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Dietary Nitrate and Physical Performance

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Abstract

Nitric oxide (NO) plays a plethora of important roles in the human body. Insufficient production of NO (for example, during older age and in various disease conditions) can adversely impact health and physical performance. In addition to its endogenous production through the oxidation of L-arginine, NO can be formed nonenzymatically via the reduction of nitrate and nitrite, and the storage of these anions can be augmented by the consumption of nitrate-rich foodstuffs such as green leafy vegetables. Recent studies indicate that dietary nitrate supplementation, administered most commonly in the form of beetroot juice, can (*a*) improve muscle efficiency by reducing the O_2 cost of submaximal exercise and thereby improve endurance exercise performance and (*b*) enhance skeletal muscle contractile function and thereby improve muscle power and sprint exercise performance. This review describes the physiological mechanisms potentially responsible for these effects, outlines the circumstances in which ergogenic effects are most likely to be evident, and discusses the effects of dietary nitrate supplementation on physical performance in a range of human populations.

Contents

1. INTRODUCTION

Athletes, both elite and recreational, are interested in using legal means to improve their performance. While the consumption of a balanced diet is important for the maintenance of general health and to fuel and recover from training, many athletes also use dietary supplements with the aim of enhancing performance during competition. Examples of these supplements include caffeine, creatine, sodium bicarbonate, beta alanine, and, most recently, inorganic nitrate (96). The evidence base for the efficacy of nutritional "ergogenic aids" is notoriously mixed, and nitrate is no exception. However, recent meta-analyses do support the suggestion that dietary nitrate, which is normally ingested by athletes in the form of beetroot juice, has the potential to enhance performance in several types of sport and exercise activities (98, 114). Moreover, unlike other supplements that are mainly of value to athletes, nitrate supplementation may also provide cardiovascular health benefits to the general (nonathletic) public via its well-described reduction of resting blood pressure (e.g., 158), and it may benefit older or diseased populations by enhancing the efficiency of locomotion, muscle O_2 delivery, and/or muscle power (164).

Inorganic nitrate is a natural component of many foods, notably green leafy vegetables, but is also used as a preservative in products such as cured meats. For many years, despite relatively weak evidence, nitrate (NO_3^-) and nitrite (NO_2^-) were considered to be carcinogens, but more recent evidence indicates that nitrate may be a key bioactive component in salad vegetables, the consumption of which is encouraged to promote human health. This has prompted a reevaluation of the risks and benefits of dietary nitrate (19).

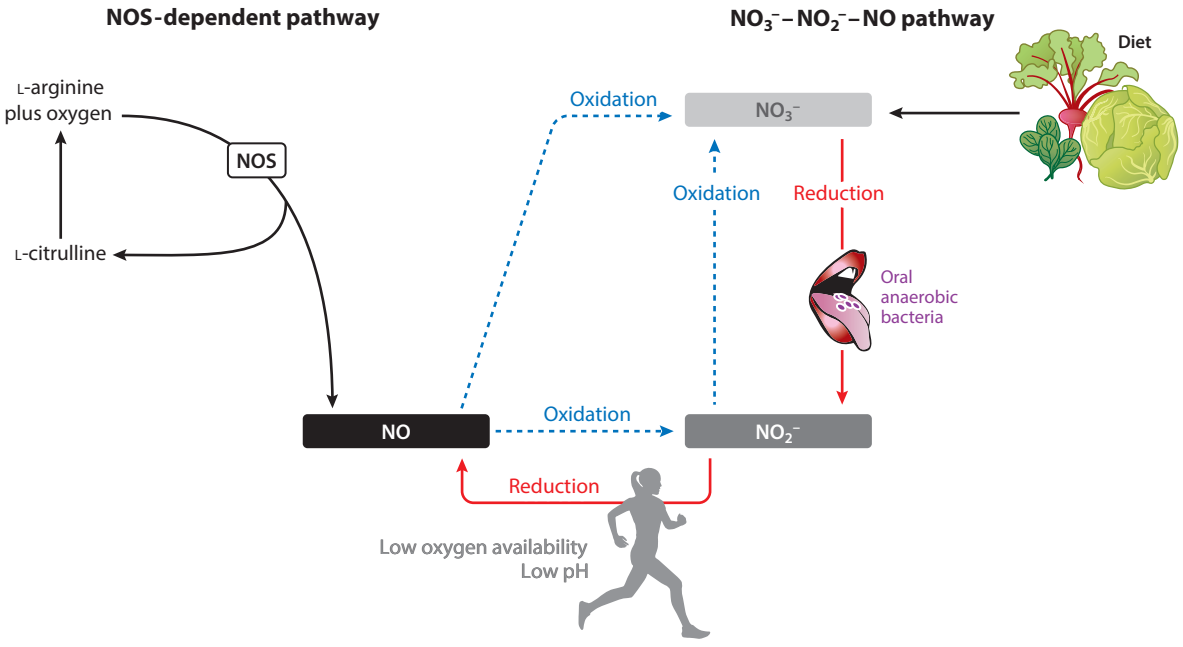

Figure 1

The pathways of nitric oxide (NO) production. In the NO synthase (NOS)–dependent pathway, L-arginine and O_2 produce NO in a reaction catalyzed by the NOS enzymes. Note that L-citrulline is coproduced with NO in this reaction before being effectively recycled into L-arginine. After its production, NO can be rapidly oxidized to form NO_2^- and NO_3^- (*dashed lines*). This endogenously produced NO_3^- can be reduced to NO_2^- by anaerobic bacteria in the oral cavity, and NO_2^- can undergo further reduction to NO in acidic and hypoxic conditions such as occurs in skeletal muscle tissue during exercise. Importantly, the consumption of foods rich in NO_3^-, such as green leafy vegetables and beetroot, can significantly elevate the body's store of NO_3^- and thus boost the production of NO via the NO_3^--NO_2^--NO pathway.

The purpose of this review is to highlight recent advances in our understanding of the role of dietary nitrate in promoting physical performance. Specifically, the review addresses the impact of nitrate ingestion on physiological systems, including the cardiovascular, metabolic, and muscle contractile systems, which are essential to exercise performance.

2. NITRIC OXIDE PRODUCTION PATHWAYS

Nitric oxide (NO), a ubiquitous free-radical gas, is involved in a wide array of signaling and regulatory functions in the human body. In particular, it is known to play a critical role in vasodilation (103), mitochondrial respiration (18), glucose and calcium (Ca^+) homeostasis (100, 155), and skeletal muscle contractility (143) and fatigue development (117). Given its importance and its short half-life, which ranges from milliseconds to seconds (depending on local conditions), the continuous production of NO is essential. Indeed, the human body possesses two complementary pathways by which to generate NO. These are the NO synthase (NOS)-dependent pathway and the NOS-independent NO_3^--NO_2^--NO pathway (**Figure 1**).

The biosynthesis of NO by NOS enzymes is well defined and has been extensively reviewed (e.g., 17) since its discovery in the late 1980s (104). Three isoforms of NOS have been identified and are typically referred to as endothelial (eNOS), neuronal (nNOS), and inducible (iNOS) NOS (17). These enzymes utilize L-arginine and O_2 to produce NO and L-citrulline in a reaction that

requires essential cofactors, including tetrahydrobiopterin (BH_4), nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMD), and calcium-calmodulin (17, 103). Although the concentration of intracellular L-arginine is far greater than required for maximal NO production via eNOS (165), evidence indicates that supplemental L-arginine can cause an increase in NO production (e.g., 156). This surprising phenomenon is commonly referred to as the arginine paradox. However, most studies in healthy humans (e.g., 4, 41, 151; cf. 7) have shown that oral supplementation with pure L-arginine does not increase NO bioavailability. After its production, NO is rapidly oxidized to form NO_2^- and NO_3^- (33), and these anions are therefore considered end-products of NOS-dependent NO synthesis.

The NO_3^--NO_2^--NO pathway, defined more recently (11, 90), involves the serial reduction of NO_3^- to NO_2^- and further to NO and other nitrogen oxides (89) (**Figure 1**). The source of NO_3^- and NO_2^- to fuel this pathway includes not only endogenous oxidation of NO produced via NOS (as described above) but also exogenous inorganic NO_3^- from the diet, particularly that derived through the ingestion of green leafy vegetables such as rocket, kale, and spinach, as well as root vegetables such as beetroot (58, 170). These vegetables typically contain over 250 mg NO_3^- per 100 g fresh weight produce (~4 mmol; 62 mg/mmol), and typical NO_3^- intakes are 31–185 mg/day in Europe and ~40–100 mg/day in the United States (58).

The serial reduction of NO_3^- requires an intricate interorgan metabolic pathway. Following ingestion, NO_3^- is absorbed by the upper gastrointestinal tract and enters the systemic circulation, with peak plasma concentrations appearing approximately 60 min post NO_3^- consumption (89). Approximately 25% of this NO_3^- is then absorbed by the salivary gland via an active transporter, sialin (124), before being concentrated (up to 20-fold) in saliva (49). A large proportion of the remaining NO_3^- is extracted by the kidneys and excreted in the urine (157). In the oral cavity, faculative anaerobic bacteria located on the dorsal surface of the tongue reduce ~20% of salivary NO_3^- to NO_2^- (38). When subsequently swallowed, a portion of this NO_2^- is reduced to NO (and other reactive nitrogen intermediates, including s-nitrosothiols) in the acidic environment of the stomach (11) in a reaction that is greatly enhanced by the presence of vitamin C and polyphenols (159). However, it is clear that some NO_2^- enters the systemic circulation, with peak plasma [NO_2^-] occurring 2–3 h post NO_3^- consumption. This NO_2^- is rapidly distributed in blood and tissue, and it can undergo a one-electron reduction to yield NO in a reaction catalyzed by deoxyhemoglobin (30), deoxymyoglobin (138), xanthine oxidase (172), aldehyde oxidase (86), cytochrome P-450 (74), the mitochondrial electron transfer complexes (75), and even NOS itself (154).

The reduction of NO_2^- to NO is greatly enhanced in conditions of hypoxia (22) and acidosis (102), and therefore NO production from this pathway can be seen as a backup system to ensure continued NO generation when the O_2-dependent NOSs may be dysfunctional. Skeletal muscle is likely to experience hypoxia and acidosis during contraction (127), implying that this pathway may be of particular importance during exercise.

3. INFLUENCE OF DIETARY NITRATE INTAKE ON INDICES OF NITRIC OXIDE BIOAVAILABILITY AND PHYSIOLOGICAL EFFECTS DURING EXERCISE

In 2007, Larsen et al. (83) made the remarkable discovery that three days of sodium nitrate ($NaNO_3$) supplementation increased the plasma [NO_2^-] and reduced the O_2 cost of submaximal cycle exercise (83). These findings were corroborated by Bailey et al. (9), who administered NO_3^- in the form of NO_3^- rich beetroot juice. Following three days of beetroot juice supplementation,

the plasma [NO_2^-] was doubled, and the increase in O_2 uptake ($\dot{V}_{O_2}$) during exercise was reduced by approximately 5% (9). The O_2 cost of cycle exercise is considered to be essentially fixed (with a 10-mL/min increase in $\dot{V}_{O_2}$ required for every 1-W increase in power output) and to be independent of factors such as age, health status, and physical fitness (65). These results imply that dietary nitrate ingestion permits more muscular work to be performed per unit time for the same energy cost, i.e., that the efficiency of skeletal muscle contraction is enhanced. That a short-term, practical dietary intervention can improve the efficiency of muscular work is both surprising and exciting (63).

Improved exercise efficiency has also been reported following acute NO_3^- treatment, having been observed 60 min following $NaNO_3$ ingestion (82) and 2.5 h following beetroot juice ingestion (150). Continuing NO_3^- supplementation for 15 days maintains the improved exercise efficiency observed following acute beetroot juice supplementation (150). This indicates that longer-term NO_3^- supplementation does not elicit greater improvements in exercise efficiency but also does not result in development of tolerance to the intervention. The reduction in $\dot{V}_{O_2}$ following NO_3^- administration is not unique to cycling exercise, having also been observed in healthy participants during walking and running (79), a two-legged knee-extensor exercise (8) (**Figure 2**), kayaking (105), and rowing (116).

Improved exercise efficiency has been consistently reported when the diets of recreationally active humans have been supplemented with NO_3^- (8, 9, 79, 81–83, 150). However, several studies have reported that NO_3^- ingestion does not significantly improve submaximal exercise efficiency in trained subjects (13, 15, 26). There is some evidence that continued endurance training over a number of years can improve cycling efficiency (31) and running economy (62). Therefore, one explanation for this discrepancy could be the greater aerobic fitness of the participants recruited in these studies (121). Moreover, the resting plasma [NO_3^-] and [NO_2^-] are higher in athletes than in nonathletic controls (67, 132). Therefore, the scope for NO_3^- ingestion to enhance muscular efficiency appears to be reduced in trained subjects owing to their already well-developed oxidative metabolic machinery and/or greater NO bioavailability.

An improved efficiency of submaximal exercise is of functional significance in that efficiency is a very important determinant of exercise tolerance and performance. Each individual has a maximum O_2 uptake ($\dot{V}_{O_2\,max}$), which is related to the maximum cardiac output (a product of maximal heart rate and stroke volume) and the capacity of skeletal muscle to extract O_2 from the arterial blood for use in mitochondrial respiration. The absolute O_2 cost for a given work rate (for example, running or cycling at a certain fixed speed) will determine the fraction of the $\dot{V}_{O_2\,max}$ required, which will, in turn, influence exercise tolerance. In athletes or recreational exercise enthusiasts, improved exercise efficiency following NO_3^- ingestion would be expected to enable the same work rate to be sustained for longer or a higher work rate to be sustained for the same duration.

Early studies in this field indicated that NO_3^- supplementation improved both metabolic efficiency and exercise tolerance in healthy humans (8, 9, 79, 150), but not necessarily in trained athletes (13). During constant-work-rate exercise, the improved exercise tolerance with NO_3^- supplementation has been in the range of 16–25% (8, 9, 78). However, the magnitude of improvement in actual exercise performance would be expected to be far smaller; indeed, using the predictions of Hopkins et al. (57), this would be expected to correspond to an improvement in exercise performance (time taken to cover a set distance) of 1–3%. This expectation was tested in the study of Lansley et al. (78), where competitive cyclists completed, on separate days, 4.0- and 16.1-km time trials (TT) following acute beetroot juice ingestion. Consistent with the experimental hypothesis, NO_3^- administration improved 4.0-km and 16.1-km TT performance by 2.8% and 2.7%, respectively (78). The cyclists were able to produce a higher power output for

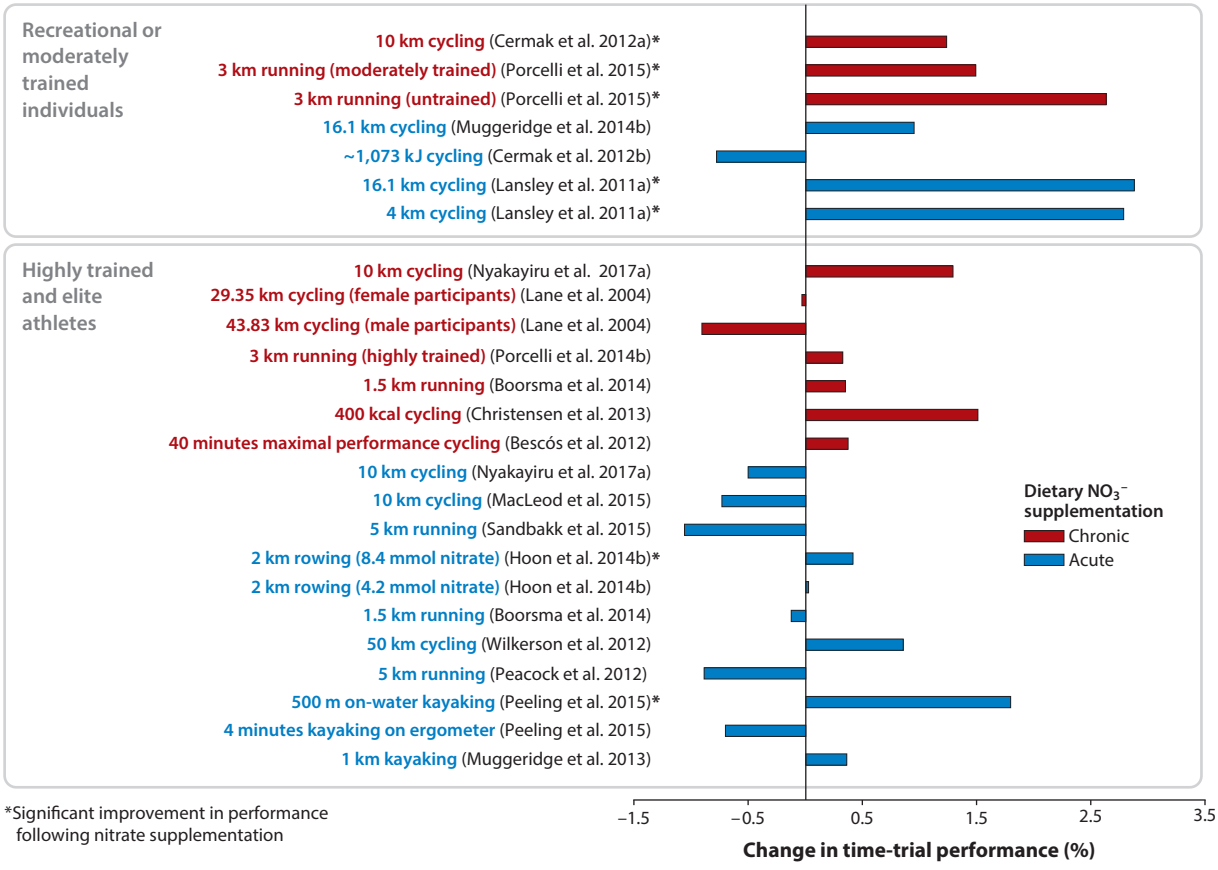

Figure 2

Percentage change in time-trial (TT) performance following acute (*blue bars*) or chronic (*red bars*) dietary NO_3^- supplementation in recreational and moderately trained athletes ($\dot{V}_{O_2\ peak}$ < 60 mL/kg/min; presented in the *top panel*) and in highly trained and elite athletes ($\dot{V}_{O_2\ peak} \geq 60$ mL/kg/min; presented in the *bottom panel*). An asterisk denotes significant improvement in performance following nitrate supplementation. Hoon et al. (56) did not report the $\dot{V}_{O_2\ peak}$ of their participants. The kayakers participating in the Peeling et al. (116) and Muggeridge et al. (105) studies had a reported $\dot{V}_{O_2\ peak}$ below 60 mL/kg/min but are presented in the "highly trained" section of this figure because they were described as highly trained or elite athletes, and/or the $\dot{V}_{O_2\ peak}$ was likely lower due to the exercise modality (i.e., kayaking versus cycling or running).

the same O_2 uptake after NO_3^- supplementation, and this resulted in a lower time to complete the TT distances.

In recent years, a plethora of research studies have investigated the potential ergogenic effects of NO_3^- supplementation (**Figure 2**). While many studies have confirmed the early observations described above, others have not, and it appears that the efficacy of NO_3^- supplementation depends on several factors, including the training status of the participants, the dose and duration of NO_3^- supplementation, and the duration, intensity, and nature of the exercise task (63). Despite the discrepancy in the literature, recent meta-analyses indicate that NO_3^- supplementation both lowers the O_2 cost of exercise (114) and improves endurance exercise performance (98) in healthy volunteers.

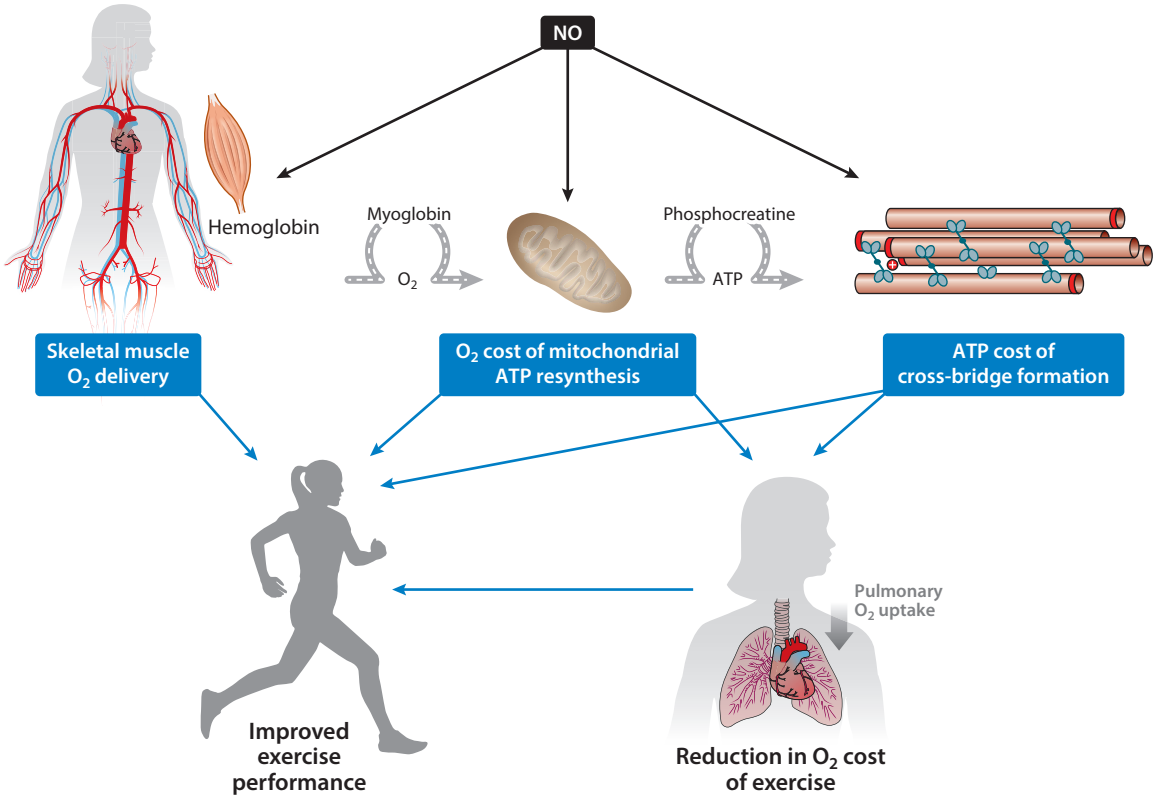

Figure 3

Potential mechanisms for the effects of elevated nitric oxide (NO) bioavailability subsequent to NO_3^- supplementation on exercise efficiency. NO-mediated improvements in both mitochondrial efficiency and contractile efficiency may contribute to changes in O_2 cost of exercise under certain conditions. Improved O_2 delivery through vasodilatory effects of NO does not alter efficiency, but it may contribute to improved performance during maximal exercise via enhanced muscle blood flow, which may be principally directed to type II muscle fibers. Abbreviation: ATP, adenosine triphosphate.

4. MECHANISTIC BASES FOR THE EFFECT OF DIETARY NITRATE ON MUSCLE EFFICIENCY

As illustrated in **Figure 3**, one potential explanation for the reduced O_2 cost of submaximal exercise following NO_3^- ingestion is a reduced ATP cost to generate a given submaximal muscle force. Alternatively, or additionally, NO_3^- may improve exercise efficiency by reducing the O_2 cost of mitochondrial ATP resynthesis (**Figure 3**).

Bailey et al. (8) investigated the mechanistic bases for the physiological effects of dietary NO_3^- supplementation using calibrated ^{31}P-magnetic resonance spectroscopy (^{31}P-MRS). This approach permitted the in vivo assessment of absolute muscle concentration changes in phosphocreatine (PCr), inorganic phosphate (P_i), and adenosine diphosphate (ADP); changes in pH; and rates of ATP turnover through PCr hydrolysis, anaerobic glycolysis, and oxidative phosphorylation during knee-extensor exercise (70, 80). The estimated rates of ATP turnover from PCr hydrolysis and oxidative phosphorylation were lower following beetroot juice supplementation, resulting in a significant reduction in the estimated total ATP turnover rate during both low- and high-intensity exercise (8). In turn, the reduced muscle ATP turnover with NO_3^- retarded

the accumulation of muscle ADP and P_i and the depletion of PCr (8). These results indicate that dietary NO_3^- supplementation improves the coupling between ATP hydrolysis and muscle force production, implicating this as an important determinant of the reduced $\dot{V}_{O_2}$ during exercise. Consistent with this notion, the changes in [ADP], [P_i], and [PCr] following NO_3^- supplementation would be predicted to reduce the stimulus for oxidative phosphorylation based on existing models of respiratory control (25, 92).

Accumulation of the metabolites ADP and P_i and depletion of the finite intramuscular PCr reserves are important contributors to muscle fatigue (3). While the intramuscular [ADP], [P_i], and [PCr] were similar at exhaustion in the NO_3^- and placebo conditions, the time taken to achieve these critical metabolite concentrations was delayed with NO_3^- supplementation, and this may explain the improved exercise tolerance. A subsequent study reported that muscle oxidative capacity ($\dot{Q}_{max}$), estimated using ^{31}P-MRS, was not significantly altered by short-term dietary NO_3^- supplementation (79). This finding was corroborated by Larsen et al. (81), who did not find changes in markers of mitochondrial density or biogenesis following NO_3^- supplementation. These results suggest that mitochondrial biogenesis does not contribute to the improvement in exercise tolerance, at least following short-term NO_3^- supplementation.

Although the results of Bailey et al. (8) showed a reduced ATP cost of muscle force production with NO_3^-, it was not possible to exclude a role for an increased mitochondrial efficiency—i.e., a higher ratio of ATP resynthesized to O_2 consumed (P/O ratio)—in reducing $\dot{V}_{O_2}$ during low-intensity exercise. To determine whether NO_3^- supplementation altered the mitochondrial P/O ratio, Larsen et al. (81) isolated mitochondria from the vastus lateralis muscle of healthy humans supplemented with $NaNO_3$. The resultant mitochondrial suspension was added to a reaction medium containing respiratory substrate to investigate parameters of mitochondrial respiration. During a submaximal rate of ADP infusion, which was selected to mimic the metabolic rate in vivo (76), the mitochondrial P/O ratio was significantly increased (81). The respiratory control ratio, which is the ratio between state 3 (coupled) and state 4 (uncoupled) respiration, was also significantly increased with $NaNO_3$ supplementation, as was the maximal rate of ATP production through oxidative phosphorylation. State 2 respiration, indicative of back leakage of protons through the mitochondrial inner membrane, and state 4 respiration were both reduced with $NaNO_3$ (81). These results elegantly demonstrated that NO_3^- supplementation reduced mitochondrial proton leakage and uncoupled respiration. The increased P/O ratio with NO_3^- supplementation was correlated with the reduction in whole body $\dot{V}_{O_2}$ during exercise (81). Taken together with the findings of Bailey et al. (8), those of Larsen et al. (81) suggest that NO_3^- supplementation improves exercise efficiency by improving the efficiency of both muscle contraction (reduced high-energy phosphate cost of force production) and mitochondrial oxidative phosphorylation (increased P/O ratio). However, it should be pointed out that the influence of NO_3^- supplementation on mitochondrial efficiency is controversial. In a recent investigation, Whitfield et al. (161) did not corroborate the findings of Larsen et al. (81) but did report increased mitochondrial hydrogen peroxide emission in humans following beetroot juice supplementation, which implies that changes in redox status might contribute in some way to the lower O_2 cost of exercise.

5. THE INFLUENCE OF DOSE AND DURATION OF NITRATE SUPPLEMENTATION ON THE PHYSIOLOGICAL EFFECTS OBSERVED

The effects of NO_3^- supplementation are somewhat dependent on the supplementation procedure adopted, particularly the dose of NO_3^- administered and the duration of supplementation. Wylie et al. (167) assessed the dose-response relationship between NO_3^- ingestion and exercise economy

and exercise tolerance. Specifically, the influence of 70, 140, and 280 mL concentrated beetroot juice [containing 4.2 mmol (260 mg), 8.4 mmol (521 mg), and 16.8 mmol (1042 mg) NO_3^-, respectively] on $\dot{V}_{O_2}$ during submaximal cycling and exercise tolerance was assessed 2.5 h post NO_3^- consumption (167). It was reported that 8.4 and 16.8 mmol NO_3^- lowered submaximal exercise $\dot{V}_{O_2}$ by 2% (trend $p = 0.06$) and 3%, respectively. Furthermore, the acute ingestion of 8.4 and 16.8 mmol NO_3^- was similarly effective (+14% and +12%, respectively) at improving exercise tolerance. However, no benefit in terms of exercise economy or exercise tolerance was evident with the acute consumption of 4.2 mmol NO_3^-. Hoon et al. (56) corroborated this dose-response relationship by showing that the acute ingestion of 8.4 mmol NO_3^-, but not 4.2 mmol NO_3^-, improved 2-km rowing performance. Other studies have also reported a significant reduction in submaximal $\dot{V}_{O_2}$ (by ~2–5%) during cycling exercise after the acute ingestion of 5.2 mmol (322 mg) (150) and 6 mmol (373 mg) (169) NO_3^-, as well as a trend for a reduction in $\dot{V}_{O_2}$ after the acute consumption of 5 mmol (310 mg) (149) and 8 mmol (496 mg) (107) NO_3^-. However, an acute dose of 3 mmol (186 mg) did not lower submaximal exercise $\dot{V}_{O_2}$ (169). In terms of exercise tolerance, an acute dose of 5.2 mmol (322 mg) NO_3^- did not improve exercise tolerance (150). Taken together, the findings from these acute NO_3^- consumption studies suggest that a NO_3^- dose of 5.0–8.5 mmol (310–527 mg) is likely to improve exercise economy, but to elicit an improvement in both exercise economy and performance, a NO_3^- dose of >8.5 mmol (>527 mg) might be necessary. It is important to note that, while these studies have administered NO_3^- in the form of a supplement (e.g., concentrated beetroot juice or a NO_3^- salt), the consumption of this amount of NO_3^- can also be achieved through the normal diet, for example, by eating ~200 g of beetroot or spinach (58).

The improvement in exercise economy observed after the acute consumption of NO_3^- has been shown to remain for up to 15 days when subjects consume 5.2 mmol (322 mg) NO_3^- per day (150) and for up to 28 days when they consume 6 mmol (372 mg) NO_3^- per day (169). In both of these studies, the improvement in exercise economy observed after 15 and 28 days of supplementation was neither greater than nor smaller than the effect observed after acute supplementation (150, 169). Furthermore, extending the supplementation (up to 28 days) of a low NO_3^- dose (~3 mmol) that is not beneficial when ingested acutely does not enhance exercise efficiency (169), suggesting that the duration of supplementation has little impact on the dose-response relationship.

Unlike the effects on exercise efficiency, improvements in exercise tolerance may be more dependent on supplementation duration. Exercise tolerance tended be improved after 15 days of supplementation with 5.2 mmol (322 mg) NO_3^- per day, but not after an acute bolus of 5.2 mmol NO_3^- (150). Furthermore, while the effects of acute NO_3^- supplementation on cycling TT performance are equivocal (24, 78) in moderately trained endurance athletes ($\dot{V}_{O_{2\,peak}}$ < 60 mL/kg/min), six days of supplementation has been shown to improve cycling TT performance (23) in this cohort. In all, these reports suggest that extending the supplementation period (at least up to four weeks) does not improve (or reduce) the effects of NO_3^- consumption on exercise economy but that exercise performance may benefit from a chronic, rather than an acute, supplementation strategy. This information should be considered by researchers and/or practitioners wishing to utilize dietary NO_3^- supplementation as a potential ergogenic aid.

6. INFLUENCE OF FITNESS ON THE EFFICACY OF NITRATE SUPPLEMENTATION

Although there is evidence that inorganic NO_3^- supplementation, particularly when administered chronically, can improve performance in moderately trained endurance athletes ($\dot{V}_{O_{2\,peak}}$ < 60 mL/kg/min), the impact of NO_3^- on highly trained or elite endurance athletes

($\dot{V}_{O_2\ peak} \geq 60$ mL/kg/min) is not promising and has been the topic of much debate (e.g., 66) (**Figure 2**). Indeed, no improvement in economy or TT performance during cycling (55, 91, 109, 163) or running (15, 77, 115, 131) exercise was found after the acute consumption of NO_3^- [dose: 6.0–19.5 mmol (372–1209 mg)]. Furthermore, studies have shown that prolonging the supplementation period to 3–8 days [4.8–19.5 mmol (298–1209 mg) NO_3^- per day] does not elicit an improvement in economy or TT performance during running (15, 121) or cycling (13, 20, 26, 109) when subjects are well trained. The reason for the lack of effect is unclear but may be related, in part, to higher eNOS- and nNOS-dependent NO synthesis (50, 97) that renders the potential for additional NO production through the NOS-independent pathway after NO_3^- supplementation redundant. As a consequence, it has been suggested that elite endurance athletes may require a higher dose of NO_3^-, compared to their less-trained counterparts, in order to elicit a positive effect. However, a lack of improvement in economy and TT performance after a very high NO_3^- dose [19.5 mmol (1209 mg)] does not lend support to this hypothesis (15).

In addition to changes in baseline NO bioavailability, highly trained endurance athletes are likely to have greater skeletal muscle capillarization (61), increased content of skeletal muscle Ca^{2+}-handling proteins (72), and/or a lower proportion of type II muscle fibers (144), all of which may reduce the effectiveness of NO_3^- supplementation compared to less-trained individuals. It is important to note that although the aforementioned studies reported no effect of NO_3^- supplementation on performance at the group mean level, some did identify individual participants who responded very positively (in terms of improvements in TT performance) to NO_3^- supplementation (e.g., 163). Further research is required to identify what factors determine an individual's responsiveness to NO_3^- ingestion.

Despite the trivial effects of dietary NO_3^- supplementation on performance in highly trained and elite endurance athletes completing cycling or running exercise, there is evidence that NO_3^- supplementation may be ergogenic in other modes of exercise. For instance, improved exercise economy has been observed in highly trained kayakers after acute consumption of 5 mmol (310 mg) (105) and 4.8 mmol (298 mg) NO_3^- (116). Furthermore, while TT performance was not improved after consumption of ~5 mmol (310 mg) NO_3^- (105, 116), an improvement in kayaking TT performance was reported after the acute ingestion of 9.6 mmol (595 mg) NO_3^- (116). In addition to kayakers, highly trained rowers show an improvement in rowing TT performance after acute ingestion of 8.4 mmol (521 mg) but not 4.2 mmol (260 mg) NO_3^- (56). It is presently unclear why individuals with highly trained upper body musculature may be more sensitive to NO_3^- supplementation. Given that the upper body musculature has been shown to contain a greater proportion of type II muscle fibers (120), it is possible that exercise modalities involving the upper body favor the aforementioned type II fiber specific effects of NO_3^- supplementation (42, 53, 64). In all, these findings suggest that while NO_3^- supplementation appears less effective at improving economy and performance in highly trained and elite athletes during cycling and running exercise, NO_3^- supplementation may provide an ergogenic effect for highly trained kayakers and rowers, although these improvements are dependent on a sufficient dose of NO_3^- being administered.

7. EFFECTS OF NITRATE SUPPLEMENTATION ON REPEATED-SPRINT EXERCISE AND MUSCLE POWER, INCLUDING MECHANISMS OF ACTION

The early applied research surrounding the ergogenic effect of dietary NO_3^- primarily comprised studies assessing the implications for performance during continuous endurance-type exercise (9, 23, 24, 78, 163). Recent advances in our understanding of how dietary NO_3^- may favorably alter the

physiological response of type II (fast-twitch) muscle to exercise has fueled several investigations into the application of NO_3^- supplementation in high-intensity, intermittent, sprint-type, and other exercise modalities that require a significant contribution of type II muscle fibers.

Wylie et al. (166) tested the effect of dietary NO_3^- on high-intensity interval exercise performance in untrained individuals. Following a very high dose of NO_3^- (29 mmol administered over 36 h), a 4.2% improvement in distance covered was observed in recreationally active team sport players during the Yo-Yo intermittent recovery test level 1 (Yo-Yo IR1), a well-established and ecologically valid test widely used to mimic the high-intensity running demands of football (10). The high NO_3^- dose greatly exceeded that adopted in studies of continuous endurance exercise (9, 23, 24, 78, 163). However, a recent study has demonstrated that a dosing regimen similar to that used to enhance continuous exercise performance (~6 mmol NO_3^-), when administered over 5 days, resulted in a similar improvement in Yo-Yo IR1 performance (3.9%) in recreationally active team sport players (146). In the same study, all-out sprint-running performance was improved over 5, 10, and 20 m (146). A period of several days of supplementation with a moderate NO_3^- dose (~8.4–12.8 mmol NO_3^-) has also been reported to be effective at enhancing short-duration, maximal-intensity exercise performance in other exercise settings. For example, 3 days of NO_3^- supplementation extended time to volitional exhaustion during repeated 15-s bouts of supramaximal intermittent knee extensor exercise (6), and 5–7 days of NO_3^- supplementation enhanced repeated 6-s sprint performance during both a short-duration (12 min) (166) and a long-duration (80 min) (148) intermittent sprint cycling protocol. However, 5 days of NO_3^- supplementation did not improve repeated 30-s and 60-s sprint cycling performance (166).

As described above for endurance exercise, the evidence for NO_3^- supplementation to enhance repeated sprint performance in trained populations is equivocal. A low dose of NO_3^- (~5 mmol) administered acutely improved maximal power during 3–4-s inertial-load cycling sprints in trained athletes (128) but had no effect on the capacity of trained athletes to perform repeated 8–10-s sprints in cycling (94) or kayaking (105). Furthermore, an acute, high NO_3^- dose (~10 mmol) did not alter 180-m sprint running performance compared to placebo in junior elite cross-country skiers (131). A low NO_3^- dose (~5.5 mmol/day) taken over six days had no effect on repeated 20-s sprint cycling performance in elite endurance athletes (26). However, six days of NO_3^- supplementation has been reported to improve high-intensity intermittent exercise performance in trained soccer players who consumed ~13 mmol NO_3^- per day (110) and in trained rowers who consumed ~5.5 mmol NO_3^- per day (14).

Interpreting the efficacy of dietary NO_3^- to enhance sprint performance and muscle power is complicated by key differences within the literature relating to supplementation strategy. As highlighted above for endurance exercise, it is likely that the effects of dietary NO_3^- on muscle contractility and sprint performance is influenced by the dose and duration of NO_3^- administration. Acutely, dietary NO_3^- may augment cyclic guanosine monophosphate (cGMP) mediated effects of NO on muscle contraction (93). A single, high-NO_3^- bolus (11.2 mmol) has been shown to improve isokinetic knee extensor torque at very high angular velocities (360° s^{-1}) (28), where the relative contribution of type II muscle to force development and power is expected to be greatest (32) (**Figure 4**). Longer-term NO_3^- supplementation may evoke structural protein-related changes in type II muscle that are sufficient to improve contractile efficiency and support greater performance during sprint-type activity. Indeed, seven days of NO_3^- supplementation increased the expression of the sarcoplasmic reticulum Ca^{2+}-handling proteins, calsequestrin and dihydropyridine, in the type II skeletal muscle of $NaNO_3$-supplemented mice (53). This adaptation to several days of NO_3^- supplementation may underpin the reported increase in contractile force at low stimulation frequencies and faster rates of force development in isolated type II muscle fibers (53) (**Figure 4**). Seven days of NO_3^- supplementation also improved force production at

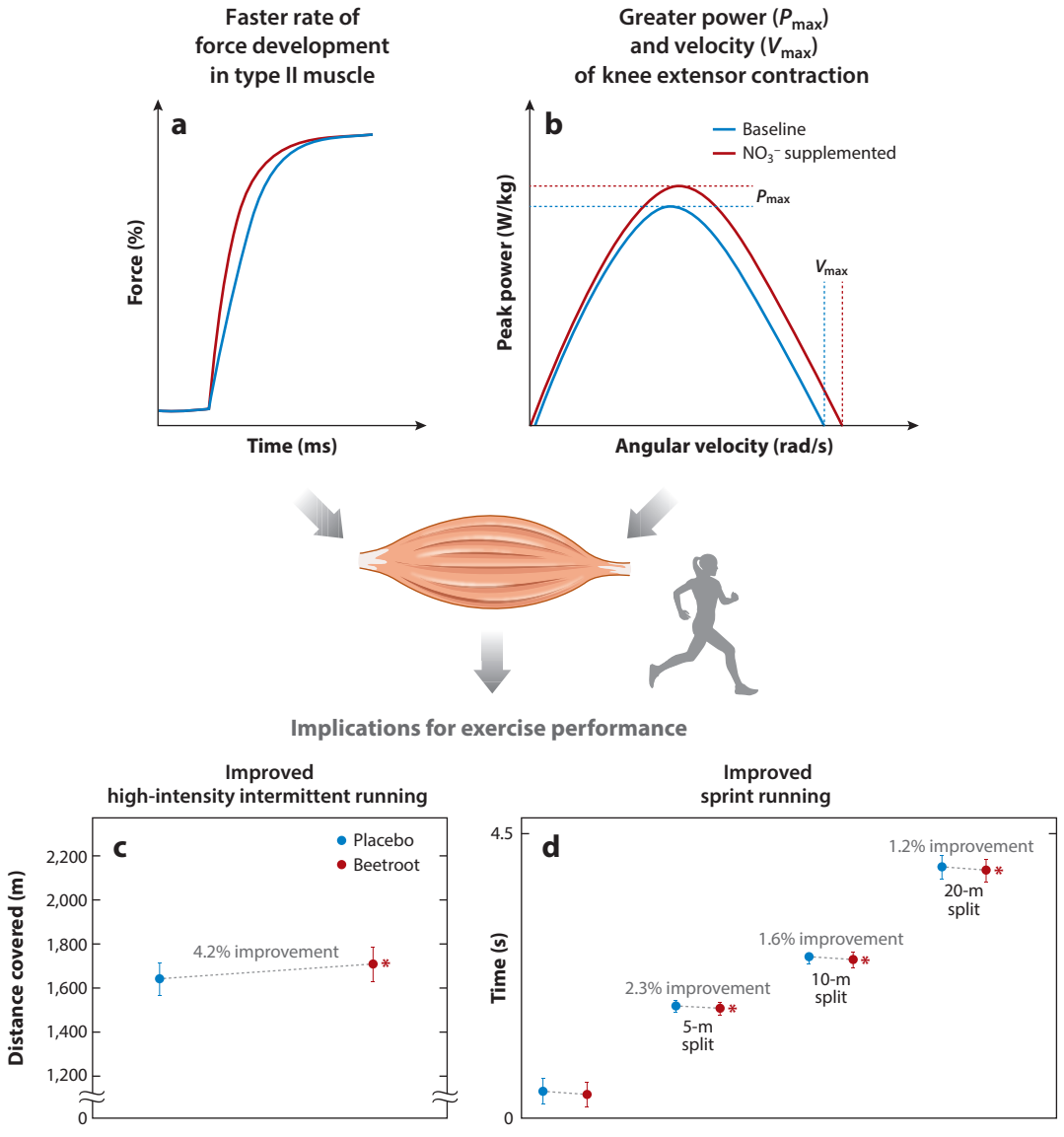

Figure 4

The reported effects of dietary NO_3^- include (*a*) faster development of muscle force (53), (*b*) greater power and velocity of contraction (28), and greater exercise performance during (*c*) high-intensity intermittent running and (*d*) short-distance sprint running. Items in red reflect the response of a NO_3^- supplemented condition. Asterisk denotes $p < 0.05$ versus placebo. Panel *c* redrawn from data presented in Reference 168, and panel *d* redrawn from data presented in Reference 146.

low stimulation frequencies in human muscle (51, 160), but, in contrast to rodent skeletal muscle, improvements in contractile function may be independent of changes in proteins associated with calcium handling (160).

Collectively, there is evidence that several days of dietary NO_3^- supplementation may enhance the performance of healthy, recreationally active individuals during activities that require the

generation of power and speed over short distances and durations (**Figure 4**). This phenomenon is likely explained by favorable changes in muscle contractility. The mechanistic bases for the reported functional changes in human muscle require further investigation, and it is currently unclear whether NO_3^- supplementation can promote sprint-type exercise performance in elite athletes.

8. INTERACTION OF NITRATE SUPPLEMENTATION WITH EXERCISE TRAINING AND DAILY PHYSICAL ACTIVITY

Many of the NO_3^- supplementation strategies reported to alter the physiological response to exercise and/or improve exercise performance in particular exercise settings involve several days of NO_3^- supplementation prior to an event. For competitive athletes, the weeks and months prior to competition typically involve demanding training regimens. Some recent evidence indicates that dietary NO_3^- supplementation during periods of exercise training may impact physiological adaptation and thus positively influence subsequent exercise performance.

Increased exposure to NO_3^- and NO_2^- has been reported to activate PGC1-α (peroxisome proliferator–activated receptor γ coactivator 1-α) (129) and adenosine monophosphate-activated protein kinase (AMPK) (101) in skeletal muscle. These proteins are known to activate signaling cascades that promote adaptive skeletal muscle remodeling to exercise training (27, 47, 87). It is possible that the NO-mediated signaling cascades generated by NO_3^- supplementation are synergistic to the molecular signaling processes stimulated by exercise training. By augmenting transcription pathways integral to skeletal muscle remodeling and by maintaining higher training intensities via the aforementioned effects on muscle contractile efficiency (8, 53, 160), dietary NO_3^- supplementation may modulate some of the physiological and exercise performance adaptations to exercise training.

Puype et al. (123) investigated the interaction of NO_3^- supplementation and exercise training on physiological outcomes. Six weeks of NO_3^- supplementation combined with endurance training in normobaric hypoxia did not enhance 30-min TT performance compared to the placebo condition. Moreover, postintervention muscle AMPK and hypoxia-inducible factor-1α mRNA content was not different between groups. These findings indicate that NO_3^- supplementation may not augment the skeletal muscle adaptive response to endurance training in hypoxia.

Given that dietary NO_3^- may specifically enhance the physiological responses of type II, and not type I, muscle fibers to exercise (42, 43, 53, 64), NO_3^- supplementation may be particularly beneficial to athletes engaging in high-intensity interval and sprint interval training (SIT). Indeed, recent evidence suggests that NO_3^- supplementation in the form of beetroot juice (147), a high-NO_3^- gel (108), and $NaNO_3$ (34) can modulate some of the physiological and performance adaptations to SIT. In particular, dietary NO_3^- supplementation enhanced the transformation toward a more oxidative muscle phenotype (type IIx → IIa) when combined with SIT in hypoxia (34) and normoxia (147). Moreover, compared to placebo, peak work rate during incremental exercise improved to a greater extent when three weeks (108) and four weeks (147) of SIT were combined with NO_3^- supplementation.

Further study is required to address (*a*) whether different NO_3^- dosing strategies combined with endurance training enhance endurance exercise performance, (*b*) whether the combination of SIT and NO_3^- supplementation evokes greater exercise performance benefits during team sport–specific high-intensity intermittent exercise and sprint sports than SIT alone, and (*c*) whether different supplementation vehicles for NO_3^- elicit comparable or disparate physiological and performance adaptations to an exercise training program.

The possible mechanisms by which the combination of engaging in exercise training and consuming a NO_3^--rich diet can promote human health and performance may extend beyond the

effects observed on skeletal muscle physiology and function. Exercise is known to promote several mechanisms that maintain brain structure and function (29, 40), and recent evidence suggests that dietary NO_3^- may also be important in the health of the aging brain (122). Indeed, Petrie et al. (118) found improved functional connections within the motor community in older adults assigned to six weeks of exercise with concurrent beetroot juice supplementation compared to those assigned to exercise with a placebo supplement. This preliminary finding indicates that the interaction of exercise and dietary NO_3^- may have important implications for neuroplasticity in older age.

In summary, when administered concurrently with exercise training in the form of SIT, dietary NO_3^- supplementation may potentiate some physiological and exercise performance adaptations by augmenting the molecular signaling response. The potential for dietary NO_3^- to be administered alongside exercise interventions to promote both physical and cognitive function should be explored further.

9. EFFECTS OF NITRATE SUPPLEMENTATION IN HYPOXIA

As enzymatic production of NO requires the presence of O_2 and essential cofactors, it has been proposed that the NO_3^--NO_2^--NO reduction pathway might compensate for impaired NO production via NOS under conditions of reduced O_2 availability (88). Augmenting the NO_3^--NO_2^--NO pathway by dietary means may therefore have potential as an ergogenic aid at high altitude. It may also have therapeutic applications in conditions where O_2 delivery to muscle is acutely or chronically reduced, such as in pulmonary, cardiovascular, and sleep disorders; during vascular surgery; or as a consequence of aging.

Exercise in hypoxia is associated with reduced muscle oxidative function and impaired exercise tolerance. Dietary NO_3^- has the potential to ameliorate these effects through enhanced O_2 delivery and/or a reduction in the O_2 cost of exercise. An early study showed that NO_3^- supplementation attenuated the muscle metabolic perturbation during high-intensity knee-extension exercise in hypoxia (14.5% O_2) and restored exercise tolerance to that observed in normoxia (152). The maximal rate of mitochondrial ATP resynthesis ($\dot{Q}_{max}$), inferred from the muscle phosphocreatine (PCr) recovery kinetics, was also restored to normoxic levels by NO_3^- supplementation (152). Since several studies have found that NO_3^-supplementation does not influence postexercise PCr recovery kinetics in normoxia (45, 79, 153), it is likely that the speeding of PCr recovery in hypoxia is primarily due to improved O_2 delivery rather than a reduced O_2 cost of ATP resynthesis. This interpretation is consistent with the finding that compared to placebo, NO_3^- supplementation increased the recovery kinetics of the T2* (effective transverse relaxation time) of the magnetic resonance signal, which reflects perfusion-related changes in oxygenation of the vascular compartment, following high-intensity knee-extension exercise in hypoxia (13% O_2) (153). NO_3^- may therefore restore the O_2 gradient between the microvasculature and the myocyte in hypoxia, thus enabling a faster rate of mitochondrial ATP resynthesis, which is reflected in a faster PCr resynthesis rate (152, 153). It should be noted that evidence for enhanced O_2 delivery does not exclude the possibility that NO_3^--induced alterations in metabolic control (153) and mitochondrial efficiency (81) also contribute to increased $\dot{Q}_{max}$ in hypoxia.

Assessment of muscle oxygenation using near infrared spectroscopy (NIRS) has provided further evidence that enhanced O_2 delivery is important in the ergogenic effects of NO_3^- supplementation in hypoxic exercise. Masschelein et al. (95) reported a 4% increase in total oxygenation index (TOI) of *m. vastus lateralis* during incremental cycle exercise in hypoxia (11% O_2) after six days of NO_3^- supplementation, and Shannon et al. (134) showed a similar increase in *m. gastrocnemius* oxygenation during a 3-km treadmill TT under 11.7% and 14% O_2 after acute NO_3^- ingestion.

In another study, three days of NO_3^- supplementation increased the TOI of *m. vastus lateralis* during cycle exercise by 3.6% compared to placebo, although this effect did not reach statistical significance (69). The arterial-venous [NO_2^-] difference has been associated with limb vasodilation and increased muscle blood flow during hypoxic exercise (37). This observation indicates that NO_2^- is utilized by the active muscle, potentially facilitating distribution of O_2 to areas of high metabolic activity (145). T2* MRI assessment of the inactive thigh muscles during single-leg knee-extension exercise (153) and NIRS assessment of *m. biceps brachii* during lower-limb cycle exercise (59) suggest that elevated circulating plasma [NO_2^-] following NO_3^- supplementation does not result in indiscriminate vasodilation in inactive muscle, but rather facilitates distribution of limited O_2 delivery to loci with the greatest O_2 demand.

The ergogenic effects of dietary NO_3^- supplementation in hypoxia have been assessed using tests of exercise capacity, reflected by time to exhaustion during constant-work-rate or incremental exercise (46, 59, 69, 95, 130, 152) and exercise performance, i.e., TTs (5, 16, 91, 106, 111, 134, 135) (**Table 1**). These studies suggest that the exercise capacity of physically active individuals is improved during hypoxic exercise (11–14.5% O_2) when NO_3^- supplementation is continued for 24 h–6 days (59, 69, 95, 152; cf. 130), but not following acute NO_3^- ingestion (46). Some studies indicate that acute NO_3^- supplementation (~6–15 mmol) can improve exercise performance in a TT (106, 134, 135), but others have found no significant ergogenic effects (5, 91, 111). Only one study has investigated the effects of chronic (three-day) NO_3^- supplementation and found that 15-km cycle TT performance was not improved in trained cyclists exercising in 11% O_2 (16). The high training status of study participants may have attenuated the ergogenic effects of NO_3^- during hypoxic TTs in some (5, 91, 111) but not all (106, 135) of these studies. It should also be considered that the acute NO_3^- supplementation employed in all but one (16) of the hypoxic TT studies has likely contributed to variable findings. It is recommended that future research assessing effects of NO_3^- on exercise performance in hypoxia should apply the recommendations on dose and duration of supplementation as outlined in Section 5.

10. EFFECTS OF NITRATE SUPPLEMENTATION IN OLDER AGE AND DISEASE STATES

A hallmark of aging and of cardiovascular and metabolic diseases is a reduced ability to produce NO through the canonical L-arginine-NOS-NO pathway (84). This manifests as a reduction in circulating [NO_3^-] and [NO_2^-] (73), which, as described in Section 2, can be recycled back into bioactive NO through the NO_3^-- NO_2^--NO pathway. Therefore, the reduction in NOS activity and the lower endogenous NO_3^- and NO_2^- stores limit the ability of these populations to produce NO. A characteristic feature of these populations is exercise intolerance that often occurs in concert with a reduced NO production during exercise (2, 84), suggesting a role for NO dysfunction in exercise intolerance. Dietary supplementation with NO_3^- may therefore represent a practical and cost-effective means to restore NO homeostasis and improve exercise tolerance in these populations (113). While the efficacy of dietary NO_3^- to reduce systemic blood pressure in older age has been extensively studied (reviewed in 140; see also 139, 142), relatively few studies have assessed the effects of NO_3^- supplementation on exercise capacity in healthy older individuals. NO_3^- supplementation with 9.6 mmol/day for three days resulted in a reduction in systemic blood pressure at rest and a speeding of the $\dot{V}_{O_2}$ response (mean response time) across the transition from standing rest to treadmill walking in 60–70-year-old men and women (68). However, NO_3^- ingestion did not affect functional capacity assessed with a 6-min walk test, nor the muscle metabolic response to low-intensity knee-extension exercise measured by ^{31}P-MRS (68). In another study, NO_3^- supplementation with 12 mmol/day for seven days did not influence

Table 1 Evidence for ergogenic effects of dietary NO_3^- supplementation in hypoxia is diverse[a]

Acute or chronic?	Study	Subjects	Nitrate intervention	Hypoxia intervention	Criterion exercise test	Ergogenic?[b]
Acute	Shannon et al. (135)	Trained and untrained	15.2 mmol BR	~15.3% O_2 (2,500 m)	1,500-m treadmill TT	✓
	Gasier et al. (46)	Healthy	15 mmol $NaNO_3$	~12.3% O_2 (4,300 m) hypobaric	T_{lim} for handgrip exercise	×
	Nybäck et al. (111)	Trained skiers	13 mmol BR	~16.8% O_2 (1,800 m)	1000-m roller-skiing TT	×
	Shannon et al. (134)	Healthy	12.5 mmol BR	~14.0% O_2 (3,000 m), ~11.7% O_2 (4,300 m)	3-km treadmill TT with 10-kg load	✓
	Arnold et al. (5)	Trained runners	7 mmol BR	~15.3% O_2 (2,500 m), ~12.7% O_2 (4,000 m)	10-km treadmill TT (2,500m) T_{lim} incremental test (4,000 m)	×
	MacLeod et al. (91)	Trained cyclists	6 mmol BR	~15.3% O_2 (2,500 m)	10-km cycle TT	×
	Muggeridge et al. (106)	Trained cyclists	~5.0 mmol BR	~15.3% O_2 (2,500 m)	16.1-km cycle TT	✓
Chronic	Vanhatalo et al. (152)	Moderately trained	1 day, 9.3 mmol/24 h	~14.5% O_2 (3,000 m)	T_{lim} for single-leg knee extension	✓
	Bourdillon et al. (16)	Trained cyclists	3 days, ~7.4 mmol/day $NaNO_3$	~11.0%O_2 (5,200 m)	15-km cycle TT	×
	Kelly et al. (69)	Physically active	3 days, 8.4 mmol/day BR	~13.1% O_2 (3,700 m)	T_{lim} at 75%Δ cycle	✓
	Masschelein et al. (95)	Healthy	6 days ~5.1 mmol/day BR	~11.0% O_2 (5,200 m)	T_{lim} incremental test cycle	✓
	Rossetti et al. (130)	Recreationally active	6 days, 6.4 mmol/day BR	~14.1% O_2 (3,100m)	T_{lim} at 80% hypoxic $\dot{V}_{O_2\ max}$ reserve with 15-kg load, treadmill	×

[a]The efficacy of supplementation may be modulated by factors such as the choice of acute or chronic supplementation regime and training status.
[b]A checkmark indicates a statistically significant positive effect on the criterion exercise test, and a cross indicates no significant effect.
Abbreviations: BR, NO_3^--rich beetroot juice; $NaNO_3$, sodium nitrate; TT, time trial; T_{lim}, time to exhaustion during a constant-work-rate task; $\dot{V}_{O_2\ max}$, maximum O_2 uptake; 75%Δ, work rate occurring at 75% of the interval between the gas-exchange threshold and $\dot{V}_{O_2\ max}$.

submaximal and maximal $\dot{V}_{O_2}$ during incremental exercise, and measures of functional capacity, including hand-grip strength; timed, repeated chair rising; and 10-m walking speed, were also not altered in 60–75-year-old men and women (141). Further studies are required to ascertain whether the positive effects of chronic NO_3^- supplementation on indices of cardiovascular health and exercise capacity reported in healthy young individuals may be reproduced in healthy older adults.

Individual responsiveness to NO_3^- supplementation may be influenced by the ability to metabolize ingested NO_3^-, including NO_3^- reduction by the commensal bacteria in the oral cavity

(36), as well as the bioactivity of NO and/or other nitrogen-containing derivatives. Several studies have shown that the vasculature of healthy older individuals is sensitive to the vasodilatory effect of dietary NO_3^- at rest, as indicated by reduced systemic blood pressure (60, 68, 125, 140). NO_3^- ingestion has also been shown to increase the compensatory vasodilator response during hypoxic, but not normoxic, exercise in older adults (21). NO_3^- may therefore have potential for improving vascular control and hyperemic response during exercise in older populations that are characterized by impaired skeletal muscle blood flow and/or reduced NO bioavailability. Further research is needed to establish whether alterations in muscle energetic and/or contractile properties observed in young adults following NO_3^- supplementation (28, 51, 81, 160) are replicated in healthy older adults.

Evidence relating to the effects of NO_3^- supplementation on exercise tolerance in older patient populations, which are affected by reduced O_2 delivery to skeletal muscle, is equivocal (for review, see 112, 164). Dietary NO_3^- has been shown to improve exercise tolerance in older (~67 years) peripheral arterial disease patients by delaying the onset of claudication pain (71), in heart failure patients (~65–69-year-olds) with preserved ejection fraction (39, 171), and in chronic obstructive pulmonary disease (COPD) patients (12). However, others have found no change in exercise capacity after NO_3^- supplementation in ~63–70-year-old patients with heart failure with reduced ejection fraction (54), COPD (137), or type II diabetes mellitus (136). Differences in supplementation doses and durations, as well as the sensitivity of criterion exercise tests, likely contribute to discrepancies between studies regarding the ergogenic effects of dietary NO_3^- in these populations. Chronic medication and/or multiple systemic and metabolic maladaptations associated with conditions such as type II diabetes, COPD, and obesity may limit NO production, NO bioavailability, and NO's ultimate bioactivity in target tissues. Further research is needed to optimize supplementation regimens and identify which disease populations may benefit from the ergogenic potential of dietary NO_3^-.

11. EFFECTS OF NITRATE SUPPLEMENTATION ON COGNITIVE FUNCTION AT REST AND DURING EXERCISE

Cognitive performance is considered to be a key determinant of sporting success (99), and athletes competing in both individual and team sports must make quick and accurate decisions while simultaneously performing exercise at varying intensities. Therefore, there is considerable interest in interventions that may improve decision-making accuracy and/or reaction time in sports settings.

Some studies have indicated that NO donors and NO_3^- supplementation may enhance cerebral perfusion (52, 122), particularly to areas responsible for executive functioning (122), and enhance coupling of cerebral blood flow to neuronal activity (1, 119). A positive effect of dietary NO_3^- on cognitive function (decision-making reaction time) was first reported in patients with type II diabetes (48). This effect has since been replicated in healthy younger individuals at rest (146) and during exercise (148) following short-term (5–7 days) NO_3^- supplementation. Wightman et al. (162) extended these findings by demonstrating that improvements in cognitive task performance following acute NO_3^- supplementation coincided with better matching of the cerebral hemodynamic response to the cognitive challenge. In contrast, Kelly et al. (68) found no effect of three days of NO_3^- supplementation on the ability of healthy older adults to complete cognitive tasks at rest, and found no changes in cerebral perfusion and metabolism, measured via ^{1}H-MRS and ^{31}P-MRS, respectively. Similarly, no changes in resting cognitive and cerebrovascular function were reported following acute NO_3^- supplementation at moderate (134) and high (85, 134) simulated altitude.

During intense exercise, as cerebral oxygenation diminishes (133), higher-order functions of the prefrontal cortex may be disengaged to avoid compromising optimal motor function (35), and

cognitive abilities are compromised (44, 126). To date, relatively few studies have investigated the influence of NO_3^- supplementation on cognitive performance during exercise. Acute dietary NO_3^- intake did not alter cognitive task performance during continuous cycling at 50% or 70% $\dot{V}_{O_2\,peak}$ (149) or during loaded treadmill walking at 30% $\dot{V}_{O_2\,peak}$ at simulated altitude (134). During high-intensity intermittent exercise, cognitive performance was unaltered following five days of NO_3^- supplementation (146). However, larger NO_3^- doses (~13 mmol) taken over seven days improved decision-making reaction time without decrements in response accuracy during a 90-min intermittent sprint-cycling protocol designed to mimic the metabolic demands of team sport (148). Importantly, the greatest differences between the NO_3^- and placebo conditions were observed in the final third of each 40-min "half" (148), when fatigue-related alterations in cerebral physiology/cognitive abilities may be greatest (44).

Taken together, these findings suggest that, at rest and perhaps during exercise, acute and short-term NO_3^- supplementation may improve cognitive task performance in healthy young adults in normoxia. Further studies should seek to measure the relationship between NO_3^- dosing strategy, cerebral perfusion, and cognitive task performance during exercise following NO_3^- supplementation.

12. CONCLUSIONS

Dietary NO_3^- supplementation has been one of the main innovations in sports nutrition over the last decade. While the mechanisms responsible for the physiological effects observed remain unclear, the effects on muscle function and performance appear to be robust, at least in non–elite athlete subjects and when an appropriate supplementation regimen is followed. One important difference between NO_3^- and other putative ergogenic aids is that the effects of NO_3^- on muscle contractility and muscle efficiency are relevant across a wide spectrum of human activity—from very short-duration contractions requiring muscle strength and power to long-duration activities requiring muscular endurance. As was highlighted in this review, NO plays many important roles that are relevant to human physical activity, including regulation of neuromuscular activity and muscle excitation–contraction coupling (via Ca^{2+} activity), mitochondrial respiration, and blood flow and its intramuscular and/or cerebral distribution. It is therefore essential that NO production is maintained at the appropriate rate to support activity, and there are certain circumstances in which it might limit muscular performance (for example, during intense exercise, in hypoxia, and in older age and specific disease conditions). The existence of the NO_3^--NO_2^--NO pathway and the capacity to increase the body reservoirs for NOS-independent NO production via increasing dietary NO_3^- intake offer the opportunity to develop ergogenic and/or therapeutic dietary interventions.

DISCLOSURE STATEMENT

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