Multidisciplinary Reviews

REVIEW ARTICLE
Published Online: October 24, 2022
https://doi.org/10.31893/multirev.2022009

Effect of acute and chronic nitrate supplementation on the performance of endurance athletes: a systematic review

Ludmila Dias dos Santos Leal[ab]* | Miller Pereira Guimarães[ac] | Yuri de Almeida Costa Campos[ad] | Wilson Cesar Abreu[e] | Sandro Fernandes da Silva[ab]

[a]Grupo de Estudos e Pesquisa em Respostas Neuromusculares (GEPREN), Universidade Federal de Lavras, MG, Brazil.
[b]Programa de Pós Graduação em Nutrição e Saúde, Universidade Federal de Lavras, MG, Brazil.
[c]Faculdade Presbiteriana Gammon (FAGAMMON), Lavras, MG, Brazil.
[d]Universidade Federal de Juiz de Fora (UFJF), MG, Brazil.
[e]Departamento de Nutrição, Universidade Federal de Lavras, MG, Brazil.

**Corresponding author: luddiasleal@hotmail.com*

Abstract Evidence that dietary supplementation with nitrate-rich beet juice (NO3-) can improve endurance exercise performance. This paper aimed critically assess the effects of acute and chronic NO3- supplementation in the performance of trained healthy adult individuals and/or athletes. This systematic review analyzed 12 randomized, blinded, crossover clinical trials with control, which investigated the effect of acute and/or chronic supplementation of NO3- through juice or fresh beet on physical performance in endurance exercises. These studies involved 185 subjects. Four of the six acute studies in this review demonstrated improvements in sports performance in running and cycling. Among the eight chronic studies, only two showed no improvement in performance. Six studies with secondary supplementation yield favorable results. Of the six studies conducted with athletes, five demonstrated increases in VO2MAX. The current literature points to the positive effects of dietary supplementation of NO3- acute and chronic in trained and/or athletes. However, there are limitations regarding research on the results of NO3- supplementation in women and if there is an optimal time for observing effects on athletic performance.

Keywords: endurance, food supplementation, nitric oxide donors, performance

1. Introduction

The use of sports supplements has been gaining strength and market share worldwide, with an ever-increasing number of consumers and extensive corroborating studies in the field (Coggan et al 2015).

Nitrate (NO_3^-) has gained prominence in sports due to its potential impact on performance (Haider and Folland 2014; Whitfield et al 2016). The ergogenic effects of NO_3^- supplementation are attributed to its ability to increase the blood concentration of nitric oxide (NO). NO is a gas molecule that modulates neurotransmission, glucose metabolism, vascular tone, mitochondrial respiration, and muscle function in general. Its peak concentration in the blood occurs two to three hours after ingestion of its precursors NO_3^- and/or nitrite (NO_2^-), which are present in foods such as beetroot (Jones 2014; Tiso and Schechter 2015). An increase in blood NO concentration can also be observed after a single session of exercise because exercise increases NO synthase activity (NOS), thus promoting increased NO synthesis, which results in vasodilation and consequently improves muscle supply nutrients and oxygen consumption (Whitfield et al 2016).

The number of people taking up endurance sports such as cycling and road running, which can benefit from NO_3^- supplementation, has increased over the past few years, mainly due to the benefits observed in the health of its practitioners and the popularization of these sports with an increased number of competitions (Maughan et al 2018a). Several supplements are used in these sports in the short-term (acute) and/or long-term (chronic); however, investigating chronic use is a greater challenge for researchers because it is difficult to control confounding variables, in addition to the high cost of conducting these studies; thus, there is a vast field of untapped research (Rothschild and Bishop 2019). Some of the main food sources of inorganic NO_3^- are mustard greens, lettuce, spinach, arugula, watercress, celery, and beetroot (Harty et al 2019). Some foods can increase NO production in the gastrointestinal tract due to their polyphenol content, such as apples or red wine (Gago et al 2007; Peri et al 2005). In addition, pomegranate juice has been shown to protect NO from oxidation while increasing its biological activity. Watermelon is a good source of L-citrulline, a precursor of NO (Jones 2014). After being consumed, the absorbed NO_3^- reaches the systemic circulation, where most of it (75%) is excreted by the kidneys, but approximately 25%

 Received: June 22, 2022 | Accepted: September 19, 2022

enters the enterosalivary system and is reduced to NO_2^- by commensal bacteria present in the oral cavity. This NO_2^- is then partially reduced to NO by stomach acids and is subsequently absorbed in the intestine. A part of this NO_2^- enters the bloodstream, and under low oxygen conditions, such as during intense exercise, it is converted into NO, with a variety of physiological results. NO_3^- not reduced in the oral cavity enters systemic circulation again (Domínguez et al 2017; Mcdonagh et al 2018).

A priori, the supported theory for NO production was that it is exclusively produced through the oxidation of the amino acid L-arginine through a reaction catalyzed by a family of NOS enzymes (Mcdonagh et al 2018). Such a theory is called endogenous/enzymatic production (Díaz et al 2013), and the enzymatic pathway of NO synthesis is dependent on O2 and most ATP generation for cellular metabolism. However, later, it was elucidated that NO production also occurs through NO_3^- and NO_2^- substrates via NO_3^--rich foods that can be serially reduced to form NO_3^--NO_2^--NO in a manner not dependent on NOS enzymes (Carlström et al 2011). In addition, supplementation with NO_3^- via beet juice has recently gained considerable attention in sports as a potent ergogenic to increase the bioavailability of NO and, consequently, benefit athlete performance (Hord et al 2009) and cause physiological benefits that can lead to physical improvements (Rojas-Valverde et al 2020) and recovery too (Costa et al 2020). The use of beet juice is promising for its potential supply of NO_3^-, which, after its metabolism, provides NO in the bloodstream and is thus able to reduce systolic pressure through mechanisms associated with vasodilation and promote decreased platelet aggregation and increased mitochondrial efficiency (Whitfield et al 2016). In particular, recent studies indicate that the increase in NO from dietary supplementation with NO_3^- can potentiate mitochondrial function, optimize oxygen consumption during exercise, and increase time to exhaustion during high-intensity exercise, thus improving performance (Cuenca et al 2018). Furthermore, dietary nitrate appears to have a positive effect on muscle strength and endurance, regardless of dosage, frequency of intake, level of training, muscle group, or type of contraction (Alvares et al 2021).

Another relevant point is the time of NO_3^- supplementation, divided into acute (≤ 2 days) and chronic (≥ 3 days) supplementation (Thomas et al 2016). The results in the literature are not consistent regarding the best form of supplementation, acute or chronic. Hypotheses demonstrate that the use of NO_3^- corroborates the performance improvement in endurance sports, mainly exhibiting acute benefits within 2 to 3 hours after consumption at concentrations ranging from 5 to 9 mmol (310 to 560 mg) (Maughan et al 2018b). Conversely, prolonged periods of NO_3^- supplementation (greater than/equal to 3 days) according to the American College of Sports Medicine (ACSM) guidelines also seem to be beneficial to athletic performance, in addition to being a positive strategy, especially for highly trained athletes, where performance gains seem to be more challenging to obtain, and promising results are observed with the use of NO_3^- (Maughan et al 2018b). Given the above, this review article aimed to critically evaluate the effects of acute and chronic dietary supplementation of NO_3^- on physiological performance in endurance athletes, evaluating the impact of acute and chronic NO_3^- supplementation via beetroot, and thus ascertain from the literature the supposed physiological mechanisms of NO_3^- supplementation.

2. Materials and Methods

2.1. Search strategy

This study was based on a systematic review of English, Spanish, and Portuguese literature using the PubMed, Scopus, PEDro, and Web of Science databases up to April 7, 2020, from the ten preceding years. The reference lists of all included articles were also searched to identify other articles. The keywords used in the study were performance, beetroot juice or beetroot and endurance exercise or athlete endurance and nitric oxide or nitrate and chronic supplementation and/or acute supplementation, and VO_{2MAX} and aerobic power.

2.2. Study selection

All studies identified during the database searches and in the reference, lists were evaluated for relevance based on the information contained in the title, abstract, and description by two independent reviewers (SFS and WCA). Initially, two independent reviewers selected the studies based on the titles, excluding those unrelated to the proposed topic. The full texts of the articles with the potential to be included in the review were retrieved for a final evaluation. A third evaluator resolved possible disagreements during the process. The reasons for exclusion were recorded for the studies considered ineligible for inclusion in the review.

2.3 Data extraction

The data were extracted by one reviewer (LDSL) and confirmed by a second reviewer (SSF) using a standardized data extraction tool developed by the authors. The following information was extracted: main characteristics of the study (authors, date of publication, journal, and sample); description of the type of test used and intensity; supplementation (type, time, and classification as acute or chronic); and description of the study results (findings).

2.4 Assessment of risk of bias/quality

Two independent reviewers (SFS and WCA) assessed the risk of bias, with a third independent reviewer making the final decision in case of disagreement (MPG). The Quality Criterion Checklist of the Academy of Nutrition and Dietetics for Primary Research was used to assess the risk of bias (Dietetics 2016). This tool evaluates ten criteria related to:

(1) the clarity of the research question
(2) bias in the selection of the study participants/subjects
(3) the comparability of the study groups
(4) whether supplement withdrawal methods were described
(5) the use of blinding
(6) whether the intervention and the benchmarks were described in detail
(7) whether the results were clearly defined and whether the measures were valid and reliable
(8) the appropriateness of the statistical analyses
(9) whether the conclusions were supported by the results and considered bias and limitations
(10) whether it was likely that study funding or conflicts of interest introduced bias.

Each of the criteria was classified as yes/low risk of bias, no/high risk of bias, or unclear; based on that, the overall quality of the study was classified as positive/low risk of bias.

2.5 Data summary

The results were described in narrative form and a table 1. The studies were grouped into chronic, acute, or combined supplementation studies.

Table 1 Multiple lines are listed for a single study if multiple performance trials were performed; one line is included for each data set contributing. NO3-, nitrate supplementation; TrTE, trials to exhaustion; TTE, time to exhaustion; TT, time trial (fixed-distance); WT, work trial. BP (Best Performance); ND (no differences); $VO_{2MÁX}$.

Reference	Participants	Exercise Parameters			NO_{3-} Supplementation			Results
		Modality	Task	[NO3-] (mmol)	Duration (days)	Timing (min)	Classification	
Lansley et al (2013)	9	Cycling	TT	6.2	1	150	Chronic	↑ TT
Murphy et al (2012)	11	Running	TT	~ 8.0	1	75	Chronic	↑ TT
Wylie et al (2013)	14	Running	TrTe	4.1	2	150	Chronic	↑ TrTe
Kent et al (2019)	12	Cycling	WT	13	1	120	Chronic	= WT
Puybe et al (2015)	22	Running	TT	5.5	24	-	Acute	↑ $VO_{2MÁX}$
Lee et al (2015)	14	Cycling	TTE	6.4	15	-	Acute	↑ TTE
Boyton et al (2014)	37	Cycling	TTE	Beetroot	7	-	Chronic	↑ $VO_{2MÁX}$
Amano et al (2018)	8	Cycling	TTE	~ 8.0	3	120	Acute	= TTE
De Castro et al (2019)	13	Running	TTE	8.4	3		Acute	↑ $VO_{2MÁX}$ and ↑ V_{PICO}
Rokkedal-Lausch et al (2019)	12	Cycling	TT and TTE	12.4	7	-	Acute	↑ TT and ↑ TTE
Vanhatalo et al (2010)	8	Cycling	TTE	5.2	1, 5 and 15	-	Acute and Chronic	↑ $VO_{2MÁX}$
Boorsma et al (2014)	8	Running	TT	6.5 and 19.5	1 and 8	150	Acute and Chronic	= $VO_{2MÁX}$

2.6 Inclusion criteria

2.6.1. Type of study

Only intervention studies using chronic (studies lasting three days or more) and acute (studies lasting up to 1 day) supplementation with fresh beet and/or beet juice and/or NO_3^- alone, according to the International Olympic Committee – IOC (Maughan et al 2018b) in humans performing endurance exercise were included.

2.6.2. Type of participants

Only studies that reported results for the population of active subjects and/or elite male or female athletes in endurance activities were selected.

2.6.3. Type of intervention

Studies that investigated or compared nutritional intervention with NO_3^- supplementation (be it in acute or chronic dose), with a time trial test protocol or time until exhaustion with the observation of aerobic parameters (maximum oxygen volume - VO_{2MAX}; power; maximum heart rate - HRmax; and metabolic thresholds), and fatigue index through subjective or non-performance protocols.

2.6.4. Benchmark

For studies with comparison groups, the comparison was a control group without intervention or another type of nutritional intervention.

2.6.5. Type of results reported

The main results were obtained from studies investigating variables related to improvement and/or worsening of performance by NO_3^- supplementation.

2.7. Exclusion criteria

Articles linking NO_3^- to coronary and pulmonary diseases and cancer were excluded. Articles with a combination of NO_3^- and another supplement were excluded, in addition to articles where the exercise protocols were resistance exercises (anaerobic), repeated articles, experimental studies, and articles that did not have free access.

2.8. Stratification of studies

A total of 178 articles related to the topic were found, of which 107 were found on the Web of Science, 10 in PEDro, 4 in PubMed, and 57 in Scopus. A priori, 40 articles were excluded because they did not have free access (n = 5), were short papers (n = 8), and had a date before 2010 (n = 27), thus resulting in 138 articles to be further refined (FIGURE 1).

2.9. Study design

All experimental studies (i.e., randomized clinical trials, pseudorandomized studies, and pre-post studies) were included. The results were described in narrative form and a table (TABLE 1). The studies were grouped according to subtitles for discussion and other studies to support the discussion on each topic.

3. Results

From a total of 178 studies identified in preliminary searches of online databases and screening of the reference list, twelve studies were included in this systematic review (Amano et al 2018; Boorsma et al 2014; Boynton et al 2014; De Castro et al 2019; Kent et al, 2019; Lansley et al 2011; Lee et al 2015; Murphy et al 2012; Puype et al 2015; Rokkedal-Lausch et al 2019; Wylie et al 2013) (Figure 1).

3.1. Characteristics of the sample

Of the 12 studies included, 185 participants (158 men and 27 women) were equivalent to 85% and 15%, respectively. The number of participants ranged from 8 (Amano et al 2018; Boorsma et al 2014; De Castro et al 2019; Vanhatalo et al 2010). A cycle ergometer (n = 7/58%) was used to perform the tests observed in the studies (Vanhatalo et al 2010; Lansley et al 2011; Boynton et al 2014; Lee et al 2015; Puype et al 2015; Amano et al 2018; Kent et al 2019).

The 12 studies targeted active subjects (n = 97/52%) or healthy elite athletes (n = 88/48%) who practiced endurance sports such as cycling, road running, and team sports. Six studies recruited elite athletes (Boorsma et al 2014; Boynton et al 2014; Kent et al 2019; Lansley et al 2011; Puype et al 2015; Rokkedal-Lausch et al 2019), and only one of these had a sample of men and women (Boynton et al 2014). The other six studies recruited active subjects (Vanhatalo et al 2010; Murphy et al

2012; Wylie et al 2013; Lee et al 2015; Amano et al 2018; De Castro et al 2019); in only three of them, the half samples contained men and women (Amano et al 2018; Murphy et al 2012; Vanhatalo et al 2010).

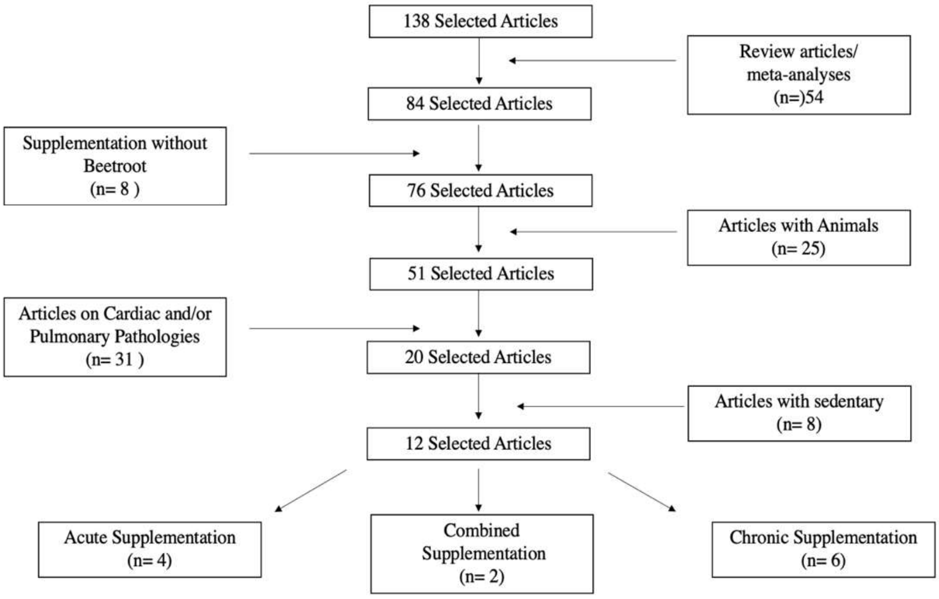

Figure 1 Flowchart of the process of choosing the articles.

3.2. Characteristics of the study

Most of the studies (*n* = 9) were crossover studies (Amano et al 2018; Boorsma et al 2014; De Castro et al 2019; Lansley et al 2011; Lee et al 2015; Murphy et al 2012; Rokkedal-Lausch a et al 2019; Vanhatalo et al 2010), randomized (n = 11) (Amano et al 2018; Boorsma et al 2014; Boynton et al 2014; De Castro et al 2019; Kent et al 2019; Lansley et al 2011; Lee et al 2015; Puype et al 2015; Rokkedal-Lausch et al 2019; Vanhatalo et al 2010; Wylie et al 2013), and double-blind (n = 7) (Amano et al 2018; Boorsma et al 2014; De Castro et al 2019; Lee et al 2015; Murphy a et al 2012; Rokkedal-Lausch et al 2019; Wylie et al 2013), involving treatment with placebo and supplementation with NO_3^-. Of the 12 studies, ten used beet juice as a tool for NO_3^- supplementation (Amano et al 2018; Boorsma et al 2014; Boynton et al 2014; Kent et al 2019; Lansley et al 2011; Lee et al 2015; Puype et al 2015; Rokkedal-Lausch et al 2019; Vanhatalo et al 2010; Wylie et al 2013), while two used whole beets (De Castro et al 2019; Murphy et al 2012). Four studies used acute supplementation (Kent et al 2019; Lansley et al 2011; Murphy et al 2012; Wylie et al 2013), while 6 used chronic supplementation et al 2015; Puype et al 2015; Rokkedal-Lausch et al 2019), and two tested combined supplementation (Boorsma et al 2014; Vanhatalo et al 2010). The average number of days for acute supplementation was one day, and the maximum number of days for chronic supplementation was eight days.

3.3. Effects of NO_3^- supplementation

The NO_3^- dosages used in the studies ranged from 4.1 to 13 mmol/day. Eight studies showed improvements in performance variables such as time, $VO_{2MAX,}$ power, strength, and endurance (Boorsma et al 2014; Boynton et al 2014; De Castro et al 2019; Lansley et al 2011; Murphy et al 2012; Rokkedal-Lausch et al 2019; Wylie et al 2013). Only three studies showed no influence of NO_3^- on performance (Amano et al 2018; Kent et al 2019; Puype et al 2015), and no significant differences were observed in the peak power at sprint output (Kent et al 2019), and there were no changes in the effect of performance during intermittent training in hypoxia (Puype et al 2015). Four studies showed lower blood pressure in the group supplemented with NO_3^- (Vanhatalo et al 2010; Murphy et al 2012; Lee et al 2015; Amano et al 2018). Regarding VO_{2MAX}, only two studies - 3 acutes and 1 combined (Kent et al 2019; Lansley et al 2011) explored this variable. Regarding chronic studies, three of the six studies provided information on the results of the variable (De Castro et al 2019; Puype et al 2015; Rokkedal-Lausch et al 2019), and the two combined studies also reported results on (Boorsma et al 2014; Vanhatalo et al 2010).

4. Discussion

This systematic review investigated the effects of acute or chronic inorganic NO_3^- supplementation on the performance of trained, healthy adult individuals and/or athletes in endurance sports. The results were expected to reveal the most appropriate duration of supplementation and whether the period affected the results in the performance of the practitioners. The current literature indicates positive effects of dietary supplementation with NO_3^- in sports, both acute and chronic dosages, acting directly and/or indirectly on the physiological parameters, with some evidence indicating benefits, namely, improved oxygen uptake during exercise and prolonged exhaustion time. In addition to caffeine, sodium bicarbonate, beta-alanine, and creatine, NO_3^- has been considered a true ergogenic resource capable of directly improving sports performance (Maughan et al 2018b).

4.1. NO_3^- *action at a muscle level on performance*

We can observe that both acute and chronic supplementation can generate positive responses to training. Several studies have shown that dietary supplementation with beets or sodium NO_3^- reduces the submaximal cost of O_2 during exercise and improves performance in exercises performed in time trial tests (Boorsma et al 2014; Cermak et al 2012; Cermak et al 2012; Christensen et al 2013). The increased blood flow, called *shear stress,* can activate membrane proteins that phosphorylate the enzyme *endothelial constitutive NOS* (eNOS), which in turn produces NO (Zago et al 2010). The stimulation of physical exercise can also directly increase the activity of the antioxidant enzyme superoxide dismutase (SOD) and/or reduce the activity of the enzyme complex NADPH oxidase, which increases the bioavailability of NO to smooth muscle cells (Bond and collaborators, 2013). In turn, NO produced in endothelial cells translocates to smooth muscles, generating cyclic guanosine monophosphate (cGMP). The formation of cGMP induces the reduction of intracellular calcium and promotes the relaxation of vascular smooth muscle in its inner layer. This relaxation reduces peripheral vascular resistance, causing vasodilation, leading to a decrease in arterial hypertension, bronchodilation, angiogenesis, mitochondrial biogenesis, glucose uptake and greater sensitivity to calcium ions (Domínguez et al 2017) (Figure 2).

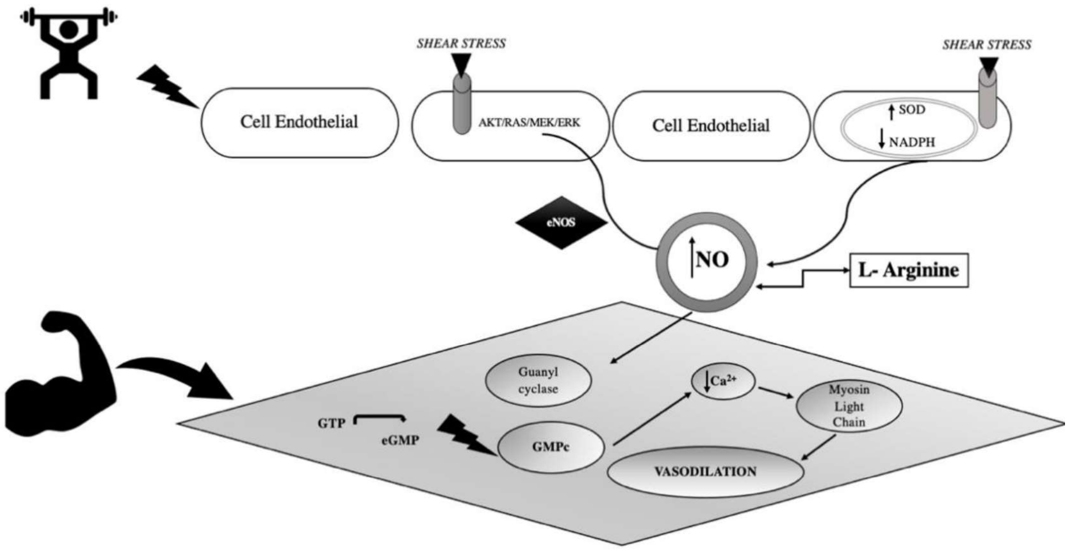

Figure 2 NO action mechanism. Increased blood flow (shear stress) caused by physical exercise triggers some protein kinase of the membrane that perhaps phosphorylate specific enzymes (eNOS) and also act in the greater expression of the antioxidant enzyme SOD-1 which, in contrast, reduces the activity of the oxidizing enzyme, NADPH oxidase. Such an effect causes greater bioavailability of NO to smooth muscle cells. such effect triggers an intra-molecular cascade, where cGMP will induce ion reduction intracellular calcium, thus promoting smooth muscle relaxation vascular resistance, reducing peripheral vascular resistance, and, consequently, reducing pressure values. In the muscle, NO interacts with the iron of the haem group of the enzyme guanylate cyclase (GC), causing changes in the conformation of this enzyme and making it active (GCa). GCa catalyses the output of two phosphate groups of the guanosine triphosphate (GTP) molecule, similar to adenosine triphosphate (ATP), forming cGMP. The increase in cGMP concentration occurs when NO activates the GC enzyme inside the cells (Faria et al 2010). However, cGMP production is interrupted seconds after removal of NO from the GC enzyme. These physical stimuli occur as the blood exerts force on the artery wall, known as shear stress (Faria et al 2010; Zago et al 2010). Furthermore, dietary nitrate, could contribute to cardiovascular health through effects on nitric oxide (NO) status (Bondonno et al 2016). *Source:* The Authors.

4.2. Acute versus chronic NO_3^- *supplementation*

Recent studies have shown that consuming NO_3^- in an acute, chronic, or combined manner can improve performance in endurance and strength exercises, resulting in less energy expenditure during activity and generating greater resistance to peripheral and central muscle fatigue, improving strength and physical performance (Bond et al 2013; Cuenca et al 2018; Vanhatalo et al 2010).

Four of the six acute studies included in this review showed improvements in sports performance in the supplemented group (Vanhatalo et al 2010; Lansley et al 2011; Murphy et al 2012; Wylie et al 2013). Only two of the eight chronic studies showed no improvement (Boorsma et al 2014; Puype et al 2015). Six studies had elite athletes in their sample group, and three obtained positive performance improvement results (Boynton et al 2014; Lansley et al 2011; Rokkedal-Lausch et al 2019). The other six studies had a sample of active subjects (Vanhatalo et al 2010; Murphy et al 2012; Wylie et al 2013; Lee et al 2015; Amano et al 2018; De Castro et al 2019). In the study of acute supplementation by Murphy et al. (2012), the intake of NO_3^- utilizing 200 g of roasted and chopped beets (~ 500 mg NO_3^-) improved endurance exercise performance. During the 5-km test on a treadmill 75 minutes after beet intake, the average running speed was higher than beet intake (12.3 ± 2.7 vs. 11.9 ± 2.6 km/hour; P = 0.06). During the last 1.8 km run, the speed was 5% faster (12.7 ± 3.0 vs. 12.1 ± 2.8 km/hour; P = 0.02) in the beet test. There were no differences in exercise heart rate between the tests; however, after 1.8 km of running, the classification of perceived effort was lower with beet supplementation (13.0 ± 2.1 vs. 13.7 ± 1.9; P = 0.04).

In the study of acute supplementation by Wylie et al. (2013) the hypothesis was that supplementation with NO_3^- via beet juice in healthy male athletes would be able to increase performance in intermittent high-intensity exercise (a yo-yo test). The plasma concentration of NO_2^- at rest was 400% higher in the group supplemented with beet juice than in the placebo group. The performance in the shuttle run test was 4.2% higher (P <0.05) for the best group (1,704 ± 304 m vs. 1,636 ± 288 m). In summary, this study suggested that NO_3^- supplementation can promote NO production through the NO_3^--NO_2^--NO pathway and thus improve performance in the shuttle run test by facilitating muscle glucose uptake and/or maintaining muscle excitability.

Some factors can impact fatigue and muscle performance during physical exercises, such as duration, intensity and nature of training, type of muscle fiber, fitness level, and environmental factors such as altitude, humidity, and temperature (Bailey et al 2012; Gross and Wolin 1995). The improvement in performance reported in the article discussed above (Wylie et al 2013) would be linked to the physiological signaling functions of NO. This, in turn, has numerous physiological signaling functions, including blood flow regulation, muscle contractility, glucose and calcium homeostasis, and mitochondrial oxygen consumption (Stamler and Meissner 2001).

Physical training and supplementation with beet juice promote increased concentrations of NO and NO_2^- in the plasma. Under conditions of low oxygen concentration, NO_2^- can be reduced to NO (Bailey et al 2012; Stamler and Meissner 2001). It is believed that the balance between production and consumption regulates the levels of NO in tissues through two pathways of endogenous production of NO in the human body (Hernández et al 2012; Cuenca et al 2018; Kramer et al 2016) (TABLE 1).

Boynton et al. (2014) observed beneficial responses from chronic NO_3^- supplementation in the performance of cyclists and runners, especially in females. They observed a significant increase in exhaled NO after consumption of the beet dose compared to placebo in all cyclists and all runners, with emphasis on the sample of female cyclists and runners (30.00 ± 7.00 ppb, 11.67 ± 2.52 ppb, p = 0.0790), (43.75 ± 40.63 ppb, 28.50 ± 28.25 ppb, p = 0.0535), respectively. Another relevant point observed in the same study was the absolute oxygen consumption at the lactate threshold. They observed significant increases after the consumption of beet compared to placebo in female runners (2.76 ± 0.25 L/min, 2.68 ± 0.25 L/min, p = 0.0250) and in the relative amount of oxygen consumption at the lactate threshold in the female group of runners (47.96 ± 4.75 ml/kg/min, 46.71 ± 4.62 ml/kg/min, p = 0.0563). Given these findings, the above study suggested that beet supplementation can improve endurance performance in trained athletes, especially women. These findings suggested that the improvements observed in the study can be reported as the action of NO_3^- on the mitochondria, with important effects on oxygen consumption during physical exercise, as described by Dominguez et al. (2017) in their study. Regarding the physiological variables, the study showed that chronic NO_3^- supplementation via beet juice improved VO_{2MAX} (46.6 ± 6.4 vs. 45.1 ± 5.8 mL kg - 1 min - 1; P = 0.022), the velocity associated with VO_{2MAX} (vVO_{2MAX}) (14.5 ± 0.8 vs 13.9 ± 1.0 km h - 1P = 0.024), and peak velocity (V_{peak}) (15.5 ± 1.1 vs 15.2 ± 1.2 km h - 1P = 0.038) in recreational runners (De Castro et al 2019).

Another study evaluated the acute and chronic effects of NO_3^- rich beet juice supplementation on VO_{2MAX} in long-distance runners. No differences were observed in VO_{2MAX} at different intensities, and there was no improvement in performance in the 1500 m time trial test. However, two individuals improved in the 1500 m tests after acute and chronic supplementation (Boorsma et al 2014).

The results presented in this review show that acute or chronic supplementation with NO_3 may benefit endurance exercise practitioners. The main benefits suggested by many studies regarding NO_3^- supplementation on improved performance in endurance exercises are an increase in time to exhaustion and a greater capacity for total oxygen uptake. However, a large portion of favorable results in physical performance found in the literature is for long-term or chronic supplementation (Amano et al 2018; Boynton et al 2014; De Castro et al 2019; Lee et al 2015; Puype et al 2015; Rokkedal-Lausch et al 2019). The hypothesis raised about the benefits of long-term supplementation is the promotion of changes in the number of mitochondria (Larsen et al 2007), in muscle contractility, and in proteins that promote improvement in skeletal muscle metabolism and mechanical efficiency (James et al 2015).

4.3. Effects of acute and chronic supplementation on physiological and physical performance variables

The monitoring of diastolic and systolic blood pressure was described in most studies (Vanhatalo et al 2010; Lansley et al 2011; Lee et al 2015; Amano et al 2018; Kent et al 2019; Rokkedal-Lausch et al 2019), demonstrating that this variable behaves more mildly in the supplemented group than in the control group. Lee et al (2015), in their study with chronic NO_3^- supplementation, observed that vascular resistance was lower in the supplemented group, suggesting that this effect may be due to vasodilation induced in the endothelium during muscle contraction. Thus, NO_3^- supplementation could potentially improve the oxygen supply and decrease cardiac work so that exercise could be performed at a specific workload for a longer time, delaying fatigue.

A study on chronic NO_3^- supplementation did not observe differences in the blood pressures of the supplemented practitioners compared to those of the control group. Regarding respiratory variables, no significant differences were found regarding blood oxygen saturation under normoxic and hypoxic conditions. However, an increase in VO_{2MAX} and ventilations per minute was observed in supplemented individuals, thus suggesting a higher proportion of aerobic capacity, thus improving the performance of the cyclists (Rokkedal-Lausch et al 2019). The study that compared chronic and acute NO_3^- supplementation observed that resting blood pressure decreased in both groups (supplemented and control) by approximately 4% in the group supplemented at the acute stage (2.5 hours after supplementation) and remained during the chronic period of the study (5 and 15 days of supplementation) (Vanhatalo et al 2010).

Heart rate was also one of the parameters measured in some studies (Murphy et al 2012; Puype et al 2015; Amano et al 2018; Kent et al 2019); however, only one study observed significant differences in lower heart rate in individuals supplemented with acute NO_3^- (Kent et al 2019). Regarding blood lactate, some studies showed divergent results (Wylie et al 2013; Puype et al 2015; De Castro et al 2019). In the acute effect study of NO_3^- supplementation by Wylie et al (2013), the blood lactate measured after two yo-yo tests was higher in the supplemented group than in the placebo group. In the study of chronic supplementation by De Castro et al (2019), blood lactate was slightly lower in the supplemented group than in the placebo group. The blood glucose parameter was also measured in some studies (Vanhatalo et al 2010; Wylie et al 2013; De Castro et al 2019). In an acute (Wylie et al 2013), a chronic (De Castro et al 2019), and a combined (Vanhatalo et al 2010) study, the concentration of blood glucose was lower in the supplemented group than in the placebo group. Puybe et al (2015), in their study of the chronic effect of supplementation, observed after biopsy of the vastus lateralis muscle that the concentration of muscle glycogen was maintained, as well as the mRNA content of HIF-1-alpha, or the phospho-AMPK/AMPK ratio. In their study with chronic supplementation, Amano et al (2018) observed the rate of sweating and cutaneous vascular responses in an environment with relatively high temperatures. Oesophageal, body, and skin temperatures were measured. The results showed normality, concluding that supplementation with NO_3^- could not change the rate of sweating or vascular responses during exercise.

4.4. Effects of NO_3^- *supplementation in elite athletes versus active subjects*

Regarding the distinction between athletes and trained individuals samples, our review shows that there is not much discrepancy regarding the positive results. Of the six studies conducted with athletes (Boynton et al 2014; Kent et al 2019; Lansley et al 2011; Puype et al 2015; Rokkedal-Lausch et al 2019; Whitfield et al 2016), five showed results of improved VO_{2MAX} and decreased times during physical tests (Boorsma et al 2014; Boynton et al 2014; Kent et al 2019; Lansley et al 2011; Rokkedal-Lausch et al 2019; Whitfield et al 2016). Regarding the studies with trained individuals, 5 showed improvement in performance during the research (De Castro et al 2019; Lee et al 2015; Murphy et al 2012; Vanhatalo et al 2010; Wylie et al 2013). Studies comparing the effects of NO_3^- supplementation in highly trained athletes and recreational athletes under the same testing conditions may contribute to elucidating the possible differences observed in these groups. Considering that the potential of NO_3^- supplementation to improve performance in groups of trained people and/or recreational practitioners is a vast field to be explored, the potential impact of NO_3^- supplementation on the performance of elite athletes also needs to be considered (Jones 2014).

Highly trained athletes are very well adapted to training; therefore, relatively few nutritional supplements have proven benefits, and the effects tend to be smaller (Burke and Peeling 2018; Wilkerson et al 2012). Training can result in individual adaptations, such as muscle and capillary increases (Andersen and Henriksson 1977; Prior et al 2004), and this effect can conserve local muscle oxygenation and positively regulate NOS enzymes (Cocks et al 2013; Mcconell et al 2007). Thus, well-trained athletes have higher baseline NO_2^- values than active individuals (Poveda et al 1997). Studies also emphasize this hypothesis when they positively link the basal NO_2^- concentration associated with incremental exercise to the performance of elite athletes (Totzeck et al 2012).

Therefore, it is suggested that the need and demand for NO_2^- and, consequently, NO are lower in elite athletes than in trained/recreational individuals during exercise. Genetic issues and chronic training positively impact mitochondrial efficiency and/or skeletal muscle contractility and, therefore, may also limit the potential of dietary NO_3^- supplementation (Guerrero et al 2005; Mogensen 2006).

Another hypothesis raised is that NO_3^- would have a greater metabolic or vascular function in type II muscle fibers than in type I muscle fibers. Therefore, elite or highly trained endurance athletes could have less benefit from NO_3^- supplementation since a higher proportion of type I muscle fibers is observed in this population (Gollnick and Matoba 1984).

In the study by Lansley et al (2011), an increase of 2.8% was observed in performance in a 4 km cycling time trial test and 2.7% in a 16.1 km test. Although the magnitude of the performance increase seems small, in reality, minor performance improvements can strongly impact the results achieved by athletes. This higher endogenous production combined with higher dietary NO_3^- consumption suggests that trained athletes have a higher NO_2^- reserve, so supplementary intake would not be effective (Jones 2014). This hypothesis was reported by Wylie et al (2013), who found no performance benefits compared to a placebo.

It was also suggested that there is a dose-response relationship between NO_3^- intake and its ergogenic properties (Wylie et al 2013). Such dosages could improve performance depending on the training status, exercise mode, and/or supplementation period (Jonvik et al 2015). Regarding the training time, there is the question of reconditioning responses in cardiovascular function, vascularization of the skeletal muscle, and mitochondrial efficiency, resulting in higher metabolism and mechanical efficiency in this specific group (Jensen et al 2004). Given this, the hypothesis that elite athletes would be less responsive to the ergogenic properties of NO_3^- than active subjects are partly linked to better metabolic adaptations and lower adaptive responses.

Another important point to be raised is the limited number of studies with elite athletes due to the difficulty of working with such a restricted and scarce group. Conducting research in the environment of major competitions is not viable for training plans, which hinders studies with this group. According to the literature, such studies generally do not reach statistical significance; however, minor variations in responses in performance, with differences even less than 1%, are important and beneficial for elite athletes. It is suggested that to detect subtle but relevant improvements in the performance of elite athletes, it is more interesting to repeatedly evaluate their performance instead of using more traditional methodological designs with groups for comparison (Boorsma et al 2014; Christensen et al 2013). However, in this review, we observed that most studies with elite athletes showed positive results in performance, thus elucidating that studies with such a group are promising and able to offer benefits to this group.

5. Final considerations

This systematic review provides evidence that there is a consistent improvement in performance in trained individuals and/or athletes after dietary supplementation with NO_3^-. Highlighting the improvement in the supply of oxygen and nutrients to the muscles and modulation in response to the performance of physical exercise, the optimization of the endurance parameters such as time and VO_{2MAX} in the different endurance sports modalities was quite clear in most of the studies discussed here. In addition, these findings reinforce that both acute and chronic supplementation with NO_3^- can have ergogenic effects in trained individuals, even at different NO_3^- doses and durations.

However, studies with larger samples that are homogeneous between sexes and with more sports modalities are needed to verify more significant effects of NO_3^- supplementation.

Conflict of Interest

The authors have no conflicts of interest related to the publication.

Funding

The research was funded by the Coordination for the Improvement of Higher Education Personnel – CAPES.

References

Alvares TS, Oliveira GVD, Volino-Souza M, Conte-Junior CA, Murias JM (2022) Effect of dietary nitrate ingestion on muscular performance: A systematic review and meta-analysis of randomized controlled trials. Critical Reviews in Food Science and Nutrition 62:5284-5306.

Amano T, Okushima D, Breese BC, Bailey SJ, Koga S, Kondo N (2018) Influence of dietary nitrate supplementation on local sweating and cutaneous vascular responses during exercise in a hot environment. European journal of applied physiology 118:1579-1588.

Andersen P, Henriksson J (1977) Capillary supply of the quadriceps femoris muscle of man: adaptive response to exercise. The Journal of physiology 270:677-690.

Bailey SJ, Vanhatalo A, Winyard PG, Jones AM (2012) The nitrate-nitrite-nitric oxide pathway: Its role in human exercise physiology. European Journal of Sport Science 12:309-320.

Bond V, Curry BH, Adams RG, Asadi MS, Millis RM, Haddad GE (2013) Effects of dietary nitrates on systemic and cerebrovascular hemodynamics. Cardiology research and practice 34:54.

Bondonno CP, Croft KD, Hodgson JM (2016) Dietary nitrate, nitric oxide, and cardiovascular health. Critical reviews in food science and nutrition 56:2036-2052.

Boorsma RK, Whitfield J, Spriet LL (2014) Beetroot juice supplementation does not improve performance of elite 1500-m runners. Medicine Science Sports Exercise 46:2326-2334.

Boynton JR, Stein TD, Wuenstel JG, Fitzgerald KA, Gregoire DM, Albrechtsen SJ (2014) Beet Shot Supplements to Improve Endurance Performance in Cyclists and Runners: 552 May 28, 2: 45 PM-3: 00 PM. Medicine & Science in Sports & Exercise 46:132.

Burke LM, Peeling P (20180 Methodologies for investigating performance changes with supplement use. International Journal of Sport Nutrition and Exercise Metabolism 28:159-169.

Carlström M, Persson AEG, Larsson E, Hezel M, Scheffer PG, Teerlink T, Lundberg JO (2011) Dietary nitrate attenuates oxidative stress, prevents cardiac and renal injuries, and reduces blood pressure in salt-induced hypertension. Cardiovascular research 89:574-585.

Cermak NM, Stinkens R, Lundberg JO, Gibala MJ, Van Loon LJ (2012) No improvement in endurance performance after a single dose of beetroot juice. International journal of sport nutrition and exercise metabolism 22:470-478.

Cermak NM, Gibala MJ, Van Loon LJ (2012) Nitrate supplementation's improvement of 10-km time-trial performance in trained cyclists. International journal of sport nutrition and exercise metabolism 22:64-71.

Christensen PM, Nyberg M, Bangsbo J (2013) Influence of nitrate supplementation on VO2 kinetics and endurance of elite cyclists. Scandinavian journal of medicine & science in sports 23:21-31.

Clifford T, Berntzen B, Davison GW, West DJ, Howatson G, Stevenson EJ (2016) Effects of beetroot juice on recovery of muscle function and performance between bouts of repeated sprint exercise. Nutrients 8:506.

Cocks M, Shaw CS, Shepherd SO, Fisher JP, Ranasinghe AM, Barker TA, Wagenmakers AJ (2013) Sprint interval and endurance training are equally effective in increasing muscle microvascular density and eNOS content in sedentary males. The Journal of physiology 591:641-656

Coggan AR, Leibowitz JL, Kadkhodayan A, Thomas DP, Ramamurthy S, Spearie CA, Peterson LR (2015) Effect of acute dietary nitrate intake on maximal knee extensor speed and power in healthy men and women. Nitric Oxide 48:16-21.

Costa MS, Toscano LT, Toscano LDLT, Luna VR, Torres RA, Silva JA, Silva AS (2022) Ergogenic potential of foods for performance and recovery: a new alternative in sports supplementation? A systematic review. Critical Reviews in Food Science and Nutrition 62:1480-1501.

Cuenca E, Jodra P, Pérez-López A, González-Rodríguez LG, Fernandes da Silva S, Veiga-Herreros P, Domínguez R (2018) Effects of beetroot juice supplementation on performance and fatigue in a 30-s all-out sprint exercise: a randomized, double-blind cross-over study. Nutrients 10:1222

De Castro TF, de Assis Manoel F, Figueiredo DH, Figueiredo DH, Machado FA (2019) Effects of chronic beetroot juice supplementation on maximum oxygen uptake, velocity associated with maximum oxygen uptake, and peak velocity in recreational runners: a double-blinded, randomized and crossover study. European Journal of Applied Physiology 119:1043-1053.

Faria TDO, Targueta GP, Angeli JK, Almeida EAS, Stefanon I, Vassallo DV, Lizardo J HDF (2010) Acute resistance exercise reduces blood pressure and vascular reactivity, and increases endothelium-dependent relaxation in spontaneously hypertensive rats. European journal of applied physiology 110:359-366.

Díaz MM, Bocanegra OL, Teixeira RR, Soares SS, Espindola FS (2013) Salivary nitric oxide and alpha-amylase as indexes of training intensity and load. International journal of sports medicine 34:8-13

Dietetics A. (2016). Evidence Analysis Manual: Steps in the Academy Evidence Analysis Process 23:90-97.

Domínguez R, Garnacho-Castaño MV, Cuenca E, García-Fernández P, Muñoz-González A, De Jesús F, Maté-Muñoz JL (2017) Effects of beetroot juice supplementation on a 30-s high-intensity inertial cycle ergometer test. Nutrients 9:1360.

Gago B, Lundberg JO, Barbosa RM, Laranjinha J (2007) Red wine-dependent reduction of nitrite to nitric oxide in the stomach. Free Radical Biology and Medicine 43:1233-1242.

Gollnick PD, Matoba H (1984) The muscle fiber composition of skeletal muscle as a predictor of athletic success: An overview. The American journal of sports medicine 12:212-217.

Gross SS, Wolin MS (1995) Nitric oxide: pathophysiological mechanisms. Annual Review of Physiology 57:37-769.

Guerrero K, Wuyam B, Mezin P, Vivodtzev I, Vendelin M, Borel JC, Pison C (2005) Functional coupling of adenine nucleotide translocase and mitochondrial creatine kinase is enhanced after exercise training in lung transplant skeletal muscle. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 289:1144-1154.

Haider G, Folland JP (2014) Nitrate supplementation enhances the contractile properties of human skeletal muscle. Medicine and science in sports and exercise 46:2234-2243.

Harty PS, Cottet ML, Malloy JK, Kerksick CM (2019) Nutritional and supplementation strategies to prevent and attenuate exercise-induced muscle damage: A brief review. Sports medicine-open 5:1-17.

Hernández A, Schiffer TA, Ivarsson N, Cheng AJ, Bruton JD, Lundberg JO, Westerblad H (2012) Dietary nitrate increases tetanic [Ca2+] i and contractile force in mouse fast-twitch muscle. The Journal of physiology 590:3575-3583.

Hord NG, Tang Y, Bryan NS (2009) Food sources of nitrates and nitrites: the physiologic context for potential health benefits. The American journal of clinical nutrition 9:1-10.

James PE, Willis GR, Allen JD, Winyard PG, Jones AM (2015) Nitrate pharmacokinetics: Taking note of the difference. Nitric Oxide 48:44-50.

Jensen L, Bangsbo J, Hellsten Y (2004) Effect of high intensity training on capillarization and presence of angiogenic factors in human skeletal muscle. The Journal of physiology 557:571-582.

Jonvik KL, Nyakayiru J, Van Loon LJ, Verdijk LB (2015) Can elite athletes benefit from dietary nitrate supplementation? Journal of applied physiology 119:759-761.

Kent GL, Dawson B, McNaughton LR, Cox GR, Burke LM,Peeling P (2019) The effect of beetroot juice supplementation on repeat-sprint performance in hypoxia. Journal of sports sciences 37:339-346.

Kramer SJ, Baur DA, Spicer MT, Vukovich MD, Ormsbee MJ (2016) The effect of six days of dietary nitrate supplementation on performance in trained CrossFit athletes. Journal of the International Society of Sports Nutrition 13:39.

Lansley KE, Winyard PG, Bailey SJ, Vanhatalo A, Wilkerson DP, Blackwell JR, Jones AM (2011) Acute dietary nitrate supplementation improves cycling time trial performance. Medicine Science Sports Exercise 43:125-1131.

Larsen FJ, Weitzberg E, Lundberg JO, Ekblom B (2007) Effects of dietary nitrate on oxygen cost during exercise. Acta physiologica 191:59-66

Lee JS, Stebbins CL, Jung E, Nho H, Kim JK, Chang MJ, Choi HM (2015) Effects of chronic dietary nitrate supplementation on the hemodynamic response to dynamic exercise. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 309:459-466.

Maughan RJ, Burke LM, Dvorak J, Larson-Meyer DE, Peeling P, Phillips. SM, Engebretsen L (2018) IOC consensus statement: dietary supplements and the high-performance athlete. International journal of sport nutrition and exercise metabolism 2:104-125.

McConell GK, Bradley SJ, Stephens TJ, Canny BJ, Kingwell BA, Lee-Young RS (2007) Skeletal muscle nNOSμ protein content is increased by exercise training in humans. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 293:821-828.

McDonagh ST, Wylie LJ, Webster JM, Vanhatalo A, Jones AM (2018) Influence of dietary nitrate food forms on nitrate metabolism and blood pressure in healthy normotensive adults. Nitric Oxide 72:66-74.

Mogensen M, Bagger M, Pedersen PK, Fernström M, Sahlin K (2006) Cycling efficiency in humans is related to low UCP3 content and to type I fibres but not to mitochondrial efficiency. The Journal of physiology 571:669-681.

Murphy M, Eliot K, Heuertz RM,Weiss E (2012) Whole beetroot consumption acutely improves running performance. Journal of the Academy of Nutrition and Dietetics 112:548-552.

Peri L, Pietraforte D, Scorza G, Napolitano A, Fogliano V, Minetti M (2005) Apples increase nitric oxide production by human saliva at the acidic pH of the stomach: a new biological function for polyphenols with a catechol group? Free Radical Biology and Medicine 39:668-681.

Poveda JJ, Riestra A, Salas E, Cagigas ML, López-Somoza C, Amado JA, Berrazueta J R (1997). Contribution of nitric oxide to exercise-induced changes in healthy volunteers: effects of acute exercise and long-term physical training. European journal of clinical investigation 27:967-971.

Prior BM, Yang HT, Terjung RL (2004). What makes vessels grow with exercise training? Journal of applied physiology 97:1119-1128.

Puype J, Ramaekers M, Van Thienen R, Deldicque L, Hespel P (2015) No effect of dietary nitrate supplementation on endurance training in hypoxia. Scandinavian journal of medicine & science in sports 25:234-241.

Rojas-Valverde D, Montoya-Rodriguez J, Azofeifa-Mora C, Sanchez-Urena B (2021) Effectiveness of beetroot juice derived nitrates supplementation on fatigue resistance during repeated-sprints: A systematic review. Critical reviews in food science and nutrition 61:3395-3406.

Rokkedal-Lausch T, Franch J, Poulsen MK, Thomsen LP, Weitzberg E, Kamavuako E N,Larsen RG (2019) Chronic high-dose beetroot juice supplementation improves time trial performance of well-trained cyclists in normoxia and hypoxia. Nitric Oxide 85:44-52.

Rothschild JA, Bishop DJ (2020) Effects of dietary supplements on adaptations to endurance training. Sports Medicine 50:25-53.

Stamler JS, Meissner G (2001) Physiology of nitric oxide in skeletal muscle. Physiological reviews 81:209-237.

Thomas DT, Erdman KA, Burke LM (2016) Position of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine: nutrition and athletic performance. Journal of the Academy of Nutrition and Dietetics 116:501-528.

Tiso M, Schechter AN (2015) Nitrate reduction to nitrite, nitric oxide and ammonia by gut bacteria under physiological conditions. PloS one 10:119-712.

Totzeck M, Hendgen-Cotta UB, Rammos C, Frommke LM, Knackstedt C, Predel HG, Rassaf T (2012) Higher endogenous nitrite levels are associated with superior exercise capacity in highly trained athletes. Nitric Oxide 27:75-81.

Vanhatalo A, Bailey SJ, Blackwell JR, DiMenna FJ, Pavey TG, Wilkerson DP, Jones AM (2010) Acute and chronic effects of dietary nitrate supplementation on blood pressure and the physiological responses to moderate-intensity and incremental exercise. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 34:76 -80.

Whitfield J, Ludzki A, Heigenhauser GJF, Senden JM, Verdijk LB,Van Loon LJ,Holloway GP (2016) Beetroot juice supplementation reduces whole body oxygen consumption but does not improve indices of mitochondrial efficiency in human skeletal muscle. The Journal of physiology 594:421-435.

Wilkerson DP, Hayward GM, Bailey SJ, Vanhatalo A, Blackwell JR, Jones AM (2012) Influence of acute dietary nitrate supplementation on 50 mile time trial performance in well-trained cyclists. European journal of applied physiology 112:4127- 4134.

Wylie LJ, Mohr M, Krustrup P, Jackman SR, Ermιdis G, Kelly J, Jones AM (2013) Dietary nitrate supplementation improves team sport-specific intense intermittent exercise performance. European journal of applied physiology 113:1673-1684.

Zago AS, Reis Silveira L, Kokubun E (2010) Effects of aerobic exercise on the blood pressure, oxidative stress and eNOS gene polymorphism in pre-hypertensive older people. European journal of applied physiology 110:825-832.