REVIEW

Factors that Moderate the Effect of Nitrate Ingestion on Exercise Performance in Adults: A Systematic Review with Meta-Analyses and Meta-Regressions

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ABSTRACT

To identify how variables such as exercise condition, supplementation strategy, participant characteristics and demographics, and practices that control oral microbiota diversity could modify the effect of inorganic nitrate ingestion (as nitrate salt supplements, beetroot juice, and nitrate-rich vegetables) on exercise performance, we conducted a systematic review with meta-analysis. Studies were identified in PubMed, Embase, and Cochrane databases. Eligibility criteria included randomized controlled trials assessing the effect of inorganic nitrate on exercise performance in healthy adults. To assess the variation in effect size, we used meta-regression models for continuous variables and subgroup analysis for categorical variables. A total of 123 studies were included in this meta-analysis, comprising 1705 participants. Nitrate was effective for improving exercise performance (standardized mean difference [SMD]: 0.101; 95% CI: 0.051, 0.151, $P < 0.001$, $I^2 = 0\%$), although nitrate salts supplementation was not as effective ($P = 0.629$) as ingestion via beetroot juice ($P < 0.001$) or a high-nitrate diet ($P = 0.005$). Practices that control oral microbiota diversity influenced the nitrate effect, with practices harmful to oral bacteria decreasing the ergogenic effect of nitrate. The ingestion of nitrate was most effective for exercise lasting between 2 and 10 min ($P < 0.001$). An inverse dose-response relation between the fraction of inspired oxygen and the effect size (coefficient: −0.045, 95% CI: −0.085, −0.005, $P = 0.028$) suggests that nitrate was more effective in increasingly hypoxic conditions. There was a dose-response relation for acute administration ($P = 0.049$). The most effective acute dose was between 5 and 14.9 mmol provided ≥150 min prior to exercise ($P < 0.001$). An inverse dose-response for protocols ≥2 d was observed ($P = 0.025$), with the optimal dose between 5 and 9.9 mmol·d^{-1} ($P < 0.001$). Nitrate, via beetroot juice or a high-nitrate diet, improved exercise performance, in particular, in sessions lasting between 2 and 10 min. Ingestion of 5–14.9 mmol·d^{-1} taken ≥150 min prior to exercise appears optimal for performance gains and athletes should be aware that practices controlling oral microbiota diversity may decrease the effect of nitrate. *Adv Nutr* 2022;13:1866–1881.

Statement of Significance:

- ▶ Hygiene practices that harm the oral microbiota may negatively impact the ergogenic effect of nitrates.
- ▶ Nitrate ingestion appears most beneficial for exercise lasting 2–10 min in duration.
- ▶ The optimal dose was 5–14.9 mmol ≥150 min prior to exercise when ingested acutely, and 5–9.9 mmol if ingestion was chronic (≥2 d).
- ▶ Beetroot juice and a high-nitrate diet provide greater ergogenic effects than nitrate salts.
- ▶ Nitrate ingestion seems to be even more effective when exercise is performed in hypoxic conditions.

Keywords: nitrate supplementation, oral microbiota, hypoxia, exercise, nitric oxide

Introduction

NO is a gaseous molecule produced endogenously from nitrate (1) and L-arginine (2), and has several functions in the human body including antigen combat (3), bactericidal function in the stomach (4), maintaining the integrity of the gastric epithelium (5), neurotransmission (6), and anti-inflammatory (7) and antioxidant (8) actions. Nonetheless, NO is known primarily for its vasodilatory capacity (9) and role in blood pressure control (10–13). Although NO can be produced from L-arginine, this pathway relies on limiting

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factors such as oxygen availability and nitric oxide synthase (NOS) enzyme activity (2). NO production via the nitrate pathway, whereby NO accrues from a nitrate-nitrite-NO reduction, has become an interesting alternative to the L-arginine pathway as it is not dependent upon oxygen or NOS activity (1).

Nitrate ingestion has been associated with better skeletal muscle contractile function (14) via calcium handling (15), in addition to a reduced oxygen cost during submaximal exercise (16). These effects have led nitrate to become an increasingly popular and researched method to improve exercise performance. Indeed, meta-analytical data over the last few years suggests that nitrate can improve exercise performance (17–20). Some of these meta-analyses have shown contrasting results regarding the effects of nitrate on different exercise conditions. For example, 1 study showed positive effects of nitrate on time-trial tasks (20), whereas another showed no effect (19). Campos et al. (17) showed that nitrate is ineffective for exercises lasting <180 s, whereas contrasting data from Senefeld et al. (20) showed a general efficacy of nitrate across a wide range of exercise durations, even those <300 s. These discrepancies may be due to large variations in the relative contribution of the energy systems during exercises lasting ≤300 s (21). Maximal exercise lasting ≤ ∼60 s is predominantly fueled by anaerobic energy sources (21), with the anaerobic and aerobic energy contribution being almost identical within the 60–120 s range. As exercise continues, it becomes increasingly more aerobic, though exercise lasting ≤300 s may still rely on an anaerobic energy contribution of ≤20% (21). More detailed separation and analysis of highly anaerobic exercises (<120 s) may provide greater insight into the types of exercise tasks most susceptible to improvements with nitrate ingestion.

Environmental condition is another important aspect for athletes to consider that can influence performance (22–24). Although studies often report environmental data, such as the fraction of inspired oxygen (FiO_2), temperature (°C), and relative humidity (%) (25, 26), no previous systematic review assessed via meta-regression whether these continuous variables could modulate the effect of nitrate. A previous meta-analysis showed that nitrate supplementation was effective when exercise was performed in hypoxia, and performance gains were not greater than when exercise was performed in normoxia (20). However, this analysis grouped all hypoxic conditions together regardless of the degree of FiO_2, which may not have allowed for detailed analysis of whether the degree of hypoxia influenced the ergogenic effect of nitrate. Since NO bioavailability is greater under conditions of low oxygen availability (27), it would be of particular interest to verify how the effect of nitrate supplementation changes relative to FiO_2.

The strategy of ingestion is another factor that may modify the effect of nitrate on exercise outcomes. Evidence shows that antioxidants present in vegetables increase NO bioavailability via nitrite-NO reduction (27, 28). Indeed, a previous study showed that nitrate-rich beetroot juice may reduce oxygen consumption during exercise by 4% compared with an equivalent dose of sodium nitrate (both contained 6 mmol nitrate) (29). Since antioxidants can increase NO production, investigating whether different sources of nitrate (i.e., nitrate salts or vegetables with high nitrate content) could influence its ergogenic effects is crucial. Additionally, it is important to evaluate whether the optimal nitrate dose differs according to the number of days of ingestion, as skeletal muscle stores nitrate (30) and nitrate and nitrite stores increase when ingestion is maintained over several days (31).

The oral microbiota has also been proposed as an important factor that may alter the effect of nitrate ingestion on exercise performance (32). Indeed, the importance of nitrate-reducing bacteria in NO bioavailability is increasingly clear (14, 33). These bacteria — mainly the genus *Actinomyces*, *Rothia*, and *Veillonella* — reside in the oral cavity and reduce nitrate to nitrite, increasing NO bioavailability (34). Govoni et al. (35) showed that antibacterial mouthwash use can decrease NO bioavailability. Subsequently, McDonagh et al. (36) showed that antibacterial mouthwash could also worsen clinical outcomes such as blood pressure. Hence, some researchers have attempted to control the use of antibacterial mouthwash and other oral hygiene practices that could theoretically modify the oral microbiota, including antibacterial toothpaste, chewing gum, and tongue scraping (37, 38). Although no study has directly investigated whether these practices affect the ergogenic effect of nitrate supplementation, it would be of interest to determine whether the lack of these recommendations in studies could dampen or nullify the ergogenic effect of nitrate.

This study aimed to determine how exercise conditions, supplementation strategies, participant characteristics and demographics, and practices that control oral microbiota diversity could modify the ergogenic effect of nitrate supplementation using a systematic review with a meta-analytical approach.

Methods

Study eligibility

This systematic review was reported according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (39). The PICOS (Population, Intervention, Comparator, Outcome, Study Design) method was employed to answer the research question (40). The target population was healthy adults of both sexes and any athletic status without pre-existing diseases. For the intervention,

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Address correspondence to JFM (e-mail: joao_mota@ufg.br).
Abbreviations used: FiO_2, fraction of inspired oxygen; NOS, nitric oxide synthase; RCT, randomized controlled trial; SMD, standardized mean difference; $\dot{V}O_2$, oxygen consumption; $\dot{V}O_{2max}$, maximum oxygen consumption; $\dot{V}O_{2peak}$, peak oxygen consumption.

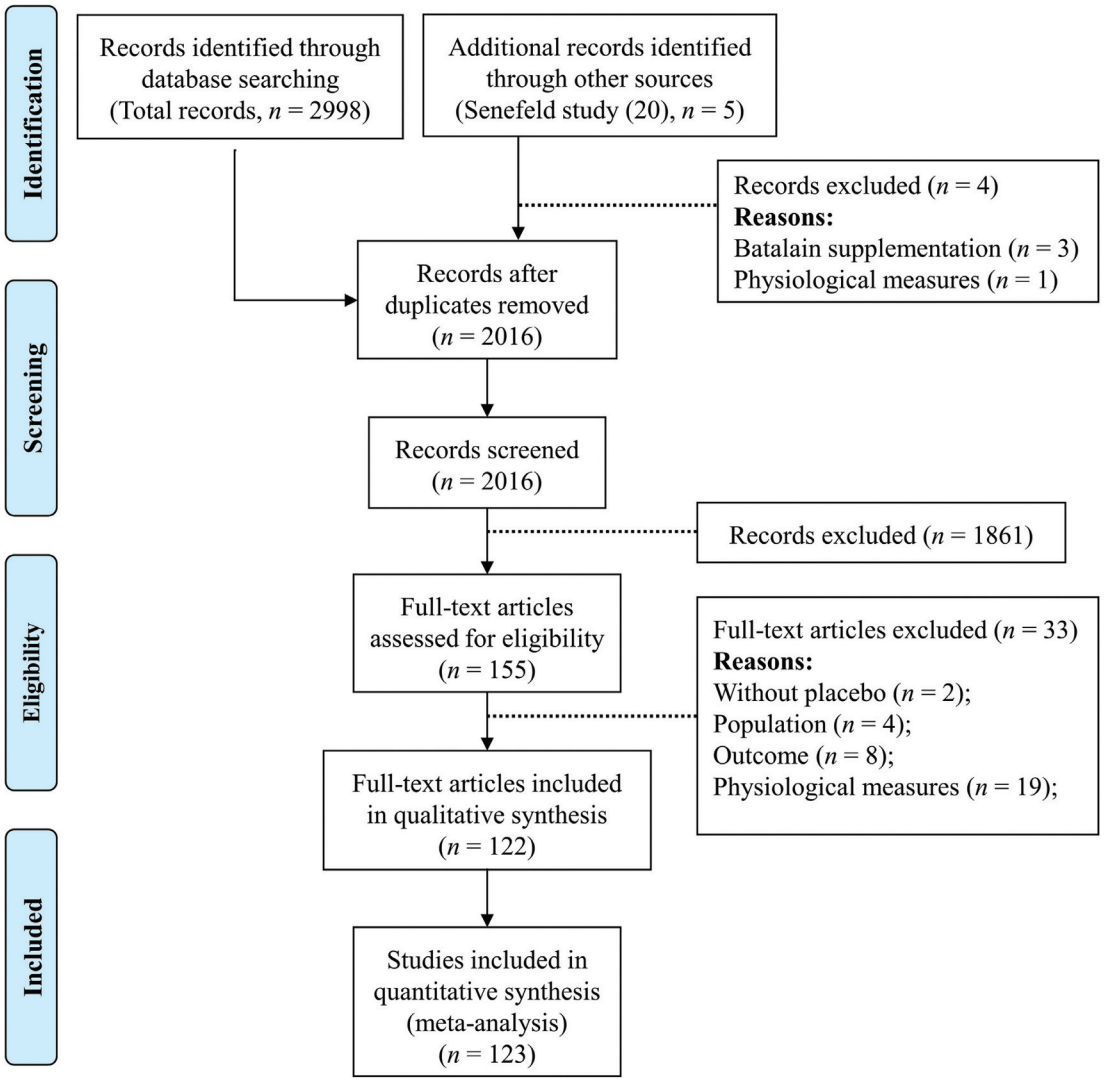

FIGURE 1 PRISMA flow chart of studies evaluated in the systematic review. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

crossover or parallel group designs assessing the effect of inorganic nitrate ingestion in any form (e.g., nitrate salts, beetroot juice, and vegetables with high nitrate content) on exercise performance were included. The comparator was a nonnitrate containing intervention (e.g., nitrate-depleted drinks and low or zero nitrate content vegetables/capsules); included studies were those where the only difference between visits/groups was the nitrate ingestion. Exercise performance (e.g., power output, exercise tolerance, and time-to-completion) was the primary outcome measure. Trials that only assessed changes in physiological parameters, such as blood pressure, heart rate, oxygen saturation, and oxygen consumption, without any performance measure were excluded.

Search strategy

A systematic search was carried out in PubMed (all fields), Embase (all fields), and Cochrane (title, abstract, keyword) databases with a filter from 1 January, 2007 to 31 December, 2020. The initial date was chosen to coincide with the first trial published relating to nitrate and physical exercise (41). The search term "nitrates" was concatenated with "athletic," "exercise," and "sports." References from a previous meta-analysis were also analyzed (20) to detect records that may not have been found via the primary search (**Figure 1**).

Data extraction and variable categorization

Two authors (KVCS and BDC) screened all citations retrieved independently in a 2-step process. The initial step consisted of screening titles and abstracts using online software (Rayyan) (42), and the second step consisted of reviewing full-text articles to confirm study selection using Foxit Reader software (Figure 1). After each stage, discrepancies were resolved via discussion. The information extracted from each study was as follows: author and year of publication, country, study design, population (number and sex), baseline

data (age, body mass, height, BMI), maximum oxygen consumption ($\dot{V}O_{2max}$) and/or peak oxygen consumption ($\dot{V}O_{2peak}$), training experience (years of training and training time per week), environmental conditions (temperature, relative humidity, and FiO_2), ingestion protocol (source [beetroot juice, sodium nitrate, potassium nitrate, gel, and diet], dose, duration, and timing of ingestion relative to exercise), practices that control oral microbiota diversity (antibacterial mouthwash, tongue scraping, antibacterial toothpaste, and chewing gum [advice to avoid brushing teeth was considered as advice for not using antibacterial toothpaste and for not tongue scraping]), exercise protocol, outcome data (mean and SD, variable, and measurement unit), and side effects. For data available only in Figures, WebPlotDigitizer (version 4.4) software was used to extract means and SDs (43). The authors were contacted by e-mail when studies did not provide enough information. A deadline of 2 mo was given for a reply; unfortunately, several data requests were not responded to and were thus not included in the meta-analysis. A solitary outcome measure was extracted per exercise test according to the hierarchy adopted by Saunders et al. (44).

Data transformation

Ingested doses were converted to a total dose in mmol using a molar mass of 62.005, 84.995, and 101.103 g/mol for nitrate, sodium nitrate, and potassium nitrate. Outcome data were converted to mean and SD when reported as mean and SE, median, and IQR or 95% CI. For this, we used the following formulae (45):

$$SD = SE \times \sqrt{n} \quad (1)$$

$$SD = \frac{\sqrt{n} \times (upper\ limit - lower\ limit)}{t\ value\ for\ a\ 95\%\ CI\ from\ study\ sample\ size \times 2} \quad (2)$$

$$SD = \frac{75th\ percentile - 25th\ percentile}{1.35} \quad (3)$$

To group baseline data from independent groups, we used these 2 formulae (45):

$$Mean = \frac{n_1 M_1 + n_2 M_2}{n_1 + n_2} \quad (4)$$

$$SD = \sqrt{\frac{(n_1 - 1)\, SD_1^2 + (n_2 - 1)\, \frac{n_1 n_2}{n_1 + n_2} \left(M_1^2 + M_2^2 - 2M_1 M_2\right)}{n_1 + n_2 - 1}} \quad (5)$$

where n = number of participants, M = mean.

Data adjustment

Timing of ingestion was adjusted with respect to the start of the exercise protocol where necessary. For example, some studies reported that participants ingested the nitrate 3 h before arriving at the laboratory. If the exercise test then started 1 h after the participant arrived at the laboratory due to various reasons (e.g., standardization of tests, pre-exercise measurements, etc.), an ingestion time of 4 h was noted.

Quality assessment

The Cochrane Collaboration's tool (ROB2) for parallel group and crossover clinical trials was used to independently assess risk of bias (46) in a 2-step process. This tool considers bias from randomization and blinding (domain 1), deviations in interventions (domain 2), baseline imbalances (domain 2 for parallel group trials), carryover effects (domain 2 for crossover trials), lack of data (domain 3), outcome measurement (domain 4), and bias in reported outcome selection (domain 5). In addition to the 5 domains of the ROB2 tool, we also assessed bias according to whether participants were familiarized to the exercise test (domain 6). Specifically, if the study did not have individuals perform a specific familiarization session of the exercise protocol, or if the participants were not already familiar with the exercise test (e.g., athletes may be familiar with exercise protocols used in training), then the sixth domain was assessed as "high risk of bias."

Each domain, as well as the final judgment, was classified as either "low risk of bias," "some concerns," or "high risk of bias." For a study to be classified as "low risk of bias," it needed to be considered "low risk" in all 6 domains. Studies were classified as "some concerns" when only 1 domain was classified as "some concerns." If the study had >1 domain assessed as "some concerns," or ≥1 domain as "high risk," the overall study classification would be "high risk of bias" (46).

Certainty of evidence

To assess the quality of evidence and strength of recommendations based upon all the meta-analyses performed here (overall and subanalyses), the GRADE (Grading of Recommendations, Assessment, Development and Evaluations) Working Group approach was employed whereby the certainty in evidence could be classified as high (⊕⊕⊕⊕), moderate (⊕⊕⊕), low (⊕⊕), or very low (⊕). Results from randomized controlled trials (RCTs) start with a high certainty of evidence (⊕⊕⊕⊕) but can be downgraded according to a 5-domain assessment: study limitations (i.e., risk of bias), inconsistency of results (heterogeneity), indirect evidence, imprecision, and publication bias (47).

The ROB2 tool was used to assess study limitations (see the quality assessment section). If 50–75% of the studies assessed were considered low risk of bias, certainty was downgraded 1 level. If <50% of the studies were considered low risk of bias, certainty was downgraded by 2 levels. The I^2 statistic was used to identify any possible heterogeneity in the effect estimates (inconsistency), where 0–25% meant "low heterogeneity," >25–50% meant "moderate heterogeneity" and >50% meant "high heterogeneity" (48). Certainty was downgraded by 1 level for "moderate heterogeneity," and by 2 levels if there was "high heterogeneity." Certainty was downgraded 1 level if the evidence was indirect (e.g., network meta-analysis) (47). In terms of imprecision, certainty was downgraded 1 level when analyses included <10 outcomes; 2 levels if there were <5 outcomes (47). Publication bias was determined using Egger's test for each meta-analyzed

outcome (**Supplemental Table 1**). If there was evidence of publication bias (i.e., $P \leq 0.05$ for Egger's test), certainty of evidence was downgraded 1 level.

Data analysis

Study outcome data were converted to standardized mean differences (SMDs) with 95% CIs using means, SDs, study sample size, and intertrial correlations for matched groups. Main analyses, as well as analyses by moderating variables, were performed using a random-effects model to account for variability across studies (e.g., different populations, exercise tests, environmental conditions, and ingestion protocols) (49). We included only RCTs in the meta-analyses. For the overall analysis, all outcome data from each study were combined to provide 1 single data point for each individual study. For the continuous meta-regressions and subgroup analyses, individual outcomes within each study were considered to allow analysis of factors that might modify the effect of nitrate (i.e., different performance tests, ingestion protocols, or environmental conditions).

Subgroup analysis.

We categorized and analyzed outcomes taking into account aerobic fitness in $\dot{V}O_{2max}$ or $\dot{V}O_{2peak}$ ($\leq$54.9 mL·kg^{-1}·min^{-1} and $\geq$55 mL·kg^{-1}·min^{-1} [55–64.9 mL·kg^{-1}·min^{-1} and $\geq$65 mL·kg^{-1}·min^{-1}]), biological sex (males, females, and males + females), nitrate source (beetroot juice, sodium nitrate, potassium nitrate, beetroot gel, and high-nitrate diet), timing of ingestion prior to exercise ($\leq$120 min, 121–149 min, 150–179 min, and $\geq$180 min), dose ($\leq$4.9 mmol, 5–9.9 mmol, 10–14.9 mmol, and $\geq$15 mmol), exercise duration ($\leq$ 120 s [$\leq$30 s, 30.1–60, $\leq$60 s, 60.1–120 s, and $\leq$120 s], 120.1–600 s [120.1–300 s and 300.1–600 s], and $>$ 600 s [600.1–900 s, 900.1–1200 s, 1200.1–1800 s, and $>$1800 s]) (21), task type (tasks to exhaustion [time to task failure, time-to-exhaustion, time to fatigue, and exercise tolerance] and time-trial tasks), environmental condition (normoxia, hypoxia, and heat [$\geq$30°C]), and practices that control oral microbiota diversity (antibacterial mouthwash, tongue scraping, antibacterial toothpaste, and chewing gum). Only acute ingestion protocols (i.e., performed only on the day of the exercise test) were used to analyze the effect of timing since skeletal muscle nitrate storage may occur with longer periods of consumption (30, 31, 50).

Continuous meta-regressions.

We performed meta-regressions using continuous variables, such as training experience (weekly training in hours and training experience in years), FiO_2 (%), temperature (°C), and relative humidity (%) of the environment to explore associations with effect sizes. Using dose as a continuous covariate and considering that timing can influence the acute effect of nitrate, 3 models were assembled: 1 model for acute ingestion ($\geq$150 min prior to exercise with $<$1 d ingestion); and 2 for long-term ingestion ($\geq$2 d and $\geq$6 d), with all outcomes in normoxic or hypoxic conditions. For the dose-response models, we identified only 2 outliers (24 and 26 mmol) (51, 52) using IQR rules in SPSS software (version 25) and subsequently removed these from the analysis.

All data analyses were performed using the Comprehensive Meta-analysis software (version 3.0). Bubble plots were created using Comprehensive Meta-analysis software (version 3.0), and all other figures using GraphPad Prism software (version 8.0). Additionally, publication bias was evaluated using Egger's regression test. Studies were removed 1 by 1 for sensitivity analysis to verify if any individual study interfered in the direction of the main nitrate effect. Next, all studies classified as "some concerns" and "high risk of bias" were removed to verify if this modified results. Additionally, the I^2 statistic was calculated to identify possible heterogeneity in the effect estimates (48). Finally, effect sizes were interpreted according to threshold values of 0.01, 0.2, 0.5, and 0.8 corresponding to very small, small, moderate, and large effects (53).

Results

Search strategy and characteristics of included studies

Studies.

We included a total of 122 articles (Figure 1), comprising 127 individual studies and 251 outcomes (**Supplemental Tables 2** and **3**). However, only RCTs were used for the meta-analyses and meta-regressions (123 studies and 243 outcomes). Of these, 5 were parallel-group trials and the remaining 118 were crossover (Supplemental Tables 1 and 2). The smallest sample comprised 9 individuals (54) and the largest 70 individuals (55). In crossover trials, the washout period ranged from 2 (56–62) to 21 d (63). Of the 123 studies, 6 involved females only; 20 both sexes; and 96 only males. This provided a total sample size of 1705 participants: 1460 males, 236 females, and 9 unidentified participants (64). The mean age range was 18–39 y; weight 60.0–87.5 kg; and BMI 21.1–27.3 kg/m^2 (65–70).

Main meta-analysis and sensitivity analysis

The main meta-analysis (**Table 1**) showed a very small significant effect of nitrate ingestion on exercise outcomes (123 studies; SMD: 0.101; 95% CI: 0.051, 0.151, P <0.001, I^2 = 0%). No study changed the significant outcome of this meta-analysis after withdrawing studies 1 by 1. The lowest estimate value, excluding Rodrígues-Fernández et al. (71), was 0.095 (122 studies; 95% CI: 0.044, 0.145, P <0.001, I^2 = 0%; Table 1); the highest estimate, excluding Flanagan et al. (72), was 0.108 (122 studies; 95% CI: 0.057, 0.158, P <0.001, I^2 = 0%; Table 1). Additionally, removing those studies classified as "some concerns" or "high risk of bias" did not change the overall result (97 studies; SMD: 0.087; 95% CI: 0.032, 0.142, P = 0.002, I^2 = 0%; Table 1).

Exercise conditions

Exercise duration.

Nitrate ingestion was effective for exercises lasting 120.1–300 s and 300.1–600 s (P <0.05, **Table 2**). However, no effect was shown for any subanalysis of exercises lasting $\leq$120 s (all P >0.05, Table 2). Similarly, no effect was shown for

TABLE 1 Main meta-analysis and sensitivity analyses for the overall effect of nitrate ingestion on exercise performance[1]

Subgroup analyses	*n*	SMD (95% CI)	*P* value	Certainty of evidence
Overall effect	123	0.101 (0.051, 0.151)	<0.001	Moderate (⊕⊕⊕)
Overall effect without Rodrígues-Fernandez et al. (71) [lowest]	122	0.095 (0.044, 0.145)	<0.001	Moderate (⊕⊕⊕)
Overall effect without Flanagan et al. (72) [highest]	122	0.108 (0.057, 0.158)	<0.001	Moderate (⊕⊕⊕)
Only low-risk RCTs	97	0.087 (0.032, 0.142)	0.002	Moderate (⊕⊕⊕)

[1] *n*, number of observations; RCT, randomized controlled trial; SMD, standardized mean difference.

exercise lasting 600.1–900 s, 900.1–1200 s, 1200.1–1800 s, and >1800 s (all $P > 0.05$, Table 2). For exercises lasting ≤10 min (i.e., 0–600 s), meta-regression showed a positive relation between exercise duration and effect size ($P < 0.05$, **Table 3**), such that larger effects were observed with longer exercise duration.

Task type.

There was a small positive effect of nitrate ingestion on time-to-exhaustion protocols ($P < 0.001$, Table 2), but not on time trials ($P = 0.139$, Table 2), with a significant difference between the 2 subgroups at the study level (Q-value = 8.326, $P = 0.016$, **Supplemental Figure 1**).

Environmental condition of exercise.

Nitrate ingestion was effective under both hypoxic and normoxic conditions ($P < 0.05$; **Figure 2** and Table 2), but not under hot conditions (i.e., all >30°C; $P > 0.05$; Table 2). Using FiO_2 as a continuous covariate, meta-regression analysis showed an inverse association between FiO_2 and effect size ($P = 0.028$, **Figure 3** and Table 3), such that smaller effect sizes were observed with decreasing FiO_2. There was no association when including temperature and humidity as continuous covariates ($P > 0.05$; Table 3).

Ingestion strategy

Ingestion protocol.

Of the 243 outcomes, 168 (69.1%) used beetroot juice as the nitrate source; 27 (11.1%) sodium nitrate; 12 (4.9%) potassium nitrate; 9 beetroot gel (3.7%); and 27 (11.1%) other sources (**Supplemental Table 1**). Analyses using categorical covariates showed that ingestion with beetroot juice and beetroot gel resulted in very small significant benefits (SMDs = 0.125 and 0.182; both $P \leq 0.05$, Table 2), whereas a high-nitrate diet showed a small ergogenic effect (SMD = 0.426; $P = 0.005$, Table 2). Sodium nitrate and potassium nitrate did not show significant performance effects (SMDs = 0.048 and −0.022; both $P > 0.05$, Table 2). Acute nitrate ingestion was effective when provided ≥150 min prior to exercise ($P < 0.001$, Table 2), but not ≤ 149 min

TABLE 2 Subgroup analyses for the effect of nitrate ingestion depending on the exercise conditions[1]

Subgroup analyses	*n*	SMD (95% CI)	*P* value	Certainty of evidence
Exercise duration, s				
≤30	13	0.06 (−0.058, 0.178)	0.322	Moderate (⊕⊕⊕)
30.1–60	9	0.106 (−0.118, 0.33)	0.353	Low (⊕⊕)
All ≤60	22	0.07 (−0.035, 0.174)	0.190	High (⊕⊕⊕⊕)
60.1–120	10	0.098 (−0.093, 0.289)	0.314	Moderate (⊕⊕⊕)
≤120	32	0.076 (−0.015, 0.168)	0.103	High (⊕⊕⊕⊕)
120.1–300	21	0.114 (0.002, 0.226)	0.047	Moderate (⊕⊕⊕)
300.1–600	43	0.200 (0.102, 0.297)	<0.001	Moderate (⊕⊕⊕)
120.1–600	64	0.163 (0.089, 0.236)	<0.001	Moderate (⊕⊕⊕)
600.1–900	6	0.000 (−0.243, 0.243)	0.999	Very low (⊕)
900.1–1200	17	0.018 (−0.119, 0.154)	0.800	High (⊕⊕⊕⊕)
1200.1–1800	16	0.069 (−0.068, 0.206)	0.322	Moderate (⊕⊕⊕)
>1800	22	0.053 (−0.075, 0.182)	0.415	High (⊕⊕⊕⊕)
All >600	61	0.043 (−0.031, 0.116)	0.256	Moderate (⊕⊕⊕)
Environmental condition				
Hypoxia	18	0.197 (0.050, 0.343)	0.008	High (⊕⊕⊕⊕)
Normoxia	224	0.106 (0.068, 0.144)	<0.001	Moderate (⊕⊕⊕)
Heat	4	0.090 (−0.214, 0.394)	0.563	Very low (⊕)
Task type				
Time-to-exhaustion	50	0.249 (0.161, 0.336)	<0.001	Moderate (⊕⊕⊕)
Time-trial	80	0.048 (−0.016, 0.111)	0.139	High (⊕⊕⊕⊕)

[1] *n*, number of observations; SMD, standardized mean difference.

TABLE 3 Meta-regressions for the effect of nitrate ingestion depending on the continuous variables[1]

Analysis	*n*	Coefficient (95% CI)	*P* value	Certainty
Environmental condition				
FiO_2, %	23	−0.045 (−0.085, −0.005)	0.028	High (⊕⊕⊕⊕)
Temperature, °C	46	0.002 (−0.011, 0.015)	0.766	High (⊕⊕⊕⊕)
Relative humidity, %	36	−0.003 (−0.009, 0.004)	0.419	High (⊕⊕⊕⊕)
Training				
Weekly training, h	55	0.002 (−0.011, 0.016)	0.740	High (⊕⊕⊕⊕)
Training experience, y	28	−0.009 (−0.032, 0.013)	0.402	High (⊕⊕⊕⊕)
Dose, mmol				
All ≥150 min and <1 d	90	0.015 (0.000, 0.031)	0.049	High (⊕⊕⊕⊕)
All doses ≥2 d	103	−0.023 (−0.043, −0.003)	0.025	Moderate (⊕⊕⊕)
All doses ≥6 d	64	−0.028 (−0.052, −0.005)	0.018	Moderate (⊕⊕⊕)
Exercise duration, s				
≤600	96	0.000 (0.000, 0.001)	0.010	Moderate (⊕⊕⊕)
Individual characteristics				
Age, y	243	−0.004 (−0.013, 0.005)	0.420	Moderate (⊕⊕⊕)
$\dot{V}O_2$, $mL \cdot kg^{-1} \cdot min^{-1}$	135	−0.001 (−0.007, 0.004)	0.624	Moderate (⊕⊕⊕)

[1] FiO_2, fraction of inspired oxygen; *n*, number of observations; $\dot{V}O_2$, oxygen consumption.

prior ($P = 0.318$, Table 2). Overall, doses of ≤4.9 $mmol \cdot d^{-1}$ ($P = 0.522$, Table 2) and ≥15 $mmol \cdot d^{-1}$ ($P = 0.303$, Table 2) were not effective to improve exercise outcomes. However, doses of 5–9.9 $mmol \cdot d^{-1}$ ($P < 0.001$, Table 2) and 10–14.9 $mmol \cdot d^{-1}$ ($P = 0.001$, Table 2) showed small positive effects. Considering ingestion strategies with a duration ≥6 d, a dose of 5–9.9 $mmol \cdot d^{-1}$ was still effective ($P < 0.001$, Table 2), but not 10–14.9 $mmol \cdot d^{-1}$ ($P = 0.311$, Table 2) or ≥15 $mmol \cdot d^{-1}$ ($P = 0.953$, Table 2). Meta-regression using dose as a continuous covariate only when ingestion was performed for ≥6 d showed an inverse dose-response effect ($P = 0.019$, Figure 3 and Table 3). A similar result was shown when ingestion was performed for ≥2 d ($P = 0.023$, Figure 3 and Table 3). There was a positive dose-response effect for acute doses with optimal timing (i.e., <1 d and ≥150 min prior; $P = 0.049$, Figure 3 and Table 3).

Participant characteristics and demographics.

There was a significant effect of nitrate ingestion when aerobic fitness was ≤54.9 $mL \cdot kg^{-1} \cdot min^{-1}$ ($P = 0.016$, **Table 4**), but not ≥55 $mL \cdot kg^{-1} \cdot min^{-1}$ ($P = 0.059$, Table 4). However, there was a significant effect of nitrate for those with aerobic fitness of 55–64.9 $mL \cdot kg^{-1} \cdot min^{-1}$ ($P = 0.036$, Table 4). Despite this, the meta-regression using $\dot{V}O_{2max}$ or $\dot{V}O_{2peak}$ as a continuous variable did not identify a relation between $\dot{V}O_2$ and effect size ($P = 0.624$, Table 3). The meta-regression showed no significant effect for weekly training in hours ($P = 0.740$, Table 3) or for training experience in years ($P = 0.4019$, Table 3) on the response to nitrate ingestion. Nitrate was effective for males ($P < 0.001$, Table 3, **Figure 4**), but not for females ($P = 0.869$, Table 4) or when both males and females were pooled in the same study ($P = 0.881$, Table 2, Figure 4). Meta-regression using age as a continuous variable did not show a relation with effect size ($P = 0.4201$, Table 3).

Practices that control oral microbiota diversity

When participants were requested to abstain from using antibacterial mouthwash, there was a very small positive effect of nitrate ingestion ($P < 0.001$, **Table 5** and Figure 2); however, meta-analysis of studies where participants were not given such instructions showed no performance improvements ($P = 0.130$, Table 5). The lack of control over other factors, such as the use of antibacterial toothpaste, tongue scraping, and chewing gum, did not alter the significant positive effect of nitrate ingestion on exercise outcomes (Table 5). However, effect sizes were marginally greater (+0.041 to + 0.085) when there was counseling to avoid these practices (antibacterial toothpaste [51 outcomes; SMD: 0.168; 95% CI: 0.09, 0.246, $P < 0.001$, $I^2 = 15.9\%$ with $P = 0.169$], tongue scraping [40 outcomes; SMD: 0.184; 95% CI: 0.09, 0.278, $P < 0.001$, $I^2 = 25\%$ with $P = 0.079$], and chewing gum [122 outcomes; SMD: 0.130; 95% CI: 0.081, 0.180, $P < 0.001$, $I^2 = 0\%$]; Figure 2 and Table 5). Further analysis showed a small positive effect when all 4 control practices were reported (37 outcomes; SMD: 0.200; 95% CI: 0.099, 0.301, $P < 0.001$ $I^2 = 29.4\%$; Table 5).

Side effects

Most studies (98 out of 127 [77.2%]) did not evaluate side effects of nitrate ingestion. Of the 29 that did, 20 studies (69%) reported that participants did not experience any side effect and 9 (31%) studies described some type of gastrointestinal discomfort.

Publication bias, risk of bias, and certainty of evidence

A funnel plot (**Figure 5**) of the main meta-analysis demonstrated asymmetry that was confirmed by a significant Egger's test score ($P = 0.002$, Table 1). This suggests that there is publication bias relating to nitrate ingestion studies. Of all 123 studies evaluated using the Cochrane tool, 97 (79%) were assessed as low risk of bias, 23 (19%) as high risk of bias,

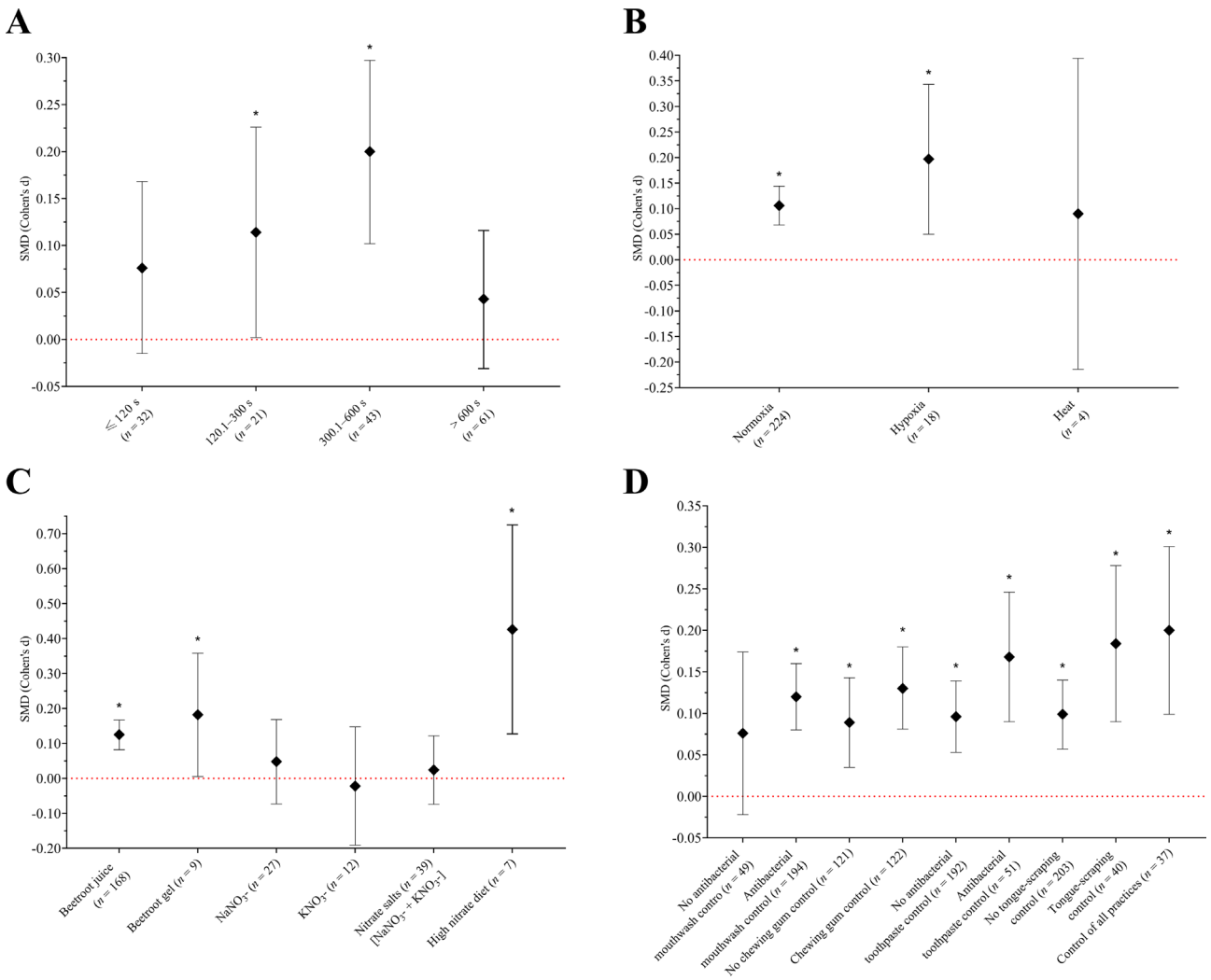

FIGURE 2 Standardized mean differences (SMDs) and 95% CIs of the effect of nitrate ingestion on exercise performance according to: A) exercise duration, B) environmental conditions, C) nitrate source, and D) oral microbiota hygiene practices. *Denotes significant improvement in subgroup after nitrate ingestion compared with the placebo group.

and 3 (2%) as some concerns (Supplemental Table 1). The certainty of evidence for each outcome analyzed ranged from very low to high (Tables 1–2, 4–5). Supplemental Table 3 shows the assessment of each domain of certainty of evidence.

Discussion

This systematic review and meta-analysis showed that inorganic nitrate ingestion leads to very small to small (0.087 to 0.426; *P* values <0.05) improvements in exercise performance in healthy adults. Several factors influenced the ergogenic effect of nitrate, including exercise conditions (exercise duration, environment, and task type), ingestion strategies (dose, timing, and source), participant characteristics and demographics ($\dot{V}O_{2max}$ and sex), and practices that control oral microbiota diversity. These data have important practical implications for physically active healthy adults aiming to ingest nitrates.

Nitrate ingestion is most effective for exercises between 2 and 10 min in duration (Figure 2). This time frame includes a potentially wide range of exercises with different requirements in terms of energy metabolism, though, in general, they might be considered moderate-intensity exercises that are predominantly fueled by aerobic energy sources (21). These may include exercise tasks such as 4 km cycling (56), 2000 m rowing (59), and 3 km running (69). Conversely, nitrate was ineffective for short-duration exercises. Most of the studies included in the ≤30-s analysis used a 30-s Wingate protocol (11 of 13 tests), which predominantly uses the anaerobic energy system (alactic component of the anaerobic energy system [ATP-PCr] and anaerobic glycolysis) (73). Our analyses also showed no effects on exercises ≤120 s, an exercise duration that still has a substantial anaerobic contribution (21). Thus, nitrate ingestion appears ineffective for exercises that have a large reliance on anaerobic energy contribution. Corroborating this assertion,

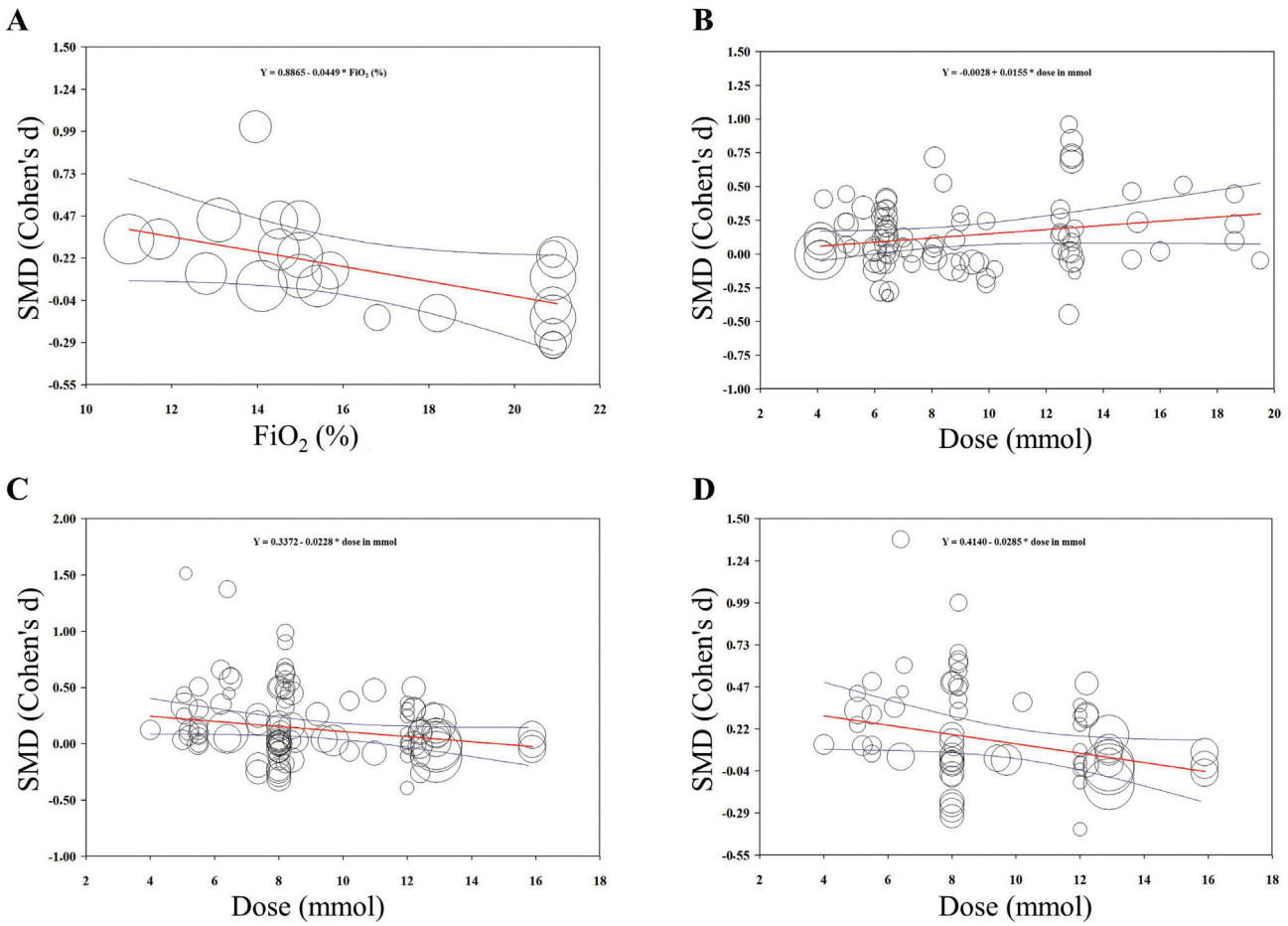

FIGURE 3 Random-effects univariate meta-regression between the standardized mean differences (SMDs) and: A) fraction of inspired oxygen (FiO_2), B) acute dose, C) dose ≥2 d, and D) dose ≥6 d. Each circle represents a study and the size of the circle reflects the influence of that study on the model (inversely proportionate to the SE of that study).

our meta-regressions showed a dose-response effect for exercise duration ≤10 min, increasing the certainty as to the efficacy of nitrate ingestion for predominantly aerobic exercise lasting ≤10 min. Surprisingly, considering the beneficial effects of nitrates on aerobic exercise lasting 2–10 min, exercise lasting >10 min was not improved with nitrate ingestion. Nonetheless, some individual studies have shown beneficial effects of nitrate on longer duration exercise, such as 10 (74) and 16 km cycling (56). Higher nitrate doses (~12.4 $mmol \cdot d^{-1}$) may be necessary during prolonged endurance exercise to attenuate the increase in oxygen consumption and spare muscle glycogen (75). However, well-controlled clinical trials on nitrate and energy metabolism are needed to elucidate how nitrate ingestion could influence energy metabolism during longer duration exercise.

Positive effects of acute nitrate ingestion were shown only when nitrates were ingested ≥150 min prior to exercise. This is somewhat aligned with peak plasma nitrite, which occurs ~120 min after 4.2 and 8.4 mmol of nitrate ingestion (76). Similarly, muscle nitrate peaks ~120 min after nitrate ingestion (30). Therefore, based on these pharmacokinetic data and our results, it is likely the peak conversion to NO may occur close to these peak times for blood and muscle nitrite, and the best time to start exercising is from 150 min onwards after nitrate ingestion.

The current data suggest that the most effective dose will depend on the total number of days of nitrate ingestion. There was a significant and positive dose-response effect on acute nitrate ingestion when analyzing ideal acute timing (i.e., ≥150 min prior) using meta-regression. Analyzing the doses categorically, the optimal acute dose ranges between 5 and 14.9 mmol, with a dose-response effect, meaning doses closer to this upper limit appear preferable. The ideal dose for nitrate ingestion ≥2 d appears to be between 5 and 10 mmol, with no evidence of an effect at doses <5 mmol or >10 mmol. There was also an apparent dose-response effect when nitrate ingestion lasted ≥2 d and ≥6 d, although this effect was inverse (i.e., less nitrate was better). Thus, to optimize performance, it appears best to ingest close to the inferior limit of 5 mmol (maximum 10 mmol) when ingestion is chronic (i.e., >1 d), whereas it appears best to ingest closer to the upper limit of 14.9 mmol

TABLE 4 Subgroup analyses for the effect of nitrate depending on the ingestion strategies[1]

Subgroup analyses	*n*	SMD (95% CI)	*P* value	Certainty of evidence
Duration, d				
1	123	0.104 (0.054, 0.154)	<0.001	High (⊕⊕⊕⊕)
2–5	43	0.110 (0.018, 0.202)	0.020	Moderate (⊕⊕⊕)
≥6	67	0.122 (0.052, 0.192)	0.001	Moderate (⊕⊕⊕)
Dose, mmol·d^{-1} (any duration in days)				
≤4.9	13	−0.055 (−0.221, 0.112)	0.522	Moderate (⊕⊕⊕)
5–9.9	135	0.136 (0.085, 0.188)	<0.001	Moderate (⊕⊕⊕)
10–14.9	60	0.116 (0.046, 0.187)	0.001	High (⊕⊕⊕⊕)
≥15	17	0.075 (−0.068, 0.218)	0.303	High (⊕⊕⊕⊕)
5–14.9	195	0.129 (0.088, 0.171)	<0.001	Moderate (⊕⊕⊕)
≤4.9 + ≥15	30	0.012 (−0.082, 0.106)	0.803	High (⊕⊕⊕⊕)
Dose, mmol·d^{-1} (<1 d)				
≤4.9	12	−0.065 (−0.24, 0.111)	0.469	Moderate (⊕⊕⊕)
5–9.9	68	0.117 (0.046, 0.187)	0.001	High (⊕⊕⊕⊕)
10–14.9	30	0.184 (0.061, 0.308)	0.003	High (⊕⊕⊕⊕)
≥15	13	0.111 (0.06, 0.281)	0.204	High (⊕⊕⊕⊕)
5–14.9	98	0.138 (0.079, 0.196)	<0.001	High (⊕⊕⊕⊕)
≤4.9 + ≥15	25	0.012 (−0.102, 0.126)	0.836	High (⊕⊕⊕⊕)
Dose, mmol·d^{-1} (only ≥6 d)				
≤4.9	1	—	—	—
5–9.9	40	0.195 (0.092, 0.297)	<0.001	Low (⊕⊕)
10–14.9	20	0.057 (−0.053, 0.166)	0.311	High (⊕⊕⊕⊕)
≥15	4	−0.008 (−0.268, 0.252)	0.953	Very low (⊕)
≥10	24	0.047 (−0.054, 0.148)	0.362	High (⊕⊕⊕⊕)
Dose, mmol·d^{-1} (only ≥2 d)				
≤4.9	1	—	—	—
5–9.9	67	0.161 (0.083, 0.238)	<0.001	Moderate (⊕⊕⊕)
10–14.9	30	0.059 (−0.036, 0.155)	0.224	High (⊕⊕⊕⊕)
≥15	4	−0.008 (−0.268, 0.252)	0.953	Very low (⊕)
≥10	34	0.051 (−0.038, 0.141)	0.263	High (⊕⊕⊕⊕)
Timing of ingestion prior to exercise, min				
≤120	19	0.117 (−0.07, 0.304)	0.219	Moderate (⊕⊕⊕)
121–149	5	−0.061 (−0.283, 0.161)	0.588	Low (⊕⊕)
≤149	24	0.076 (−0.073, 0.226)	0.318	Moderate (⊕⊕⊕)
≥150	92	0.123 (0.064, 0.183)	<0.001	High (⊕⊕⊕⊕)
150–179	40	0.139 (0.054, 0.223)	0.001	High (⊕⊕⊕⊕)
≥180	52	0.109 (0.026, 0.191)	0.010	High (⊕⊕⊕⊕)
Nitrate source				
Beetroot juice	168	0.125 (0.082, 0.167)	<0.001	Moderate (⊕⊕⊕)
Sodium nitrate	27	0.048 (−0.073, 0.168)	0.438	High (⊕⊕⊕⊕)
Potassium nitrate	12	−0.022 (−0.191, 0.147)	0.797	High (⊕⊕⊕⊕)
Beetroot gel	9	0.182 (0.005, 0.358)	0.044	Low (⊕⊕)
High-nitrate diet	7	0.426 (0.127, 0.725)	0.005	Very low (⊕)
Other sources	20	−0.002 (−0.198, 0.195)	0.984	Moderate (⊕⊕⊕)
Nitrate salts	39	0.024 (−0.074, 0.122)	0.629	High (⊕⊕⊕⊕)
Aerobic fitness, mL·kg^{-1}·min^{-1}				
≤54.9	64	0.095 (0.018, 0.173)	0.016	Moderate (⊕⊕⊕)
≥55	71	0.071 (−0.003, 0.145)	0.059	High (⊕⊕⊕⊕)
55–64.9	42	0.102 (0.007, 0.197)	0.036	High (⊕⊕⊕⊕)
≥65	29	0.024 (−0.093, 0.142)	0.684	High (⊕⊕⊕⊕)
Sex				
Males only	206	0.144 (0.103, 0.186)	<0.001	Moderate (⊕⊕⊕)
Females only	7	−0.018 (−0.23, 0.194)	0.869	Low (⊕⊕)
Mixed sex	29	−0.004 (−0.089, 0.081)	0.925	Moderate (⊕⊕⊕)
Females only + mixed sex	36	−0.006 (−0.085, 0.073)	0.881	Moderate (⊕⊕⊕)

[1] *n*, number of observations; SMD, standardized mean difference.

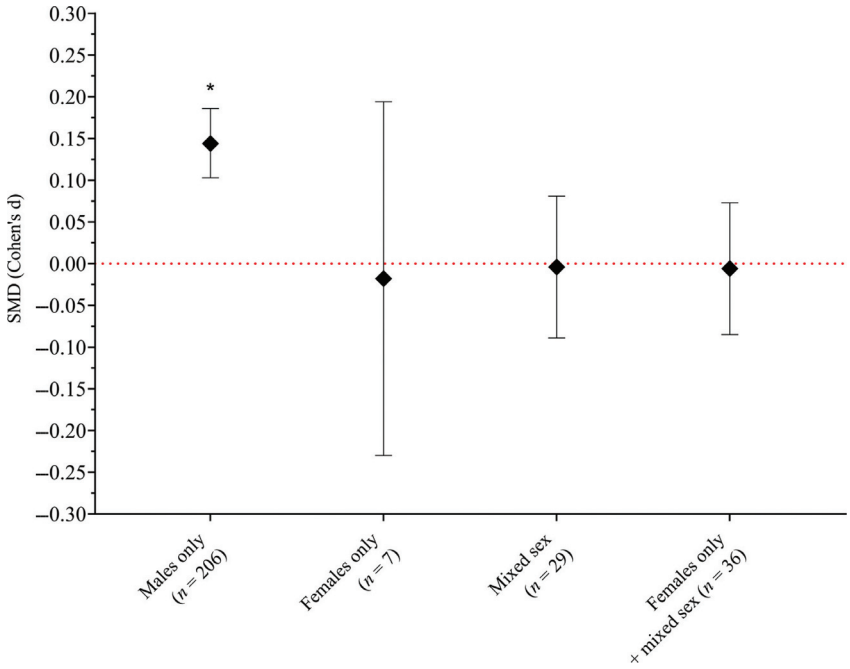

FIGURE 4 Standardized mean differences (SMDs) and 95% CIs of the effect of nitrate ingestion on exercise performance according to sex. *Denotes significant improvement in subgroup after nitrate ingestion compared with the placebo group.

(with a minimum of 5 mmol) when ingestion is acute (<1 d).

Beneficial performance effects were shown only with beetroot juice, beetroot gels, and a high-nitrate diet, but not with nitrate salts (e.g., $NaNO_3$ and KNO_3). Several studies with nitrate salts used exercise durations less likely to be improved with nitrate (e.g., <2 min and >10 min), which may have contributed to this result (51, 65, 69, 77–80). It is also possible that the absence of phytochemicals in nitrate salts contributed to this, as antioxidants can help to reduce nitrite to NO (27). Unlike nitrate salts, vegetables with high nitrate content (i.e., >1000 $mg \cdot kg^{-1}$ fresh matter, such as red beetroot, radish, spinach, cress, and rocket) (81) and their derivative products have phytochemicals that can help increase NO production, such as betalains present in beetroot which has significant antioxidant activity (82). Commercial beetroot juice tested in studies (38, 83) has ascorbic acid as a natural preservative. Previous studies have demonstrated positive effects of betalains themselves on sports performance (84, 85). Thus, it is difficult to conclude that the effect of nitrate ingestion through vegetables with high nitrate content and their derivative products is exclusively due to the nitrate, but rather due to the synergy between these compounds (29, 86). Nonetheless, our data suggest that nitrate ingestion through vegetables with high nitrate content or a high-nitrate diet is the best way to improve exercise performance. It is currently unclear what threshold of nitrate allows us to define a diet as a "high-nitrate diet." Estimates of dietary intake in the American population (age 18–70 y) are that individuals ingest ~1.8 $mmol \cdot d^{-1}$ of nitrate (87). Porcelli et al. (2016) (88) investigated the effect of a high-nitrate diet on plasma nitrate and nitrite, and exercise performance. The control diet contained ~2.9 $mmol \cdot d^{-1}$ of nitrate, whereas the high-nitrate diet contained more than twice this amount (~8.2 $mmol \cdot d^{-1}$), leading to an almost 6-fold increase in plasma nitrate (127 compared with 23 μM) and a near 50% increase in plasma nitrite (350 compared with 240 nM). A high-nitrate diet ranged from 5.47 to 12.4 $mmol \cdot d^{-1}$ across studies, whereas the control diet typically contained 0.1 to 2.9 $mmol \cdot d^{-1}$ of nitrate (88–90).

Antibacterial mouthwash use can decrease the amount of oral nitrate-reducing bacteria and almost annul nitrate-reducing capacity (35). This leads to lower nitrite concentrations in blood and saliva (35), which reduces NO bioavailability. Chronic antibacterial mouthwash use is associated with poor clinical outcomes related to NO metabolism (e.g., blood pressure), which demonstrates that antibacterial mouthwash use can worsen NO metabolism and, consequently, its related outcomes (91). Therefore, it is not surprising that a lack of antibacterial mouthwash control eliminated the beneficial effect of nitrate ingestion on exercise performance. Although the lack of control of other harmful practices to the oral microbiota (tongue scraping, antibacterial toothpaste, and chewing gum) did not hinder the ergogenic effect of nitrate, effect sizes were 75, 46, and 86% greater when antibacterial toothpaste, chewing gum, and tongue-scraping practices were discouraged (Figure 2). Further experimental studies are needed to determine the minimum time required between oral hygiene practices and nitrate ingestion to avoid reduction of NO bioavailability.

Nitrate ingestion was effective in both normoxic and hypoxic conditions, although effects sizes were marginally

TABLE 5 Subgroup analyses for the effect of nitrate ingestion depending on the oral microbiota practices[1]

Subgroup analyses	*n*	SMD (95% CI)	*P* value	Certainty of evidence
No antibacterial mouthwash control	49	0.076 (–0.022, 0.174)	0.130	Moderate (⊕⊕⊕)
Antibacterial mouthwash control	194	0.120 (0.080, 0.160)	<0.001	Moderate (⊕⊕⊕)
No chewing gum control	121	0.089 (0.035, 0.143)	0.001	Low (⊕⊕)
Chewing gum control	122	0.130 (0.081, 0.180)	<0.001	Moderate (⊕⊕⊕)
No antibacterial toothpaste control	192	0.096 (0.053, 0.139)	<0.001	Moderate (⊕⊕⊕)
Antibacterial toothpaste control	51	0.168 (0.090, 0.246)	<0.001	Moderate (⊕⊕⊕)
No tongue-scraping control	203	0.099 (0.057, 0.140)	<0.001	Moderate (⊕⊕⊕)
Tongue-scraping control	40	0.184 (0.090, 0.278)	<0.001	Moderate (⊕⊕⊕)
Control of all practices	37	0.200 (0.099, 0.301)	<0.001	Low (⊕⊕)

[1] *n*, number of observations; SMD, standardized mean difference.

greater (∼86%) in hypoxia with no statistical difference between subgroups. Subsequent meta-regression showed an inverse association between FiO_2 and effect size, suggesting greater efficacy of nitrate with decreased oxygen availability. These results may be explained by the greater activity of enzymes that convert nitrate to nitrite during local hypoxia in skeletal muscle (27), meaning greater NO bioavailability would be expected in more hypoxic conditions. These outcomes corroborate with individual studies that have shown an increased efficacy of nitrates in hypoxia versus normoxia (62) and suggest that nitrate may be a useful ergogenic aid for individuals exercising at altitude.

The current meta-analysis supports previous work (20) in showing nitrate ingestion appears to be effective only for males. The lack of an effect in females is possibly due, at least in part, to the low number of studies that included a stand-alone group of females, as well as heterogeneity in practices that control oral microbiota diversity, ingestion strategies, and exercise conditions. Most studies that included females (either females only or grouped with males) had some form of suboptimal study design as determined by the current meta-analysis. For example, several used a low (≤4.9 mmol) (55, 70, 92, 93) or high (≥15 mmol) (52, 80, 94) acute dose; others ingested <150 min before the exercise test (70, 92, 93, 95–99) or supplemented nitrate salts (80, 96). Additionally, in some cases the duration of exercise was ≤60 s (37, 100) or >600 s (52, 55, 70, 93–95, 101), or used time-trial tasks (25, 52, 55, 94, 95, 99, 101–105). Furthermore, ovarian hormones may influence sports performance, particularly during the follicular phase of the menstrual cycle (106). The reduction of estrogen during the follicular phase may reduce its neuroexcitatory effects (107), besides negatively influencing energy metabolism (108), decreasing exercise performance. However, most studies did not control for menstrual cycle phase. Thus, the lack of effect in females may also have been due, in part, to hormonal differences between the nitrate and placebo visits. Further studies on females should address these limitations using more optimal study designs. Future studies may wish to use lower doses in females considering that higher doses appear ineffective

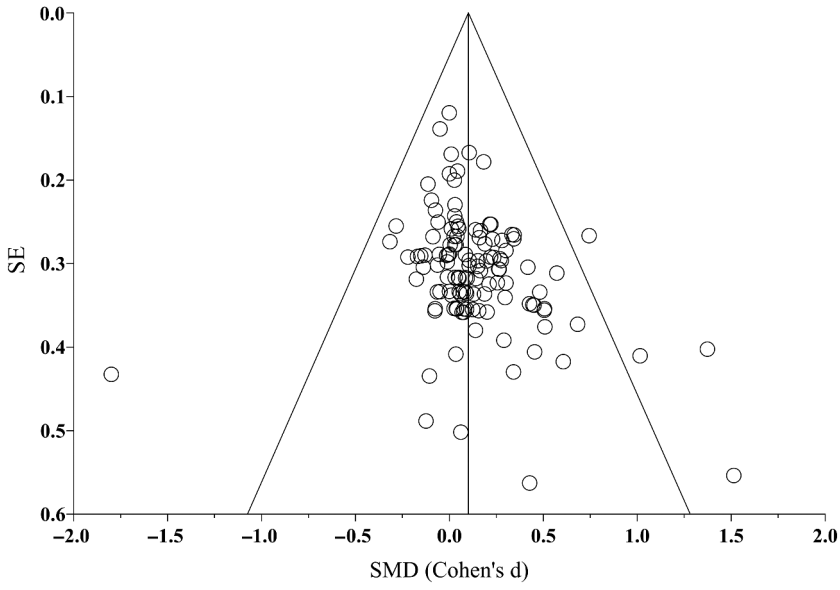

FIGURE 5 Funnel plot: SE by standard differences in means. SMD, standardized mean difference.

(≥15 mmol when acute and ≥10 mmol when ≥2 d), and that females usually have lower body mass and lean mass in comparison to males. Specifically, it would be interesting to employ an optimal dosing strategy of 5–10 mmol nitrate within beetroot juice provided >150 min prior to a time-to-exhaustion task lasting 120–600 s with females. This would provide the greatest chance of an ergogenic effect and provide an excellent model within which to test whether nitrate ingestion is truly effective in females. It is also important that studies control or measure the hormonal status of their female participants.

The results shown here are in accordance with previous findings showing that athletes with greater aerobic fitness benefit less from nitrate ingestion (20). Indeed, our analysis showed that nitrate ingestion was not effective for athletes with aerobic fitness ≥65 $mL \cdot kg^{-1} \cdot min^{-1}$. This may be, in part, because more trained athletes have greater expression of NOS enzymes (109), which could possibly elevate their capacity to produce NO through the arginine pathway. Thus, athletes may already have optimal NO production that is not further increased via nitrate ingestion, though direct experimental comparisons between well-trained and nontrained individuals are required to fully elucidate these claims.

One of the primary strengths of this meta-analysis is that we accounted for several covariates, which enabled an in-depth analysis of effect size variation, allowing us to enhance general knowledge in this growing field. On the other hand, we included all studies in the current analysis, including those with a high risk of bias. This was done to examine factors that may modify the effect of nitrate ingestion. Furthermore, the withdrawal of all studies classified as high risk of bias did not change the result of this meta-analysis, strengthening our conclusions. Additionally, the certainty of evidence for each meta-analyzed result was evaluated, showing a high degree of certainty for most of the analyses performed. Another important limitation is that very few studies specifically set out with the aim of controlling all the moderating factors investigated here. Thus, most studies will likely have contained one type of limitation with their experimental design. Nonetheless, our analysis is strengthened by the large number of included studies and these results provide us with an insight into whether these factors moderate the effect of nitrates across a large body of evidence. Further work should experimentally confirm the influence of these factors, and studies may wish to control for some of these moderators.

Our findings can help to guide clinicians and athletes in their decision-making regarding nitrate ingestion. The optimal acute dose appears to be between 5 and 15 mmol when taken acutely ≥150 min prior to exercise, or between 5 and 10 mmol when ingestion is taken for ≥2 d. Vegetables with high nitrate content and derived products (such as beetroot juice) should be prioritized because of the nutrients and phytochemicals present, which may act synergistically with nitrate to increase NO production and improve exercise performance. Exercise tasks lasting 120–600 s appear most malleable to improvements with nitrate ingestion. Individuals should avoid antibacterial oral hygiene products prior to ingesting nitrates as this can reduce the NO bioavailability leading to a subsequent loss in the ergogenic benefits.

Twenty-eight studies did not report whether they prohibited antibacterial mouthwash use throughout the study, an important control factor since this can abolish the nitrate effect. Only 9.5% of the included studies implemented all types of practices that control oral microbiota diversity (antibacterial mouthwash + tongue scraping + antibacterial toothpaste + chewing gum). Future studies should determine the influence of these oral hygiene practices on the ergogenic effects of nitrate, with others ensuring that measures influencing the oral microbiota are avoided to ensure they do not modify the effect of nitrate ingestion. Further studies looking to evaluate what types of exercise are most improved with nitrate ingestion should employ appropriate acute (5–15 mmol with ≥150 min prior to exercise) or chronic (≥2 d with 5–10 mmol) dosing strategies to optimize the chance of an ergogenic effect. More studies with females are essential to determine whether sex differences truly exist. Most studies (77.2%) did not report any measures of side effects, though ∼30% of those that did reported some type of gastrointestinal discomfort. Given the lack of reporting of side effects, there is a large risk of bias associated with these studies and future work should monitor the incidence and intensity of associated side effects. This could be evaluated using a gastrointestinal tolerance questionnaire (110). Furthermore, it is of common interest that future studies employ sufficient sample sizes (i.e., sample-size calculation), and report better control of randomization, familiarization, and blinding. This would contribute to a higher proportion of studies evaluated as low risk of bias.

In conclusion, nitrate ingestion is an effective ergogenic intervention to improve exercise performance, albeit effect sizes were very small to small. Meta-regressions and subgroup analyses identified several factors that may modify the effect size, including exercise condition (exercise duration, exercise environment, and task type), ingestion strategies (dose, timing, and source), population characteristics and demographics, and practices that control oral microbiota diversity. These data have important practical implications for athletes, clinicians, and future studies.

Acknowledgments

The authors' responsibilities were as follows—all authors contributed to the conception and study design; KVCS and BDC: evaluated studies for inclusion, were responsible for screening, data extraction, and resolved discrepancies; KVCS: was responsible for the analysis and interpretation of data; KVCS and BDC: carried out risk of bias assessment; ACG, JFM, and BS: revised the manuscript critically; and all authors: read and approved the final manuscript.

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