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Ergogenic Effect of Nitrate Supplementation: A Systematic Review and Meta-analysis

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ABSTRACT

SENEFELD, J. W., C. C. WIGGINS, R. J. REGIMBAL, P. B. DOMINELLI, S. E. BAKER, and M. J. JOYNER. Ergogenic Effect of Nitrate Supplementation: A Systematic Review and Meta-analysis. *Med. Sci. Sports Exerc.*, Vol. 52, No. 10, pp. 2250–2261, 2020. Although over 100 studies and reviews have examined the ergogenic effects of dietary nitrate (NO_3^-) supplementation in young, healthy men and women, it is unclear if participant and environmental factors modulate the well-described ergogenic effects—particularly relevant factors include biological sex, aerobic fitness, and fraction of inspired oxygen (F_IO_2) during exercise. To address this limitation, the literature was systematically reviewed for randomized, crossover, placebo-controlled studies reporting exercise performance outcome metrics with NO_3^- supplementation in young, healthy adults. Of the 2033 articles identified, 80 were eligible for inclusion in the meta-analysis. Random-effects meta-analysis demonstrated that exercise performance improved with NO_3^- supplementation compared with placebo (d = 0.174; 95% confidence interval (CI), 0.120–0.229; $P < 0.001$). Subgroup analyses conducted on biological sex, aerobic fitness, and F_IO_2 demonstrated that the ergogenic effect of NO_3^- supplementation was as follows: 1) not observed in studies with only women (n = 6; d = 0.116; 95% CI, −0.126 to 0.358; P = 0.347), 2) not observed in well-trained endurance athletes (≥65 $mL \cdot kg^{-1} \cdot min^{-1}$; n = 26; d = 0.021; 95% CI, −0.103 to 0.144; P = 0.745), and 3) not modulated by F_IO_2 (hypoxia vs normoxia). Together, the meta-analyses demonstrated a clear ergogenic effect of NO_3^- supplementation in recreationally active, young, healthy men across different exercise paradigms and NO_3^- supplementation parameters; however, the effect size of NO_3^- supplementation was objectively *small* (d = 0.174). NO_3^- supplementation has more limited utility as an ergogenic aid in participants with excellent aerobic fitness that have optimized other training parameters. Mechanistic research and studies incorporating a wide variety of subjects (e.g., women) are needed to advance the study of NO_3^- supplementation; however, additional descriptive studies of young, healthy men may have limited utility. **Key Words:** DIETARY NITRATE, EXERCISE PERFORMANCE, SEX DIFFERENCES, BEETROOT JUICE, NITRIC-OXIDE

The study of the ergogenic effects of dietary nitrate/beetroot (NO_3^-) supplementation has been a prominent topic in human performance for the last decade. As previously reviewed (1), early studies demonstrate that NO_3^- supplementation improves exercise tolerance in healthy humans with blunted effects in trained athletes (2). Although it is generally well accepted that NO_3^- supplementation may improve exercise tolerance in healthy, young men (1,3–18), there is substantial variability within and between studies, and previous systematic reviews have failed to find a (significant) performance-enhancing effect with NO_3^- supplementation under some conditions (7,13,17). Indeed, nearly 70% of studies examining the potential performance-enhancing effects of NO_3^- supplementation do not observe a difference in performance with NO_3^- supplementation compared with placebo. The variability of the ergogenic effects of NO_3^- supplementation is likely due to several descriptive factors including aerobic fitness, dose and timing of NO_3^- supplementation (19–21), environmental factors (e.g., hypoxia) (22), biological sex (23), and interindividual variability in pharmacodynamics and dose–response relationships (19–21). Thus, the primary purposes of this systematic review are to determine the magnitude of the potential ergogenic effect of NO_3^- supplementation and the influence of the aforementioned descriptive factors that contribute to variability of the ergogenic effects of NO_3^- supplementation.

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Nitric oxide has long been recognized for vasculoprotective effects (24), effects on mitochondrial respiration (25), and effects on fatigue development (26). In addition to the biosynthesis of endogenous nitric oxide (24), exogenous dietary sources of NO_3^- (e.g., green leafy vegetables and beetroot) can markedly increase the bioavailability of nitric oxide (1,27–29). After ingestion of a NO_3^- supplement, plasma nitrate levels peak after 1–2 h and plasma nitrite levels peak after 2–3 h, both levels gradually return to baseline after about 24 h (30). Thus, it is not surprising that NO_3^- supplementation has been shown to enhance exercise performance in some instances. For example, in 2009, it was shown that beetroot ingestion of 5.5 mmol NO_3^- per day for 6 d improved time to exhaustion during intense cycling exercise in eight young, healthy men compared with placebo (NO_3^-: 675 ± 203 s vs placebo: 585 ± 145 s, $P < 0.05$) (31). Similarly, beetroot ingestion of ~6.2 mmol NO_3^- in nine competitive male cyclists improved performance by ~3% during laboratory-based simulated cycling races using a 16.1-km fixed-distance time trial compared with a placebo (NO_3^-: 1614 ± 108 s vs placebo: 1662 ± 126 s, $P < 0.01$) (32). Despite the growing number of studies demonstrating augmented performance with NO_3^- supplementation, there are twice as many studies demonstrating no performance-enhancing effect of NO_3^- supplementation.

Thus, several important questions persist regarding the potential ergogenic effect of NO_3^- supplementation: 1) what is the magnitude and effect size of the potential ergogenic effect? 2) are there sex differences? (23) 3) what is the role of aerobic fitness? (6) 4) what is the role of the fraction of inspired oxygen (F_iO_2)? (22) and 5) what are the optimal dosage, duration, and timing of NO_3^- supplementation? Thus, the purpose of this systematic review and meta-analysis was to examine these five questions. The information garnered may better inform appropriate dosing for future studies and optimal use of NO_3^- supplementation as an ergogenic aid for athletes and coaches.

METHODS

Methods of the analysis and inclusion criteria were specified *a priori* and conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses checklist (33).

Literature search. Studies were initially identified from a search of the online PubMed database through August 2019. Keywords used in the search included the following: (exercise) AND [(beetroot juice) OR (beetroot) OR (beet) OR (nitrite) OR (nitrate) OR (nitrate supplement) OR (dietary nitrate)], using the following limits: humans and English. The references of all eligible studies were also reviewed to identify other potentially eligible studies that may have been missed using the approaches outline previously. Only published material was used.

Identification and study selection. In order to be considered eligible for inclusion, studies had to meet the following criteria: 1) all subjects were previously healthy, and mean age of study participants was between 18 and 40 yr; 2) the study must have used a single or double-blind, within-subject crossover, placebo-controlled study, with a randomized or counterbalanced study design; and 3) results had to be reported for both nitrate supplement and placebo as mean ± SD or SE. If the required performance data were provided in figure format but not numerical text, authors were contacted to obtain the numerical data. If the data could not be provided, the study was excluded to avoid potential bias due to estimation of values.

Selected studies were uploaded into a screening tool (Covidence). Three independent investigators (C.C.W., J.W.S., and R.J.R.) screened the titles and abstracts of all studies identified by the search methodologies to determine potential eligibility. Studies that did not have an abstract, along with studies that were deemed potentially eligible, had their full text reviewed in order to determine if they met the criteria for inclusion in the meta-analysis. In addition, reference lists of included studies were carefully inspected, and any relevant articles not initially captured in the systematic search but met the inclusion criteria were included. Disagreement was resolved by consensus.

Quality assessment. Risk of bias was assessed with the Cochrane Risk of Bias (34). This standardized appraisal tool consists of seven components: 1) sequence generation, 2) allocation concealment, 3) blinding of participants and personnel, 4) blinding of outcome assessors, 5) incomplete outcome data, 6) selective outcome reporting, and 7) other sources of bias. For each included study, components were rated as "high," "low," or "unclear" risk of bias based on the detail definitions and standardized criteria provided by the quality assessment tool (34). Because the present systematic review included randomized or counterbalanced, single- or double-blind, crossover, placebo-controlled, within-group study design, components 1, 2, 3, and 4 were all considered "low risk of bias." Risk of bias was conducted independently by two authors (J.W.S. and R.J.R.), and disagreements were resolved by review of a third author (C.C.W.). No studies were excluded based on the quality assessment. To assess publication bias in the included studies, we used visual inspection of the funnel plot and the Egger's regression test to statistically quantify funnel plot asymmetry (35).

Data extraction and analysis. Participants' characteristics (number, sex, age, and aerobic fitness ($\dot{V}O_{2peak}$)) were identified from the selected studies. Exercise type (cycling, handgrip, kayaking, knee extension, roller skiing, rowing, running, swimming), task end-criteria (time trial, work trial, time to exhaustion, trials to exhaustion, fatigue index, or distance trial), NO_3^- supplementation (daily amount, total amount, and timing relative to exercise initiation), and performance outcome metric were extracted. Data including means and SD were extracted independently by two authors (J.W.S. and R.J.R.), and disagreements were resolved by review of a third author (C.C.W.). To account for differences in exercise tasks (e.g., time trial (in seconds) vs distance trial (in meters)), all data were transformed so that a positive mean difference denoted "better performance with NO_3^- supplementation" and a negative mean difference denoted "better performance with placebo." For the exercise tasks in which a smaller value indicates "better performance" (time trial, fatigue index), the mean performance metrics for placebo and

NO_3^- supplementation were replaced with the arithmetic opposite values. Using the example cycling time trial from Lansley and colleagues (32), values of −1614 and −1662 s were input for NO_3^- and placebo, respectively, such that the standardized mean difference (SMD) would be a positive value indicating "better performance with NO_3^- supplementation."

Narrative synthesis. Initially, a narrative synthesis of studies was conducted. Studies were first grouped based on participant sex (men, mixed, or women only) and F_iO_2 during exercise bout (normoxia or hypoxia), then the studies were listed in alphabetical order based on the last name of the first author, and then the studies were listed in chronological order based on publication year from the earliest to most recent. This summary table is provided a supplemental content (Table, Supplemental Digital Content 1, Included studies characteristics and result, http://links.lww.com/MSS/B962).

Meta-analysis and subgroup analyses. To support the narrative synthesis, a meta-analysis of pooled data and subgroup analyses were conducted. Initially, effect sizes were calculated for each study using a general inverse variance and weighted using Cohen's *d* for differences in performance outcome between placebo and NO_3^- supplement. Thresholds for very small, small, moderate, and large effect sizes were 0.15, 0.2, 0.5, and 0.8, respectively. Data were pooled with both fixed-effects (inverse-variance method) and random-effects (DerSimonian and Laird method [36]) models. Although both models returned similar main effects, we only reported the results of the random-effects analyses. The *a priori* level of significance for all comparisons was $P < 0.05$. Pooled data are presented as (Cohen's *d* SMD (95% confidence intervals, or 95% CI); *z*-statistic, *P* value) unless otherwise indicated. Comprehensive Meta-Analysis Software (Biostat) version 3.3.070 was used for all analyses.

RESULTS

Study selection. A total of 2033 articles were identified in the initial search, and duplicates were removed ($n = 2$). After screening titles and abstracts, 1830 articles were deemed ineligible for inclusion. Of the remaining 201 full-text studies, 77 studies were included. The references of the included 77 studies were then carefully inspected (3723 references, including 1729 unique references), 41 articles were added to the full-text screening (242 total), and an additional 8 articles were deemed eligible for inclusion. In total, 162 studies were excluded for various reasons including the following: no reported performance outcome data ($n = 40$), no placebo ($n = 20$), between-group study design ($n = 15$), incorrect subject population (e.g., older adults, patient population) ($n = 34$), no exhaustive exercise included in the study ($n = 36$), or an inappropriate control/placebo performance trial ($n = 17$). Thus, 80 studies were included in the narrative synthesis and meta-analyses. Within these 80 studies, 2 presented data from men and women separately, 6 presented data from different NO_3^- supplementation separately, 7 presented data from different exercises separately, and 8 presented data from different ambient oxygen concentrations separately, resulting in 111 data sets (2,19,31,32,37–115). A schematic of the search stratagem is presented in Figure 1.

Study characteristics: narrative review. A summary of the 80 included studies and the 113 data sets is provided in the supplemental content (Table, Supplemental Digital Content 1, Included studies characteristics and result, http://links.lww.com/MSS/B962). Studies included were published between 2009 and 2019. A total of 1,179 men and 156 women were included in the selected studies; most data sets (79%; $n = 90$) were composed of men only, 16% ($n = 17$) included both men and women, and 5% ($n = 6$) included exclusively women. The primary performance metrics of studies included time to complete a fixed distance (time trial; $n = 52$), time to complete a relative total work (work trial; $n = 5$), maximal distance covered in a fixed time period (distance trial; $n = 6$), reduction in maximal strength or power during a task (fatigue index; $n = 13$), endurance time maintaining a submaximal task (time-to-exhaustion (TTE); $n = 32$), or number of trials of a submaximal task to exhaustion (trials to exhaustion; $n = 4$). NO_3^- supplementation was performed between 40 and 210 min before exercise initiation with a concentration between 1. and –28.7 mmol for 1–15 d resulting in a cumulative NO_3^- of 4.2–208 mmol. Most studies did not observe a difference in performance (76 of 111 studies; 68%), but no studies reported worsened performance with NO_3^- supplementation compared with placebo. Generally, most studies examined sustained, endurance-style exercise; however, several studies incorporate single-sprint exercises (e.g., 500-m kayak time trial; $n = 5$) or repeated sprint exercises (e.g., three sequential Wingate tests; $n = 8$). Most studies incorporating "sprint" exercises are encompassed within the "300[s] or less" exercise time.

Pooled analysis. Considering all studies included in the quantitative synthesis (Fig. 2), exercise performance was improved (faster time, longer distance, lower fatigue index, or more trials) with NO_3^- supplementation compared with placebo with negligible NO_3^- (0.174 (0.120–0.229); $z = 6.299$, $P < 0.001$). Although significant, the effect size was very small ($d < 0.2$); thus, it is not surprising that only ~32% studies demonstrated significant improved performance with NO_3^- supplementation compared with placebo. There is substantial variability in the response to NO_3^- supplementation (21), indicating that other factors may be contributing to the change in performance other than NO_3^- supplementation. Factors that may contribute to variability of the effects of NO_3^- supplementation include interindividual differences, such as biological sex and aerobic fitness and interstudy differences, such as performance parameters (ambient oxygen concentration, exercise time, and exercise type), and/or NO_3^- supplementation parameters (daily dose, dosing period, or timing). Thus, subanalyses were undertaken to examine each identified, potential source of variability. Statistical power analyses indicated that approximately six data sets are required within each category for subanalyses. To reduce the potential effects of learning, all studies included familiarization procedures for

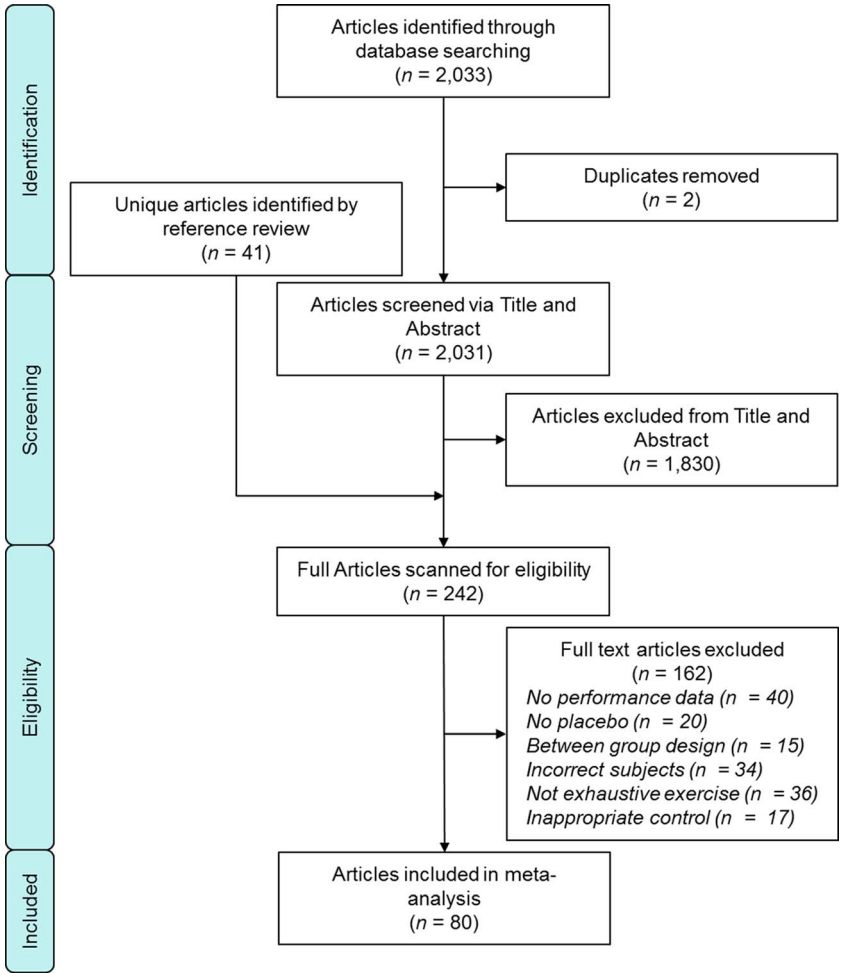

FIGURE 1—Flowchart of the study selection.

the exhaustive exercise bout and 95% of the studies included a "control" session with completion of the exhaustive exercise *without* supplementation (placebo or NO_3^-).

Subgroup analysis: biological sex and aerobic fitness. Comparison of men only, mixed sex (men and women), and women only studies revealed a blunted overall effect of NO_3^- supplementation for women compared with mixed sex and men only studies (Fig. 3A). As has been suggested by Wickham and colleagues (112) in a recent investigation and review (23), women are heavily underrepresented in this field of research as is generally observed in biomedical research (116). Six studies that examined women only or presented data separately for women found no effect of NO_3^- supplementation on exercise performance (P = 0.347). However, a study examining kayak performance in elite, international-level athletes found that NO_3^- supplementation improved 500-m time-trial performance for five women (P = 0.004) but not 4-min distance trial performance for six men (P = 0.110) (95). As reviewed previously (23), although there are physiologically based sex differences that could potentially reduce the efficacy of NO_3^- supplementation as an ergogenic aid for women, there is a clear underrepresentation of women that should be addressed in future investigations.

A priori, $\dot{V}O_{2peak}$ was delineated increments of 5 mL·kg·min^{-1} resulting in six categories (<45, 45–49.9,…,65+ mL·kg·min^{-1}). However, the lowest $\dot{V}O_{2peak}$ category was underpowered and highly variable (n = 4; d = 0.168; 95% CI, −0.134 to 0.469; P = 0.276), and thus was combined with the next lowest category (45–49.9 mL·kg·min^{-1}). Comparison across $\dot{V}O_{2peak}$ demonstrates that the ergogenic effect of NO_3^- supplementation is observed across a large range of $\dot{V}O_{2peak}$ values (~40–65 mL·kg·min^{-1}); however, the ergogenic effect is not observed in highly fit athletes ($\dot{V}O_{2peak}$ > 64.9 mL·kg^{-1}·min^{-1}; Fig. 3B). Although a recent systematic review suggested that the data were inconclusive (6), with the inclusion of additional data, the role of $\dot{V}O_{2peak}$ can be more clearly observed. These data are in agreement with a study from Porcelli and colleagues (96). In a cohort of 21 men with low, moderate, and high $\dot{V}O_{2peak}$ (~40 vs ~50 vs ~70 mL·kg^{-1}·min^{-1}), 3-km running time was improved with a 6-d supplementation of 5.5 mmol·d^{-1} of NO_3^- compared with placebo for men with low (~3% improvement) and moderate (~1.5% improvement) but not high $\dot{V}O_{2peak}$ (0.2% improvement) (96). Based on these data, the ergogenic effect of NO_3^- supplementation is not observed in highly trained athletes with likely optimal training adaptations

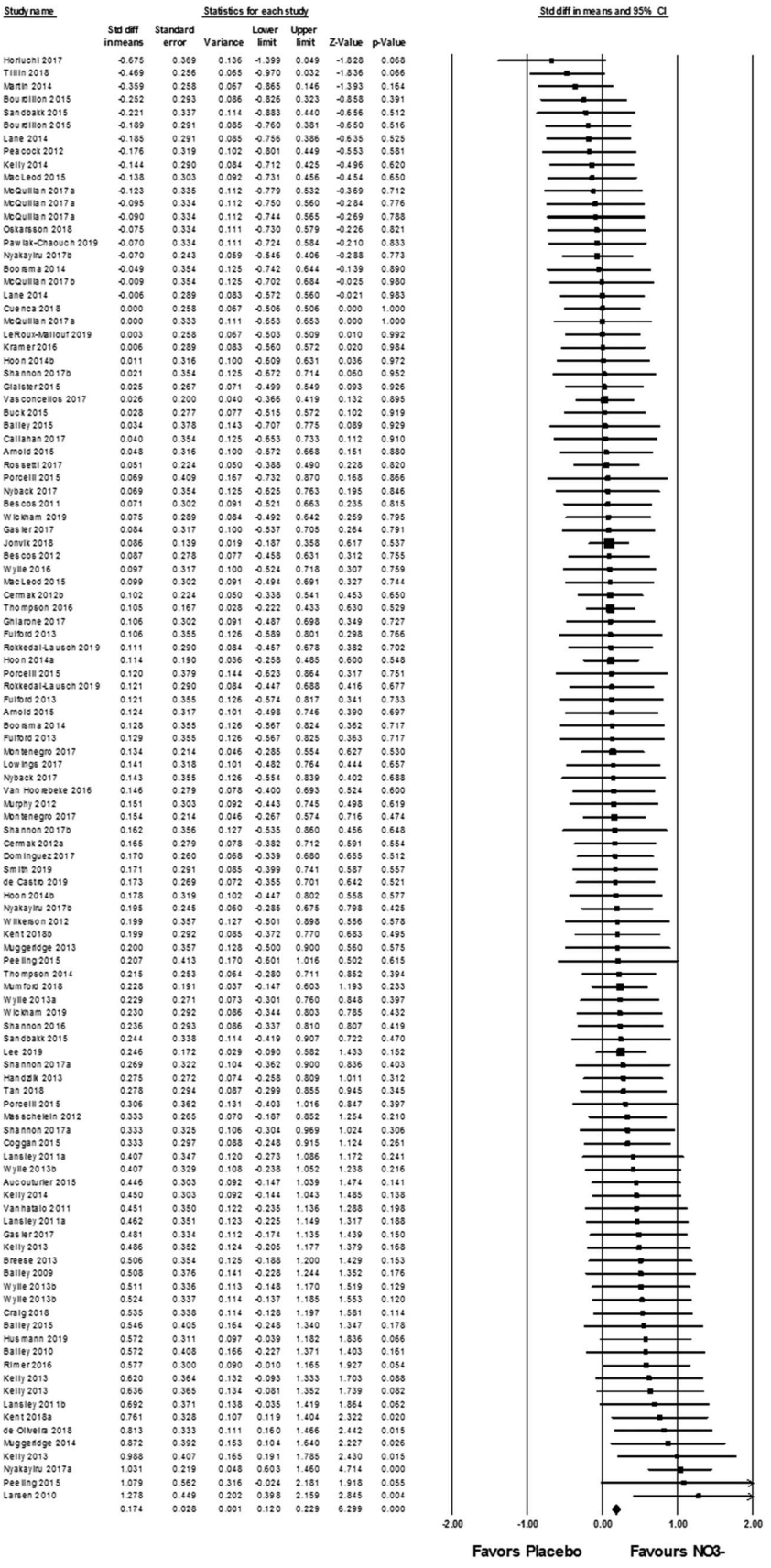

FIGURE 2— Forest plot displaying random-effects meta-analysis of exercise performance after placebo or NO_3^- supplementation. The vertical line represents the mean overall effect. Symbol size reflects weight of the effect for each individual study. Symbols on the left of the continuous black line at 0 show better exercise performance after placebo supplementation, whereas studies on the right of the black line demonstrate better exercise performance after NO_3^- supplementation.

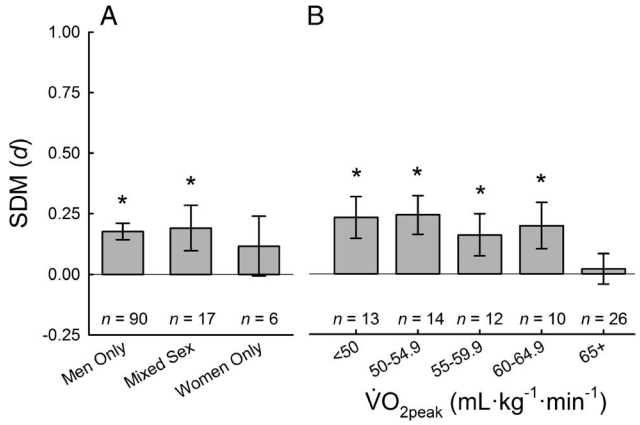

FIGURE 3—Subgroup analysis of biological sex and aerobic fitness. SMD of NO_3^- supplementation compared with placebo for biological sex of included participants (A) and $\dot{V}O_{2peak}$ (B) calculated used random-effects meta-analyses. * denotes better performance after NO_3^- supplementation compared with placebo, $P < 0.05$.

($\dot{V}O_{2peak}$ > 64.9 mL·kg^{-1}·min^{-1}). However, in moderately trained and untrained men without optimized fitness, a significant ergogenic effect of NO_3^- supplementation is observed.

Subgroup analysis: exercise parameters. Comparison of hypoxic and normoxic conditions revealed a similar overall effect of NO_3^- supplementation for both conditions (Fig. 4A). In line with a previous review from Shannon and colleagues (22), these data suggest that NO_3^- supplementation is a promising ergogenic aid for exercising in low-O_2 environments. However, these data do not suggest that the ergogenic effects of NO_3^- supplementation are greater in hypoxia than normoxia, as previously suggested (22). Albeit, these data are primarily from simulated altitude using normobaric hypoxia with short hypoxic exposure times, and future investigations at terrestrial altitude (hypobaric hypoxia) may be warranted, as formerly suggested (22).

Comparison across different exercise parameters (time and type) revealed heterogeneous results for exercise type and limited effect of NO_3^- supplementation in long-duration exercise (1000 s or more). As previously reviewed (8,9), long-duration exercise that by virtue is lower intensity than short-duration exercise likely minimizes hypoperfusion of metabolically active tissue during exercise and reduces the requirement for NO production through the reduction of nitrite. This physiological rationale likely underlies the finding that NO_3^- supplementation is more beneficial for short-duration exercise (<15 min) than long-duration exercise (Fig. 4B). Most studies (~80%) used cycling or running as exercise modalities, and the ergogenic effect of NO_3^- supplementation was not different between running and cycling. However, for the other exercise types (handgrip, kayaking, knee extension, roller skiing, rowing, and swimming), there was markedly less data and more heterogeneity due to small sample sizes (Fig. 4C). These data may suggest that NO_3^- supplementation has larger effects in small muscle exercise (handgrip), which is likely limited by peripheral factors (tissue perfusion and metabolic accumulation) rather than cardiac output, compared with whole body or large muscle exercise.

Previous systematic reviews have demonstrated an ergogenic effect of NO_3^- supplementation that is dependent on the criteria for exercise termination. As examples, both Hoon et al. (7) and McMahon et al. (13) demonstrated a significant ergogenic effect of NO_3^- supplementation for TTE exercise but not time trials or graded-exercise performance tests. The current data demonstrated a consistent ergogenic effect of NO_3^- supplementation across all exercises regardless of criteria for exercise termination, including the following: time trials (n = 52; 0.086 (0.002–0.0173); z = 2.000, P = 0.045), distance trials (n = 6; 0.318 (0.136–0.499); z = 3.430, P = 0.001), fatigue index tasks (n = 13; 0.175 (0.036–0.313); z = 2.473, P = 0.013), and TTE tasks (n = 32; 0.324 (0.213–0.436); z = 5.690, P < 0.001). In line with findings from Hoon et al.

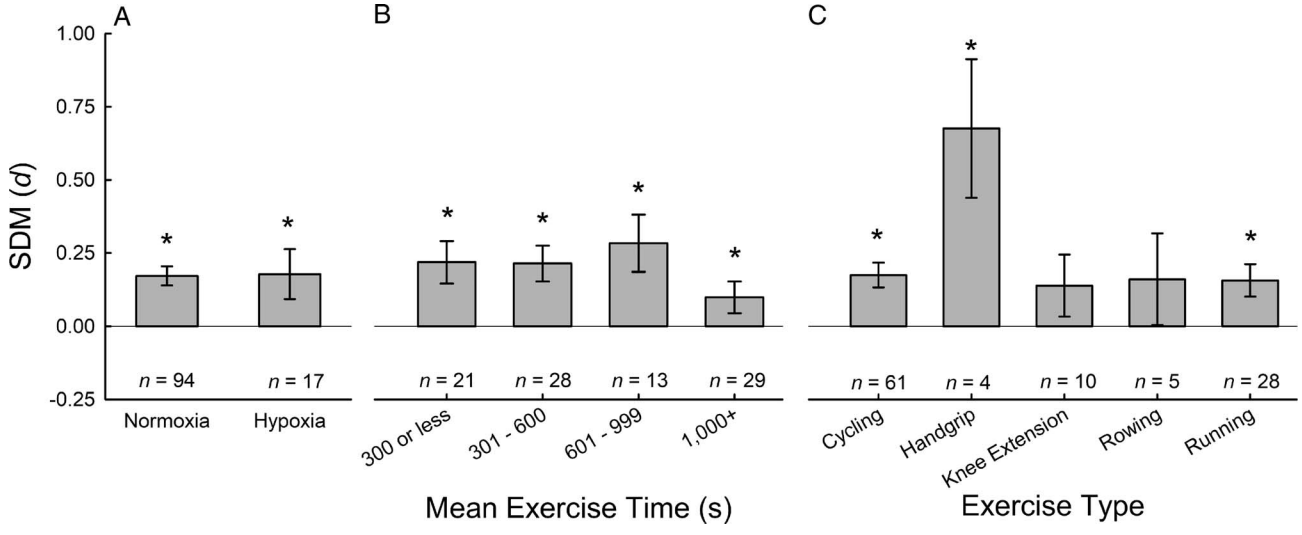

FIGURE 4—Subgroup analysis of exercise parameters. SMD of NO_3^- supplementation compared with placebo for F_IO_2 (normoxia vs hypoxia; A), mean exercise time (B), and exercise type (C) calculated used random-effects meta-analyses. * denotes better performance after NO_3^- supplementation compared with placebo, $P < 0.05$.

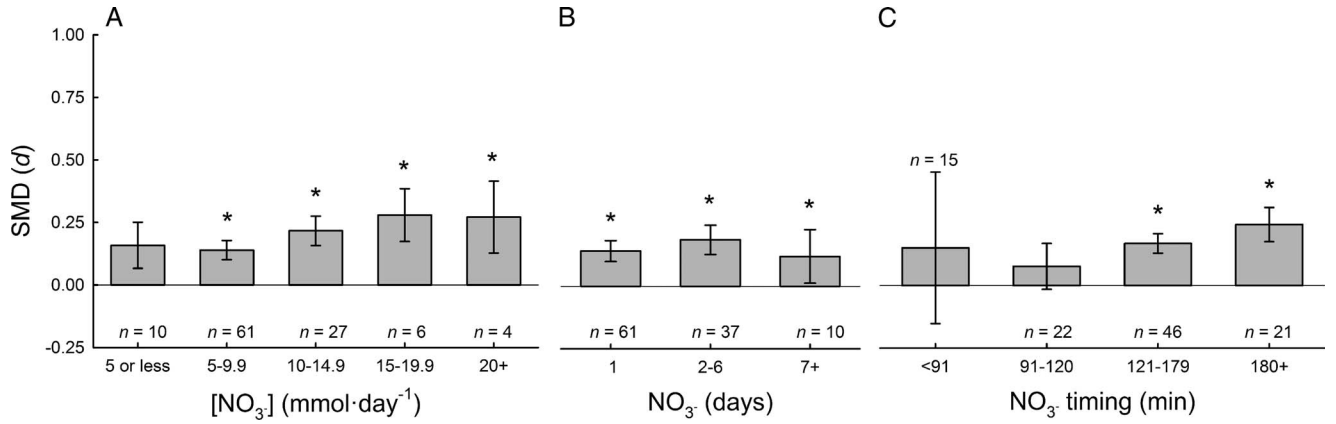

FIGURE 5—Subgroup analysis of NO_3^- supplementation parameters. SMD of NO_3^- supplementation compared with placebo for different daily concentrations of NO_3^- supplementation (A), different number of days of NO_3^- supplementation (B), and different timing of NO_3^- supplementation relative to commencement of exercise (C) calculated used random-effects meta-analyses. * denotes better performance after NO_3^- supplementation compared with placebo, $P < 0.05$.

(7) and McMahon et al. (13), the smallest effect size was observed for studies utilizing time-trial performances and the largest effect size was observed for TTE protocols.

Subgroup analysis: NO_3^- dosage and timing. The influences of NO_3^- supplementation parameters were also explored. The ergogenic effect of NO_3^- supplementation was not different between daily concentrations of NO_3^- supplementation greater than 5 mmol·d^{-1} (range, 5.1–28.7 mmol·d^{-1}); however, there was no ergogenic effect with low NO_3^- supplementation (range, 1.6–5.0 mmol·d^{-1}; $P = 0.085$). The ergogenic effect of NO_3^- supplementation was not different between the number of days of NO_3^- supplementation (range, 1–15 d). However, the ergogenic effect of NO_3^- supplementation was different with the timing of NO_3^- supplementation relative to exercise performance (range, 5–210 min before exercise; Fig. 5. The optimal timing of NO_3^- supplementation is 2–3.5 h before the onset of exercise. Dr. Jones' groups has previously examined the pharmacokinetics and dose response of NO_3^- supplementation on plasma [NO_3^-], and determined that the timing of the peak plasma [NO_3^-] is dependent on the NO_3^- dose ingested (19). The potential interaction of NO_3^- dose and timing was explored in these data; however, no significant interaction was evidenced. Based on these data, the optimal ergogenic effect of NO_3^- supplementation is with the following parameters of NO_3^- supplementation: 1) any dose between 5.1 and ~25 mmol·d^{-1}, 2) at least 1 d of supplementation, and 3) ingestion of NO_3^- 2 – 3.5 h before initiation of exercise.

Based on these data, a secondary pooled analysis was performed after removing the studies that administered NO_3^- in an inadequate dose (≤5 mmol·d^{-1}; $n = 10$) or with insufficient time to adequately metabolize the NO_3^- before exercise (<91 min before exercise; $n = 15$). The results of this secondary pooled analysis are similar to our primary pooled analysis (0.185 (0.125–0.244); $z = 6.102$, $P < 0.001$). Similarly, removal of these 25 data sets with ineffective NO_3^- supplementation did not change interpretations of any of the subgroup analyses but marginally increased Cohen's d SDM by ~0.02.

Despite the heterogeneity with NO_3^- supplementation parameters, a vast proportion of studies used a similar type of commercial NO_3^- supplementation and placebo from *Beet it* (James White Drinks, Ipswich, United Kingdom). The placebo is created by passage of the beetroot juice, before pasteurization, through a column containing Purolite A520E ion-exchange resign, which selectively removes NO_3^- ions (39). Thus, the placebo is an identical version of the beetroot juice in appearance and taste, with negligible levels of NO_3^- ions.

Risk of bias. Publication bias was assessed using a funnel plot (Fig. 6). Visual inspection of the funnel plot shows that three studies fall below 95% CI and three studies are above 95% CI. Egger's regression test suggests that there is no significant asymmetry of the plot (intercept = 1.18, $P = 0.07$).

DISCUSSION

This systematic review incorporated 80 studies investigating exercise performance after ingestion of NO_3^- supplementation

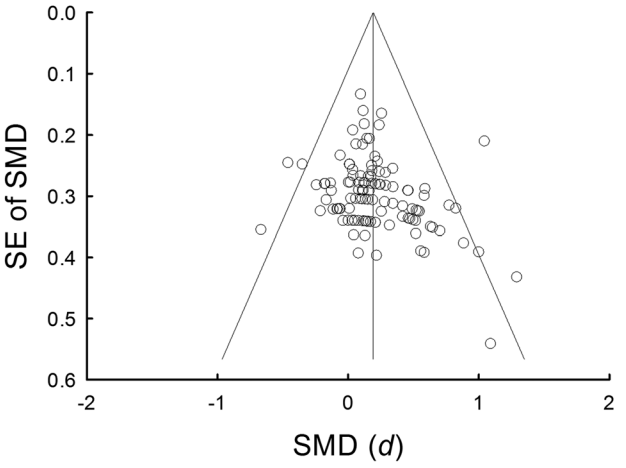

FIGURE 6—Funnel plot of the SE and standardized effect for each study. The angled lines define the area including the 95% CI of the SMD, and the vertical line defines the middle of the funnel at the mean SMD. Visual inspection of the funnel plot shows that three studies fall below 95% CI and three studies are above 95% CI.

or placebo supplement with negligible NO_3^- content in ~1300 subjects. Contrary to much of the literature (~2/3 studies), there was a clear ergogenic effect of NO_3^- supplementation across many different exercises modalities in normoxic and hypoxic conditions in young men, but not women or elite athletes ($\dot{V}O_{2peak}$ > 64.9 mL·kg^{-1}·min^{-1}). In agreement with previous recommendations, the ergogenic effect of NO_3^- supplementation is limited in highly trained athletes and lower-intensity/long-duration exercise (>15 min) (8,9). The optimal NO_3^- supplementation was between 5 and ~25 mmol·d^{-1} ingested 2–3.5 h before exercise. These data provide robust support of previous contentions from individual studies (19,53) and narrative reviews (1,9,117) considering optimal NO_3^- supplementation parameters. Thus, the general parameters for "successful" NO_3^- supplementation gleaned from our analysis are not novel; however, the current meta-analyses substantiate previous claims from leading experts regarding "best practices" for NO_3^- supplementation. These data may also be useful in designing studies that consider the effects of NO_3^- supplementation on other modes of exercise in other demographic groups.

The effect size of NO_3^- supplementation was objectively small (d = 0.174) even with the removal of studies administering suboptimal NO_3^- supplementation (d = 0.185), and this small effect size likely explains the large number of studies (~68%) that do not observe an ergogenic effect of NO_3^- supplementation on exercise performance. Although the effect size is small, these data demonstrate a quantitative and repeatable enhancement of exercise performance by ~3% (e.g., 48 s in 16.1-km cycling time trial [32]) across many different exercise modalities and performances. In the context of athletic competition, the ~3% ergogenic effect of NO_3^- supplementation may be highly meaningful and is not dissimilar to the potential ergogenic effect of new running shoes with embedded carbon-fiber plates (e.g., Nike Next%) (118). Thus, although the effect size of NO_3^- supplementation is small, these data suggest NO_3^- as a viable ergogenic aid.

Inorganic versus organic nitrates. Although often underreported, it is nearly ubiquitous for studies to use inorganic nitrate for supplementation for enhanced exercise performance. The differences between organic and inorganic nitrate are related to their underlying chemical structure, with organic nitrates primarily used in medicine (e.g., glyceryl trinitrate) and inorganic nitrates are primarily found in plants, particularly green leafy vegetables and beetroot plants (119). As reviewed previously, the pharmacokinetic properties of organic versus inorganic nitrates are markedly different, and within the current data, all studies collated utilized inorganic nitrate supplementation (119). Thus, the effect of organic nitrate supplementation as an ergogenic aid is unknown but may be limited by developed tolerance and potential endothelial dysfunction with prolonged use (119,120).

Oral microbiome. The ergogenic potential of NO_3^- supplementation is largely dependent on the reduction of concentrated NO_3^- to nitrite (NO_2^-), which is regulated by anaerobic bacteria in the oral cavity (121–123). Although the oral microbiome may be disturbed by many oral substances (e.g., antibiotics, antibacterial mouthwash, gum chewing, etc.) (124), only about 50% of studies reported controlled environments for the oral microbiome; for example, "subjects were asked to abstain from using antibacterial mouthwash and chewing gum…" (115) Thus, variability in the oral microbiome may contribute to the observed variability in the efficacy of NO_3^- supplementation. Furthermore, recent studies have demonstrated that under controlled conditions, the reduction of NO_3^- to NO_2^- in biological fluids varies substantially within individuals across repeated visits (121). The large variability in the performance-enhancing effects of NO_3^- supplementation may be due to the profound biological variability of the oral microbiome (121), and this postulation warrants future investigation. One approach to reduce the potential impact of the variability of oral microbiome is to provide NO_3^- supplementation for several days before an exercise test, which has been shown to increase abundance of some bacteria capable of NO_3^- reduction (123), which may optimize the effectiveness of NO_3^- supplementation.

Sex differences. As previously reviewed (23), there is a clear underrepresentation of women in the study of NO_3^- supplementation and more generally in science (116). In the current data, women account for ~10% of the total sample size, and there was no ergogenic effect of NO_3^- supplementation. The absence of an ergogenic effect of NO_3^- supplementation in women is likely spurious because of a dearth of studies including women, and the sex bias in studies of NO_3^- supplementation has created a field that is ripe with opportunities for future study. A recent review from Wickham and Spriet (23) explicitly provides a strong rationale for potential sex differences in response to NO_3^- supplementation and highlights areas for future scientific inquiry.

CONCLUSIONS

This systematic review and meta-analysis clearly demonstrates a ~3% performance-enhancing effect after optimal NO_3^- supplementation for healthy, young men that is often not observed in individual studies likely because of the small effect size. Importantly, these data support previous assertions from narrative reviews regarding optimal NO_3^- supplementation parameters and highlight a dearth of studies including women. The performance-enhancing effect of NO_3^- supplementation was not observed with administration of low doses of NO_3^- (≤5 mmol·d^{-1}) or NO_3^- dose within 90 min before exercise, or in participants with excellent $\dot{V}O_{2peak}$ values (>64.9 mL·kg·min^{-1}). Thus, NO_3^- supplementation was demonstrated to be an effective ergogenic aid for young, healthy men; however, additional mechanistic research and studies incorporating a wide variety of subjects (e.g., women) are warranted to advance the study of NO_3^- supplementation as an ergogenic aid.

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