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Artigo de Revisão

# Suplementação de nitrato dietético, desempenho aeróbico e potencial de ganhos marginais – uma breve revisão

# Dietary nitrate supplementation, aerobic performance and potential marginal gains - a brief review

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**Resumo:** Nos últimos anos, a suplementação de nitrato, obtido principalmente via suco de beterraba, tornou-se um dos recursos ergogênicos nutricionais mais populares na nutrição esportiva. Supõe-se que o nitrato aumente a biodisponibilidade de óxido nítrico, melhorando a entrega de oxigênio muscular e o desempenho aeróbico. No entanto, esses efeitos da suplementação com nitrato parecem estar relacionados ao nível de aptidão física dos indivíduos. Contudo, em atletas, a suplementação de nitrato exerce ganhos marginais no desempenho aeróbico que podem definir a vitória. Esta revisão tem como objetivo apresentar uma breve atualização sobre os efeitos ergogênicos da suplementação de nitrato no desempenho aeróbico e como o nível de condicionamento físico pode influenciar os efeitos do nitrato. Os mecanismos pelos quais a suplementação de nitrato melhora o desempenho aeróbico e as implicações práticas para ganhos marginais da suplementação de nitrato em um ambiente competitivo também são discutidos.

**Palavras-chave:** Suco de beterraba; Recursos ergogênicos; Nitrato, Desempenho esportivo; Suplemento.

**Abstract**: In recent years, nitrate supplementation, obtained primarily via beetroot juice, has become one of the most popular nutritional ergogenic resources in sports nutrition. The nitrate is supposed to increase nitric oxide bioavailability, improving muscle oxygen delivery and aerobic performance. However, these effects of nitrate supplementation seems to be related to the physical fitness level of individuals. In athletes, nitrate supplementation exerts marginal gains in aerobic performance which may define victory. This review aims to present a brief update on the ergogenic effects of nitrate supplementation in aerobic performance, and how the physical fitness level might influence the nitrate effects. The mechanisms by which nitrate supplementation improves aerobic performance and practical implications for marginal gains from nitrate supplementation in a competitive setting, are also discussed.

**Key words:** Beetroot Juice; Ergogenic; Nitrate; Sports performance; Supplement.

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## 1. Introduction

Ergogenic resources that, directly and/or indirectly, maximize physical and competitive performance have been widely investigated in the last decades. They are used to improve energy use efficiency, to recovery body composition, and resistance to peripheral and central fatigue. Nutritional ergogenic resources are obtained by modulating the dietary composition and/or supplementation use (intake of a nutrient or dietary component above the usual)[1]. Nutritional ergogenic resources improve athletes´ adaptations to physical training and may represent the difference between victory and defeat in a competition.

The International Society of Sports Nutrition considers a nutrient/dietary component ergogenic when: (1) the basic science behind the supposed ergogenic agent presents theoretical evidence; (2) it is legal and safe, and (3) there is robust scientific evidence to support this effectiveness [2]. To date, only five nutritional ergogenic resources are recognized by the International Association of Athletics Federations[3] and International Olympic Committee[4]: beta -alanine, sodium bicarbonate, caffeine, creatine, and nitrate.

In recent years, nitrate ($NO_3^-$) supplementation, obtained via beetroot juice or inorganic nitrate, has become one of the most popular nutritional ergogenic resources in sports nutrition. $NO_3^-$ is supposed to increase nitric oxide (NO) bioavailability improving muscle oxygen delivery and aerobic performance[5,6]. NO is a free radical that plays a pivotal role in cellular functions with worthwhile effects to exercise performance, such as vasodilatation and improvements in mitochondrial respiration, glucose uptake, and skeletal muscle contractility. Because the NO molecule is highly unstable, there is a constant need for its regeneration[7,8].

The spotlight on beetroot juice occurred almost accidentally. It resulted from a search for a natural food source for $NO_3^-$ supplementation to substitute the nitrate salts that are not approved for use in many countries[5]. Beetroot and green leafy vegetables such as spinach, arugula, watercress, lettuce, celery, radish, and chard are the food sources with the highest levels of $NO_3^-$ (250mg/100g) [9]. After their intake, $NO_3^-$ is reduced to nitrite ($NO_2^-$) and subsequently to NO, which becomes available to tissues. The NO synthesis through the NO-synthase enzyme (NOS) is oxygen-dependent, whereas $NO_2^-$ reduction to NO is potentiated by acidosis and hypoxia. Therefore, this last pathway for NO synthesis is preferred during exercise **(Fig. 1)**[8,10].

Nutritional supplements developed to increase the NO availability focus on the NOS-dependent pathway. Thereby, supplements with arginine amino acid as their base became popular. However, scientific evidence has shown that, although plasma arginine increases after intake of these supplements, no significant increase in NO production is observed in response to exercise[2,11]. The first study that highlighted the benefits of $NO_3^-$ dietary intake on aerobic performance was a randomized, double-blind, placebo-controlled crossover clinical trial conducted by Larsen et al.[12]. The trial evaluated nine well-trained men which performed a graded exercise testing on a cycle ergometer (45 to 80% of the peak oxygen consumption - $VO_{2peak}$) after sodium $NO_3^-$ supplementation (0.1 mmol/kg$^-$

$^{1}$/day) or placebo for 3 days. The previous supplementation reduced oxygen consumption without an increase in lactate concentration during the submaximal aerobic physical test, indicating that energy production had become more efficient.

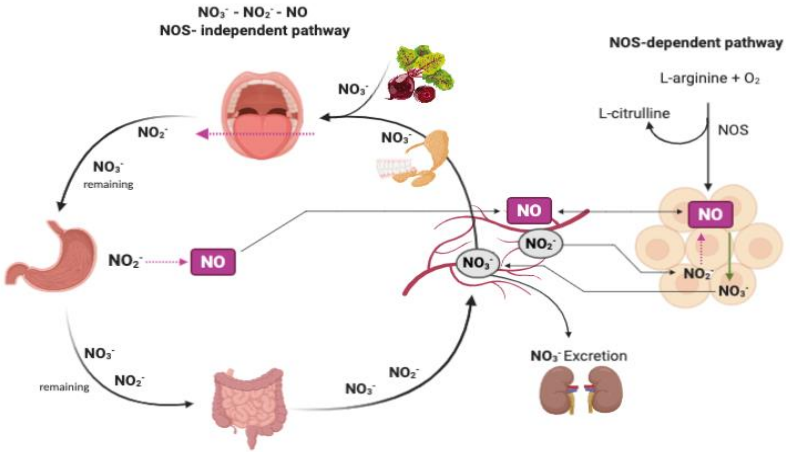

**Figure 1 -** The pathways of nitric oxide (NO) production.

Legend: In the **NO synthase (NOS)–dependent pathway**, L-arginine and $O_2$ produce NO in a reaction catalyzed by the NOS enzymes. After its production, NO can be rapidly oxidized to form $NO_2^-$ and $NO_3^-$. In the **NOS-independent pathway,** the ingestion of $NO_3^-$ from dietary sources is swallowed and rapidly absorbed across the upper gastrointestinal tract into the systemic circulation, where it mixes with endogenous $NO_3^-$. Approximately 70% of ingested $NO_3^-$ is excreted by kidneys whereas 25% of the circulating $NO_3^-$ is actively taken up by the salivary glands. When salivary $NO_3^-$ is secreted into the oral cavity with dietary $NO_3^-$, anaerobic bacteria reduce $NO_3^- \rightarrow NO_2^-$ via $NO_3^-$ reductase enzymes. $NO_2^-$ -rich saliva is then swallowed and further reduce → to NO nonenzymatically in the acidic stomach in a reaction that is greatly enhanced by the presence of vitamin C and polyphenols. Some $NO_2^-$ escapes acidic reduction in the stomach and enters the system circulation. However, most of the remaining $NO_2^-$ and $NO_3^-$ are absorbed in the intestine and directly enter the systemic circulation, generating NO in blood and tissues[10]. Figure created with BioRender.com.

The amount of $NO_3^-$ supplementation used in the studies from Larsen et al.[12] was similar to that found in 150 to 250g of $NO_3^-$ vegetable sources. According to classical knowledge, oxygen consumption is fixed for each submaximal workload with a minimal inter-individual variation. Thus, the discovery that a dietary component could influence oxygen consumption was unexpected[8,13]. In subsequent years, it was also investigated whether $NO_3^-$, specifically from beetroot, would be exclusively responsible for the reduction in oxygen consumption during submaximal exercise since this plant contains other bioactive compounds such as bioflavonoids and carotenoids[14]. Lansley et al.[15] did not observe a reduction in $VO_2$ consumption during submaximal exercise when individuals were previously supplemented with beetroot juice placebo (depleted in $NO_3^-$), confirming that $NO_3^-$ is the primary constituent responsible for the physiological effects of beetroot juice supplementation.

Although extremely popular, the increase in aerobic performance induced by $NO_3^-$ supplementation was not seen in highly trained subjects and elite athletes[16,17]. It seems that the effects of $NO_3^-$ supplementation are influenced by training status (aerobic fitness) and muscle fiber type ratio [18]. Therefore, this brief review aims to present (1) a synthesis of the studies evaluating the effect of $NO_3^-$ supplementation on aerobic performance; (2) the mechanisms by which $NO_3^-$ supplementation favors aerobic performance according to physical fitness level and (3) practical implications for marginal gains from $NO_3^-$ supplementation in a competitive setting.

## 2. Methods

A literature search was performed in English, until July 31th 2022, independently by the authors using MEDLINE, ScienceDirect, Web of Science, and Google Scholar databases. The search was not restricted by date. Three blocks of concepts were used (these keywords were used individually and/or combined): the first, with terms related to the effect of $NO_3^-$ supplementation and the mechanisms by which $NO_3^-$ supplementation favors aerobic performance ($NO_3^-$, nitrate, "beetroot juice", supplementation, aerobic, performance, ergogenic); the second, with terms related to the type of the study design (trial, random); and the third, terms related to the group of interest ("physical fitness", "aerobic fitness", "training status", "highly trained subjects", "elite athletes"; competition). The articles were selected by two reviewers (CSS and DRM), initially based on reading of the title, then on reading of the abstracts, and subsequently the full articles. In case of disagreement between the two reviewers, a third reviewer had the final decision on inclusion (EAE). The bibliographic references of the studies found in these databases were also reviewed. In order to clarify the available scientific evidence regarding the effect of $NO_3^-$ supplementation on aerobic performance, the authors inclusion articles of systematic review with meta-analysis published so far and summarized the results. For the other topics, relevant studies were combined and analyzed to provide an overview of the available research, besides with practical implications for marginal gains from $NO_3^-$ supplementation in a competitive setting.

## 3. Results

**Chart 1** presents the summary of the meta-analysis studies evaluating the effect of $NO_3$- supplementation on aerobic performance. In addition, we present the characteristics of the physical exercise test protocols used in clinical trials to evaluate the effect of $NO_3^-$ supplementation as follows: (1) time trial test: exercise is performed in the shortest time possible over a predetermined distance; (2) graded-exercise test: exercise is performed with a systematic and linear increase in workload over time until the individual is unable to maintain or tolerate the exercise and (3) exhaustion test (open-ended test): exercise is performed at constant intensity until the individual reaches voluntary fatigue.

Hoon et al.[19] and McMahon, Leveritt e Pavey[20] analyzed the effect of $NO_3^-$ supplementation on aerobic performance during different physical exercise test protocols in women and men (age ranged from 16.7 to 64 years). Both authors observed that $NO_3^-$ supplementation was ergogenic only when aerobic performance was assessed by the exhaustion test. The analyses presented by these authors need to be considered carefully since the ergogenic effect of $NO_3^-$ did not consider the physical fitness level. Therefore, grouping all individuals regardless of physical fitness level may have led to an incorrect interpretation since $NO_3^-$ supplementation does not seem to benefit well-trained individuals[21,22,23]. In the meta-analysis carried out by Van De Walle e Vukovich[24], $NO_3^-$ supplementation was ergogenic, improving aerobic performance in both graded-exercise and exhaustion tests. However, when the effects of supplementation were assessed according to the level of physical fitness (well-trained, recreationally active, athletes and untrained subjects - defined by each study), the aerobic performance was significantly increased only in untrained individuals.

Campos et al.[16] evaluated whether the effect of $NO_3^-$ supplementation on aerobic performance was dependent on the individual's physical fitness levels (adult subjects). The authors assessed 64 trials with well-trained subjects ($VO_{2máx}$ 61.1±1.8 ml $kg^{-1}$ $min^{-1}$) and 43 trials with non-athletes subjects ($VO_{2max}$ 50.5±1.8 ml $kg^{-1}$ $min^{-1}$). The $NO_3^-$ supplementation was ergogenic only in non-athletes with superior effects in non-athletes performing the long-duration test (> 180 seconds, characterized by a predominance of the aerobic pathway) than in non-athletes performing the long-term exhaustion test. To consolidate these results, the authors evaluated the effect of $NO_3^-$ supplementation on aerobic performance according to 5 levels of physical fitness. Individuals classified at the lowest levels (1 and 2) showed a significant increase in performance after the supplementation. However, this increase did not occur in individuals at the highest levels (4 and 5).

The most recent review by Senefeld et al.[25] included an evaluation of the effects of $NO_3$- on individuals with different fitness levels as part of their secondary analyses. The authors assessed 80 trials with healthy subjects (age ranged from 18 to 40 years). The pooled analysis considering all studies included in the quantitative synthesis revealed a very small effect size (0.174; CI95% 0.120 – 0.229, $p < 0,001$) of $NO_3^-$ supplementation on aerobic performance. Subgroup analyses demonstrated that the ergogenic effect of $NO_3^-$ supplementation on aerobic fitness was not observed in well-trained endurance athletes (≥65 ml $kg^{-1}$ $min^{-1}$). Although the small effect size in the pooled analysis, for the authors, an enhancement of exercise performance by ~3% with NO3- supplementation in the context of a competitive setting may be highly meaningful (e.g., 48 seconds in 16.1 km cycling time trial across many different exercise modalities and performances[15]) and is not dissimilar to the potential ergogenic effect of new running shoes with embedded carbon fiber plates [26].

In general, results from meta-analysis studies demonstrate that the ergogenic effects of $NO_3^-$ supplementation are more likely observed in less-trained than in highly-trained individuals. In non-athlete subjects, these effects seem to be more pronounced when aerobic performance is assessed by the exhaustion test. Exhaustion tests are considered more accurate than graded-exercise tests in evaluating exercise tolerance. However, exhaustion tests do not apply to most sports competitions that require athletes to complete a specific course as fast as possible. These results suggest a strong link between the subjects' training status and the ergogenic effects of $NO_3^-$ supplementation.

**Chart 1** – Summary of meta-analysis studies that evaluated the ergogenic effect of $NO_3^-$ supplementation on aerobic performance.

| Reference | Subjects and Nitrate protocol | Experimental design | Main results |
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| Hoon et al.[19] | **Subjects:** 184 well-trained and recreationally fit<br>**supplementation:** predominant of beetroot juice<br>**Dose**: between 5.0 and 9 mmol<br>**Dose duration:** acute (<1 day) and prolonged period (until 15 days) | **Effect of $NO_3^-$ supplementation assessed according to performance in physical exercise test protocols:**<br>- time trial test: 8 clinical trials<br>- exhaustion test: 4 clinical trials<br>- graded-exercise testing: 5 clinical trials | $NO_3^-$ supplementation was ergogenic when aerobic performance was assessed in the **exhaustion test:** effect size 0.79; CI95% 0.23 – 1.35 ($p = 0.006$). |
| McMahon, Leveritt e Pavey[20] | **Subjects:** 581 ($VO_{2max} > 28.1$ ml $kg^{-1}$ $min^{-1}$)<br>**$NO_3^-$ supplementation:** predominant of beetroot juice<br>**Dose**: between 4.1 and 19.5 mmol<br>**Dose duration:** acute (<1 day) and prolonged period (until 15 days) | **Effect of $NO_3^-$ supplementation assessed according to performance in physical exercise test protocols:**<br>- time trial test: 28 clinical trials<br>- exhaustion test: 22 clinical trials<br>- graded-exercise testing: 8 clinical trials<br>**Effect of $NO_3^-$ supplementation assessed according to:**<br>**Exercise type:** cycling and others<br>**$NO_3^-$ supplementation:** beetroot juice and others<br>**Dose duration:** acute (< 6 hours) e and prolonged period (≥ 6 hours) | $NO_3^-$ supplementation was ergogenic when aerobic performance was assessed in the **exhaustion test:** effect size 0.33; CI95% 0.15 – 0.50 ($p<0,01$). |

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| | | **Dose:** <6.5 mmol and ≥6.5 mmol<br>**Fitness level (**$VO_{2max}$**):** low (< 44.0 ml kg$^{-1}$ min$^{-1}$) e high (> 45.0 ml kg$^{-1}$ min$^{-1}$) | |
| Van De Walle e Vukovich[24] | **Subjects:** 324 (well-trained, recreationally active, athletes and untrained - defined by each study)<br>**$NO_3^-$ supplementation:** predominant of beetroot juice<br>**Dose**: between 4.2 and 19.5 mmol<br>**Dose duration:** acute (<1 day) and prolonged period (until 15 days) | **Effect of $NO_3^-$ supplementation assessed according to performance in physical exercise test protocols:**<br>- time trial test: 38 clinical trials<br>- exhaustion test: 22 clinical trials<br>- graded-exercise testing: 16 clinical trials<br>**The effect of $NO_3^-$ supplementation was assessed according to the level of physical fitness:**<br>Trained and untrained (defined by each author) | $NO_3^-$ supplementation was ergogenic when aerobic performance was assessed in the **exhaustion test** and **graded-exercise testing**: effect size 0.28 (CI 95%: 0.08 – 0.47; p = 0.006) and **untrained:** effect size 0.32 (CI95%: 0.10 – 0.53; p = 0.004). |
| Campos et al.[16] | **Subjects:** 662 non-athletes ($VO_{2máx}$ 50.5±1.8 ml kg$^{-1}$ min$^{-1}$) and athletes ($VO_{2máx}$ 61.1±1.8 ml kg$^{-1}$ min$^{-1}$)<br>**$NO_3^-$ supplementation:** predominant of beetroot juice<br>**Dose**: between 4.0 and 19.5 mmol<br>**Dose duration:** acute (<1 day) and prolonged period (until 15 days) | **Effect of $NO_3^-$ supplementation assessed according to:**<br>**Physical fitness:** non-athletes (43 clinical trials) and athletes (61 clinical trials)<br>**Test duration**<br>Short: < 180 seconds (non-athletes: 18 clinical trials and athletes: 17 clinical trials)<br>Long: ≥ 180 seconds (non-athletes: 25 clinical trials and athletes: 44 clinical trials)<br>**Physical exercise test protocols (untrained):**<br>- time trial test: 4 clinical trials<br>- exhaustion test: 14 clinical trials | $NO_3^-$ supplementation was ergogenic in:<br>- non-athletes: effect size 0.25; CI95% 0.11 – 0.38 ($p< 0.05$);<br>**- non-athletes to long-duration tests:** effect size 0.33; CI95% 0.15 – 0.51 (p< 0,05)<br>**- non-athletes to long-duration, exhaustion test:** effect size 0.47; CI95% 0.23 – 0.71($p< 0.05$)<br>Percentage of trials reporting increased |

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| | | - graded-exercise testing: 5 clinical trials<br>**Physical fitness level:**<br>Level 1: VO$_{2max}$ <45.0 ml/kg/min<br>Level 2: VO$_{2max}$ between 45.0 and 54.9 ml/kg/min<br>Level 3: VO$_{2max}$ between 55.0 and 64.9 ml/kg/min<br>Level 4: VO$_{2max}$ between 65.0 and 71.0 ml/kg/min<br>Level 5: VO$_{2max}$ > 71.0 ml/kg/min | aerobic performance in individuals classified into different fitness level:<br>Level 1: 50%<br>Level 2: 56%<br>Level 3: 37%<br>level 4 and 5: did not significantly increase |
| Senefeld et al.[25] | **Subjects:** 1.335 healthy, age between 18 and 40 years and VO$_{2máx}$ between <45 and > 65 ml kg$^{-1}$ min$^{-1}$<br>**NO$_3^-$ supplementation:** predominant of beetroot juice<br>**Dose**: between 1.0 and 28.7 mmol<br>**Dose duration:** acute (40 and 210 min before exercise initiation) and prolonged period (until 15 days) | **Effect of NO$_3^-$ supplementation assessed according to:**<br>**Biological sex:** 1.179 men and 156 women<br>**Physical fitness level:**<br>Level 1: VO$_{2max}$ <50.0 ml/kg/min<br>Level 2: VO$_{2max}$ between 50.0 and 54.9 ml/kg/min<br>Level 3: VO$_{2max}$ between 55.0 and 59.9 ml/kg/min<br>Level 4: VO$_{2max}$ between 60.0 and 64.9 ml/kg/min<br>Level 5: VO$_{2max}$ > 65.0 ml/kg/min<br>**Fraction of inspired oxygen (F$_i$O$_2$) during exercise:** comparison of hypoxic and normoxic conditions<br>**Mean exercise time:**<br>< 300 seconds<br>301 – 600 seconds<br>601 – 999 seconds<br>> 1000 seconds<br>**Exercise type:** cycling, handgrip, knee extension, rowing and running | NO$_3^-$ supplementation was ergogenic:<br>**Pooled analysis considering all studies:** the effect size was very small – 0.174; CI95% 0.120 – 0.229, $p < 0{,}001$)<br>**Biological sex:** not observed in studies with only women<br>**Physical fitness level:** not observed in well-trained endurance athletes (VO$_{2max}$ > 65.0 ml/kg/min)<br>**F$_i$O$_2$:** not modulated by hypoxia vs normoxia<br>**Mean exercise time:** revealed heterogeneous results for exercise type and limited effect in long-duration exercise (> 1000 seconds).<br>**NO$_3^-$ dosage and timing:** |

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| | | **$NO_3^-$ dosage and timing** | any dose between 5.1 and ~25 mmol·d$^{-1}$; at least 1 day of supplementation; ingestion 2 – 3.5 hours before initiation of exercise |

## 4. Discussion

### Mechanisms associated with improvements in aerobic performance after $NO_3^-$ supplementation

The mechanisms by which $NO_3^-$ supplementation might improve performance are still debated. Previous studies have reported that dietary supplementation with sodium nitrate or $NO_3^-$ -rich beetroot juice:

- elevates plasma [$NO_2^-$] providing oxygen through a pathway independent of NO synthase[6,27];
- increases the store of $NO_3^-$ and $NO_2^-$ in skeletal muscle with levels far exceeding those observed in the blood[28,29];
- reduces oxygen uptake during skeletal muscle contractions[12,15,30,31];
- may improves calcium ($Ca^{2+}$) handling in the skeletal muscles through pathways involving calsequestrin1 and dihydropyridine receptor[32,33,34];
- increases oxygen transport across active muscle fibres[6];
- increases blood flow to skeletal muscle and slows the reduction of oxygen partial pressure in microvascular tissues[34,35];

Taken together, these studies indicate $NO_3^-$ as a potential supplementation to improve the balance between muscle oxygen delivery and metabolic demand, particularly in the contracting skeletal muscles where the acidic and hypoxic milieu might compromise NOS-derived NO, but potentiate $NO_2^-$-derived NO production[6].

Nevertheless, the increase in NO availability does not always lead to an improvement in performance in all individuals. What would explain the absence of ergogenic properties of beetroot juice supplementation in highly trained subjects and elite athletes? Compared to less trained athletes, these subjects may have higher baseline $NO_2^-$ levels but lower rising in plasma $NO_3^-$ and $NO_2^-$ after $NO_3^-$ supplementation[36,37]. One possible explanation for this higher baseline $NO_2^-$ levels and this blunted responsiveness to $NO_3^-$ supplementation in well-trained athletes might be their greater habitual energy intake which is associated with more healthy eating patterns, including ($NO_3^-$-rich) vegetable consumption. Despite the popularity of beetroot juice, many other $NO_3^-$-rich vegetables exist, such as spinach, rocket salad (arugula), and other green leafy vegetables.

The ingestion of such vegetables increase $NO_3^-$ and $NO_2^-$ bioavailability just as effectively as beetroot juice[38]. However, Jonvik et al.[39] reported a modest $NO_3^-$ intake and a significant inter-individual variation in $NO_3^-$ consumption (19-525 mg/d) in elite Dutch athletes. Furthermore, the intake was similar to that reported in general population.

It seems the ergogenic effect of $NO_3^-$ supplementation is somehow related to the fiber type ratio in skeletal muscles[18]. In experimental animals, $NO_3^-$ supplementation increased the development of strength in type II muscle fiber (MF) (fast-twitch glycolytic fibers type IIx - ) but not in type I MF (slow-twitch oxidative fibers)[32]. Compared to type I MF, type II MF has a lower blood supply and a lower oxygen delivery[40]. Long-duration high-intensity exercise and intermittent high-intensity exercise increase muscle hypoxia and acidosis, especially in type II MF[41] which may disrupt the function of NOS-derived NO and favor the $NO_3^-$– $NO_2^-$–NO pathway[42]. The $NO_3^-$ supplementation does not seem to improve performance in highly trained cyclists[22,43], runners[21,44] and cross-country skiers[23]. These endurance-trained athletes seem to have a higher type I MF ratio than non-trained, recreationally active athletes[18,45], physically inactive population and elderly[46]. In contrast, the supplementation improved performance in highly trained kayakers and rowers[47,48]. In fact, it is recognized that upper body muscles (e.g., biceps brachii, triceps brachii, deltoid, trapezius, or latissimus dorsi) have a higher type II MF ratio than type I MF[49,50,51]. For example, highly trained athletes in rowing[52] and kayaking[53] develop hypertrophy and have increased type II MF ratio[54]. The specific adaptations in type II MF and the high aerobic capacity of these athletes may explain the lower ergogenicity of DN supplementation in some highly trained athletes[38].

## $NO_3^-$ supplementation and marginal gains in athletes

The International Association of Athletics Federations[3] and the International Olympic Committee[4] recognize that $NO_3^-$ supplementation may increase the performance in exhaustion tests (open-ended test) and in time trial tests (sports modalities lasting <40 minutes) by 4-25% and 1-3%, respectively. Ergogenic effects are generally seen within 2–3 hours following a $NO_3^-$ bolus of 310–560 mg and prolonged periods of $NO_3^-$ intake (> 3 days), nevertheless, for elite athletes and exercises lasting <12 minutes the evidence are limited.

The effects of $NO_3^-$ supplementation in elite athletes are not yet completely elucidated, especially due to methodological limitations that hinder the detection of small but relevant effects. The main problem is that a large sample size of elite athletes is difficult to obtain. Also, from the perspective of more ecologically valid situations (e.g., sports competitions), an enhancement on performance less than 1% may define the winner. Thus, such small differences in performance are challenging to detect in a laboratory setting[38,55].

In **Chart 2**, we present some clinical trials investigating the effect of $NO_3^-$ supplementation on athlete´s aerobic performance during time trial tests (some clinical trials included in the meta-analyses discussed in the previous session). In some clinical trials, $NO_3^-$ supplementation did not improve performance on time trial tests (no statistical differences were detected between supplemented and non-supplemented subjects). However, the individuals supplemented with $NO_3^-$ presented a time to complete the time trial tests 0.7 seconds to 1.2 minutes lower than the placebo group. Considering a competitive setting, a difference of a few seconds, even if not statistically significant, has clinical relevance. To exemplify the importance of this small difference, we presented in the last column in Chart 2, the time and the corresponding distances of the first places' athletes in some competitions from the Brazilian Olympic games in 2016[56]. As shown, a small difference in time defined the winner.

Another result that drew attention due to the tiny margin of difference came from the traditional Brazilian São Silvestre marathon in 2019. In the final meters of the men's race, the Ugandan athlete Jacob Kiplimo surpassed the Kenyan athlete Kibiwott Kandie and crossed the finish line at the first place with just 1 second difference to the second place (42'59'' versus 43'00'')[57].

**Chart 2 –** The ergogenic effects of $NO_3^-$ supplementation on athletes' aerobic performance assessed in time trial tests.

| **Reference** | **Subjects** | **Nitrate protocol** | **Test distance** | **Main result** | **Brazilian Olympics (2016)** |
|---|---|---|---|---|---|
| Callahan et al.[58] | Cyclists<br>$VO_2$ de 65.2 ± 4.2 ml $kg^{-1}$ $min^{-}$ | 3 days<br>Dose 5,0 mmol<br>Pre-test dose: 60 min | 4 km time trial<br>Cycle ergometer | Supplementation: 337.4±17.1 sec<br>**(↓ 0.7 sec)**<br>Placebo: 338.1± 18.0 sec | **Cycling track 4 km (team pursuit men)**<br>1º: 03:51:94 minutes<br>**(↓ 4 seconds from 2nd place)**<br>2º: 03:55:39 minutes<br>3º: 03:55:60 minutes |
| McQuillan et al.[59] | Cyclists<br>$VO_2$ de 63 ± 4 ml $kg^{-1}$ $min^{-}$ | 8 days<br>Dose 4 mmol<br>Pre-test dose: 120 min | 4 km time trial<br>Cycle ergometer | Supplementation: 343.6±14.3 sec<br>**(↓ 1.2 sec)**<br>Placebo: 344.8± 14.0 sec | |
| Nyakayiru et al.[60] | Cyclists and triathletes<br>$VO_2$ de 65 ± 4 ml $kg^{-1}$ $min^{-}$ | 6 days<br>Dose 12.9 mmol<br>Pre-test dose: 240 min | 10 km time trial<br>Cycle ergometer | Supplementation: 1004±61 sec<br>**(↓ 16 sec)**<br>Placebo: 1017± 71 sec | **Athletics 10 km (men)**<br>1º: 27:05:17 minutes<br>**(↓ 47 milliseconds from 2nd place)**<br>2º: 27:05:64 minutes<br>3º: 27:06:26 minutes<br>4º: 27:06:27 minutes<br>5º: 27:08:92 minutes |
| Shannon et al.[44] | Distance runners and triathletes | 1 day<br>Dose 12.5 mmol | 10 km time trial<br>treadmill | Supplementation: 2643.1±324.1 sec<br>**(↓ 6 sec)** | |

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| | $VO_2$ de 62.1 ± 8.1 ml $kg^{-1}$ $min^{-}$ | Pre-test dose: 180 min | | Placebo:<br>2649.9±319.8 sec | |
| Mosher et al.[43] | Cyclists<br>$VO_2$ de 60.8 ± 7.4 ml $kg^{-1}$ $min^{-}$ | 3 days<br>Dose 12.8 mmol<br>Pre-test dose: 180 min | 40km time trial<br>Cycle ergometer | Supplementation:<br>4098.0±209.8 sec<br>**(↓ 63.9 sec)**<br>Placebo:<br>4161.9± 263.3 sec | **Cycling road 54.6 km (men)**<br>1º: 01:12:15 hour<br>**(↓ 47 milliseconds from 2nd place)**<br>2º: 01:13:02 hour<br>3º: 01:13:17 hour<br>4º: 01:13:21 hour<br>5º: 01:13:25 hour |
| Wilkerson et al.[61] | Cyclists<br>$VO_2$ de 63 ± 8 ml $kg^{-1}$ $min^{-}$ | 1 day<br>Dose 6.2 mmol<br>Pre-test dose: 150 min | 80km time trial<br>Cycle ergometer | Supplementation:<br>136.7±5.6 min<br>**(↓ 1.2 min)**<br>Placebo:<br>137.9± 6.4 min | |

Legend: $VO_{2max}$: maximal oxygen uptake.

## Pratical Applications

Therefore, considering that such few time difference defines the winner in a competitive setting, how to investigate the effects of $NO_3^-$ supplementation in elite athletes? For Jonvik et al.[38], to detect small but relevant differences, it would be preferred to measure time performance repetitively in the same athlete instead of comparing time performance in different groups. Accordingly, some studies found no statistical effects of $NO_3^-$ supplementation in elite athletes but someones seemed to respond very positively to $NO_3^-$ supplementation[21,22].

In sports science, researchers have been discussing alternatives to the probability value (p-value) that varies depending on the sample size and the magnitude of the association. Some authors suggest the approach based on the Bayesian method and adaptations to make statistical inference based on the magnitude of quantifying and interpreting effects and calculating probabilities to make decisions based on chances of benefits and risks. These authors encourage the use of these methods, which are appropriate for analyzing small samples and provide indications about the 'minimum clinical difference' between sample groups[62,63]. Moreover, the authors argue that the statistical inference method allows using small samples to form larger samples which contribute to meta-analysis studies. Instead, some researchers consider this method controversial and do not recommend its use[64,65]. Although this discussion is far from over, we cannot forget that statistical inference can help to form conclusions, but it cannot replace the reasoning and the approximation with clinical relevance.

For Hlinský, Kumstát e Vajda[17], it is evident that translating athlete-$NO_3^-$ supplementation outcomes into practical interventions requires determining their translational potential. For Close, Kasper e Morton[66], elite sport is dynamic, unpredictable and often chaotic, thus, the results not always can be interpreted by a two-way ANOVA or predicted from the controlled laboratory environment. In this sense, these authors recently proposed an excellent 9 step framework, that may assist practitioners in the proper evaluation of sports nutrition research and applying the findings into practice.

In addition, in the real world, athletes and coaches have understood the impact of small margins of difference in a competitive setting and also have found advantages in aerobic performance with these small margins. Therefore, could be the dietary $NO_3^-$ supplementation a strategy to maximize an athlete's performance through marginal gains?

## 5. Conclusion

Dietary $NO_3^-$ supplementation promotes ergogenic effects on aerobic performance of individuals with less physical fitness, mainly when evaluated in stress tests until exhaustion (open-ended test). In athletes, although scientific evidence demonstrates that $NO_3^-$ supplementation did not lead to statistically significant effects on aerobic performance, more controlled studies are needed in which the clinical significance of small differences is considered. In practice, these small differences may define the winner in a competitive setting.

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