Nitrates

Technical Documents
Developed by INDI/SNIG for the Irish Sports Council

2014
Nitrates

Pubmed (Medline) and SPORT Discus were searched for all human studies published in peer reviewed journals in the last 5 years (2009-2014). The terms searched were “nitrates,” “nitrates AND sport,” and “nitrates AND exercise.” Studies included in this review adhered to the following criteria:

Inclusion criteria
- Human studies published in English
- Healthy subjects
- Original investigations or reviews assessing the use of nitrates and exercise
- Peer reviewed journals, reviews and position statements on nitrate consumption and exercise

Exclusion Criteria
- The use of nitrates in diseased populations
- Qualitative studies assessing the prevalence of supplement use in both the general and athletic population

The searches revealed 44 results (PubMed) and 53 results (SPORT Discus). After title and abstract review 18 original articles that assessed the use of nitrates in sport and/or exercise settings were retrieved for review. A summary of this nitrate literature is available in Table 2.

Introduction

Nitrates are polyatomic ions (NO₃⁻) which belong to a compound group containing nitrogen and oxygen which is ingested through dietary sources and is also produced in vivo (Australian Institute of Sport 2014). Green leafy vegetables such as spinach, beetroot, celery and lettuce are particularly high in nitrate content. Vegetables are categorised by nitrate level and content in Table 1.
### Table 1: Classification of vegetable by nitrate level and content

<table>
<thead>
<tr>
<th>NITRATE LEVEL</th>
<th>NITRATE CONTENT PER Kg</th>
<th>VEGETABLE TYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very high</td>
<td>&gt;2500mg</td>
<td>Celery, cress, chervil, lettuce, red beetroot, beetroot juice, spinach, rocket (rucola)</td>
</tr>
<tr>
<td>High</td>
<td>1000-2500mg</td>
<td>Celeriac, chinese cabbage, endive, fennel, kohlrabi, leek, parsley</td>
</tr>
<tr>
<td>Moderate</td>
<td>500-1000mg</td>
<td>Cabbage, dill, turnip, savoy cabbage, carrot juice</td>
</tr>
<tr>
<td>Low</td>
<td>200-500mg</td>
<td>Broccoli, carrot, cauliflower, cucumber, pumpkin, chicory</td>
</tr>
<tr>
<td>Very low</td>
<td>&lt;200mg</td>
<td>Artichoke, asparagus, broad bean, eggplant, garlic, onion, green bean, mushroom, pea, pepper, potato, summer squash, sweet potato, tomato, watermelon, capsicum</td>
</tr>
</tbody>
</table>

Adapted from: (Santamaria 2006) and (Australian Institute of Sport 2014)

It is thought that ingested nitrates are converted to nitrites and nitric oxide especially in conditions of acidosis and hypoxia (Hoon et al. 2013). Nitric oxide is a gaseous signalling molecule and is formed from the conventional L-arginine – nitric oxide pathway and also by the independent nitrate – nitrite – nitric oxide pathway requiring bacteria on the tongue to initially convert dietary nitrate to nitrite (Figure 1). When combined, this dual pathway facilitates nitric oxide production across many physiological conditions (Bailey et al. 2012).
Nitric oxide is known to act as a vasodilator by acting on the smooth muscle of arterioles. It is produced within the endothelium of these arterioles and as such aids intrinsic control of blood flow (Kenney, Wilmore and Costill 2012). Nitrate supplementation has been shown to influence exercise efficiency, muscle contractility (via enhanced cross-bridging and calcium availability), mitochondrial respiration and glucose uptake as well as vasodilation (Bailey et al. 2012). The end result is an increased efficiency or reduced oxygen cost at a given exercise intensity (Jones, Vanhatalo and Bailey 2013).

Figure 1: The dual pathway for nitric oxide synthesis in humans

![Diagram of nitric oxide synthesis pathways](image-url)

Figure adapted from: (Bailey et al. 2012)

**The health benefits claims of consuming nitrate supplements**

First and foremost athletes should strive for a diet that incorporates all major food groups to achieve both macronutrient (carbohydrates, proteins and fats) and micronutrient (vitamins and minerals) requirements. Supplements should not be seen as a quick fix and a substitute for a poor diet.

A nitrate rich diet may have numerous cardiovascular and other health benefits and as such aid lifelong health. However in some cases nitrate supplementation may be a
practical and cost effective option as it has been shown to improve exercise performance in healthy and clinical populations (Bailey et al. 2012).

The following populations have been reported to benefit from nitrate supplementation:

- Individuals requiring a pharmacological agent to treat cardiovascular pathologies (Kapil, Webb and Ahluwalia 2010) (Kenjale et al. 2011)
- Populations with high blood pressure (Vanhatalo et al. 2010), (Hobbs et al. 2012)
- Individuals with high levels of oxidative stress (Hord, 2011)
- Populations wishing to improve vascular compliance (Bahra et al. 2012)

The athletic benefits of consuming nitrate supplements

A summary of studies using nitrate supplementation is shown below (Table 2). The majority of studies used a nitrate-rich beetroot juice supplement but some used whole beetroot (Murphy et al. 2012) or sodium nitrate (Bescós et al. 2012, Larsen et al. 2010), while potassium nitrate capsules (Peacock et al. 2012) and topical gels have also been used (Bloomer et al. 2012)

The consumption of nitrates has been studied as a potential aid to enhance exercise performance. Short term dietary supplementation has been shown to increase plasma nitrate/nitrite levels and reduce the oxygen cost of exercise (Lansley et al. 2011) (Vanhatalo et al. 2010, Bailey et al. 2009, Bailey et al. 2010). However other studies have shown no (or insignificant) performance increases despite elevated plasma nitrate/nitrite levels (Bescós et al. 2012, Christensen, Nyberg and Bangsbo 2013, Wilkerson et al. 2012a).

A recent review and meta-analysis by Hoon et al. (2013) revealed that some studies used acute (single bolus protocols) while others used chronic supplementation and nitrate dosage varies from 300 – 600mg nitrate. Protocols vary from acute (single bolus 2 – 2.5 hours prior to exercise) (Vanhatalo et al. 2010; Wilkerson et al. 2012a; Bescós et al. 2011; Engan et al. 2012) to chronic dosage (4 - 6 days) (Lansley et al. 2011) and up to 15 days (Vanhatalo et al. 2010).

The mode of exercise used in studies varied from severe intensity cycling (Wylie et al. 2013a) to 50 mile TT performance (Wilkerson et al. 2012b). Some studies used submaximal endurance cycling at set percentages of maximal power (Cermak, Gibala and van Loon. 2012) while other researchers used combined arm and leg
cranking (Larsen et al. 2010). Incremental treadmill (Lansley et al. 2011) and high intensity step exercise protocols (Bailey et al. 2009, Bailey et al. 2010) were used while other researchers used arm curl isometric force exercises (Bloomer et al. 2012).

The training status of subjects also varied, from healthy active subjects (Vanhatalo et al. 2010, Bahra et al. 2012, Breese et al. 2013) to recreationally fit (Bescós et al. 2012)(Murphy et al. 2012). Elite level cyclists (Christensen, Nyberg and Bangsbo 2013), cross-country skiers (Peacock et al. 2012) and trained divers (Engan et al. 2012) have all been used as experimental groups.

**Nitrate products and dosage**

- “Beet it” (James White, U.K.) organic beetroot juice contains 90% organic beetroot juice and 10% organic apple juice. Protocol: 1 x 70 ml serving 1 – 12 hours before exercise or on a loading basis, 1 x 70 ml dose/day 1 – 2 weeks prior to competition. (1 x 70 ml dose contains 0.3g (300mg) of dietary nitrate)

- “Beet it sport bar” (James White, U.K.) 60g beetroot and oat (50% oats) flapjack. Protocol: 1 x 60g bar 1 – 12 hours prior to exercise. 1 x 60g bar contains 0.4g (400mg) dietary nitrate.

- “Beet it sport pro-elite shot” (James White, U.K.) 70ml concentrated beetroot juice (and lemon juice) sports shot. Protocol: 1 x 70 ml shot 1 – 12 hours before exercise or on a loading basis, 1 x 70 ml dose/day 1 – 2 weeks prior to competition. (1 x 70 ml dose contains 0.4g (400mg) of dietary nitrate)

(James White Drinks 2014)

**Concerns/adverse effects with nitrate supplementation**

- Caution is advised regarding the uncontrolled use of nitrates/nitrite salts. Although nitrate is non-toxic, nitrite (salts) can cause harm, even at lower doses. The LD$_{50}$ for nitrite is ~ 100 - 200mg/kg and a rapid reaction with haemoglobin in the blood can cause acute toxicity and may lead to methemoglobinemia (reduced ability of red blood cells to release oxygen to tissue leading to tissue hypoxia). Higher doses of nitrite salts (especially if combined with vasodilatory drugs) may cause hypotension (Lundberg, Larsen and Weitzberg 2011)

- Concentrated beetroot juice may cause mild gastrointestinal discomfort (Australian Institute of Sport 2014)
• Harmless (pink) discoloration of urine (beeturia) may occur as a result of nitrate (beetroot/beetroot juice) supplementation (Bailey et al. 2012, Vanhatalo et al. 2010)

• Caution should be shown with using antibacterial mouthwashes as rinsing the mouth with an antibacterial mouthwash destroys oral cavity bacteria and reduces greatly the conversion of nitrate to nitrite in saliva (Govoni et al. 2008)
Table 2: Summary of nitrate literature

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects</th>
<th>Dose</th>
<th>Intervention</th>
<th>Outcome</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Wylie et al. 2013b)</td>
<td>14 (male) recreational team-sport players</td>
<td>490mL conc. nitrate rich beetroot juice (~4.1mmol NO₃⁻ per 70ml) Vs 490mL nitrate depleted placebo juice</td>
<td>Double-blind RCT, crossover design. Variables: Pre and post Yo-Yo IR1 performance, resting plasma nitrite, blood lactate, mean blood glucose and plasma K⁺ levels</td>
<td>Positive</td>
<td>Short-term dietary nitrate sup. can improve intermittent exercise performance in recreationally active male adults.</td>
</tr>
<tr>
<td>(Wilkerson et al. 2012a)</td>
<td>8 (male) well trained cyclists</td>
<td>500mL nitrate-rich beetroot juice (~6.2 mmol NO₃⁻ per 500mL) Vs 500mL nitrate-depleted beetroot juice</td>
<td>Single-blind RCT, crossover design. Variables: 50 mile TT (post beverage ingestion) time, plasma NO₂⁻, mean VO₂, BP, mean PO and blood lactate levels</td>
<td>Negative</td>
<td>Acute dietary sup. with NO₃⁻ rich beetroot juice did not give stat. sig. results in 50 mile TT performance</td>
</tr>
<tr>
<td>(Lansley et al. 2011)</td>
<td>9 (male) physically active</td>
<td>500mL nitrate-rich beetroot juice (~6.2 mmol NO₃⁻ per 500mL) Vs 500mL nitrate-depleted beetroot juice</td>
<td>Double-blind RCT, crossover design. 10 test days over 4 – 5 weeks. Incremental treadmill and knee ext. trials. Variables: Plasma NO₂⁻, BP, HR, blood lactate, VO₂ dynamics and time to task failure</td>
<td>Positive</td>
<td>4–6 days of dietary sup. with NO₃⁻ rich beetroot juice increased plasma NO₂⁻ and reduced SBP. It also reduced the O₂ cost of exercise and time to task failure at running and knee ext. exercise</td>
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### Table 2 con/td: Summary of nitrate literature

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<td>(Wylie et al. 2013a)</td>
<td>10 (male) healthy subjects</td>
<td>70mL (4.2 mmol NO$_3^-$), 140mL (8.4 mmol NO$_3^-$) or 280mL (16.4 mmol NO$_3^-$) conc. beetroot juice Vs nitrate-depleted beetroot juice</td>
<td>Balanced crossover design. Dose response. Variables: Plasma NO$_2^-$, $O_2$ uptake, time to task failure at moderate and severe int. cycling exercise.</td>
<td>Positive (140mL and 280mL) Nitrate</td>
<td>140mL and 280mL beetroot juice reduce steady state $O_2$ uptake and increase time to task failure</td>
</tr>
<tr>
<td>(Christensen, Nyberg and Bangsbo 2013)</td>
<td>10 (male) elite level cyclists</td>
<td>500mL nitrate-rich beetroot juice (0.5g nitrate/day) Vs 500mL isocaloric placebo</td>
<td>Single-blind RCT crossover design. Variables: Plasma NO$_2^-$, VO$_2$ kinetics, endurance capacity and RST performance</td>
<td>Negative</td>
<td>6 day nitrate-rich supplementation leads to elevated plasma NO$_2^-$, but no sig. changes in VO$_2$ kinetics, endurance capacity and RST performance</td>
</tr>
<tr>
<td>(Bescós et al. 2012)</td>
<td>13 (male) non-professional cyclists and triathletes</td>
<td>10mg/kg/body mass sodium nitrate Vs 10 mg/kg/body mass sodium chloride placebo</td>
<td>Double-blind RCT crossover design. Variables: Plasma NO$_2^-$, metabolic gas analysis, urinary nitrate/nitrite in 40 min incremental cycle ergometry tests</td>
<td>Negative</td>
<td>3 days of sodium nitrate supplementation does not improve performance of endurance athletes in 40 min cycle ergometry tests</td>
</tr>
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<td>(Larsen et al. 2010)</td>
<td>9 (7 male; 2 female) healthy, non-smoking subjects</td>
<td>0.1mmol/kg/day sodium nitrate (dose = 100-300g of nitrate-rich vegetables) Vs sodium nitrate placebo</td>
<td>Double-blind RCT crossover design. Variables: Plasma NO\textsuperscript{2–}, plasma amino acids, nitrate/nitrite kinetics, VO\textsubscript{2}, BP, in combined incremental arm and leg cranking exercise to exhaustion</td>
<td>Positive</td>
<td>Sodium nitrate supplementation decreases VO\textsubscript{2max} at max. combined arm and leg exercise with trend towards increased performance</td>
</tr>
<tr>
<td>(Cermak, Gibala and van Loon. 2012)</td>
<td>12 (male) cyclists or triathletes</td>
<td>140ml/day conc. beetroot juice (~8 mmol/day NO\textsubscript{3}⁻) Vs nitrate depleted beetroot juice placebo</td>
<td>Double-blind repeated measure crossover design. Variables: Plasma NO\textsubscript{2–}, mean VO\textsubscript{2} at 45% and 65%W\textsubscript{max}, BP, RER and trial time in submaximal endurance cycling (at 45% and 65%W\textsubscript{max})</td>
<td>Positive</td>
<td>6 days of 140 ml dietary nitrate supplementation lowers VO\textsubscript{2} during submax. exercise and improves 10K TT perf. in trained end. cyclists.</td>
</tr>
<tr>
<td>(Bloomer et al. 2012)</td>
<td>14 (male) resistance trained</td>
<td>2-nitrooxy ethyl 2-amino 3-methylbutanoate gel; mixed in tea tree oil Vs tea tree oil placebo over 7 days</td>
<td>Double blind RCT, crossover design. Variables: Plasma NO\textsubscript{2–}, blood lactate, perceived muscle pump, arm circumference and ex. performance in arm curl isometric force and muscular endurance exercises</td>
<td>Non-sig. positive</td>
<td>The use of nitrite topical gel over 7 days provides a (6.2%), nonsig. effect on repetitions performed (esp. @ 50% 1RM)</td>
</tr>
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<tr>
<td>(Breese et al. 2013)</td>
<td>9 healthy, physically active subjects</td>
<td>140ml/day conc. beetroot juice (~8 mmol NO₃⁻) Vs nitrate depleted beetroot juice placebo over 6 days</td>
<td>Double-blind RCT crossover design. Variables: Plasma NO₂⁻, VO₂ kinetics, muscle deoxyhaemoglobin kinetics and time to task failure at double-step ex. protocol</td>
<td>Positive</td>
<td>Dietary sup. with NO₃⁻ rich beetroot juice speeds VO₂ kinetics and enhances exercise tolerance during severe-intensity exercise (from an elevated met rate).</td>
</tr>
<tr>
<td>(Peacock et al. 2012)</td>
<td>10 (male) junior cross-country skiers</td>
<td>1g potassium nitrate gel capsule (9.9 mmol NO₃⁻) Vs 1g maltodextrin placebo capsule</td>
<td>Double-blind RCT crossover design. Variables: Plasma NO₂⁻, nitric oxide metabolites, O₂ response, RER and exercise performance in 5K endurance running</td>
<td>Negative</td>
<td>Acute ingestion of potassium nitrate did not enhance 5k TT performance and did not reduce O₂ cost of submax. running in elite cross-country skiers</td>
</tr>
<tr>
<td>(Vanhatalo et al. 2010)</td>
<td>8 (5 male, 3 female) healthy active subjects</td>
<td>500mL nitrate-rich beetroot juice/day (5.2 mmol NO₃⁻) Vs 500mL low calorie juice cordial placebo</td>
<td>Double-blind RCT crossover design. Variables: Plasma NO₂⁻, BP, O₂ cost, Wpeak and work rate in mod. intensity and ramp incremental exercise</td>
<td>Positive</td>
<td>Dietary nitrate sup. reduces BP and O₂ cost of submax. exercise over 15 days</td>
</tr>
</tbody>
</table>
**Table 2 con/td: Summary of nitrate literature**

<table>
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<tr>
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<tr>
<td>(Murphy et al. 2012)</td>
<td>11 (5 male, 6 female) recreationally fit subjects</td>
<td>Whole beetroot (~500mg nitrate) Vs nitrate free placebo (cranberry relish)</td>
<td>Double-blind placebo controlled crossover design. Variables: BP, HR, RPE and exercise performance in 5K treadmill TT</td>
<td>Positive</td>
<td>Whole beetroot consumption improved 5 km running performance in healthy adults.</td>
</tr>
<tr>
<td>(Engan et al. 2012)</td>
<td>12 (9 male, 3 female) breath hold divers</td>
<td>70mL conc. nitrate rich organic beetroot juice/day (5.0 mmol NO$_3^-$) Vs 70mL nitrate free placebo</td>
<td>Double-blind RCT, crossover design. Variables: Spirometry, SaO$_2$, HR and BP in submax. apneas and apneic duration</td>
<td>Positive</td>
<td>Max. apneic duration was extended by 11% following acute dietary sup. with NO$_3^-$ rich beetroot juice (reduced metabolic cost)</td>
</tr>
<tr>
<td>(Vanhatalo et al. 2011)</td>
<td>9 (7 male, 2 female) recreationally fit subjects</td>
<td>0.75L nitrate rich beetroot juice (9.3 mmol NO$_3^-$) Vs 0.75L nitrate depleted beetroot juice placebo</td>
<td>Double-blind RCT. Variables: Plasma NO$_2^-$, muscle metabolites, HR, BP, SaO$_2$, in hypoxic/normoxia prone position ergometry trials at high/low intensity</td>
<td>Positive</td>
<td>Dietary nitrate sup. reduced muscle met. changes during high-int. ex in hypoxia and restored exercise tolerance to that obs. in normoxia</td>
</tr>
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<tr>
<td>(Bescós et al. 2011)</td>
<td>11 (male) cyclists and triathletes</td>
<td>10mg/kg/body mass sodium nitrate Vs 10mg/kg/body mass sodium chloride placebo</td>
<td>Double-blind RCT crossover design. Variables: Plasma NO\textsuperscript{2}, VO\textsubscript{2} data, blood sampling and power outputs at submax. and max. cycle ergometry trials</td>
<td>Positive (reduced VO\textsubscript{2peak})</td>
<td>Acute sodium nitrate sup. significantly reduces VO\textsubscript{2peak} while maintaining CV and work parameters</td>
</tr>
<tr>
<td>(Bailey et al. 2010)</td>
<td>7 (male) healthy subjects</td>
<td>500mL/day nitrate-rich beetroot juice (5.1mmol/day NO\textsubscript{3}⁻) Vs 500mL nitrate-depleted placebo</td>
<td>Double-blind RCT crossover design. Variables: Plasma NO\textsuperscript{2}, VO\textsubscript{2} data and muscle metabolites in low and high intensity step exercise tests</td>
<td>Positive</td>
<td>Dietary nitrate sup. allows enhanced contractile efficiency (reduced O\textsubscript{2} cost - reduced ATP and muscle metabolites) during knee extensor exercise</td>
</tr>
<tr>
<td>(Bailey et al. 2009)</td>
<td>8 (male) healthy subjects</td>
<td>500mL/day nitrate-rich beetroot juice (5.5 mmol/day) Vs 500mL nitrate-negligible blackcurrant cordial placebo</td>
<td>Double-blind RCT crossover design. Variables: Plasma NO\textsuperscript{2}, VO\textsubscript{2} data, O\textsubscript{2} kinetics and BP in moderate and severe intensity step exercise tests</td>
<td>Positive</td>
<td>Dietary nitrate sup. reduces O\textsubscript{2} cost at mod. intensity and slows O\textsubscript{2} slow component with increased TTE at severe intensity during step exercise tests</td>
</tr>
</tbody>
</table>
Possible contamination

- With any nutritional supplement there is always the risk of contamination in the manufacturing process, which could lead to a supplement containing substances that are not listed on the label.
- Athletes are advised to check that any supplement they choose to take has been tested for containing any banned substances.
- The legal clause ‘strict liability’ means that an athlete is responsible for any and all substances that may appear in his or her urine or blood in a doping test.
- It is always advisable to seek professional advice from a Sports Dietitian/Nutritionist regarding any nutrition supplement.

Conclusion

Nitrates are available through dietary sources which will have varying levels of nitrate content. Supplementation is available as beetroot juice, sodium nitrate, potassium nitrate and topical gels. Acute and chronic supplementation protocols have been used in exercise studies. The dosage, timing of dose and mode of exercise are all varied despite the fact that many of these are double-blind randomized control trials. Some of these studies have shown significant improvements in exercise performance, a reduced oxygen cost and increased task time to exertion. Other studies have shown insignificant or no performance improvement despite elevated plasma nitrate/nitrite levels. It is likely that factors including age, diet, health, training level and exercise duration and intensity will impact on an individual’s response to dietary nitrate supplementation. Further research is needed to unequivocally state that nitrate supplementation improves exercise performance. However, a vegetable –rich diet may well potentiate the nitrate-nitrite-nitric oxide pathway and aid lifelong health.

References:


Cermak, N.M., Gibala, M.J. and van Loon, L.J.C. 2012. Nitrate supplementation's improvement of 10-km time-trial performance in trained cyclists. United States: HUMAN KINETICS PUBL INC.


