“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 (NO3−) 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 NO3− supplementation during exposure to simulated and terrestrial altitude. Previous investigations at simulated altitude indicate that NO3− supplementation may reduce the oxygen cost of exercise, elevate arterial and tissue oxygenation, improve muscle metabolic function, and enhance exercise capacity/performance. Conversely, current evidence suggests that NO3− supplementation does not augment the training response at simulated altitude. Few studies have evaluated the effects of NO3− at terrestrial altitude. Current evidence indicates potential improvements in endothelial function at terrestrial altitude following NO3− supplementation. No effects of NO3− 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 (NO3−) 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 NO3− supplementation has no effect on performance-related adaptations, although further investigations are warranted.

Additional studies are required to confirm whether the beneficial effects of NO3− supplementation that have been demonstrated at simulated altitude also manifest at terrestrial altitude.

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 (PO₂), such that the high-altitude environment is hypoxic relative to sea level (normoxia). Exposure to this hypoxic environment decreases the amount of oxygen (O₂) reaching the lungs, blood, and ultimately tissue [2, 3]. Consequently, maximal O₂ consumption (VO₂max) decreases [4, 5]. The absolute VO₂ 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 VO₂max 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 O₂ 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 O₂ delivery and the metabolic demands of muscle and cerebral tissue when O₂ 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 O₂ 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 (NO₃⁻) and nitrite (NO₂⁻) through the recently elucidated NO₃⁻–NO₂⁻–NO pathway [37]. Consumption of NO₃⁻-rich foods such as leafy green vegetables and beetroot or NO₃⁻-salts (e.g., sodium [Na]- or potassium [K⁺]-NO₃⁻) rapidly and substantially elevates plasma [NO₃⁻] (square brackets denote concentration) [38]. Exogenous NO₃⁻ mixes with endogenously derived NO₃⁻, which is an oxidation product of the L-arginine NOS pathway, in the blood and can be slowly reduced to NO₂⁻ by the enzyme xanthine oxidoreductase [39]. A more rapid pathway for NO₃⁻ reduction also exists, which is facilitated through recirculation of NO₃⁻ into the mouth via the salivary glands [40]. Here, oral bacteria reduce NO₃⁻ to NO₂⁻ via reductase enzymes [41]. A portion of the bacterially generated NO₂⁻ 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 NO₃⁻ reduction are all considerably enhanced in hypoxic conditions [37]. The NO₃⁻–NO₂⁻–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 NO₂⁻ on skeletal muscle and mitochondrial function and in hypoxia-induced vasodilation [50]. Thus, any effects of NO₃⁻ consumption might, at least in part, be related to NO₂⁻-dependent signaling, rather than being solely attributable to NO [50].

Interestingly, recent evidence from studies conducted at sea level suggests that NO₃⁻ supplementation can reduce resting blood pressure (BP) [51–53], lower the O₂ cost of exercise [54–56], and augment both exercise capacity and performance [55, 57–59]. Logically, greater effects of NO₃⁻ supplementation might be expected in hypoxia, given the particularly important role that NO plays in this
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.

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.

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 [FIO₂] whilst maintaining sea-level barometric pressure), and hypobaric hypoxia (i.e., the altitude PO₂ is replicated by lowering the barometric pressure whilst the FIO₂ is unchanged from sea-level values). Terrestrial altitudes ranged from 1300 to 5300 m. Reported simulated altitudes were ~2500–5000 m (FIO₂ 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 VO$_2$ consequent to NO$_3^-$ supplementation with VO$_2$ ~5% lower during cycle ergometry exercise in normoxia. This effect was unexpected, as the VO$_2$ 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. VO$_2$ was ~4% lower during cycle ergometry exercise at 45% peak O$_2$ uptake (VO$_{2peak}$) in extreme normobaric hypoxia (F$\text{O}_2$ 11%, ~5000 m). Subsequent studies have reported VO$_2$ 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$\text{O}_2$ 11–15.4%, ~2500–5000 m). Interestingly, one study reported lower steady-state VO$_2$ in normobaric hypoxia (F$\text{O}_2$ 13.1%, ~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 VO$_2$ during acute exercise in hypobaric hypoxia [65]. In contrast to some evidence obtained in normobaric hypoxia, NO$_3^-$ supplementation did not influence VO$_2$ 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 (VO$_{2max}$ at 1600 m altitude: ~61 ml·kg$^{-1}$·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 VO$_2$ during sub-maximal exercise in normobaric hypoxia (F$\text{O}_2$ 11–15.4%, ~2500–5000 m) in highly-trained individuals (VO$_{2max}$: ~60 ml·kg$^{-1}$·min$^{-1}$) [66–68]. Moreover, a recent investigation at sea level reported a significant inverse relationship between VO$_{2peak}$ and the reduction in VO$_2$ consequent to NO$_3^-$ supplementation [58]. However, direct comparison of the effects of NO$_3^-$ ingestion on VO$_2$ in normobaric and hypobaric hypoxia is warranted to exclude the influence of other potentially confounding methodological variables.

The reduction in VO$_2$ 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 VO$_2$ 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 VO$_2$ 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 VO$_2$. 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 VO$_2$ [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 NO₃⁻-rich beetroot juice on mitochondrial efficiency despite a reduced VO₂. These disparate results may be related to the form of NO₃⁻ administered (i.e., NaNO₃ vs. beetroot juice), although direct comparison is required to eliminate other methodological discrepancies [80]. Interestingly, one recent study [81] reported greater reductions in VO₂ following beetroot juice versus NaNO₃ supplementation in normoxia, possibly as a consequence of the beetroot juice polyphenols and antioxidants enhancing NO generation, further suggesting different effects of these two NO₃⁻-containing supplements.

3.2 O₂ Uptake Kinetics

Normoxic studies have reported faster phase II VO₂ kinetics during the transition from moderate to severe [82], but not unloaded to moderate [55, 82, 83], intensity exercise following NO₃⁻ supplementation. The apparently greater effects of NO₃⁻ during the moderate to severe intensity transition might be related to type II muscle fiberspecific effects of NO₃⁻ [61, 77, 84], given greater relative activation of these fibers during this transition [82]. In hypoxia, VO₂ kinetics are typically slowed compared with normoxia [36, 85]. Interestingly, Kelly et al. [36] observed faster phase II VO₂ kinetics during the step transition from unloaded to moderate-intensity but not moderate- to severe-intensity exercise in normobaric hypoxia [36]. Conversely, NO₃⁻ supplementation did not influence VO₂ kinetics in normoxia in this study [36]. The precise reasons why NO₃⁻ had no effect on VO₂ kinetics during the moderate to severe transition in hypoxia, or either transition in normoxia, are unclear. Nevertheless, the speeding of VO₂ 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 O₂ deficit [86].

3.3 Arterial O₂ Saturation

NO₃⁻ supplementation has been reported to result in a small (1–4%) increase in arterial O₂ saturation (SₐO₂), 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 NO₃⁻ on SₐO₂ during acute exercise in a hypobaric hypoxic chamber (~3500 m).

Only one study has examined the effects of NO₃⁻ supplementation on SₐO₂ at terrestrial altitude [87]. Hennis et al. [87] observed no effect of NO₃⁻ on SₐO₂ 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 NO₃⁻ as a substrate for NO generation. NO₃⁻ 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 NO₃⁻ supplementation on SₐO₂ when O₂ availability is restricted. NO₃⁻ supplementation has been reported to elevate SₐO₂ during static [88] and dynamic [89] apneas. Conversely, Schiffer et al. [90] observed lower SₐO₂ values and decreased static apnea duration following NO₃⁻ supplementation. However, NO₃⁻ supplementation tended to elevate SₐO₂ 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’), NO₃⁻ supplementation strategy, and pre-apnea hyperventilation procedures. Nevertheless, the evidence that NO₃⁻ supplementation can elevate SₐO₂ in certain apnea situations, particularly during dynamic apnea, provides support for the increase in SₐO₂ observed during exercise in hypoxia [29, 30, 64, 68].

Mechanistically, the elevated SₐO₂ reported during exercise in hypoxia following NO₃⁻ supplementation may be due to reduced muscle O₂ extraction consequent to improvements in the efficiency of muscle contraction [62] and/or mitochondrial respiration [70]. Additionally, in one study, NO₃⁻ supplementation increased pulmonary ventilation (VE) in conjunction with an elevated SₐO₂ during exercise in hypoxia [68]. Given a higher VE has previously been associated with reduced arterial desaturation [91], it is possible that an increased VE following NO₃⁻ supplementation might also play a role in elevating SₐO₂. An elevated SₐO₂ could have important implications for hypoxic exercise performance, as discussed in Sect. 4. Theoretically, an elevated SₐO₂ could also help attenuate acute mountain sickness [92], although current studies have reported no significant effect of NO₃⁻ on acute mountain sickness [29, 87].

3.4 Tissue Oxygenation

Masschelein et al. [29] reported an increase in the nearinfrared spectroscopy (NIRS)-derived tissue oxygenation index (TOI) of the vastus lateralis during cycle ergometry exercise in normobaric hypoxia (FIO₂ 11%, ~5000 m) following NO₃⁻ supplementation. Kelly et al. [36] also reported a tendency towards elevated TOI during
moderate-intensity exercise in normobaric hypoxia (FIO2 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 O2 efficiency consequent to NO3−. Alternatively, the increased TOI could reflect a greater O2 delivery to the muscle due to elevated tissue blood flow [93]. As both hypoxia [94] and NO3− [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 PO2 [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+, Pi, 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 NO3− supplementation might ameliorate the undesirable effects of hypoxia on muscle metabolic function.

NO3− supplementation attenuated the rate of change in muscle [PCr], [Pi], and pH as assessed via 31P-magnetic resonance spectroscopy (31P-MRS) during leg extension exercise in normobaric hypoxia (FIO2 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 (FIO2 13%, ~3500 m), the faster PCr recovery kinetics with NO3− 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 NO3− supplementation may indicate a greater delivery of freshly oxygenated blood to the muscle, increasing the O2 driving pressure into the muscle cells, and resulting in faster oxidative ATP resynthesis and PCr regeneration [99]. NO3− 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 NO3− supplementation, with the magnitude of the decline inversely correlated with plasma [NO2−]. 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, NO3− supplementation may enhance both muscle energetics and O2 delivery during hypoxic exercise, which might partly underpin the improvements in exercise performance discussed in Sect. 4.

3.6 Cardiovascular Response

In normoxia, NO3− supplementation has been reported to reduce BP [51–53] and enhance vascular compliance [101] in humans and improve cardiac contractility in rodents [102]. Dietary NO3− 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 NO3− supplementation. The reduced BP following NO3− supplementation has been attributed to increased NO2− and NO generation and subsequent vasodilation [52, 53]. Recently, Ingram et al. [104] reported pulmonary and arterial vasodilation after NO2− infusion in normobaric hypoxia (FIO2 12%, ~4100 m) but not normoxia. The greater activity of NO2− 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 [NO2−] had returned to baseline values. This suggests possible storage and metabolism of NO2−, 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 NO3− supplementation might also be more likely in hypoxia than in normoxia, although direct comparison is required.

In acute normobaric hypoxia (FIO2 ~11.6%, ~4600 m), NO3− supplementation neither influenced the hemodynamic response nor augmented cardiac unloading in healthy men [103]. Similarly, NO3− had no effect on the hypoxia-induced hyperemic response to handgrip exercise in young adults (mean ± standard deviation [SD] age 25 ± 1 years) exercising in normoxia and acute normobaric hypoxia (FIO2 titrated to achieve ~80% SIO2) [105]. Interestingly, despite similar increases in plasma [NO2−], NO3− supplementation significantly increased the vasodilation and blood flow response to hypoxic exercise in older participants.
(mean ± 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 ± 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 (FIO$_2$ 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 (FIO$_2$ 11%, ∼5000 m). Cycle ergometry TTE was reduced by 36% in hypoxia versus normoxia. However, NO$_3^-$ supplementation attenuated ∼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 VO$_2$ is predominately 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 (FIO$_2$ 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 ± SD VO$_2$peak: 58.3 ± 6.3 ml·kg$^{-1}$·min$^{-1}$) exercising in normobaric hypoxia (FIO$_2$ 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 (FIO$_2$ 15%, ∼2500 m) following NO$_3^-$ supplementation in participants across a range of different fitness levels (VO$_2$max range: 47.1–76.8 ml·kg$^{-1}$·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 | Supplementation protocol | Performance assessment | Trial results | Cohen’s d |
|------------|-----------------------------|---------------------------|------------------------|--------------|-----------|
| Vanhatalo et al. [11] | 7 M, 2 F; moderately trained (\(\dot{V}O_{2\text{peak}}, \text{NS}\)) | 750 ml BRJ (9.3 mmol NO\(_3^-\)) 24 h prior, with last 250 ml dose 150 min pre-exercise | Leg extension TTE (\(F_{\text{O}_2}\) 14.5%, \(\sim 2800\) m) | Normoxia: 471 ± 200 s  Hypoxia (PLA): 393 ± 169 s \(^b\)  Hypoxia (NIT): 477 ± 200 s \(^b\) | 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 (\(\dot{V}O_{2\text{peak}}, 61.7 \pm 2.1\) ml kg\(^{-1}\) min\(^{-1}\)) | \(~500\) ml d\(^{-1}\) BRJ (0.7 mmol kg\(^{-1}\) day\(^{-1}\) / \(\sim 5\) mmol day\(^{-1}\) NO\(_3^-\)) consumed daily for 6 days, with last 500 ml dose 60–120 min pre-exercise | Incremental cycle ergometry TTE (\(F_{\text{O}_2}\) 11%, \(\sim 5000\) m) | Normoxia vs. hypoxia PLA vs. hypoxia NIT: 2.69  Normoxia vs. hypoxia NIT: 2.47  Hypoxia PLA vs. hypoxia NIT: 0.33 |
| Muggeridge et al. [30] | 9 M; trained (\(\dot{V}O_{2\text{peak}}\) at simulated altitude: 51.9 ± 5.6 ml kg\(^{-1}\) min\(^{-1}\)) | 70 ml concentrated BRJ (\(~5\) mmol NO\(_3^-\)) consumed daily for 3 days, with last 140 ml dose consumed 150 min pre-exercise | 16.1 km cycle ergometry TT (\(F_{\text{O}_2}\) 15%, \(\sim 2500\) m) | Hypoxia (PLA): 1702 ± 45 s  Hypoxia (NIT): 1664 ± 42 s \(^b\) | Hypoxia PLA vs. hypoxia NIT: 0.87 |
| Kelly et al. [36] | 12 M; physically active (\(\dot{V}O_{2\text{peak}}, 58.3 \pm 6.3\) ml kg\(^{-1}\) min\(^{-1}\)) | 70 ml concentrated BRJ (\(~5\) mmol NO\(_3^-\)) consumed daily for 3 days, with last 140 ml dose consumed 150 min pre-exercise | Cycle ergometry TT at 75% between GET and \(\dot{V}O_{2\text{peak}}\) (\(F_{\text{O}_2}\) 13.1%, \(\sim 3500\) m) | Normoxia (PLA): 431 ± 139 s  Normoxia (NIT): 412 ± 139 s  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 (\(\dot{V}O_{2\text{max}}, 66 \pm 7\) ml kg\(^{-1}\) min\(^{-1}\)) | 70 ml concentrated BRJ (\(~7\) mmol NO\(_3^-\)) consumed daily for 3 days, with last 140 ml dose consumed 150 min pre-exercise | 10,000 m treadmill TT (\(F_{\text{O}_2}\) 15.4%, \(\sim 4000\) m) | Normoxia (PLA): 2862 ± 233 s  Hypoxic (NIT): 2874 ± 265 s | Hypoxia PLA vs. hypoxia NIT: 0.05  Hypoxic (NIT): 402 ± 80 s |
| Arnold et al. [67] | 10 M; well-trained (\(\dot{V}O_{2\text{max}}, 66 \pm 7\) ml kg\(^{-1}\) min\(^{-1}\)) | 70 ml concentrated BRJ (\(~7\) mmol NO\(_3^-\)) consumed daily for 3 days, with last 140 ml dose consumed 150 min pre-exercise | Incremental treadmill TT (\(F_{\text{O}_2}\) 12.8%, \(\sim 4000\) 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 (\(\dot{V}O_{2\text{peak}}, 67.5 \pm 5.8\) ml kg\(^{-1}\) min\(^{-1}\)) | 70 ml concentrated BRJ (\(~6\) mmol NO\(_3^-\)) consumed daily for 3 days, with last 140 ml dose consumed 150 min pre-exercise | 10 km cycle ergometry TT (\(F_{\text{O}_2}\) 15%, \(\sim 2500\) m) | Normoxia (PLA): 954 ± 47 s  Hypoxia (NIT): 961 ± 54 s  Hypoxia PLA vs. hypoxia NIT: 1.29  Hypoxia PLA vs. hypoxia NIT: 1.01  Hypoxia PLA vs. hypoxia NIT: 0.10 |
| Bourdillon et al. [68] | 12 M; well-trained (\(\dot{V}O_{2\text{max}}\) NS) | 0.1 mmol kg\(^{-1}\) day\(^{-1}\) NaNO\(_3^-\) for 3 days | 15 km cycle ergometry TT (\(F_{\text{O}_2}\) 11%, \(\sim 5000\) m) | Normoxia (PLA): 1597 ± 96 s  Hypoxia (NIT): 1581 ± 63 s  Hypoxia (PLA): 2155 ± 687 s \(^b\)  Hypoxia (NIT): 2005 ± 309 s \(^b\) | Hypoxia PLA vs. hypoxia NIT: 0.29  Hypoxia PLA vs. hypoxia NIT: 1.78  Hypoxia PLA vs. hypoxia NIT: 1.14  Hypoxia PLA vs. hypoxia NIT: 0.20 |
performance in well-trained athletes (mean ± SD \( \overline{\text{VO}_2}\text{peak} \) 67.5 ± 5.8 ml·kg\(^{-1}\)·min\(^{-1}\)) exercising in either normobaric hypoxia (F\(_{\text{IO2}} \sim 15\%, \sim 2500\) m) or normoxia. Likewise, Bourdillon et al. [68] observed no significant difference in 15-km cycle ergometry TT performance in extreme normobaric hypoxia (F\(_{\text{IO2}} 11\%, \sim 5000\) m) or normoxia following NO\(_3^−\) supplementation. However, there was a non-significant tendency towards improved performance (~151 s faster) in hypoxia with NO\(_3^−\) ingestion. Similarly, Arnold et al. [67] reported no effect of NO\(_3^−\) ingestion on 10-km TT running performance in moderate normobaric hypoxia (F\(_{\text{IO2}} 15.4\%, \sim 2500\) m) or incremental running performance in extreme normobaric hypoxia (F\(_{\text{IO2}} 12.8\%, \sim 4000\) m) in highly trained runners (mean ± SD \( \overline{\text{VO}_2}\text{max} \) 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 NO\(_3^−\) supplementation [31]. However, it should be noted that none of these variables alone can entirely explain the apparent ergogenic effect of NO\(_3^−\) 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 NO\(_3^−\) on exercise performance [117–122]. Notably, Porcelli et al. [58] suggested that untrained and moderately trained (\( \overline{\text{VO}_2}\text{max} <60 \text{ ml·kg}^{-1} \cdot \text{min}^{-1} \)) individuals are more likely to benefit from NO\(_3^−\) supplementation than endurance-trained athletes (\( \overline{\text{VO}_2}\text{max} >60 \text{ ml·kg}^{-1} \cdot \text{min}^{-1} \)). 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 NO\(_3^−\) supplementation [59], bringing into question this relationship. Nevertheless, as evident in Shannon et al. [64], who observed beneficial effects of NO\(_3^−\) 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 NO\(_3^−\) supplementation. Given that NO\(_3^−\) supplementation appears to be more effective in hypoxia than in normoxia [123], it is possible that individuals who may not benefit from NO\(_3^−\) supplementation in normoxia (or at low/moderate altitudes) might derive an ergogenic effect in extreme hypoxia where the \( \text{NO}_3^−\rightarrow \text{NO}_2^−\rightarrow \text{NO} \) 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$_3^-$ supplementation. 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 VO$_2$ 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\textsubscript{IO₂} 12.5%, ~4000 m). Improvements in VO\textsubscript{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\textsubscript{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)-1α messenger RNA (mRNA) content, and glycogen breakdown during the TT. More recently, De Smet et al. [139] explored the effects of NO\textsubscript{3}− on adaptations to sprint interval training (SIT) in normobaric hypoxia (F\textsubscript{IO₂} 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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{3}− compared with both training individuals given a placebo and NO\textsubscript{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\textsubscript{3}− but not in the other conditions.

Based on the findings of these studies, it is possible that NO\textsubscript{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 (VO\textsubscript{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 (VO\textsubscript{2max}: ~42–50 ml·kg⁻¹·min⁻¹), who may exhibit a greater response to training with NO\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{3}− supplementation has been demonstrated to reduce pulmonary VO\textsubscript{2} and, in some cases, elevate S\textsubscript{O₂} 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\textsubscript{2} environment. Current evidence also suggests that NO\textsubscript{3}− supplementation can improve muscle energetics during exercise in normobaric hypoxia via effects on tissue O\textsubscript{2} delivery and consumption and might alter cardiovascular responses to normobaric and hypobaric hypoxia. In
contrast, whilst NO₃⁻ 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 NO₃⁻ 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 NO₃⁻ supplementation as a potential aid to “beet-ing” the mountain.

Compliance with Ethical Standards

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