Fiber Type-Specific Effects of Dietary Nitrate

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JONES, A.M., S.K. FERGUSON, S.J. BAILEY, A. VANHATALO, and D.C. POOLE. Fiber type-specific effects of dietary nitrate. *Exerc. Sport Sci. Rev.*, Vol. 44, No. 2, pp. 53–60, 2016. *Dietary nitrate supplementation increases circulating nitrite concentration, and the subsequent reduction of nitrite to nitric oxide is promoted in hypoxic environments. Given that PO_2 is lower in Type II compared with Type I muscle, this article examines the hypothesis that the ergogenicity of nitrate supplementation is linked to specific effects on vascular, metabolic, and contractile function in Type II muscle.* **Key Words:** nitrite, nitric oxide, supplementation, exercise, hypoxia, performance, fast-twitch fibers

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Key Points

- Nitric oxide (NO) is involved in an array of physiological processes that regulate skeletal muscle function. Dietary nitrate supplementation increases plasma nitrite concentration and therefore NO bioavailability. Nitrate supplementation may enhance exercise economy and performance, although its efficacy appears to be related both to the endurance training status of the participants and to the type of exercise being performed.
- We present evidence that nitrate supplementation may be particularly effective at augmenting physiological responses in type II (fast-twitch) muscle. The relatively low O_2 tension surrounding type II fibers may promote the reduction of nitrite to NO, thereby improving: local perfusion, fatigue resistance, fiber contractility and, ultimately, whole body exercise performance.
- This targeted effect of nitrate supplementation on type II muscle may have important implications for improving performance during high-intensity (intermittent) exercise in healthy adults and for helping to restore functional capacity in senescent and patient populations where increased reliance on type II muscle fibers contributes to compromised exercise tolerance.

INTRODUCTION

Nitric oxide (NO) is essential to life. It is a gaseous signaling molecule that is produced continuously to enable or facilitate a wide range of physiological processes including neurotransmission, vasodilatation, angiogenesis, cellular respiration, mitochondrial biogenesis, and muscular contraction. A failure to produce or use sufficient NO occurs in senescence and is a characteristic of many cardiovascular and metabolic diseases. The production of NO often is attributed exclusively to the canonical pathway in which the semi-essential amino acid ʟ-arginine is oxidized in a reaction catalyzed by the NO synthase (NOS) family of enzymes. The products of this reaction, nitrite (NO_2^-) and nitrate (NO_3^-), were thought originally to have no physiological relevance. However, it is now appreciated that, under certain physiological conditions (*e.g.*, in low-O_2 environments that may exist in some tissues such as contracting skeletal muscle), NO_2^- may undergo a one-electron reduction to re-form NO.

Dietary supplementation with the NOS pathway substrate, ʟ-arginine, seems to have a very limited effect on NO-related physiological processes (37). In contrast, consuming foodstuffs such as green leafy vegetables (spinach, lettuce, rocket/arugula) and beetroot, which are rich in inorganic NO_3^-, substantially increases the circulating plasma $[NO_3^-]$ and $[NO_2^-]$, thereby increasing the potential for O_2-independent NO synthesis (24). A crucial step in this NO_3^--NO_2^--NO pathway is the conversion of NO_3^- to NO_2^- by facultative bacteria residing in the mouth. It has been shown repeatedly that dietary NO_3^- intake results in a significant reduction of resting blood pressure for several hours, consistent with an NO-mediated peripheral vasodilatation. Other physiological effects of NO_3^- ingestion also have been reported, including a reduction in the O_2 cost of submaximal exercise, at least in nonelite athletes ((2,4,22,23) cf. (19)). This effect, which may

arise as a consequence of a reduced adenosine triphosphate (ATP) cost of muscle force production (2) or to enhanced mitochondrial efficiency (22), is surprising given that this variable has been considered to be essentially unaltered by factors such as subject health and age, as well as aerobic fitness and training status, at least for cycle exercise. Improved muscle efficiency and/or exercise economy would be anticipated to result in enhanced exercise performance, and this has led to many recreational and elite athletes consuming NO_3^--rich products, usually beetroot juice, precompetition and/or to support training (18). However, although NO_3^- supplementation has been reported to be performance enhancing in multiple studies involving recreationally active or moderately trained subjects (*e.g.*, (2,4,41,42)), it seems that elite endurance athletes do not benefit to the same extent (*e.g.*, 6,9, see 18 for review).

Although there are several factors that might explain the difference in responsiveness to NO_3^- supplementation between elite and nonelite subjects, including dose and duration of NO_3^- supplementation as well as genetic or training effects on NOS activity, muscle oxygenation, and mitochondrial function, likely differences in muscle fiber type composition between these populations also might be important (18). Although there is considerable heterogeneity both between and within muscle fiber types, compared with Type I (slow-twitch) fibers, Type II (fast-twitch) fibers differ in terms of myofibrillar protein content and calcium handling, typically

have lower mitochondrial and capillary density, and, therefore, rely relatively more on nonoxidative compared with oxidative pathways of ATP resynthesis (7). The lower O_2 availability in Type II muscle, reflected in O_2 tension (PO_2), in addition to possible differences in contractile function and efficiency characteristics, results in greater fatigability compared with Type I muscle. The predominant muscle fiber type in a given muscle reflects its function. For example, across muscles in the human leg, the soleus may be approximately 88% Type I fibers, whereas the rectus femoris may be only approximately 35% Type I fibers (17). Moreover, in elite athletes, endurance runners may have approximately 70% Type I fibers whereas sprinters may have approximately 40% Type I fibers in the quadriceps femoris (33).

In this article, we explore the hypothesis (schematized in Fig. 1) that the efficacy of dietary NO_3^- supplementation may be linked to specific physiological effects on Type II muscle fibers.

PHYSIOLOGICAL HYPOXIA AND THE EFFICACY OF NITRATE SUPPLEMENTATION

Because our hypothesis that Type II muscle fibers may be especially *targeted* by NO_3^- supplementation rests, at least in part, on differences in intramyocyte PO_2 between fiber types, we first consider the role of systemic and local O_2 availability on NO production and the efficacy of NO_3^- supplementation on physiological function in hypoxia.
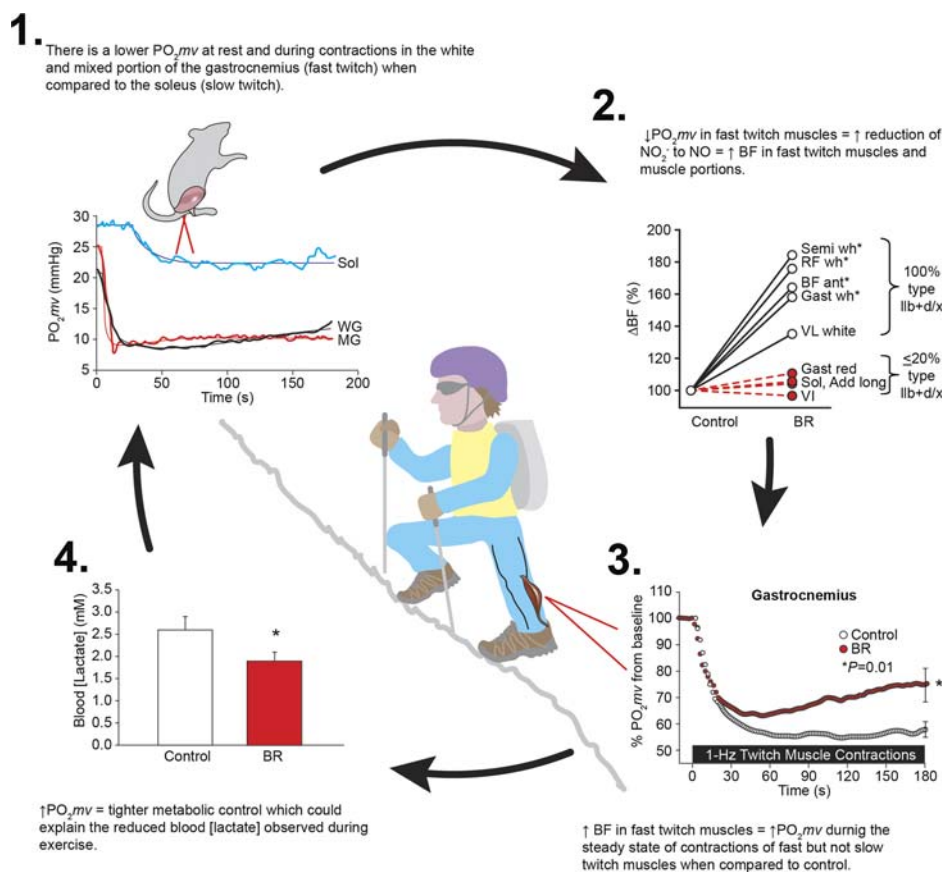
Figure 1. Proposed mechanisms by which dietary NO_3^- supplementation improves exercise performance. 1. Fast-twitch Type II muscles have a substantially lower microvascular PO_2 (PO_2mv) at rest and during contractions than their slow-twitch Type I counterparts (5). 2. This likely facilitates the reduction of NO_2^- to NO preferentially within fast-twitch Type II muscles, thus, increasing $\dot{Q}O_2$ in these tissues (12). 3. Preferential increases in $\dot{Q}O_2$ result in a greater $\dot{Q}O_2/\dot{V}O_2$ ratio in fast-twitch but not slow-twitch muscles (13). 4. This ultimately results in tighter metabolic control and thus a reduced accumulation of fatigue-associated metabolites such as blood lactate (12).

Nitric oxide bioavailability and bioactivity are dictated by an intricate balance between NO and O_2. Dependent on the metabolic rate and the matching of tissue O_2 delivery ($\dot{Q}O_2$), PO_2 fluctuates from approximately 40 mm Hg (5% O_2) at rest to as low as approximately 2 mm Hg (~0.4% O_2) during strenuous exertion (32). Nitric oxide production via NOS enzymes requires the presence of O_2. Given the role of NO as a *master regulator* of blood flow and cellular respiration, it is intuitive that a backup system is needed to guarantee NO bioavailability under the low-PO_2 conditions, which are native to skeletal muscle. Indeed, NO can be produced alternatively via the NOS-independent, single-electron reduction of NO_2^-, which is obtained through dietary NO_2^- and NO_3^- as well as via oxidation of endogenous NO (24). Importantly for maintenance of NO bioavailability, NO_2^- reduction to NO is stimulated by low PO_2 and low pH (24). Therefore, the therapeutic potential of the NO_3^- reduction pathway to compensate for dysfunctional NOS is substantial.

There is convincing evidence from *in vitro* experiments that physiologically critical NO-mediated signaling pathways that regulate skeletal muscle $\dot{Q}O_2$ and cellular bioenergetics are O_2 dependent (35). Low physiological PO_2 in active muscle stimulates erythrocyte-derived NO-mediated vascular relaxation, which serves to match $\dot{Q}O_2$ to local metabolic rate, whereas high PO_2 triggers vasoconstriction. NO regulates myocyte calcium flux via S-nitrosylation of thiols contained in ryanodine receptor (RyR1) Ca^{2+} channels. These are activated only only by NO at low physiological PO_2 (~10 mm Hg), such that low PO_2 in active muscle increases contractility, and high physiological PO_2 has the opposite effect. NO also may regulate mitochondrial respiration by reversible inhibition of cytochrome c oxidase in competition with O_2, such that greater inhibition by NO would occur at low physiological PO_2. It has been proposed that, collectively, the actions of NO_2^- and/or NO on O_2 consumption and vasodilatation might enable a more precise local matching of blood flow to metabolic rate (35). It is important to note here that although it is likely that the effects of dietary NO_3^- largely are mediated by NO, it is possible that NO_2^- and/or other bioactive NO_3^--derived molecules also play a role in the physiological effects observed after NO_3^- supplementation (1).

Restricted $\dot{Q}O_2$ to skeletal muscle reduces maximal oxidative metabolic rate (Q_{max}), which is reflected in a slowing of muscle phosphocreatine (PCr) recovery kinetics after exercise. For the same metabolic rate, there is a greater muscle metabolic perturbation (*e.g.*, greater fall in muscle PCr concentration during exercise performed in hypoxia compared with normoxia). *In vivo* evidence suggests that the O_2 demand of exercising muscle may be reduced during normoxic exercise after dietary NO_3^- supplementation ((2,4,22,23,36) cf. (19)), which might be consequent to an increased phosphate/oxygen ratio of mitochondrial respiration (22) and/or a reduced ATP cost of force production (2). Given that NO (and/or NO_2^-) has the potential to improve $\dot{Q}O_2$ as well as reduce the O_2 demand for a fixed work rate, dietary NO_3^- supplementation might be particularly important for improving skeletal muscle functional capacity under conditions where O_2 availability is low, irrespective of whether this is considered in terms of environmental, systemic, or local tissue O_2 availability.

The influence of dietary NO_3^- on exercise tolerance in healthy humans under hypoxic conditions has been investigated using manipulation of the inspired O_2 fraction. It was first shown that 24 h of NO_3^- supplementation (9.3 mmol·NO_3^-) reduced the extent of muscle metabolic perturbation during high-intensity knee-extension exercise and enhanced exercise tolerance in moderate hypoxia (14.5% O_2) ((38) Fig. 2). Dietary NO_3^- supplementation also abolishes the reduction in the rate of pH-independent PCr recovery, which typically is observed in hypoxia, and essentially restores muscle Q_{max} to values similar to those observed in normoxia ((38,39) Fig. 2). The speeding of Cr recovery was linked to improved muscle oxygenation during the immediate postexercise period in hypoxia but not in normoxia (assessed using effective transverse relaxation time weighted magnetic resonance signal) in healthy young adults (39). These data, therefore, suggest that, in hypoxia, dietary NO_3^- supplementation may facilitate NO production and enable greater muscle oxygenation, offsetting the deleterious effects of hypoxia on muscle function.

During whole body exercise in hypoxia, NO_3^- supplementation has been shown to reduce the O_2 cost of submaximal exercise by approximately 4% to 7% (19,25,27). Moreover, *muscle oxygenation*, defined as the total oxygenation index derived from near-infrared spectroscopy (NIRS), was elevated by 4% to 5% at rest and during both submaximal and maximal-intensity exercise after NO_3^- supplementation in hypoxia (11% O_2) (25). This may reflect the lower $\dot{V}O_2$ observed (25) or be indicative of NO-mediated enhanced bulk $\dot{Q}O_2$ and/or
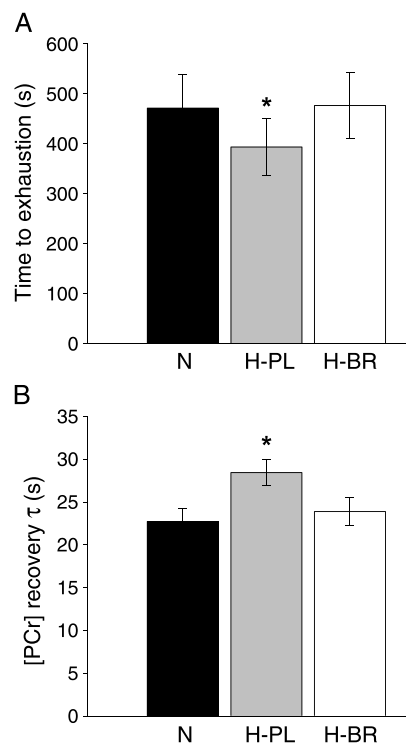
Figure 2. Time to exhaustion during high-intensity knee-extension exercise (A) and the muscle (PCr) recovery time constant (τ, B) in normoxia (*N*), in hypoxia with a placebo supplement (*H-PL*), and in hypoxia with a dietary nitrate supplement (*H-BR*). *Different from N and H-BR, $P < 0.05$. Error bars indicate SE. The figure has been drawn based on data reported in Vanhatalo *et al.* (38).

microvascular O_2 distribution during hypoxic exercise. These same factors may underpin the faster pulmonary $\dot{V}O_2$ kinetics after NO_3^- supplementation reported during exercise in hypoxia but not normoxia (19). Dietary NO_3^- ingestion also attenuated the hypoxia-induced reduction in exercise tolerance during both severe-intensity constant work rate exercise (by 9% (19)) and exhaustive incremental exercise (by 5% (25)). It seems that, for the same NO_3^- dose, NO_3^- supplementation is more effective during whole body exercise in hypoxia than in normoxia, with the improvement in high-intensity exercise tolerance being related to the reduced O_2 cost of submaximal exercise (19) perhaps because this would permit a better preservation of arterial O_2 saturation. Importantly, the beneficial effects by NO_3^- ingestion on exercise tolerance in hypoxia may translate to improved exercise performance; in one study, a 16.1-km cycling time trial performance was reported to be improved by approximately 2% by NO_3^- supplementation compared with placebo under 15% inspired O_2 (27).

The available evidence indicates that elevating NO bioavailability through a simple dietary intervention may particularly be beneficial in hypoxic conditions such as occurs during exposure to high altitude and in conditions where $\dot{Q}O_2$ is impaired, including various cardiovascular, pulmonary, and sleep disorders. Although the potentially greater physiological effects of NO_3^- supplementation pertain to when an individual exercises at altitude or breathes a gas containing a low fraction of O_2 at sea level (19), it is important to note that the ratio between local muscle blood flow and metabolic rate is heterogeneous even *within* skeletal muscle, and that Type II muscles and muscle fibers operate at a lower PO_2 than do Type I muscles and muscle fibers (5,26). It, therefore, is conceivable that the physiological effects of NO_2^- are more manifest in Type II than Type I muscle. In the next section, we consider the potential influence of *local* hypoxia on NO bioavailability and the efficacy of NO_3^- supplementation.

MUSCLE FIBER TYPE SELECTIVITY OF NITRATE SUPPLEMENTATION: ANIMAL STUDIES

Skeletal muscles are highly heterogeneous in terms of their structure and function. For instance, muscle activation patterns, fatigability, fiber type composition, oxidative/glycolytic enzyme activities, vascularity, and vascular control broadly range among and within muscles (20). In humans, bipedalism has facilitated the additional flexibility of preferentially activating muscle groups in either the arms or legs and distributing the available (and potentially limited) cardiac output such that prodigious blood flows can be achieved when smaller muscle masses are activated. Through experimental evidence procured in humans, and especially animals, for the past few decades, our appreciation of heterogeneity with respect to blood flow among and within muscles has undergone a radical revision.

It is now recognized that both spatial and temporal heterogeneity of blood flow is of fundamental importance in the matching of increases in $\dot{Q}O_2$ to local metabolic requirements ($\dot{V}O_2$) (20). The ability to drive O_2 from the capillary into the myocyte and, thus, to the mitochondria is dependent on the driving pressure (*i.e.*, microvascular PO_2, $PmvO_2$) and the

resultant pressure gradient. Because $PmvO_2$ principally is determined by the $\dot{Q}O_2/\dot{V}O_2$ ratio and because $PmvO_2$ determines blood-myocyte O_2 flux and also influences metabolic control via intracellular PO_2, it is intuitive that, unless $\dot{Q}O_2/\dot{V}O_2$ is regulated extremely tightly both spatially and temporally, $\dot{V}O_2$ kinetics may be slowed and muscle function will be suboptimal, thereby reducing exercise tolerance (30). By the same token, if the $\dot{Q}O_2/\dot{V}O_2$ ratio, and thus $PmvO_2$, can be increased at any given work rate, exercise performance may be enhanced.

Dietary Nitrate Supplementation, Type II Muscle, and Fatigue

Exercise tolerance is dictated by a plethora of physiological and physiochemical interactions that ultimately determine the fatigability of skeletal muscle. Of the potential mechanisms of fatigue, the recruitment of higher-order motor units that elicit contraction of fast-twitch Type II muscles is especially pertinent herein. Muscles predominantly composed of Type II fibers regulate their $PmvO_2$ very differently from their slow-twitch (Type I) counterparts. Specifically, significantly lower blood flow (relative to $\dot{V}O_2$) results in a much lower contracting $PmvO_2$ (*i.e.*, ~10 vs 20 mm Hg; Fig. 1), ultimately forcing greater reliance on fractional O_2 extraction in Type II compared with Type I muscle (5,26). The heterogeneous fiber type distribution evident in the rat and the ability to measure skeletal muscle vascular function across discrete muscle fiber types provide a powerful research tool to examine the impact of dietary NO_3^- on muscles of varying fiber type. It is important to note that human skeletal muscle also exhibits appreciable heterogeneity in fiber type (17,33), and that murine models may provide important insight into fiber-specific vascular control (20).

Given the propensity for a very low PO_2 environment and possibly greater acidity to reduce NO_2^- to NO, Ferguson *et al.* (12) tested the hypothesis that dietary NO_3^- preferentially would increase blood flow and thus $PmvO_2$ in exercising muscles composed of Type II fibers. In rats running at approximately 70% $\dot{V}O_{2max}$, beetroot juice ingestion (1 mmol·NO_3^{--1}·kg^{-1} for 5 d) elevated blood flow to muscles and muscle portions composed of 66% or more Type II b+d/x muscle fibers (12) in concert with a lower blood lactate level. As expected, these selective vascular effects were found to raise $PmvO_2$ in Type II, but not Type I, muscles during contractions (13) providing the mechanistic basis for the reduced blood lactate level (Fig. 1).

Mechanistic Considerations

The preferential fiber type effects of NO_3^- supplementation is important for vascular control because NO synthesis via the NOS family of enzymes requires O_2 and consequently may become dysfunctional at the low PO_2 extant in contracting muscle. This may be especially true in Type II muscle given their inherently lower PO_2 compared with Type I muscle (5,26). Thus, the reduction of NO_2^- to NO acts as a useful backup to maintain NO homeostasis and hyperemia in situations where NOS function is compromised. Indeed, it recently has been reported that bolus infusion of NO_2^- offsets acute pharmacological NOS inhibition and restores cardiovascular function to

control values (11), thus supporting the complementary role of the NO_3^--NO_2^--NO pathway.

Another potential means by which dietary NO_3^- may elicit its beneficial effects involves its impact on skeletal muscle calcium-handling proteins. For instance, Hernández et al. (16) demonstrated that 7 d of supplementation with NO_3^--enriched water resulted in an increased level of calsequestrin 1 and the dihydropyridine receptor, which ultimately increased the rate of muscle force development. Crucially, these effects were evident in the extensor digitorum longus (predominantly Type II) but not soleus (predominantly Type I) muscles, further supporting fiber type-specific effects of NO_3^- supplementation. Collectively, these results demonstrate that NO_3^- supplementation has the ability to impact skeletal muscle vascular and metabolic function during contractions, via both blood flow-dependent and -independent mechanisms.

From a performance standpoint, NO_3^--targeted effects likely take center stage during high-intensity exercise given that Type II fibers are recruited to a greater extent in the heavy-intensity to severe-intensity exercise domains (21). These higher-intensity domains result in an increased $\dot{V}O_2$/$\dot{Q}O_2$ ratio (decreased $\dot{Q}O_2$/$\dot{V}O_2$ and $PmvO_2$ (26)), which further exacerbates the tissue hypoxia within Type II muscles, ultimately resulting in increased accumulation of ADP, $[P_i]$, $[K^+]$, and $[H^+]$, all of which are associated with fatigue development. As previously mentioned, these low-PO_2/pH environments are ripe for reduction of NO_2^- to NO and thus likely facilitate the increase in $\dot{Q}O_2$ (and potentially reduced $\dot{V}O_2$) observed after NO_3^- supplementation. Moreover, the increased $PmvO_2$ and any reduced $\dot{V}O_2$ may raise the intracellular PO_2, which will act to reduce the intracellular perturbation of the aforementioned metabolites, thereby resulting in a tighter metabolic control. This is especially important when considering that increased intracellular PO_2 reduces the ADP and nicotinamide adenine dinucleotide concentrations necessary to drive a given ATP production, consequently suppressing glycolysis and glycogen depletion. Finally, if $\dot{Q}O_2$ is limiting $\dot{V}O_2$ kinetics, it is quite possible that NO_3^- supplementation will help alleviate the O_2 deficit by speeding $\dot{V}O_2$ kinetics in the first minutes of exercise (30).

## ROLE OF TYPE II MUSCLE FIBER RECRUITMENT IN EFFICACY OF NITRATE SUPPLEMENTATION IN HUMANS

Since the seminal findings of Larsen et al. (23), most, although not all, studies have reported improvements in exercise economy and/or fatigue resistance in moderately trained participants ($\dot{V}O_{2peak}$, $\leq$55 mL·kg^{-1}·min^{-1}) after NO_3^- supplementation ((2,4,23,41) cf. (19)). Conversely, although some well-trained endurance athletes do exhibit improvements in exercise economy or performance, it seems that the ergogenicity of NO_3^- supplementation is attenuated in this population (e.g., (6,9)). These observations are encapsulated in the recent study by Porcelli et al. (31), in which NO_3^- supplementation lowered $\dot{V}O_2$ during moderate-intensity running and improved 3-km running performance in participants with low ($\dot{V}O_{2peak}$, <45 mL·kg^{-1}·min^{-1}) and moderate ($\dot{V}O_{2peak}$, <45–57 mL·kg^{-1}·min^{-1}), but not high ($\dot{V}O_{2peak}$,

>63 mL·kg^{-1}·min^{-1}), aerobic fitness, with the reductions in moderate-intensity $\dot{V}O_2$ and 3-km completion time being positively correlated with $\dot{V}O_{2peak}$. As previously reviewed (18), many NO_3^- supplementation studies may not have provided a sufficient NO_3^- dose and/or duration of supplementation to elicit physiological effects (41). It is plausible that elite athletes, in whom NOS activity, plasma $[NO_2^-]$, and muscle oxygenation will all be enhanced by intensive training, might benefit from greater and/or more protracted NO_3^- supplementation (18). However, given that high-level endurance athletes would, in general, be expected to have a lower percentage of Type II muscle fibers (33) and that NO_3^- treatment seems to preferentially augment physiological responses in Type II muscle fibers in animal models (as described in the previous section), it is tempting to speculate that the efficacy of NO_3^- supplementation to improve physiological and functional responses in human participants might be linked to the population of muscle fibers recruited during exercise. Accordingly, recent research has sought to elucidate the potential for preferential effects of NO_3^- on human Type II skeletal muscle in vivo.

Influence of Muscle Fiber Type on the Effectiveness of Nitrate Supplementation in Humans

To examine the potential for a greater effectiveness of NO_3^- supplementation in human Type II muscle, recent studies have used experimental models to capitalize on differences in the power-velocity relationship between fiber types and the hierarchical recruitment of fiber types as force requirements are increased. Bailey et al. (3) observed greater muscle oxyhemoglobin concentration using NIRS, faster pulmonary $\dot{V}O_2$ kinetics, and extended severe-intensity exercise tolerance (+22%) after NO_3^- supplementation when cycling at 115 rpm, but not 35 rpm (Fig. 3). These findings are consistent with other recent in vivo data that indicated that peak knee extensor torque was increased at an angular velocity of 360 degrees·s^{-1} but not at angular velocities of 90, 180, and 270 degrees·s^{-1} after NO_3^- supplementation (10). Assuming that the proportional contribution of Type II muscle fibers to force production is greater at higher contraction frequencies or velocities, these findings support the notion of enhanced $\dot{Q}O_2$ and/or contractile function of Type II muscle in vivo after NO_3^- supplementation (3,10), consistent with observations in murine models (12,13,16).

Further support for a targeted effect of NO_3^- ingestion on human Type II muscle was provided in the study by Breese et al. (8). These authors administered an initial step work rate increment from a low-intensity baseline (20 W) to a moderate-intensity work rate (<gas exchange threshold; L→M), where predominantly Type I muscle fibers are recruited (21), and a subsequent step to a severe-intensity work rate (>critical power; M→S), where a greater recruitment of Type II muscle fibers has been reported (21). These double-step tests were completed after both NO_3^- and placebo supplementation. Compared with placebo, NO_3^- resulted in faster pulmonary $\dot{V}O_2$ and muscle deoxyhemoglobin kinetics (reflective of faster muscle O_2 extraction kinetics) in M→S but not L→M (Fig. 3). In addition, exercise tolerance during M→S was extended by 22% after NO_3^- compared with placebo ingestion.
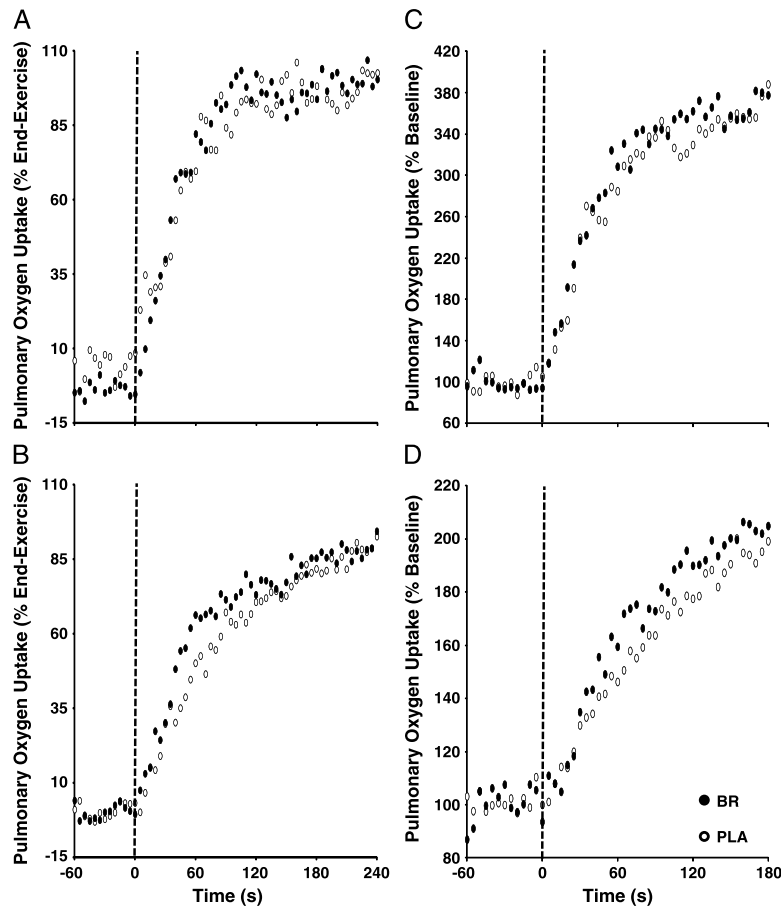
Figure 3. Pulmonary oxygen uptake responses in a representative individual during a step increment from a low-intensity baseline to a moderate-intensity work rate (A), a step increment from a moderate-intensity work rate to a severe-intensity work rate (B), as well as step increments from a low-intensity baseline to a severe-intensity work rate when cycling at a low pedal cadence (35 rpm; C) or high pedal cadence (115 rpm; D). Responses after NO_3^--rich beetroot juice supplementation (BR) are shown in *filled circles*, with the responses after NO_3^--depleted beetroot juice (PLA) shown in *open circles*. Notice the faster adjustment of pulmonary oxygen uptake when the proportional contribution of Type II muscle to force production would be expected to be greater (B and D) compared with settings where the proportional contribution of Type II muscle to force production would be expected to be lower (A and C). Data redrawn from Bailey *et al.* (3) and Breese *et al.* (8).

Although necessarily speculative, taken together, these findings suggest that the physiological effects evoked by NO_3^- supplementation may be enhanced in Type II muscle fibers.

It is well known that short-duration high-intensity intermittent exercise mandates a significant recruitment of Type II muscle fibers. Accordingly, improved performance during such exercise would be hypothesized if NO_3^- supplementation was particularly effective at augmenting physiological processes in Type II muscle. However, the effect of NO_3^- supplementation on performance during short-duration high-intensity intermittent exercise is controversial (9,28,34,40,42). From the existing studies, it seems that when participants received only an acute NO_3^- bolus (28), or when elite endurance cyclists completed repeated cycle sprints after 6 d of NO_3^- supplementation (9), performance was not enhanced. The lack of an ergogenic effect with NO_3^- supplementation in these studies might be attributable to an insufficient supplementation dose and/or duration to elicit the skeletal muscle and mitochondrial remodeling that underpins some of the positive physiological responses reported elsewhere (16,22) and to the lower percentage of Type II fibers in the locomotor muscles of elite endurance athletes (33). An interesting exception in this regard is that NO_3^- ingestion resulted in significantly improved exercise economy in a laboratory exercise test and significantly improved on-water time trial performance in elite kayakers (29). This is intriguing in relation to the hypothesis advanced in the current article because the upper body musculature typically has a higher fraction of Type II muscle fibers (17). However, other factors such as muscle volume, intramuscular tension, and perfusion pressure also may impact on the efficacy of NO_3^- supplementation in exercise of this type. On the other hand, when moderately trained team-sport athletes undergo short-term dietary NO_3^- supplementation, short-duration high-intensity intermittent exercise performance generally has been found to be enhanced (34,40,42). Although further research is required, initial observations support an ergogenic effect after short-term NO_3^- supplementation in moderately trained team-sport athletes, but not necessarily after acute NO_3^- ingestion and/or when elite endurance athletes complete short-duration high-intensity intermittent exercise.

Functional and Clinical Implications and Future Directions

The capacity to complete repeated bouts of high-intensity exercise is linked to the metabolic homeostasis of Type II

muscle fibers and is an important determinant of performance in team sports. Therefore, the apparent improvements in pulmonary $\dot{V}O_2$ kinetics (3,8), skeletal muscle metabolic homeostasis (2,14), perfusion (12), oxygenation (3,13), force production (10,16), and fatigue resistance (2–4,8,34,42) with NO_3^- when Type II muscle fiber recruitment is likely to be greater might be expected to improve performance in team sports or other exercise settings that mandate a significant recruitment of Type II muscle. It also is noteworthy that many patient groups including those with peripheral artery disease, chronic obstructive pulmonary disease, and heart failure have a higher proportion of Type II muscle fibers (*e.g.*, (15)). Given the emerging evidence to support augmented physiological benefits in Type II compared with Type I skeletal muscle, NO_3^- supplementation might represent a simple dietary intervention to improve skeletal muscle perfusion, contractile function, and fatigue resistance in some patient populations.

To further our understanding of these specific effects, future investigations using a multidisciplinary approach should resolve the impact of dietary NO_3^- supplementation on the $\dot{Q}O_2/\dot{V}O_2$ relationship across the spectrum of fast- versus slow-twitch muscles. In particular, simultaneous measurement of $\dot{Q}O_2$ and PO_2mv (potentially by combining the phosphorescence quenching with the radiolabeled microsphere technique) in murine muscles of varying fiber type would afford calculation of skeletal muscle $\dot{V}O_2$ and shed further light on the impacts of NO_3^- supplementation on muscle vascular and metabolic control. In humans, important unresolved questions include whether 1) dietary NO_3^- supplementation increases skeletal muscle $[NO_3^-]$ and $[NO_2^-]$, 2) NO_2^- is "consumed" by contracting skeletal muscle (as determined by measurements of arterial-venous difference) and this correlates with changes in either $\dot{Q}O_2$ or $\dot{V}O_2$, 3) the energetics of isolated myocytes and/or mitochondria are altered on exposure to NO_3^- and NO_2^-, and 4) NO_3^- supplementation impacts the heterogeneity of local blood flow to metabolic rate as assessed with time-resolved multichannel NIRS (20). With specific regard to the potential fiber type sensitivity of NO_3^- supplementation highlighted in this article, future investigations might usefully compare the physiological responses to NO_3^- ingestion of athletes specializing in power and endurance sports (within whom fiber type distribution would be expected to be different) and/or characterize responders/nonresponders to NO_3^- supplementation on the basis of directly measured muscle fiber type.

SUMMARY

Nitric oxide is involved in an array of physiological processes that regulate cardiovascular and muscle function. Because the activity of NOS can decline in low-O_2 environments, various disease states, and in older age, the NO_3^--NO_2^--NO pathway represents an essential backup system for NO generation. Dietary NO_3^- supplementation increases plasma $[NO_2^-]$ and therefore NO bioavailability. Assuming a sufficient dose and duration of supplementation, the effectiveness of NO_3^- in positively altering exercise economy and performance seems to be related both to the aerobic fitness or endurance training status of the subject population and to the type of exercise being considered (18). In this article, we have presented evidence consistent with the hypothesis that NO_3^- may be particularly effective at augmenting

physiological responses in Type II muscle (Fig. 1). The relatively low $PmvO_2$ therein may promote the reduction of NO_2^- to NO, thereby increasing local perfusion and $\dot{Q}O_2/\dot{V}O_2$, improving fatigue resistance, augmenting fiber contractility, and ultimately enhancing whole body exercise tolerance or performance. This targeted effect of NO_3^- supplementation on Type II muscle may have important implications for improving performance during intense exercise in healthy adults and for helping to restore functional capacity in senescent and patient populations, where increased reliance on Type II muscle fibers contributes to compromised aerobic capacity and exercise intolerance.

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