The effect of dietary nitrate supplementation on endurance exercise performance in healthy adults: A Systematic Review and Meta-Analysis

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Running Head: Dietary Nitrate Supplementation and Endurance Exercise Performance

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Key Points

Findings from this meta-analysis highlight the positive ergogenic effect of dietary nitrate supplementation on endurance exercise capacity.

Further randomised controlled trials are required to determine the true ergogenic effect of dietary nitrate supplementation on exercise performance.

Abstract

BACKGROUND: Recent research into the use of dietary nitrates and their role in vascular function has led to it becoming progressively more popular amongst athletes attempting to enhance performance. ***OBJECTIVE:*** The objective of this review was to perform a systematic review and meta-analysis of the literature to evaluate the effect of dietary nitrate (NO_3^-) supplementation on endurance exercise performance. An additional aim was to determine whether the performance outcomes are affected by potential moderator variables. ***DATA SOURCES:*** Relevant databases such as Cochrane Library, Embase, PubMed, Ovid, Scopus and Web of Science were searched for the following search terms 'nitrates OR nitrate OR beetroot OR table beet OR garden beet OR red beet AND exercise AND performance' from inception to October 2015. ***STUDY SELECTION:*** Studies were included if a placebo versus dietary nitrate-only supplementation protocol was able to be compared, and if a quantifiable measure of exercise performance was ≥ 30 seconds (for a single bout of exercise or the combined total for multiple bouts). ***STUDY APPRAISAL AND SYNTHESIS:*** The literature search identified 1038 studies, with 47 (76 trials) meeting the inclusion criteria. Data from the 76 trials was extracted for inclusion in the meta-analysis. A random-effects meta-analysis was conducted for time trial (TT) (n = 28), time to exhaustion (TTE) (n = 22), and graded-exercise test (GXT) (n = 8) protocols. Univariate meta-regression was used to assess potential moderator variables (exercise type, dose duration, NO_3^- type, study quality, fitness level, and percentage nitrite change). ***RESULTS:*** Pooled analysis identified a trivial, but non-significant effect in favour of dietary NO_3^- supplementation (effect size (ES) = -0.10, 95% Cl = -0.27-0.06, $p > 0.05$). TTE trials had a small to moderate statistically significant effect in favour of dietary NO_3^- supplementation (ES = 0.33, 95% Cl = 0.15-0.50, $p < 0.01$). GXT trials had a small, but non-significant effect in favour of dietary NO_3^- supplementation in GXT performance measures (ES = 0.25, 95% Cl = -0.06-0.56, $p > 0.05$).

No significant heterogeneity was detected in the meta-analysis. No statistically significant effects were observed from the meta-regression analysis. ***CONCLUSION:*** Dietary NO_3^- supplementation is likely to elicit a positive outcome when testing endurance exercise capacity; whereas, dietary NO_3^- supplementation is less likely to be effective for time-trial performance. Further work is needed to understand the optimal dosing strategies, which population is most likely to benefit, and under which conditions dietary nitrates are likely to be most effective for performance.

1. Introduction

Through dietary manipulation, a number of different macronutrient and micronutrients have been identified as having the capacity to enhance exercise performance [1]. These nutritional ergogenic aids allow athletes to reach beyond the abilities achieved from training alone, and could be the difference between victory or defeat. For this reason, exploring and evaluating the efficacy of nutritional ergogenic aids is a valuable process to undertake [2]. Recent research into the use of dietary nitrates and their role in vascular function has led to it becoming progressively more popular amongst athletes attempting to enhance performance. Other physiological processes that might be altered to provide an ergogenic effect due to nitrate ingestion include skeletal muscle contractility and mitochondrial efficiency, glucose homeostasis, and respiration [3].

Green leafy and root vegetables constitute the primary dietary source of nitrate (NO_3^-). Vegetables with a very high NO_3^- concentration (> 250mg/100g) include spinach, rocket, cress, lettuce, celery, radish, Swiss chard, chervil, and red beetroot [4]. Once ingested, dietary NO_3^- is reduced to nitric oxide (NO) via the NO_3^--nitrite-NO pathway, increasing the level of NO in the blood and tissues [5]. NO is a potent signalling molecule that plays a key role in vasodilation by relaxing smooth muscle and subsequently improving blood circulation.

The first study to observe the benefits of dietary NO_3^- ingestion on exercise was performed by Larsen et al. [6]. The randomised double-blind placebo-controlled crossover study involved nine, well-trained male subjects performing progressive work rate cycling after chronic sodium NO_3^- supplementation (0.1 mmol·kg^{-1}/day for three days). NO_3^- ingestion resulted in a significantly lower oxygen (O_2) cost of exercise at work rates ranging from 45-80% peak oxygen uptake ($\dot{V}O_{2peak}$), without an increase in blood lactate concentration, resulting in enhanced exercise efficiency. The amount of NO_3^- supplemented by Larsen et al. [6] resembled the amount found in 150 - 250g of NO_3^--rich vegetables. Their findings were unexpected because it is generally considered that the O_2 cost of exercise at a given work rate was a fixed quantity among individuals, particularly during cycling [7, 8].

The observation by Larsen et al. [6] instigated further studies investigating the effect of dietary NO_3^- supplementation on exercise performance. Reported physiological changes include reduced blood pressure [9, 10], enhanced muscle deoxyhemoglobin kinetics [11], reduced adenosine triphosphate (ATP) utilisation and phosphocreatine (PCr) degradation resulting in enhanced muscle contractile efficiency [9], reduced O_2 cost of submaximal exercise [12-14], and improved exercise performance [15, 9, 16, 12]. However, a number of studies have found dietary NO_3^- supplementation to have no effect on performance [17-22]. The variability in findings may be due to different study designs, protocols, or participant characteristics but this has not been systematically evaluated.

A previous systematic review and meta-analysis conducted in 2013 examined the effects of dietary NO_3^- supplementation on endurance exercise performance [23]. After examining 17 studies, the meta-analysis concluded that dietary NO_3^- supplementation had a minor benefit on time trial (TT) performance (ES = 0.11, $p > 0.05$, n = 9); moderate effect on time to

exhaustion (TTE) trials (ES = 0.79, $p < 0.01$, n = 3); and a slight benefit on graded-exercise test (GXT) performance (ES = 0.23, $p > 0.05$, n = 7). Due to the small number of studies, Hoon et al. [23] concluded that more research was necessary to determine the overall effect of dietary NO_3^- supplementation on endurance performance.

The purpose of this systematic review and meta-analysis was to update, critically evaluate, and summarise the methodological quality of the literature on dietary NO_3^- supplementation and endurance exercise performance. A secondary aim was to determine whether the performance outcomes are affected by potential moderator variables such as exercise type and duration, protocol, dose duration and amount, NO_3^- type, subject's level of fitness, and change in nitrite (NO_2^-). The results may help to further our understanding of the influence dietary NO_3^- supplementation has on performance, with the purpose of providing clear usage recommendations to augment participant performance.

2. Methods

We conducted and reported this systematic review in accordance with the guidelines outlined in the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement [24].

2.1 Search strategy

The following databases were systematically searched, and limited to English language: Cochrane Library, Embase, PubMed, Ovid, Scopus and Web of Science, from inception to October 2015. The following search terms and Medical Subject Headings (MeSH) were used to source pertinent peer-reviewed journals: nitrates (MeSH) OR nitrates (All Fields) OR nitrate (All Fields) OR beetroot (All Fields) OR table beet (All Fields) OR garden beet (All Fields) OR red beet (All Fields) AND exercise (MeSH) OR exercise (All Fields) AND performance (All Fields). The search was supplemented by manually cross-matching reference lists, key author searches, and citation searching of all retrieved papers to potentially identify additional studies.

2.2 Inclusion and Exclusion Criteria

Selection criteria for all relevant articles was determined by two researchers (NM and ML). Only full-text primary source articles published in peer-reviewed journals utilising a randomised placebo-controlled crossover design were included. Other specific eligibility criteria were: (i) participants had to be healthy, human adolescents or adults (age ≥ 16 years); (ii) studies had to evaluate dietary NO_3^- supplementation such as nitrate-rich vegetable sources or beetroot juice; (iii) studies evaluating multiple supplements were included only if the placebo vs. nitrate-only supplementation protocol was able to be compared; (iv) studies had to include an outcome of a quantifiable measure of exercise performance lasting ≥ 30 seconds (for a single bout of exercise or the combined total for multiple bouts).

2.3 Data Extraction and Analysis

Two researchers (NM and ML) independently assessed the retrieved title and abstract with clearly irrelevant studies excluded. Full papers of abstracts potentially eligible for inclusion were then screened (NM and ML). Differences in opinion were resolved through discussion and consensus with a third reviewer (TP).

2.4 Data Extraction

Data was extracted using a standardised form. The primary outcome measures in this review were changes in exercise performance after dietary NO_3^- supplementation. Data on participant characteristics (sex, age, training status, and maximal rate of oxygen uptake ($\dot{V}O_{2max/peak}$ - when reported), intervention protocol (dose and delivery method), study methodology, exercise protocol (type, duration and exercise assessment), percentage difference between NO_3^- and placebo, significant performance effect, and trial results were extracted systematically by one researcher (NM) and substantiated by a second (ML). The effect of dietary NO_3^- supplementation was calculated at the end of the exercise assessment, as [($mean_{nitrate}$ – $mean_{placebo}$) ÷ $mean_{placebo}$ x 100]. If a study included an additional NO_3^- protocol or exercise assessment it was extracted separately and included as another trial. A time trial was defined as a timed race over a specified course or distance. A time to exhaustion trial was defined as a single step increment in work rate that is continued until exhaustion. A graded-exercise performance test was defined as a multiple step or continuous ramp incremental test until exhaustion.

2.5 Quality assessment

The studies were assessed for quality using the Physiotherapy Evidence Database (PEDro) scale [25]. PEDro scale items and operational definitions of each item are given in the Electronic Supplementary Material Appendix S1. The PEDro scale was used because of its ability to objectively and reliably assess a randomised controlled trial's (RCT) internal validity [25]. Each article was independently analysed by two reviewers (NM and ML) using the 11-item checklist to yield a maximum score of 10. The kappa value signifying the level of agreement between reviewers was k = 0.94. Differences in opinion concerning the scoring of an article were settled via discussion with a third reviewer (TP).

2.6 Statistical analysis

Data synthesis was descriptive, with detailed tabular summaries presented. For the primary outcomes of TT performance (n = 28), TTE (n = 22), and GXT (n = 8), we were able to consistently extract data across studies to allow a quantitative summary using a meta-analysis (where the performance outcome could be measured in seconds). Trials that could not be measured in seconds were excluded from the meta-analysis due to the quantitative differences [6, 10, 14, 32, 41, 49, 53, 55, 70]. Despite the difference in physiological stressors between the hypoxic and normoxic trials, a sub-analysis was not undertaken due to the

small number of hypoxic trials (n = 6; TT = 3, TTE = 3). We compared absolute changes and calculated a standardised mean difference (95% confidence intervals) for each study.

Heterogeneity was investigated by reviewing study populations, methods, and interventions, and by using the χ^2 test for homogeneity and the I^2 statistic. A random effects model for the meta-analysis was used unless statistical heterogeneity was identified (χ^2 test, $p \leq 0.05$, or $I^2 \geq 50\%$). The random effects model was applied because of the considerable variability in several experimental factors (e.g., test and dose duration, dose amount) across trials. However, random and fixed effects models produced the same results. Hedges' g was used to determine potential bias due to the reasonably small sample sizes prevalent across the studies [26]. Effect sizes were interpreted using Cohen's definitions of trivial (< 0.2), small (0.2-0.3), moderate (0.5) and large (> 0.8) [27]. Analysis was conducted using Review Manager 5.0 (Nordic Cochrane Centre, Copenhagen, Denmark).

The level of agreement between reviewers evaluating the study quality was assessed using Cohen's kappa statistics using SPSS for Windows, Version 23.0 (Armonk, NY: IBM Corp.). The kappa values were interpreted using the ranges suggested by Landis and Koch [28] of < 0.00 = poor, 0.00 – 0.20 = slight, 0.21 – 0.40 = fair, 0.41 – 0.60 = moderate, 0.61 – 0.80 = substantial, 0.81 – 1.00 = almost perfect.

Eight trial features were identified as potential moderator variables. The analysis included dichotomous data of exercise type (cycling or other), test duration (≥ 10 mins or < 10 mins), dose duration (acute (< 6 hours) or chronic (repeated doses ≥ 6 hours apart)), NO_3^- type (beetroot juice or other), NO_3^- dose (< 6.5 mmol or ≥ 6.5 mmol), and study quality (< 9 or ≥ 9 (assessed using the PEDro scale)). Fitness level ($\dot{V}O_{2max}$) and percentage nitrite change were analysed using continuous data. Univariate meta-regression was used to assess the association between each potential moderating variable and TT and TTE performance outcomes. Univariate meta-regression was not used for trials utilising graded-exercise performance tests as there were fewer than 10 studies.

As outcomes were continuous, we assessed for publication bias using Egger's test and by visual inspection of funnel plots, with a p-value of > 0.10 considered statistically significant (publication bias was not assessed for the GXT, for which there were fewer than 10 studies) [29, 30].

3. Results

The bibliographic search yielded 1,038 articles (Figure 1) for preliminary screening of titles and abstracts, with 62 full-text articles retrieved, and 47 identified as meeting the inclusion criteria.

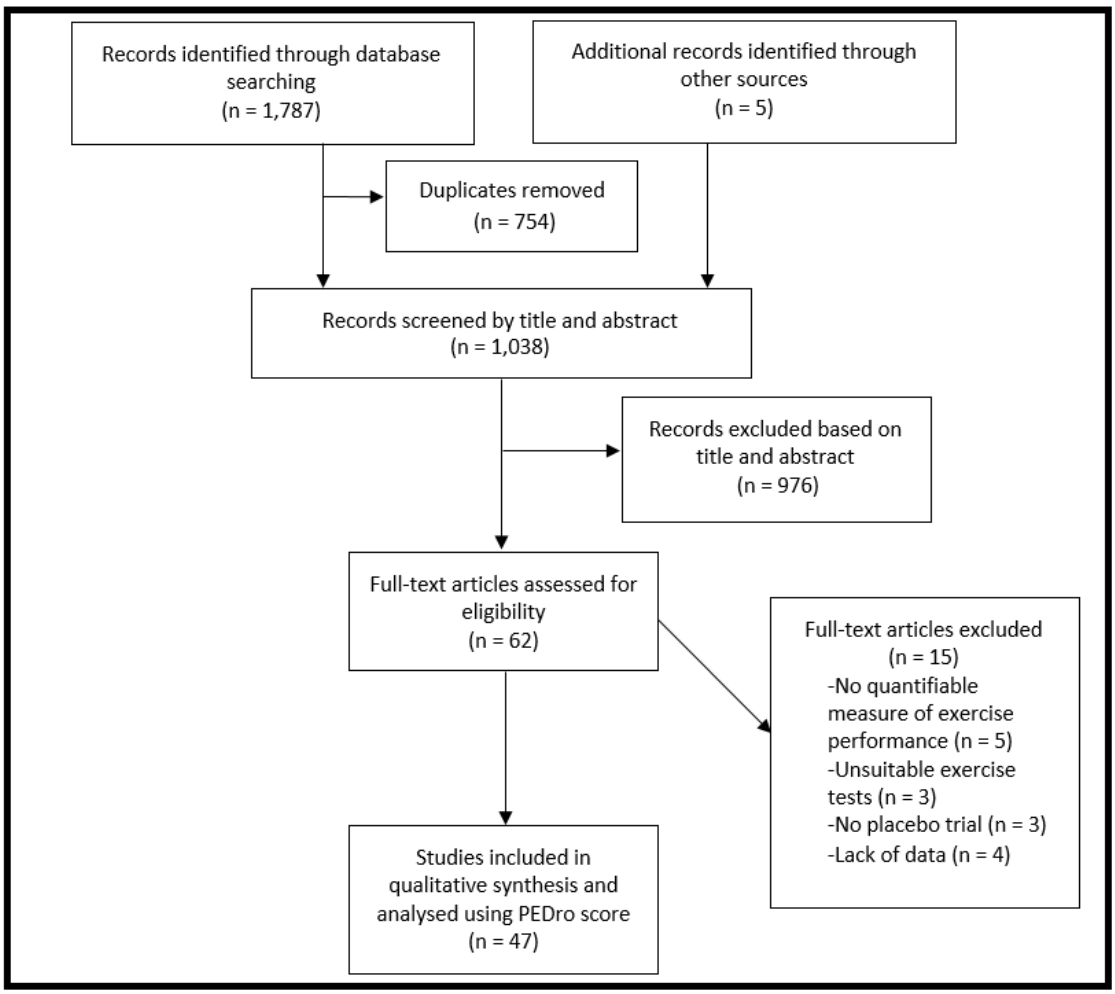

Figure 1:

Flowchart of study selection. PEDro = Physiotherapy evidence database scale

3.1 Study characteristics

The characteristics of each study and the physiological changes are summarised in Table 1. Multiple studies utilised more than one category of participants: dose-response trials [31-33]; different distances [16]; different exercise intensities [34-37]; acute (< 6 hours) or chronic (repeated doses ≥ 6 hours apart) [22, 10]; hypoxia vs normoxia [38, 39]; sex [40, 41]; different exercise protocols [42]; or level of fitness [14]. Consequently, these studies were reported as two or more trials, raising the total number of cross-over trials to 76 across 47 publications, each with a NO_3^- and placebo condition.

The studies were published between 2007 [6] and 2015 [43]. Three types of performance assessments were utilised across the studies, with 38 examining the effect of dietary NO_3^- supplementation vs. placebo on exercise time/distance (TT – summarised in Table 1), 22 trials using a TTE protocol (TTE – summarised in Table 2), and 16 included a graded-exercise performance test (GXT – summarised in Table 3). Sixty-one trials showed improved performance after dietary NO_3^- supplementation, 29 of which were statistically significant ($p < 0.05$), and in one study, decreased performance was observed

following NO_3^- supplementation [44]. Following dietary NO3- supplementation, 20 of the 22 TTE trials showed a mean improvement in performance (16 of which showed significant improvements), as did 27 of the 38 TTs and 14 of the 16 GXTs (of which 7 and 5 were significant improvements, respectively).

Cycling was the most common method of exercise, utilised in 44 of the 76 trials. Fourteen opted for treadmill running [14, 18, 19, 22, 35, 36, 42, 74], 6 utilised field running [14, 43, 58], 3 used kayaking [13, 41], 3 used rowing [33, 48], 3 used resistance training in the form of knee extensions [9, 36, 45], and 1 each for underwater diving [46], walking [49], and arm/leg crank [51]. Eight trials investigated exercise performance in hypoxic conditions [45, 46, 12, 47, 38, 42, 39]. Exercise duration ranged from 1.5 to 137 minutes. All studies included a NO_3^- and placebo group.

3.2 Characteristics of subjects

In total, 581 participants (494 males, 87 females) participated in the included studies. The mean ages ranged from 16.7 [48] to 64 [49] years. Fifty-nine trials had male only participants, 4 trials had exclusively women subjects [40, 41, 50, 43], and 13 trials had both sexes [51, 10, 45, 46, 18, 11, 49, 44, 35]. The number of participants involved in the trials ranged from 5 [41] to 28 [32], with a mean sample size of 10.8 ± 4.

$\dot{V}O_{2max}$ values were reported in 53 trials, with values ranging between 28.2 and 81.1 mL kg^{-1} min^{-1}. Porcelli et al. [14] implemented 2 trials with participants classified in the "low aerobic fitness" group (28.1- 44.1 mL kg^{-1} min^{-1}). The remaining 51 trials included participants with a $\dot{V}O_{2max}$ > 45 mL kg^{-1} min^{-1}, and ranged from "physically active" and "well-trained", right up to "elite" international level athletes.

3.3 Nitrate administration

The trials utilised a variety of dietary NO_3^- supplementation types. The majority opted for beetroot juice (n=58; 76%) as the source of NO_3^- delivery, 6 used NO_3^- water [14], 4 used sodium NO_3^- [6, 51-53], 3 utilised pomegranate extract [35], 3 used potassium NO_3^- [19, 54, 55], and 1 trial each for NO_3^- gel [56] and beetroot portions [18]. There was a large variability in the amount of NO_3^- given per dose, with doses ranging from 4.1mmol [32] – 19.5mmol per day [22].

The intervention period ranged from 30 minutes to 15 days prior to testing. Forty trials had an acute invention protocol, whereas 36 trials utilised a chronic dietary NO_3^- supplementation protocol.

3.4 Methodological quality of studies

The mean PEDro score was 8.8 ± 1.1 out of 10. All 47 studies reviewed scored a moderate to high score of 7 and above. Thirty-nine of the 47 studies reported blinding of both the assessors and participants, and received a perfect 10 score, 4 studies scored

8 out of 10 as they failed to blind therapists and assessors thus opting for a single-blind crossover study design [13, 32, 56, 41], and the remaining 4 studies scored 7 out of 10 due to a lack of allocation concealment and single-blind crossover studies [10, 12, 20, 21]. Overall, the study quality was deemed to be good to excellent.

Table 1 Summary of studies examining the effect of NO_3^- on time trial performance.

Reference	PEDro score	Sample size and sex	Fitness level ($\dot{V}O_{2max/peak}$, mL·kg·min^{-1} [mean ± SD])	NO_3^- dose and duration	Exercise protocol	Percentage NO_3^-/ NO_2^- change	Trial result (mean ± SD)	% Difference
Lansley et al. 2011 [16]	10	9 M	Well-trained cyclists ($\dot{V}O_{2peak}$ 56 ± 5.7)	500mL BR (~6.2 mmol of NO_3^-) Acute	4km TT Cycle ergometer	139% ↑ NO_2^-*	TT N: 376.2 ± 21 s P: 387 ± 25.2 s	2.79*
Lansley et al. 2011 [16]	10	9 M	Well-trained cyclists ($\dot{V}O_{2peak}$ 56 ± 5.7)	500mL BR (~6.2 mmol of NO_3^-) Acute	16.1km TT Cycle ergometer	139% ↑ NO_2^-*	TT N: 1614 ± 108 s P: 1662 ± 126 s	2.89*
Bescós et al. 2012 [53]	10	13 M	Cyclists and triathletes	$NaNO_3$ (10mg·kg^{-1} – day) Chronic	40-min TT Cycle ergometer	79% ↑ NO_2^-*	TT N: 26.4 ± 1.1 km P: 26.3 ± 1.2 km	0.38
Bond et al. 2012 [48]	10	14 M	Well-trained junior rowers	500mL/day BR (5.5 mmol of NO_3^- /day) Chronic	6x500m maximal TT Rowing ergometer	Not reported	TT (1-6) N: 89.4 ± 3.2s P: 90.19 ± 2.9s	0.88
Cermak et al. 2012 [57]	10	13 M	Well-trained cyclists and triathletes ($\dot{V}O_{2max}$ 58 ± 2)	140mL BR (~8 mmol of NO_3^-/day) for 6 days Chronic	10km TT Cycle ergometer	1906.67% ↑ NO_3^-*	TT N: 953 ± 75.7 s P: 965 ± 75.7 s	1.24*
Cermak et al. 2012 [17]	10	20 M	Well-trained cyclists and triathletes ($\dot{V}O_{2max}$ 60 ± 1)	140mL BR (8.7 mmol of NO_3^-) Acute	~1h cycling at 75% W_{max} (energy expenditure based) TT Cycle ergometer	96% ↑ NO_2^-*	TT N: 3930 ± 295.2 s P: 3900 ± 295.2 s	-0.77
Murphy et al. 2012 [18]	10	11 Both	Recreationally fit	200g Beetroot portions (≥500mg NO_3^-) Acute	5km TT Treadmill	Not measured	TT N: 1541 ± 380 s P: 1581 ± 382 s	2.53
Peacock et al. 2012 [19]	10	10 M	Junior-elite cross-country skiers ($\dot{V}O_{2max}$ 69.6 ± 5.1)	1g KNO_3^- (9.9 mmol – 614mg NO_3^-) Acute	5km TT treadmill	127% ↑ NO_2^-*	TT N: 1005 ± 53 s P: 996 ± 49 s	-0.9

Wilkerson et al. 2012 [20]	7	8 M	Well-trained cyclists ($\dot{V}O_{2max}$ 63 ± 8)	500mL/day BR (~6.2 mmol of NO_3^-) 2.5h prior to exercise Acute	50 mile TT Cycle ergometer	25% ↑ NO_2^-*	TT N: 8202 ± 336 s P: 8274± 384 s	0.87
Christensen et al. 2013 [21]	7	10 M	Elite cyclists ($\dot{V}O_{2max}$ 72.1 ± 4.5)	500mL/day BR (~8 mmol of NO_3^- /day) 6 days. Chronic	~400kcal (15-20min) cycling TT	297% ↑ NO_3^-*	TT: N: 1100 ± 163 s P: 1117 ± 167 s	1.52
Kelly et al. 2013 [49]	10	12 Both	Older participants (> 60 yrs.)	2x 70mL BR (~9.6 mmol of NO_3^- /day) for 3 days. Chronic	6-min walk test TT	418% ↑ NO_2^-*	TT N: 682 ± 89 m P: 667 ± 86 m	2.25
Muggeridge et al. 2013 [13]	8	8 M	Trained kayakers ($\dot{V}O_{2max}$ 49 ± 6.1)	70mL BR (~5 mmol of NO_3^-) Acute	1km TT kayak ergometer	32% ↑ NO_2^-*	TT N: 276 ± 14.1 s P: 277 ± 14.1 s	0.36
Boorsma et al. 2014 [22]	10	8 M	Elite 1500m runners ($\dot{V}O_{2max}$ 80 ± 5)	210mL BR (19.5 mmol of NO_3^-) Acute	1500m TT on indoor track	(Chronic > acute*)	TT N: 250.7 ± 4.3 s P: 250.4 ± 7 s	-0.12
Boorsma et al. 2014 [22]	10	8 M	Elite 1500m runners ($\dot{V}O_{2max}$ 80 ± 5)	Days 1+8: 210mL BR (19.5 mmol of NO_3^-/day) Days 2-7: 140mL BR (13.0 mmol of NO_3^-/day) Chronic	1500m TT on indoor running track	(Chronic > acute*)	TT N: 250.5 ± 6.2 s P: 251.4 ± 7.6 s	0.36
Hoon et al. 2014 [32]	8	28 M	Trained cyclists	N150: 70mL BR (4.1 mmol of NO_3^-) 2.5h prior to exercise Acute	4-min TT Cycle ergometer	22% ↑ NO_2^-*	TT1 N150: 402 ± 47 W P: 396 ± 57 W	1.52
Hoon et al. 2014 [32]	8	28 M	Trained cyclists	N75: 70mL BR (4.1 mmol of NO_3^-) 75mins prior to exercise Acute	4-min TT Cycle ergometer	70% ↑ NO_2^-*	TT1 N75: 403 ± 52 W P: 396 ± 57 W	1.77
Hoon et al. 2014 [32]	8	28 M	Trained cyclists	N-Top: 70mL BR (4.1 mmol of NO_3^-) 2.5h prior to exercise Acute	4-min TT Cycle ergometer	38% ↑ NO_2^-*	TT1 N-Top: 400 ± 48 W P: 396 ± 57 W	1.01
Hoon et al. 2014 [32]	8	28 M	Trained cyclists	N150: 70mL BR (4.1 mmol of $NO3^-$) 225 mins prior to exercise Acute	4-min TT Cycle ergometer	Not reported	TT2 N150: 396 ± 46 W P: 397 ± 56 W	-0.25

Hoon et al. 2014 [32]	8	28 M	Trained cyclists	N75: 70mL BR (4.1 mmol of NO_3^-) 2.5h mins prior to exercise Acute	4-min TT Cycle ergometer	Not reported	TT2 N75: 396 ± 54 W P: 397 ± 56 W	-0.25
Hoon et al. 2014 [32]	8	28 M	Trained cyclists	N-Top: 70mL BR (4.1 mmol of NO_3^-) 225 mins prior to exercise +35mL immediately after TT1 Acute	4-min TT Cycle ergometer	Not reported	TT2 N-Top: 396 ± 45W P: 397 ± 56 W	-0.25
Hoon et al. 2014 [33]	10	10 M	Highly trained	70mL BR (4.2 mmol of NO_3^-) Acute	2000m TT Rowing ergometer	Not reported	TT N: 383.4 ± 8.7s P: 383.5 ± 9s	0.03
Hoon et al. 2014 [33]	10	10 M	Highly trained	140mL BR (8.4 mmol of NO_3^-) Acute	2000m TT Rowing ergometer	Not reported	TT N: 381.9 ± 9s P: 383.5 ± 9s	0.42
Kokkinoplitis and Chester 2014 [70]	10	7 M	Healthy	70mL of BR (0.4 g NO_3^- /day). 3h prior to exercise Acute	5 x 6-sec sprints interspersed with 30-sec recovery Treadmill	Not measured	TT N: 4133.5 ± 674.4 W P: 3938.3 ± 603.1 W	4.96
Lane et al. 2014 [40]	10	12 M	Competitive cyclists ($\dot{V}O_{2peak}$ 71.6 ± 4.6)	2 separate doses of 140mL BR (8.4 mmol of NO_3^-). 1x ~8-12h prior to exercise 1x – 130-mins prior to exercise Chronic	43.83km TT Cycle ergometer	Not reported	TT N: 3845.03 ± 196.15 s P: 3813.39 ± 170.09 s	-0.91
Lane et al. 2014 [40]	10	12 F	Competitive cyclists ($\dot{V}O_{2peak}$ 59.9 ± 5.1)	2 separate doses of 140mL BR (8.4 mmol of NO_3^-). 1x ~8-12h prior to exercise 1x – 130-mins prior to exercise Chronic	29.35km TT Cycle ergometer	Not reported	TT N: 3101.06 ± 159.51 P: 3100.10 ± 151.71	-0.03
Muggeridge et al. 2014 [47]	10	9 M	Trained cyclists ($\dot{V}O_{2peak}$ (at altitude) 51.9 ± 5.8)	70mL BR (~5 mmol of NO_3^-) 3h prior to exercise Acute	16.1km TT Cycle ergometer	242% ↑ NO_2^-*	TT N: 1664 ± 42 s P: 1702 ± 45 s	2.23*
Muggeridge et al. 2014 [56]	8	9 M	Trained cyclists and triathletes ($\dot{V}O_{2max}$ 53.1 ± 4.4)	2x60mL NO_3^- gel (~8.1 mmol of NO_3^-) Acute	16.1km TT Cycle ergometer	61.6% ↑ NO_2^-*	TT N: 1455 ± 47 s P: 1469 ± 52 s	0.95

Peeling et al. 2014 [41]	8	6 M	National-level kayakers ($\dot{V}O_{2peak}$ 57.15 ± 2.8)	70mL BR (~4.8 mmol of NO_3^-) Acute	4-min TT kayak ergometer	Not measured	TT N: 989 ± 31 mtrs P: 982 ± 36 mtrs	-0.71
Peeling et al. 2014 [41]	8	5 M	International-level kayakers ($\dot{V}O_{2peak}$ 47.8 ± 3.7)	2x70mL BR (~9.6 mmol of NO_3^-) Acute	500m TT Kayak	Not measured	TT N: 114.6 ± 1.5 s P: 116.7 ± 2.2 s	1.8*
Porcelli et al. 2014 [14]	10	8 M	Participants with a low fitness level ($\dot{V}O_{2peak}$ range 28.2-44.1)	500mL/day NO_3^- containing water (~5.5 mmol of NO_3^- /day) 6 days. Chronic	3km TT on a running track	Not reported	TT N: 886 ± 74 s P: 910 ± 82 s	2.64*
Porcelli et al. 2014 [14]	10	7 M	Participants with a moderate fitness level ($\dot{V}O_{2peak}$ range 45.5-57.1)	500mL/day NO_3^- containing water (~5.5 mmol of NO_3^- /day) 6 days. Chronic	3km TT on a running track	Not reported	TT N: 723 ± 90 s P: 734 ± 93 s	1.5*
Porcelli et al. 2014 [14]	10	6 M	Participants with a high fitness level ($\dot{V}O_{2peak}$ range 63.9-81.1)	500mL/day NO_3^- containing water (~5.5 mmol of NO_3^- /day) 6 days. Chronic	3km TT on a running track	Not reported	TT N: 627 ± 30 s P: 629 ± 28 s	0.32
Sandbakk et al. 2014 [54]	10	9 M	Junior-elite cross-country skiers ($\dot{V}O_{2max}$ 69.3 ± 5.8)	1g KNO_3^- (~9.9 mmol NO_3^-) Acute	5km TT on indoor running track	120.1% ↑ NO_2^-*	TT N: 1016 ± 52s P: 1005 ± 47s	-1.09
Arnold et al. 2015 [42]	10	10 M	Well-trained competitive runners ($\dot{V}O_{2peak}$ 66 ± 7)	70mL of BR (~7 mmol of NO_3^-). 2.5h prior to exercise Acute	10km TT Treadmill	675% ↑ NO_2^-*	TT N: 2862 ± 233 s P: 2874 ± 265 s	0.42
Buck et al. 2015 [43]	10	13 F	Team-sport trained	70mL of BR (6 mmol of NO_3^-). 3h prior to exercise Acute	Simulated team-game circuit. With 6 x 20-m repeated-sprint set performed at the start, half-time and end Running	891% ↑ NO_3^-*	TT (sprints) N: 69.84 ± 4.94 s P: 69.97 ± 4.17 s	0.19
Glaister et al. 2015 [50]	10	14 F	Well-trained cyclists and triathletes ($\dot{V}O_{2max}$ 52.3 ± 4.9)	70mL BR (~7.3 mmol of NO_3^-) 2.5h prior to exercise Acute	20km TT Cycle ergometer	223.7% ↑ NO_2^-*	TT N: 2119.8 ± 90 s P: 2122.2± 102 s	0.11

MacLeod et al. 2015 [39]	10	11 M	Trained cyclists ($\dot{V}O_{2peak}$ 67.5 ± 5.8)	70mL of BR (6.5 mmol of NO_3^-). 2.5h prior to exercise Acute	10km TT (normoxia) Cycle ergometer	441% ↑ NO_3^-*	TT N: 961 ± 54 s P: 954 ± 47 s	-0.73
MacLeod et al. 2015 [39]	10	11 M	Trained cyclists ($\dot{V}O_{2peak}$ 67.5 ± 5.8)	70mL of BR (6.5 mmol of NO_3^-). 2.5h prior to exercise Acute	10km TT (hypoxia) Cycle ergometer	441% ↑ NO_3^-*	TT N: 1018 ± 52 s P: 1023 ± 49 s	0.49

* = significantly different from placebo (as reported within studies; $p < 0.05$)

$\dot{V}O_{2max}$ = maximal oxygen uptake $\dot{V}O_{2peak}$ = peak oxygen uptake PEDro = physiotherapy evidence database scale TT = time trial N = NO_3^- P = placebo

BR = beetroot juice M = male F = female s = seconds W = watts km·h-1 = kilometres per hour W_{max} = maximal power

N-Top = NO_3^- top up ↑ = increase

Table 2 Summary of studies examining the effect of NO_3^- on time to exhaustion performance.

Reference	PEDro score	Sample size and sex	Fitness level ($\dot{V}O_{2max}/_{peak}$, $mL \cdot kg \cdot min^{-1}$ [mean ± SD])	NO_3^- dose and duration	Exercise protocol	Percentage NO_3^-/ NO_2^- change	Trial result (mean ± SD)	% Difference
Bailey et al. 2009 [15]	10	8 M	Recreationally fit ($\dot{V}O_{2max}$ 49 ± 5)	500mL/day BR (5.5 mmol of NO_3^- /day) for 6 days Chronic	SI TTE Cycling ergometer	96% ↑ NO_2^-*	TTE N: 675 ± 203 s P: 583 ± 145 s	15.78*
Bailey et al. 2010 [9]	10	7 M	Recreationally fit	500mL/day BR (5.1 mmol of NO_3^- /day) for 6 days Chronic	2-legged HI (30% MVC) knee-extension TTE	137% ↑ NO_2^-*	TTE N: 734 ± 290 s P: 586 ± 212 s	25.26*
Lansley et al. 2011 [36]	10	9 M	Physically active ($\dot{V}O_{2max}$ 55 ± 7)	500mL/day BR (~6.2 mmol of NO_3^- /day) for 4 days Chronic	SI run TTE Treadmill	104% ↑ NO_2^-*	TTE N: 522 ± 108 s P: 456 ± 90 s	14.47*
Vanhatalo et al. 2011 [45]	10	9 Both	Recreationally fit	750mL/day BR (9.3 mmol of NO_3^-) in 3 equal doses (24h, 12h, 2.5h prior to exercise) Chronic	Knee extension TTE	50% ↑ NO_2^-*	TTE N: 477 ± 200 s P: 393 ± 169 s	21.37*
Engan et al. 2012 [46]	10	12 Both	Well-trained apnea divers	70mL BR (~5.0 mmol of NO_3^-) 2.5h prior to exercise Acute	Apnea TTE	Not measured	TTE N: 278 ± 64 s P: 250 ± 58 s	11.2*
Breese et al. 2013 [11]	10	9 Both	Recreationally active ($\dot{V}O_{2max}$ M: 48.4 ± 6, F: 46.4 ± 9)	140mL BR (~8 mmol of NO_3^-/day) for 6 days Chronic	SI TTE Cycling ergometer	435% ↑ NO_2^-*	TTE N: 635 ± 258 s P: 521 ± 158 s	21.88*
Handzlik and Gleeson 2013 [68]	10	14 M	Well-trained ($\dot{V}O_{2max}$ 63 ± 10)	70mL BR (4 mmol of NO_3^-) 2.5h prior to exercise. Another 70mL BR (4 mmol of NO_3^-) 75mins prior to exercise Acute	Cycling (80% VO_{2max}) TTE Cycle ergometer	Not reported	TTE N: 1240 ± 994 s P: 1003 ± 480 s	23.63

Kelly et al. 2013 [34]	10	9 M	Habitually active ($\dot{V}O_{2max}$ 54.5 ± 7.5)	500mL/day BR (~8.2 mmol of NO_3^- /day) for 5 days Chronic	TTE (60% peak power) Cycle ergometer	208.7% ↑ NO_2^-*	TTE N: 696 ± 120 s P: 593 ± 68 s	17.37*
Kelly et al. 2013 [34]	10	9 M	Habitually active ($\dot{V}O_{2max}$ 54.5 ± 7.5)	500mL/day BR (~8.2 mmol of NO_3^- /day) for 5 days Chronic	TTE (70% peak power) Cycle ergometer	156.3% ↑ NO_2^-*	TTE N: 452 ± 106 s P: 390 ± 86 s	15.9*
Kelly et al. 2013 [34]	10	9 M	Habitually active ($\dot{V}O_{2max}$ 54.5 ± 7.5)	500mL/day BR (~8.2 mmol of NO_3^- /day) for 5 days Chronic	TTE (80% peak power) Cycle ergometer	181.2% ↑ NO_2^-*	TTE N: 294 ± 50 s P: 263 ± 50 s	11.79*
Kelly et al. 2013 [34]	10	9 M	Habitually active ($\dot{V}O_{2max}$ 54.5 ± 7.5)	500mL/day BR (~8.2 mmol of NO_3^- /day) for 5 days Chronic	TTE (100% peak power) Cycle ergometer	227.6% ↑ NO_2^-*	TTE N: 182 ± 37 s P: 166 ± 20 s	9.64
Wylie et al. 2013 [31]	10	10 M	Recreationally active	70mL BR (~4.2 mmol of NO_3^-) 2.5h prior to exercise Acute	SI TTE Cycle ergometer	Not reported	TTE N: 508 ± 102 s P: 470 ± 81 s	8.09
Wylie et al. 2013 [31]	10	10 M	Recreationally active	140mL BR (~8.4 mmol of NO_3^-) 2.5h prior to exercise Acute	SI TTE Cycle ergometer	Not reported	TTE N: 570 ± 153 s P: 498 ± 113 s	14.46*
Wylie et al. 2013 [31]	10	10 M	Recreationally active	280mL BR (~16.8 mmol of NO_3^-) 2.5h prior to exercise Acute	SI TTE Cycle ergometer	Not reported	TTE N: 552 ± 117 s P: 493 ± 114 s	11.97*
Kelly et al. 2014 [38]	10	12 M	Physically active ($\dot{V}O_{2peak}$ 58.3 ± 6.3)	140mL BR (~8.4 mmol of NO_3^- /day) 2.5h prior to exercise. For 3 days. Chronic	SI cycling TTE (hypoxia) Cycle ergometer	242% ↑ NO_2^-*	TTE N: 214 ± 43 s P: 197 ± 28 s	8.63*
Kelly et al. 2014 [38]	10	12 M	Physically active ($\dot{V}O_{2peak}$ 58.3 ± 6.3)	140mL BR (~8.4 mmol of NO_3^- /day) 2.5h prior to exercise. For 3 days. Chronic	SI cycling TTE (normoxia) Cycle ergometer	557% ↑ NO_2^-*	TTE N: 412 ± 139 s P: 431 ± 124 s	-4.41
Martin et al. 2014 [44]	10	16 Both	Moderately trained - team sport ($\dot{V}O_{22max}$ M: 57.4 ± 8, F: 47.2 ± 8)	70mL BR (~5 mmol of NO_3^-) 2h prior to exercise Acute	8-sec sprints interspersed with 30-sec active rest TTE Cycle erogometer	Not reported	HIIST N: 104 ± 40 s P: 120 ± 48 s	-13.33
Thompson et al. 2014 [69]	10	16 M	Recreationally active ($\dot{V}O_{2max}$ 47.3 ± 6.3)	500mL BR (~5 mmol of NO_3^- /day) 1.5h prior to exercise Acute	1x TTE (~90% VO_{2max}) Cycle ergometer	79% ↑ NO_2^-*	TTE N: 185 ± 122 s P: 160 ± 109 s	15.63*

Trexler et al. 2014 [35]	10	19 Both	Highly active ($\dot{V}O_{2max}$ 51.3 ± 9.4)	1000mg pomegranate extract 0.5h prior to exercise Acute	TTE (90% PV) Treadmill	Not reported	TTE N: 387.9 ± 199.2 s P: 346 ± 162.5 s	12.11*
Trexler et al. 2014 [35]	10	19 Both	Highly active ($\dot{V}O_{2max}$ 51.3 ± 9.4)	1000mg pomegranate extract 0.5h prior to exercise Acute	TTE (100% PV) Treadmill	Not measured	TTE N: 170.8 ± 66.3 s P: 159.3 ± 62.3 s	7.22*
Trexler et al. 2014 [35]	10	19 Both	Highly active ($\dot{V}O_{2max}$ 51.3 ± 9.4)	1000mg pomegranate extract 0.5h prior to exercise Acute	TTE (110% PV) Treadmill	Not measured	TTE N: 108.8 ± 45.1 s P: 104.4 ± 40.1 s	4.21
Aucouturier et al. 2015 [59]	8	12 M	Healthy ($\dot{V}O_{2peak}$ 46.6 ± 3.4)	500mL/day BR (~340mg of NO_3^- /day) for 3 days. 3h prior to exercise Chronic	15-sec sprints interspersed with 30-sec recovery Cycle ergometer	108% ↑ NO_2^-*	TTE N: 1176 ± 486 s P: 984 ± 360 s	19.51*

* = significantly different from placebo (as reported within studies; $p < 0.05$)

$\dot{V}O_{2max}$ = maximal oxygen uptake $\dot{V}O_{2peak}$ = peak oxygen uptake PEDro = physiotherapy evidence database scale TTE = time to exhaustion N = NO_3^- P = placebo

BR = beetroot juice M = male F = female s = seconds W = watts km·h-1 = kilometres per hour HI = high-intensity SI = severe-intensity

PV = peak velocity MVC = maximal voluntary contraction HIIST = high-intensity interval sprint training ↑ = increase

Table 3 Summary of studies examining the effect of NO_3^- on graded exercise performance.

Reference	PEDro score	Sample size and sex	Fitness level ($\dot{V}O_{2max/peak}$, mL·kg·min^{-1} [mean ± SD])	NO_3^- dose and duration	Exercise protocol	Percentage NO_3^-/NO_2^- change	Trial result (mean ± SD)	% Difference
Larsen et al. 2007 [6]	10	9 M	Well-trained cyclists or triathletes ($\dot{V}O_{2peak}$ 55 ± 3.7)	$NaNO_3$ (0.1 mmol/kg /day) for 3 days Chronic	Incremental TTE Cycle ergometer	82% ↑ NO_2^-*	Maximal work capacity N: 360.6 ± 32.8 W P: 358.9 ± 32.3 W	0.47
Larsen et al. 2010 [51]	10	9 Both	Recreationally fit ($\dot{V}O_{2max}$ 3.72 ± 0.33 L·kg·min−1)	$NaNO_3$ (0.1 mmol/kg/day) for 2 days Chronic	Combined arm + leg crank (separate ergometers) Incremental TTE	133% ↑ NO_2^-*	TTE N: 563 ± 90.1 s P: 524 ± 93.7 s	7.44
Vanhatalo et al. 2010 [10]	7	8 Both	Recreationally fit ($\dot{V}O_{2max}$ 47 ± 8)	500mL/day BR (5.2 mmol of NO_3^-) 2.5h prior to exercise Acute	Incremental TTE Cycle ergometer	36% ↑ NO_2^-*	TTE N: 325 ± 71 W P: 322 ± 68 W	0.93
Vanhatalo et al. 2010 [10]	7	8 Both	Recreationally fit ($\dot{V}O_{2max}$ 47 ± 8)	500mL/day BR (5.2 mmol of NO_3^- /day) for 5 days Chronic	Incremental TTE Cycle ergometer	Not reported	TTE N: 328 ± 68 W P: 323 ± 67 W	1.55
Vanhatalo et al. 2010 [10]	7	8 Both	Recreationally fit ($\dot{V}O_{2max}$ 47 ± 8)	500mL/day BR (5.2 mmol of NO_3^- /day) for 15 days Chronic	Incremental TTE Cycle ergometer	46% ↑ NO_2^-*	TTE N: 331 ± 68 W P: 323 ± 68 W	2.48*
Bescós et al. 2011 [52]	10	11 M	Cyclists and triathletes ($\dot{V}O_{2peak}$ 65.1 ± 6.2)	$NaNO_3$ (10mg of NO_3^-/kg/day) 3h prior to exercise Acute	Incremental TTE Cycle ergometer	15.77% ↑ NO_2^-*	TTE N: 416 ± 106.1 s P: 409 ± 89.5 s	1.71
Lansley et al. 2011 [36]	10	9 M	Physically active ($\dot{V}O_{2max}$ 55 ± 7)	500mL/day BR (~6.2 mmol of NO_3^- /day) for 6 days Chronic	Incremental knee extension TTE	104% ↑ NO_2^-*	Knee TTE N: 510 ± 48 s P: 492 ± 54 s	3.66*
Masschelein et al. 2012 [12]	7	15 M	Physically active ($\dot{V}O_{2peak}$ 61.7 ± 2.1)	~500mL BR (0.07 mmol of NO_3^-) /kg/day) for 6 days. 1-2h prior to exercise Chronic	Incremental TTE Cycle ergometer	39% ↑ NO_2^-*	TTE N: 597 ± 85.2 s P: 568 ± 89.1 s	5.11*

Wylie et al. 2013 [58]	10	14 M	Team sport trained ($\dot{V}O_{2max}$ 52 ± 7)	2x 70mL BR (~8.2 mmol of NO_3^-) morning prior. 2x 70mL BR (~8.2 mmol of NO_3^-) evening prior 2x 70mL BR (~8.2 mmol of NO_3^-) 2.5h prior to exercise 1x 70mL BR (~4.1 mmol of NO_3^-) 1.5h prior to exercise Chronic	Yo-Yo IR1 TTE test	395% ↑ NO_2^-*	TTE N: 1704 ± 304 s P: 1638 ± 288 s	4.03*
Porcelli et al. 2014 [14]	10	8 M	Participants with a low aerobic fitness ($\dot{V}O_{2peak}$ range 28.2-44.1)	500mL/day NO_3^- containing water (~5.5 mmol of NO_3^- /day) 5 days. 3.5 ± 0.5 h prior to exercise Chronic	Incremental TTE treadmill	Not reported	Peak speed N: 14.5 ± 0.8 km·h^{-1} P: 14.4 ± 1.2 km·h^{-1}	0.69*
Porcelli et al. 2014 [14]	10	7 M	Participants with a moderate aerobic fitness ($\dot{V}O_{2peak}$ range 45.5-57.1)	500mL/day NO_3^- containing water (~5.5 mmol of NO_3^- /day) 5 days. 3.5 ± 0.5 h prior to exercise Chronic	Incremental TTE treadmill	Not reported	Peak speed N: 17.7 ± 1.9 km·h^{-1} P: 17.4 ± 1.9 km·h^{-1}	1.72*
Porcelli et al. 2014 [14]	10	6 M	Participants with a high aerobic fitness ($\dot{V}O_{2peak}$ range 63.9-81.1)	500mL/day NO_3^- containing water (~5.5 mmol of NO_3^- /day) 5 days. 3.5 ± 0.5 h prior to exercise Chronic	Incremental TTE treadmill	Not reported	Peak speed N: 20.0 ± 0.9 km·h^{-1} P: 20.0 ± 1.4 km·h^{-1}	0
Arnold et al. 2015 [42]	10	10 M	Well-trained competitive runners ($\dot{V}O_{2peak}$ 66 ± 7)	70mL of BR (~7 mmol of NO_3^-). 2.5h prior to exercise Acute	Incremental step TTE Treadmill	675% ↑ NO_2^-*	TTE N: 402 ± 80 s P: 393 ± 62 s	2.29
Bailey et al. 2015 [37]	10	7 M	Recreationally active	Days 1-3 and 6-7: 70mL of BR (6.2 mmol of NO_3^-) once in the morning and in the evening Days 4-5 and 8-9: 140mL of BR (12.4 mmol of NO_3^-) 2.5h prior to exercise and 70mL of BR (6.2 mmol of NO_3^-) 2h after exercise Chronic	SI step test (35 rpm) Cycle ergometer	179% ↑ NO_2^-*	TTE N: 344 ± 74 s P: 341 ± 99 s	0.88

Bailey et al. 2015 [37]	10	7 M	Recreationally active	<u>Days 1-3 and 6-7</u>: 70mL of BR (6.2 mmol of NO_3^-) once in the morning and in the evening <u>Days 4-5 and 8-9</u>: 140mL of BR (12.4 mmol of NO_3^-) 2.5h prior to exercise and 70mL of BR (6.2 mmol of NO_3^-) 2h after exercise Chronic	SI step test (115 rpm) Cycle ergometer	179% ↑ NO_2^-*	<u>TTE</u> N: 362 ± 137 s P: 297 ± 79 s	21.89*
Carpentier et al. 2015 [55]	10	13 M	Healthy ($\dot{V}O_{2peak}$ 46.8 ± 1.1)	450mL/day NO_3^- solution (~450mg of NO_3^-/day) for 6 days. 2h prior to exercise Chronic	Incremental step TTE (85% VO_{2max}) Cycle ergometer	Not measured	<u>TTE</u> N: 178 ± 15 W P: 179 ± 15 W	0.56

* = significantly different from placebo (as reported within studies; $p < 0.05$)

$\dot{V}O_{2max}$ = maximal oxygen uptake $\dot{V}O_{2peak}$ = peak oxygen uptake PEDro = physiotherapy evidence database scale TTE = time to exhaustion N = NO_3^- P = placebo

BR = beetroot juice M = male F = female s = seconds W = watts km·h-1 = kilometres per hour SI = severe-intensity

IR1 = intermittent recovery test level 1 ↑ = increase rpm = revolutions per minute

3.5 Meta-Analysis

3.5.1 Time trial performance

Following data pooling from 28 trials, the standardised mean difference was -0.10 (95% CI -0.27 - 0.06), providing a trivial, but non-significant effect in favour of dietary NO_3^- supplementation in TT performance measures ($p > 0.05$) as shown in Fig. 2. There was no heterogeneity displayed among these studies ($I^2 = 0\%$; Q = 7.46, df = 27, p = 1.00), utilising a random effects analysis

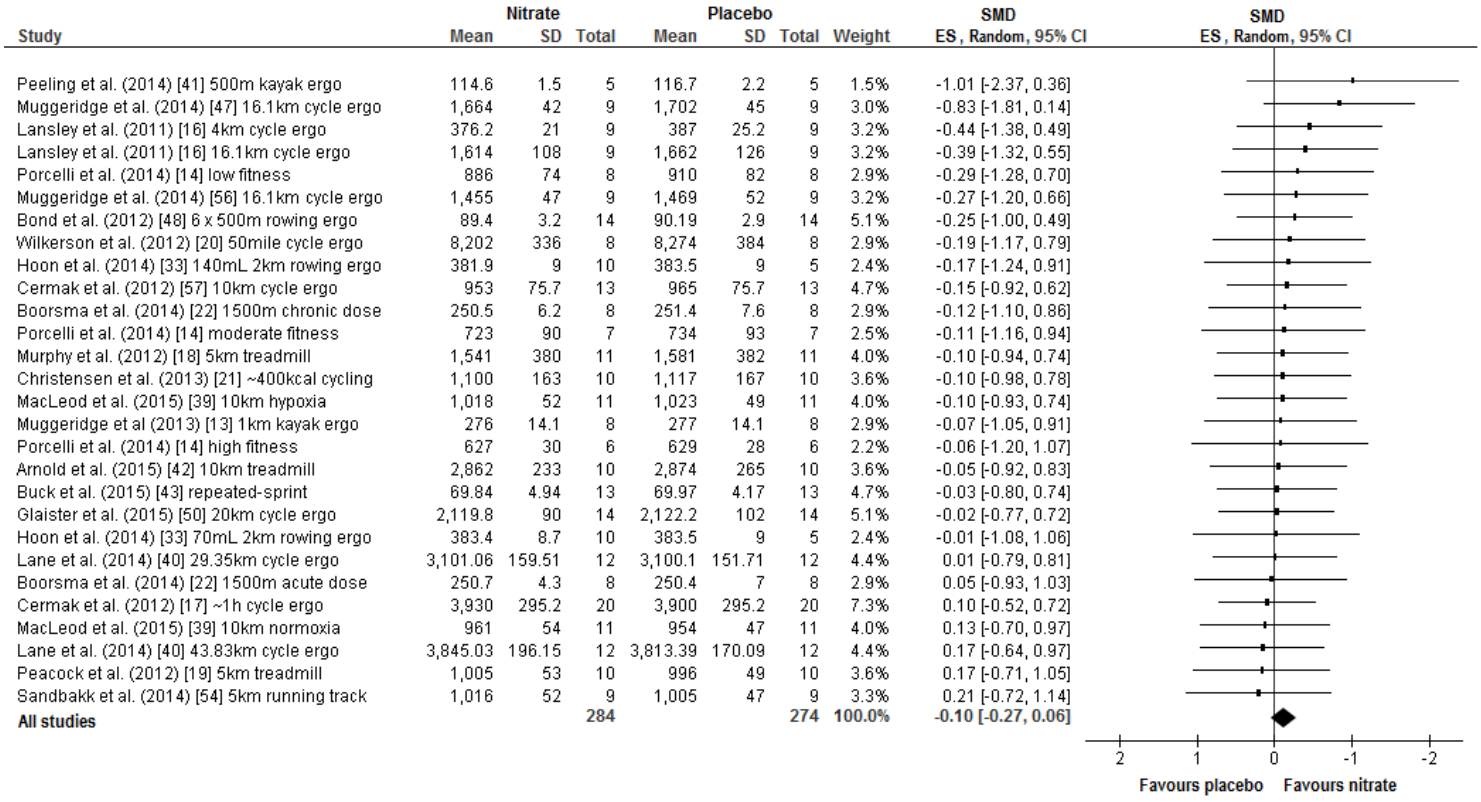

Figure 2: Effect size forest plot for the effect of dietary NO_3^- supplementation on time trial performance (means ± 95% confidence intervals). *ES* effect size, *SD* standard deviation, *CI* confidence interval, *SMD* standardised mean difference, *ergo* ergometer, *kcal* kilo calorie

3.5.2 Time to exhaustion

The standardised mean difference from 22 trials was 0.33 (95% CI 0.15 - 0.50), indicating a small to moderate statistically significant effect in favour of dietary NO_3^- supplementation in TTE performance measures ($p < 0.01$) as shown in Fig. 3. There was no heterogeneity displayed among these studies ($I^2 = 0\%$; Q = 9.82, df = 21, p = 0.98) utilising a random effects analysis.

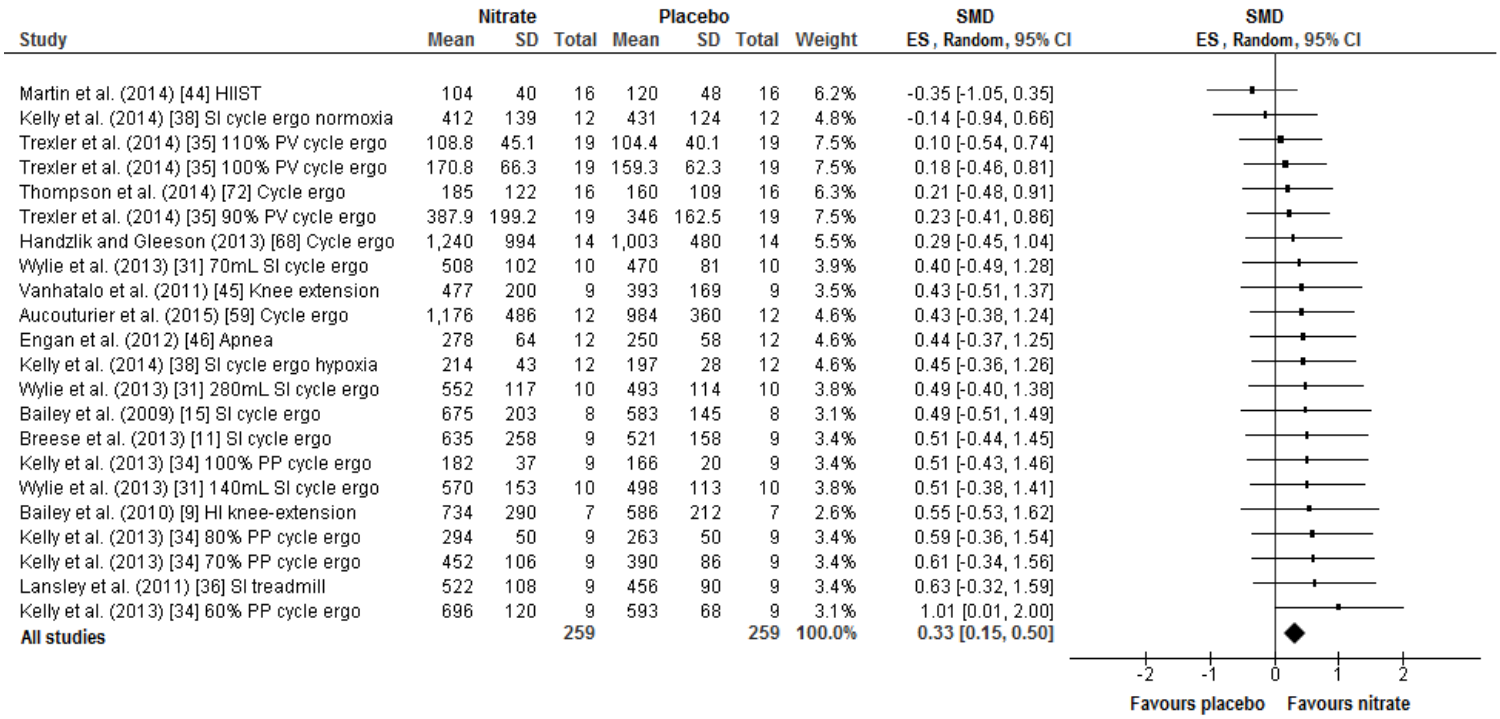

Figure 3: Effect size forest plot for the effect of dietary NO_3^- supplementation on time to exhaustion performance (means ± 95% confidence intervals). *ES* effect size, *SD* standard deviation, *CI* confidence interval, *SMD* standardised mean difference, *ergo* ergometer, *PV* peak velocity, *HIIST* high-intensity interval sprint training, *SI* severe-intensity, *HI* high-intensity, *PP* peak power

3.5.3 Graded-exercise performance test

The standardised mean difference from 8 trials was 0.25 (95% CI -0.06 - 0.56), providing a small, but non-significant effect in favour of dietary NO_3^- supplementation in GXT performance measures ($p > 0.05$) as shown in Fig. 4. There was no heterogeneity displayed among these studies ($I^2 = 0\%$; $Q = 0.90$, $df = 7$, $p = 1.00$) utilising a random effects analysis.

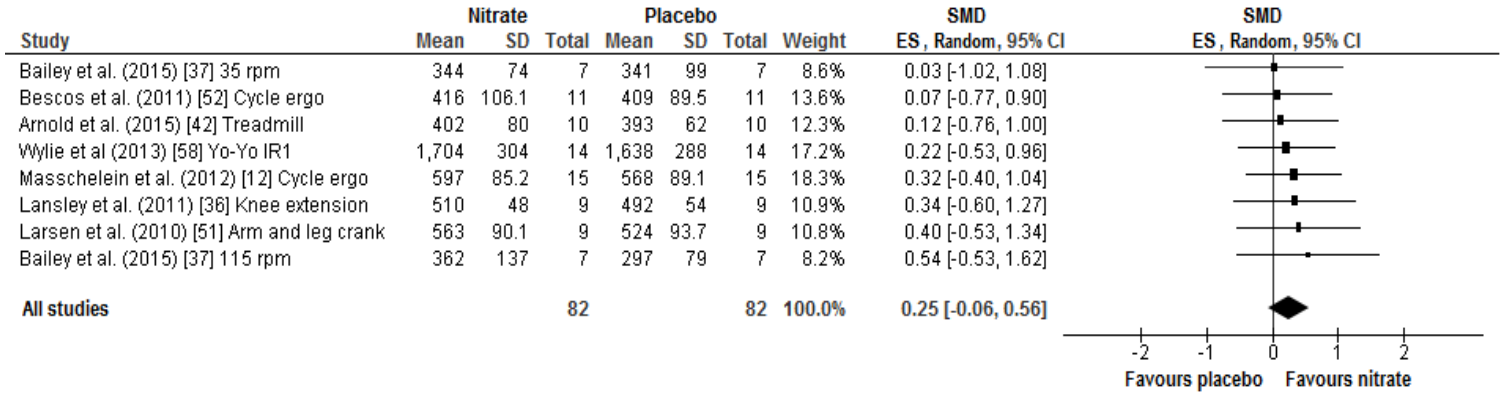

Figure 4: Effect size forest plot for the effect of dietary NO_3^- supplementation on graded-exercise test performance (means ± 95% confidence intervals). *ES* effect size, *SD* standard deviation, *CI* confidence interval, *SMD* standardised mean difference, *ergo* ergometer, *rpm* revolutions per minute, *IR1* intermittent recovery test level 1

Publication bias was assessed by visual inspection of the funnel plot of standard error verses ES for both TT (Fig. 5) and TTE (Fig. 6), with minor asymmetrical inverted distributions prominent for both plots. For both TT performance and TTE, there was evidence of publication bias, Egger's test <0.02 and <0.001 respectively, suggesting small study bias.

3.5.4 Meta-regression analyses

There was no statistically significant effects observed from the meta-regression analysis. Data from the analyses of moderator variables are presented in Tables 4 and 5. A positive trend towards significance (p = 0.11) was seen in trials implementing a chronic dosage regime in the TTE protocol.

Table 4 Time trial univariate meta-regression

Trial feature	Classification	Number of trials	SMD (95% CI)	Z-value	p-value
Dichotomous outcomes					
Exercise type	Other	15			
	Cycling	13	-0.013 (-0.35, 0.32)	0.08	0.94
Test duration	< 10 mins	9			
	≥ 10 mins	19	0.12 (-0.24, 0.49)	0.66	0.51
Dose duration	Acute	19			
	Chronic	9	0.02 (-0.33, 0.37)	0.11	0.92
NO_3^- type	Other	7			
	Beetroot	21	-0.10 (-0.53, 0.33)	0.47	0.64
NO_3^- dose	< 6.5 mmol	11			
	≥ 6.5 mmol	16	0.23 (-0.12, 0.58)	1.29	0.20
Continuous outcomes					
Fitness level	$\dot{V}O_{2max}$	23	0.008 (-0.010, 0.026)	0.87	0.39
% NO_2^- change		11	-0.0001 (-0.0016, 0.0015)	0.10	0.92

SMD = standardised mean difference, NO_2^- = nitrite, CI = confidence interval

Table 5 Time to exhaustion univariate meta-regression

Trial feature	Classification	Number of trials	SMD (95% CI)	Z-value	p-value
Dichotomous outcomes					
Exercise type	Other	7			
	Cycling	15	0.05 (-0.31, 0.41)	0.27	0.78
Test duration	< 10 mins	16			
	≥ 10 mins	6	0.25 (-0.17, 0.67)	1.18	0.24
Dose duration	Acute	10			
	Chronic	12	0.29 (-0.064, 0.67)	1.59	0.11
NO_3^- type	Other	3			
	Beetroot	19	0.22 (-0.20, 0.64)	1.03	0.30
NO_3^- dose	< 6.5 mmol	8			
	≥ 6.5 mmol	11	0.17 (-0.23, 0.57)	0.84	0.40
Continuous outcomes					
Fitness level	$\dot{V}O_{2max}$	16	0.003 (-0.045, 0.046)	0.01	0.99
% NO_2^- change		13	-0.0008 (-0.0025, 0.0009)	0.95	0.34

SMD = standardised mean difference, NO_2^- = nitrite, CI = confidence interval

3.6 Adverse Events

Information on adverse events was reported in 6 of the 47 studies. Bailey et al. [15], Bailey et al. [9], Vanhatalo et al. [10] and Wylie et al. [31] reported beeturia (red urine) and red stools. Hoon et al. [32] reported the withdrawal of one subject due to a beetroot juice intolerance. Hoon et al. [33] reported slight gastrointestinal symptoms immediately after beetroot juice ingestion across the exercise trials, while another reported minor discomfort before one trial. Both occurrences were resolved prior to performance tests. Peeling et al. [41] measured NO_3^- ingestion and its effects on gut sensation. The results showed a lower level of gut distress after a double dose beetroot juice (~9.6 mmol of NO_3^-) when compared to the placebo protocol. No major adverse events were reported across the 47 studies.

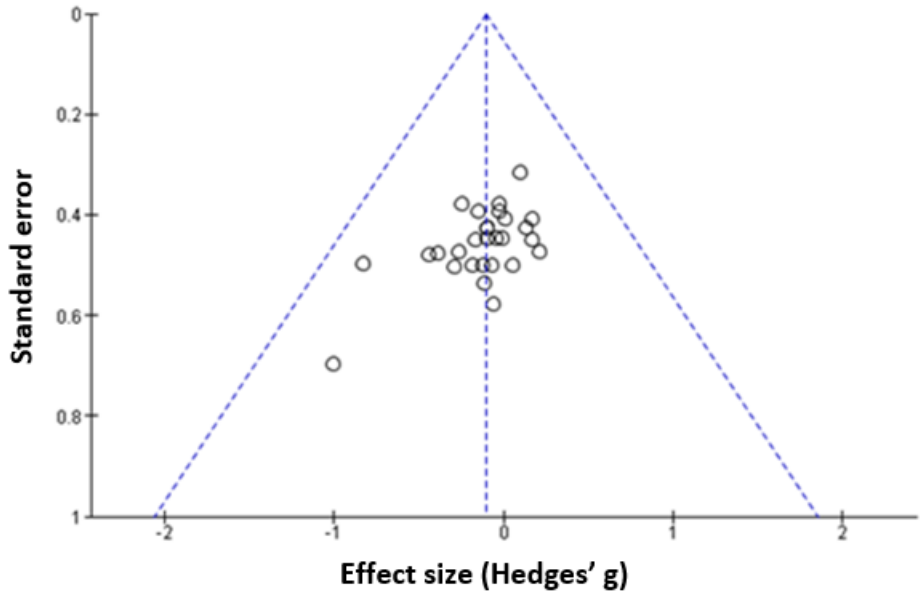

Figure 5: Funnel plot of Hedges' g effect size versus study standard error - outcome: time trial.

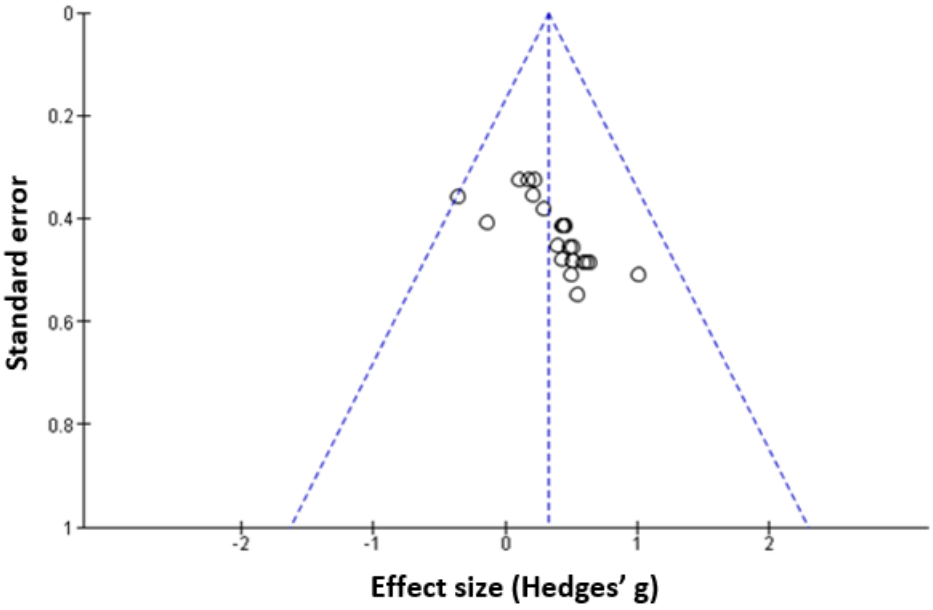

Figure 6: Funnel plot of Hedges' g effect size versus study standard error - outcome: time to exhaustion.

4. Discussion

The primary aim of this study was to perform a systematic review and meta-analysis to determine the efficacy of dietary NO_3^- supplementation on endurance exercise performance. The pooled analysis for nitrate's

influence on TTE showed a significantly greater ES when compared to a placebo control. However, the small effects of dietary NO3- supplementation on TT and GXT performance were not statistically significant. The main conclusion of this meta-analysis was the differing effects dietary NO_3^- supplementation had on TT and TTE protocols.

The findings of this meta-analysis are similar to that of a previous meta-analysis of the impact of dietary NO_3^- supplementation's on exercise performance. In Hoon et al.'s [23] meta-analysis TT protocols had an ES of -0.11 (n = 9) compared to an ES of -0.12 (n = 24) in the present study. In addition, Hoon et al.'s [23] meta-analysis of GXT protocols had an ES of 0.23 (n = 7) compared to an ES of 0.25 (n = 8) in the present study. Hoon et al. [23] also found that dietary NO_3^- supplementation has a statistically significant effect on TTE protocols (ES = 0.79; n = 3). Similarly, the results of the current meta-analysis suggest dietary NO_3^- supplementation is more likely to affect TTE protocols (ES = 0.33; n = 22). The larger number of trials in the current meta-analysis reinforce the findings reported by Hoon et al. [23] and strengthens the evidence for dietary NO_3^- supplementation. This review and quantitative analysis provides an important contribution to the literature and suggests that there is clear evidence that dietary NO_3^- supplementation can boost aerobic exercise capacity measured by TTE protocols.

This enhanced exercise performance in TTE protocols is likely due to the reduced whole-body O_2 cost of constant-work-rate exercise following dietary NO_3^- supplementation [15, 51, 76]. Bailey et al. [9] reported that the decrease in O_2 cost correlates with a reduced ATP cost of muscle force production, creating a reduction in the phosphocreatine degradation, as well as a reduced accumulation of adenosine diphosphate and inorganic phosphate concentration during low and high-intensity exercise (knee extensions) after beetroot juice supplementation when compared to a placebo. Moreover, beetroot juice supplementation significantly reduced muscle ATP hydrolysis during both low and high-intensity exercise bouts. The authors speculated that the possible mechanisms behind the in vivo decrease in O_2 cost of exercise following NO_3^- supplementation is predominantly a result of a reduction in total ATP cost of muscle force production, and not an increase in mitochondrial phosphate/O_2 ratio. Alternatively, Jones [60] suggests that the decreases in steady-state $\dot{V}O_2$ and phosphocreatine after dietary NO_3^- supplementation could potentially be due to the simultaneous improvement of mitochondrial efficiency and muscle oxygenation. These findings suggest a fatigue protocol

such as TTE may be more suited for dietary NO_3^- studies looking at the physiological mechanisms affecting performance and exercise capacity.

The ergogenic effect of dietary NO_3^- was more apparent when TTE tests were used as the main outcome measure. Protocols involving exercising until exhaustion have been suggested to have a greater variability than TT tests [61, 62]. In a study by Saris et al. [61], times to exhaustion across five trials resulted in a high coefficient of variation (CV) of 26.6%, with an individual CV range from 17.4% to 39.5%. In the same study, two time-trial protocols produced a CV of 3.5% and 3.4%, and the individual CV ranged from 1.7% to 5.8% and 0.8% to 5.8% respectively. Thus it initially appears surprising that the ergogenic effects were significant only when the more variable TTE measures were used. However, Amann et al. [63] found similar sensitivities between TT and TTE protocols suggesting TTE protocols are a valid option when determining the effects of an intervention on endurance performance. Jeukendrup et al. [64] suggested the difference in the variability between protocols could be attributed to differences in the influence of psychological factors such as motivation and monotony on the outcome measure and that TTE protocols measure endurance capacity rather than exercise performance, which is better measured by TT protocols. Clearly further research is required to determine why the present analysis shows a greater ergogenic effect of dietary NO_3^- when TTE protocols are used as the outcome measure rather than TT protocols. It is worth noting that a TT protocol has been suggested to be the most appropriate and reliable choice for an intervention resembling “real-life” endurance exercise performance (63) and therefore, these protocols may be the most ecologically valid option when assessing the impact of dietary NO_3^- supplementation on performance [65, 66].

Despite not being statistically significant, the 0.8% improvement in TT performance following dietary NO_3^- supplementation may be meaningful for athletes. To put this into perspective, the difference between first and twelfth place in the 10000m men’s running final at the 2012 London Olympics was only 0.66% [67]; therefore, it is still prudent to recommend dietary NO_3^- supplementation to aid endurance exercise performance, when small improvements in performance can be particularly meaningful. In addition, using dietary NO_3^- supplementation to increase TTE during training may result in the completion of more intervals, enhancing those physiological adaptations that improve TT performance.

Moderator variables, including exercise type, exercise duration, dose duration, NO_3^- type, dose amount, study quality, fitness level ($\dot{V}O_{2max}$), and percentage NO_2^- change, did not appear to have any significant interactions on the effects of dietary NO_3^- supplementation on exercise performance.

A chronic dosage regime appears to show a trend towards a slightly better performance outcome than acute on the TTE protocol (ES: 0.29; p = 0.11). Interestingly, there were two studies that directly compared chronic and acute doses on performance. Vanhatalo et al. [10] found that chronic ingestion (15 days) of beetroot juice had a greater effect on peak power output, gas exchange threshold, and blood pressure compared to an acute dosage (2.5 hours prior to testing). Boorsma et al. [22] also reported a slight improvement in TT performance after a chronic dosing protocol (8 days), whereas participants consuming an acute dose (2.5 hours prior to testing) of dietary NO_3^- did not improve exercise efficiency or performance. Taking into account the results from the meta-analysis and also these studies, it would appear that chronic dosing may be more likely to produce a benefit; however, further research is needed to understand what length of dietary NO_3^- supplementation period elicits the best outcome.

Interestingly, level of fitness did not influence the ergogenic effect of dietary NO_3^- supplementation according to the continuous variable meta-regression, however, the subjects involved in the trials had a similar fitness level; therefore, we were unable to determine confidently the effect training status has on the response to dietary NO_3^- supplementation. The only study to directly compare individual aerobic fitness levels with dietary NO_3^- supplementation observed positive improvements in sedentary and moderately trained individuals, but not highly trained subjects [14]. Further research should be specifically targeted towards the level of fitness variable before definitive conclusions can be made regarding its effect on the dietary NO_3^- supplementation response.

A potential limitation of this meta-analysis is the possible effect of publication bias with the suggestion of small study bias. However, these types of studies typically employ small sample sizes. Thus, there may be other sources of funnel plot asymmetry, e.g. true heterogeneity and chance [30]. Although studies included in the meta-analysis showed no statistical heterogeneity, they still varied considerably in study design. Differences in exercise mode, dose duration and amount, mode of NO_3^- delivery, test duration, and NO_3^- type along with a lack of repetition when measuring these variables made it difficult to draw conclusions and make interpretations from the results. Additionally, univariate meta-regression does have limitations that

can diminish its ability to make valid conclusions. The main limitation of the uni-variate approach is that potential moderators cannot be assessed in isolation in trials with large numbers of characteristics. The findings of this meta-analysis demonstrate that there is enough evidence to suggest dietary NO_3^- supplementation can improve endurance exercise performance; however, more experimental trials need to be conducted with a research focus on potential moderator variables to provide definitive conclusions and recommendations for dietary NO_3^- supplementation and its effect on endurance exercise performance.

With respect to moderator variables, future research might also be designed to isolate the ergogenic effect of nitrate ingestion for individuals possessing different muscle fibre type proportions. For example, research conducted by Hernandez et al. [71] on the effect of dietary NO_3^- ingestion observed an enhanced contractile force in fast-twitch muscles in the NO_3^- supplemented mice. The results translate to an activation of fast-twitch muscle fibres at a lower frequency but still achieving the same force after dietary NO_3^- supplementation, therefore, a reduced effort required to perform a given task. Dietary NO_3^- supplementation appears to be particularly effective at improving physiological responses in type II muscle [11, 37, 71, 72] and can lead to increased force production at higher contraction velocities [73], and improved performance during short-duration high-intensity intermittent exercise [74, 75] when type II muscle fibre recruitment is high. This provides an interesting avenue for future research investigating the effects of dietary NO_3^- supplementation on performance during intermittent and power exercise tests.

5. Conclusion

In summary, the findings of this systematic review and meta-analysis provide convincing evidence that dietary NO_3^- supplementation is likely to elicit a positive outcome when testing endurance exercise capacity, but is less likely to be effective for TT performance. The design of the test protocol selection may influence the conclusion regarding the ergogenic effect of dietary NO_3^- supplementation. Further work is needed to understand the optimal dosing strategies, which population is most likely to benefit, and under which conditions dietary nitrates are likely to be most effective for enhancing performance.

Compliance with Ethical Standards

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Conflicts of interest

Nicholas McMahon, Michael Leveritt and Toby Pavey declare they have no conflicts of interest relevant to the content of this review.

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Electronic Supplementary Material Appendix S1. PEDro scale criteria and operational definitions

PEDro scale	
1. eligibility criteria were specified	no ☐ yes ☐ where:
2. subjects were randomly allocated to groups (in a crossover study, subjects were randomly allocated an order in which treatments were received)	no ☐ yes ☐ where:
3. allocation was concealed	no ☐ yes ☐ where:
4. the groups were similar at baseline regarding the most important prognostic indicators	no ☐ yes ☐ where:
5. there was blinding of all subjects	no ☐ yes ☐ where:
6. there was blinding of all therapists who administered the therapy	no ☐ yes ☐ where:
7. there was blinding of all assessors who measured at least one key outcome	no ☐ yes ☐ where:
8. measures of at least one key outcome were obtained from more than 85% of the subjects initially allocated to groups	no ☐ yes ☐ where:
9. all subjects for whom outcome measures were available received the treatment or control condition as allocated or, where this was not the case, data for at least one key outcome was analysed by "intention to treat"	no ☐ yes ☐ where:
10. the results of between-group statistical comparisons are reported for at least one key outcome	no ☐ yes ☐ where:
11. the study provides both point measures and measures of variability for at least one key outcome	no ☐ yes ☐ where:

The PEDro scale is based on the Delphi list developed by Verhagen and colleagues at the Department of Epidemiology, University of Maastricht [77].

Criterion	Operational Definition
All criteria	**Points are awarded only when a criterion is clearly satisfied**. If on a literal reading of the trial report, it is possible that a criterion was not satisfied, a point should not be awarded for that criterion.
Criterion 1	This criterion is satisfied if the report describes the source of subjects and a list of criteria used to determine who was eligible to participate in the study.
Criterion 2	A study is considered to have used random allocation if the report states that allocation was random. The precise method of randomization need not be specified. Procedures such as coin tossing and dice rolling should be considered random. Quasi-randomization allocation procedures such as allocation by hospital record number or birth date, or alternation, do not satisfy this criterion.
Criterion 3	Concealed allocation means that the person who determined if a subject was eligible for inclusion in the trial was unaware, when this decision was made, of which group the subject would be allocated to. A point is awarded for this criterion, even if it is not stated that allocation was concealed, when the report states that allocation was by sealed opaque envelopes or that allocation involved contacting the holder of the allocation schedule who was "off-site."
Criterion 4	At a minimum, in studies of therapeutic interventions, the report must describe at least one measure of the severity of the condition being treated and at least one (different) key outcome measure at baseline. The rater must be satisfied that the groups' outcomes would not be expected to differ, on the basis of baseline differences in prognostic variables alone, by a clinically significant amount. This criterion is satisfied even if only baseline data of subjects completing the study are presented.
Criteria 4, 7–11	Key outcomes are those outcomes that provide the primary measure of the effectiveness (or lack of effectiveness) of the therapy. In most studies, more than one variable is used as an outcome measure.
Criteria 5–7	Blinding means the person in question (subject, therapist, or assessor) did not know which group the subject had been allocated to. In addition, subjects and therapists are only considered to be "blind" if it could be expected that they would have been unable to distinguish between the treatments applied to different groups. In trials in which key outcomes are self-reported (e.g., visual analog scale, pain diary), the assessor is considered to be blind if the subject was blind.
Criterion 8	This criterion is satisfied only if the report explicitly states both the number of subjects initially allocated to groups and the number of subjects from whom key outcome measurements were obtained. In trials in which outcomes are measured at several points in time, a key outcome must have been measured in more than 85% of subjects at one of those points in time.
Criterion 9	An intention-to-treat analysis means that, where subjects did not receive treatment (or the control condition) as allocated and where measures of outcomes were available, the analysis was performed as if subjects received the treatment (or control condition) they were allocated to. This criterion is satisfied, even if there is no mention of analysis by intention to treat, if the report explicitly states that all subjects received treatment or control conditions as allocated.
Criterion 10	A between-group statistical comparison involves statistical comparison of one group with another. Depending on the design of the study, this may involve comparison of 2 or more treatments or comparison of treatment with a control condition. The analysis may be a simple comparison of outcomes measured after the treatment was administered or a comparison of the change in one group with the change in another (when a factorial analysis of variance has been used to analyse the data, the latter is often reported as a group time interaction). The comparison may be in the form hypothesis testing (which provides a P value, describing the probability that the groups differed only by chance) or in the form of an estimate (eg, the mean or median difference, a difference in proportions, number needed to treat, a relative risk or hazard ratio) and its confidence interval.
Criterion 11	A point measure is a measure of the size of the treatment effect. The treatment effect may be described as a difference in group outcomes or as the outcome in (each of) all groups. Measures of variability include standard deviations, standard errors, confidence intervals, interquartile ranges (or other quartile ranges), and ranges. Point measures and/or measures of variability may be provided graphically (e.g., standard deviations may be given as error bars in a figure) as long as it is clear what is being graphed (e.g., as long as it is clear whether error bars represent standard deviations or standard errors). Where outcomes are categorical, this criterion is considered to have been met if the number of subjects in each category is given for each group.