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The Effect of Dietary Nitrate on the Contractile Properties of Human Skeletal Muscle: A Systematic Review and Meta-Analysis

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ABSTRACT

The propose of this study was to systematically review the current literature and meta-analyse the effects of dietary nitrate (NO_3^-) supplementation on the contractile properties of skeletal muscle. A literature search of three databases was conducted in June 2021, with 19 studies meeting the inclusion criteria. Studies were included if a placebo versus dietary NO_3^--only supplementation protocol was used in healthy human, assessed muscle contraction or activities that was < 3 minutes in duration and focused on the lower-body. For the meta-analysis, a pooled standardised mean difference (SMD) was determined for maximum voluntary contraction (MVC) ($n = 11$), cycling, running and inertial load squad peak power output (PPO) ($n = 8$), mean power output (MPO) ($n = 6$) and time to PPO ($n = 4$). NO_3^- supplementation demonstrated a small improvement in PPO (SMD = 0.25, $P = 0.030$) and MPO (SMD = 0.28, $P = 0.030$) when compared to the placebo. NO_3^- also resulted in an enhanced time to PPO (SMD = -0.78, $P < 0.001$). There was no clear effect of NO_3^- on isometric MVC (SMD = 0.03, $P = 0.758$). This review reports that NO_3^- supplementation may have potential to enhance PPO, MPO and time to PPO during dynamic exercise, which may transfer to brief explosive actions commonly observed in sporting activities. Due to the variability in studies, we encourage researchers to use this work to explore areas where evidence in lacking and standardize the study design and procedures.

KEY TEACHING POINTS

- Findings from this meta-analysis highlight the potential positive ergogenic effect of dietary NO3-supplementation on PPO, MPO and time to PPO during short duration (<10 s) dynamic exercise.
- NO3- supplementation might be considered as an ergogenic aid when executing power-based actions (e.g., 100 m sprinter or weightlifter).
- This review highlights that further research is required to address some of the contrasting findings presented here using a standardised procedure to allow for improved synthesis.

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Sports nutrition; supplements; functional foods; muscle performance; ergogenic aid

Introduction

Nitric oxide (NO) is a gaseous signaling molecule produced mainly through the oxidation of semi-essential amino acid, L-arginine, by NO synthase and through reducing nitrite (NO_2^-) to nitrate (NO_3^-). NO production can also occur through the reduction of NO_3^- and NO_2^- via anaerobic bacteria that populate the oral cavity (1). The consumption of NO_3^- rich food, such as green leafy vegetables and beetroot, can increase NO synthesis via NO_3^-- NO_2^--NO pathway (1), and this pathway is suggested to be particularly effective under hypoxic and acidic conditions such as that observed in the skeletal muscle during a contraction (2).

The effect of NO_3^- supplementation, most commonly in the form of beetroot juice (BRJ), has been reported to be performance enhancing in low- and moderately-trained participants (3), but not highly endurance-trained participants (4). One explanation for this difference may be due to variances in the muscle fiber type composition between these groups (4). Recent evidence has demonstrated that acute and chronic NO_3^- supplementation improves muscle contractile force and rate of force/torque development (RFD/RTD) during isokinetic knee extension (5). Using a rat model, improvements in contractile force and RFD have been found in predominately fast-twitch but not slow-twitch muscles (6). Whilst such findings might be explained by an increase in NO_3^- concentration

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in the muscle through protein-mediated transport sialin (7), the effect of NO_3^- supplementation on a range of muscle contraction types (i.e., isometric and isokinetic) has been independently investigated. It has been hypothesized that improvements may be associated with increased Ca^{2+} release or sensitivity in fast-twitch fibers by increased NO bioavailability (5). An increased in NO concentration through consumption of dietary NO_3^- would allow Ca^{2+} channels (ryanodine receptors) of the sarcoplasmic reticulum (SR) to remain in an open state, particularly under hypoxic conditions, thus may serves to improve to muscle contractility during exercise (8–10).

Research on the effects of NO_3^- supplementation on muscle performance is building, with findings offering some mechanistic insight, though these are largely limited to isolated studies of small samples that has used a range of exercises, and single or multiple muscle group exercises. Furthermore, there appears to be a degree of variability in the results across studies largely due to the participants training status, sex and muscle fiber composition. Differences also exists in the exercise type (i.e. single- or multi-joint, isometric or isokinetic, whole-body repeated sprits), fatiguing protocols and dosing strategy. Therefore, drawing firm conclusions from the individual studies is difficult. However, given that the potential effect of NO_3^- supplementation on muscle contractile properties, such as force, velocity and power (11, 12), is directly relevant to exercise and sport performance, a systematic review and meta-analysis of this research will support researchers, athletes and nutritionists using NO_3^- supplementation. For example, this review will seek to answer 1). Does NO_3^- improve force, power or velocity of muscle contraction? 2). What supplement strategies and testing protocols are commonly used? 3). What is the quality of evidence that currently exists? Therefore, the aim of this study was to systematically review the current literature on the effect of dietary NO_3^- supplementation on muscle contractility and apply meta-analysis techniques where appropriate.

Methods

We conducted and reported this systematic review in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (13).

Search strategy

The online databases Pubmed, Google Scholar and Web of Science were used to conduct the search, which was conducted on the 30th June 2021 by two independent researchers (OE and ND). Results were limited to peer-review research published after 2007 as this was the earliest known work based on exercise and performance. The following key words and Medical Subject Headings (MeSH) were used to obtain relevant articles: dietary nitrate (MeSH) OR beetroot (All fields) AND exercise OR muscle (All fields) contract* (All fields) OR power (All Fields) OR force (All Fields) OR torque (All Fields) OR strength (All fields). To ensure the search was up to date, the same two authors (OE and ND) subsequently reviewed the references lists of those articles included as well as searched the 'in-press' sections of relevant journals.

Inclusion and exclusion criteria

The inclusion criteria that were applied within this study focused on four key areas; the paper characteristics, the study design and participants, the supplement used, and muscle contraction type. Research was limited to primary research published in peer-reviewed journals and written in English. Only those study that used a double-bind, randomized, placebo-controlled, crossover design were included. Studies were limited to healthy human participants aged over 16 years. Supplement included inorganic dietary NO_3^- and those with multiple sources of NO_3^-. The review included any type of muscle contraction and those using acute or chronic supplementation strategies. Studies excluded included those that focused on the upper-body musculature, recovery and/or medium- to long-duration exercise performance (>3 minutes), those using clinical populations with one or more non-communicable disease and those that did not include a measurement of muscle properties (i.e. maximal voluntary contraction (MVC), maximal voluntary torque, power, force or RFD/RTD).

Study selection

After removing duplicates using Endnote (X8, Thomson Reuters, Philadelphia, USA), articles were initially screened based on the title and abstract independently by two authors (OE and ND). For those deemed potentially suitable, the full paper was retrieved and screened against the inclusion criteria. Papers deemed not suitable at this stage were removed with justification provided by each author. Where any uncertainly was apparent, this was resolved by discussion between the same two authors. An overview of this process is provided in Figure 1.

Data extraction

The lead author (OE) extracted all information from the relevant articles using a standardized form, which was cross-checked by another author (ND). The information extracted included the sample size, participant characteristics (sex, age, stature, body mass), the supplement dose and strategy, the testing protocol, and the mean and SD for each outcome. If data were presented in graphical form with no mean and SD available, this was requested from the authors. If there was no response, these data were extracted from the figure using digitizer software (Engauge Digitizer 12.1, Digitizer.sf.net).

Quality assessment

All studies were assessed for quality using the Physiotherapy Evidence Database (PEDro) scale (14). Each article was assessed independently by two authors (OE and ND), with

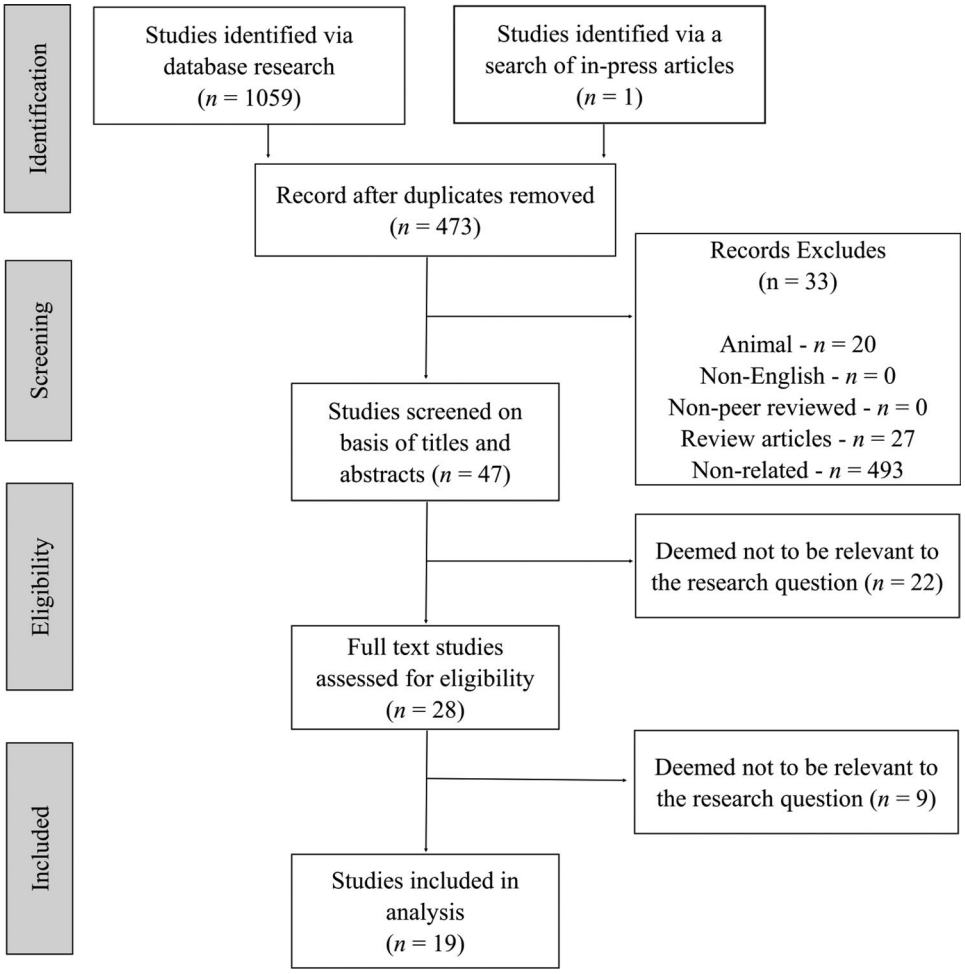

Figure 1. Flow diagram of the search strategy.

each article scored out of a maximum of 10, and the agreement between assessors determined using a kappa statistic ($k \geq 0.94$; substantial agreement). Differences of opinion were discussed until a resolution was found. Based on the total score, articles were categorized as poor (≤3), fair (4–5), and high (6–10) quality, with those considered 'poor 'omitted.

Statistical analysis

Data synthesis initially took form of a descriptive analysis of the results, with a detailed summary of each study provided. For the meta-analysis, the standardized mean difference (SMD, Hedges g) was determined for each key dependent outcome variable, including maximum voluntary contraction (MVC) ($n = 11$), cycling and running peak power output (PPO) ($n = 8$), mean power output (MPO) ($n = 6$) and time to PPO ($n = 4$). Variables such as RFD and fatigue measures were omitted from the meta-analysis due to the limited number of studies and high degree of heterogeneity. The presence of statistical heterogeneity was determined by the I^2 statistic and Chi-square Cochran's Q statistic (15). I^2 values of 25%, 50%, and 75% represented low, medium, and high heterogeneity (X^2 test, $P < 0.05$, or I^2 C = 50%), respectively (16) and a *P value* from the Q statistic of ≤ 0.10 considered to display significant heterogeneity (17). A random effects model was constructed for the meta-analysis to account for the potential variability in several experimental factors such as test type, dosing strategy and experimental conditions. SMD across studies was calculated and interpreted using the following descriptors: <0.20, trivial; 0.21-0.60, small, 0.61-1.2, moderate, 1.2-2.0, large; > 2.0, very large (18). Analysis was performed using R Studio with metafor package.

Results

Study characteristics and quality

Nineteen studies were included and used a randomized, double-blind, crossover study design. The placebo used varied across studies (Table 1), although 14 of the 19 studies used NO_3^--depleted beetroot juice (BRJ). One study used placebo bar, one powdered beetroot, two used blackcurrant cordial, and another used modified beverage which was isocaloric and isonitrogenous. Blinding of assessors and participants was, for the most part, achieved through use of drink bottles that were identical in size, brand, material and color. No information was provided for placebo bar and therefore information on blinding was unknown.

Table 1. Summary of studies examining the effects of dietary NO_3^- on human skeletal muscle contractile properties.

	Reference	Participants	Supplementation protocol	Placebo	Testing protocol	NO indices	Findings
1	Fulford et al. (19)	Healthy young men ($n=8$)	10.2 mmol·d^{-1} of NO_3^- 2.5 h before testing or daily for 5 or 15 d including 2.5 h before testing	Depleted BRJ	Isometric knee extension	↑Plasma NO_2^-	↔ MVC ↔FI
2	Haider & Folland (25)	Healthy young men ($n=19$)	9.7 mmol·d^{-1} of NO_3^- for 7 d including 2.5 h before testing	Black-currant juice cordial	Isometric knee extension (voluntary and electrically stimulated)	NM	↔ MVC ↑ Peak F_{tw} ↑ F_{tw} at 10 Hz ↔ F_{tw} at 100 Hz ↑ RFD during electrical simulation
3	Kokkinoplitis & Chester (30)	Healthy men ($n=7$)	4.2 mmol·d^{-1} of NO_3^- 2.5 h before testing	Black-currant juice cordial	Isokinetic knee extension and repeated sprints (5 × 6 s)	NM	↔ F at 1.05 and 4.19 rad·s^{-1} ↔ PPO in repeated sprints
4	Coggan et al. (31)	Healthy young and middle-aged men ($n=7$) and women ($n=5$)	11.2 mmol·d^{-1} of NO_3^- 3 h before testing	Depleted BRJ	Isometric and isokinetic knee extension	↑Breath NO	↔ MVC, ↔ F at 1.67, 3.14, and 4.71 rad·s^{-1}, ↑ F at 6.28 rad·s^{-1}, ↑ P_{max}, ↑ V_{max} ↔FI
5	Hoon et al. (29)	Healthy young men ($n=12$ and women ($n=6$)	8.8 mmol·d^{-1} of NO_3^- for 3 d plus 17.6 mmol NO_3^- 2–4 h before testing	Depleted BRJ	Isometric knee extension (voluntary and electrically stimulated)	NM	↔ MVC ↔ Peak F_{tw} ↔ F_{tw} at 10–100 Hz ↔ RFD during electrical simulation ↑ initial F in fatigued condition
6	Flanagan et al. (32)	Resistance-trained men ($n=14$)	0.5 mmol·d^{-1} of NO_3^- for 3 d	Placebo bar	Isometric box squat EMG	NM	↑Mean MVC peak EMG amplitude ↔ Number of Repetitions
7	Rimer et al. (26)	Collegiate team sport and endurance athletes (males $n=13$); females $n=2$)	11.2 mmol·d^{-1} of NO_3^- 2.5–3 h before testing	Depleted BRJ	Inertial load and cycling	NM	↑ PPO in repeated sprints, ↔ PPO in 30 s cycling ↑ RPM_{opt} ↔FI
8	Domínguez et al. (20)	Healthy young men ($n=15$)	5.6 mmol·d^{-1} of NO_3^- 3 h before testing	Powdered BR	Wingate test	NM	↑ PPO, ↔MPO ↑MPO at 0-15 s ↓ time to PPO ↔FI
9	Bender et al. (27)	Active adolescent males ($n=12$)	12.9 mmol·d^{-1} of NO_3^- 2.5 h before testing	Depleted BRJ	Repeated (n = 4) Wingate test	NM	↔PPO ↔MPO ↔FI
10	Tillin et al. (34)	Healthy active males ($n=17$)	12.9 mmol NO_3^- for 7 days	Depleted BRJ	Isometric knee extension (voluntary and electrically stimulated)	↑Plasma NO_3^- ↑Plasma NO_2^-	Unfatigued condition, ↔MVC ↔F_{tw} at 10–50 Hz ↔RFD during electrical simulation ↔FI Fatigued condition, ↔MVC ↑F_{tw} at 20;50 Hz ↑ RFD during electrical simulation
11	Coggan et al. (33)	Healthy men ($n=13$) and women ($n=7$)	11.2 mmol·d^{-1} of NO_3^- 2.5 h before testing	Depleted BRJ	Isometric and isokinetic knee extension	↑Plasma NO_3^- ↑Plasma NO_2^-	↑ P_{max}, ↑ V_{max} ↔FI
12	Jonvik et al. (21)	Recreational ($n=20$), competitive ($n=22$), and elite ($n=10$) male ($n=29$) and female ($n=23$) athletes	12.9 mmol·d^{-1} of NO_3^- 7 d including 3 h before testing	Depleted BRJ	Repeated (n = 3) Wingate tests	↑Plasma NO_3^- ↑Plasma NO_2^-	↔PPO, ↔MPO ↓ time to PPO
13	Cuenca et al. (22)	Healthy resistance-trained men ($n=15$)	6.4 mmol·d^{-1} of NO_3^- 3 h before testing	Depleted BRJ	Wingate test	NM	↔ PPO ↔MPO ↑MPO at 0-15 s ↓ time to PPO ↔FI

Table 1. (Continued)

	Reference	Participants	Supplementation protocol	Placebo	Testing protocol	NO indices	Findings
14	Lee et al. (28)	Recreationally active female ($n=9$) and males ($n=26$)	4 mmol of NO_3^- 12 h before testing And 4 mmol of NO_3^- 3 h before testing	Modified beverage	Isokinetic knee extension	NM	↔ MVC ↔ rate of muscle fatigue
15	Husmann et al. (23)	Recreationally active males ($n=12$)	6.5 mmol·d^{-1} of NO_3^- for 5 d	Depleted BRJ	Single leg isokinetic knee extension	NM	↓ΔMVC ↓ΔF_{tw100}
16	Coggan et al. (35)	Healthy (nondiabetic) elderly men ($n=6$) and women ($n=6$)	13.4 mmol·d^{-1} of NO_3^- 3 h before testing	Depleted BRJ	Isometric and isokinetic knee extension	↑Breath NO ↑Plasma NO_3^- ↑Plasma NO_2^-	↑ P_{max}, ↑ V_{max} ↔ FI ↑ F at 6.28 rad·s−1,
17	Jodra et al. (24)	Resistance trained male ($n=15$)	6.4 mmol·d^{-1} of NO_3^- 3 h before testing	Depleted BRJ	Wingate test	NM	↔PPO ↔MPO ↓ time to PPO
18	Rodríguez-Fernández et al. (36)	Active males (n = 18)	12.9 mmol NO_3^- 2.5 h before testing	Depleted BRJ	Inertial load squats	NM	↑PPO ↑MPO
19	Jonvik et al. (37)	Recreationally active males ($n=15$)	12.9 mmol·d^{-1} of NO_3^- 6 d including 3 h before testing	Depleted BRJ	Isokinetic knee extension	↑Plasma NO_3^- ↑Plasma NO_2^-	↔ MVC, ↔ P_{max} ↔ FI

↔, unchanged;↑, significantly increased;↓, significantly decreased; *ΔMVC*, the % change in MVC from pre- to post-exercise; ΔF_{tw100}, the %changes in F_{tw} at 100 Hz from pre- to post-exercise; *EMG*, electromyography; *F*, force; *FI*, fatigue index; F_{tw}, twitch force; *MPO*, mean power output during cycling; *MVC*, maximal voluntary contraction; *NM*, not measured; NO_2^-, nitrite; NO_3^-, nitrate; P_{max}, maximal power during knee extension; *PPO*, peak power output during cycling; *RFD*, rate of force development; RPM_{opt}, pedaling cadence resulting in PPO; V_{max}, maximal velocity of knee extension.

Randomization

Six studies reported that participants were randomly allocated by using counter-balanced fashion (19–24). Four studies also reported that an independent researcher who did not participate in data collection allocated the participants to the supplements (25–28), while one other used a digital sequence for randomization (29). Eight studies did not describe their randomization protocol (30–37).

Quality assessment

The studies included in this systemic review were high quality with many scoring 10/10. All studies were placebo controlled, randomized crossover experiments with low selection/detection bias, double-blinded, and with low performance bias (Supplementary material). However, we highlight that whilst all studies used a randomized approach, information on how this was achieved was not disclosed in eight studies.

Participant characteristics

Participants' characteristics are summarized in Table 1. The total sample of participants across all 19 studies was 371 (313 men, 58 women). Seven studies used recreational, competitive and elite team-sport, resistance or endurance athletes, eleven studies included recreationally active participants, and one study included healthy, minimally active, elderly participants. Participants' age ranged between 16 to 71 years. Twelve studies included only men participants while seven included men and women.

NO_3^- supplementation

Eighteen studies used inorganic BRJ and one used NO_3^--rich bar as NO_3^- supplementation with doses varying between 5.6 mmol·d^{-1} and 17.6 mmol·d^{-1}. The supplementation period used included acute supplementation (2.5–3 h before, $n=11$), a 3-day period ($n=1$), a 4-day period ($n=1$), a 5-day period ($n=1$), and a 6-day period ($n=1$). Three studies used a 7-day period. One study investigated the effects of acute (2.5 h before), long term (5 days) and chronic (15 days) supplementation.

Characteristics of nitric oxide measures

Eight studies used various methods to analyze changes in NO_3^- or NO_2^- after dietary NO_3^- supplementation, while all others did not (Table 1).

Outcomes

Nitric oxide indices

All studies ($n=8$) that analyzed NO indices following dietary NO_3^- supplementation observed 65% to 1180% greater NO_2^- compared to placebo.

Isometric exercise

Force at maximum voluntary contraction

Eight independent studies analyzed the effect of NO_3^- supplementation on peak force during MVC, whilst one only explored the muscle activity during an MVC. The study by Fulford et al. (19) analyzed MVC after acute, short and chronic supplementation period and was therefore included as independent observations. In that study,

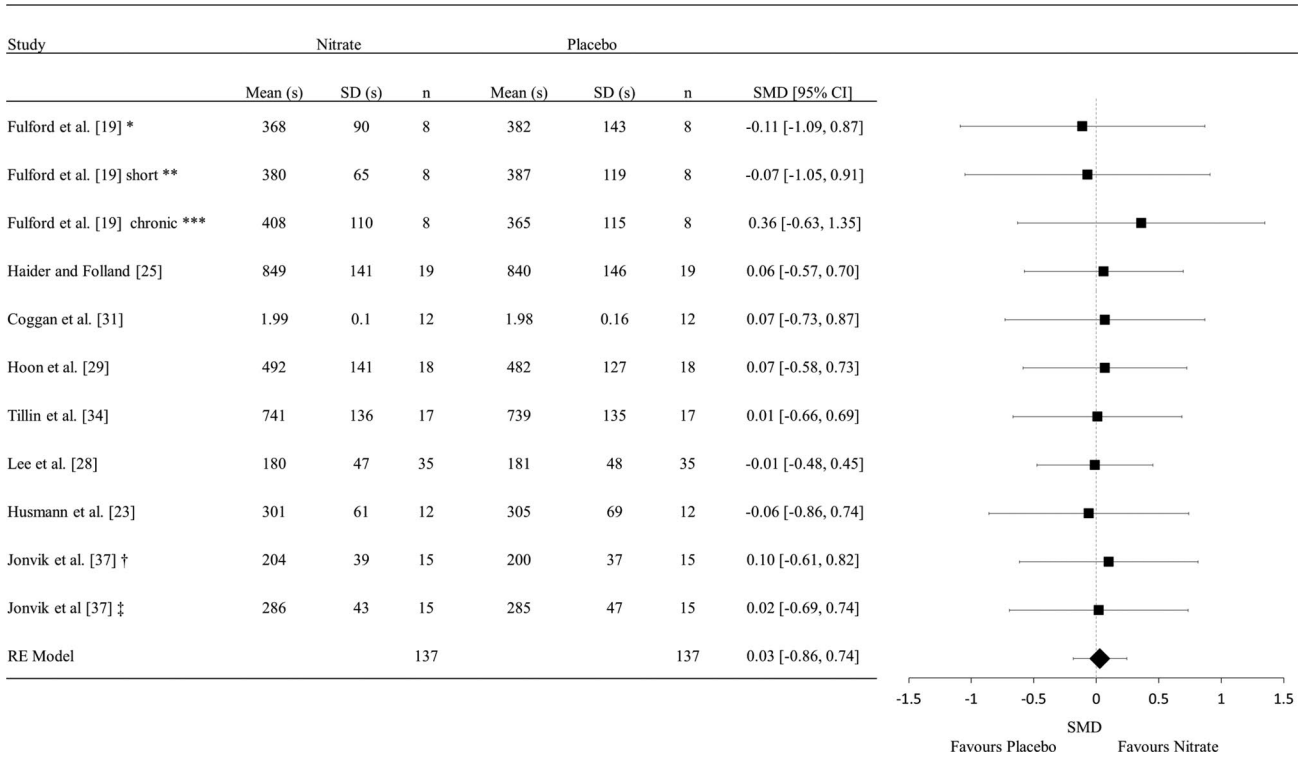

Figure 2. The overall standardized mean difference for the effect of dietary NO_3^- supplementation on MVC (means ± 95% CIs). *SMD* standardized mean difference, *SD* standard deviation, *CI* confidence interval. * acute, ** short-term, *** chronic. † MVC at a knee angle of 30°, †† MVC at a knee angle of 60°.

no changes in force were observed when supplementing 2.5 h, 5 days or 15 days of before the MVC assessments using 10.2 mmol·d^{-1} of NO_3^- supplementation. The study by Jonvik et al. (37) analyzed MVC at 30° and 60° knee flexion and were therefore included as independent observations. In that study, no changes in force were observed either at 30° and 60° knee flexion. Similarly, no change in force during an MVC was observed when varying the dose and duration of NO_3^- supplementation (Table 1). The overall standardized mean difference for force at MVC was considered trivial (SMD = 0.03, 95% CI −0.86, 0.74, $P = 0.758$; Figure 2). No heterogeneity was observed (I^2 = 0%, Q = 0.6721, $P = 0.99$).

In addition to maximal force during an MVC, Haider and Folland (25) found no change in explosive force (in Newtons also) measured during 15 voluntary isometric knee extension contractions across 0 to 150 ms following 7 days of NO_3^- or placebo supplementation (i.e., NO_3^- = ~317 ± 133 N. Placebo = 299 ± 145 N; $P = 0.467$). Similarly, Tillin et al. (34) found that there was no difference in the impulse recorded at 0-50 (NO_3^- = 1.58 ± 0.52 *cf.* placebo = 1.52 ± 0.59 N·s^{-1}), 50-100 (NO_3^- = 15.2 ± 3.6 *cf.* placebo = 15.1 ± 4.2 N·s^{-1}) and 100-150 ms (NO_3^- = 39.4 ± 7.6 *cf.* placebo = 39.4 ± 8.9 N·s^{-1}) in an unfatigued condition ($P = 0.903$).

Response to involuntary contraction via electrical stimulation

Haider and Folland (25) reported a 7% greater peak twitch force (F_{tw}) after NO_3^- compared with placebo (SMD = 0.56, $P = 0.008$) and that force was greater after NO_3^- compared with placebo at 20 ms (14.2%, SMD = 0.59, $P = 0.029$) and at 50 ms (7.2%, SMD = 0.56, $P = 0.048$) after force onset. The same authors also reported an improvement of 2% in the F_{tw} response to low-frequency (10 Hz) twitch stimulation (SMD = 0.63, $P = 0.048$), but not high-frequency (100 Hz) (SMD = 0.12, $P = 0.66$). Further, no change in RFD (SMD = 0.04, $P = 0.702$) or explosive force, measured during the rising phase for the force-time curve (i.e. at 50 ms) was observed.

In the study by Haider and Folland (25), who used supramaximal octet stimulation, they reported improvements of 3–15% in force (SMD = 0.52, $P = 0.023$) but non-significant effects on time to reach octet peak force (SMD = 0.33, $P = 0.167$) after NO_3^- supplementation. Hoon et al. (29) reported no change in peak F_{tw}, F_{tw} across frequencies (10 to 100 Hz), or RFD following 3 days of NO_3^- supplementation of 8.8 mmol·d^{-1} plus 17.6 mmol·d^{-1} on the day of the assessment. Tillin et al. (34) also observed no differences in F_{tw} at any frequency (10 and 50 Hz) or RFD with twitch stimulation in the fatiguing or unfatigued conditions ($P > 0.05$) after 7 days of NO_3^- supplementation with 12.9 mmol·d^{-1}. However, in fatigued condition, they found improvements in the F_{tw} (SMD = 0.46, $P = 0.110$) and RFD (SMD = 0.83, $P = 0.011$) when considering the 20:50 Hz ratio.

Isokinetic exercise

In a recent meta-analysis (38), which include 4 out of 5 studies assessing muscle function during isokinetic dynamometry in the present study, a trivial effect was reported

for knee extension peak torque production at various velocities (1.05, 1.57, 3.14, 4.19, 4.71, and 6.28 rad·s^{-1}) (SMD = 0.01, CI: −0.18, 0.19; I^2: 0%; $P = 1.00$) after acute NO_3^- supplementation. In a more recent study, which is in the present review, using the 6-day dosing strategy, Jonvik et al. (37) reported no difference in isokinetic knee extension power at any velocities (1.05, 2.09, 3.14 and 5.24 rad·s^{-1}, $P = 0.33$) in recreationally active males.

Response to fatiguing exercise

Fulford et al. (19) reported no interaction effect for NO_3^- supplementation when given acutely (2.5 h), in short-term (5 days) and chronically (15 days) for fatigue index (FI), determined as the percentage of between the first 10 to last during 50 MVCs. Likewise, Tillin et al. (34) found no difference in FI, though the FI for force-time integral (0 – 150 ms) was lower after 7 days of NO_3^- supplementation (SMD = 0.51, $P = 0.039$). Hoon et al. (29) reported no differences in initial force during the first contraction of the fatigue test that involved electrically stimulated 64 contraction, in total 102.4 s. In the same study using the same fatigue protocol under restricted blood flow condition, the reduction in the initial force was 8% lower at 80 and 102 s ($P < 0.01$) following 3 days of NO_3^- supplementation compared to placebo. Husman et al. (23) reported a lower percentage reduction maximal voluntary torque between pre- to post-exercise in NO_3^- trial compared with placebo trial (SMD = 0.66, $P < 0.001$). Similarly, there was a lower percentage changes evoked twitch torque at 100 Hz between pre- to post-exercise in NO_3^- trial compared to placebo (SMD = 0.91, $P = 0.001$).

In studies by Coggan et al. (31, 34), no differences were found in FI, which was determined as the ratio of between the first 3 to last during 50 isokinetic contractions at 3.14 rad·s^{-1}, following acute NO_3^- supplementation in healthy and elderly adults. Jonvik et al. (37) also reported no difference in FI during 30 isokinetic contractions at 3.14 rad·s^{-1}, following a 6-day NO_3^- supplementation period. Rimmer et al. (26) found no change in the mean percentage drop in power per second (placebo: −2.2 ± 0.4%·s^{-1} vs. NO_3^-: −2.0 ± 0.2%·s^{-1}; $P = 0.22$) during the 30 s isokinetic trial in collegiate team and endurance athletes. During a repeated Wingate test that involved 4 × 20 s efforts, Bender et al. (27) observed no effect of acute NO_3^- supplementation on the percentage change from peak to minimum power. Similarly, Domínguez et al. (20) and Cuenca et al. (22) observed no difference in the percentage change in power output during a 30 s Wingate test following NO_3^- supplementation using doses of 12.9 mmol·d^{-1} (46% *cf.* 46%) and 5.6 mmol·d^{-1} (49% *cf.* 46%) in resistance trained and healthy men, respectively.

Dynamic exercise performance outcomes

Peak power output

Three studies reported an improvement of 5.4% ($P = 0.034$) in healthy men (20), and 3.8% ($P = 0.049$) and 4.4% ($P = 0.039$) in resistance-trained men (22, 26) in PPO during a Wingate test following 5.6 mmol·d^{-1} and 6.4 mmol·d^{-1} of NO_3^- supplementation, respectively. Rimmer et al. (26) found an increase in mean relative PPO across repeated sprints (4 × 3-4 s) on an inertial-load cycle after acute NO_3^- supplementation of 11.2 mmol·d^{-1} (6.0 ± 2.6%) compared to placebo (2.0 ± 3.8%) (SMD = 1.21, $P = 0.014$), whilst no effect was found in PPO during 30 s 'all-out' effort. PPO did not change over three Wingate test (3 × 30 sec) in either recreational, competitive or elite sprint athletes (21). Similarly, no effect was found in PPO across four Wingate tests (4 × 20 s) following acute ingestion of 12.9 mmol·d^{-1} NO_3^- (27). In another recent study using the acute single dose dosing strategy, no effect was observed in mean PPO across five 6 s running sprints in healthy males (30). When all studies assessing fatigue were pooled, the point estimate SMD for PPO was considered small (SMD = 0.25, 95% CI 0.02, 0.48, $P = 0.034$, Figure 3). Heterogeneity was: I^2 = 0%, Q = 3.06, $P = 0.88$).

Mean power output

Two studies reported an improvement of 6.7% ($P = 0.048$) (20) and by 4.3% ($P = 0.017$) (22) in MPO during the first 15 s of the 30 s Wingate test after acute supplementation of NO_3^-. However, the MPO across the entire Wingate test (30 s) indicated minimal difference (20, 22, 24) after acute supplementation of NO_3^-. Similarly, there was no difference in the pattern of change in MPO over three (3 × 30 sec) (19) or four (4 × 20 s) (27) repeated Wingate tests following acute or chronic supplementation of NO_3^-. The overall SMD for MPO was considered small (SMD = 0.28, 95% CI 0.03, 0.53, $P = 0.030$, Figure 3) with a low degree of heterogeneity (I^2 = 0%, Q = 2.66, $P = 0.75$).

Time to peak power

An improvement in time to PPO of ~1.2% to 18% ($P = 0.002$ to 0.055) was observed in healthy men and resistance trained men after acutely ingesting NO_3^- (20, 22, 24). Similarly, time to PPO improved by ~2.8% over three Wingate test (3 × 30 s) following 7 days of NO_3^- supplementation of 12.9 mmol·d^{-1} ($P = 0.007$) (21). The overall SMD for time to PPO was considered moderate (SMD = −0.78, 95% CI −1.14 to −0.43, $P < 0.001$, Figure 3) with a small degree of heterogeneity (I^2 = 23.5%, Q = 3.9933, $P = 0.262$).

Discussion

This systematic review and meta-analysis aimed to assess the effectiveness of NO_3^-supplementation on skeletal muscle contractility in healthy humans. The overall quality of studies in the present review was deemed high with all scoring 10/10, all down to the use of a placebo-controlled, double-blind, randomized trial. However, eight studies did not disclose information on the randomization approach. Across the studies included, participants' activity levels ranged from minimally active to highly active elite-level

Study	Nitrate			Placebo			
	Mean (W)	SD (W)	n	Mean (W)	SD (W)	n	SMD [95% CI]
PPO							
Kokkinopltis & Chester [30]	4133	674	7	3938	603	7	0.29 [-0.77, 1.34]
Rimer et al. [26]	1173	255	15	1185	249	15	-0.05 [-0.76, 0.67]
Dominiguez et al. [20]	866	143	15	817	136	15	0.34 [-0.38, 1.06]
Bender et al. [27]	750	52	12	708	48	12	0.81 [-0.02, 0.55]
Jonvik et al. [21]	1518	59	52	1510	54	52	0.14 [-0.24, 0.53]
Cuenca et al. [22]	881	135	15	848	134	15	0.24 [-0.48, 0.96]
Jorda et al. [24]	869	53	15	839	76	15	0.45 [-0.28, 1.17]
Rodriguez-Fernandez et al. [36]	2302	472	18	2196	346	18	0.25 [-0.41, 0.91]
RE Model			124			124	0.25 [0.01, 0.50]
MPO							
Dominiguez et al. [20]	648	105	15	614	94	15	0.33 [-0.39, 1.05]
Bender et al. [27]	605	40	12	587	39	12	0.44 [-0.37, 1.20]
Jonvik et al. [21]	810	24	52	807	23	52	0.13 [-0.26, 0.51]
Cuenca et al. [22]	666	100	15	641	91	15	0.25 [-0.46, 0.97]
Jorda et al. [24]	650	45	15	643	61	15	0.13 [-0.59, 0.84]
Rodriguez-Fernandez et al. [36]	1356	307	28	1140	272	18	0.73 [0.05, 1.40]
RE Model			109			109	0.21 [-0.06, 0.47]
Time to PPO							
Dominiguez et al. [20]	7.3	1.2	15	8.0	1.5	15	-0.48 [-1.21, 0.24]
Jonvik et al. [21]	2.7	0.1	52	2.8	0.3	52	-0.59 [-0.98, 0.21]
Cuenca et al. [22]	7.3	0.9	15	8.9	1.4	15	-1.32 [-2.11, -0.53]
Jorda et al. [24]	7.3	0.9	15	643	1.5	15	-1.10[-1.87, -0.43]
RE Model			97			97	-0.78 [-1.14, -0.43]

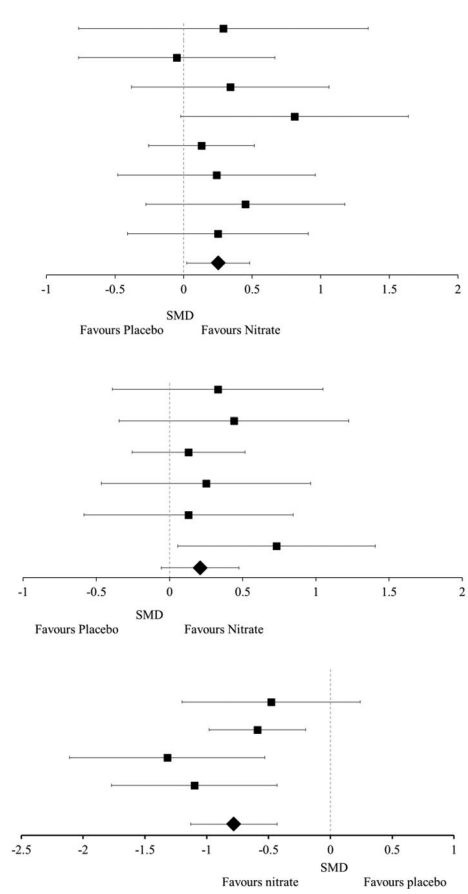

Figure 3. The overall standardized mean difference for the effect of dietary NO_3^- supplementation on PPO, MPO and time to PPO (means ± 95% CIs). *SMD* standardized mean difference, *SD* standard deviation, *CI* confidence interval.

participants. The main results indicate that NO_3^- supplementation demonstrated a small improvement in PPO and MPO during exercise when compared with a placebo. The ingestion of NO_3^- resulted in an enhanced time to PPO as demonstrated by a moderate SMD and narrow confidence intervals. There was no clear effect of NO_3^- on isometric MVC. Based on a previous meta-analysis (38) and the findings of this review, NO_3^- supplementation had a trivial effect on P_{max} during isokinetic exercise. There was a mixed effect on F_{tw} and no effect on FI in 8 out of 11 studies that included this metric. Collectively, the current literature demonstrates that NO_3^- supplementation may have a potential to enhance muscle contractility during a short duration (<10 s) dynamic exercise in highly active and resistance-trained individuals.

In total, PPO, MPO and time to PPO was evaluated in 8, 6 and 4 studies, respectively, using healthy active men to elite-level athletes. Five studies used a Wingate protocol whilst another used running-based protocol, and the remaining two studies used inertial cycling or squat protocol. Although only one study directly measured plasma NO_3^- and NO_2^- concentration, the small but systematic effect of NO_3^- on PPO, MPO and time to PPO likely suggests that all studies increased NO_3^-induced NO production. The increase in PPO, MPO and time to PPO during Wingate and running sprints might be due to the elevated NO_3^- and fast-twitch muscle fibers preference of NO_3^- supplementation. Based on a previous animal model, the excitation-contraction coupling was enhanced in fast-twitch fibers only following NO_3^- supplementation (6). Hence, improved time to PPO with NO_3^- supplementation may be a consequence of a targeted effect of NO_3^- on fast-twitch fibers (4), given that the recruitment of fast-twitch fibers is greater during short-duration high-intensity exercise (39–42). Such effect can also explain the findings of several other studies, including increased PPO during consecutive 3-4 s cycling sprints trials (26), greater MPO (20, 22), and greater P_{max} and V_{max} when assessed at velocities of 4.71 and 6.28 rad·s^{-1} (31, 33, 35). Further, the fast-twitch preference of NO_3^- may have resulted in the improved involuntary muscle contraction when blood flow was restricted in the study by Hoon et al. (29), where greater recruitment of fast-twitch fibers is observed (42). Taken together, our finding supports and extends to the findings of previous systematic reviews (43) and meta-analysis (44) that have reported that NO_3^- supplementation can enhance muscle power and performance in healthy adults and trained individuals.

The observed improvement in PPO and time to PPO, and MPO could be associated with the attenuation of ATP cost and PCr degradation as well as accumulation of metabolites (45). As the required energy during the activities above is mostly supplied by anaerobic pathways (~75%) and free ATP and PCr (46, 47), it is possible that NO_3^- plays an important role during the initial part of activity (first 5–10 s) (48). Furthermore, NO_3^--induced reductions in ATP cost for force production may improve neuromuscular efficiency, due to a reduced motor unit (MU) activity required to produce a given contractile force. Indeed, Flanagan et al. (32) reported NO_3^- supplementation lowered MU firing rate and enhanced peak EMG amplitude during box squat exercise. However, further studies are required to provide insight into the potential impact of NO_3^- on MU activation and muscle function. Recent studies also point that NO_3^- supplementation may increase Ca^{2+} handling or sensitivity (6, 49), which might go some way to explaining the small increase in PPO and MPO, and moderate increase in time to PPO observed in our meta-analysis when supplementing with NO_3^-. Although the mechanisms of action remain to be established, it is likely that the proposed mechanisms work concomitantly to produce the robust physiological effects related to NO_3^- supplementation.

Eight studies in the present meta-analysis evaluated isometric MVC force, using healthy and/or recreational individuals, except one that used resistance-trained men. Of the 8 studies, half of them reported plasma NO_3^- or/and NO_2^- concentrations or breathing NO level whereas others did not measure any NO indices. As such, before exploring the results, we highlight that future research ought to include a measure of plasma NO_3^- or NO_2^- to support overall interpretation of the result. The present meta-analysis showed there was a trivial effect of NO_3^- supplementation on MVC, with most studies having wide confidence intervals that encompassed a null effect due to small sample sizes, variability in procedures and the supplementation strategy. These results might also be reflection of the larger inter-individual variability in muscle fiber composition, especially in the quadriceps which was used in all studies (50) where it has been reported that fast-twitch fibers can vary by 20 to 80% (51). It is also important to note that whilst seven studies used BRJ, only one used NO_3^--rich bar as NO_3^- supplementation. Since, other nutrients in the bar may have resulted in the effects observed by Flanagan et al. (32). Nonetheless, our results indicate a consistent pattern for MVC suggesting it is unaffected by NO_3^- supplementation, potentially due to there being no effect on force at firing frequency greater than 20 Hz that can be inferred from an MVC.

It has been reported that NO_3^- supplementation reduces muscle fatigue during fatiguing contraction when blood flow is restricted (29) and is more effective at enhancing skeletal muscle contractility in fatigued muscle (34). However, the narrative synthesis of results in the present review relating to FI indicated that NO_3^- has no effect regardless of the difference in activity type, protocol and method used to determine FI. The most obvious reason for this disparate result is that Hoon et al. (29) and Tillin et al. (34) have employed electrically stimulated involuntary contraction, which is hard to translate to voluntary contractions due to some major differences between involuntary and voluntary contractions (i.e., randomized and ordered MU recruitment). Regarding to the lack of effect of NO_3^- on FI, some of the possible reasons suggested by the respective authors relate variances in muscle fiber composition, inadequate sample size and lack of statistical power, the use efforts lasting greater than 10 s (27), and that NO_3^- might better maintain ATP stores and reduced the cost of its synthesis during metabolism (22). Inter-individual differences, such as plasma NO_2^- concentration (52, 53) and potential of sex-related differences (33, 54), might have also contributed inter-individual differences in the effects of dietary NO_3^- on FI. For example, the effect of NO_3^- supplementation was more apparent in women than men in study by Coggan et al. (35). However, a recent meta-analysis has revealed that NO_3^- supplementation had no benefit in exercise performance in women (43). Given that there is a lack of mechanistic work in females regarding NO_3^- supplementation and women have a higher fatigue resistance than men (55), it might be still possible that sex differences may influence in the impact of NO_3^- supplementation on muscle fatigue resistance. Future research might seek to better determine the role of NO_3^- on the various dimensions of fatigue and understanding the mechanical changes at the muscle as well as addressing possible inter-individual, particularly sex-related, differences in effects of NO_3^- supplementation on fatigue.

Studies that investigated the effects of NO_3^- supplementation on involuntary contraction in healthy human reported contrasting findings. However, such contrasts are difficult to elucidate given the small number of studies in this area and difference in the supplementation protocols. Further, across the three studies, one used blackcurrant as a placebo (25) that raised question over performance bias and the role of other nutrients, whereas the others used NO_3^--depleted BRJ (22, 29, 34). The differences in placebo condition between studies might have also caused potential awareness of the supplement by the participants due to issues around blinding taste and texture.

Practical recommendation for sport-specific performance

While the International Olympic Committee consensus (56) highlights the effect of NO_3^-supplementation is apparent in sport-specific tests lasting 10–40 min, this present review indicates that supplementation of NO_3^- may also be effective for exercise lasting ≤ 10 seconds. Such findings might be extrapolated to different aspects of various exercise modality that rely on PPO, MPO and time to PPO such as diving from blocks, sprinting from blocks, and snatching a bar during weightlifting. Therefore, NO_3^- supplementation might be considered as an ergogenic aid for power-based exercise and/or athletes (e.g., 100 m sprinter or weightlifter). However, further investigations are warranted to determine if the muscle contractility-enhancing effects of NO_3^- would transfer in the context of sport specific performance.

The present review did not attempt to assess the impact of dose or dosing strategy, however, most of the studies used a dose of between 5.6 mmol and 13.4 mmol in the present review which aligns with the current recommendation of 5–9 mmol (56). Cited studies in this review that reported benefits of NO_3^- also generally employed acute (2-3 hours prior to exercise) supplementation procedure. While previous meta-analyses on the effect of NO_3^- supplementation on muscular performance have revealed no differences between acute and chronic supplementation (43, 44), ≥ 3 days supplementation is recommended for trained athletes (4). Taken together, the present review aligns with current literature suggest that dose of NO_3^- supplementation should be the first consideration and then duration of supplementation can be employed according to individual response to NO_3^- during training and competition. Further, the dose and dosing strategy of NO_3^- supplementation is in its infancy, and its impact on the mechanical function of the skeletal muscle during muscle contraction is required further investigations.

Conclusion

This review reports that NO_3^- supplementation may have a potential to enhance PPO, MPO and time to PPO during short duration (< 10 s) dynamic exercise, which may transfer to brief explosive actions. While this would suggest that NO_3^- supplementation may enhance performance in training or competitions where quick, short and explosive movements are performed (e.g., weightlifting, track and field, team sports), practical applicability of these results remains somewhat questionable because just few studies included in the present systematic review and meta-analysis when concerning specific outcomes. Due to the variability in studies, we encourage researchers to use this piece of work to explore areas where evidence in lacking and standardize their study design and supplementation procedures.

Author contributions

OE designed the review. OE and ND conducted the searches and completed the two-phase screening process. OE extracted the data, which was cross-checked by ND. OE and ND independently assessed quality of each article. OE and ND performed all statistical analysis and interpreted the results. OE and ND wrote the manuscript with critical input from MC. All authors read and approved the final manuscript.

Compliance with ethical standards

Disclosure statement

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Data availability statement

The data extracted sheet that support the findings of this study are available from the corresponding author on request.

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