Abstract
Several recent studies indicate that supplementation of the diet with inorganic nitrate results in a significant reduction in pulmonary $O_2$ uptake during sub-maximal exercise, an effect that appears to be related to enhanced skeletal muscle efficiency. The physiological mechanisms responsible for this effect are not completely understood but are presumably linked to the bioconversion of ingested nitrate into nitrite and thence to nitric oxide. Nitrite and/or nitric oxide may influence muscle contractile efficiency perhaps via effects on sarcoplasmic reticulum calcium handling or actin-myosin interaction, and may also improve the efficiency of mitochondrial oxidative phosphorylation. A reduced $O_2$ cost of exercise can be observed within 3 h of the consumption of 5–6 mmol of nitrate, and this effect can be preserved for at least 15 days provided that the same ‘dose’ of nitrate is consumed daily. A reduced $O_2$ cost of exercise following nitrate supplementation has now been reported for several types of exercise including cycling, walking, running, and knee extension exercise. Dietary nitrate supplementation has been reported to extend the time to exhaustion during high-intensity constant work rate exercise by 16–25% and to enhance cycling performance over 4, 10, and 16.1 km by 1–2% in recreationally active and moderately trained subjects. Although nitrate appears to be a promising ‘new’ ergogenic aid, additional research is required to determine the scope of its effects in different populations and different types of exercise.

The Nitrate-Nitrite-Nitric Oxide Pathway

Nitric oxide (NO) is an important physiological signaling molecule that can modulate skeletal muscle function through its role in the regulation of blood flow, contractility, glucose and calcium homeostasis, and mitochondrial respi-
Until quite recently, it was considered that NO was generated solely through the oxidation of the amino acid L-arginine in a reaction catalyzed by NO synthase (NOS), and that nitrite (NO$_2^-$) and nitrate (NO$_3^-$) were inert by-products of this process [2]. However, it is now clear that these metabolites can be recycled back into bioactive NO under certain physiological conditions [3, 4]. The reduction of NO$_3^-$ to NO$_2^-$ and subsequently of NO$_2^-$ to NO may be important as a means to increase NO production when NO synthesis by the NOS enzymes is impaired [5] and in conditions of low oxygen availability, as may occur in skeletal muscle during exercise.

In addition to being created through the NOS-catalyzed production of NO from L-arginine, tissue concentrations of NO$_3^-$ and NO$_2^-$ can be increased by dietary means. Vegetables account for 60–80% of the daily NO$_3^-$ intake in a Western diet [6] with green leafy vegetables such as lettuce, spinach and beetroot being particularly rich in NO$_3^-$ [7] (table 1). Ingested inorganic NO$_3^-$ is rapidly absorbed from the gut and passes into the systemic circulation with peak plasma [NO$_3^-$] being observed approximately 60 min after ingestion [4]. While some 60% of the systemic NO$_3^-$ is excreted in the urine [4], 25% passes into the enteralcirculation and becomes highly concentrated in the saliva [8]. In the mouth, facultative anaerobic bacteria on the surface of the tongue reduce NO$_3^-$ to NO$_2^-$ [9]. This NO$_2^-$ is swallowed and reduced to NO and other reactive nitrogen intermediates within the acidic environment of the stomach [10, 11]. However, some NO$_2^-$ is absorbed to increase circulating plasma [NO$_2^-$] with the peak concentration being attained 2–3 h following NO$_3^-$ ingestion [8, 12]. Therefore, dietary NO$_3^-$ supplementation represents a practical method to increase circulating plasma [NO$_2^-$] and thus NO bioavailability. This has been demonstrated after ingestion of sodium nitrate (NaNO$_3$) [8 and 13–15], potassium nitrate [16], as well as NO$_3^-$-rich beetroot juice [17–22]. Interestingly, the characteristic rise in plasma [NO$_2^-$] following an oral NO$_3^-$ bolus is largely abolished by the use of antibacterial mouthwash [23], indicating that the reduction of NO$_3^-$ to NO$_2^-$ in humans is critically dependent on the oral bacterial NO$_3$ reductases.

Table 1. Nitrate content (mg/100 g fresh weight) of selected vegetables

<table>
<thead>
<tr>
<th>Nitrate content</th>
<th>Vegetable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very high (&gt;250)</td>
<td>beetroot, spinach, lettuce, rocket, celery, cress, chervil</td>
</tr>
<tr>
<td>High (100–250)</td>
<td>celeriac, fennel, leek, endive, parsley</td>
</tr>
<tr>
<td>Medium (50–100)</td>
<td>cabbage, savoy cabbage, turnip, dill</td>
</tr>
<tr>
<td>Low (20–50)</td>
<td>broccoli, carrot, cauliflower, cucumber, pumpkin</td>
</tr>
<tr>
<td>Very low (&lt;20)</td>
<td>asparagus, aubergine, onion, mushroom, pea, pepper, potato, sweet potato, tomato</td>
</tr>
</tbody>
</table>
The final step in the $\text{NO}_3^-$-$\text{NO}_2^-$-$\text{NO}$ pathway is the one electron reduction of $\text{NO}_2^-$ to $\text{NO}$. This reaction is potentiated in hypoxic [24] and acidic [25] environments such as those which may exist in skeletal muscle during exercise [26]. The existence of an alternative NO generation pathway is important as it promotes NO synthesis under conditions that would otherwise limit the production of NO from NOS, ensuring that NO synthesis can occur across a wide range of cellular $O_2$ tensions. It is important to note, however, that $\text{NO}_2^-$ may itself induce physiological effects independent of its reduction to NO [27].

The purpose of this paper is to provide a brief review of the available literature which supports a role for dietary nitrate supplementation in enhancing exercise performance in healthy humans. Given the importance of NO in vascular and metabolic control, there are sound theoretical reasons why augmenting NO bioavailability might be important in optimizing skeletal muscle function during exercise. Recent evidence indicates that elevating plasma $[\text{NO}_2^-]$ through dietary nitrate supplementation is associated with enhanced muscle efficiency, fatigue resistance and performance. The mechanistic bases for this effect are considered and practical recommendations for nitrate supplementation by athletes are provided.

**Nitrate and Exercise**

In 2007, Larsen et al. [15] reported that 3 days of NaNO$_3$ supplementation increased plasma $[\text{NO}_2^-]$ and reduced the $O_2$ cost of sub-maximal cycle exercise. Blood [lactate], heart rate and minute ventilation ($V\text{E}$) were not significantly altered. These findings were highly surprising because it is well established that the $O_2$ cost of exercising at a given sub-maximal power output is essentially ‘fixed’. For example, during cycle ergometry, it is expected that pulmonary $O_2$ uptake ($V\text{O}_2$) will increase by approximately 10 ml per minute for every additional watt of external power output. The efficiency of exercise is considered to be independent of age, health status and physical fitness and, prior to the study of Larsen et al. [15], had been reported to be essentially unaffected by a variety of acute or chronic interventions [28]. Long-term endurance training may elicit some improvements in exercise efficiency, and it is known that efficiency is an important determinant of endurance exercise performance [29]. The results of Larsen et al. [15] were therefore exciting because they suggested that a short-term dietary intervention might improve exercise efficiency and have the potential to enhance performance.

The initial findings of Larsen et al. [15] were corroborated in the study of Bailey et al. [17] in which NO$_3^-$ was administered in the form of beetroot juice.
Following 3 days of beetroot juice supplementation, the plasma \([\text{NO}_2^-]\) was doubled, the steady-state \(\dot{V}_\text{O}_2\) during moderate-intensity exercise was reduced, and the \(\dot{V}_\text{O}_2\) ‘slow component’ during severe-intensity exercise was attenuated (fig. 1). As highlighted above, it is striking that a short-term, natural dietary intervention can improve the efficiency of muscular work.

The reduction in steady-state \(\dot{V}_\text{O}_2\) after NO\(_3^-\) supplementation was of the order of 5% in the studies of Larsen et al. [15] and Bailey et al. [17] in which supplementation was continued for 3–6 days. A similar reduction in steady-state \(\dot{V}_\text{O}_2\) during moderate-intensity cycle ergometry has been reported following acute NO\(_3^-\) treatment: 60 min following NaNO\(_3^-\) administration [14] and 2.5 h following beetroot juice ingestion [21]. The improved exercise efficiency was sustained when NO\(_3^-\) supplementation was continued for 15 days [21] (fig. 2). This indicates that longer term NO\(_3^-\) supplementation does not elicit greater improvements in exercise efficiency but also, importantly, that tolerance to the intervention does not develop (at least up to 15 days). The reduction in \(\dot{V}_\text{O}_2\) following NO\(_3^-\) administration is not unique to cycling exercise, having also been observed during two-legged knee-extensor exercise [16] and treadmill walking and running [19]. Importantly, no reduction in \(\dot{V}_\text{O}_2\) was observed compared to a control
Fig. 2. The group mean \( \dot{V_O}_2 \) profiles during moderate-intensity exercise across 15-day supplementation periods with beetroot juice (BR) and placebo (PL) in comparison to pre-supplementation baseline (filled circles). Open circles indicate BR-supplemented trials in a–c and PL-supplemented trials in d–f. Error bars are omitted for clarity.
condition when the subjects were supplemented with a placebo beetroot juice that had been depleted of NO₃ using an ion-exchange resin [19]. This confirms that NO₃ is the key ‘active’ ingredient responsible for the physiological changes observed following beetroot juice supplementation. It does not rule out, however, a synergistic role for other components of beetroot juice such as antioxidants and polyphenols, which may facilitate the reduction of NO₃ to NO₂ and NO [30, 31]. Collectively, these results indicate that the reduced \( \dot{V}_{O_2} \) following NO₃ supplementation is reproducible and can be observed across a range of different supplementation regimens and exercise modalities.

**Exercise Performance**

Plasma [NO₂] has recently been identified as an important correlate of exercise tolerance in healthy humans [32, 33]. Given that NO₃ supplementation increases plasma [NO₂], this intervention may therefore have the potential to improve exercise tolerance. This hypothesis was tested in the study of Bailey et al. [17]. Plasma [NO₂] was doubled and exercise tolerance was enhanced by 16% following NO₃-rich beetroot juice supplementation, suggesting that NO₃ supplementation may indeed be ergogenic. Subsequent experiments have reported improvements in exercise tolerance of 25% during two-legged knee-extensor exercise [18], and of 15% during treadmill running [19] following 6 days of beetroot juice supplementation. Improved incremental exercise performance has also been noted following 6 days of beetroot juice supplementation during single-legged knee extension exercise [19] and after 15 days of beetroot juice supplementation during cycle exercise [21]. A trend for an improved exercise tolerance (+7%) during combined incremental arm and leg exercise was reported following 2 days of NaNO₃ supplementation [14]. This observation was made in concert with a reduced \( \dot{V}_{O_2\text{max}} \) (−3%) which indicated that the subjects were more efficient even at maximal exertion following NO₃ supplementation [14]. Incremental exercise performance was not significantly different (+2%) in trained athletes following acute NaNO₃ administration, despite a 4% statistically significant reduction in \( \dot{V}_{O_2\text{max}} \) [34]. It is important to note that a reduction in \( \dot{V}_{O_2\text{max}} \) is not always observed following NO₃ supplementation [17, 21]. It is possible that the influence of NO₃ supplementation on \( \dot{V}_{O_2\text{max}} \) may be dependent on the exercise modality and/or the training status of the subjects.

It is well documented that exercise performance is compromised in hypoxia relative to normoxia. In this regard, it is noteworthy that Vanhatalo et al. [35] recently reported that nitrate supplementation with beetroot juice restored muscle performance in hypoxia (14% inspired O₂; equivalent to 4,000 m altitude) to
that observed in the normoxic control condition. Specifically, in hypoxia, nitrate supplementation resulted in a 20% extension of the time to exhaustion during high-intensity knee extensor exercise. Vanhatalo et al. [35] also reported that nitrate supplementation improved muscle oxidative function in hypoxia, suggesting that muscle oxygenation may have been enhanced. Consistent with this interpretation, Kenjale et al. [36] reported that beetroot juice supplementation resulted in a 17–18% longer time to claudication pain and peak walking time during incremental exercise in patients with peripheral arterial disease. The authors attributed the enhanced performance to NO$_2^-$-related improvement in peripheral tissue oxygenation. Collectively, these results have potential performance implications for athletes competing at altitude and for improving functional capacity in clinical conditions where tissue O$_2$ supply may be compromised.

As summarized above, during constant work rate exercise, the improved exercise tolerance following NO$_3^-$ supplementation has been reported to be in the range of 16–25% [17–19]. However, the magnitude of improvement in ‘actual’ exercise performance would be expected to be far smaller; indeed, using the predictions of Hopkins et al. [37], a ∼20% improvement in time to exhaustion would be expected to correspond to an improvement in exercise performance (time taken to cover a set distance) of 1–2%. This hypothesis was tested in the study of Lansley et al. [20], where competitive but sub-elite cyclists completed, on separate days, 4.0- and 16.1-km time trials following acute beetroot juice ingestion. Consistent with the experimental hypothesis, NO$_3^-$ administration improved 4.0- and 16.1-km time trial performance by ∼2.7% compared to the placebo condition [20] (fig. 3). These improvements in exercise performance were...
consequent to the maintenance of a higher mean power output (+5–6%) and an increase in the power output/\(\dot{V}_O_2\) ratio. Therefore, trained subjects were able to produce a higher power output for the same oxidative energy turnover (i.e. the inverse of a lower \(\dot{V}_O_2\) for the same power output), resulting in an improved exercise performance following NO\(_3\) supplementation. The improved cycle time trial performance following nitrate supplementation reported by Lansley et al. [20] has recently been corroborated by Cermak et al. [38]. These authors reported that 6 days of beetroot juice supplementation (8 mmol/day) significantly reduced \(\dot{V}_O_2\) at two sub-maximal work rates and improved mean power output and 10 km time trial performance (by 1.2%) in trained cyclists.

An improved exercise efficiency has been consistently reported when recreationally active humans (\(\dot{V}_{O_2}\text{max}\) values typically between 45–55 ml · kg\(^{-1}\) · min\(^{-1}\)) have been supplemented with NO\(_3\) [13–15, 17–21]. However, Bescós et al. [34] recently reported that acute NaNO\(_3\) ingestion did not significantly improve sub-maximal exercise efficiency in trained subjects (\(\dot{V}_{O_2}\text{max}\) of 65 ml · kg\(^{-1}\) · min\(^{-1}\)). It is important to note that plasma [NO\(_2\)] was only increased by 16% in this study, whereas previous studies have observed far greater increases in plasma [NO\(_2\)] following NO\(_3\) supplementation, of as much as 100% [13–15, 17–21]. The resting plasma [NO\(_3\)] and [NO\(_2\)] is higher in athletes [39, 40], which may reduce the scope for NO\(_3\) supplementation to improve exercise efficiency in this population. Alternatively, more highly trained individuals may require a larger NO\(_3\) dose to elicit similar changes in plasma [NO\(_2\)] and exercise efficiency to those observed in recreationally active participants. It should also be considered that highly trained subjects are likely to have both higher NOS activity and greater mitochondrial and capillary density, which might limit the development of hypoxia and acidosis in skeletal muscle during exercise. Further research is needed to elucidate the influence of NO\(_3\) supplementation on exercise efficiency in athletes.

**Mechanisms**

The reduced O\(_2\) cost of exercise following nitrate supplementation is not associated with an elevated blood [lactate] [15, 17], suggesting that there is no compensatory increase in anaerobic energy production as might be expected if oxidative metabolism were somehow inhibited. This indicates that nitrate supplementation results in a ‘real’ improvement in muscle efficiency. Theoretically, a lower O\(_2\) cost of exercise for the same power output could result from: (1) a lower adenosine triphosphate (ATP) cost of muscle contraction for the same force production (i.e. improved muscle contractile efficiency), or (2) a lower O\(_2\)
consumption for the same rate of oxidative ATP resynthesis [i.e. improved metabolic (mitochondrial) efficiency].

Bailey et al. [18] investigated the first of these possibilities using calibrated \(^{31}\)P-magnetic resonance spectroscopy. This procedure permitted the in vivo assessment of absolute muscle concentration changes in phosphocreatine ([PCr]), inorganic phosphate ([Pi]), and adenosine diphosphate ([ADP]), as well as pH. The ATP supply contributed by PCr hydrolysis, anaerobic glycolysis and oxidative phosphorylation during knee-extensor exercise was also calculated. The estimated ATP turnover rates from PCr hydrolysis and oxidative phosphorylation were lower following 6 days of beetroot juice supplementation, and contributed to a significant reduction in the estimated total ATP turnover rate during both low- and high-intensity exercise [18]. It is known that the ATP turnover rate in contracting myocytes is determined, in the large part, by the activity of actomyosin ATPase and \(\text{Ca}^{2+}\)-ATPase [41]. NO has been shown to slow myosin cycling kinetics [42] and to reduce \(\text{Ca}^{2+}\)-ATPase activity [43]. As such, elevated NO production following beetroot juice supplementation may have reduced skeletal muscle ATP turnover by reducing the activity of actomyosin ATPase and/or \(\text{Ca}^{2+}\)-ATPase. The intramuscular accumulation of ADP and Pi, and the extent of PCr depletion, were also blunted following \(\text{NO}_3^-\) supplementation [18]. The smaller changes in [ADP], [Pi] and [PCr] following \(\text{NO}_3^-\) supplementation would be predicted to reduce the stimuli for increasing oxidative phosphorylation [44, 45].

The accumulation of metabolites such as [ADP] and [Pi], and the rate of depletion of the finite intramuscular [PCr] reserves, are important contributors to muscle fatigue development [46, 47]. While the intramuscular [ADP], [Pi] and [PCr] were similar at exhaustion in the \(\text{NO}_3^-\) and placebo conditions in the study of Bailey et al. [18] and also Vanhatalo et al. [35], the time taken to achieve these critical concentrations was delayed following \(\text{NO}_3^-\) supplementation, and this, in part, may explain the improved exercise tolerance. In line with these data, dietary \(\text{NO}_3^-\) supplementation has been shown to reduce the development of the \(\dot{V}_{\text{O}_2}\) ‘slow component’ during high-intensity exercise such that the attainment of the \(\dot{V}_{\text{O}_2,\text{max}}\) is delayed and the tolerable duration of exercise is extended [17]. It should be noted that while the improved muscle efficiency and reduced metabolic perturbation may be responsible for the enhanced exercise tolerance observed following nitrate supplementation, it is possible that the intervention results in a simultaneous improvement in \(\dot{V}_\text{O}_2\) delivery to muscle loci that are most ‘hypoxic’ [35, 36]. If true, then this, too, might contribute to improved exercise performance.

The second possibility – that nitrate supplementation enhances mitochondrial efficiency – was recently examined by Larsen et al. [13]. These authors
isolated mitochondria from the vastus lateralis muscle of healthy humans supplemented with NaNO₃. The resultant mitochondrial suspension was added to a reaction medium containing the substrates pyruvate and malate, allowing mitochondrial respiration to be investigated. With a submaximal rate of ADP infusion, the mitochondrial P/O ratio (the amount of ADP administered divided by O₂ consumed) was significantly increased [13]. The respiratory control ratio, which is the ratio between state 3 (coupled) and state 4 (uncoupled) respiration, was also significantly increased with NaNO₃ supplementation, as was the maximal rate of ATP production through oxidative phosphorylation. State 2 respiration, indicative of back leakage of protons through the inner mitochondrial membrane, and state 4 respiration were both reduced with NaNO₃ [13]. Therefore, these data indicated that NO₃⁻ supplementation reduced proton leakage and uncoupled respiration, which increased the mitochondrial P/O ratio. The increased P/O ratio following NO₃⁻ supplementation was correlated with the reduction in whole body O₂ during exercise [13]. Taken together with the findings of Bailey et al. [18], it appears that NO₃⁻ supplementation may improve exercise efficiency by improving the efficiency of both muscle contraction (reduced ATP cost of force production) and mitochondrial oxidative phosphorylation (increased P/O ratio).

**Practical Recommendations**

The available evidence indicates that dietary supplementation with 5–7 mmol nitrate (approximately 0.1 mmol/kg body mass) results in a significant increase in plasma [nitrite] and associated physiological effects including a lower resting blood pressure, reduced pulmonary O₂ uptake during sub-maximal exercise and enhanced exercise tolerance or performance [13–15, 17–21, 35, 36, 38]. This ‘dose’ of nitrate can readily be achieved through the consumption of 0.5 l of beetroot juice (or an equivalent high-nitrate foodstuff). Following a 5- to 6-mmol ‘bolus’ of nitrate, plasma [nitrite] typically peaks within 2–3 h and remains elevated for a further 6–9 h before declining towards baseline [22]. Therefore, it is recommended that nitrate is consumed approximately 3 h prior to competition or training. A daily dose of a high-nitrate supplement is required if plasma [nitrite] is to remain elevated.

Although nitrate supplementation appears to hold promise as an ergogenic aid, it is important to recognize that there is still much that we do not know. Firstly, most of the published studies to date have involved recreational or moderately trained subjects, and it is not known if nitrate supplementation substantially elevates plasma [NO₂⁻] or is ergogenic in elite athletes. Secondly, while the
ingestion of 5–6 mmol of nitrate appears to be effective, studies are ongoing to determine the ‘dose-response’ relationship between nitrate supplementation and changes in exercise efficiency and performance; this will shed light on the ‘optimal’ loading regimen for performance enhancement. Thirdly, while nitrate supplementation appears to be ergogenic in continuous maximal activity of 5–25 min duration, possible effects on short-term high-intensity exercise, intermittent exercise, and long-term endurance exercise performance have not been investigated. Finally, it is presently unclear if, and in what ways, sustained dietary nitrate supplementation might impact upon adaptations to training: on the one hand, increased NO bioavailability might simulate mitochondrial biogenesis and angiogenesis; on the other hand, nitrate has anti-oxidant properties that might blunt cellular adaptations.

It is important to note that dietary or environmental exposure to nitrate has historically been considered to be harmful to human health due to a possible increased risk of gastric cancer [48]. More recent evidence challenges this view and indicates that nitrate ingestion (at least through dietary means) may instead confer benefits to health [49, 50]. Until more is known, it is recommended that athletes wishing to explore possible ergogenic effects of nitrate supplementation employ a natural, rather than pharmacological, approach [51].

**Disclosure Statement**

The author declares that no financial or other conflict of interest exists in relation to the content of the chapter.

**References**


Questions and Answers

**Question 1:** Beetroot supplementation has some unwanted side effects: what are they?

**Answer:** Well, it can stain the body fluids and give the urine, saliva, and stool a pink/red/purple color. It is not really an unwanted or harmful side effect, but you just need to be aware that it is going to change color of certain excretions.
That said, at least you know then that it has been effective in actually being absorbed into your body.

Question 2: Could you explain the mechanism by which nitrate can improve performance?

Answer: Yes, we think that when the nitrate is taken through a foodstuff initially it gets converted into nitrite in the body. So, the nitrate is swallowed and then transported into the enterosalivary circulation, and it reenters the mouth in the saliva where the bacteria in the mouth reduce the nitrate to nitrite. The nitrite is subsequently swallowed and then can be absorbed into the bloodstream. So, the plasma nitrite concentration is increased following beetroot juice or other forms of dietary nitrate supplementation. The nitrite is then transported in the blood and can be broken down further into nitric oxide in areas where it is most needed. We think that this is important in performance terms because nitric oxide has been shown to regulate muscle blood flow and also to regulate muscle contraction energetics and possibly mitochondrial efficiency as well. So, there are a number of reasons by which nitrate (as long as it is turned into nitric oxide) could be useful to physiological function and to exercise performance.

Question 3: What is your practical recommendation for beetroot juice supplementation in athletes?

Answer: We are still experimenting with the appropriate dose in order to get the maximum response. The studies that we have done so far involve the consumption of around 6 mmol nitrate. That is contained in about a half a liter of normal beetroot juice, although that can vary from company to company. It is possible now to buy beetroot juice shots which are a concentrated form of beetroot juice which contains the same amount of nitrate but in a reduced volume of fluid. An important thing is that the nitrate is consumed about 2 or 3 h before exercise is commenced, because that gives sufficient time for the nitrate to be converted into nitrite.

Question 4: Should athletes consume it over a long or a short time period?

Answer: That is another very good question. We do not know the answer to that question right now. We know there is an acute effect. If you take nitrate in the diet, then 2 or 3 h later your blood pressure will be a bit lower; if you exercise, your oxygen uptake will also be slightly lower, and your performance may be slightly improved. And we can continue to supplement for up to 15 days, which is as far as we have gone in our studies, and we do not lose that effectiveness. But, that said, we do not know whether continued nitrate supplementation may or may not enhance adaptations to training.