The effects of nitrate ingestion on high-intensity endurance time-trial performance: A systematic review and meta-analysis

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**A B S T R A C T**

Background/Objective: Dietary nitrate ingestion extends endurance capacity, but data supporting endurance time-trial performance are unclear. This systematic review and meta-analysis evaluated the evidence for dietary nitrate supplementation to improve high-intensity endurance time-trial performance over 5–30 min on the premise that nitrate may alleviate peripheral fatigue over shorter durations.

Methods: A systematic literature search and data extraction was carried out following PRISMA guidelines and the PICOS framework within five databases: PubMed, ProQuest, ScienceDirect, Scopus and SPORT-Discus. Search terms used were: (nitrate OR nitrite OR beetroot) AND (high intensity OR all out) AND (time trial or total work done) AND performance.

Results: Twenty-four studies were included. Fifteen studies applied an acute supplementation strategy (4.1 mmol–15.2 mmol serving on one day), eight chronic supplementation (4.0 mmol–13.0 mmol per day over 3–15 days), and one applied both acute and chronic supplementation (8.0 mmol on one day and over 15 days). Standardised mean difference for time-trial ranging from 5 to 30 min showed an overall trivial effect in favour of nitrate (Hedges’g = 0.15, 95% CI -0.00 to 0.31, Z = 1.95, p = 0.05). Subgroup analysis revealed a small, borderline effect in favour of chronic nitrate intervention (Hedges’g = 0.30, 95% CI -0.00 to 0.59, Z = 1.94, p = 0.05), and a non-significant effect for acute nitrate intervention (Hedges’g = 0.10, 95% CI -0.08 to 0.28, Z = 1.11, p = 0.27).

Conclusion: Chronic nitrate supplementation improves time-trial performance ranging from 5 to 30 min.

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1. Introduction

Nitrate-containing foods or juices (including beetroot, spinach, rocket salad and celery) are among the list of supplements recognised by the International Olympic Committee Consensus Statement 2018 for their potential benefits to improve performance."To date, beetroot juice is the most widely used source of dietary nitrate evaluated for performance effects. Beetroot juice contains a high amount of polyphenols and ascorbic acid, which are thought to play certain roles in nitrate reduction and metabolism to nitric oxide — a signalling molecule considered to contribute to numerous physiological functions important for exercise metabolism including enhanced function of type II muscle fibres; a reduced adenosine triphosphate cost of muscle force production; increased efficiency of mitochondrial respiration; and vasodilation and increased blood flow to the muscle."5–11 After Larsen and colleagues12 made the discovery in 2007 that sodium nitrate reduced the oxygen cost of submaximal cycling, many studies have since confirmed that beetroot juice provides similar benefits on improving endurance exercise performance.3,4,13–15 However, the ingestion of other sources of dietary nitrate, including via increased dietary consumption of fresh fruits and vegetables, have also been examined in relation to exercise performance.16–18

The overall impact of nitrate supplementation on endurance exercise has been outlined in four previous systematic reviews17 and meta-analyses.16,18–20 In two earlier meta-analyses, favourable effects of nitrate supplementation on time to exhaustion tests were observed.16,18 However, for the analysis of time trial performance both Hoon and colleagues (nine trials) and McMahon and colleagues (28 trials) found trivial non-significant benefits in favour
of nitrate. More recently, Senefeld and colleagues examined the effects of nitrate on athletic performance and found consistent ergogenic effects across all areas of athletic performance. This included a subgroup analysis of 52 studies looking at time-trial and 6 studies looking at distance trials (maximal distance covered in a fixed time). However, the analysis of time and distance trials included single sprint events (e.g., 500 m kayaking) which are less than 300 s in length. Certainly, the most recent systematic review and meta-analysis from Gao and colleagues included 73 studies and noted similar conclusions to earlier meta-analyses. Nitrate supplementation improved power output, time to exhaustion and distance travelled but there was no significant difference in perceived exertion, time-trial performance or work done. Collectively these analyses suggest a clear extension of endurance capacity with dietary nitrate supplementation but the effect on endurance performance is less certain. However, a recent systematic review from Lorenzo Calvo and colleagues (27 studies) suggested that nitrate may improve time to exhaustion and race time-trial efforts in the region of between 5 and 30 min, although this was not subject to statistical analysis. Several individual studies support the hypothesis that nitrate improves short duration exercise performance, particularly the effects of nitrate in these conditions comes from a recent systematic review by Tan and colleagues of 18 studies which suggested that nitrate supplementation may improve outcomes in explosive events. The review showed that the power and velocity of certain explosive resistance exercises, along with sprint time, power output and total work in sprint studies may be affected. A separate meta-analysis has also shown improvements in muscle power with isolated sprints during cycling or isokinetic knee extensions and it is suggested that nitrate and nitrite can be stored in muscle tissue, potentially contributing to these improvements in power observed. Thus, it seems clear that nitrate supplementation has greater potential to improve performance with higher intensity work under conditions where there is competition between oxygen demand and supply. However, there is no clear demonstration of the impact of nitrate supplementation during higher intensity endurance time-trials. Certainly, there is data to support the suggestion that the mechanism of fatigue may differ depending on the length of a time-trial; with greater peripheral fatigue constraining shorter high-intensity time-trials and central fatigue predominating with longer, lower-intensity time-trials. It is therefore possible that previous meta-analyses, which comprehensively grouped measures of endurance time-trial performance together, have failed to observe clear effects because of the inclusion of longer duration time-trials where peripheral fatigue within the muscle was not the primary impediment to performance. When coupled with the variance in supplementation strategy and exercise protocols/modality the optimal duration of the ergogenic effect of nitrate supplementation may have been missed.

Although studies and meta-analyses suggest ergogenic effects of nitrates on endurance exercise capacity and high-intensity exercise performance, evidence for a strong effect on time-trial performance is lacking. Given the hypothesised ergogenic effects of nitrate in conditions of low oxygen availability this systematic review and meta-analysis was conducted with the primary aim of examining the acute and chronic ergogenic effect(s) of dietary nitrate on high-intensity endurance time-trial performance. Specifically, the effects of nitrate ingestion/supplementation on performance in time-trials of shorter lengths (for example 1.5–5 km running and 10 km cycling in duration from 5 to 30 min) were considered. However, to further support the hypothesis we compare the outcomes with longer time trials of 30–60 min in length.

2. Materials and methods

The present review followed the guidelines set out by PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) and included the PICOS (population, intervention, comparison, outcome, study design) framework for data extraction. The process of title/abstract and full-text screening was done using Covidence software (Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia).

2.1. Search strategy

The systematic literature search included five databases: PubMed, ProQuest, ScienceDirect, Scopus and SPORTDiscus. The search terms used were: (nitrate OR nitrite OR beetroot) AND (high intensity OR all out) AND (time trial OR total work done) AND performance. The search was filtered according to the last ten years of up-to-date publications and evidence, from January 1, 2011 to February 7, 2022. Upon completion of the search, the full search yields were imported, screened and analysed on the Covidence software. A total of 7 duplicates were eliminated, and 227 studies remained for screening and review. There were 15 additional studies that were identified through a high-intensity interval training systematic review as the studies had reported time-trial as part of the performance outcomes, and four systematic reviews on time-trial or endurance performance. Fig. 1 summarises the identification of studies using the PRISMA Flow Diagram.

2.2. Inclusion criteria

The inclusion criteria of this systematic review were defined using the PICOS model framework (Population: Active adults 18–45 years old; Intervention: Nitrate supplementation; Comparison: Same conditions with Placebo or control group; Outcome: Exercise time-trial performance measure (time taken to complete a set amount of work or distance); Study design: Randomised crossover (repeated measures) or parallel group designs). The seven inclusion criteria were: (1) full article; (2) nitrate and placebo/control intervention; (3) precise information on dosage and ingestion timing; (4) assessed and reported short length time-trial performance measures in the range of 5–60 min exercise; (5) employed a randomised crossover (repeated measures) or parallel-group design; (6) healthy active adults 18–45 years old; (7) article published in English. Types of athletic level, gender, or ethnicity were not considered as part of the inclusion criteria.

The screening of the articles and data extraction was conducted by two independent reviewers — the first researcher screened the
articles and extracted the data while the second researcher verified the information accordingly in order to ensure accuracy. Both researchers screened the title, abstract, and full papers independently to assess the eligibility criteria. Differences in opinion and included/excluded papers were resolved through discussion and consensus with the third researcher. After elimination of duplicates and screening of inclusion criteria, a total of 24 studies were identified for review and data extraction.

2.3. Data extraction and analysis

The data extraction process was conducted by the first researcher manually using a standardised form (Microsoft Excel, 2008) and the information was cross-checked by the second researcher. Disagreement was resolved by discussion and consensus with input from the third researcher. Data extraction included information on the authors, year of publication, sample size, sex, age, exercise level, nitrate dosage, supplement source, study design, exercise protocol and primary outcome. The included studies were then grouped by the primary outcome for meta-analysis.

2.4. Quality assessment

The assessment of the quality of the studies included in this review was measured using the Physiotherapy Evidence Database (PEDro) scale. The PEDro scale provides a reliable assessment of internal validity. The eligibility of each article was assessed independently by two reviewers using an 11-item checklist. The maximum score on the PEDro scale is 10 (item 1 on eligibility criteria does not contribute to the total score). The risk of bias was assessed with the Revised Cochrane risk-of-bias tool for randomised trials (RoB 2). The RoB 2 tool provides a framework to assess the risk of bias in study findings within five domains: (1) bias arising from the randomisation process; (2) bias due to deviations from intended interventions; (3) bias due to missing outcome data; (4) bias in the measurement of the outcome; and (5) bias in the selection of the reported result. Methodological quality and risk of bias were assessed by two researchers independently. Differences in study quality, methodological quality and risk of bias were resolved through discussion and consensus and clarified with the third author if necessary.

2.5. Statistical analysis

The data on participants and performance are reported as mean and standard deviation. The level of agreement between researchers on study quality was evaluated using Cohen’s kappa statistic. Meta-analysis was performed using Review Manager (RevMan) version 5.4 (The Cochrane Collaboration, 2020). A random-effects model was applied to compute the standardised mean difference between intervention and placebo. Statistical significant was accepted at $p < 0.05$. The overall effect (95% Confidence Interval (CI)) and $I^2$ values (percentage of total variation among studies) were calculated by RevMan. Effect sizes are described as trivial (<0.2), small (<0.5), moderate (<0.8), and large (>0.8). $I^2$ values were guided as follows: (1) might not be important (0%–40%); (2) may represent moderate heterogeneity (30%–60%); (3) may represent substantial heterogeneity (50%–90%); and (4) considerable heterogeneity (75%–100%).

3. Results

3.1. Internal validity and risk of bias

The mean study quality assessed with the PEDro scale showed a score of 8.9 ± 1.0 out of 10 (Table 1). Three studies did not report randomisation procedures, although double-blind designs were
employed.24,29,46 Three studies used randomised and crossover designs but did not apply the double-blind method.15,32,35 Twenty-one studies reported a double-blind design, with one of these studies declaring a limitation in the blinding process as the supplementation (beetroot vs placebo (cranberry)) did not taste identical.56 However, this was explained as a placebo effect as participants were not aware of which supplementation was expected to impact performance.56 Most studies did not explain clearly how the blinding process was achieved effectively. The level of agreement between reviewers was $k = 1.00$ (Kappa value), which can be interpreted as perfect agreement.27 There was no disagreement between the reviewers in classifying studies using the RoB 2. Twenty-one articles were considered ‘low risk’, and three articles were regarded as having ‘some concerns’ in the randomisation process. No studies were considered at ‘high risk’ of bias (Figs. 2 and 3).

### 3.2. Participant and study characteristics

A summary of the participants’ characteristics, study design, exercise protocol and the primary outcome of the 24 studies included in this systematic review are provided in Table 2. The 24 studies included 335 participants — 25.1% (n = 84) females, 74.9% (n = 251) males. The largest sample size was 70,34, and the smallest was 5.25 The mean age range of participants was from 18.0 to 38.7 ± 9.2 years.21,30 The participants consisted of recreational exercisers (n = 172), competitive/trained individuals (n = 148) and elite athletes (n = 15). All studies included placebo and nitrate intervention groups. Fifteen studies applied acute supplementation of beetroot on markers of performance, whilst eight studies used a chronic supplementation strategy (beetroot juice provided on multiple exercise intervals). This is equivalent to approximately 10-km running/skating/skiing or 20-km cycling or less although there can be considerable variation dependent on athletic standard. Three (one acute and two chronic) out of 24 included studies contained preload exercise before the time-trial.14,21,30 It is postulated that nitrate supplementation provides an ergogenic impact in the shorter distance (high work rate) than longer distance (lower work rate) time-trials.45 There were 19 studies for time-trial protocols ranging from 5 to 30 min and 6 studies for time-trial protocols ranging from 5 to 30 min and 6 studies for time-trial protocols ranging from 5 to 30 min and 6 studies for time-trial protocols ranging from 5 to 30 min and 6 studies for.
protocols ranging from 30 to 60 min. One study concluded that chronic supplementation of beetroot juice (8.4 mmol nitrates per day for three days) increased mean velocity in the first half of a 10-km running time-trial. However, there was no statistical improvement in overall 10-km performance. Cycling time-trials of different distances ranged between 4.0 and 20.0 km (4.0, 10.0, 16.1, 20.0 km), and pre-defined total work were included. Eight studies employed running time-trials with distances ranging from 1.5 km to 10.0 km (1.5, 2.0, 5.0, 10.0 km), whilst three studies used other exercise modalities, including skiing and rowing.

Time taken to complete the task was the primary outcome extracted for meta-analysis. Nine studies (37.5%) showed significantly improved time-trial performance after nitrate supplementation (0.6%–3.2%), while 15 studies (62.5%) found no significant difference when compared to placebo (Table 2).

3.4. Nitrate supplementation

The nitrate dosage ranged from 4.1 mmol to 15.2 mmol per serving for acute studies and 4.0 mmol–13 mmol per day for chronic intervention studies. Total nitrate intake in chronic studies ranged from 15.0 mmol provided over three days to 120.0 mmol over 15 days. Twenty studies supplemented with beetroot as a nitrate source, while 4 studies used potassium nitrate or nitrate gel.

3.5. Meta-analysis

Fig. 4 displays the forest plot comparing the effect of acute and chronic nitrate supplementation on time-trial performance ranging from 5 to 30 min with 25 effects (17 acute, 8 chronic) from 19 studies. The standardised mean difference for time-trial showed an overall trivial effect in favour of nitrate intervention (Hedges’g = 0.15, 95% CI -0.00 to 0.31, Z = 1.95, p = 0.05). Random effects analysis displayed trivial heterogeneity among studies (I2 = 0%; p = 1.00). Subgroup analysis on the effect of nitrate supplementation revealed a small, borderline significant effect in favour of chronic nitrate intervention (Hedges’g = 0.30, 95% CI -0.00 to 0.59, Z = 1.94, p = 0.05), but a trivial non-significant effect in favour of acute nitrate intervention (Hedges’g = 0.10, 95% CI -0.08 to 0.28, Z = 1.11, p = 0.27). There was no or trivial heterogeneity between subgroups (I2 = 14.6%, P = 0.28). Conversely, the pooled data of time-trial performance between 30 and 60 min (6 effects from 6 studies – 3 acute and 3 chronic) showed no significant effect in favour of nitrate intervention (Hedges’g = 0.13, 95% CI -0.20 to 0.47, Z = 0.80, p = 0.43) (Fig. 5). Similarly, both acute and chronic supplementation over this time trial distance showed non-significant effects after nitrate intervention. The publication bias was assessed using funnel plots for 300–1800s exercise and 1801–3600s exercise (Please see Fig. S1 in Supplementary File). Visual inspection of the plots showed that all studies were within 95% CI.
Table 2
Summary of the included studies assessing the effect of acute and chronic dietary nitrate supplementation on time-trial performance ranging from 5 to 60 min.

| Study     | Year | Sample size (n) | Age (years) | Exercise level and fitness, VO_{peak,max} (ml/kg/min) | A/C | Nitrates supplementation | Study design                                      | Exercise protocol | Primary Outcome |
|-----------|------|-----------------|-------------|------------------------------------------------------|-----|--------------------------|-------------------------------------------------|------------------|-----------------|
| Arnold et al.\(^{40}\) | 2015 10 | 37.0 ± 13.0 | Well-trained competitive male runners. VO_{max} = 66.0 ± 7.0 | A/B | Beetroot 7 mmol (70 ml) and 4 mmol (140 ml) | Randomised, repeated measures, crossover, double-blind | 10-km treadmill running time-trial | No significant improvement in time-trial performance (BR: 2862 ± 233 vs. PL: 2874 ± 265, p = 0.6). Improved time-trial performance Males (BR: 432.7 ± 52.9 vs. PL: 436.6 ± 52.7, p < 0.05). Females (BR: 575.1 ± 68.6 vs. PL: 580.7 ± 67.0, p < 0.05). |
| Casado et al.\(^{21}\) | 2021 14 M 10 F | 38.7 ± 9.2 M; 36.6 ± 8.2 F | Long-distance club runners A | Beetroot 12.8 mmol (140 ml) | Randomised, crossover, double-blind | 2-km running time-trial | No significant improvement (BR: 575 ± 90 vs. PL: 2122.2 ± 102, p > 0.05). No significant different in 4.2 mmol time-trial (BR: 383.4 ± 8.7 vs. PL: 383.5 ± 9 s) and, 8.4 mmol time-trial (BR: 381.9 ± 9 vs. PL: 383.5 ± 9 s). No significant improvement (BR: 1588.47 ± 263.93 vs. PL: 1587.69 ± 260.00, p = 0.875). Improved 4-km performance by 2.8% (PL: 6.45 ± 0.42 vs BR: 6.27 ± 0.35 min, p < 0.05) and, 16.1-km performance by 2.7% (PL: 27.7 ± 2.1 vs BR: 26.9 ± 1.8 min, p < 0.01). |
| Glaister et al.\(^{40}\) | 2015 14 | 31.0 ± 7.0 | Well-trained, competitive, female athletes | A | Beetroot 7.3 mmol (70 ml) | Randomised, counterbalanced, double-blind | 20-km cycling time-trial | No significant improvement (BR: 2119 ± 90 vs. PL: 2122 ± 102, p > 0.05). |
| Hoom et al.\(^{25}\) | 2014 10 | 20.6 ± 2.5 | Highly trained men | A | Beetroot 4.2 mmol (70 ml) and 8.4 mmol (140 ml) | Randomised, placebo-controlled, crossover | 2000-m time-trial with rowing ergometer | No significant different in 4.2 mmol time-trial (BR: 383.4 ± 8.7 vs. PL: 383.5 ± 9 s) and, 8.4 mmol time-trial (BR: 381.9 ± 9 vs. PL: 383.5 ± 9 s). No significant improvement (BR: 1588.47 ± 263.93 vs. PL: 1587.69 ± 260.00, p = 0.875). Improved 4-km performance by 2.8% (PL: 6.45 ± 0.42 vs BR: 6.27 ± 0.35 min, p < 0.05) and, 16.1-km performance by 2.7% (PL: 27.7 ± 2.1 vs BR: 26.9 ± 1.8 min, p < 0.01). |
| Hurst et al.\(^{24}\) | 2020 70 | 33.3 ± 12.3 | Recreational runners (38 male, 32 female) | A | Beetroot 4.1 mmol (70 ml) | quasi-randomised, placebo-controlled, double-blind | 5-km running time-trial | No significant improvement (BR: 1588.47 ± 263.93 vs. PL: 1587.69 ± 260.00, p = 0.875). Improved 4-km performance by 2.8% (PL: 6.45 ± 0.42 vs BR: 6.27 ± 0.35 min, p < 0.05) and, 16.1-km performance by 2.7% (PL: 27.7 ± 2.1 vs BR: 26.9 ± 1.8 min, p < 0.01). |
| Lansley et al.\(^{22}\) | 2011 9 | 21.0 ± 4.0 | Club-level competitive male cyclists. VO_{peak} = 56.0 ± 5.7 | A | Beetroot 6.2 mmol (500 ml) | Randomised, crossover, double-blind | 4-km and 16.1-km cycling TT. | No significant improvement (BR: 1588.47 ± 263.93 vs. PL: 1587.69 ± 260.00, p = 0.875). Improved 4-km performance by 2.8% (PL: 6.45 ± 0.42 vs BR: 6.27 ± 0.35 min, p < 0.05) and, 16.1-km performance by 2.7% (PL: 27.7 ± 2.1 vs BR: 26.9 ± 1.8 min, p < 0.01). |
| MacLeod et al.\(^{13}\) | 2015 11 | 29.3 ± 5.1 | Trained male cyclists. | A | Beetroot 6.5 mmol (70 ml) | Randomised, placebo-controlled, crossover, double-blind | 10-km cycling time-trial | No significant improvement in time-trial performance Normoxia (BR: 961 ± 54 vs. PL: 954 ± 47, p > 0.05). Hypoxia (BR: 1018 ± 52 vs. PL: 1023 ± 49, p > 0.05). |
| Mugggeridge et al.\(^{23}\) | 2014 9 | 28.0 ± 8.0 | Male trained cyclists. VO_{peak} = 51.9 ± 5.8 | A | Beetroot 5 mmol (70 ml) | Randomised cross-over, double-blind | 16.1-km cycling time-trial | Significant improvement in time-trial performance (BR: 1664 ± 42 vs PL: 1702 ± 45, p = 0.021). |
| Mugggeridge et al.\(^{56}\) | 2015 9 | 36.0 ± 6.0 | Nine male trained-cyclists and triathletes. VO_{max} = 53.1 ± 4.4 | A | Nitrate gels (2 × 60 ml gels, 8.1 mmol nitrate) | Randomised, counterbalanced placebo-controlled | 10 min submaximal steady-state cycling followed by a 16.1 km TT 2 × 5-km treadmill running time-trials in random sequence. | No significant improvement in time-trial performance under sham light (BR: 1455 ± 47 vs. PL: 1469 ± 52 s). Time-trial for the full 5 km was marginally faster after beetroot consumption as compared to placebo (BR: 1541 ± 380 vs PL: 1581 ± 382 s). Time to complete the TT was unaffected by supplementation in both N (BR: 297 ± 29 vs PL: |
Table 2 (continued)

| Study                  | Year | Sample size (n) | Age (years) | Exercise level and fitness, VO2peak/max (ml/kg/min) | A/ C Nitrates supplementation | Study design | Exercise protocol | Primary Outcome |
|------------------------|------|-----------------|-------------|---------------------------------------------------|-------------------------------|--------------|-------------------|-----------------|
| Peacock et al.²⁰       | 2012 | 18 years old    | Male junior elite cross-country skiers. VO2max = 69.6 ± 5.1 | A Potassium nitrate (614 mg nitrate), around 9.9 mmol | Randomised, counter-balanced, double-blind | 5-km running time trial on an indoor track | No significant difference in 5-km time-trial performance (BR: 1005 ± 53 vs PL: 996 ± 49 s, p = 0.12). |               |
| Peeling et al.²⁴       | 2015 | 25.0 ± 2.8      | International-level female kayakers. VO2peak = 47.8 ± 3.7 | A Beetroot 9.9 mmol (2 x 70 ml) | Crossover, double-blind | 500-m time-trial kayak | Improved time-trial performance by 1.7% (BR: 114.6 ± 1.5 vs PL: 116.7 ± 2.2 s, p < 0.005). |               |
| Shannon et al.²⁹       | 2016 | 24.4 ± 4.3      | Six competitive male runners/triathletes, four recreational and two physically active. VO2max ranging from 47.1 to 76.8 | A Beetroot 15.2 mmol (138 ml) | Randomised, counterbalanced, double-blind | Steady-state moderate-intensity running and a 1500-m running TT in a normobaric hypoxic chamber (H2O ~ 15%). | Four exercise performance tests comprised a 10 min warm-up followed by a 1500 or 10,000 m run and a 1500-m running TT. Performance in the 1500 m TT was significantly faster in BR vs. PL (BR: 319.6 ± 38.2 s vs. PL: 325.7 ± 38.8 s, p < 0.05), but was no significant difference in 10,000 m TT performance (BR: 2643.1 ± 324.1 s vs PL: 2649.9 ± 319.8 s, p > 0.05). |               |
| Shannon et al.³⁵       | 2017 | 28.3 ± 5.8      | Trained male runners or triathletes. VO2max = 62.3 ± 8.1 | A Beetroot 12.5 mmol (140 ml) | Randomised, double blind | Four exercise performance tests comprised a 10 min warm-up followed by a 1500 or 10,000 m run and a 1500-m running TT. Performance in the 1500 m TT was significantly faster in BR vs. PL (BR: 319.6 ± 38.2 s vs. PL: 325.7 ± 38.8 s, p < 0.05), but was no significant difference in 10,000 m TT performance (BR: 2643.1 ± 324.1 s vs PL: 2649.9 ± 319.8 s, p > 0.05). |               |
| Callahan et al.²⁹      | 2017 | 34.0 ± 7.0      | Well-trained male cyclists. VO2max = 65.2 ± 4.2 | C Beetroot 5 mmol/day for 3 days (15g beetroot crystals), 5 mmol top up dose 1 h pre-trial | Placebo-controlled, double-blind | 4-km cycling time-trial | No significant difference in 4-km time-trial performance (BR: 337.4 ± 17.1 s vs PL: 338.1 ± 18 s, p > 0.05). |               |
| Cermak et al.¹⁴        | 2012 | 31.0 ± 3.0      | Male cyclists engaged in regular cycling training (10 h/week) and had a training history of ~10 years. VO2peak = 58.0 ± 2.0, Wmax = 342.0 ± 10.0 W. | C Beetroot 8 mmol/day (2 x 70ml) for 6 days | Randomised, Repeated-measures, crossover, double-blind | 60-min of submaximal cycling (2 x 30 min at 45% and 65% Wmax, respectively), followed by a 10-km time-trial. | Time-trial performance improved by 1.24% (BR: 953 ± 18 vs PL: 965 ± 18 s, p < 0.005). |               |
| Christensen et al.¹⁵   | 2013 | 29.0 ± 4.0      | Highly trained male cyclists. VO2max = 72.1 ± 4.5 | C Beetroot 8.06 mmol/day for 6 days | Randomised, crossover | VO2 kinetics (3 x 6 min at 2980 ± 28.0 W, endurance (120 min preload followed by a 400-kcal cycling time-trial), Three 10-km running tests. | No significant different in time-trial performance (BR: 1100 ± 163 vs PL: 1117 ± 167 s, p > 0.05). |               |
| de Castro et al.³⁸      | 2019 | 27.8 ± 3.4      | Male recreational runners. VO2max: 45.4 ± 5.5 | C Beetroot 8.4 mmol/day for 3 days | Randomised, Placebo-controlled, crossover, double-blind | Three 10-km running tests. | No significant difference in 10-km running time performance (BR: 50.1 ± 5.3 s vs. PL: 51.0 ± 5.1 min, p = 0.301). No significant difference in acute supplementation |               |
| Jo et al.²⁸            | 2019 | 23.4 ± 2.0 (C), 22.2 ± 2.5 (A) | Healthy, recreationally active men and women | A & C Nitrates supplement 8 mmol (A) and | Randomised, placebo controlled, double-blind, parallel design | 8 km simulated cycling time-trial | (continued on next page) |               |
There is evidence that dosage of nitrate ingested is an important component related to performance efficacy, with low doses of nitrate less effective. For this reason, we completed a secondary analysis examining the effect only of dosages >6 mmol per serving. Seventeen outcome effects were included (11 acute and 6 chronic). The standardised mean difference for time-trial including all dosages was 0.19, 95% CI 0.02 to 0.68, Z = 1.80, p = 0.07 (please see Supplementary File, Fig. S2). However, for the subgroup analysis there was a significant effect for studies employing chronic nitrate intervention (Hedges’g = 0.35, 95% CI -0.02 to 0.39, Z = 2.07, p = 0.04) but not acute (Hedges’g = 0.09, 95% CI -0.17 to 0.34, Z = 0.68, p = 0.50) ingestion of nitrate.

4. Discussion

Several previous meta-analyses have found a significant effect of nitrate supplementation on time to exhaustion and total distance travelled during endurance exercise but the effects of nitrate on endurance time-trial outcomes were less certain. Evidence from animal models and human studies suggests that nitric oxide production is facilitated in low pH and low oxygen environments. As exercise intensity and peripheral oxygen demand in maximal efforts is directly associated with the duration of any endurance time trials of between 5 and 30 min, a chronic nitrate supplementation strategy exhibited a significant improvement in favour of nitrate supplementation on endurance time trials of between 5 and 30 min in length. Conversely, in time trials of 30–60 min no significant improvement was noted (6 time-trial effects). When separated by the type of study in time trials between 5 and 30 min, a chronic nitrate supplementation strategy exhibited a significant effect on time-trial outcomes whereas no clear independent effect was observed with acute nitrate supplementation (Fig. 4). Chronic supplementation of nitrate is likely to elevate blood nitrate concentrations significantly based on previous evidence that excess nitrate and
Nitrite can be preserved and stored in the blood and tissue as a NO reservoir, ready to be reduced to bioactive NO under physiological hypoxia or low pH, thereby improving exercise performance. In contrast, a recent study by Kadach and colleagues challenges this idea as they did not find a statistically significant higher nitrate concentration in muscle than in the bloodstream following nitrate intake. However, interestingly, nitrite concentration was more elevated in the muscle than in the bloodstream potentially elevating NO availability to improve exercise performance. Several studies have reported that nitrate supplementation improves endurance performance by reducing the oxygen cost of exercise. However, moderate (1500 m) and intermediate
(10,000 m) length treadmill running time-trials have reported no reduction in oxygen consumption. Thus, it was suggested that the ergogenic effect of nitrate supplementation likely contributes to the improvement in performance over these shorter distances by other physiological mechanisms, as type II muscle fibres may be positively impacted by nitrate supplementation. This observation is aligned with a study in exercising rats that nitrate raises blood flow and oxygen delivery to type II muscle fibres, simultaneously decreasing muscle metabolic perturbations. In addition, high-intensity exercise performance may be enhanced via elevated type II muscle calcium ion handling and muscle contractile function. Based on these observations, and a report from a recent systematic review, we hypothesised that these physiological changes may lead to an improvement in time-trial performance over shorter distance events ranging from 5 to 30 min. Certainly, peripheral fatigue has been shown to be greater during shorter intensity self-paced time-trials, as evidenced by reduced potentiated twitch of knee extensors. Conversely, we did not observe any improvement in time-trials within a range of 30–60 min, and previous observation suggests that greater central fatigue is associated with these longer time-trials, as evidenced by greater reductions in voluntary activation measured by motor nerve and cortical stimulation. If these prior observations related to fatigue in association with length of time-trial performance are correct, and if nitrate supplementation does improve elements of muscle recruitment or contraction over shorter distances, then it provides a plausible mechanism for the hypothesis and findings of the present analysis — that nitrate is effective for shorter duration high-intensity endurance time trials.

In contrast, another study concluded that nitrate supplementation through beetroot supplementation improved 10 km cycling time-trial performance due to higher oxygen consumption and aerobic capacity. It was suggested that higher oxygen consumption might be via augmented vascular control and improved muscle blood flow redistribution with beetroot or nitrate supplementation. However, Rokkedal-Lausch and colleagues suggested that many previous time-trial studies did not use an optimised supplementation strategy, including variation in concentration/dosage and different nitrate sources (sodium nitrate, . However, they suggested that chronic supplementation seems to be more ergogenic with a nitrate dosage >8 mmol per day, which is aligned with the outcomes of the present meta-analysis. Furthermore, the higher supplementation dosage may be required for well-trained athletes due to inherent adaptations through intensive training. Another consideration here is that evidence suggests that low dose nitrate supplementation is less effective than higher doses. We performed a secondary analysis to test this, including only studies with nitrate supplementation >6 mmol. There was a borderline effect for all outcomes measures (acute and chronic) included (p = 0.07) and a significant effect for only chronic outcomes. Given the smaller number of observations we believe that the data are reassuring and supportive of our overall main analysis.

There are strengths and limitations to our analysis. The number of studies and effects (6 effects) included in the longer time-trials of 30–60 min was considerably less than those in the shorter time trials of 5–30 min (25 effects). Nonetheless, our main hypothesis that nitrate may serve to enhance performance in the shorter duration, higher intensity endurance time trials is still supported by our analysis and previous meta-analyses have collectively shown no effect when data across all time-trial studies are pooled. Secondly, our study analysed time-trial performances of 5–30 min in length based on a previous observation from a systematic review suggesting improved performance over this time with nitrate ingestion. As stated, a possible mechanism for why this occurs is that the ergogenic benefits of nitrate are more profound with lower pH and oxygen availability in shorter time trials where peripheral fatigue is an issue. However, it is important to note literature suggests that even within the 5–30 min time frame the metabolic and neuromuscular determinants of fatigue may differ. Thus, the precise time frame for improvements in performance with nitrate supplementation may be varying depending on factors such as training status of the individual and the event. Thirdly, the studies included in our analysis primarily employed trained/competitive or elite athletes. It is often difficult to recruit individuals of sufficient athletic calibre to research studies because of the impact on their regular training. However, they are needed to make worthwhile comparisons of reliable performance outcomes between the intervention and placebo without interference from simple training/learning effects. Thus, we believe that the present data are important to those involved in regular athletic training and competition. This is further supported by the fact that it is difficult to observe clear differences in many of the individual studies in our
analysis which are conducted in well-trained and elite individuals but probably undermined. Teasing out small improvements in athletic performance with nutritional supplementation is difficult and this probability is reduced further as an individual reaches their maximal training load and adaptive and genetic potential. Indeed a previous review examining athletic performance and nitrate supplementation found that out of 80 studies reviewed only 32% demonstrated significant performance improvement with nitrate supplementation compared with placebo. Thus, we believe that this meta-analysis provides a clearer direction on the effect of nitrate ingestion on high-intensity endurance time-trials.

5. Conclusion

Findings from the present systematic review and meta-analysis suggest that chronic nitrate supplementation improves time-trial performance (small, borderline significant effect) ranging from 5 to 30 min in duration but with no clear effect beyond this. Future research can evaluate the optimal supplementation approach applicable to high-intensity endurance exercise and examine in more detail the direct relationship between distance covered and the ergogenic effect of the supplementation.

Author contributions

Conceptualisation, T.H.W. and S.F.B.; methodology, T.H.W., A.S., S.F.B.; formal analysis, T.H.W. and A.S.; writing—original draft preparation, T.H.W.; writing—review and editing, T.H.W., A.S. and S.F.B. All authors have read and agreed to the published version of the manuscript.

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Institutional review board statement

Not applicable.

Informed consent statement

Not applicable.

Declaration of competing interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jesf.2022.06.004.

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