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A single-dose nitrate-producing dietary supplement affects cardiorespiratory endurance and muscular fitness in healthy men: A randomized controlled pilot trial

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Abstract

Introduction: The main aim of this pilot study was to examine the effects of a single-dose intervention with a novel nitrate-producing formulation (MagNOVOx™) on biomarkers of cardiorespiratory endurance and muscular fitness in 12 healthy men.
Methods: The study participants (age = 22.7 ± 2.8 years, height = 184.1 ± 5.7 cm, and weight = 82.5 ± 8.4 kg) were randomly allocated to receive either a single dose of MagNOVOx™ or a placebo (inulin) in a cross-over design. The primary outcome for this study was the change in running time to exhaustion evaluated at baseline (before supplementation) and post-intervention.
Results: Time to exhaustion was improved after the intervention in 8 out of 11 participants (72.7%) who received MagNOVOx™, and in 1 out of 11 participants (9.1%) who received placebo ($p = 0.004$), and MagNOVOx™ outcompeted placebo in terms of improving leg press performance ($p < 0.01$). No significant differences between MagNOVOx™ and placebo were found for blood pressure responses ($p > 0.05$).
Conclusion: These promising findings should be further corroborated in medium- and long-term trials, and different populations, while the exact mechanism of MagNOVOx™ requires additional physiological studies.

Keywords

Nitrate, ergogenic, time to exhaustion, blood pressure

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Introduction

Dietary nitrate supplementation emerges as a practical exercise performance-enhancing strategy during the past few years. Various synthetic products and herbal extracts containing nitrates were demonstrated to reduce the oxygen cost of exercise during heavy all-out workloads,[1] improve perceived exertion and anaerobic power,[2] limit an age-related decline in muscle function,[3] and attenuate muscle fatigue in time-to-exhaustion exercise trials.[4] The mechanisms that may be responsible for these effects involve an augmented production of nitric oxide (NO), a ubiquitous physiological signaling molecule that plays many essential roles in vascular and metabolic control. This includes possible effects of NO on the sarcoplasmic reticulum calcium ATPase or the actin–myosin ATPase that can stimulate mitochondrial respiration,[5] increased inhibition of cytochrome c oxidase that result in a downregulation of adenine nucleotide translocase and improved mitochondrial efficiency,[6] and/or increased myoplasmic free $Ca^{2}+$ concentration followed by increased contractile force.[7] However, several studies failed to confirm the beneficial effects of dietary nitrates on exercise performance.[8,9] This perhaps happens due to a considerable heterogeneity among products used in terms of treatment duration, the amount of nitrates administered/available per serving,

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and dietary nitrates sources.[10] The fact that the nitrate supplement market continues to grow[11] may thus expose a consumer to many products of dubious potency. Therefore, any innovative nitrate-containing product must be carefully scrutinized for efficacy and safety before advancing it to the athletic community. In this randomized controlled preliminary trial, we examined the effects of a single-dose intervention with a novel nitrate-producing mixture (MagNOVOx™) on biomarkers of cardiorespiratory endurance and muscular fitness in healthy men.

Methods

Participants

A total of 12 healthy young men (age = 22.7 ± 2.8 years, height = 184.1 ± 5.7 cm, and weight = 82.5 ± 8.4 kg) signed an informed consent to participate in this double-blind, placebo-controlled cross-over trial voluntarily (Figure 1). The study was approved by the local Institutional Review Board (IRB) at the University of Novi Sad, with the study procedures structured in line with the Declaration of Helsinki–Seventh Revision. The sample size ($n = 12$) was computed using G*Power (Mac Version 3.1.9.3, Heinrich Heine University, Düsseldorf, Germany), with the effects size fixed at 0.50, alpha error probability 0.05, power 0.80, for two supplement groups (MagNOVOx™ and placebo), and three measurements of study outcomes. The primary outcome was the change in running time to exhaustion evaluated at baseline and post-intervention. Inclusion criteria encompassed age ⩾18 years, healthy body mass index (i.e. from 18.5 to 24.9 kg/m^2), and no major chronic diseases or acute disorders. Exclusion criteria included the history of dietary supplement use within 4 weeks before the study commences.

Experimental intervention

The study participants were randomized to receive either a single dose of MagNOVOx™ or a placebo (inulin) in a cross-over design. A random allocation sequence was established by a computer-generated list of random numbers. Both participants and testing personnel were blinded to participants' study treatments. A wash-out period of 5 days was pre-specified to exclude any residual effects of interventions across study periods since the half-life of nitrate is 5–8 h, and acute studies with a single dose of dietary nitrate have observed a return to baseline levels within 24 h.[12] Each intervention was administered in the morning (08:00–09:00), after fasting for 12 h overnight. The participants were asked to ingest an intervention (two capsules) with 250 mL of water about 5 min before exercise performance testing, with ingestion supervised by study personnel. MagNOVOx™ and placebo capsules were similar in appearance, smell, and taste. MagNOVOx™ was supplied by ThermoLife International LLC (Phoenix, AZ, USA). Participants were required to maintain their usual lifestyle (including nutrition and physical activity) and to abstain from using other dietary supplements or pharmacological agents during the trial. Exercise performance biomarkers were assessed at baseline and after each intervention. Measurements were taken between 08:00 and 12:00, with participants asked not to participate in any exercise over the previous 24 h. Cardiorespiratory endurance was evaluated by an incremental test until exhaustion (3-min warm-up walk at 6 km/h followed by running at 8 km/h with progressive workload increment rate of 1.5 km/h every 60 s until exhaustion),[13] with gas exchange data collected throughout the test using a breath-by-breath metabolic system (Quark CPET, COSMED, Rome, Italy). Maximal oxygen uptake (VO_{2max}) was defined as the attainment of at least two of the following four criteria: (1) a leveling-off VO_2 despite an increase in the velocity, (2) peak respiratory exchange ratio (RER) ⩾1.10, (3) peak heart rate (HR) ⩾95% of the age-predicted maximal HR (HR_{max}), and (4) ratings of perceived exertion at the end of test ⩾19. HR was measured during the test using a surface electrode chest strap (Polar S810, Polar Electro, Kempele, Finland). Muscular strength in the upper and lower body was assessed through a one-repetition maximum test (1-RM) for the supine free-weight bench press and seated leg press exercise, respectively.[14] Blood pressure was measured with an automatic device (OMRON Hem 907XL IntelliSense, Tokyo, Japan) at rest and immediately after each exercise. In addition, participants were instructed to report any adverse effects (e.g. palpitations, gut disturbances, and headache) of either intervention through an open-ended questionnaire. Pre-participation familiarization with exercise testing was conducted 1 week before the study, with two familiarization sessions for strength exercises and one session for a cardiopulmonary exercise test. All participants were assessed on the same day, with the tests performed in the same order.

Statistical analyses

Data were initially tested with the Shapiro–Wilk test for the normality of distribution and Bartlett's test for the homogeneity of the variances. Two-way mixed model analysis of variance (ANOVA) with repeated measures was used to establish whether any significant differences existed between participants' responses over time of intervention. In the event of a significant F-ratio, post hoc analyses were performed with Tukey's honest significant difference test employed to identify the differences between individual sample pairs. The significance level was set at $p \leqslant 0.05$. The data were analyzed using the statistical package IBM SPSS Statistics for Mac, version 21 (IBM Corporation, Armonk, NY, USA).

Results

Eleven volunteers ($n = 11$) completed both trials, with one participant was lost during the intervention due to reasons not

Table 1. Changes in exercise performance outcomes during the trial ($n = 11$).

	Baseline	At follow-up		p^a
		MagNOVOx™	Placebo	
Time to exhaustion (min)	7.0 ± 1.1	7.3 ± 1.2[b]	7.1 ± 1.0	0.18
VO_{2max} (mL/kg/min)	40.9 ± 4.4	42.4 ± 4.6[b]	41.3 ± 4.5	0.13
VO_{2ANT} (% VO_{2max})	80.4 ± 9.2	82.8 ± 5.6	81.0 ± 9.3	0.48
$Velocity_{max}$ (km/h)	17.8 ± 1.7	18.4 ± 1.8[b]	18.1 ± 1.7	0.34
$Velocity_{ANT}$ (km/h)	11.1 ± 1.4	12.4 ± 2.0[b]	11.5 ± 1.8	0.08
HR_{max} (bpm)	195 ± 6	194 ± 7	197 ± 6	0.28
HR_{ANT} (bpm)	168 ± 13	166 ± 10	167 ± 13	0.94
Bench press 1-RM (kg)	91.7 ± 21.6	94.1 ± 24.4	92.5 ± 22.8	0.12
Leg press 1-RM (kg)	295.5 ± 48.1	331.5 ± 55.3[b]	297.6 ± 53.5	<0.01

VO_{2max}: maximal oxygen uptake; VO_{2ANT}: oxygen uptake at anaerobic threshold (ANT); HR_{max}: maximum heart rate; HR_{ANT}: heart rate at ANT; 1-RM: one-repetition maximum.
Values are mean ± SD.
[a]Indicates *p*-values from two-way mixed ANOVA (treatment vs time interaction).
[b]Indicates significant difference baseline versus follow-up at $p \leq 0.05$ for each intervention.

connected to the study itself. No participants reported any side effects and adverse events of either intervention. Changes in exercise performance outcomes were depicted in Table 1. The primary outcome (time to exhaustion) was improved after the intervention in 8 out of 11 participants (72.7%) who received MagNOVOx™ and in 1 out of 11 participants (9.1%) who received placebo ($p = 0.004$). Two-way ANOVA revealed no significant differences between MagNOVOx™ and placebo for most exercise performance outcomes evaluated ($p > 0.05$). However, it appears that the single dose of MagNOVOx™ outcompeted the placebo in terms of improving leg press performance ($p < 0.01$). In addition, MagNOVOx™ induced a significant prolongation of time to exhaustion as compared to the baseline (time increment = 24 s, 95% confidence interval (CI) from –37 to 85; $p = 0.04$), an improvement in VO_{2max} (1.5 mL/kg/min on average, 95% CI from –2.5 to 5.5; $p = 0.03$), an increase in peak running velocity (percent change = 3.4%, 95% CI from –5.3 to 12.1; $p = 0.02$), and an augmentation of lower body strength (12.2%, 95% CI from –3.4 to 27.8; $p < 0.01$). The effects sizes for MagNOVOx™ intervention were <0.5 (medium effect) for most exercise performance outcomes, except for medium-to-large effects reported for running velocity at anaerobic threshold ($d = 0.75$), and leg press 1-RM performance ($d = 0.69$). Placebo induced no changes in performance outcomes at follow-up ($p > 0.05$).

Blood pressure values before and immediately after each test were shown in Table 2. Two-way ANOVA revealed no significant differences between MagNOVOx™ and placebo for blood pressure responses ($p > 0.05$). However, MagNOVOx™ induced a significant drop in diastolic blood pressure after running (–4 mm Hg, 95% CI from –16 to 8; $p = 0.04$) and leg press test (–6 mm Hg on average, 95% CI from –15 to 3; $p = 0.04$) as compared to the baseline, while placebo induced no changes in blood pressure outcomes at follow-up ($p > 0.05$).

Table 2. Changes in blood pressure (mm Hg) during the trial ($n = 11$).

	Baseline	At follow-up		p^a
		MagNOVOx™	Placebo	
Running test				
Pre-test				
Systolic	119 ± 6	117 ± 10	120 ± 7	0.22
Diastolic	71 ± 9	69 ± 8	72 ± 10	0.13
Post-test				
Systolic	165 ± 16	161 ± 13	165 ± 17	0.47
Diastolic	88 ± 15	84 ± 12[b]	87 ± 14	0.09
Bench press				
Pre-test				
Systolic	125 ± 13	127 ± 12	126 ± 14	0.88
Diastolic	76 ± 10	73 ± 11	76 ± 10	0.17
Post-test				
Systolic	133 ± 15	130 ± 16	132 ± 15	0.61
Diastolic	78 ± 11	78 ± 9	78 ± 10	0.95
Leg press				
Pre-test				
Systolic	120 ± 10	117 ± 11	118 ± 9	0.38
Diastolic	73 ± 6	69 ± 7	72 ± 6	0.20
Post-test				
Systolic	130 ± 11	130 ± 13	132 ± 13	0.83
Diastolic	81 ± 9	75 ± 11[b]	80 ± 10	0.10

Values are mean ± SD.
[a]Indicates *p*-values from two-way mixed ANOVA (treatment vs time interaction).
[b]Indicates significant difference baseline versus follow-up at $p \leq 0.05$ for each intervention.

Discussion

In this randomized controlled preliminary trial, we demonstrated the beneficial effects of supplemental MagNOVOx™ on selected biomarkers of cardiorespiratory endurance and

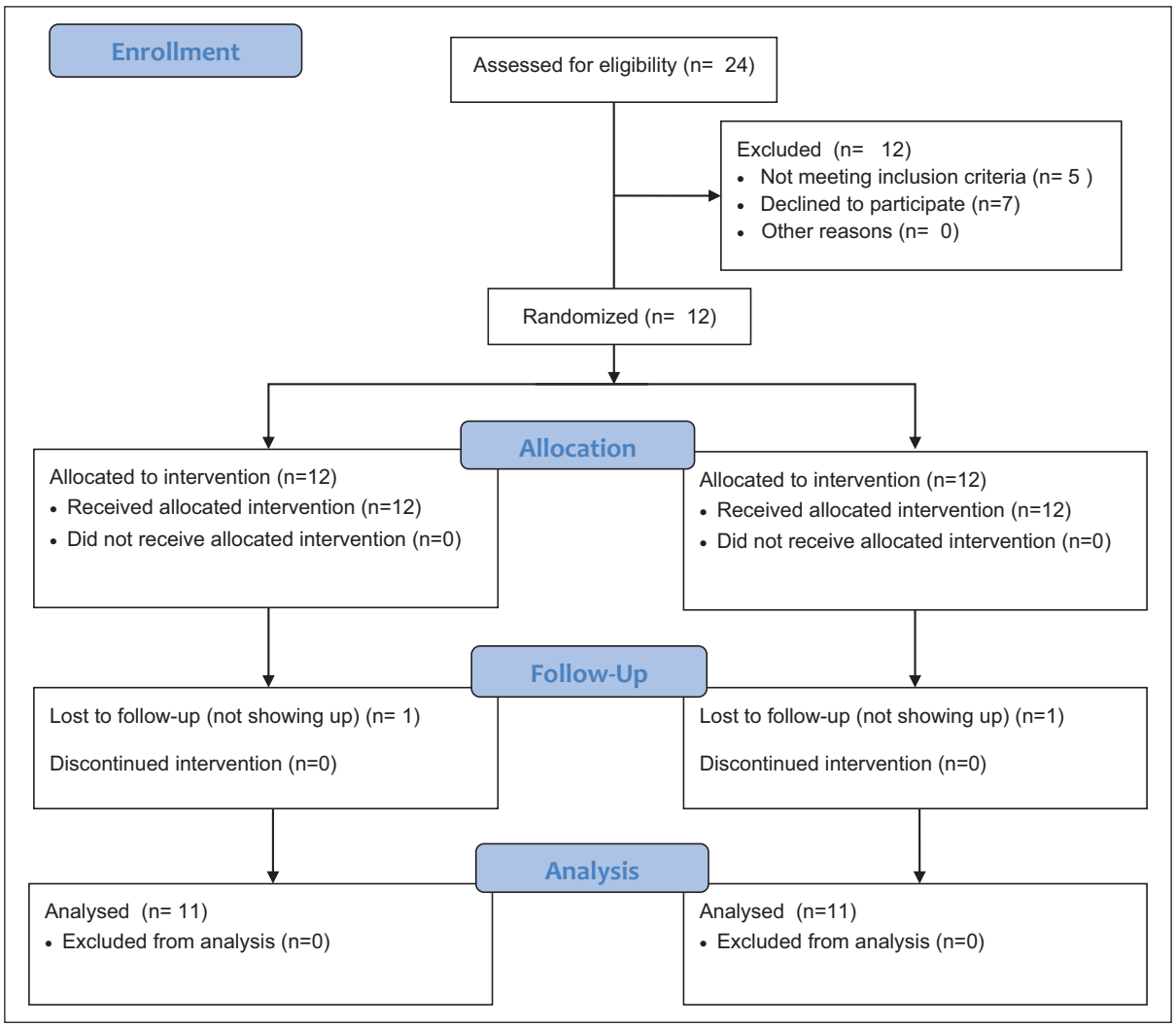

Figure 1. CONSORT 2010 flow diagram.

muscular fitness in healthy young men. It appears that a single dose of MagNOVOx™ administered immediately before exercise significantly extended the duration of the running test to exhaustion (accompanied by improved peak running velocity), suggesting a greater endurance and/or fatigue resistance induced by the intervention. Also, the nitrate-producing mixture notably improved the maximal performance of leg press muscles (e.g. quadriceps, hamstrings, gluteus maximus, and gastrocnemius), thus advancing MagNOVOx™ as an ergogenic agent for isometric muscle strength. These promising findings should be further corroborated in long-term trials and different populations, while the exact mechanism of MagNOVOx™ requires additional physiological studies.

A performance-enhancing potential of dietary nitrates is well established (for a detailed review, see Jones et al.[10]). Dietary nitrate supplementation seems to improve muscle efficiency by reducing the oxygen cost of submaximal exercise and enhance skeletal muscle contractile function, with those effects could lead to improved endurance exercise performance and muscle power. This perhaps happens due to an enhancement of nitric oxide (NO) production, with dietary nitrates first converted into nitrites and finally to NO, a ubiquitous bioactive gas that plays many essential roles in vascular and metabolic control during exercise.[15] Improved exercise tolerance illustrated by extended running time to exhaustion found after MagNOVOx™ intervention in this study corroborates previous trials with dietary nitrates.[4,16] Other studies, however, typically used a multi-day supplementation regimen and time-trial protocols with constant-work-rate exercise, while we used a single pre-exercise nitrate supplement dosing in incremental test until exhaustion. Besides, other nitrate preparations take approximately 60 min to reach the maximal effect,[10] while MagNOVOx™ works rather rapidly by being administered 5 min before an exercise session. Following MagNOVOx™, the participants improved maximal oxygen uptake and maximal running velocity, thus being able to work more efficiently and sustain

high-intensity exercise for a prolonged period without fatigue. This might be due to NO-mediated vasodilation and improved oxygen delivery to the active muscles that could contribute to improved performance during submaximal and maximal exercise, while augmented mitochondrial efficacy might also play a role.[10]

However, improved performance for lower body strength after MagNOVOx™ intake could be attributable to NO-driven effects on ATP cost of cross-bridge formation that might enable better contractile efficiency and superior muscle force. A trend for better performance has been demonstrated for upper body strength, yet it has not reached statistical significance; this might be owing to a smaller amount of muscle mass able to respond to NO generated by MagNOVOx™ intervention. Although MagNOVOx™ affected exercise performance, its impact on pre- and post-exercise blood pressure measurements appears to be relatively nominal; this is probably a consequence of a rather short duration of the intervention. In addition, favorable safety profiles of single-dose MagNOVOx™ found in this trial affirm its possible use in the athletic population, yet additional studies are needed to evaluate the long-term safety of this novel supplemental mixture.

Several limitations have to be considered when the study findings are interpreted. The study population included only young, healthy men; therefore, it remains unknown whether MagNOVOx™ impacts age, gender, or health status. The short-term safety of MagNOVOx™ in terms of patient- and clinician-reported outcome measures (e.g. clinical enzymes and biomarkers, and self-reporting adverse events) have to be additionally addressed in future studies. Besides, no information has been provided regarding the efficacy (and safety) of medium- and long-term supplementation with MagNOVOx™. Finally, with a limited number of clinical tests employed in this trial, the mechanism(s) of MagNOVOx™ could not be reliably determined.

Conclusion

In conclusion, this study demonstrated favorable effects of novel nitrate formulation (MagNOVOx™) regarding cardiorespiratory endurance and muscular fitness in young men and invites for the collection of additional information to address research gaps. A single-dose MagNOVOx™ has been proven as a fast-track, safe, and effective intervention in this population.

Author contributions

N.T. conducted the research, analyzed the data and performed the statistical analysis, and revised the paper. V.S. conducted the research, analyzed the data and performed the statistical analysis, and revised the paper. L.R. conducted the research, analyzed the data and performed the statistical analysis, and revised the paper. P.A. conducted the research, analyzed the data and performed the statistical analysis, and revised the paper. J.B. conducted the research, analyzed the data and performed the statistical analysis, and revised the paper. S.M.O. designed the research (project conception, development of overall research plan, and study oversight), analyzed the data and performed the statistical analysis, wrote the paper draft, and had primary responsibility for final content. All authors read and approved the final manuscript.

Data sharing

Data described in the manuscript will be made available upon request pending application and approval.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

The study was approved by the local Institutional Review Board at the University of Novi Sad (46-06-01/2020-1), with the study procedures structured in line with the Declaration of Helsinki (Seventh Revision).

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Informed consent

Written informed consent was obtained from all subjects before the study.

Trial registration

This randomized clinical trial is in the process of registration.

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