation of satellite cells (26), and antioxidant properties at physiological levels, as well as pro-oxidant effects at supraphysiological levels (e.g., following eccentric contraction) (27).

Given the potential benefits of nitrate, particularly its vasodilatory properties, several studies have investigated its effects on exercise performance. Comprehensive reviews of these studies provide strong evidence that 300-600 milligrams of nitrate can serve as a performance enhancer in time trials and high-intensity interval training lasting 12 to 40 minutes. Although research on the effects of this supplement on other types of exercises with varying durations is limited, the findings are promising (28).

Based on the physiological effects of nitrate, its supplementation can have varying impacts on delayed onset muscle soreness (DOMS), either alleviating or exacerbating symptoms. Previous studies, however, have primarily utilized beetroot juice supplements, which are rich in various bioactive compounds, thus complicating the ability to distinguish the direct effects of nitrate. Furthermore, nitrate has predominantly been researched as a recovery supplement following exercise. In the present study, we investigated the acute effects of potassium nitrate as a pre-exercise ergogenic aid on key DOMS indices, including muscle tenderness, muscle force, and joint range of motion, thereby addressing existing gaps and providing new evidence for nitrate supplementation and its relationship with DOMS.

## Methods

### Study design

This study is a double-blind, randomized, crossover clinical trial conducted at the Department of Sports and Exercise Medicine at Imam Khomeini Hospital in Tehran, Iran, during 2023. The proposal for this study was prepared and constructed in accordance with the Consolidated Standards of Reporting Trials (CONSORT) 2025 statement and the

latest CONSORT extension for randomized crossover trials, published in 2019 (29, 30). The study was ethically evaluated and approved by the institutional review board (ethical approval ID: IR.TUMS.IKHC.REC.1400.206) and subsequently registered in the Iranian Registry of Clinical Trials (study ID: IRCT20210512051273N1).

### Study population

The target population for this study consisted of individuals aged 18-40 years with a sedentary lifestyle. The inclusion criteria, aside from age, included the absence of any significant physical or mental illness that could potentially disrupt the study process, a sedentary lifestyle (defined as engaging in less than 3 days of physical activity for 30 minutes per week during the last 3 months), no history of addiction to drugs, narcotics, or tobacco, no pregnancy or breastfeeding, and no pain or injury in the biceps brachii muscle of the non-dominant upper extremity. Exclusion criteria included the development of any illnesses during the study, the use of any type of medication during the week preceding the study, the consumption of any ergogenic or sports supplements within one month prior to or during the study, a history of any musculoskeletal injury within three months before participation, and the consumption of foods containing more than 50 milligrams of nitrate per 100 grams within 24 hours prior to entering the study.

Participants were recruited through public notifications and advertisements placed on hospital bulletin boards, social media, and word-of-mouth referrals. Interested potential participants underwent a preliminary eligibility assessment via telephone interviews to confirm their age and sedentary lifestyle, and they were provided with detailed information about the study. Eligible individuals who met the initial criteria were invited for an in-person evaluation at the research facility. The researchers assessed the subjects' eligibility and obtained written informed consent.

### Interventions

This experiment comprised three stages. Stage 1 involved the initial assessment of the subjects. Stages 2 and 3 employed a classic two-treatment, two-period randomized crossover design (AB/BA) and included the administration of the intervention protocols as well as the assessment of their impact on the subjects. The subjects were randomly allocated to either sequence NP (potassium nitrate in Stage 2 (Period 1), followed by placebo in Stage 3 (Period 2)) or PN (placebo in Stage 2 (Period 1), followed by potassium nitrate in Stage 3 (Period 2)), with a wash-out period between stages (Figure 1).

Potassium nitrate was selected as an intervention due to its capacity to provide an isolated source of inorganic nitrates with a standardized dosage, thereby minimizing the confounding effects of other bioactive substances commonly found in nitrate-rich foods and extracts.

The details of each stage were as follows:

- *The first stage:* The purpose of this stage was to obtain the demographic, anthropometric, and muscular strength characteristics of the participants by determining their one repetition maximum (1-RM). To achieve this, the height

	1 <sup>st</sup> Stage	Wash-out	2 <sup>nd</sup> Stage	Wash-out	3 <sup>rd</sup> Stage
Day 1	Obtaining Anthropometric and Demographic Data + Determining 1-RM	> 7 days	AROM, IF, Tenderness Measurement + Biceps Curl	> 7 days	AROM, IF, Tenderness Measurement + Biceps Curl
Day 3			AROM, IF, Tenderness Measurement		AROM, IF, Tenderness Measurement
Day 5			AROM, IF, Tenderness Measurement		AROM, IF, Tenderness Measurement

*Figure 1.* The study protocol comprised multiple stages separated by periods exceeding 7 days. In Stage 1, anthropometric and demographic data, as well as one-repetition maximum (1-RM), were collected. In Stages 2 and 3, active range of motion (AROM), isometric force, and tenderness were assessed on Day 1 (baseline) during a biceps-curl session, with reassessment of the same outcomes on Days 3 (48 hours later) and 5 (96 hours later).

and weight of each participant were measured, and a dumbbell weighing 10% of the participant's body weight was utilized. The 1-RM was calculated using the Brzycki formula ( $1\text{-RM} = 100 \times \text{weight} / (102.78 - 2.78 \times \text{number of repetitions to failure})$ ), based on the number of biceps curl repetitions performed by the non-dominant arm until volitional fatigue (31).

- *The second and third stages:* The protocols for these two stages were identical and were conducted over two separate weeks, with a minimum wash-out period of seven days between them. Participants were instructed to abstain from foods containing more than 50 milligrams of nitrates per 100 grams for 24 hours prior to the first visit of each stage (a list of these foods was provided to the participants) (32). Additionally, participants were advised to refrain from using mouthwash during this period. Compliance with the dietary nitrate restriction and avoidance of mouthwash use was assessed through self-report by the participants at the beginning of each stage visit.

Either 1000 mg of potassium nitrate (approximately 600 mg nitrate and 400 mg potassium, produced by Ghatran Shimi Co., Tehran, Iran) or 1000 mg of stevia (an artificial plant sweetener produced by Kamvar Co., Isfahan, Iran) was prepared by independent staff responsible for randomization. Each volunteer consumed one capsule orally with a cup of water exactly three hours before commencing the eccentric exercise protocol, aimed at enhancing NO bioavailability during the time when the pathomechanisms of DOMS occur, under the supervision of the study team. Both capsules were identical in appearance to maintain blinding. Participants and study staff, including outcome assessors, were blinded to the capsule content, ensuring a double-blind design. Blinding was maintained until data analysis, at which point the capsule contents were revealed to the study staff.

During the initial visit (day 1) of the second and third stages, we measured the active range of motion (ROM) of the elbow joint, the isometric contraction force of the non-dominant biceps brachii at 90 degrees of elbow flexion, and the tenderness of this muscle.

Following the completion of the measurements, participants were familiarized with the exercise protocol and engaged in a warm-up by performing six repetitions of biceps curls with their non-dominant arm using a dumbbell weighing 30% of their 1-RM. Subsequently, participants were instructed to perform three sets of ten repetitions of biceps curls with a dumbbell weighing 120% of their 1-RM, also with the non-dominant arm, allowing for 60 seconds of rest between sets. During the concentric phase of each biceps curl (i.e., elbow flexion), the operator assisted in completing the movement within approximately 1–2 seconds, while the eccentric phase (i.e., elbow extension) was executed by the participant alone in a controlled and deliberate manner over approximately 3–4 seconds. This repetition tempo was standardized and applied uniformly across all subjects to ensure an equal eccentric load.

In addition to day 1, outcome assessments were conducted on Day 3 and Day 5 to reflect the typical time course of muscle damage induced by exercise. Pain and impairment generally develop within 24 hours, peak at 24-72 hours, and resolve within 5-7 days following eccentric exercise.

### Outcomes

All outcome measurements were conducted in a standardized sequence: 1. isometric force of the biceps brachii, 2. tenderness assessment, and 3. elbow joint active range of motion. This sequence was maintained consistently across all participants and study visits. Additionally, all measurements were performed during morning sessions between

9:00 AM and 11:00 AM to minimize variability related to diurnal fluctuations in muscle function and perceived soreness.

- **Isometric force of biceps brachii muscle:** The isometric force of the biceps brachii was measured using a handheld dynamometer (Danesh Salar Iranian Co., Tehran, Iran). After flexing the elbow to 90 degrees, the posterior aspect of the elbow was positioned against the edge of the examination table, and the probe of the dynamometer was placed on the volar wrist crease. The subject was instructed to exert maximum flexion force on the dynamometer's probe while the operator held the probe steadily. The force exerted was displayed on the dynamometer's monitor in kilogram-force (Kgf) units.

- **Tenderness of biceps brachii muscle:** To measure biceps brachii tenderness, we employed a method proposed and validated by Lau et al. (33). A wooden ball with a diameter of 3 cm was positioned 3 cm above the elbow crease. While the wooden ball remained in place, a sphygmomanometer cuff was wrapped around the subject's arm and inflated to 250 mm Hg. The subject was instructed to rate the pain experienced under the wooden ball by marking a line on a visual analogue scale (VAS) ranging from 1 to 100.

- **Elbow active range of motion:** Active range of motion (ROM) was assessed by calculating the difference between the full active flexion and full active extension angles of the elbow. These angles were measured using a manual goniometer (Model SH5201, Saehan Corporation, Masan, South Korea).

#### Sample Size

The sample size for this study was calculated based on data from a study that examined the effects of beetroot juice supplementation (as a nitrate source) on indices of muscle damage following eccentric exercise (34). The outcome measure used for this purpose was Maximal Isometric Voluntary Contraction (MIVC), which quantifies isometric force. The two sets of means  $\pm$  SD for sample size calculation were  $594 \pm 134$  and  $510 \pm 183$ .

Given that the power of the study and the error probability were set at 0.8 and 0.05, respectively, the sample size was calculated based on the comparison of means from two dependent samples, utilizing G\*Power Version 3.1 software. In the absence of reported within-subject correlation, a moderate within-subject correlation ( $r=0.50$ ) was assumed for the paired calculations in G\*Power.

The estimated sample size was 32 participants in total. Accounting for a potential 25% dropout rate (8 participants), the total sample size was adjusted to 40, resulting in 20 participants for the crossover study.

#### Randomization

Participants were randomized using the block randomization method. The allocation sequence was generated by a computer in R utilizing the blockrand package, with variable block sizes of 2, 4, and 6. During the randomization process, participants were assigned to either the group receiving the control protocol first or the group receiving the

intervention protocol first, designated as PN and NP, respectively (P = placebo, N = potassium nitrate). The allocation ratio of PN to NP was 1:1 within each block.

#### Allocation Concealment and Blinding

Allocation concealment was ensured by an independent individual who was not involved in participant enrollment, intervention administration, or outcome assessment. This individual maintained the allocation sequence and distributed the study capsules according to this sequence. Participants, enrollment staff, and outcome assessors remained blinded to the treatment assignments throughout both periods. The study capsules were labeled and packaged uniformly to prevent unblinding. The allocation sequence was not disclosed until the conclusion of the data analysis.

#### Statistics

The normal distribution of data was assessed using the Shapiro-Wilk test, which indicated a non-normal distribution. To analyze the longitudinal repeated measures data, we employed the LD-F2 model implemented in the nparLD package in R software version 4.5.0. This model accommodates two within-subject factors (days 1, 3, 5 and intervention types, i.e., potassium nitrate and placebo) and calculates rank means for each condition. The Wald-type statistic (WTS), a nonparametric factorial repeated measures design, and the ANOVA-type statistic (ATS), which provides additional inferential checks, were utilized to test main and interaction effects.

Statistical significance was established at  $P < 0.05$ . When significant effects were observed, pre-specified pairwise comparisons were performed using nonparametric Wilcoxon tests with Bonferroni correction. These comparisons were restricted to the primary analytic time points (Day 3 and Day 5) and included both between-treatment comparisons and within-treatment comparisons versus baseline (Day 1). Any additional pairwise comparisons were regarded as exploratory.

Given the use of a crossover design, crossover-specific effects were assessed prior to the aggregation of data from the two treatment sequences. Sequence effects were evaluated by incorporating sequence (NP vs PN) as a between-subject factor in the nonparametric repeated measures design and analyzing the main effect of sequence using WTS/ATS. Period effects (stage 2 vs stage 3) were assessed by including period as a design factor and examining the main effect of period (WTS/ATS) while controlling for treatment and sequence. Considering the nitrate half-life (approximately 5 hours) and the return of nitrate/nitrite to baseline within about 24 hours following acute ingestion (35), a minimum 7-day washout period was deemed sufficient to minimize carryover. Carryover effects were also analyzed for Day 3 and Day 5 by comparing period sums between sequences (NP vs PN) using Wilcoxon rank-sum tests.

The primary efficacy analysis was conducted on a per-protocol population, which included only participants who completed both periods and had outcome data for both treatments.

## Results

A total of 20 volunteers (9 males, 11 females) participated in the study. Three volunteers withdrew after Stage 1 and prior to randomization/allocation. Seventeen participants were randomized into the two sequences (NP = 8 and PN = 9) and completed Stage 2. One participant in the PN group withdrew during the washout period following Stage 2 and before the commencement of Stage 3. This withdrawal was not related to the study intervention or procedures, and the participant declined to continue. Consequently, sixteen participants completed both periods and were included in the analyses (NP = 8 and PN = 8) (Figure 2).

Baseline demographic and anthropometric variables did not differ significantly between the NP and PN groups (Table 1).

Before pooling data across sequences, we evaluated crossover-specific effects. Sequence, period, and carry-over effects were tested within a nonparametric repeated-

measures framework and were found to be statistically insignificant (all  $P > 0.05$ ), thereby supporting the aggregation across sequences.

After aggregating data from both the NP and PN groups, we employed a framework for data analysis. Regardless of the treatment sequence, t1, t2, and t3 corresponded to the times of the first, second, and third visits of the week when participants consumed potassium nitrate, while t4, t5, and t6 corresponded to the times of the first, second, and third visits of the week when participants consumed placebo. The detailed measurement results for each outcome variable (muscle force, muscle tenderness, and elbow ROM) at all three time points (day 1, day 3, and day 5) are presented in Table 2.

### Force

In the group receiving potassium nitrate, muscle force declined from a mean of 15.45 Kgf (IQR: 6.82) on day 1 to 14.40 Kgf (IQR: 7.00) on day 3, before increasing back to

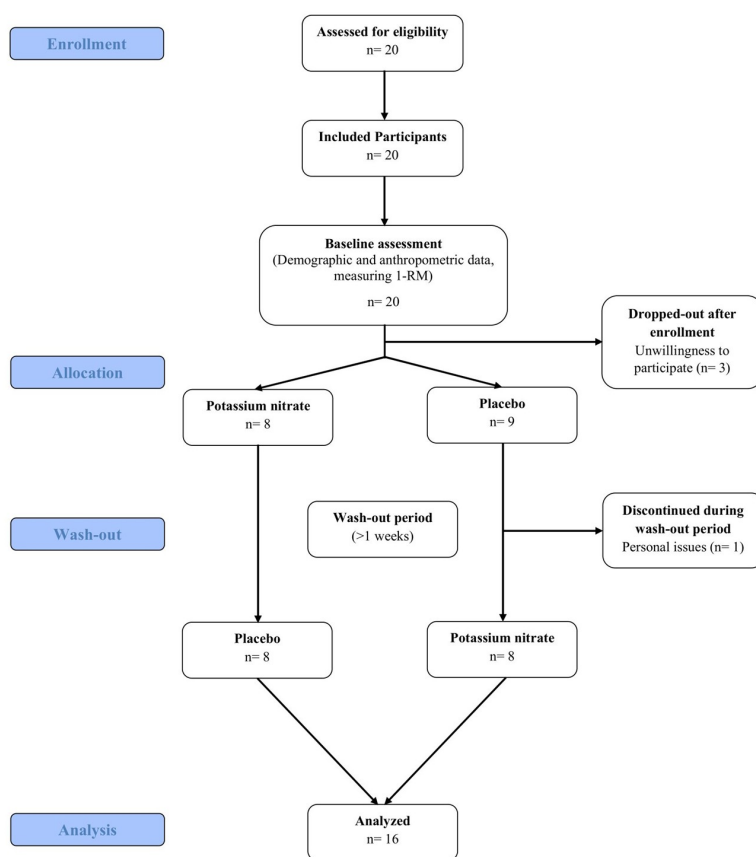


Figure 2. This figure represents the CONSORT flow diagram of the study.

Table 1. Baseline characteristics of participants. Continuous variables are presented as mean  $\pm$  SD and were compared between sequences using independent-samples t-tests; categorical variables were analyzed using Fisher's exact test.

Characteristics		NP	PN	P-value
Sex	Female	5	4	$P > 0.05$
	Male	3	4	
Age (Years)		27.62 $\pm$ 6.25	29 $\pm$ 5.85	0.62
Weight (kg)		60.75 $\pm$ 5.01	59.75 $\pm$ 5.22	0.71
Height (cm)		165.25 $\pm$ 5.75	162.5 $\pm$ 13.5	0.53

**Table 2.** presents the outcome measures over time along with the respective p-values for time effect, treatment effect, and time-treatment interaction. P-values for time, treatment, and time-treatment effects were derived from nonparametric repeated-measures analysis (nparLD; WTS/ATS). Measurements are reported as median (IQR), where WTS refers to Wald-type Statistics and ATS refers to ANOVA-type Statistics.

Variables	Measurements						P- Values					
							Time Effect		Treatment Effect		Time – Treatment Interaction	
	t <sub>1</sub>	t <sub>2</sub>	t <sub>3</sub>	t <sub>4</sub>	t <sub>5</sub>	t <sub>6</sub>	WTS	ATS	WTS	ATS	WTS	ATS
Force (Kgf)	15.45 (6.82)	14.4 (7)	14.9 (6.3)	13.50 (6.95)	12.40 (6.55)	15 (8.80)	0.06	0.07	0.52	0.58	0.21	0.23
Tenderness (VAS 1-100)	20 (39)	26.50 (45.5)	22 (36.75)	21 (36.75)	49.50 (26.25)	32 (42.75)	<0.001	<0.001	<0.001	<0.001	0.05	0.06
Range of Motion (Degrees)	136 (15)	127.50 (25)	129.50 (30)	140 (15)	139 (29)	134.50 (24)	0.20	0.25	0.12	0.14	0.20	0.25

14.90 Kgf (IQR: 6.30) on day 5. The mean change in the potassium nitrate group from day 1 to day 5 was -0.55 Kgf. In the placebo group, the force on day 1 was 13.50 Kgf (IQR: 6.95), slightly decreased to 12.40 Kgf (IQR: 6.55) by day 3, and then increased to 15.00 Kgf (IQR: 8.80) by day 5. The mean change from day 1 to day 5 was 1.50 Kgf (Figure 3A).

Statistical analyses showed no significant effects of treatment, time or time-treatment interaction (all  $P>0.05$ ) (Table 2).

On average, potassium nitrate was not more effective than placebo in increasing muscle force.

### Tenderness

In the Potassium nitrate group, pain levels were recorded at 20 (IQR: 39.00) on day 1, increased to 26.5 (IQR: 45.50) on day 3, and subsequently decreased to 22 (IQR: 36.75) on day 5. In the placebo group, pain levels rose from a median of 21 (IQR: 36.75) on day 1 to 49.5 (IQR: 26.25) on day 3, before decreasing to 32 (IQR: 42.75) by day 5 (Figure 3B).

Nonparametric repeated-measures models revealed significant main effects of both time and treatment (WTS and ATS,  $P<0.001$  for both), alongside a borderline significant time-treatment interaction (WTS,  $P=0.05$ ; ATS,  $P=0.06$ ). Post-hoc Wilcoxon tests confirmed a significantly greater level of pain on day 3 compared with baseline in the placebo group ( $P=0.01$ , Bonferroni-corrected) and a significantly lower level of pain on day 5 in the potassium nitrate group compared with placebo ( $P=0.03$ ) (Table 2).

In the paired nitrate-placebo comparison, the Hodges-Lehmann estimate (nitrate – placebo) was -9.0 VAS points at Day 3 and -12.5 VAS points at Day 5, with corresponding rank-biserial correlations (effect sizes) of -0.54 and -0.77, respectively.

Based on these results, potassium nitrate significantly reduced pain compared to placebo, indicating a potential trend suggesting that the effect may vary over time between groups.

### Range of motion

The decrease in range of motion in the potassium nitrate group was from a median of 136° (IQR: 15) on day 1 to 127.5° (IQR: 25) on day 3, followed by a slight recovery to 129.5° (IQR: 30) on day 5. The mean change in the potassium nitrate group from day 1 to day 5 was -6.50°. In the

placebo group, ROM was 140° (IQR: 15) on day 1, 139° (IQR: 29) on day 3, and 134.5° (IQR: 24) on day 5. The mean change in the placebo group from day 1 to day 5 was -5.50° (Figure 3C).

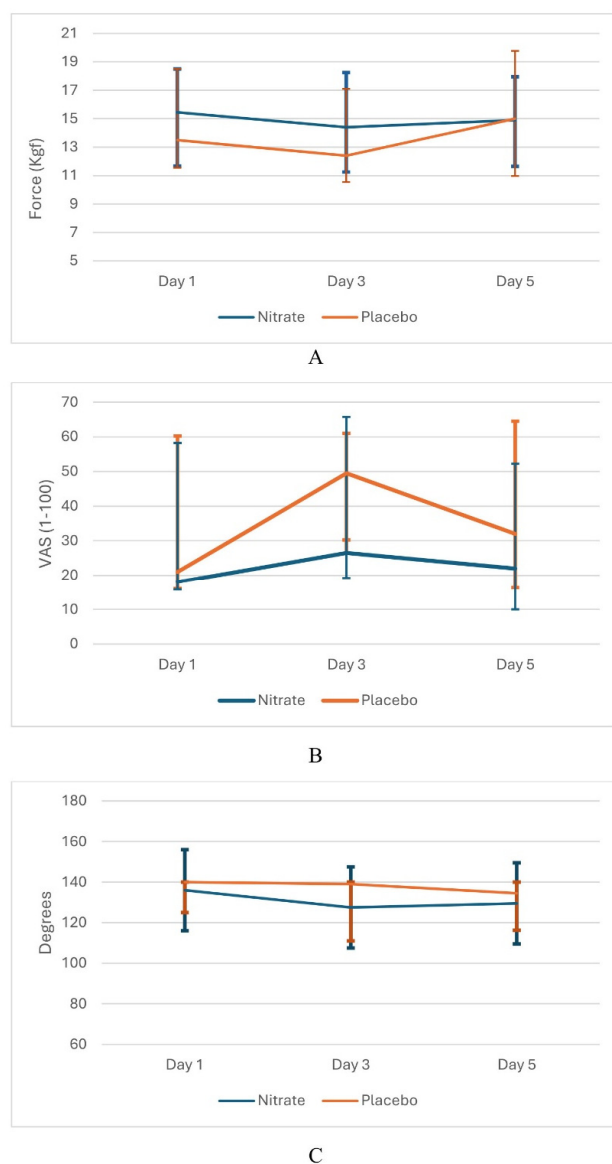
No significant effects of time, treatment, or interaction were observed (all  $P>0.05$ ) (Table 2).

Based on the results, potassium nitrate supplementation had no statistically significant effect on the active range of motion following muscle-damaging exercise.

### Discussion

This randomized crossover trial demonstrated that a single dose of potassium nitrate supplementation prior to eccentric exercise had a beneficial effect on delayed onset muscle soreness (DOMS). Specifically, nitrate significantly reduced muscle tenderness compared with placebo, particularly on day 5 of recovery, while no effects were observed on isometric force or elbow range of motion (ROM). The time-treatment interaction for tenderness was borderline; therefore, the consistency of the treatment effect over time should be interpreted cautiously and may be sensitive to sample size and analytical choices. In the placebo group, DOMS symptoms were more pronounced, with tenderness increasing from day 1 to day 3, whereas this pattern was not observed in the nitrate condition. These findings suggest that nitrate supplementation may alleviate the pain component of DOMS, although it does not appear to influence the associated declines in muscle strength or joint ROM, both of which followed a typical decline after exercise and showed only partial recovery by day 5.

The absence of a measurable effect on isometric force and elbow ROM can be attributed to several factors. Firstly, the elements influencing impairments in strength and ROM following eccentric exercise—such as structural disruption, excitation-contraction coupling, and swelling—may not be significantly modified by a dose of nitrates administered prior to exercise, despite the observed reduction in pain perception. Secondly, the subtle differences between conditions may not be readily detectable using the assessment methods employed in this study, which may lack the sensitivity required to measure small changes in strength with a handheld dynamometer, an effort-dependent tool that may be constrained by measurement error, and universal goniometry, which may also be prone to considerable measurement error.



*Figure 3.* illustrates the temporal changes in (A) isometric force (kgf), (B) muscle tenderness (VAS 1–100), and (C) elbow active range of motion (degrees) for potassium nitrate versus placebo on Days 1, 3, and 5. The values are presented as median, with error bars indicating the interquartile range. Kgf refers to Kilogram Force, and VAS denotes Visual Analogue Scale.

The findings of this study are partially consistent with previous research conducted by Clifford et al. in 2016 and 2017, which investigated the effects of beetroot juice containing dietary nitrate following various eccentric activities. Their results indicated that beetroot juice consistently reduced post-exercise muscle pain, although it had sporadic or negligible effects on other measures, such as isometric strength and countermovement jump performance. Clifford et al. also reported greater pain relief with beetroot juice compared to sodium nitrate, potentially due to the synergistic effects of polyphenols and betaine present in beetroot (34, 36-38). These compounds possess antioxidant and osmoprotective properties, which may work in conjunction

with nitric oxide pathways to mitigate oxidative stress and membrane disruption following eccentric contractions (39).

Recent trials involving nitrate-rich beetroot juices support the potential role of nitrate/NO mechanisms in the recovery from exercise-induced muscle damage. Hemmatifar et al. (2023) found that two days of beetroot juice supplementation following exercise-induced muscle damage (EIMD) in female volleyball players resulted in a decrease in perceived muscle soreness and swelling; however, it did not affect explosive performance measures compared to placebo. This aligns with our findings that potassium nitrate supplementation influenced only the pain and tenderness aspects of DOMS, without impacting isometric force or range of motion (40).

Similarly, Salem et al. (2025) found that short-term beetroot juice supplementation, which provides dietary nitrate, improved high-intensity resistance exercise performance, increased muscle oxygenation, and was associated with decreased upper limb DOMS at 24-48 hours, as well as a quicker recovery of neuromuscular function compared to placebo. Although their study demonstrated performance benefits that exceeded our findings, these differences may be attributed to the multi-day supplementation protocol, the nature of the exercise stimulus (multi-muscle resistance training bouts versus the isolated muscle damage protocol), and the time point used to assess performance recovery (41).

Our results also partially align with the overall findings of the review by Gamonales et al. in 2022, which compiled 15 studies and concluded that nitrate supplementation is effective in improving post-exercise pain and reducing muscle force. However, the consistency and magnitude of these effects were found to be dependent on dosage, timing, and type of exercise (42).

The alignment between our results and the cited studies supports the hypothesis that nitrate-generated nitric oxide increases muscle perfusion and enhances the elimination of inflammatory mediators, leading to a subsequent reduction in soreness, although it does not restore force-generating capacity. Furthermore, the improved muscle force observed in the study by Gamonales et al. (in contrast to our findings) is theorized to result from decreased leakage of calcium from the sarcoplasmic reticulum and improved muscle oxygenation (42).

Conversely, a review by Jones et al. in 2022, which analyzed 9 studies to investigate the effects of beetroot juice on DOMS indices, found that beetroot juice not only has a positive effect on post-exercise muscular pain but also enhances muscle isometric strength and functional movements, such as the counter movement jump. This finding somewhat contrasts with the results of the present study. The explanation for this disparity may lie in methodological differences (43).

The present study extends existing research on nitrate-enriched supplements and muscle soreness by utilizing a purified nitrate compound (potassium nitrate) and an ergogenic administration model for nitrate supplementation. The focus on a single muscle group (upper limb, biceps brachii) may yield more specific results compared to a multi-muscle group model.

Limitations must be acknowledged. Measurement errors may have influenced data recording; however, to mitigate this risk, all tests were conducted by a single researcher using the same equipment for all participants. The supplement also contained potassium in addition to nitrate, which may have exerted additional effects on the measured outcomes through known or unknown mechanisms. Furthermore, the relatively small number of completers may have affected the study's power to detect small effects, particularly regarding muscle force and range of motion; therefore, the null results should be interpreted with caution. Additionally, because the participants were young sedentary adults, the generalizability of these findings to athletes, older adults, or individuals with musculoskeletal or metabolic

conditions may be limited. We evaluated only a single pre-exercise dose; thus, the effects of different dosing strategies remain uncertain. Only functional measures (strength and range of motion) and self-reported pain were assessed. No blood or biochemical markers of muscle damage or inflammation were measured, which restricts our understanding of the underlying biological mechanisms. Pain-related outcomes are inherently subjective and may be influenced by individual pain tolerance, potentially contributing to variability, particularly in a modest sample.

## Conclusion

This study found that 1 gram of potassium nitrate supplementation prior to eccentric exercise significantly reduced muscle tenderness, suggesting a potential role in alleviating delayed onset muscle soreness (DOMS). However, no significant effects were observed on isometric muscle force or joint range of motion. Further research with larger sample sizes and varying dosing protocols is recommended to elucidate the broader effects of nitrate on DOMS-related outcomes.

## Acknowledgment

N/A.

## Conflict of Interests

The authors declare that they have no competing interests.

## Authors' Contributions

All authors contributed to the conception and design, data analysis and interpretation, drafting of the article, critically revising it for significant intellectual content, and granting final approval for the version to be published.

## Ethical Considerations

The study was ethically evaluated and approved by Imam Khomeini Hospital Complex Ethics Committee (ethical approval ID: IR.TUMS.IKHC.REC.1400.206).

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## Data Availability

The data supporting our findings is available upon request.

## AI Use Statement

The authors did not use artificial intelligence or AI-assisted technologies in the preparation of this manuscript.

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