The Effect of Nitrate Supplementation on Exercise Tolerance and Performance: A Systematic Review and Meta-Analysis

Gavin Van De Walle
South Dakota State University

Follow this and additional works at: https://openprairie.sdstate.edu/etd

Part of the Exercise Physiology Commons, Nutrition Commons, and the Sports Sciences Commons

Recommended Citation
https://openprairie.sdstate.edu/etd/1127
THE EFFECT OF NITRATE SUPPLEMENTATION ON EXERCISE TOLERANCE AND PERFORMANCE: A SYSTEMATIC REVIEW AND META-ANALYSIS

BY

GAVIN VAN DE WALLE

A thesis submitted in partial fulfillment of the requirements for the Master of Science
Major in Nutrition and Exercise Science – Specialization in Exercise Science
South Dakota State University
2017
THE EFFECT OF NITRATE ON EXERCISE TOLERANCE AND PERFORMANCE:
A SYSTEMATIC REVIEW AND META-ANALYSIS

This thesis is approved as a credible and independent investigation by a candidate for the Master of Science in Nutrition and Exercise Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Matthew Vukovich, Ph.D.
Thesis Advisor

Matthew Vukovich, Ph.D.
Head, Department of Nutrition and Exercise Sciences

Dean, Graduate School
ACKNOWLEDGEMENTS

First and foremost, I would like to thank my thesis advisor Dr. Matthew Vukovich for his indispensable advice, knowledge, and support not only on this thesis, but throughout my graduate career. I would also like to thank Dr. Kendra Kattelmann and Dr. Tom Stenvig for their valuable time, positive feedback, and recommendations for this thesis.

Finally, I would like to extend my gratitude to my parents for providing me with unequivocal support and encouragement throughout my academic career. So many times I debated on quitting, yet they kept me on course every time I wanted to veer off. This can be summed up in a single quote my father has exhaustively told me (and will continue to tell me), “Keep your eye on the ball.”

The authors declare no conflict of interest. Authorship Acknowledgements: The study was designed by GV and MV; data were collected and analyzed by GV and MV; data interpretation and manuscript preparation were undertaken by GV and MV. Both authors approved the final version of the paper.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>µmol</td>
<td>MICROMOLE</td>
</tr>
<tr>
<td>ATP</td>
<td>ADENOSINE TRIPHOSPHATE</td>
</tr>
<tr>
<td>BR</td>
<td>BEETROOT JUICE</td>
</tr>
<tr>
<td>CA&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>CALCIUM</td>
</tr>
<tr>
<td>CI</td>
<td>CONFIDENCE INTERVAL</td>
</tr>
<tr>
<td>ES</td>
<td>EFFECT SIZE</td>
</tr>
<tr>
<td>GET</td>
<td>GAS EXCHANGE THRESHOLD</td>
</tr>
<tr>
<td>GXT</td>
<td>GRADED EXERCISE TEST</td>
</tr>
<tr>
<td>H</td>
<td>HOUR</td>
</tr>
<tr>
<td>KG</td>
<td>KILOGRAM</td>
</tr>
<tr>
<td>KNO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>POTASMIUM NITRATE</td>
</tr>
<tr>
<td>M</td>
<td>METERS</td>
</tr>
<tr>
<td>MCV</td>
<td>MAXIMUM VOLUNTARY ISOMETRIC CONTRACTION</td>
</tr>
<tr>
<td>MIN</td>
<td>MINUTE</td>
</tr>
<tr>
<td>ML</td>
<td>MILILITER</td>
</tr>
<tr>
<td>MMOL</td>
<td>MILLIMOL</td>
</tr>
<tr>
<td>n</td>
<td>SAMPLE SIZE</td>
</tr>
<tr>
<td>N</td>
<td>NITRATE TRIAL</td>
</tr>
<tr>
<td>NaNO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>SODIUM NITRATE</td>
</tr>
<tr>
<td>NO</td>
<td>NITRIC OXIDE</td>
</tr>
<tr>
<td>NO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>NITRITE</td>
</tr>
<tr>
<td>NO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>NITRATE</td>
</tr>
<tr>
<td>NOS</td>
<td>NITRIC OXIDE SYNTHASE</td>
</tr>
<tr>
<td>P</td>
<td>PLACEBO TRIAL</td>
</tr>
<tr>
<td>SD</td>
<td>STANDARD DEVIATION</td>
</tr>
<tr>
<td>SE</td>
<td>STANDARD ERROR</td>
</tr>
<tr>
<td>SMD</td>
<td>STANDARDIZED MEAN DIFFERENCE</td>
</tr>
<tr>
<td>TT</td>
<td>TIME TRIAL</td>
</tr>
<tr>
<td>VO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>MAXIMAL OXYGEN UPTAKE</td>
</tr>
<tr>
<td>WMAX</td>
<td>PEAK POWER OUTPUT</td>
</tr>
</tbody>
</table>
ABSTRACT

THE EFFECT OF NITRATE SUPPLEMENTATION ON EXERCISE TOLERANCE AND PERFORMANCE: A SYSTEMATIC REVIEW AND META-ANALYSIS

GAVIN VAN DE WALLE

2017

The purpose of this paper was to systematically review the current literature and evaluate the overall efficacy of nitrate supplementation on exercise tolerance and performance by meta-analysis. Studies were eligible for inclusion if they met the following criteria: 1) were an experimental trial published in an English peer-reviewed journal; 2) compared the effects of inorganic nitrate consumption with a non-bioactive supplement control or placebo; 3) used a quantifiable measure of exercise performance; and 4) was carried out in apparently healthy participants without disease. A total of 29 studies were identified that investigated the effects of nitrate supplementation on exercise tolerance or performance in accordance with the criteria outlined. Analysis using TTE as the outcome variable revealed a significant effect of nitrate supplementation on exercise tolerance ES 0.28 (95% CI: 0.08 – 0.47; \( P = 0.006 \)) compared to placebo. Analysis using time to complete a specific distance as the outcome variable revealed no significant effect of nitrate supplementation on exercise performance ES -0.05 (95% CI: -0.28 – 0.17; \( P = 0.64 \)) compared to placebo. Nitrate supplementation is likely to improve exercise tolerance and capacity but not exercise performance. More research is required to determine the optimal dose and duration of nitrate supplementation. It would also be important to consider the type of athlete performing the exercise, and the duration,
intensity and mode of the exercise performed as these factors are likely to influence the
efficacy of nitrate supplementation.
INTRODUCTION

Nitric oxide (NO) is a signaling molecule that plays an important role in several cellular functions including vasodilation, cellular respiration and angiogenesis. (36) Elevated NO availability may positively augment oxygen and nutrient delivery to the working muscle, thereby lowering the ATP cost of muscle contractile force production and oxygen costs of aerobic exercise. The mechanisms attributed to these effects are linked to mitochondrial respiration and biogenesis. (36) This gives athletes reason to believe that enhancing NO bioavailability may favorably influence exercise performance. To this effect, several “NO stimulating supplements” (e.g., L-arginine, arginine-alpha-ketoglutarate) have been marketed to the fitness community as NO boosters claiming to improve performance. However, these supplements have little effect on NO-related physiological processes or exercise performance. (43)

More recently, nitrate (NO\(^3\)) supplementation via beetroot juice or as salt (NaNO\(^3\)) has gained popularity as a means of increasing NO bioavailability. A meta-analysis by Hoon et al. reported a significant benefit of nitrate supplementation on performance for time-to-exhaustion (TTE) tests and a small but insignificant benefit on performance for time-trial (TT) and graded exercise tests. (GXT) (19) The authors, however, did not perform sub-analyses on parameters likely to affect the efficacy of nitrate supplementation such as training status, nitrate dose and duration. To this effect, the ergogenic effects of nitrate supplementation appears to selectively benefit primarily recreationally active and moderately trained individuals with minimal benefit, if any, observed in well-trained individuals. (7, 9, 16, 32) Interestingly, there may be individual variability in the response to nitrate supplementation, resulting in responders and non-
responders based on individual variances. (9, 16, 42) Therefore the purpose of this paper was to systematically review the current literature and evaluate the overall efficacy of nitrate supplementation on exercise tolerance and performance by meta-analysis. We also investigated whether the effect of nitrate on effect size (ES) on was modified by training status, supplementation duration and dose.

MATERIALS AND METHODS

Studies were eligible for inclusion if they met the following criteria: 1) were an experimental trial published in an English peer-reviewed journal; 2) compared the effects of inorganic nitrate consumption with a non-bioactive supplement control or placebo; 3) used a quantifiable measure of exercise performance; and 4) was carried out in apparently healthy participants without disease.

Procedures

A systematic search of the literature was conducted in reference to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. To carry out this review, the computer databases: PubMed, Science Citation index of Web of Knowledge, EBSCOhost, and ProQuest, were searched up until the 18th of May 2016. The keywords used as search terms were “nitrate” AND “exercise.” The reference lists of the retrieved articles were subsequently screened for additional articles that were of relevance as described by Greenhalgh and Peacock. A total of 1986 studies were evaluated based on the keywords searched. An additional eight studies were then identified as potentially being eligible for inclusion for a total of 1993 studies screened for inclusion. Of the studies reviewed, 35 were determined to potentially be relevant
based on the abstracts. The full text of these articles were then screened in which 29 were identified for inclusion (see Fig. 1). Table 1 summarizes the studies.

Figure 1. Flow diagram of search strategy
<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects, n</th>
<th>Nitrate supplementation</th>
<th>Exercise Test</th>
<th>Outcome</th>
<th>Trial result (mean ± SD)</th>
</tr>
</thead>
</table>
| Arnold et al., 2015  | Well-trained runners, n = 10 (men) | 7 mmol single dose as BR, 2.5 h pre-test | Incremental treadmill TTE @ 4000 m (a) 10 km treadmill TT @ 2500 m (b) | Did not alter TTE or time to complete a 10-km TT. | N = 402 ± 80 (a)  
P = 393 ± 62  
N = 2862 ± 233s (b)  
P = 2874 ± 265s |
| Aucouturier et al., 2015 | Recreationally active, n = 12 (men) | 5.5 mmol/day for 3 days as BR | 15-s exercise periods at 170% of maximal aerobic power using a cycle ergometer. | Enhanced exercise tolerance at supramaximal intensity. | N = 26.1 ± 10.7 reps  
P = 21.8 ± 8.0 reps |
| Bailey et al., 2009  | Recreationally active, n = 8 (men) | 5.5 mmol/day for 6 days as BR | Cycling TTE @ 70% between GET and VO2 max | Increased TTE by 16% compared to placebo. | N = 675 ± 203s  
P = 583 ± 145s |
| Bailey et al., 2010  | Recreationally active, n = 7 (men) | 5.1 mmol/day for 6 days as BR | Leg extension TTE @ 15% and 30% MVC | Increased TTE by 25% compared to placebo. | N = 734 ± 288s  
P = 586 ± 212s |
| Bailey et al., 2015  | Recreationally active, n = 7 (men) | 8.4 mmol/day for 9 days as BR | Cycling TTE @ 80% between GET and VO2 max 115 RPM 35 RPM | Extended exercise tolerance by 22% when cycling at a high, but not a low pedal cadence. | N = 362 ± 137s (a)  
P = 297 ± 79s  
N = 344 ± 74s (b)  
P = 341 ± 99s |
| Bescos et al., 2011  | Well-trained cyclists and triathletes, n = 11 (men) | 10 mg/kg single dose as NaNO3−, 3 h pre-test | Cycle incremental TTE | No improvement in the tolerance of high-intensity cycling. | N = 416 ± 106s  
P = 409 ± 90s |
<table>
<thead>
<tr>
<th>Study Authors, Year</th>
<th>Participants</th>
<th>Treatment</th>
<th>Exercise Protocol</th>
<th>Performance</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bescos et al., 2012</td>
<td>Well-trained cyclists and triathletes n = 13 (men)</td>
<td>10 mg/kg for 3 days as NaNO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>40-min cycle distance TT</td>
<td>No improvement in 40-min distance TT performance.</td>
<td>N = 26.4 ± 1.1 km P = 26.3 ± 1.2 km</td>
</tr>
<tr>
<td>Boorsma et al., 2014</td>
<td>Elite distance runners n = 8 (men)</td>
<td>19.5 mmol single dose as BR (a) 2.5 h pre-test 13 mmol/d for 8 days as BR (b)</td>
<td>1500-m TT Acute (a) 1500-m TT Chronic (b)</td>
<td>No improvement in TT performance after acute or chronic supplementation.</td>
<td>N = 250.7 ± 4.3s (a) P = 250.4 ± 7.0s N = 250.5 ± 6.2s (b) P = 251.4 ± 7.6s</td>
</tr>
<tr>
<td>Bourdillon et al., 2015</td>
<td>Well-trained cyclists n = 12 (men)</td>
<td>0.1 mmol/kg for 3 days as NaNO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>15- km TT in normoxia 15- km TT in normobaric hypoxia</td>
<td>No improvement in TT performance during exercise in normoxia and hypoxia.</td>
<td>N = 1597 ± 96s (a) P = 1581 ± 63s N = 2155 ± 687 (b) P = 2005 ± 309s</td>
</tr>
<tr>
<td>Breese et al., 2013</td>
<td>Recreationally active n = 9 (4 men, 5 women)</td>
<td>8 mmol/d for 6 days as BR</td>
<td>Cycling TTE @ 70% between GET and VO2 max.</td>
<td>Increased TTE cycling by 22% over placebo.</td>
<td>N = 635 ± 258s P = 521 ± 158s</td>
</tr>
<tr>
<td>Cermak et al., 2012(1)</td>
<td>Trained cyclists and triathletes n = 20 (men)</td>
<td>8.7 mmol single dose as BR 2.5 h pre-test</td>
<td>1-h TT</td>
<td>No improvement TT performance compared to placebo.</td>
<td>N = 65.5 ± 4.9min P = 65.0 ± 4.9min</td>
</tr>
<tr>
<td>Cermak et al., 2012(2)</td>
<td>Trained cyclists and triathletes n = 12 (men)</td>
<td>8 mmol for 6 days as BR</td>
<td>10-km TT</td>
<td>Time to complete TT was 1.2% lower with BR.</td>
<td>N = 953 ± 72.7s P = 965 ± 72.7s</td>
</tr>
<tr>
<td>Study</td>
<td>Subject Category</td>
<td>Dose Details</td>
<td>Test Details</td>
<td>Results Summary</td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>-------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Hoon et al., 2014</td>
<td>Well-trained rowers</td>
<td>4.2 mmol single dose as BR (a) 2 h pre-test 8.4 mmol single dose as BR (b) 2 h pre-test</td>
<td>2000-m rowing ergometer T</td>
<td>A high but not moderate dose improved rowing performance.</td>
<td></td>
</tr>
<tr>
<td>Lansley et al., 2011</td>
<td>Trained cyclists</td>
<td>6.2 mmol single dose as BR 2.5 h pre-test</td>
<td>4-km cycling TT 16.1-km cycling TT</td>
<td>Improved 4-km and 16.1 km completion time by 2.8% and 2.7% over placebo.</td>
<td></td>
</tr>
<tr>
<td>Larsen et al., 2010</td>
<td>Healthy subjects</td>
<td>0.1 mmol/kg for 2 days as NaNO₃⁻ Incremental arm crank and cycle TTE</td>
<td></td>
<td>A trended increase in TTE was noted.</td>
<td></td>
</tr>
<tr>
<td>MacLeod et al., 2015</td>
<td>Trained cyclists</td>
<td>6.5 mmol single dose as BR 2.5 h pre-test</td>
<td>10-km TT normoxia 10-km TT normobaric hypoxia</td>
<td>No improvement in TT performance during exercise in normoxia and hypoxia.</td>
<td></td>
</tr>
<tr>
<td>Masschelein, et al., 2012</td>
<td>Physically active</td>
<td>0.07 mmol/kg for 6 days as BR 2.5 h pre-test</td>
<td>Cycle incremental TTE @ 5000 m</td>
<td>Increased TTE by 5%.</td>
<td></td>
</tr>
<tr>
<td>Muggeridge et al., 2013</td>
<td>Well-trained cyclists</td>
<td>5 mmol single dose as BR 3 h pre-test</td>
<td>16.1 km TT @2500m Stimulated altitude 15% O₂</td>
<td>Improved 16.1 km TT performance by 2.9%</td>
<td></td>
</tr>
<tr>
<td>Murphy et al., 2012</td>
<td>Healthy subjects</td>
<td>8 mmol as whole BR 1.25 h pre-test</td>
<td>5 km running TT</td>
<td>Increased velocity by 3%</td>
<td></td>
</tr>
</tbody>
</table>

N = 383.4 ± 8.7s (a) P = 383.5 ± 9.0s N = 381.9 ± 9.0s (b) P = 383.5 ± 9.0s
N = 6.27 ± 0.35min (a) P = 6.45 ± 0.42min N = 26.9 ± 1.8min (b) P = 27.7 ± 2.1min
N = 563 ± 90s P = 524 ± 93s
N = 961 ± 54s (a) P = 954 ± 47s N = 1018 ± 52s (b) P = 1023 ± 49s
N = 597 ± 85s P = 568 ± 89s
N = 1664 ± 42s P = 1702 ± 45s
N = 12.3 ± 2.7 km/h P = 11.9 ± 2.6 km/h
<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Interventions</th>
<th>Outcome Measures</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peacock et al., 2012</td>
<td>Well-trained cross-country skiers $n = 10$ (men)</td>
<td>9.9 mmol single dose as KNO$_3^-$ (pill) 2.5 h pre-test</td>
<td>5 km running TT</td>
<td>No effect on exercise performance.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N = 1005 ± 53s, P = 966 ± 49s</td>
</tr>
<tr>
<td>Peeling et al., 2014</td>
<td>National-level kayak athletes $n = 6$ (men) International-level kayak athletes $n = 5$ (women)</td>
<td>4.8 mmol single dose as BR (a) 2.5 h pre-test 9.6 mmol single dose as BR (b) 2 h pre-test</td>
<td>7 x 4 min step test kayak (a) ergometer 500m TT paddle (b)</td>
<td>(a) Distance covered was (+0.7%) greater in BR trial.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N = 989 ± 31m (a), P = 982 ± 36m</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N = 114.6 ± 1.5s (b), P = 116.7 ± 2.2s</td>
</tr>
<tr>
<td>Porcelli et al., 2015</td>
<td>Distance runners $n = 21$ (men) low trained $n = 8$ Mod trained $n = 7$ High trained $n = 6$</td>
<td>5.5 mmol for 6 days as NaNO$_3^-$ 2.5 km treadmill TT</td>
<td>Low and mod trained subjects improved task 1%-4% faster. No benefit in high trained.</td>
<td>N = 886 ± 74s (a), P = 910 ± 82s</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N = 723 ± 90s (b), P = 734 ± 93s</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N = 627 ± 30s (c), P = 629 ± 28s</td>
</tr>
<tr>
<td>Thompson et al., 2014</td>
<td>Recreationally active $n = 16$ (men)</td>
<td>5 mmol as BR 1.5 h pre-test</td>
<td>TTE at 90% VO2peak</td>
<td>TTE was 16% longer with nitrate compared to placebo.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N = 185 ± 122s, P = 160 ± 109s</td>
</tr>
<tr>
<td>Thompson et al., 2015</td>
<td>Recreational team-sport players $n = 16$ (men)</td>
<td>12.8 mmol for 7 days as BR Final dose ingested 2.5 h pre-test</td>
<td>Intermittent sprint test</td>
<td>Increased total work done by 3.5%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N = 123 ± 19KJ, P = 119 ± 17KJ</td>
</tr>
</tbody>
</table>
Vanhatalo et al. 2010  Recreationally active  
\( n = 8 \) (5 men, 3 women)  
4.84 mmol single dose as BR (a)  
4.84 mmol for 5 days as BR (b)  
4.84 mmol for 15 days as BR (c)  
Cycle incremental TTE  
Peak power output was higher after 15 days of BR supplementation but not 2.5 h or 5 days.  
\( N = 325 \pm 71 \) Wmax (a)  
\( P = 322 \pm 68 \) Wmax  
\( N = 328 \pm 68 \) Wmax (b)  
\( P = 323 \pm 67 \) Wmax  
\( N = 331 \pm 68 \) Wmax (c)  
\( P = 323 \pm 68 \) Wmax

Vanhatalo et al., 2011 Recreationally active  
\( n = 9 \) (7 men, 2 women)  
9.3 mmol 24h, 12h, and 2.5 h as BR  
Leg extension TTE @ 14.5% O2  
Increased high-intensity exercise by 21%  
\( N = 477 \pm 200 \) s  
\( P = 393 \pm 169 \) s

Wilkerson et al., 2012 Well-trained cyclists  
\( n = 8 \) (men)  
6.1 mmol single dose as BR  
2.5 h pre-test  
50-mile cycle TT  
Mean reduction in time of 0.8% or 1.2 min.  
\( N = 136.7 \pm 5.6 \) min  
\( P = 137.9 \pm 6.4 \) min

Wylie et al., 2013 Recreationally active  
\( n = 10 \) (men)  
4.2 mmol single dose as BR (a)  
8.4 mmol single dose as BR (b)  
16.8 mmol single dose as BR (c)  
2.5 h pre-test  
Cycling TTE @ 75% between GET and VO2 max  
Increased time to exhaustion with 8.4 and 16.8 mmol by 14% and 12% but not 4.2 mmol.  
\( N = 508 \pm 102 \) s (a)  
\( P = 470 \pm 81 \) s  
\( N = 570 \pm 153 \) s (b)  
\( P = 498 \pm 113 \) s  
\( N = 552 \pm 117 \) s (c)  
\( P = 493 \pm 114 \) s

Wylie et al., 2013 Recreational team-sport players  
\( n = 14 \) (men)  
8.2 mmol day before in morning  
8.2 mmol day before in evening  
8.2 mmol 2.5 hr. pre  
4.1 mmol 1.5 pre  
Yo-Yo intermittent recovery test  
Improved distance covered in intermittent exercise by 4.2%  
\( N = 1704 \pm 304 \) m  
\( P = 1636 \pm 288 \) m

Note: BR – beetroot juice; GET – gas exchange threshold; MCV – maximum voluntary isometric contract; N – nitrate trial; P – placebo trial; TT – time trial; TTE – time to exhaustion; Wmax – peak power output.
Studies were individually coded for the following variables: subject’s characteristics by group including sex and training status; supplement intervention including nitrate source, dose, delivery, and duration; and type of exercise test data. Outcome variables were coded for tolerance and performance variables. Exercise tests using TTE and GXT trials were classified as tests of exercise tolerance; whereas, TT were classified as tests of exercise performance. A TTE trial was defined as a single step increment in work rate continued to exhaustion. A GXT was defined as a multiple or continuous ramp incremental TTE. A TT was defined as the time to complete a specific distance or course. Studies that included more than one supplement intervention, tolerance or performance variable were coded as separate results.

Using a continuous measure, the variance within each intervention group was calculated as the standardized mean difference (SMD) between nitrate and placebo performance outcomes using the Hedges g statistic under the fixed effects model (17) which was calculated using the following formula (Eq. 1):

\[
g = \frac{\bar{x}_1 - \bar{x}_2}{S_{\text{pooled}}}
\]

The effect sizes were based on Cohen’s definition of small (≤ 0.2), moderate (~0.5), and large (≥ 0.8). A negative ES for an exercise performance variable suggests an ergogenic effect. Where standard error (SE) was reported, the standard deviation (SD) was calculated as \( SD = SE \times \sqrt{n} \) where \( n \) represents the sample size.

Statistical Analyses

Meta-analyses were performed using hierarchical data structures, with a sample bias adjustment for small samples. The observations were weighted by the inverse of the
reported sampling variance. It was assumed the effects varied between studies and the
total effect is the weighted average of the effects reported. Separate analyses were
performed for exercise tolerance (TTE and GXT) and performance tests (TT). When
sample size was not limited, subgroup analyses were performed on the following factors:
training status (classified as “untrained” or “trained” populations as defined by author),
supplement dose (low ≤ 7 mmol or high > 8 mmol), duration (short ≤ 3 days or long > 3
days) and experimental conditions (hypoxia). Statistical analyses were performed using
MedCalc for Windows, version 16.4 (MedCalc Software, Ostend, Belgium) Data are
presented as SMD for the performance outcomes with 95% Confidence Interval (CI), and
are presented in forest plots. The SMD was considered statistically significant if the value
of 0 was not within the 95% CI. The marker size is relative to study weight and the
pooled effects are represented using a diamond in which the location represents the ES
and the width reflects the precision of the estimate.

RESULTS

Exercise Tolerance

The analysis on exercise tolerance compromised 200 subjects and 20 ES, nested
within 15 studies. Analysis using TTE and GXT as the outcome variables revealed a
significant impact of nitrate supplementation on exercise tolerance ES 0.28 (95% CI: 0.08
– 0.47; \( P = 0.006 \)) (see Fig. 2). Subgroup analysis on training status showed a significant
effect of nitrate supplementation on exercise tolerance in untrained subjects ES 0.32
(95% CI: 0.10 – 0.53; \( P = 0.004 \)) (see Fig. 2a) but not trained ES 0.1 (95% CI: -0.41 –
0.61; \( P = 0.70 \)) (see Fig. 2b.) Subgroup analysis by dose showed that a larger nitrate dose
significantly improved exercise tolerance ES 0.27 (95% CI: 0.01 – 0.53; \( P = 0.04 \)) but not
a smaller dose ES 0.28 (95% CI: -0.03 – 0.58; \( P = 0.08 \)). Surprisingly, the supplementation duration had little impact on exercise tolerance, with a longer supplementation duration showing a similar, albeit non-significant, effect ES 0.32 (95% CI: -0.03 – 0.68; \( P = 0.07 \)) as a shorter duration ES 0.25 (95% CI: 0.02 – 0.49; \( P = 0.04 \)).
Figure 2. Forest plot of the impact of nitrate supplementation on exercise tolerance.
Figure 2a. Forest plot of the impact of nitrate supplementation on exercise tolerance in untrained subjects.
Figure 2b. Forest plot of the impact of nitrate supplementation on exercise tolerance in trained subjects.
Exercise Tolerance in Hypoxia

The analysis on exercise tolerance in hypoxia compromised 34 subjects and three ES, nested within three studies. Analysis using TTE and GXT as the outcome variables revealed a small but insignificant effect of nitrate supplementation on exercise tolerance in hypoxia compared to placebo ES 0.29 (95% CI: -0.19 – 0.77; \( P = 0.23 \)).

Exercise Performance

The analysis on exercise performance compromised 153 subjects and 16 ES, nested within 11 studies. Analysis using TT as the outcome variable revealed no significant impact of nitrate supplementation on exercise performance ES -0.05 (95% CI: -0.28 – 0.17; \( P = 0.64 \)) (see Fig. 3). Subgroup analysis on trained subjects revealed no significant effect ES -0.04 (95% CI: -0.28 – 0.20; \( P = 0.8 \)) (see Fig. 3a) and a small, insignificant effect ES -0.21 (95% CI: -0.93 – 0.51; \( P = 0.6 \)) in untrained subjects (see Fig. 3b). No significant effect was found following the subgroup analyses by supplementation dose or duration. The effect size for larger dose was ES 0.03 (95% CI: -0.27 – 0.34; \( P = 0.84 \)) while the effect size for the smaller dose was ES -0.16 (95% CI: -0.50 – 0.18; \( P = 0.35 \)). A longer supplementation duration had an effect size of ES -0.16 (95% CI: -0.59 – 0.28; \( P = 0.48 \)) while a shorter duration showed an ES -0.02 (95% CI: -0.28 – 0.25; \( P = 0.9 \)).
Figure 3. Forest plot of the impact of nitrate supplementation on exercise performance.
Figure 3a. Forest plot of the impact of nitrate supplementation on exercise performance in trained subjects.
Figure 3b. Forest plot of the impact of nitrate supplementation on exercise performance in untrained subjects.
Exercise Performance in Hypoxia

The analysis on exercise performance in hypoxia compromised 42 subjects and four ES, nested within four studies. Analysis using TT as the outcome variable revealed no significant impact of nitrate supplementation on exercise performance in hypoxia compared to placebo ES \(-0.12\) (95% CI: -0.56 – 0.31; \(P = 0.58\)).

Heterogeneity and Inconsistency

No significant heterogeneity or inconsistency was observed for exercise tolerance [\(Q 3.39\) (\(P = 1.00\)), \(I^2 0.00\%\)] or exercise performance [\(Q 8.22\) (\(P = 0.91\)), \(I^2 0.00\%\)].

DISCUSSION

The primary purpose of this study was to systematically review the current literature and evaluate the overall efficacy of nitrate supplementation on exercise tolerance and performance by meta-analysis. The pooled analysis for the effect of nitrate supplementation on exercise tolerance using TTE and GXT protocols showed a small, significant ES compared to placebo. However, there was no significant effect of nitrate supplementation on exercise performance using TT protocols.

Hoon et al. analyzed 17 studies investigating the effect of nitrate on exercise performance in 2012. (19) Several trials on the effects of nitrate supplementation on exercise tolerance and performance have since been conducted warranting an updated systematic review and meta-analysis. Moreover, sub-analyses of factors likely to influence the efficacy of nitrate supplementation (i.e. training status) were not performed. There were also instances where Hoon et al. may have utilized SE in the calculation of ES rather than SD in a few of the studies.
Constant or graded work-rate tests continued to the point of exhaustion were the primary modality used to assess exercise tolerance. The demands of these tests are not always applicable to competitive sports which generally require athletes to complete a specific distance as quick as possible. However, the increased TTE demonstrated by Bailey et al. 2009 of 15% may translate to a meaningful 1.0% improvement in TT performance. (6, 20) Therefore, this potential improvement in TT performance may go undetected which may explain why the current meta-analysis found no significant benefit of nitrate supplementation relative to placebo on exercise performance.

Athlete Training Status

A secondary purpose of this meta-analysis was to determine if the ES of nitrate supplementation on exercise tolerance and performance was modified by athlete training status. Results from our subgroup analysis suggested that training status may influence the effectiveness of nitrate supplementation on exercise tolerance and performance. Of the 20 ES compromising the exercise tolerance analysis, only three were classified as trained while the remaining 17 were considered untrained. In contrast, of the 16 ES comprising the exercise performance analysis, only two were classified as untrained while the remaining 14 were considered trained.

The lack of effect in trained athletes may be due to the fact that under hypoxic and ischemic conditions, the reduction of nitrate to NO is enhanced. To this effect, training increases muscle capillarity which preserves muscle oxygenation and upregulates NOS activity under most conditions resulting in a decreased reliance on the nitrate - nitrite - NO pathway. (1) Another possibility is that well-trained endurance athletes are largely
adapted to their specialist discipline. This might limit the potential ergogenic effects of supplemental nitrate on mitochondrial efficiency or skeletal muscle contractility.

Finally, because training enhances the production of NO via the NOS pathway, well-trained individuals have higher baseline levels of nitrite than untrained individuals. (28) On this note, Poveda et al. found that plasma nitrite was 158% higher in endurance athletes compared to untrained control subjects (4.9 vs 1.9 µM, respectively) (35). It would also be expected that athletes consume a varied diet – including nitrate-rich vegetables – to meet caloric needs. Collectively, this means that increased endogenous production of nitrite and dietary consumption of nitrate-rich foodstuffs would leave additional nitrate through supplementation of little benefit.

Indeed, Porcelli et al., evaluated the effects of nitrate supplementation on running performance in subjects with varying levels of aerobic fitness. (34) Subjects were divided into three groups based on their VO2peak: low aerobic fitness, VO2peak range, 28.2-44.1 mL/kg/min; moderate aerobic fitness, VO2peak range, 45.5-57.1 mL/kg/min; and high aerobic fitness, VO2peak range, 63.9-81.7 mL/kg/min. Following six days of nitrate supplementation (5.5 mmol), low and moderate aerobically trained subjects completed a 3-km TT 1%-4% faster, where no improvement was found in high aerobically trained subjects compared to placebo.

**Nitrate Supplementation Dose**

Because trained athletes have higher baseline plasma nitrite and nitrate levels, it has been suggested that a larger dose would be needed to elicit ergogenic effects. (21) Wylie et al., investigated the dose-response relationship between nitrate supplementation and the physiological effects associated with exercise. (44) Utilizing three different
nitrate doses, they found that plasma nitrate and nitrite increased in a dose dependent manor up to 16.8 mmol of nitrate. It was found that 8.4 and 16.8 mmol, but not 4.2 mmol of nitrate, administered 2.5 h pre-test increased TTE by 14% and 12% respectively during severe-intensity exercise. These results suggest no benefit of a small (4.2 mmol) or no further benefit with a larger (16.8 mmol) dose, at least in recreationally active men. Similarly, in a study using well-trained rowers, Hoon et al. found that a high dose (8.4 mmol) resulted in a probable improvement in 2000-m rowing performance but not a smaller (4.2 mmol) dose. (18) Subgroup analysis by dose of our meta-analysis suggested similar findings showing that a larger nitrate dose had a significant effect on exercise tolerance ES 0.28 (95% CI: 0.04 – 0.52; $P = 0.02$) but not a smaller dose ES 0.27 (95% CI: -0.01 – 0.57; $P = 0.06$) despite similar ES. However, subgroup analysis on the determinants of nitrate dose on exercise performance found no significant differences between a smaller or larger dose.

Nitrate Supplementation Duration

The bioavailability of nitrate from beetroot is around 100% and plasma concentrations of nitrate peak around one to two hours ($T_{\text{max}}$ of 1.7 ± 0.5 h) with a plasma half-life of ~6 h ($T_{1/2}$ of 6.1 ± 0.9 h) demonstrating the acute effects of nitrate supplementation. (41) However, it is suggested that longer durations of exposure to nitrate supplementation may favorably modify intracellular calcium handling and enhance mitochondrial protein expression. (25) Vanhatalo 2010 et al. looked at the effects of acute and prolonged beetroot juice supplementation (up to 15 days) containing 4.84 mmol of nitrate per day. (39) When compared with placebo, ramp test performance remained unchanged 2.5 h and five days post nitrate supplementation, but there was a
significant increase in peak power output at the gas exchange threshold (GET) after 15
days of beetroot supplementation. However, it is unknown whether longer-term (<15
days) nitrate supplementation may increase (or attenuate) the physiological benefits
compared to short-term (≤3 days). Studies included in the present meta-analysis used
acute (2-3 h pre-test), short-term (3 days) and longer-term (up to 15 days) nitrate duration
supplementation. Subgroup analysis of nitrate supplementation duration showed little
difference between longer duration (>3 days) compared to shorter duration (≤3 days) in
exercise tolerance. Similarly, no significant differences in exercise performance were
found with longer duration compared to shorter duration nitrate supplementation.

Simulated Altitude and Hypoxia

When sea-level residents are exposed to acute environmental hypoxic conditions,
pulmonary NO significantly decreases suggesting a maladaptive response. (12)
Theoretically, increasing blood flow to improve oxygen delivery could offset the low
supply of oxygen in the air. To this effect, it has been suggested that supplementing with
inorganic nitrate may exert ergogenic effects in hypoxic conditions. At high altitude, the
l-arginine pathway is unable to optimally generate NO, making the nitrate - nitrite - NO
pathway important. (4) This pathway can facilitate NO production through an increase in
generation and reduction of nitrate and nitrite via deoxyhemoglobin and
deoxymyoglobin; (36) both of which are more available when blood oxygen saturation is
decreased. However, while several studies favored nitrate over placebo, pooled analysis
showed no significant benefit on exercise tolerance or performance under hypoxic
conditions – most likely due to the wide confidence intervals. Similar to normoxic
conditions, individual training status appears to influence the effectiveness of nitrate
supplementation on exercise tolerance and performance. Vanhatalo et al., 2011 was among the first to report that nitrate supplementation has the potential to negate the ergolytic effects of exercise tolerance in a hypoxic environment compared to the same exercise in normoxia. (40) More specifically, the recreationally active subjects increased TTE during high-intensity knee extensor exercise by 21% with nitrate supplementation. Masschelein et al. 2012 reported that under hypoxic conditions (stimulated 5,000 m altitude) TTE during a cycle incremental test was decreased by 36%. (27) Following nitrate supplementation this ergolytic effect was eliminated by 5% compared to placebo. In regards to the effects of nitrate supplementation on exercise performance in hypoxia, the majority of studies (2, 10, 26) suggest no benefit with one study showing improved performance. (30) Nonetheless, using nitrate prior to high-intensity training or events may represent an effective strategy to at least offset some of the deleterious effects of a hypoxic environment on exercise performance.

Limitations

In certain analyses, only three to four studies were used to determine ES. Theoretically, only two studies are needed to conduct a meta-analysis. With that said however, this low number of observations may lead to inaccurate estimates in which careful interpretation of the results is warranted.

Furthermore, although no significant heterogeneity was detected in the studies included for the meta-analysis, there were considerable differences in study design. Some studies asked subjects to refrain from consuming nitrate-rich foods throughout the duration of the study; whereas other studies did not restrict the consumption of nitrate-rich foods to subjects. On this note, it is possible that restricting habitual dietary intake of
nitrate-rich foods lowers baseline plasma nitrite thereby augmenting the effects observed when nitrate is then supplemented.

For example, individuals following a vegetarian or vegan dietary pattern have low levels of intramuscular creatine as they don’t consume striated tissue foods; the primary dietary source of creatine for humans. But with creatine supplementation, the ergogenic effects of creatine on sports performance are more pronounced in these individuals. (13) Conversely, those who consume high amounts of meat products may already have high levels of intramuscular creatine and therefore may not respond as greatly to supplementation. Furthermore, differences in exercise mode, duration and intensity in addition to individual subject training status made it challenging to interpret the results and devise practical recommendations.

This meta-analysis shows that nitrate supplementation increases tolerance and efficiency to high-intensity constant and maximal incremental exercise, which may increase exercise performance. Doses ranging from 5 to 9 mmol of nitrate seem to be the most effective and can be taken as either a single bolus or as multiple doses (up to 15 days). This amount (5-9 mmol) can easily be met through a normal diet consisting of vegetables, with beetroot, spinach and rocket (rogula) representing the richest sources of dietary nitrate. And while, nitrate salts (NaNO₃⁻ and KNO₃⁻) are equally effective in elevating plasma nitrite and nitrate levels, natural sources should remain the primary vessel for those looking to explore the physiological effects of nitrate associated with exercise. It would also be important to consider the type of athlete performing the exercise, the duration, intensity and mode of the exercise performed as these factors are likely to influence the efficacy of nitrate supplementation.
LITERATURE CITED


ACKNOWLEDGMENTS

The authors declare no conflict of interest. *Authorship Acknowledgements*: The study was designed by GV and MV; data were collected and analyzed by GV and MV; data interpretation and manuscript preparation were undertaken by GV and MV. Both authors approve the final version of the paper.