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

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ABSTRACT

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 SPORTDiscus. 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.

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[1] for their potential benefits to improve performance.[2–6] 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[7] – 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.[8–11] After Larsen and colleagues[12] 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 reviews[17] 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

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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).[20] 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.[16,18,19] 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.[16,18,19] Collectively these analyses suggest a clear extension of endurance capacity with dietary nitrate supplementation but the effect on endurance time-trial 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.[17] Several individual studies support the hypothesis that nitrates improve short duration time-trials over this duration[14,21–26] but this finding is not consistent.[15,27–35] A variety of interindividual factors (e.g. participant sex and fitness levels) and interstudy differences (e.g., exercise type and dosage and timing of nitrate supplementation) contribute to the variability in the study outcomes observed. Indeed, in one previous meta-analysis it was noted that only ~32% of 80 studies examining the effects of nitrate on athletic performance demonstrated significant improvement.[20] Thus, meta-analysis is required to draw clearer conclusions on the ergogenic effect of nitrate ingestion on performance.

Based on the present understanding of nitrate metabolism, some consideration should be given to the potential for improved endurance performance in time-trials of shorter duration. It is thought that nitric oxide production is facilitated in low pH and low oxygen environments which occur during higher intensity exercise. Certainly, in animal models nitrate supplementation can affect the contractile function and blood flow to type II muscle fibres,[9,36,37] which are recruited during high intensity work. Further support for an effect 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.[38] A separate meta-analysis has also shown improvements in muscle power with isolated sprints during cycling or isokinetic knee extensions[39] and it is suggested that nitrate and nitrite can be stored in muscle tissue, potentially contributing to these improvements in power observed.[11,40] 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.[41] 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 examples 1.5–5 km running and 10 km cycling in duration from 5 to 30 min) were considered.[17,41] 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)[42,43] and included the PICOS (population, intervention, comparison, outcome, study design) framework for data extraction.[44] 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[21–24,27,28,32,33,35,45–50] that were included in the selection process. These studies were identified through a high-intensity interval training systematic review[51] as the studies had reported time-trial as part of the performance outcomes, and four systematic reviews on time-trial or endurance performance.[16–19] 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

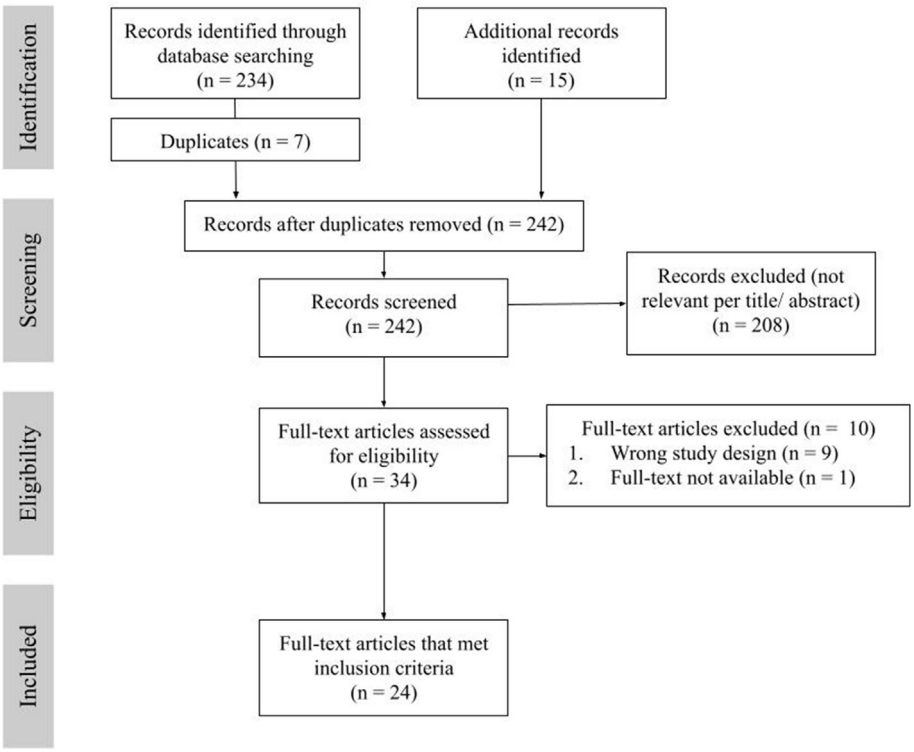

Fig. 1. Selection process based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) flowchart for time-trial performance.

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.[52] 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).[53] 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.[54,55] 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).[54] The 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%).[54]

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

Table 1
PEDro scores for the 24 included studies.

Study ID	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	Total
Arnold et al., 2015	x	x	x	x	x	x	x	x	x	x	x	**10**
Callahan et al., 2017	o	o	x	x	x	x	x	x	x	x	x	**9**
Casado et al., 2021	x	x	x	x	x	x	x	x	x	x	x	**10**
Cermak et al., 2012	o	x	x	o	x	x	x	x	x	x	x	**9**
Christensen et al., 2013	o	x	x	o	x	o	o	o	x	x	x	**6**
de Castro et al., 2019	x	x	x	x	x	x	x	x	x	x	x	**10**
Glaister et al., 2015	x	x	x	o	x	x	x	x	x	x	x	**9**
Hoon et al., 2014	x	x	x	x	o	o	o	x	x	x	x	**7**
Hurst et al., 2020	x	x	x	x	x	x	x	o	x	x	x	**9**
Jo et al., 2019	x	x	x	0	x	x	x	x	x	x	x	**9**
Kent et al., 2018	o	o	x	x	x	x	x	x	x	x	x	**9**
Kramer et al., 2016	x	x	x	x	x	x	x	x	x	x	x	**10**
Lansley et al., 2011	x	x	x	o	x	x	x	x	x	x	x	**9**
MacLeod et al., 2015	x	x	x	x	x	x	x	x	x	x	x	**10**
McQuillan et al., 2017	o	x	x	o	x	x	x	x	x	x	x	**9**
Muggeridge et al., 2014	x	x	x	x	x	x	x	x	x	x	x	**10**
Muggeridge et al., 2015	x	x	x	x	x	o	o	x	x	x	x	**8**
Murphy et al., 2012	x	x	x	o	x	x	o	x	x	x	x	**8**
Nybäck et al., 2017	o	x	x	o	x	x	x	o	x	x	x	**8**
Peacock et al., 2012	o	x	x	x	x	x	x	x	x	x	x	**10**
Peeling et al., 2015	x	o	o	x	x	x	x	x	x	x	x	**8**
Rokkedal-Lausch et al., 2019	x	x	x	o	x	x	x	x	x	x	x	**9**
Shannon et al., 2016	o	x	x	o	x	x	x	x	x	x	x	**9**
Shannon et al., 2017	o	x	x	o	x	x	x	x	x	x	x	**9**
Score for each point	**59%**	**86%**	**100%**	**55%**	**91%**	**91%**	**82%**	**82%**	**100%**	**100%**	**100%**	**8.9**

x: Criterion met; o: Criterian not met; P1: Eligibility criteria were specified; P2: Randomised allocation of subjects; P3: Allocation was concealed; P4: Groups were similar at baseline regarding most important indicators; P5: Blinding of subjects; P6: Blinding of therapists; P7: Blinding of assessors; P8: Measures of key outcome >85% participants; P9: Subjects received the allocated treatment; P10: Between-group stats comparisons reported for at least one key outcome; P11: Study provides both point measures and measures of variability.

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.[57] 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).

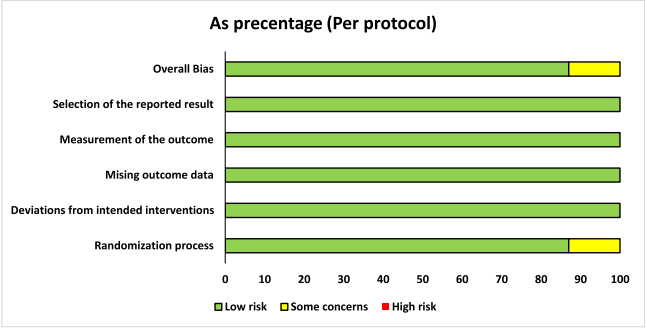

Fig. 2. Risk of bias chart for the 24 studies included in the review. Three studies (~12.5%) included were considered to have 'some concerns' related to the randomisation process and overall bias.

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.[24] 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 >1 day). One study applied both acute and chronic supplementation.[28] Nine studies reported more than one condition and outcome.[21,22,26,28,31,33,35,45,46] The multiple outcomes were included separately in the meta-analysis; with a total of 31 outcome measures in all (20 acute and 11 chronic supplementation).

3.3. Time-trial protocols

As stated in the inclusion criteria, the time-trial protocols were limited 5–60 min exercise (5–30 min and 30–60 min were analysed separately). 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.[14,15,25] 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

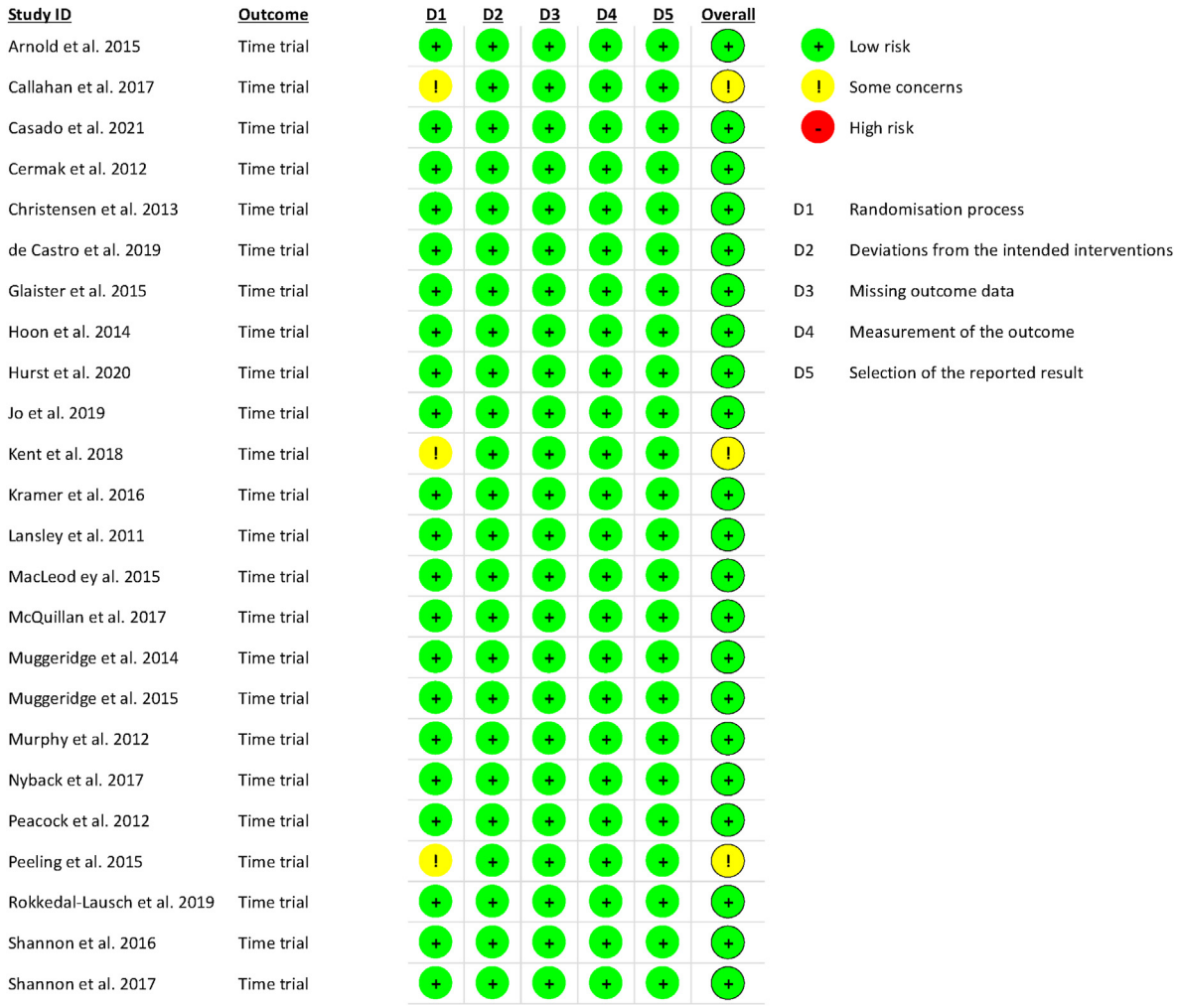

Fig. 3. Summary of the risk of bias over five domains for the 24 included studies.

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.[58] However, there was no statistical improvement in overall 10-km performance.[58] Cycling time-trials of different distances ranged between 4.0 and 20.0 km (4.0, 10.0, 16.1, 20.0 km),[14,22,23,26,28,29,32,33,46,49,50] and pre-defined total work were included.[15,46] 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),[21,25,30,34,45,48,56,58] whilst three studies used other exercise modalities, including skiing [31]and rowing.[27,35]

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 ($I^2 = 0\%$; $p = 1.00$). 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 ($I^2 = 14.6\%$, $P = 0.28$). 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, $VO_{2peak/max}$ (ml/kg/min)	A/C	Nitrate supplementation	Study design	Exercise protocol	Primary Outcome
Arnold et al.[48]	2015	10	37.0 ± 13.0	Well-trained competitive male runners. VO_{2max} = 66.0 ± 7.0	A	Beetroot 7 mmol (70 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 s, p = 0.6).
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	Improved time-trial performance Males (BR: 432.7 ± 52.9 vs. PL: 436.6 ± 52.7 s, p < 0.05), Females (BR: 575.1 ± 68.6 vs. PL: 580.7 ± 67.0 s, p < 0.05).
Glaister et al.[49]	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: 2122.2 ± 102 s, p > 0.05).
Hoon et al.[35]	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).
Hurst et al.[34]	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).
Lansley et al.[22]	2011	9	21.0 ± 4.0	Club-level competitive male cyclists. VO_{2peak} = 56.0 ± 5.7	A	Beetroot 6.2 mmol (500 ml)	Randomised, crossover, double-blind	4-km and 16.1-km cycling TT.	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).
Study	**Year**	**Sample size (n)**	**Age (years)**	**Exercise level and fitness,** $VO_{2peak/max}$ (ml/kg/min)	**A/C**	**Nitrate supplementation**	**Study design**	**Exercise protocol**	**Primary Outcome**
MacLeod et al.[33]	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 in time-trial performance Normoxia (BR: 961 ± 54 vs. PL: 954 ± 47 s, p > 0.05), Hypoxia (BR: 1018 ± 52 vs. PL: 1023 ± 49 s, p > 0.05).
Muggeridge et al.[23]	2014	9	28.0 ± 8.o	Male trained cyclists. VO_{2peak} = 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 (BR: 1664 ± 42 vs. PL: 1702 ± 45 s, p = 0.021).
Muggeridge et al.[32]	2015	9	36.0 ± 6.0	Nine male trained-cyclists and triathletes. VO_{2max} = 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	No significant improvement in time-time under sham light (BR: 1455 ± 47 vs. PL: 1469 ± 52 s).
Murphy et al.[56]	2012	11	25.0 ± 4.0	Recreationally fit men (n = 5) and women (n = 6). 5 ± 1 days/week of moderate to vigorous-intensity exercise.	A	Baked beetroot (200g with ≥500 mg nitrate)	Randomised, Placebo controlled, crossover, double-blind	2 x 5-km treadmill running time-trials in random sequence.	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).
Nybäck et al.[31]	2017	8	21.8 ± 2.8 (M), 20.7 ± 1.2 (F)	Competitive cross-country skiers. 5 male VO_{2max} = 71.5 ± 4.7, 3 female VO_{2max} = 58.4 ± 2.5	A	Beetroot 13 mmol	Randomised, counter-balanced, double blind	Performed 2 x 6-min submaximal exercise bouts and a 1000-m skiing TT on a	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, $VO_{2peak/max}$ (ml/kg/min)	A/C	Nitrate supplementation	Study design	Exercise protocol	Primary Outcome
								treadmill in N (20.9% O_2) or H (16.8% O2).	295 ± 29 s, p = 0.216, and H (BR: 305 ± 28 vs. PL: 301 ± 28 s, p = 0.358).
Study	**Year**	**Sample size (*n*)**	**Age (years)**	**Exercise level and fitness,** $VO_{2peak/max}$ (ml/kg/min)	**A/C**	**Nitrate supplementation**	**Study design**	**Exercise protocol**	**Primary Outcome**
Peacock et al.[30]	2012	10	18 years old	Male junior elite cross-country Skiers. VO_{2max} = 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	5	25.0 ± 2.8	International-level female kayakers. VO_{2peak} = 47.8 ± 3.7	A	Beetroot 9.9 mmol (2 × 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 < 0.05).
Shannon et al.[25]	2016	12	24.4 ± 4.3	Six competitive male runners/triathletes, four recreational and two physically active. VO_{2max} 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 (FIO2 ~ 15%).	BR improved TT performance in all 12 participants by an average of 3.2% (BR: 331.1 ± 45.3 vs. PL: 341.9 ± 46.1 s, p < 0.001).
Shannon et al.[45]	2017	8	28.3 ± 5.8	Trained male runners or triathletes. VO_{2max} = 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 treadmill running TT.	Performance in the 1500 m TT was significantly faster in BR vs. PL (BR: 319.6 ± 36.2 vs. 325.7 ± 38.8 s, p < 0.05), but was no significant difference in 10,000 m TT performance (BR: 2643.1 ± 324.1 vs. PL: 2649.9 ± 319.8 s, p > 0.05).
Study	**Year**	**Sample size (*n*)**	**Age (years)**	**Exercise level and fitness,** $VO_{2peak/max}$ (ml/kg/min)	**A/C**	**Nitrate supplementation**	**Study design**	**Exercise protocol**	**Primary Outcome**
Callahan et al.[29]	2017	8	34.0 ± 7.0	Well-trained male cyclists. VO_{2max} = 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 in 4-km time trial performance (BR: 337.4 ± 17.1 vs PL 338.1 ± 18 s, p > 0.05).
Cermak et al.[14]	2012	12	31.0 ± 3.0	Male cyclists engaged in regular cycling training (10 h/week) and had a training history of ~10 years. VO_{2peak} = 58.0 ± 2.0, [Wmax] = 342.0 ± 10.0 W.	C	Beetroot 8 mmol/day (2 × 70ml) for 6 days	Randomised, Repeated-measures, crossover, double-blind	60-min of submaximal cycling (2 × 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	8	29.0 ± 4.0	Highly trained male cyclists. VO_{2max} = 72.1 ± 4.5	C	Beetroot 8.06 mmol/day for 6 days	Randomised, crossover	VO_2 kinetics (3 × 6 min at 298.0 ± 28.0 W), endurance (120 min preload followed by a 400-kcal cycling time-trial).	No significant different in time-trial performance (BR: 1100 ± 163 vs. PL: 1117 ± 167 s, p > 0.05).
de Castro et al.[58]	2019	14	27.8 ± 3.4	Male recreational runners. $VO_{2max=}$45.4 ± 5.9	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 vs. PL: 51.0 ± 5.1 min, p = 0.391).
Jo et al.[28]	2019	15 M, 14 F	23.4 ± 2.0 (C), 22.2 ± 2.5 (A)	Healthy, recreationally active men and women	A & C	Nitrate supplement 8 mmol (A) and	Randomised, placebo controlled, double-blind, parallel design	8 km simulated cycling time-trial	No significant difference in acute supplementation

(*continued on next page*)

Table 2 (*continued*)

Study	Year	Sample size (*n*)	Age (years)	Exercise level and fitness, $VO_{2peak/max}$ (ml/kg/min)	A/C	Nitrate supplementation	Study design	Exercise protocol	Primary Outcome
						8 mmol/day for 15 days (C).			(BR:1050 ± 144 vs. PL: 1074 ± 168 s, p > 0.05). Chronic supplementation improved time-trial performance significantly (BR: 1014 ± 96 vs. PL: 1074 ± 102, p < 0.05).
Study	**Year**	**Sample size (*n*)**	**Age (years)**	**Exercise level and fitness,** $VO_{2peak/max}$ (ml/kg/min)	**A/C**	**Nitrate supplementation**	**Study design**	**Exercise protocol**	**Primary Outcome**
Kent et al.[46]	2018	12	26.6 ± 4.4	Male endurance-trained cyclists. VO_{2peak} 65.8 ± 5.5	C	Beetroot (6.5 mmol for 2 days and 13 mmol on the final day)	Repeated-measures, double-blind	Cycling time-trial (14 kJ/kg) in hot (35 °C, 48% relative humidity) and euthermic (21 °C, 52%) conditions.	BR supplementation has no significant effect on cycling TT performance. Euthermic (BR: 53:09 ± 04:35 vs. PL: 54:01 ± 04:05 min, p = 0.380), Hot (BR: 56:50 ± 05:08 vs. PL: 58:30 ± 04:48 min, p = 0.178).
Kramer et al.[27]	2016	12	23.0 ± 5.0	Male CrossFit athletes, VO_{2peak} 48.5 ± 7.0	C	Potassium nitrate 8 mmol/day for 6 days	Randomised, double-blind, crossover design	2-km rowing time trial	No significant difference in time-trial performance (BR: 459.73 ± 23.93 vs. PL: 459.87 ± 24.85, p > 0.05).
McQuillan et al.[50]	2017	8	26.0 ± 8.0	Well-trained male cyclists. V_{O2peak} = 63.0 ± 4.0	C	Beetroot 4 mmol/day (70 ml) for 8 days	Randomised, Placebo-controlled, crossover, double-blind	4-km cycling time-trial	Time-trial performance likely beneficial with BR (BR: 343.6 ± 14.3 vs. PL: 344.8 ± 14 s).
Rokkedal-Lausch et al.[26]	2019	12	29.1 ± 7.7	Well trained male cyclists. VO_{2max} = 66.4 ± 5.3	C	Beetroot 12.4 mmol/day (140 ml) for 7 days	Randomised, counter balanced-crossover, double blind	10-km cycling TT performance N and H.	Chronic BR supplementation improves 10-km TT performance in both Normoxia (PL: 890.1 ± 16 vs. BR: 884.5 ± 16 s, p = 0.024) and, Hypoxia (PL: 945.6 ± 16 vs. BR: 939.5 ± 16 s, p = 0.001).

A: acute; C: chronic; s: seconds; min: minutes; SIE/SIT: sprint interval exercise; m: meter; M: males; F: female; TTE: time to exhaustion; Wmax: peak power; WR: work rate; TT: Time-trial; BR: Nitrate/beetroot; PL: placebo; N: normoxia; H: normobaric hypoxia; ES: Cohen's effect sizes; All data are mean ± standard deviation.

There is evidence that dosage of nitrate ingested is an important component related to performance efficacy, with low doses of nitrate less effective.[20] 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 studies did not reach significance in favour of nitrate intervention (Hedges'g = 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 (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[16,18,20] have found a significant effect of nitrate supplementation on time to exhaustion tests 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.[9] As exercise intensity and peripheral oxygen demand in maximal efforts is directly associated with the duration of any event, we hypothesised that nitrate supplementation may be effective in shorter, high-intensity endurance time-trial events ranging from 5 to 30 min in duration. The 19 studies (25 time-trial effects) included in the present meta-analysis showed a borderline 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

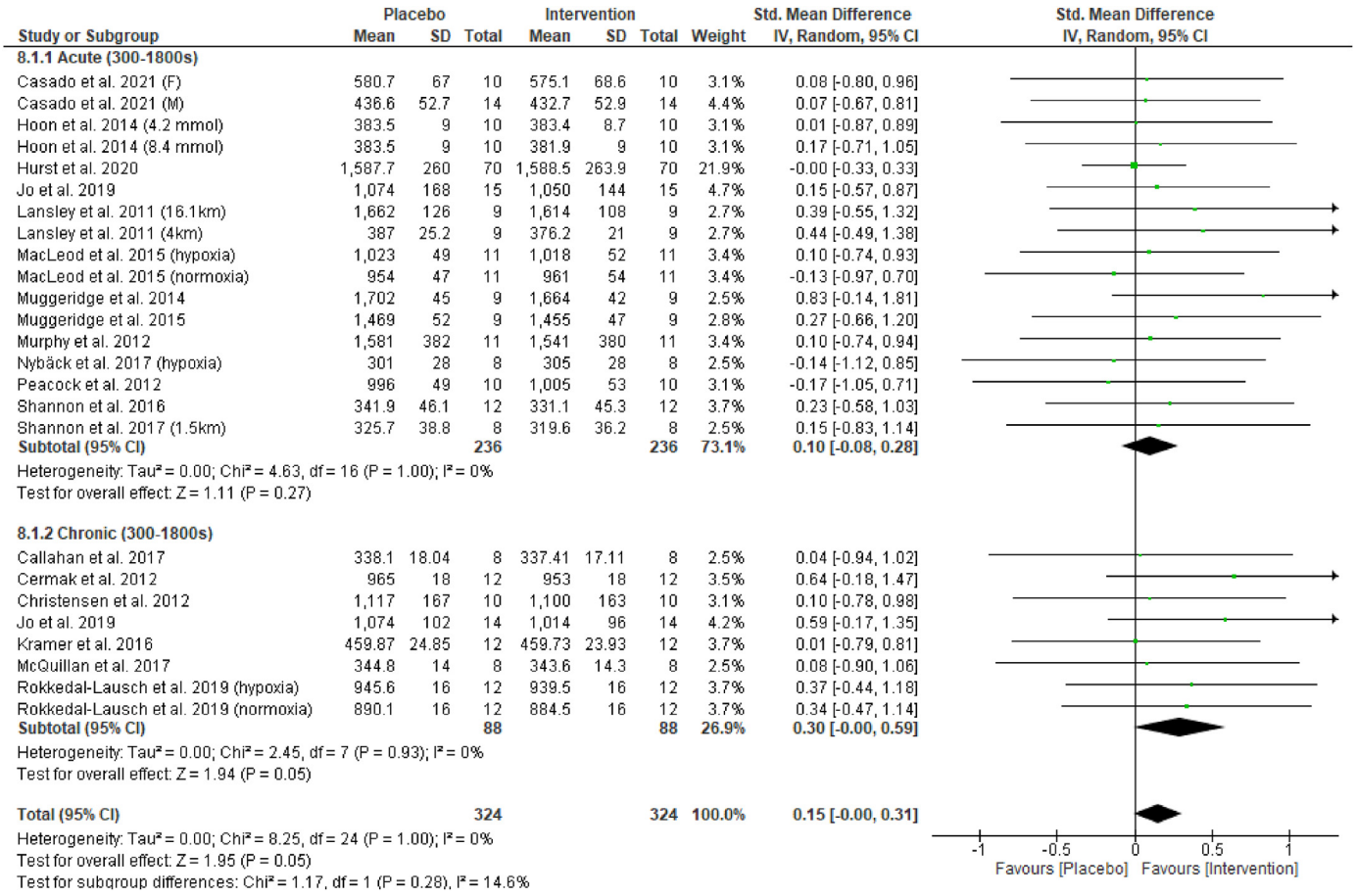

Fig. 4. Forest plot showing effects of acute and chronic nitrate supplementation on time-trial performance ranging from 5 to 30 min.

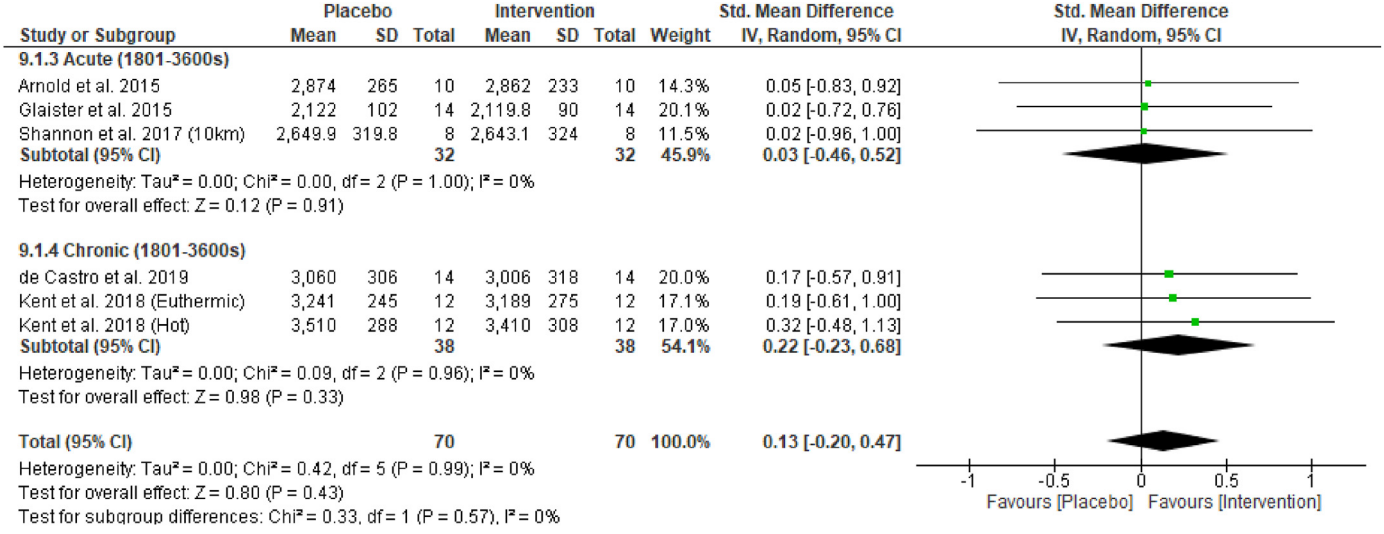

Fig. 5. Forest plot showing effects of acute and chronic nitrate supplementation on time-trial performance between 30 and 60 min.

nitrite can be preserved and stored in the blood and tissue as a NO reservoir,[9] 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.[40] However, interestingly, nitrite concentration was more elevated in the muscle than in the bloodstream[40] potentially elevating NO availability to improve exercise performance.[41]

Several studies have reported that nitrate supplementation improves endurance performance by reducing the oxygen cost of exercise.[3,12,13,59] However, moderate (1500 m) and intermediate

(10,000 m) length treadmill running time-trials have reported no reduction in oxygen consumption.[45] 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.[45] 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.[37] In addition, high-intensity exercise performance may be enhanced via elevated type II muscle calcium ion handling and muscle contractile function.[60] Based on these observations, and a report from a recent systematic review,[17] 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.[41] 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.[41] 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.[26] It was suggested that higher oxygen consumption might be via augmented vascular control and improved muscle blood flow redistribution with beetroot or nitrate supplementation.[26,61] However, Rokkedal-Lausch and colleagues further suggested that the discrepancy could be contributed by the training level of the participants where the study included well-trained athletes.[26] Not all studies have shown an ergogenic effect when trained athletes performed 10–15 km cycling, 10 km running and 10 km roller-skiing time-trials in hypoxia after beetroot supplementation.[31,33,48,61,62] However, this finding is not consistent and at least two studies have found a positive effect on 1.5 km running and 16.1 km cycling performance.[23,25] Jones and colleagues further explained that the discrepancy could be caused by methodological limitations, relatively smaller sample size and lack of sport-specific tests in studies of elite or well-trained athletes. Moreover, only subtle improvements in performance may be expected in studies of elite athletes who may already have high levels of blood nitrate or nitrite concentrations from training induced nitric oxide synthase upregulation which catalyses endogenous nitric oxide production through the conversion of L-arginine to L-citrulline.[9] One criticism here is that the definition of elite, well-trained or recreational within the published papers analysed is dependent on a competitive level which is not uniform across countries where the studies are conducted.

As noted, several previous meta-analyses have found no effect of nitrate supplementation on time-trial performance.[16,18,19] The one exception here was a meta-analysis by Senefeld and colleagues of 52 time-trial outcomes but this included single sprint events (e.g., 500 m kayaking) which are less than 300 s in length.[20] The finding of the present study adds to these previous analyses by examining a specific time-period of endurance exercise from 5 to 30 min. Nonetheless, as with previous analyses, many of the individual studies included showed non-significant effects. A previously stated a variety of interindividual factors and interstudy differences contribute to the variability in individual study outcomes. For example, an examination of Table 2 reveals multiple modes of exercise (running, track running, cycling, rowing ergometry, kayaking) and different conditions (euthermic, hot, hypoxic) have been employed across the available studies, along with the different training levels already mentioned. These factors are in addition to the variability in nitrate dosage (please see next paragraph). Thus, our findings are important because they suggest that one of the important factors for consideration in supplementation is duration of the sporting event, irrespective of the mode of exercise.

In this review, the acute nitrate dosage ranged from 4.1 mmol to 15.2 mmol per serving. The chronic dosage ranged from 4.0 mmol to 13 mmol per day over three to 15 days of supplementation. A clearer effect was seen in our meta-analysis for chronic supplementation on performance compared to acute supplementation over the shorter time period of 5–30 min. 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).[7,26] However, they suggested that chronic supplementation seems to be more ergogenic with a nitrate dosage >8 mmol per day,[26] 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.[9,26] 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.[16,18,19] Secondly, our study analysed time-trial performances of 5–30 min in length based on a previous observation from a systematic review[17] 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.[63] Thus, the precise time frame for improvements in performance with nitrate supplementation may be vary 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 underpowered. 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.[64] 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.[20] . 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

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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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