




REVIEW ARTICLE

“Beet-ing” the Mountain: A Review of the Physiological and Performance Effects of Dietary Nitrate Supplementation at Simulated and Terrestrial Altitude

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Abstract Exposure to altitude results in multiple physiological consequences. These include, but are not limited to, a reduced maximal oxygen consumption, drop in arterial oxygen saturation, and increase in muscle metabolic perturbations at a fixed sub-maximal work rate. Exercise capacity during fixed work rate or incremental exercise and time-trial performance are also impaired at altitude relative to sea level. Recently, dietary nitrate (NO_3^-) supplementation has attracted considerable interest as a nutritional aid during altitude exposure. In this review, we summarise and critically evaluate the physiological and performance effects of dietary NO_3^- supplementation during exposure to simulated and terrestrial altitude. Previous investigations at simulated altitude indicate that NO_3^- supplementation may reduce the oxygen cost of exercise, elevate arterial and tissue oxygen saturation, improve muscle metabolic function, and enhance exercise capacity/performance. Conversely, current evidence suggests that NO_3^- supplementation does not augment the training response at simulated altitude. Few studies have evaluated the effects of NO_3^- at terrestrial altitude. Current evidence indicates potential improvements in endothelial function at terrestrial altitude following NO_3^- supplementation. No effects of

NO_3^- supplementation have been observed on oxygen consumption or arterial oxygen saturation at terrestrial altitude, although further research is warranted. Limitations of the present body of literature are discussed, and directions for future research are provided.

Key Points

The findings of this review demonstrate that dietary nitrate (NO_3^-) supplementation may reduce the oxygen cost of exercise, elevate arterial and tissue oxygenation, improve muscle metabolic function, and enhance exercise capacity/performance at simulated altitude.

Current preliminary evidence from training studies conducted at simulated altitude suggests that NO_3^- supplementation has no effect on performance-related adaptations, although further investigations are warranted.

Additional studies are required to confirm whether the beneficial effects of NO_3^- supplementation that have been demonstrated at simulated altitude also manifest at terrestrial altitude.

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1 Introduction

Every year, millions of individuals ascend to altitude for recreational, occupational, and sporting purposes [1]. Reasons for ascending to altitude include, amongst others, tourism, hiking and mountaineering, skiing, military

deployment, sports training camps, and athletic competition. With increasing altitude, there is a decrease in barometric pressure and a concomitant decline in the partial pressure of oxygen (PO_2), such that the high-altitude environment is hypoxic relative to sea level (normoxia). Exposure to this hypoxic environment decreases the amount of oxygen (O_2) reaching the lungs, blood, and ultimately tissue [2, 3]. Consequently, maximal O_2 consumption ($\dot{V}\text{O}_{2\text{max}}$) decreases [4, 5]. The absolute $\dot{V}\text{O}_2$ necessary to maintain a given sub-maximal work rate is typically the same at sea level and altitude up to ~ 4300 m, although it may be slightly reduced at higher altitudes (e.g., 5260 m) consequent to alterations in substrate oxidation [6]. Nevertheless, the relative percentage of $\dot{V}\text{O}_{2\text{max}}$ utilized is higher at altitude relative to sea level [7]. As such, muscle metabolic perturbations are increased [8–11]. Furthermore, exercise time to exhaustion (TTE) and time-trial (TT) performance are impaired at altitude compared with sea level [7]. Prolonged altitude exposure results in acclimatization, comprising multiple renal, cardio-pulmonary, and hematological adaptations that act to increase the delivery of O_2 to the tissue [12]. However, these adaptive changes can take days or weeks to fully manifest, and sea-level exercise performance/capacity is never fully attained, even with prolonged acclimatization [7].

Nitric oxide (NO) is a pleiotropic signaling molecule and a regulator of multiple physiological processes, many of which are altered by hypoxia, including mitochondrial function [13, 14], cerebral and muscle tissue blood flow [15–17], muscle metabolism [10, 11], and endothelial function [18, 19]. NO plays a fundamental role in the response to hypoxia and has been implicated in hypoxia-induced vasodilation, a mechanism designed to ensure appropriate matching between O_2 delivery and the metabolic demands of muscle and cerebral tissue when O_2 availability is low [15, 20]. Elevated levels of NO [21], alongside hematological (e.g., erythropoiesis and increased hemoglobin mass) and ventilatory (e.g., increased hypoxic ventilatory response) changes [22], have been associated with successful adaptation and acclimatization to altitude in lowlanders and altitude residents. In particular, Tibetan highlanders who have adapted to the genetic selection pressures of living at high altitude, manifest greatly increased plasma and pulmonary NO bioavailability [18, 23, 24]. Such adaptations afford distinctive metabolic [25] and microcirculatory [18] benefits that translate into an extraordinary capacity to cope with the challenges of hypoxia relative to unacclimatized lowlanders. Conversely, prior to acclimatization, lowlanders exhibit suppressed NO bioavailability with altitude exposure, and both failure to acclimatize and high-altitude illness have been associated with insufficient NO generation in hypoxia [26–28].

Given the importance of NO during hypoxic exposure, it has been widely speculated that increasing NO bioavailability via dietary supplementation might confer beneficial effects in individuals ascending to altitude [29–32]. Endogenous NO production occurs via two distinct and uniquely different pathways that may be targeted to increase NO generation. First, NO can be produced via oxidation of the semi-essential amino acid L-arginine. This reaction is catalyzed by the NO synthase (NOS) enzymes and requires O_2 as a co-substrate. In vitro evidence suggests that NO generated via the L-arginine NOS pathway is suppressed in hypoxia [33], although in vivo evidence is less clear [34–36]. Alternatively, NO can be generated via the reduction of nitrate (NO_3^-) and nitrite (NO_2^-) through the recently elucidated NO_3^- – NO_2^- –NO pathway [37]. Consumption of NO_3^- -rich foods such as leafy green vegetables and beetroot or NO_3^- -salts (e.g., sodium [Na]- or potassium [K^+]- NO_3^-) rapidly and substantially elevates plasma [NO_3^-] (square brackets denote concentration) [38]. Exogenous NO_3^- mixes with endogenously derived NO_3^- , which is an oxidation product of the L-arginine NOS pathway, in the blood and can be slowly reduced to NO_2^- by the enzyme xanthine oxidoreductase [39]. A more rapid pathway for NO_3^- reduction also exists, which is facilitated through recirculation of NO_3^- into the mouth via the salivary glands [40]. Here, oral bacteria reduce NO_3^- to NO_2^- via reductase enzymes [41]. A portion of the bacterially generated NO_2^- is transformed into NO in the stomach [42, 43]. However, the majority enters systemic circulation and may be further reduced to NO via enzymatic and non-enzymatic catalysts [37, 44] including, amongst others, xanthine oxidoreductase [45, 46], deoxygenated hemoglobin [47] and myoglobin [48], and cytochrome c oxidase (COX) [49]. Importantly, these pathways for NO generation via NO_2^- reduction are all considerably enhanced in hypoxic conditions [37]. The NO_3^- – NO_2^- –NO pathway is therefore of particular importance in hypoxia and is viewed as an important “back-up” system for maintaining and/or enhancing NO bioavailability and signaling in this environment (Fig. 1). It should be noted that recent evidence suggests a possible direct role of NO_2^- on skeletal muscle and mitochondrial function and in hypoxia-induced vasodilation [50]. Thus, any effects of NO_3^- consumption might, at least in part, be related to NO_2^- -dependent signaling, rather than being solely attributable to NO [50].

Interestingly, recent evidence from studies conducted at sea level suggests that NO_3^- supplementation can reduce resting blood pressure (BP) [51–53], lower the O_2 cost of exercise [54–56], and augment both exercise capacity and performance [55, 57–59]. Logically, greater effects of NO_3^- supplementation might be expected in hypoxia, given the particularly important role that NO plays in this

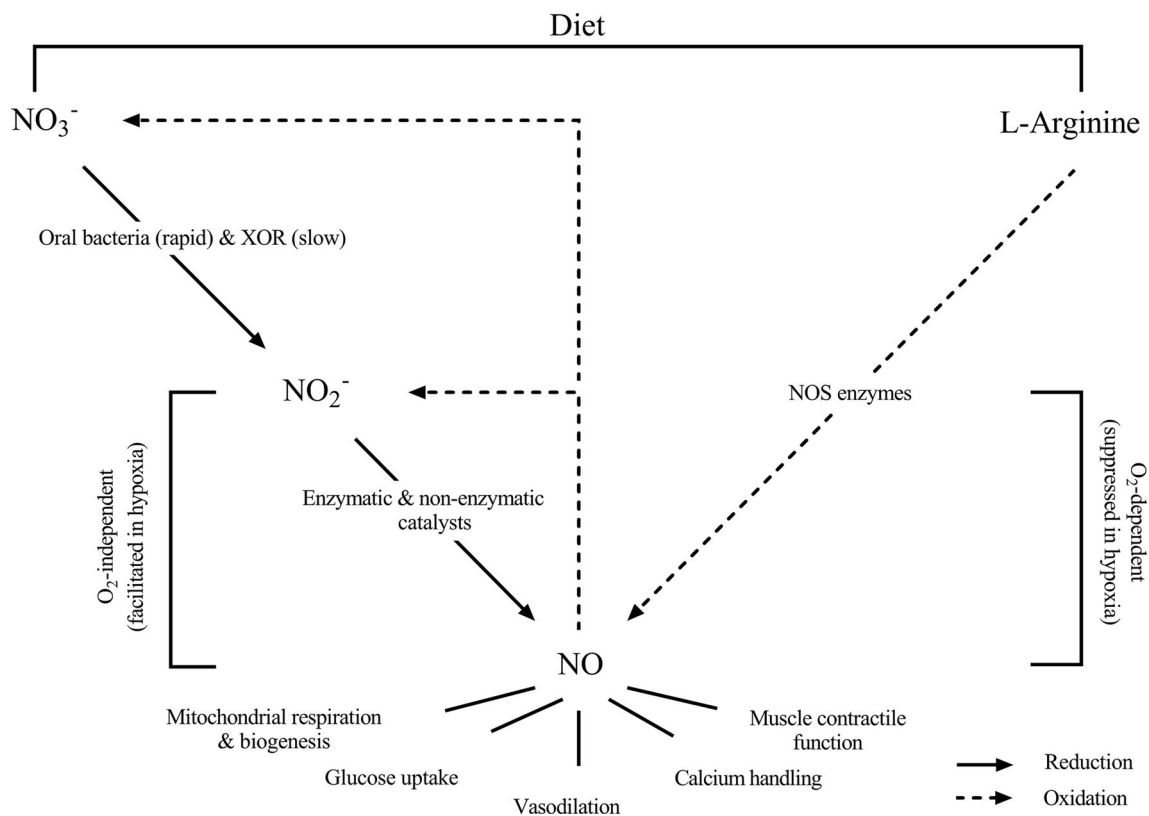


Fig. 1 A schematic representation of the metabolic pathways for nitric oxide (NO) generation in the human body (adapted from Jones [32], with permission). The ‘traditional’ pathway for NO generation involves oxidation of the semi-essential amino acid L-arginine, in a reaction which requires the availability of O₂ and is catalysed via the NO synthase (NOS) enzymes. This O₂-dependent reaction is suppressed in hypoxia. Alternatively, NO can be generated via the nitrate (NO₃⁻)–nitrite (NO₂⁻)–NO pathway. Here, NO₃⁻ from

dietary sources or produced as an oxidation product of the L-arginine pathway is reduced into NO₂⁻ via oral bacteria (a rapid pathway) or via the enzyme xanthine oxidoreductase (XOR) (a slower pathway). NO₂⁻ can subsequently be reduced into NO via multiple catalysts, particularly under conditions of low O₂ availability. This second pathway may serve to maintain or enhance NO signalling under conditions of hypoxia, such as that experienced at an altitude

environment and that NO production via the L-arginine NOS pathway is suppressed yet NO₂⁻ reduction is enhanced in hypoxia. Furthermore, a reduced O₂ cost of exercise is likely to be especially meaningful for individuals exercising in a low-O₂ environment.

Although several recent articles have been published reviewing the potential benefits of dietary NO₃⁻ supplementation at sea level [31, 32, 59–61], there is presently no review of the literature focusing on its specific applications for individuals exercising at altitude, despite a considerable and broad body of evidence emerging in the area. The purpose of the present review is to address this need by summarizing and critically evaluating the findings of recent research exploring the effects of NO₃⁻ supplementation on cardiopulmonary and metabolic responses to exercise and exercise performance at altitude. The potential effects of NO₃⁻ supplementation on adaptations to altitude training are also explored. Throughout the review, key limitations of the present body of evidence will be discussed and directions for future research suggested.

2 Study Selection and Characteristics

Studies were identified for this review by searching online journals and the databases PubMed and SPORTDiscus. This review includes studies that assessed the effects of NO₃⁻ supplementation during both acute (~1–5 h) and chronic (1–28 days) altitude exposure. We included studies conducted at terrestrial altitude, simulated altitude via normobaric hypoxia (i.e., the altitude PO₂ is replicated by lowering the fraction of inspired O₂ [F_IO₂] whilst maintaining sea-level barometric pressure), and hypobaric hypoxia (i.e., the altitude PO₂ is replicated by lowering the barometric pressure whilst the F_IO₂ is unchanged from sea-level values). Terrestrial altitudes ranged from 1300 to 5300 m. Reported simulated altitudes were ~2500–5000 m (F_IO₂ 11–15.4%) in normobaric hypoxia and 3500 m in a hypobaric chamber. The dose of NO₃⁻ ranged from ~5 to 15.2 mmol·day⁻¹. Supplementation duration ranged from an acute dose 1.5–3 h pre-trial, to chronic loading for up to 11 days.

3 Physiological Effects of Nitrate (NO_3^-) Supplementation in Hypoxia

3.1 Oxygen (O_2) Consumption During Steady-State Constant Work-Rate Exercise

Larsen et al. [54] first reported a reduction in steady-state $\dot{V}\text{O}_2$ consequent to NO_3^- supplementation with $\dot{V}\text{O}_2$ \sim 5% lower during cycle ergometry exercise in normoxia. This effect was unexpected, as the $\dot{V}\text{O}_2$ requirement of a given workload is typically regarded as unchangeable, but has since been confirmed in multiple subsequent investigations in normoxia [38, 55–57, 62, 63].

Masschelein et al. [29] confirmed the O_2 -sparing effect of NO_3^- supplementation during exercise in normobaric hypoxia. $\dot{V}\text{O}_2$ was \sim 4% lower during cycle ergometry exercise at 45% peak O_2 uptake ($\dot{V}\text{O}_{2\text{peak}}$) in extreme normobaric hypoxia (F_1O_2 11%, \sim 5000 m). Subsequent studies have reported $\dot{V}\text{O}_2$ reductions of 4–10% (similar magnitudes to those reported in normoxia) during cycle ergometry [29, 30, 36] and treadmill running exercise [64] across a range of simulated altitudes (F_1O_2 11–15.4%, \sim 2500–5000 m). Interestingly, one study reported lower steady-state $\dot{V}\text{O}_2$ in normobaric hypoxia (F_1O_2 13.1%, \sim 3500 m) but not normoxia [36] following NO_3^- supplementation. This suggests that NO_3^- supplementation might be especially effective at reducing the O_2 cost of exercise in hypoxia, possibly due to greater conversion of NO_2^- into NO and/or more effective modulation of O_2 -consuming cellular processes.

To the authors' knowledge, only one study has reported the effects of NO_3^- supplementation on $\dot{V}\text{O}_2$ during acute exercise in hypobaric hypoxia [65]. In contrast to some evidence obtained in normobaric hypoxia, NO_3^- supplementation did not influence $\dot{V}\text{O}_2$ during cycle ergometry exercise in a hypobaric hypoxic chamber simulating 3500 m altitude. These inconsistent findings are puzzling but might be related to the high training status of the participants ($\dot{V}\text{O}_{2\text{max}}$ at 1600 m altitude: \sim 61 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; see Sect. 4 for details of how training status may modulate the effects of NO_3^- supplementation) rather than the exercise environment. Supporting this notion, three recent studies have reported no effect of NO_3^- supplementation on $\dot{V}\text{O}_2$ during sub-maximal exercise in normobaric hypoxia (F_1O_2 11–15.4%, \sim 2500–5000 m) in highly-trained individuals ($\dot{V}\text{O}_{2\text{max}}$: $>$ 60 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) [66–68]. Moreover, a recent investigation at sea level reported a significant inverse relationship between $\dot{V}\text{O}_{2\text{peak}}$ and the reduction in $\dot{V}\text{O}_2$ consequent to NO_3^- supplementation [58]. However, direct comparison of the effects of NO_3^- ingestion on $\dot{V}\text{O}_2$ in normobaric and hypobaric hypoxia is

warranted to exclude the influence of other potentially confounding methodological variables.

The reduction in $\dot{V}\text{O}_2$ subsequent to NO_3^- supplementation is unlikely to be related to a shift towards anaerobic adenosine triphosphate (ATP) generation, as blood [lactate] [54], muscle pH [69], and glycolytic ATP turnover [62] are typically unchanged following NO_3^- ingestion. Instead, it has been suggested that the lower $\dot{V}\text{O}_2$ reported after NO_3^- supplementation might be related to improvements in the efficiency of muscle force generation [62] and/or mitochondrial respiration [70].

In the study by Bailey et al. [62], NO_3^- supplementation attenuated intramuscular phosphocreatine (PCr), adenosine diphosphate (ADP), and inorganic phosphate (P_i) perturbations and lowered $\dot{V}\text{O}_2$ during leg-extension exercise in normoxia, suggesting a lower ATP cost of muscle force generation [62]. The ATP cost of muscle force production is primarily accounted for by cross-bridge cycling and calcium (Ca^{2+}) handling [71, 72]. Given NO has been demonstrated to slow cross-bridge cycling kinetics [73, 74] and inhibit Ca^{2+} -ATPase activity [75, 76], the lower ATP requirement of muscle force generation following NO_3^- supplementation may be related to a NO-mediated reduction in the ATP cost of one or both of these processes [62]. Recently, Hernandez et al. [77] reported increased muscle force generation and elevated expression of the Ca^{2+} handling protein calsequestrin 1 and the dihydropyridine receptor in murine type II muscle fibers following NO_3^- supplementation. It was proposed that similar effects in humans might allow muscle activation at a lower frequency for an equivalent force production, thus decreasing motor unit recruitment and lowering the ATP cost (and therefore improving the efficiency) of force production.

Larsen et al. [70] reported improvements in mitochondrial efficiency following NO_3^- supplementation. Supplementation with NaNO_3^- for 3 days increased the phosphate/ O_2 (P/O) ratio of mitochondria harvested from the vastus lateralis of healthy men, an effect that was significantly correlated with the reduced in vivo $\dot{V}\text{O}_2$. The authors reported reduced expression of the ATP/ADP translocase protein (ANT) and a tendency towards reduced expression of uncoupling protein 3 (UCP3). Such effects may have reduced proton leak during oxidative phosphorylation and could account for the improved P/O ratio and lower pulmonary $\dot{V}\text{O}_2$ [70]. It was proposed that these changes to protein expression may manifest as a consequence of NO competitively inhibiting O_2 binding to COX [78], which may be detected by the mitochondria as hypoxia, activating signaling pathways that downregulate ANT [70]. Given greater inhibition of COX would be expected when PO_2 is low, it is possible that this mechanism may be more active in hypoxia than in normoxia [61].

In contrast to the findings of Larsen et al. [70], Whitfield et al. [79] reported no effect of NO_3^- -rich beetroot juice on mitochondrial efficiency despite a reduced $\dot{V}\text{O}_2$. These disparate results may be related to the form of NO_3^- administered (i.e., NaNO_3^- vs. beetroot juice), although direct comparison is required to eliminate other methodological discrepancies [80]. Interestingly, one recent study [81] reported greater reductions in $\dot{V}\text{O}_2$ following beetroot juice versus NaNO_3^- supplementation in normoxia, possibly as a consequence of the beetroot juice polyphenols and antioxidants enhancing NO generation, further suggesting different effects of these two NO_3^- -containing supplements.

3.2 O_2 Uptake Kinetics

Normoxic studies have reported faster phase II $\dot{V}\text{O}_2$ kinetics during the transition from moderate to severe [82], but not unloaded to moderate [55, 82, 83], intensity exercise following NO_3^- supplementation. The apparently greater effects of NO_3^- during the moderate to severe intensity transition might be related to type II muscle fiber-specific effects of NO_3^- [61, 77, 84], given greater relative activation of these fibers during this transition [82]. In hypoxia, $\dot{V}\text{O}_2$ kinetics are typically slowed compared with normoxia [36, 85]. Interestingly, Kelly et al. [36] observed faster phase II $\dot{V}\text{O}_2$ kinetics during the step transition from unloaded to moderate-intensity but not moderate- to severe-intensity exercise in normobaric hypoxia [36]. Conversely, NO_3^- supplementation did not influence $\dot{V}\text{O}_2$ kinetics in normoxia in this study [36]. The precise reasons why NO_3^- had no effect on $\dot{V}\text{O}_2$ kinetics during the moderate to severe transition in hypoxia, or either transition in normoxia, are unclear. Nevertheless, the speeding of $\dot{V}\text{O}_2$ kinetics during the unloaded to moderate-intensity exercise transition might be beneficial by reducing the time required to reach a steady state and thus minimizing the incurred O_2 deficit [86].

3.3 Arterial O_2 Saturation

NO_3^- supplementation has been reported to result in a small (1–4%) increase in arterial O_2 saturation (S_aO_2), as assessed via pulse oximetry, in some [29, 30, 64, 68] but not all [36] studies during acute exposure to normobaric hypoxia. Carriker et al. [65] observed no effects of NO_3^- on S_aO_2 during acute exercise in a hypobaric hypoxic chamber (~3500 m).

Only one study has examined the effects of NO_3^- supplementation on S_aO_2 at terrestrial altitude [87]. Hennis et al. [87] observed no effect of NO_3^- on S_aO_2 during an 11-day trek to Mount Everest Base Camp (~5300 m). The

lack of effect in this study may be related to the chronic hypoxic exposure and gradual altitude ascent. Here, endogenous NO generation may be elevated with acclimatization [21], thus potentially diminishing the reliance on exogenous NO_3^- as a substrate for NO generation. NO_3^- might therefore be more effective during acute altitude exposure prior to acclimatization, where NO bioavailability is reduced. However, this awaits direct investigation.

Apnea (i.e., breath holding) investigations provide further insight into the effects of NO_3^- supplementation on S_aO_2 when O_2 availability is restricted. NO_3^- supplementation has been reported to elevate S_aO_2 during static [88] and dynamic [89] apneas. Conversely, Schiffer et al. [90] observed lower S_aO_2 values and decreased static apnea duration following NO_3^- supplementation. However, NO_3^- supplementation tended to elevate S_aO_2 during a maximal dynamic apnea. These conflicting results might be related to methodological differences between studies, including the type of apnea (e.g., static vs. dynamic, underwater vs. 'dry'), NO_3^- supplementation strategy, and pre-apnea hyperventilation procedures. Nevertheless, the evidence that NO_3^- supplementation can elevate S_aO_2 in certain apnea situations, particularly during dynamic apnea, provides support for the increase in S_aO_2 observed during exercise in hypoxia [29, 30, 64, 68].

Mechanistically, the elevated S_aO_2 reported during exercise in hypoxia following NO_3^- supplementation may be due to reduced muscle O_2 extraction consequent to improvements in the efficiency of muscle contraction [62] and/or mitochondrial respiration [70]. Additionally, in one study, NO_3^- supplementation increased pulmonary ventilation ($\dot{V}E$) in conjunction with an elevated S_aO_2 during exercise in hypoxia [68]. Given a higher $\dot{V}E$ has previously been associated with reduced arterial desaturation [91], it is possible that an increased $\dot{V}E$ following NO_3^- supplementation might also play a role in elevating S_aO_2 . An elevated S_aO_2 could have important implications for hypoxic exercise performance, as discussed in Sect. 4. Theoretically, an elevated S_aO_2 could also help attenuate acute mountain sickness [92], although current studies have reported no significant effect of NO_3^- on acute mountain sickness [29, 87].

3.4 Tissue Oxygenation

Masschelein et al. [29] reported an increase in the near-infrared spectroscopy (NIRS)-derived tissue oxygenation index (TOI) of the vastus lateralis during cycle ergometry exercise in normobaric hypoxia (F_1O_2 11%, ~5000 m) following NO_3^- supplementation. Kelly et al. [36] also reported a tendency towards elevated TOI during

moderate-intensity exercise in normobaric hypoxia ($F_{I}O_2$ 13.1%, ~ 3500 m). The TOI reflects the ratio between absolute values of oxygenated and total haemoglobin plus myoglobin, and therefore provides a measure of local oxygenation status. Masschelein et al. [29] interpreted the increased TOI as reflecting a greater O_2 efficiency consequent to NO_3^- . Alternatively, the increased TOI could reflect a greater O_2 delivery to the muscle due to elevated tissue blood flow [93]. As both hypoxia [94] and NO_3^- [95] have been reported to increase tissue blood flow, this second explanation cannot be excluded. Given the possible influence of skin blood flow (which increases for thermoregulatory purposes during exercise) on the NIRS signal [96], caution is also advised when interpreting these data. Additional information on the strengths and limitations of NIRS is presented elsewhere [93, 97].

3.5 Muscle Metabolism

Exposure to hypoxia reduces both arterial and intracellular PO_2 [3]. This has a deleterious effect on muscle metabolic function, reducing the capacity for oxidative ATP resynthesis [8, 14], increasing the degradation of limited metabolic substrates including muscle glycogen and PCr and elevating the accumulation of fatigue-associated metabolites such as H^+ , P_i , and ADP [9–11]. These factors potentially contribute towards the decreased exercise tolerance in hypoxia [98]. Intriguingly, Vanhatalo et al. [11, 99] provide evidence to suggest that NO_3^- supplementation might ameliorate the undesirable effects of hypoxia on muscle metabolic function.

NO_3^- supplementation attenuated the rate of change in muscle [PCr], $[P_i]$, and pH as assessed via ^{31}P -magnetic resonance spectroscopy (^{31}P -MRS) during leg extension exercise in normobaric hypoxia ($F_{I}O_2$ 14.5%, ~ 2800 m) [11]. Muscle PCr recovery, reflective of muscle oxidative capacity, was restored to normoxic values. In a follow-up study in more severe hypoxia ($F_{I}O_2$ 13%, ~ 3500 m), the faster PCr recovery kinetics with NO_3^- supplementation were associated with an accelerated effective transverse relaxation time ($T2^*$), as obtained via magnetic resonance imaging (MRI) [99]. The $T2^*$ signal is a result of both blood flow to the area under investigation and the oxygenation status of the blood and tissue. Consequently, an increase in blood volume and/or the proportion of oxygenated blood increases the $T2^*$ signal [100]. The authors speculated that the accelerated $T2^*$ recovery following NO_3^- supplementation may indicate a greater delivery of freshly oxygenated blood to the muscle, increasing the O_2 driving pressure into the muscle cells, and resulting in faster oxidative ATP resynthesis and PCr regeneration [99]. NO_3^- supplementation also improved mitochondrial efficiency, as indicated by an increased

muscle phosphorylation potential (indicative of the proton motive force) and lower Gibb's free energy (ΔG) [99]. Interestingly, resting [PCr] was reduced consequent to NO_3^- supplementation, with the magnitude of the decline inversely correlated with plasma $[NO_2^-]$. It was speculated that this might be related to reversible S-nitrosation of creatine kinase (CK), and could serve to enhance mitochondrial sensitivity to ADP stimulation. Thus, NO_3^- supplementation may enhance both muscle energetics and O_2 delivery during hypoxic exercise, which might partly underpin the improvements in exercise performance discussed in Sect. 4.

3.6 Cardiovascular Response

In normoxia, NO_3^- supplementation has been reported to reduce BP [51–53] and enhance vascular compliance [101] in humans and improve cardiac contractility in rodents [102]. Dietary NO_3^- might also provide a means of altering the complex and multifaceted cardiovascular response to hypoxia.

Some [11, 64], but not all [68, 103], previous studies have reported BP reductions during acute normobaric hypoxic exposure following NO_3^- supplementation. The reduced BP following NO_3^- supplementation has been attributed to increased NO_2^- and NO generation and subsequent vasodilation [52, 53]. Recently, Ingram et al. [104] reported pulmonary and arterial vasodilation after NO_2^- infusion in normobaric hypoxia ($F_{I}O_2$ 12%, ~ 4100 m) but not normoxia. The greater activity of NO_2^- reductases, including xanthine oxidoreductase, deoxygenated hemoglobin, and myoglobin, and COX in hypoxia [37] may partly explain this effect. Interestingly, Ingram et al. [104] observed pulmonary vasodilation in hypoxia after plasma $[NO_2^-]$ had returned to baseline values. This suggests possible storage and metabolism of NO_2^- , and NO in the extravascular tissue could contribute to hypoxia-induced vasodilation [104]. It is possible, based on the findings of Ingram et al. [104], that the capacity to elicit vasodilation and BP effects with NO_3^- supplementation might also be more likely in hypoxia than in normoxia, although direct comparison is required.

In acute normobaric hypoxia ($F_{I}O_2 \sim 11.6\%$, ~ 4600 m), NO_3^- supplementation neither influenced the hemodynamic response nor augmented cardiac unloading in healthy men [103]. Similarly, NO_3^- had no effect on the hypoxia-induced hyperemic response to handgrip exercise in young adults (mean \pm standard deviation [SD] age 25 ± 1 years) exercising in normoxia and acute normobaric hypoxia ($F_{I}O_2$ titrated to achieve $\sim 80\% S_aO_2$) [105]. Interestingly, despite similar increases in plasma $[NO_2^-]$, NO_3^- supplementation significantly increased the vasodilation and blood flow response to hypoxic exercise in older participants

(mean \pm SD age 64 ± 2 years). The magnitude of the effect was such that age-related differences in compensatory vasodilation were abolished with NO_3^- supplementation, suggesting therapeutic utility of NO_3^- in older populations exercising in hypoxia [105] or potentially those afflicted with clinical conditions involving tissue hypoxia [106].

Limited evidence is available regarding the effects of NO_3^- on cardiovascular parameters at terrestrial altitude. Bakker et al. [19] and Hennis et al. [87] reported no effects of NO_3^- supplementation on BP during prolonged treks at terrestrial altitude. However, in the study by Bakker et al. [19], acute NO_3^- ingestion offset the decline in endothelial function assessed via flow-mediated vasodilation (FMD) in healthy young participants (mean \pm SD age 25 ± 5 years) during an expedition in Nepal (28 days >2500 m, including a peak of 3700 m). FMD has traditionally been viewed as reflecting NO-dependent vasodilation of the smooth muscle in response to acute hyperemia following circulatory occlusion [107, 108]. The hyperemia that manifests following occlusion is believed to increase G-protein expression of phosphokinase A and subsequently increase activity of endothelial NOS (eNOS) [108]. NO is then generated via eNOS catabolism of L-arginine, which ultimately results in smooth muscle relaxation and vasodilation [108]. Based on this conventional view, Bakker et al. [19] primarily attributed the beneficial effects of NO_3^- supplementation on FMD to elevated NO bioavailability via the NO_3^- - NO_2^- -NO pathway and subsequent effects on smooth muscle. However, there exists considerable debate about the NO-dependency of this technique, with some authors supporting [109–111] and others questioning [112–115] the role of NO in FMD assessment. Further exploration may therefore be warranted to understand the precise mechanisms through which NO_3^- supplementation influences FMD.

4 Hypoxic Exercise Performance

Several studies have examined the effects of NO_3^- ingestion on exercise capacity/performance in acute normobaric hypoxia (Table 1). The effects of NO_3^- supplementation on exercise capacity/performance in simulated altitude via hypobaric hypoxia and at terrestrial altitude have yet to be investigated. Vanhatalo et al. [11] were the first to study the effects of NO_3^- supplementation during exercise in normobaric hypoxia ($F_{\text{I}}\text{O}_2$ 14.5%, ~ 2800 m) and observed a 21.4% increase in leg-extension TTE with NO_3^- supplementation. This improvement effectively restored exercise duration to normoxia values. Whilst exhaustive leg-extension exercise is not directly representative of athletic performance, these data suggest that NO_3^- supplementation might offer a means of ameliorating the ergolytic effects of hypoxia on exercise performance.

Masschelein et al. [29] confirmed the beneficial effects of NO_3^- supplementation in extreme normobaric hypoxia ($F_{\text{I}}\text{O}_2$ 11%, ~ 5000 m). Cycle ergometry TTE was reduced by 36% in hypoxia versus normoxia. However, NO_3^- supplementation attenuated $\sim 5\%$ of this ergolytic effect. The magnitude of this effect is substantially lower than that reported by Vanhatalo et al. [11], although it is likely of practical relevance to an athlete and may be accounted for by methodological differences. Notably, in the study by Vanhatalo et al. [11], exercise was conducted in moderate hypoxia involving a small muscle group, whereas Masschelein et al. [29] had participants complete whole-body exercise in severe hypoxia. In the former, a beneficial effect of NO_3^- on muscle O_2 efficiency may result in a direct and substantial improvement in performance, given muscles are over perfused and muscle $\dot{V}\text{O}_2$ is predominantly limited by mitochondrial O_2 consumption rather than delivery of O_2 to the muscle [11]. In the latter, muscle O_2 consumption is limited primarily by impaired O_2 diffusion, consequent to the drop in arterial PO_2 , such that greater O_2 efficiency may result in smaller performance improvements [29]. Additionally, constant-load TTE tests, as employed by Vanhatalo et al. [11], are typically more sensitive than incremental TTE tests for detecting improvements in exercise capacity [29, 116].

Muggeridge et al. [30] first assessed the effects of NO_3^- supplementation on TT performance rather than TTE in normobaric hypoxia ($F_{\text{I}}\text{O}_2$ 15%, ~ 2500 m). Performance in a pre-loaded (i.e., preceded by a steady-state period) 16.1-km cycle ergometry TT was significantly faster (2.2%) following NO_3^- supplementation. Kelly et al. [36] reported similar beneficial effects of NO_3^- on severe-intensity cycle ergometry TTE in physically active males (mean \pm SD $\dot{V}\text{O}_{2\text{peak}}$: 58.3 ± 6.3 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) exercising in normobaric hypoxia ($F_{\text{I}}\text{O}_2$ 13.1%, ~ 3500 m). Interestingly, NO_3^- supplementation did not influence exercise tolerance in normoxia in this study [36], supporting the notion that the ergogenic effects of NO_3^- might be particularly pronounced in conditions of low O_2 availability. Most recently, Shannon et al. [64] reported a significant improvement in pre-loaded 1500-m running TT performance in normobaric hypoxia ($F_{\text{I}}\text{O}_2$ $\sim 15\%$, ~ 2500 m) following NO_3^- supplementation in participants across a range of different fitness levels ($\dot{V}\text{O}_{2\text{max}}$ range: 47.1–76.8 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). All 12 participants in that study were faster with NO_3^- supplementation than with placebo, with an average improvement in 1500 m TT performance of 3.2%.

However, not all studies have observed a positive effect of NO_3^- supplementation on hypoxic exercise performance. MacLeod et al. [66] reported no effects of NO_3^- supplementation on 10-km cycle ergometry TT

Table 1 The effects of nitrate supplementation on exercise performance in hypoxia

References	Participant characteristics ^a	Supplementation protocol	Performance assessment	Trial results ^a	Cohen's <i>d</i>
Vanhatalo et al. [11]	7 M, 2 F; moderately trained ($\dot{V}O_{2\max}$ NS)	750 ml BRJ (9.3 mmol NO_3^-) 24 h prior, with last 250 ml dose 150 min pre-exercise	Leg extension TTE ($\text{F}_{\text{I}O_2}$ 14.5%, ~2800 m)	Normoxia: 471 ± 200 s Hypoxia (PLA): 393 ± 169 s ^b Hypoxia (NIT): 477 ± 200 s ^c	Normoxia vs. hypoxia PLA: 0.42 Normoxia vs. hypoxia NIT: 0.03 Hypoxia PLA vs. hypoxia NIT: 0.45
Masschelein et al. [29]	15 M; healthy, physically active ($\dot{V}O_{2\text{peak}}$: 61.7 ± 2.1 ml·kg ⁻¹ ·min ⁻¹)	~500 ml·d ⁻¹ BRJ (0.7 mmol·kg ⁻¹ ·day ⁻¹ / ~5 mmol·day ⁻¹ NO_3^-) consumed daily for 6 days, with last 500 ml dose 60–120 min pre-exercise	Incremental cycle ergometry TTE ($\text{F}_{\text{I}O_2}$ 11%, ~5000 m)	Normoxia: 888 ± 143 s Hypoxia (PLA): 568 ± 89 s ^b Hypoxia (NIT): 597 ± 85 s ^{bc}	Normoxia vs. hypoxia PLA: 2.69 Normoxia vs. hypoxia NIT: 2.47 Hypoxia PLA vs. hypoxia NIT: 0.33
Muggenidge et al. [30]	9 M; trained ($\dot{V}O_{2\text{peak}}$ at simulated altitude: 51.9 ± 5.8 ml·kg ⁻¹ ·min ⁻¹)	70 ml concentrated BRJ (~5 mmol NO_3^-) consumed 180 min pre-exercise	16.1 km cycle ergometry TT ($\text{F}_{\text{I}O_2}$ 15%, ~2500 m)	Hypoxia (PLA): 1702 ± 45 s Hypoxia (NIT): 1664 ± 42 s ^c	Hypoxia PLA vs. hypoxia NIT: 0.87
Kelly et al. [36]	12 M; physically active ($\dot{V}O_{2\text{peak}}$: 58.3 ± 6.3 ml·kg ⁻¹ ·min ⁻¹)	2 × 70 ml·day ⁻¹ concentrated BRJ (~8.4 mmol·day ⁻¹ NO_3^-) consumed daily for 3 days, with last 140 ml dose consumed 150 min pre-exercise	Cycle ergometry TTE at 75% between GET and $\text{VO}_{2\text{peak}}$ ($\text{F}_{\text{I}O_2}$ 13.1%, ~3500 m)	Normoxia (PLA): 431 ± 124 s Normoxia (NIT): 412 ± 139 s Hypoxia (PLA): 197 ± 28 s ^b Hypoxia (NIT): 214 ± 43 s ^{bc}	Normoxia PLA vs. normoxia NIT: 0.14 Normoxia PLA vs. hypoxia PLA: 2.60 Normoxia PLA vs. hypoxia NIT: 2.34 Hypoxia PLA vs. hypoxia NIT: 0.47
Arnold et al. [67]	10 M; well-trained ($\dot{V}O_{2\max}$: 66 ± 7 ml·kg ⁻¹ ·min ⁻¹)	70 ml concentrated BRJ (~7 mmol NO_3^-) consumed 150 min pre-exercise	10,000 m treadmill TT ($\text{F}_{\text{I}O_2}$ 15.4%, ~2500 m)	Hypoxia (PLA): 2862 ± 233 s Hypoxic (NIT): 2874 ± 265 s	Hypoxia PLA vs. hypoxia NIT: 0.05
Arnold et al. [67]	10 M; well-trained ($\dot{V}O_{2\max}$: 66 ± 7 ml·kg ⁻¹ ·min ⁻¹)	70 ml concentrated BRJ (~7 mmol NO_3^-) consumed 150 min pre-exercise	Incremental treadmill TTE ($\text{F}_{\text{I}O_2}$ 12.8%, ~4000 m)	Hypoxia (PLA): 393 ± 62 s Hypoxic (NIT): 402 ± 80 s	Hypoxia PLA vs. hypoxia NIT: 0.13
MacLeod et al. [66]	11 M; well-trained ($\dot{V}O_{2\text{peak}}$: 67.5 ± 5.8 ml·kg ⁻¹ ·min ⁻¹)	70 ml concentrated BRJ (~6 mmol NO_3^-) consumed 120 min pre-exercise	10 km cycle ergometry TT in normoxia and hypoxia ($\text{F}_{\text{I}O_2}$ ~15%, ~2500 m)	Normoxia (PLA): 954 ± 47 s Normoxia (NIT): 961 ± 54 s Hypoxia (PLA): 1023 ± 49 s ^b Hypoxia (NIT): 1018 ± 52 s ^b	Normoxia PLA vs. normoxia NIT: 0.14 Normoxia PLA vs. hypoxia PLA: 1.44 Normoxia PLA vs. hypoxia NIT: 1.29 Hypoxia PLA vs. hypoxia NIT: 0.10
Bourdillon et al. [68]	12 M; well-trained ($\dot{V}O_{2\max}$ NS)	0.1 mmol·kg ⁻¹ ·day ⁻¹ NaNO_3^- for 3 days	15 km cycle ergometry TT in normoxia and hypoxia ($\text{F}_{\text{I}O_2}$ 11%, ~5000 m)	Normoxia (PLA): 1597 ± 96 s Normoxia (NIT): 1581 ± 63 s Hypoxia (PLA): 2155 ± 687 s ^b Hypoxia (NIT): 2005 ± 309 s ^b	Normoxia PLA vs. normoxia NIT: 0.20 Normoxia PLA vs. hypoxia PLA: 1.14 Normoxia PLA vs. hypoxia NIT: 1.78 Hypoxia PLA vs. hypoxia NIT: 0.29

Table 1 continued

References	Participant characteristics ^a	Supplementation protocol	Performance assessment	Trial results ^a	Cohen's <i>d</i>
Shannon et al. [64]	12 M; spectrum of untrained to well-trained ($\dot{V}O_{2\max}$ 62.1 ± 9.3 ml·kg ⁻¹ ·min ⁻¹)	140 ml concentrated BRJ (15.2 mmol NO ₃ ⁻) consumed 180 min pre-exercise	1500 m treadmill TT in hypoxia (F _I O ₂ ~15%, ~2500 m)	Hypoxia (PLA): 342 ± 46 s Hypoxia (NIT): 331 ± 45 s ^c	Hypoxia PLA vs. hypoxia NIT: 0.24

BRJ beetroot juice, F female, F_IO₂ fraction of inspired oxygen, GET gas exchange threshold, M male, NIT nitrate, NS not specified, PLA placebo, TT time-trial, TTE time to exhaustion, $\dot{V}O_{2\max}$ maximal oxygen uptake, $\dot{V}O_{2\text{peak}}$ peak oxygen uptake

^a Values are mean ± standard deviation
^b Significantly different to normoxia
^c Significantly different to PLA

performance in well-trained athletes (mean ± SD $\dot{V}O_{2\text{peak}}$ 67.5 ± 5.8 ml·kg⁻¹·min⁻¹) exercising in either normobaric hypoxia (F_IO₂ ~15%, ~2500 m) or normoxia. Likewise, Bourdillon et al. [68] observed no significant difference in 15-km cycle ergometry TT performance in extreme normobaric hypoxia (F_IO₂ 11%, ~5000 m) or normoxia following NO₃⁻ supplementation. However, there was a non-significant tendency towards improved performance (~151 s faster) in hypoxia with NO₃⁻ ingestion. Similarly, Arnold et al. [67] reported no effect of NO₃⁻ ingestion on 10-km TT running performance in moderate normobaric hypoxia (F_IO₂ 15.4%, ~2500 m) or incremental running performance in extreme normobaric hypoxia (F_IO₂ 12.8%, ~4000 m) in highly trained runners (mean ± SD $\dot{V}O_{2\max}$ 66 ± 7 ml·kg⁻¹·min⁻¹).

These conflicting results might be explained by methodological differences between investigations. At present, some evidence exists to suggest that the training status of participants, exercise protocol, supplementation protocol, and exercise environment (i.e., the degree and duration of hypoxic exposure) can all moderate the effects of NO₃⁻ supplementation [31]. However, it should be noted that none of these variables alone can entirely explain the apparent ergogenic effect of NO₃⁻ supplementation (or lack thereof), and it is the interaction between these and other factors that appears to be important.

In several normoxic studies, high aerobic fitness has been suggested as a possible reason for minimal effects of NO₃⁻ on exercise performance [117–122]. Notably, Porcelli et al. [58] suggested that untrained and moderately trained ($\dot{V}O_{2\max} < 60$ ml·kg⁻¹·min⁻¹) individuals are more likely to benefit from NO₃⁻ supplementation than endurance-trained athletes ($\dot{V}O_{2\max} > 60$ ml·kg⁻¹·min⁻¹). However, given the cross-sectional nature of this study and relatively modest sample size (fitness-stratified sub-groups of six to eight participants), these data should be interpreted with caution. Moreover, a recent meta-analysis found no influence of aerobic fitness on the response to NO₃⁻ supplementation [59], bringing into question this relationship. Nevertheless, as evident in Shannon et al. [64], who observed beneficial effects of NO₃⁻ in participants across a spectrum of fitness levels during exercise in moderate normobaric hypoxia, a high training status does not entirely preclude an ergogenic effect of NO₃⁻ supplementation. Given that NO₃⁻ supplementation appears to be more effective in hypoxia than in normoxia [123], it is possible that individuals who may not benefit from NO₃⁻ supplementation in normoxia (or at low/moderate altitudes) might derive an ergogenic effect in extreme hypoxia where the NO₃⁻–NO₂⁻–NO pathway is enhanced yet NOS activity is suppressed.

NO_3^- supplementation appears to be most effective during short-duration high-intensity exercise, although data are presently limited regarding the effects of NO_3^- on this type of exercise at altitude. During short-duration high-intensity activity, the cellular environment is likely to be particularly hypoxic and acidic, potentiating NO_2^- reduction into NO [49, 124]. Moreover, type II muscle is likely to be highly activated, and compelling evidence from murine models suggests that NO_3^- supplementation might solely increase perfusion to [84, 95] and contractile function/force generation of [77] type II muscle. Whilst a precise ‘cut-off’ duration or exercise intensity for the effects of NO_3^- supplementation to be meaningful has yet to be established and will likely differ depending on other experimental factors, it appears that NO_3^- supplementation is usually beneficial for exercise tests lasting <30 min [55, 57, 58, 62, 63, 125, 126], but usually not longer [117, 118, 120, 127], at least in normoxia. Exercise at altitude, particularly mountaineering and hiking, can include sustained periods of relatively low-intensity activity. The effects of NO_3^- supplementation on key physiological and functional (e.g., walking distance in a given time period) parameters during this type of exercise warrant further study, especially as only a small proportion of people ascending to altitude are highly trained athletes conducting high-intensity exercise. Interestingly, Kuennen et al. [128] reported a lower O_2 cost of exercise during a simulated desert march in the heat (41 °C) consequent to NO_3^- supplementation. Given the similarity of this exercise mode to altitude hiking (i.e., relatively low-intensity walking whilst carrying a backpack), it is possible that NO_3^- supplementation might also reduce O_2 consumption during altitude hiking, although this has yet to be investigated. The effect of NO_3^- supplementation at lower altitudes (<2500 m >sea level) also remains unexplored yet is of interest given physiological functioning may be influenced by altitudes as low as 300–800 m [4].

Finally, normoxic studies suggest the supplementation strategy might influence the ergogenic effects of NO_3^- . First, there is evidence to indicate a possible dose response to NO_3^- supplementation [38, 129, 130]. Likewise, some effects of NO_3^- , particularly those requiring changes in protein expression [70, 77], may require several days to occur, suggesting chronic loading may be more effective than acute supplementation [59, 63]. Interestingly, Flueck et al. [81] reported greater reductions in $\dot{V}\text{O}_2$ with beetroot juice than with NaNO_3^- supplementation, which could be related to greater NO_2^- reduction into NO with beetroot juice consequent to the polyphenols and/or antioxidants. The potential influence of these moderators awaits investigation in hypoxia.

4.1 Simulated Versus Terrestrial Altitude

To date, few studies have explored the efficacy of NO_3^- supplementation during exposure to terrestrial altitude [19, 87, 131], presumably because of the high financial costs and logistical difficulties associated with such investigations. Instead, researchers have typically administered inspired hypoxic gas during exercise or conducted exercise in normobaric or (to a lesser degree) hypobaric hypoxic chambers. There is ongoing debate about potential differences in the physiological response to normobaric hypoxia and terrestrial altitude [132], including potential disparities in NO metabolism [133]. Further investigations into the effects of NO_3^- at terrestrial altitude and/or contrasting the effects of NO_3^- supplementation between terrestrial and simulated altitude is warranted. It seems likely that NO_3^- supplementation could enhance exercise performance at terrestrial altitude, given exercise performance is predominantly limited by the low PO_2 in both normobaric and hypobaric hypoxia [29]. Furthermore, NO_3^- supplementation has been reported to improve endothelial function at terrestrial altitude, suggesting the potential to alter peripheral vascular function in this environment [19]. Given the large degree of “noise” associated with field-based testing, future studies may require large sample sizes to ensure sufficient power to detect any meaningful physiological changes with NO_3^- supplementation.

5 Training in Hypoxia

There remains a degree of ambiguity over the performance effects of NO_3^- supplementation during exercise in hypoxia, as discussed in Sect. 4. Nevertheless, it is possible that NO_3^- supplementation could help augment the physiological and performance adaptations to training in hypoxia, if the maintainable work rate during training sessions was enhanced or prolonged. Moreover, NO has been implicated in the adaptation to hypoxia [21] and appears to play a role in skeletal muscle hypertrophy and fiber-type transitions [134] and endothelial adaptations [135], but not mitochondrial biogenesis [136, 137], following exercise training. Increasing NO bioavailability via NO_3^- supplementation might therefore enhance some of the adaptations to hypoxic training. Conversely, it is also possible that NO_3^- might suppress hypoxic training adaptations by limiting the drop in arterial and muscle O_2 saturation, factors that could serve as “signals” for adaptation to hypoxic training [138].

Puype et al. [138] found no effect of NO_3^- supplementation on the physiological and performance adaptations to 6 weeks of high-intensity endurance training

(5×30 min·week⁻¹ cycle ergometry at 4–6 mmol·l⁻¹ blood [lactate]) in normobaric hypoxia ($F_{I}O_2$ 12.5%, ~4000 m). Improvements in $\dot{V}O_{2max}$, power output at the onset of blood lactate accumulation (OBLA; 4 mmol·l⁻¹ blood [lactate]) and average power output during a 30-min TT in normoxia were similar in NO_3^- and placebo conditions. Likewise, the pre- to post-hypoxic training change was similar between groups for muscle adenosine monophosphate-activated kinase (AMPK) protein content and phosphorylation, hypoxia-inducible factor (HIF)-1 α messenger RNA (mRNA) content, and glycogen breakdown during the TT. More recently, De Smet et al. [139] explored the effects of NO_3^- on adaptations to sprint interval training (SIT) in normobaric hypoxia ($F_{I}O_2$ 15%, ~2500 m) versus separate unsupplemented normoxic and hypoxic training groups. Participants completed 3×4 –6 maximal 30 s cycle ergometry sprints per week over a 5-week period. The proportion of type IIa fibers was significantly increased in the hypoxic NO_3^- group, whereas the proportion of type IIx fibers was significantly decreased. By contrast, a significant decrease in the proportion of type IIx fibers in the unsupplemented groups did not lead to significant increases in other fiber-type groups. These findings were coupled with a tendency towards greater improvements in normoxic 30-s sprint performance in the hypoxic NO_3^- training group relative to the hypoxic unsupplemented training group. Conversely, performance in a 30-min TT completed in normoxia increased by similar amounts in all groups. Taken together, the results of Puype et al. [138] and De Smet et al. [139] suggest that NO_3^- supplementation consumed during 5–6 weeks of endurance or sprint training in hypoxia has minimal effects on physiological adaptations or on exercise performance in normoxia.

Two studies have also recently explored the effects of NO_3^- supplementation on the adaptations to SIT in normoxia [140, 141]. Muggeridge et al. [140] reported greater improvements in maximal work rate during incremental exercise and indices of repeated high-intensity performance in participants who ingested NO_3^- versus placebo prior to SIT sessions (3×4 –6 maximal 15 s cycle ergometry sprints per week, for 3 weeks). Thompson et al. [141] also demonstrated greater improvements in peak work rate during incremental exercise in individuals who performed SIT (3 – 4×4 –5 maximal 30 s cycle ergometry sprints per week for 4 weeks) with NO_3^- compared with both training individuals given a placebo and NO_3^- -supplemented individuals who did not train. Interestingly, and supporting the findings of De Smet et al. [139], Thompson et al. [141] showed a decrease in the proportion of type IIx muscle fibers in the vastus lateralis in participants completing SIT with NO_3^- but not in the other conditions.

Based on the findings of these studies, it is possible that NO_3^- may elicit similar muscle fiber-type changes when consumed parallel to training in normoxia and hypoxia but may be more effective at enhancing performance adaptations to normoxic than hypoxic training. However, this requires direct investigation given the multifarious methodological differences between studies. In particular, Puype et al. [138] and De Smet et al. [139] in hypoxia recruited moderately trained participants ($\dot{V}O_{2max}$: ~51–60 ml·kg⁻¹·min⁻¹), whereas Muggeridge et al. [140] and Thompson et al. [141] in normoxia recruited individuals with lower aerobic fitness ($\dot{V}O_{2max}$: ~42–50 ml·kg⁻¹·min⁻¹), who may exhibit a greater response to training with NO_3^- .

The approach adopted in the above studies is similar to most training-intervention studies, whereby a standardized exercise session is utilized throughout the intervention, either with or without an overload component [142–145]. This strategy is seldom adopted by ‘real-world’ athletes, who instead perform a wide variety of training sessions [146–148]. Interestingly, anecdotal reports suggest that some elite endurance athletes at high-altitude training camps selectively use NO_3^- supplements (e.g., beetroot juice) in an attempt to maximize performance in some, but not all, training sessions. This approach, first highlighted by Professor Andrew Jones of Exeter University, UK [31, 32], may allow athletes to benefit from conducting key training sessions at higher exercise intensities. Conversely, low-intensity high-mileage sessions are performed unsupplemented, thus maximizing the hypoxic stimulus for adaptation [31, 32]. Periodizing NO_3^- supplementation in hypoxia has yet to be evaluated scientifically but may offer valuable psychological advantages to athletes regardless of the potential physiological merits.

6 Summary and Conclusions

NO_3^- supplementation is emerging as a promising nutritional aid, with potentially beneficial applications for the wide variety of individuals ascending to altitude each year. In this review, NO_3^- supplementation has been demonstrated to reduce pulmonary $\dot{V}O_2$ and, in some cases, elevate S_aO_2 in normobaric hypoxia—effects that may be attributable to improvements in the efficiency of muscle contraction and/or mitochondrial respiration, and are of functional relevance for individuals exercising in a low- O_2 environment. Current evidence also suggests that NO_3^- supplementation can improve muscle energetics during exercise in normobaric hypoxia via effects on tissue O_2 delivery and consumption and might alter cardiovascular responses to normobaric and hypobaric hypoxia. In

contrast, whilst NO_3^- supplementation might influence some of the physiological responses to hypoxic training, current evidence suggests that this does not translate into improved exercise performance in normoxia. Given that the majority of published investigations have explored the effects of NO_3^- supplementation in simulated altitude, which is often regarded as an incomplete surrogate of “true” altitude, future studies at terrestrial altitude are necessary. Researchers are encouraged to further probe the myriad potentially beneficial effects of NO_3^- supplementation as a potential aid to “beet-ing” the mountain.

Compliance with Ethical Standards

Funding No sources of funding were used to assist in the preparation of this article.

Conflicts of interest Oliver Shannon, Kerry McGawley, Linn Nybäck, Lauren Duckworth, Matthew Barlow, David Woods, Mario Siervo, and John O’Hara have no conflicts of interest relevant to the content of this review.

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References

- Dumont L, Lysakowski C, Tramèr MR, et al. Controversies in altitude medicine. *Travel Med Infect Dis.* 2005;3:183–8.
- Richardson RS, Duteil S, Wary C, et al. Human skeletal muscle intracellular oxygenation: the impact of ambient oxygen availability. *J Physiol.* 2006;571:415–24.
- Calbet JA, Lundby C. Air to muscle O_2 delivery during exercise at altitude. *High Alt Med Biol.* 2009;10:123–34.
- Wehrin JP, Hallén J. Linear decrease in $\text{VO}_{2\text{max}}$ and performance with increasing altitude in endurance athletes. *Eur J Appl Physiol.* 2006;96:404–12.
- MacInnis MJ, Nugent SF, MacLeod KE, et al. Methods to estimate $\text{VO}_{2\text{max}}$ upon acute hypoxia exposure. *Med Sci Sports Exerc.* 2015;47:1869–76.
- Lundby C, Calbet JA, Sander M, et al. Exercise economy does not change after acclimatization to moderate to very high altitude. *Scand J Med Sci Sports.* 2007;17:281–91.
- Fulco CS, Rock PB, Cymerman A. Maximal and submaximal exercise performance at altitude. *Aviat Space Environ Med.* 1998;69:793–801.
- Magalhães J, Ascensão A, Soares JMC, et al. Acute and severe hypobaric hypoxia increases oxidative stress and impairs mitochondrial function in mouse skeletal muscle. *J Appl Physiol.* 2005;99:1247–53.
- Linnarsson D, Karlsson J, Fagraeus L, et al. Muscle metabolites and oxygen deficit with exercise in hypoxia and hyperoxia. *J Appl Physiol.* 1974;36:399–402.
- Hogan MC, Richardson RS, Haseler LJ. Human muscle performance and PCr hydrolysis with varied inspired oxygen fractions: a ^{31}P -MRS study. *J Appl Physiol.* 1999;86:1367–73.
- Vanhatalo A, Fulford J, Bailey SJ, et al. Dietary nitrate reduces muscle metabolic perturbation and improves exercise tolerance in hypoxia. *J Physiol.* 2011;589:5517–28.
- Mason NP. The physiology of high altitude: an introduction to the cardio-respiratory changes occurring on ascent to altitude. *Curr Anaesth Crit Care.* 2000;11:34–41.
- Clerc P, Rigoulet M, Leverve X, et al. Nitric oxide increases oxidative phosphorylation efficiency. *J Bioenerg Biomembr.* 2007;39:158–66.
- Solaini G, Baracca A, Lenaz G, et al. Hypoxia and mitochondrial oxidative metabolism. *Biochim Biophys Acta.* 2010;1797:1171–7.
- Van Mil AHM, Spilt A, Van Buchem MA, et al. Nitric oxide mediates hypoxia-induced cerebral vasodilation in humans. *J Appl Physiol.* 2002;92:962–6.
- Hackett PH, Roach RC. High-altitude illness. *N Engl J Med.* 2001;345:107–14.
- Joyner MJ, Casey DP. Muscle blood flow, hypoxia, and hypoperfusion. *J Appl Physiol.* 2014;116:852–7.
- Erzurum SC, Ghosh S, Janocha AJ, et al. Higher blood flow and circulating NO products offset high-altitude hypoxia among Tibetans. *Proc Natl Acad Sci.* 2007;104:17593–8.
- Bakker E, Engan H, Patrician A, et al. Acute dietary nitrate supplementation improves arterial endothelial function at high altitude: a double-blinded randomized controlled cross over study. *Nitric Oxide.* 2015;50:58–64.
- Diesen DL, Hess DT, Stamler JS. Hypoxic vasodilation by red blood cells. *Circ Res.* 2008;103:545–53.
- Levett DZ, Fernandez BO, Riley HL, et al. The role of nitrogen oxides in human adaptation to hypoxia. *Sci Rep.* 2011;1:109. doi:10.1038/srep00109.
- Bärtsch P, Saltin B. General introduction to altitude adaptation and mountain sickness. *Scand J Med Sci Sports.* 2008;18:1–10.
- Beall CM, Laskowski D, Strohl KP, et al. Pulmonary nitric oxide in mountain dwellers. *Nature.* 2001;414:411–2.
- Hoit BD. Nitric oxide and cardiopulmonary hemodynamics in Tibetan highlanders. *J Appl Physiol.* 2005;99:1796–801.
- Marconi C, Marzorati M, Sciuto D, et al. Economy of locomotion in high-altitude Tibetan migrants exposed to normoxia. *J Physiol.* 2005;569:667–75.
- Droma Y, Hanaoka M, Ota M, et al. Positive association of the endothelial nitric oxide synthase gene polymorphisms with high-altitude pulmonary edema. *Circulation.* 2002;106:826–30.
- Bailey DM, Dehnert C, Luks AM, et al. High-altitude pulmonary hypertension is associated with a free radical-mediated reduction in pulmonary nitric oxide bioavailability. *J Physiol.* 2010;588:4837–47.
- Beall CM, Laskowski D, Erzurum SC. Nitric oxide in adaptation to altitude. *Free Radic Biol Med.* 2012;52:1123–34.
- Masschelein E, Van Thienen R, Wang X, et al. Dietary nitrate improves muscle but not cerebral oxygenation status during exercise in hypoxia. *J Appl Physiol.* 2012;113:736–45.
- Muggeridge DJ, Howe CCF, Spendiff O, et al. A single dose of beetroot juice enhances cycling performance in simulated altitude. *Med Sci Sports Exerc.* 2014;46:143–50.
- Jones AM. Influence of dietary nitrate on the physiological determinants of exercise performance: a critical review. *Appl Physiol Nutr Metab.* 2014;39:1019–28.
- Jones AM. Dietary nitrate supplementation and exercise performance. *Sports Med.* 2014;44:35–45.
- Fish JE, Matouk CC, Yeboah E, et al. Hypoxia-inducible expression of a natural *cis*-antisense transcript inhibits endothelial nitric-oxide synthase. *J Biol Chem.* 2007;282:15652–66.
- Shaul PW, Wells LB, Horning KM. Acute and prolonged hypoxia attenuate endothelial nitric oxide production in rat

- pulmonary arteries by different mechanisms. *J Cardiovasc Pharmacol.* 1993;22:819–27.
35. Leuenberger UA, Johnson D, Loomis J, et al. Venous but not skeletal muscle interstitial nitric oxide is increased during hypobaric hypoxia. *Eur J Appl Physiol.* 2008;102:457–61.
 36. Kelly J, Vanhatalo A, Bailey SJ, et al. Dietary nitrate supplementation: effects on plasma nitrite and pulmonary O₂ uptake dynamics during exercise in hypoxia and normoxia. *Am J Physiol.* 2014;307:920–30.
 37. Lundberg JO, Weitzberg E, Gladwin MT. The nitrate–nitrite–nitric oxide pathway in physiology and therapeutics. *Nat Rev Drug Discov.* 2008;7:156–67.
 38. Wylie LJ, Kelly J, Bailey SJ, et al. Beetroot juice and exercise: pharmacodynamic and dose–response relationships. *J Appl Physiol.* 2013;115:325–36.
 39. Jansson EA, Huang L, Malkey R, et al. A mammalian functional nitrate reductase that regulates nitrite and nitric oxide homeostasis. *Nat Chem Biol.* 2008;4:411–7.
 40. Qin L, Liu X, Sun Q, et al. Sialin (SLC17A5) functions as a nitrate transporter in the plasma membrane. *Proc Natl Acad Sci.* 2012;109:13434–9.
 41. Hyde ER, Andrade F, Vaksman Z, et al. Metagenomic analysis of nitrate-reducing bacteria in the oral cavity: implications for nitric oxide homeostasis. *PLoS One.* 2014;9(3):e88645. doi:10.1371/journal.pone.0088645.
 42. Benjamin N, O'Driscoll F, Dougall H, et al. Stomach NO synthesis. *Nature.* 1994;368:502.
 43. Lundberg JO, Weitzberg E, Lundberg JM, et al. Intra-gastric nitric oxide production in humans: measurements in expelled air. *Gut.* 1994;35:1543–6.
 44. Hunault CC, van Velzen AG, Sips AJAM, et al. Bioavailability of sodium nitrite from an aqueous solution in healthy adults. *Toxicol Lett.* 2009;190:48–53.
 45. Godber BL, Doel JJ, Sapkota GP, et al. Reduction of nitrite to nitric oxide catalyzed by xanthine oxidoreductase. *J Biol Chem.* 2000;275:7757–63.
 46. Cantu-Medellin N, Kelley EE. Xanthine oxidoreductase-catalyzed reduction of nitrite to nitric oxide: insights regarding where, when and how. *Nitric Oxide.* 2013;35:19–26.
 47. Cosby K, Partovi KS, Crawford JH, et al. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. *Nat Med.* 2003;9:1498–505.
 48. Huang Z, Shiva S, Kim-Shapiro DB, et al. Enzymatic function of hemoglobin as a nitrite reductase that produces NO under allosteric control. *J Clin Invest.* 2005;115:2099–107.
 49. Castello PR, David PS, McClure T, et al. Mitochondrial cytochrome oxidase produces nitric oxide under hypoxic conditions: implications for oxygen sensing and hypoxic signalling in eukaryotes. *Cell Metab.* 2006;3:277–87.
 50. Affourtit C, Bailey SJ, Jones AM, et al. On the mechanism by which dietary nitrate improves human skeletal muscle function. *Front Physiol.* 2015;6:211. doi:10.3389/fphys.2015.00211.
 51. Larsen FJ, Ekblom B, Sahlin K, et al. Effects of dietary nitrate on blood pressure in healthy volunteers. *N Engl J Med.* 2006;335:2792–3.
 52. Webb AJ, Patel N, Loukogeorgakis S, et al. Acute blood pressure lowering, vasoprotective and anti-platelet properties of dietary nitrate via bioconversion to nitrite. *Hypertension.* 2008;51:784–90.
 53. Siervo M, Lara J, Ogbonmwan I, et al. Inorganic nitrate and beetroot juice supplementation reduces blood pressure in adults: a systematic review and meta-analysis. *J Nutr.* 2013;143:818–26.
 54. Larsen FJ, Weitzberg E, Lundberg JO, et al. Effects of dietary nitrate on oxygen cost during exercise. *Acta Physiol.* 2007;191:59–66.
 55. Bailey SJ, Winyard P, Vanhatalo A, et al. Dietary nitrate supplementation reduces the O₂ cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. *J Appl Physiol.* 2009;107:1144–55.
 56. Pawlak-Chaouch M, Boissière J, Gamelin FX, et al. Effect of dietary nitrate supplementation on metabolic rate during rest and exercise in human: a systematic review and a meta-analysis. *Nitric Oxide.* 2016;53:65–76.
 57. Cermak NM, Gibala MJ, van Loon LJ. Nitrate supplementation's improvement of 10-km time-trial performance in trained cyclists. *Int J Sport Nutr Exerc Metab.* 2012;22:64–71.
 58. Porcelli S, Ramaglia M, Bellistri G, et al. Aerobic fitness affects the exercise performance responses to nitrate supplementation. *Med Sci Sports Exerc.* 2015;47:1643–51.
 59. McMahon NF, Leveritt MD, Pavey TG. The effect of dietary nitrate supplementation on endurance exercise performance in healthy adults: a systematic review and meta-analysis. *Sports Med.* 2017;47(4):735–56. doi:10.1007/s40279-016-0617-7.
 60. Clements WT, Lee SR, Bloomer RJ. Nitrate ingestion: a review of the health and physical performance effects. *Nutrients.* 2014;6:5224–64.
 61. Jones AM, Ferguson SK, Bailey SJ, et al. Fiber-type specific effects of dietary nitrate. *Exerc Sport Sci Rev.* 2016;44:53–60.
 62. Bailey SJ, Fulford J, Vanhatalo A, et al. Dietary nitrate supplementation enhances muscle contractile efficiency during knee-extensor exercise in humans. *J Appl Physiol.* 2010;109:135–48.
 63. Vanhatalo A, Bailey SJ, Blackwell JR, et al. Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate-intensity and incremental exercise. *Am J Physiol.* 2010;299:1121–31.
 64. Shannon OM, Duckworth L, Barlow MJ, et al. Dietary nitrate supplementation enhances high-intensity running performance in moderate normobaric hypoxia, independent of aerobic fitness. *Nitric Oxide.* 2016;30:59–63.
 65. Carriker CR, Mermier CM, McLain TA, et al. Effect of acute dietary nitrate consumption on oxygen consumption during submaximal exercise in hypobaric hypoxia. *Int J Sport Nutr Exerc Metab.* 2015;26:315–22.
 66. MacLeod KE, Nugent SF, Barr SI, et al. Acute beetroot juice supplementation does not improve cycling performance in normoxia or moderate hypoxia. *Int J Sport Nutr Exerc Metab.* 2015;25:359–66.
 67. Arnold JT, Oliver SJ, Lewis-Jones TM, et al. Beetroot juice does not enhance altitude running performance in well-trained athletes. *Appl Physiol Nutr Metab.* 2015;40:590–5.
 68. Bourdillon N, Fan JL, Uva B, et al. Effect of oral nitrate supplementation on pulmonary hemodynamics during exercise and time trial performance in normoxia and hypoxia: a randomized controlled trial. *Front Physiol.* 2015;6:288. doi:10.3389/fphys.2015.00288.
 69. Fulford J, Winyard PG, Vanhatalo A, et al. Influence of dietary nitrate supplementation on human skeletal muscle metabolism and force production during maximum voluntary contractions. *Eur J Physiol.* 2013;465:517–28.
 70. Larsen FJ, Schiffer TA, Borniquel S, et al. Dietary inorganic nitrate improves mitochondrial efficiency in humans. *Cell Metab.* 2011;13:149–59.
 71. Bergstrom M, Hultman E. Energy cost and fatigue during intermittent electrical stimulation of human skeletal muscle. *J Appl Physiol.* 1988;65:1500–5.
 72. Barclay CJ, Woledge RC, Curtin NA. Energy turnover for Ca²⁺ cycling in skeletal muscle. *J Muscle Res Cell Motil.* 2007;28:259–74.
 73. Galler S, Hilber K, Göbesberger A. Effects of nitric oxide on force-generating proteins of skeletal muscle. *Eur J Physiol.* 1997;434:242–5.

74. Heunks LM, Cody MJ, Geiger PC, et al. Nitric oxide impairs Ca^{2+} activation and slows cross-bridge cycling kinetics in skeletal muscle. *J Appl Physiol.* 2001;91:2233–9.
75. Ishii T, Sunami O, Saitoh N, et al. Inhibition of skeletal muscle sarcoplasmic reticulum Ca^{2+} -ATPase by nitric oxide. *FEBS Lett.* 1998;440:218–22.
76. Viner RI, Williams TD, Schöneich C. Nitric oxide-dependent modification of the sarcoplasmic reticulum Ca-ATPase: localization of cysteine target sites. *Free Radic Biol Med.* 2000;29:489–96.
77. Hernandez A, Schiffer TA, Ivarsson N, et al. Dietary nitrate increases tetanic $[\text{Ca}^{2+}]_i$ and contractile force in mouse fast-twitch muscle. *J Physiol.* 2012;590:3575–83.
78. Brown GC, Cooper CE. Nanomolar concentrations of nitric oxide reversibly inhibit synaptosomal respiration by competing with oxygen at cytochrome oxidase. *FEBS Lett.* 1994;356:295–8.
79. Whitfield J, Ludzki A, Heigenhauser GJF, et al. Beetroot juice supplementation reduces whole body oxygen consumption but does not improve indices of mitochondrial efficiency in human skeletal muscle. *J Physiol.* 2016;594:421–35.
80. Kemp GJ. Beetroot juice supplementation reduces the oxygen cost of exercise without improving mitochondrial efficiency: but how? *J Physiol.* 2016;594:253.
81. Flueck JL, Bogdanova A, Mettler S, et al. Is beetroot juice more effective than sodium nitrate? The effects of equimolar nitrate dosages of nitrate-rich beetroot juice and sodium nitrate on oxygen consumption during exercise. *Appl Physiol Nutr Metab.* 2016;41:421–9.
82. Breese BC, McNarry MA, Marwood S, et al. Beetroot juice supplementation speeds O_2 uptake kinetics and improves exercise tolerance during severe-intensity exercise initiated from an elevated metabolic rate. *Am J Physiol.* 2013;305:1441–50.
83. Lansley KE, Winyard PG, Fulford J, et al. Dietary nitrate supplementation reduces the O_2 cost of walking and running: a placebo-controlled study. *J Appl Physiol.* 2011;110:591–600.
84. Ferguson SK, Holdsworth CT, Wright JL, et al. Microvascular oxygen pressures in muscles comprised of different fiber types: impact of dietary nitrate supplementation. *Nitric Oxide.* 2015;48:38–43.
85. Hughson RL, Kowalchuk JM. Kinetics of oxygen uptake for submaximal exercise in hyperoxia, normoxia, and hypoxia. *Can J Appl Physiol.* 1995;20:198–210.
86. Burnley M, Jones AM. Oxygen uptake kinetics as a determinant of sports performance. *Eur J Sport Sci.* 2007;7:63–79.
87. Hennis PJ, Mitchell K, Gilbert-Kawai E, et al. Effects of dietary nitrate supplementation on symptoms of acute mountain sickness and basic physiological responses in a group of male adolescents during ascent to Mount Everest Base Camp. *Nitric Oxide.* 2016;60:24–31.
88. Engan HK, Jones AM, Ehrenberg F, et al. Acute dietary nitrate supplementation improves dry static apnea performance. *Respir Physiol Neurobiol.* 2012;182:53–9.
89. Patrician A, Schagatay E. Dietary nitrate enhances arterial oxygen saturation after dynamic apnea. *Scand J Med Sci Sports.* 2017;27(6):622–6. doi:10.1111/sms.12684.
90. Schiffer TA, Larsen FJ, Lundberg JO, et al. Effects of dietary inorganic nitrate on static and dynamic breath-holding in humans. *Respir Physiol Neurobiol.* 2013;185:339–48.
91. Benoit H, Busso T, Castells J, et al. Influence of hypoxic ventilatory response on arterial O_2 saturation during maximal exercise in acute hypoxia. *Eur J Appl Physiol.* 1995;72:101–5.
92. Baumgartner RW, Bärtsch P, Maggiorini M, et al. Enhanced cerebral blood flow in acute mountain sickness. *Aviat Space Environ Med.* 1994;65:726–9.
93. Ferrari M, Mottola L, Quaresima V. Principles, techniques, and limitations of near infrared spectroscopy. *Can J Appl Physiol.* 2004;29:463–87.
94. Calbet JL, Boushel R, Rådegran G, et al. Determinants of maximal oxygen uptake in severe acute hypoxia. *Am J Physiol.* 2003;284:291–303.
95. Ferguson SK, Hirai DM, Copp SW, et al. Impact of dietary nitrate supplementation via beetroot juice on exercising muscle vascular control in rats. *J Physiol.* 2013;591:547–57.
96. Tew GA, Ruddock AD, Saxton JM. Skin blood flow differentially affects near-infrared spectroscopy-derived measures of muscle oxygen saturation and blood volume at rest and during dynamic leg exercise. *Eur J Appl Physiol.* 2010;110:1083–9.
97. Neary JP. Application of near infrared spectroscopy to exercise sports science. *Can J Appl Physiol.* 2004;29:488–503.
98. Allen DG, Lamb GD, Westerblad H. Skeletal muscle fatigue: cellular mechanisms. *Physiol Rev.* 2008;88:287–332.
99. Vanhatalo A, Jones AM, Blackwell JR, et al. Dietary nitrate accelerates post-exercise muscle metabolic recovery and O_2 delivery in hypoxia. *J Appl Physiol.* 2014;117:1460–70.
100. Jacobi B, Bongartz G, Partovi S, et al. Skeletal muscle BOLD MRI: from underlying physiological concepts to its usefulness in clinical conditions. *J Magn Reson Imaging.* 2012;35:1253–65.
101. Bahra M, Kapil V, Pearl V, et al. Inorganic nitrate ingestion improves vascular compliance but does not alter flow-mediated dilation in healthy volunteers. *Nitric Oxide.* 2012;26:197–202.
102. Pironti G, Ivarsson N, Yang J, et al. Dietary nitrate improves cardiac contractility via enhanced cellular Ca^{2+} signaling. *Basic Res Cardiol.* 2016;111:34.
103. Lefferts WK, Hughes WE, White CN, et al. Effect of acute nitrate supplementation on neurovascular coupling and cognitive performance in hypoxia. *Appl Physiol Nutr Metab.* 2016;41:133–41.
104. Ingram TE, Pinder AG, Bailey DM, et al. Low-dose sodium nitrite vasodilates hypoxic human pulmonary vasculature by a means that is not dependent on a simultaneous elevation in plasma nitrite. *Am J Physiol.* 2010;298:331–9.
105. Casey DP, Treichler DP, Ganger CT, et al. Acute dietary nitrate supplementation enhances compensatory vasodilation during hypoxic exercise in older adults. *J Appl Physiol.* 2015;118:178–86.
106. Kenjale AA, Ham KL, Stabler T, et al. Dietary nitrate supplementation enhances exercise performance in peripheral arterial disease. *J Appl Physiol.* 2011;110:1582–91.
107. Harris RA, Nishiyama SK, Wray DW, et al. Ultrasound assessment of flow-mediated dilation. *Hypertension.* 2010;55:1075–85.
108. Thijssen DHJ, Black MA, Pyke KE, et al. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *Am J Physiol.* 2011;300:2–12.
109. Green DJ, Jones H, Thijssen D, et al. Flow-mediated dilation and cardiovascular event prediction: does nitric oxide matter? *Hypertension.* 2011;57:363–9.
110. Green D. Point: Flow-mediated dilation does reflect nitric oxide-mediated endothelial function. *J Appl Physiol.* 2005;99:1233–4.
111. Green DJ, Dawson EA, Groenewoud HMM, et al. Is flow-mediated dilation nitric oxide mediated? A meta-analysis. *Hypertension.* 2014;63:376–82.
112. Tschakovsky ME, Pyke KE. Counterpoint: Flow-mediated dilation does not reflect nitric oxide-mediated endothelial function. *J Appl Physiol.* 2005;99:1235–7.
113. Pyke K, Green DJ, Weisbrod C, et al. Nitric oxide is not obligatory for radial artery flow-mediated dilation following release of 5 or 10 min distal occlusion. *Am J Physiol.* 2010;298:119–26.
114. Parker BA, Tschakovsky ME, Augeri AL, et al. Heterogenous vasodilator pathways underlie flow-mediated dilation in men and women. *Am J Physiol.* 2011;301:1118–26.

115. Wray DW, Witman MAH, Ives SJ, et al. Does brachial artery flow-mediated vasodilation provide a bioassay for NO? *Hypertension*. 2013;62:345–51.
116. Whipp BJ, Ward SA. Quantifying intervention-related improvements in exercise tolerance. *Eur Respir J*. 2009;33:1254–60.
117. Wilkerson DP, Hayward GM, Bailey SJ, et al. Influence of acute dietary nitrate supplementation on 50 mile time trial performance in well-trained cyclists. *Eur J Appl Physiol*. 2012;112:4127–34.
118. Cermak NM, Res P, Stinkens R, et al. No improvement in endurance performance after a single dose of beetroot juice. *Int J Sport Nutr Exerc Metab*. 2012;22:470–8.
119. Peacock U, Tjønnå AE, James P, et al. Dietary nitrate does not enhance running performance in elite cross-country skiers. *Med Sci Sports Exerc*. 2012;44:2213–9.
120. Bescós R, Ferrer-Roca V, Galilea PA, et al. Sodium nitrate supplementation does not enhance performance of endurance athletes. *Med Sci Sports Exerc*. 2012;44:2400–9.
121. Christensen PM, Nyberg M, Bangsbo J. Influence of nitrate supplementation on VO₂ kinetics and endurance of elite cyclists. *Scand J Med Sci Sports*. 2013;23:e21–31.
122. Lowings S, Shannon OM, Deighton K, et al. Effect of dietary nitrate supplementation on swimming performance in trained swimmers. *Int J Sport Nutr Exerc Metab*. 2017:1–24. doi:10.1123/ijsnem.2016-0251.
123. Kelly J, Fulford J, Vanhatalo A, et al. Effects of short-term dietary nitrate supplementation on blood pressure, O₂ uptake kinetics, and muscle and cognitive function in older adults. *Am J Physiol*. 2013;304:73–83.
124. Modin A, Björne H, Herulf M, et al. Nitrite-derived nitric oxide: a possible mediator of “acidic-metabolic” vasodilation. *Acta Physiol Scand*. 2001;171:9–16.
125. Lansley KE, Winyard PG, Bailey SJ, et al. Acute dietary nitrate supplementation improves cycling time trial performance. *Med Sci Sports Exerc*. 2011;43:1125–31.
126. Bond H, Morton L, Braakhuis AJ. Dietary nitrate supplementation improves rowing performance in well-trained rowers. *Int J Sport Nutr Exerc Metab*. 2012;22:251–6.
127. Lane SC, Hawley JA, Desbrow B, et al. Single and combined effects of beetroot juice and caffeine supplementation on cycling time trial performance. *Appl Physiol Nutr Metab*. 2014;39:1050–7.
128. Kuennen M, Jansen L, Gillum T, et al. Dietary nitrate reduces the O₂ cost of desert marching but elevates the rise in core temperature. *Eur J Appl Physiol*. 2015;115:2557–69.
129. Wylie LJ, Ortiz de Zavallos J, Isidore T, et al. Dose-dependent effects of dietary nitrate on the oxygen cost of moderate-intensity exercise: acute vs. chronic supplementation. *Nitric Oxide*. 2016;57:30–9.
130. Hoon MW, Jones AM, Johnson NA, et al. The effect of variable doses of inorganic nitrate-rich beetroot juice on simulated 2,000-m rowing performance in trained athletes. *Int J Sports Physiol Perform*. 2014;9:615–20.
131. Martin DS, Gilbert-Kawai ET, Meale PM, et al. Design and conduct of “Xtreme Alps”: a double-blind, randomised controlled study of the effects of dietary nitrate supplementation on acclimatisation to high altitude. *Contemp Clin Trials*. 2013;36:450–9.
132. Coppel J, Hennis P, Gilbert-Kawai E, et al. The physiological effects of hypobaric hypoxia versus normobaric hypoxia: a systematic review of crossover trials. *Extreme Physiol Med*. 2015;4:2. doi:10.1186/s13728-014-0021-6.
133. Faiss R, Pialoux V, Sartori C, et al. Ventilation, oxidative stress, and nitric oxide in hypobaric versus normobaric hypoxia. *Med Sci Sports Exerc*. 2013;45:253–60.
134. Smith LW, Smith JD, Criswell DS. Involvement of nitric oxide synthase in skeletal muscle adaptation to chronic overload. *J Appl Physiol*. 2002;92:2005–11.
135. Kingwell BA, Sherrard B, Jennings GL, et al. Four weeks of cycle training increases basal production of nitric oxide from the forearm. *Am J Physiol*. 1997;272:1070–7.
136. Wadley GD, McConell GK. Effect of nitric oxide synthase inhibition on mitochondrial biogenesis in rat skeletal muscle. *J Appl Physiol*. 2007;102:314–20.
137. Wadley GD, Choate J, McConell GK. NOS isoform-specific regulation of basal but not exercise-induced mitochondrial biogenesis in mouse skeletal muscle. *J Physiol*. 2007;585:253–62.
138. Puype J, Ramaekers M, Van Thienen R, et al. No effect of dietary nitrate supplementation on endurance training in hypoxia. *Scand J Med Sci Sports*. 2014;25:234–41.
139. De Smet S, Van Thienen R, Deldicque L, et al. Nitrate intake promotes shift in muscle fiber type composition during sprint interval training in hypoxia. *Front Physiol*. 2016;7:233. doi:10.3389/fphys.2016.00233.
140. Muggeridge DJ, Sculthorpe N, James PE, et al. The effects of dietary nitrate supplementation on the adaptations to sprint interval training in previously untrained males. *J Sci Med Sport*. 2017;20:92–7.
141. Thompson C, Wylie LJ, Blackwell JR, et al. Influence of dietary nitrate supplementation on physiological and muscle metabolic adaptations to sprint interval training. *J Appl Physiol*. 2016;122(3):642–52. doi:10.1152/jappphysiol.00909.2016.
142. Gaesser GA, Wilson LA. Effects of continuous and interval training on the parameters of the power-endurance time relationship for high-intensity exercise. *Int J Sports Med*. 1988;9:417–21.
143. Laursen PB, Shing CM, Peake JM, et al. Interval training program optimization in highly trained endurance cyclists. *Med Sci Sports Exerc*. 2002;34:1801–7.
144. Burgomaster KA, Howarth KR, Phillips SM, et al. Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. *J Physiol*. 2008;586:151–60.
145. Seiler S, Jørranson K, Olesen BV, et al. Adaptations to aerobic interval training: interactive effects of exercise intensity and total work duration. *Scand J Med Sci Sports*. 2013;23:74–83.
146. Esteve-Lanao J, San Juan AF, Earnest CP, et al. How do endurance runners actually train? Relationship with competition performance. *Med Sci Sports Exerc*. 2005;37:496–504.
147. Seiler S. What is best practice for training intensity and duration distribution in endurance athletes? *Int J Sports Physiol Perform*. 2010;5:276–91.
148. Tønnessen E, Sylta Ø, Haugen TA, et al. The road to gold: training and peaking characteristics in the year prior to a gold medal endurance performance. *PLoS One*. 2014;9(7):e101796. doi:10.1371/journal.pone.0101796.