International Journal of Sport Nutrition and Exercise Metabolism, 2013, 23, 522-532

INTERNATIONAL JOURNAL OF
SPORT NUTRITION AND
EXERCISE METABOLISM
www.IJSNEM-Journal.com
SCHOLARLY REVIEW

The Effect of Nitrate Supplementation on Exercise Performance in Healthy Individuals: A Systematic Review and Meta-Analysis

Matthew W. Hoon, Nathan A. Johnson, Phillip G. Chapman, and Louise M. Burke

The purpose of this review was to examine the effect of nitrate supplementation on exercise performance by systematic review and meta-analysis of controlled human studies. A search of four electronic databases and cross-referencing found 17 studies investigating the effect of inorganic nitrate supplementation on exercise performance that met the inclusion criteria. Beetroot juice and sodium nitrate were the most common supplements, with doses ranging from 300 to 600 mg nitrate and prescribed in a manner ranging from a single bolus to 15 days of regular ingestion. Pooled analysis showed a significant moderate benefit (ES = 0.79, 95% CI: 0.23–1.35) of nitrate supplementation on performance for time to exhaustion tests (p = .006). There was a small but insignificant beneficial effect on performance for time trials (ES = 0.11, 95% CI: –0.16–0.37) and graded exercise tests (ES = 0.26, 95% CI: –0.10–0.62). Qualitative analysis suggested that performance benefits are more often observed in inactive to recreationally active individuals and when a chronic loading of nitrate over several days is undertaken. Overall, these results suggest that nitrate supplementation is associated with a moderate improvement in constant load time to exhaustion tasks. Despite not reaching statistical significance, the small positive effect on time trial or graded exercise performance may be meaningful in an elite sport context. More data are required to clarify the effect of nitrate supplementation on exercise performance and to elucidate the optimal way to implement supplementation.

Keywords: ergogenic aids, endurance, nitric oxide, time trial, sport supplements

Nitrate (NO_3^-) is a naturally occurring anion in the human body, initially believed to be an inert by-product of nitric oxide metabolism (Lundberg et al., 2008). Although nitric oxide has various roles that are vital to normal body function including neurotransmission (Vincent, 2010), vascular control (Kelm & Schrader, 1990), mitochondrial respiration (Brown, 1999), and skeletal muscle contraction (Reid, 2001), it was originally believed that nitrate itself had little specific activity. It has since been established that nitrate may be reduced to its antecedents nitrite and nitric oxide in vivo, particularly in environments of hypoxia and acidosis. Research investigating the physiological actions of nitrate has reported effects such as improvement of vascular compliance (Bahra et al., 2012), reduction of blood pressure (Larsen et al., 2007), and attenuation of oxidative stress (Carlström et al., 2011) following consumption. Given these properties, nitrate is commonly used as a pharmacological agent to treat a host of cardiovascular pathologies (Butler & Feelisch, 2008).

Hoon and Johnson are with the Dept. of Exercise and Sports Science, University of Sydney, Strathfield, Australia. Chapman is with the School of Exercise Science, Australian Catholic University, Strathfield, Australia. Burke is with the Dept. of Sports Nutrition, Australian Institute of Sport, Canberra, Australia.

In addition to its therapeutic use, nitrate supplementation has recently been studied for its potential to enhance exercise performance. One of the earliest investigations of the effects of inorganic nitrate ingestion on exercise physiology was conducted by Larsen and colleagues, who examined pulmonary and cardiovascular responses during progressive load cycling (Larsen et al., 2007). They reported the novel finding that ingestion of sodium nitrate was associated with a reduction in the oxygen cost of cycling (i.e., an improvement of gross efficiency). This finding was significant in view of the traditional belief that exercise efficiency is resistant to significant change, particularly as efficiency has been found to be similar across training status (Moseley et al., 2004). Given that efficiency/economy is considered a key predictor of endurance exercise performance (Joyner & Coyle, 2008), there is potential for nitrate supplements to be used as ergogenic aids in endurance based activities. However, the results of subsequent investigations of nitrate supplementation on exercise performance have been inconsistent, with some showing a benefit (Bailey et al., 2010; Bailey et al., 2009; Bond et al., 2012; Cermak et al., 2012a; Lansley et al., 2011a, 2011b; Masschelein et al., 2012) but others showing no significant effect (Bescós et al., 2012a, 2011; Cermak et al., 2012b; Larsen et al., 2007, 2010; Murphy et al., 2012; Peacock et al., 2012; Wilkerson et al. 2012) when compared with placebo.

Therefore the purpose of this study was to systematically review the available data and evaluate the overall efficacy of nitrate supplementation on endurance exercise performance in healthy populations by meta-analysis.

Methods

A systematic literature search was conducted by one researcher (MH) to identify studies investigating nitrate supplementation and exercise performance. Online scientific databases searched from inception to August 2012 included Medline (Ovid), SportDiscus, Science citation index of Web of Knowledge, and PubMed. The keywords employed in the search were *nitrate* and *exercise*. Reference lists of all retrieved papers were manually searched for potentially eligible papers.

Inclusion and Exclusion Criteria

Inclusion and exclusion criteria were determined *a priori* by two researchers (MH and NJ). Only intervention studies in peer-reviewed journals were considered. Other article types such as book sections, opinion articles, observational studies, and abstracts without adequate data and reviews were not included. Study participants were required to be apparently healthy adults with no reported known disease, while nonhuman studies were excluded. Studies were required to employ at least one trial involving nitrate supplementation and a control or placebo trial in which no active supplement was given. Only studies using inorganic nitrate were included for review, due to the reported differences in pharmacokinetic properties of inorganic versus organic nitrate (Omar et al., 2012). Trials employing the use of additional supplements likely to affect performance were excluded. Trials were also required to employ a quantifiable measure of exercise performance.

Selection of Studies

Following removal of duplicates, the title and abstract of the remaining references were screened independently against the eligibility criteria by two researchers (MH and PC). Where information was insufficient, further screening of methods and results was undertaken. Disagreements concerning the eligibility of a paper were settled by discussion or consultation with a third researcher (NJ).

Data Extraction

The outcome measures assessed in this review were measures of exercise capacity or performance. This included graded exercise tests to exhaustion, time to exhaustion and time/distance trials. Data on participant characteristics (sex, training status), nutritional intervention (nitrate dose and delivery method), and exercise test data were extracted independently by two researchers (MH and PC). Where required, means and standard deviations were calculated using appropriate equations ($SE = SD/\sqrt{n}$; Hozo et al., 2005). If studies included more than one appropriate data set (such as an additional nitrate supplementation trial or exercise assessment), these were extracted and analyzed as a separate result.

Analyses and Meta-Analyses

The between-trial standardized mean difference (nitrate vs. placebo) and 95% confidence intervals (CI) were determined using Comprehensive Meta-analysis software (Version 2, Biostat, Englewood NJ, 2005). Given the small sample sizes in each study ($n < 20$), Hedges' g was selected as the measure of effect size (Hedges, 1981), with interpretations of magnitude based on Cohen's definitions of small (0.2), moderate (0.5) and large (0.8) effects (Cohen, 1988). Between study variability was assessed using the I^2 measure of inconsistency (Higgins et al., 2003) and pooled estimates of the effect of nitrate supplementation versus placebo on exercise performance (using effect size) were then calculated using a fixed-effects model. Subanalysis (determined a priori) was performed based on the type of exercise assessment employed in each study.

Results

Descriptive Data

The initial search of electronic databases yielded 2,776 results in total. An additional 11 studies were included following a search of reference lists in retrieved manuscripts. Following the removal of duplicates and elimination of papers based on eligibility criteria, 17 studies remained (Figure 1). In total 184 subjects (170 male, 14 female) participated in the 17 studies (Table 1).

Nine studies recruited subjects who reported regularly participating in a structured exercise training (of these 7 were classified as "highly trained" athlete populations) and 8 recruited "recreationally fit" populations. All studies were controlled trials which employed a randomized crossover design involving a placebo condition and at least one condition using nitrate supplementation. Two studies employed more than one exercise test in their investigations. Lansley et al. (2011a) assessed performance in both a 4 km time trial and a separate 16 km time trial following intervention. Vanhatalo et al. (2010) implemented a chronic supplementation strategy and assessed performance on days 1, 5, and 15 of intervention. These trials were included into analysis. Multiple types of exercise assessments were employed, with five studies using a graded exercise test to exhaustion (GXT), four employing a constant work rate time to exhaustion protocol (TTE) and eight studies examining the effect of supplementation versus placebo on endurance exercise time/distance (i.e., time trial [TT]). One study employed a repeated time trial design, where 6 × 500 m time trials were performed as quickly as possible and the average of these reported. This was therefore considered as a time trial for exercise assessment sub analysis. The majority

Table 1 Summary of Included Studies Examining the Effect of Nitrate Supplementation Versus Placebo on Exercise Test Performance

Reference	Subjects, *n* (male)	Nitrate protocol	Exercise assessment	Trial result ± *SD*
Time Trials				
Bescós et al. (2012a)	well-trained cyclists 13 (13)	10 mg nitrate per kg body mass a day (as $NaNO_3$) for 3 days	40-min cycle distance trial	N: 26.4 ± 1.1 km[a] P: 26.3 ± 1.2 km
Bond et al. (2012)	well-trained junior rowers 14 (14)	500 ml beetroot juice (~340 mg NO_3^-) per day for 6 days	6 × 500 m rowing ergometer trials	N: 0.4% ± 1.0% (95% CI) improvement vs P
Cermak et al. (2012a)	well-trained cyclists 13 (13)	140 ml beetroot juice concentrate/day (~500 mg NO_3^-) for 6 days	10 km cycle time trial	N: 953 ± 18 s P: 965 ± 18 s
Cermak et al. (2012b)	well-trained cyclists 20 (20)	140 ml beetroot juice concentrate (~550 mg NO_3^-), 150 min before exercise	energy expenditure based time trial	N: 65.5 ± 1.1 min P: 65.0 ± 1.1 min
Lansley et al. (2011a; i)	moderately trained cyclists 9 (9)	500 ml beetroot juice (~350mg NO_3^-), 150 min before exercise	4 km cycle time trial	N: 6.27 ± 0.35 min P: 6.45 ± 0.42 min
Lansley et al. (2011a; ii)	as above	as above	16 km cycle time trial	N: 26.9 ± 1.8 min P: 27.7 ± 2.1 min
Murphy et al. (2012)	healthy, recreationally fit 11 (15)	200 g whole beetroot (~500 mg NO_3^-), 75 min before exercise	5 km treadmill time trial	N: 12.3 ± 2.7 km/h[a] P: 11.9 ± 2.6 km/h
Peacock et al. (2012)	well-trained cross country skiers 10 (10)	1 g KNO_3 (614 mg NO_3^-), 150 min before exercise	5 km running time trial	N: 1005 ±53 s P: 996 ± 49 s
Wilkerson et al. (2012)	well-trained cyclists 13 (13)	500 ml beetroot juice (~380 mg NO_3^-), 150 min before exercise	50 mi cycle time trial	N: 136.7 ± 5.6 min P: 137.9 ± 6.4 min
Time to Exhaustion Tests				
Bailey et al. (2009)	healthy, recreationally fit 8 (8)	500 ml beetroot juice (~340 mg NO_3^-) a day for 6 days	cycling TTE @ 70% between GET and VO_{2max}	N: 675 ± 203 s P: 583 ± 145 s

(continued)

Table 1 *(continued)*

Reference	Subjects, *n* (male)	Nitrate protocol	Exercise assessment	Trial result ± *SD*
Bailey et al. (2010)	healthy, recreationally fit 7 (7)	500 ml beetroot juice (~300 mg NO_3^-) a day for 6 days	leg extension TTE @ 30% MVC	N: 734 ± 109 s P: 586 ± 80 s
Lansley et al. (2011b)	healthy, recreationally fit 9 (9)	500 ml beetroot juice (~380 mg) a day for 6 days	treadmill time to exhaustion @ 75% between GET and VO_{2max}	N: 8.7 ± 1.8 min P: 7.6 ± 1.5 min
Vanhatalo et al. (2011)	healthy, recreationally fit 9 (7)	750 ml beetroot juice (~580 mg NO_3^-), 24 h prior, with last 250 ml 2.5 h pre exercise	leg extension TTE @ 14.5% O_2	N: 477 ± 200 s P: 393 ± 169 s
Graded Exercise Tests				
Bescós et al. (2011)	well-trained cyclists 11 (11)	10 mg nitrate per kg body mass (as $NaNO_3$) 3 hr before exercise	cycle incremental TTE	N: 416 ± 32 s P: 409 ± 27 s
Larsen et al. (2007)	moderately trained cyclists 9 (9)	0.1 mmol (6.2 mg) NO_3^- per kg body mass per day (as NaNO3) for 3 days, last dose 60 min before exercise	cycle incremental time to exhaustion	N: 360.6 ± 32.8 W_{max} P: 358.9 ± 32.3 W_{max}
Larsen et al. (2010)	healthy 9 (7)	0.1 mmol (6.2 mg) NO_3^- per kg body mass per day (as $NaNO_3$) for 2 days	arm crank and cycle incremental time to exhaustion	N: 563 ± 30 s P: 524 ± 31 s
Masschelein et al. (2012)	healthy, recreationally fit 15 (15)	0.07 mmol (4.4 mg) NO_3^- per kg body mass per day (as beetroot juice) for 6 days	cycle incremental TTE @ simulated 5,000m altitude	N: 597 ± 22 s P: 568 ± 23 s
Vanhatalo et al. (2010)	healthy, recreationally fit 9 (5)	500 ml beetroot juice (~300 mg NO_3^-) 2.5 h before exercise	cycle incremental TTE	N: 325 ± 71 W_{max} P: 322 ± 68 W_{max}
as above	as above	500 ml beetroot juice (~300 mg NO_3^-) per day for 5 days	as above	N: 328 ± 68 W_{max} P: 323 ± 67 W_{max}
as above	as above	500 ml beetroot juice (~300 mg NO_3^-) per day for 15 days	as above	N: 331 ± 68 W_{max} P: 323 ± 68 W_{max}

Note. TTE—time to exhaustion; W_{max} = peak power; N = nitrate trial; P = placebo trial; NR = not reported; GET = gas exchange threshold; MVC = maximal voluntary contraction.

[a]Greater number denotes improved performance.

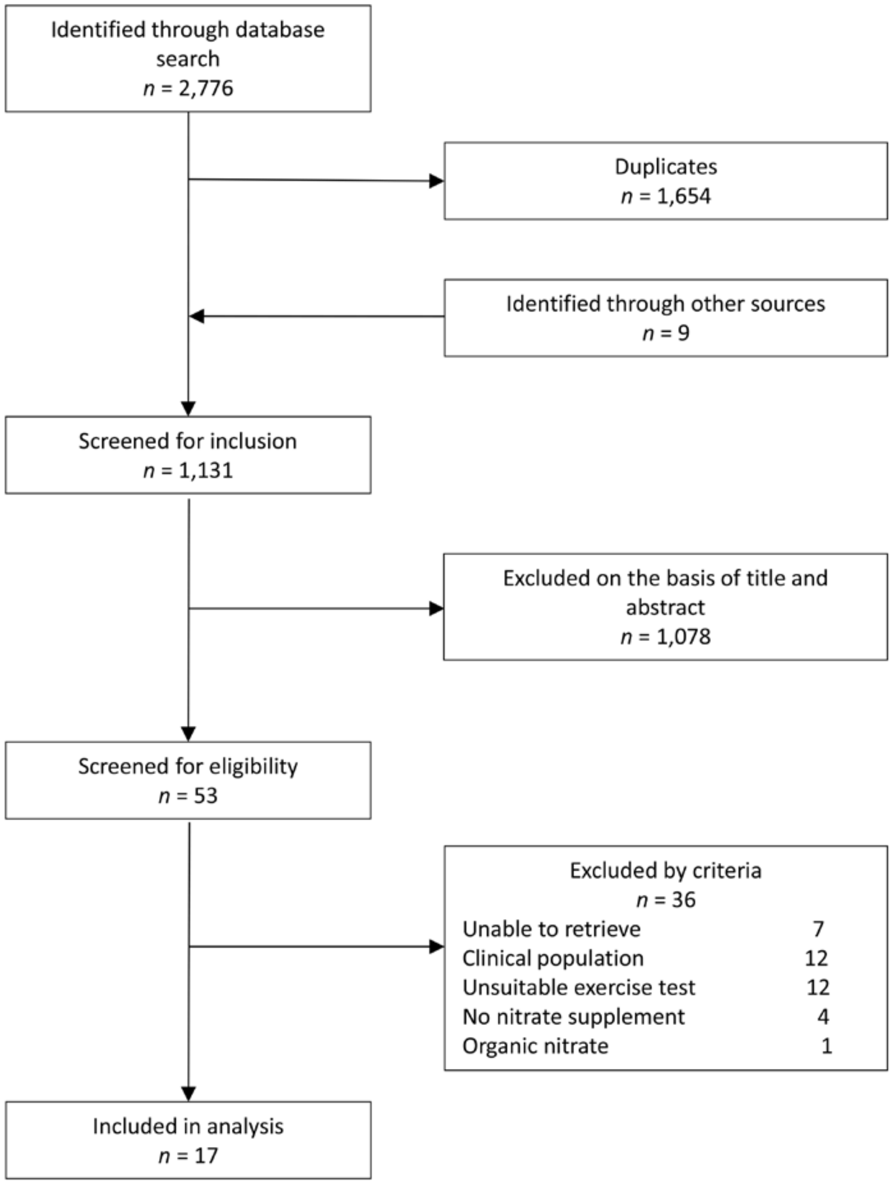

Figure 1 — Search strategy and results.

(*n* = 10) of the 12 studies based on TTE or TT protocols employed an aerobic exercise test involving a mean exercise time ≥7 min at an estimated *severe* intensity, whereas only two studies used *extreme* intensity exercise (Jones & Poole, 2005). Although a majority of studies were conducted in a normoxic environment, two studies investigated exercise capacity in hypoxia.

Nitrate Supplementation

A majority of studies (*n* = 12) used beetroot as the source of nitrate delivery, with 11 studies prescribing beetroot juice and one study providing whole beetroots to participants. The remainder of studies delivered nitrate through chemical means, either as sodium nitrate (*n* = 5) or potassium nitrate (*n* = 1). The supplementation protocol also varied across studies, with 8 studies examining the effect of acute supplementation (supplement given 75–180 min before exercise) and 10 employing a chronic dosing schedule which ranged from several boluses over a 24 hr period before exercise, to 15 days of nitrate loading (Table 1).

Adverse Events

There were few reports of adverse events as a result of nitrate supplementation. Several papers noted incidents of beeturia (discoloration of urine) following consumption of beetroot juice specifically (Bailey et al., 2010; Bailey et al., 2009; Vanhatalo et al., 2010); however, no major health consequences were reported in any study.

Effect of Nitrate Supplementation on Exercise Capacity and Performance

Methodological heterogeneity (I^2; i.e., the percentage of the total variability in the set of effect sizes due to true heterogeneity) for studies when grouped by exercise assessment (time trial, time to exhaustion and graded exercise) was low (<25%). This indicates that it is appropriate to pool study results for meta-analysis as any between study differences are likely due to sampling error rather than differences in design. The pooled corrected effect size from studies examining time trial performance was 0.11 (95% CI: -0.16–0.37) indicating a small effect in favor of nitrate supplementation over placebo, with six of the nine trials reporting an improvement in performance under the nitrate condition, however this was not statistically significant ($p = .43$; Figure 2a). The two studies assessing exercise performance in hypoxia were not included in the pooled effect size calculation due to the dissimilarity in physiological stress. However the individual effects of these studies are presented in Figure 2d. The pooled effect size for the graded exercise tests to exhaustion in normoxia was 0.26 (–0.10–0.62) in favor of nitrate supplementation ($p = .16$; Figure 2b). The three studies that assessed time to exhaustion at a fixed work rate (in normoxia) all reported favorable results under nitrate supplementation, with a combined pooled effect size of 0.79 (0.23–1.35, $p = .006$), representing a moderate effect (Figure 2c).

Discussion

This is the first systematic review with meta-analyses to examine the efficacy of nitrate supplementation on exercise performance in healthy populations. The results of our analyses show that when compared with a placebo control, nitrate supplementation did not significantly affect time trial performance or performance during graded exercise testing. However, pooled analysis showed that nitrate supplementation increased performance during time-to-exhaustion exercise protocols by a moderate degree.

This systematic review and meta-analyses combined 17 studies involving a total of 184 participants. Nine of the studies examined supplementation in trained cohorts while the remainder employed healthy untrained subjects. Exercise performance in these studies was examined in multiple ways, namely fixed intensity and graded exercise tests to exhaustion and time trial tasks. Overall, the literature demonstrated a wide variation in the manner nitrate was supplemented. Several sources of nitrate were employed, ranging from pharmacological (sodium and potassium nitrate) to natural products (beetroot juice, vegetables). The bioavailability of nitrate from each of these substances has yet to be investigated, so it is difficult to ascertain if the nitrate source influences its potency. It is important to note that all studies included in this analysis used the inorganic form of nitrate for

Study	Hedges's *g* (95% CL)	*p*-value
Peacock et al. 2012	–0.17(–1.01 to 0.67)	.694
Wilkerson et al. 2012	0.19(–0.74 to 1.12)	.691
Cermak et al. 2012a	0.65(–0.12 to 1.41)	.098
Bescos et al. 2012a	–0.08(–0.83 to 0.66)	.825
Lansley et al. 2011a	0.44(–0.45 to 1.34)	.330
Lansley et al. 2011b	0.39(–0.50 to 1.28)	.390
Cermak et al. 2012b	–0.47(–1.08 to 0.15)	.138
Murphy et al. 2012	0.15(–0.66 to 0.95)	.724
Bond et al. 2012	0.30(–0.42 to 1.03)	.414
Combined	**0.11 (–0.1 6 to 0.37)**	**.427**

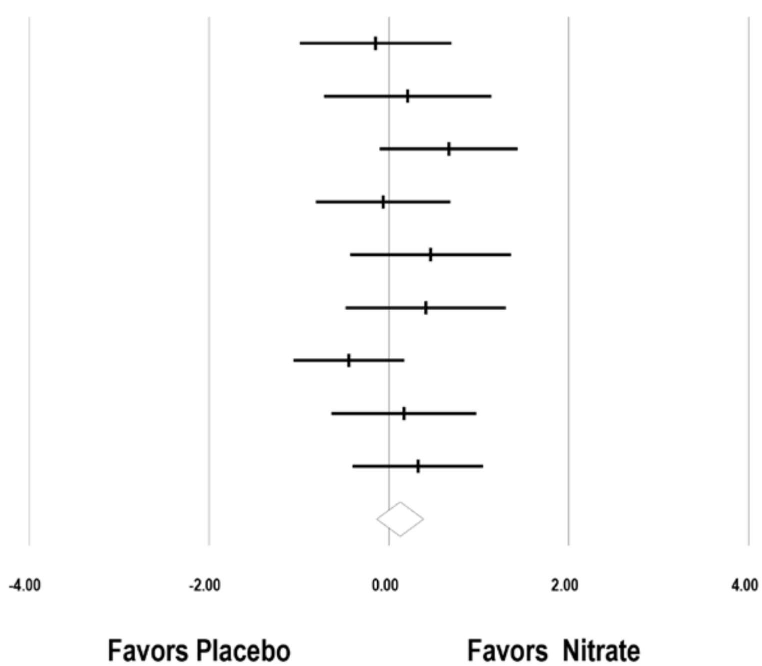

Figure 2a — Effect of nitrate supplementation on time trial performance

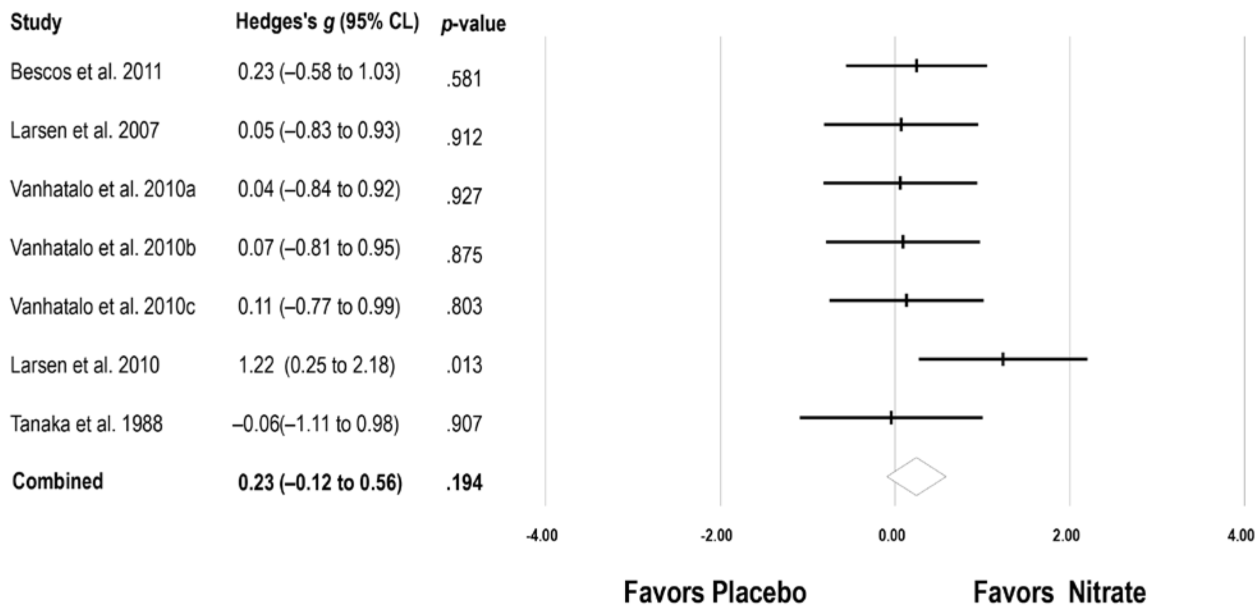

Figure 2b — Effect of nitrate supplementation on graded exercise test performance

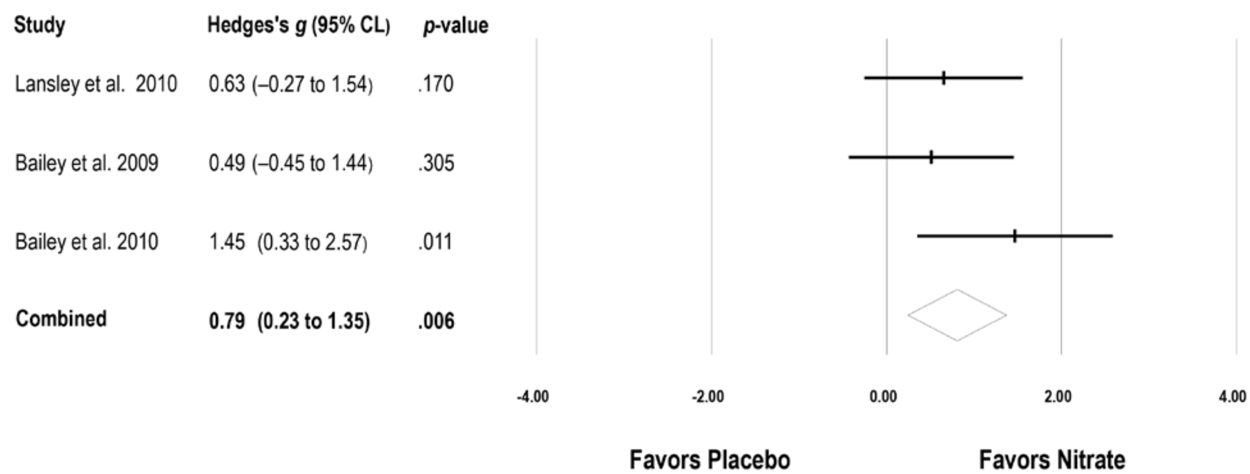

Figure 2c — Effect of nitrate supplementation on time to exhaustion tasks

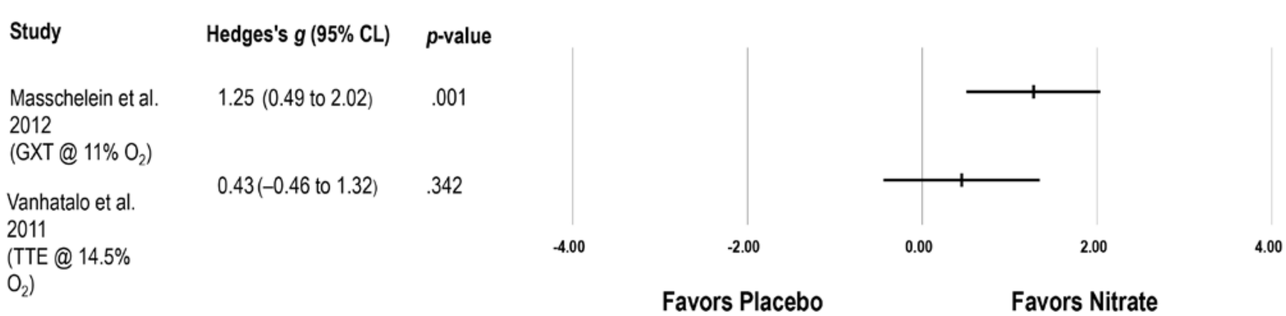

Figure 2d — Effect size estimates of nitrate supplementation versus a placebo control in studies assessing exercise capacity in hypoxia. GXT = graded exercise test; TTE = time to exhaustion

supplementation. Organic nitrate is most commonly found in pharmaceutical agents (e.g., glyceryl trinitrate), which may not be appropriate for use as a sport supplement. Despite their similar physiological effects, organic and inorganic nitrate possess different chemical structures and pharmacokinetics (Omar et al., 2012). Furthermore, continual use of organic nitrates may result in tolerance and may increase the risk of endothelial dysfunction (Abrams, 2002).

The dose and timing of supplementation was also highly variable across studies. A majority ($n = 11$) implemented a chronic dosing protocol, supplementing subjects with multiple boluses of nitrate 24 hr to 15 days before exercise. However several studies demonstrated either an improvement in performance (Lansley et al., 2011a) or exercise efficiency (Bescós et al., 2011; Wilkerson et al., 2012) arising from an acute dose of nitrate 75–150 min before exercise, suggesting effects may occur in a relatively short time frame. Overall, a multiple day dosing strategy may be more efficacious for improving exercise performance, as a greater proportion of chronic supplementation trials favored nitrate supplementation (11 of 12) compared with the acute studies (6 of 8). This notion was supported by Vanhatalo et al. (2010), who directly compared the effects of acute and chronic supplementation. No detectable improvement in performance during a graded exercise test was found following an acute (2.5 hr before exercise) 0.5 L dose of beetroot juice, however following 5 and 15 days of supplementation (0.5 L of beetroot juice per day), peak power and power at gas exchange threshold improved with duration (days) of supplementation. Nevertheless, only the results at 15 days of supplementation demonstrated significance.

The studies included in our pooled effect size analysis were relatively homogenous in study design. All were conducted in a randomized crossover fashion and used a placebo trial. Time trial type assessments are generally accepted as the best indicator of real world performance, due to their close resemblance to the demands of competition (Hopkins et al., 2001). The majority of these studies assessed severe intensity endurance performance with time trials lasting 15–138 min. Analysis of these studies found a mixed response to nitrate, with four of seven studies favoring supplementation. Only two studies assessed nitrate supplementation in time trials of similar or shorter duration i.e., in the extreme intensity domain (Jones & Poole, 2005). The outcomes of these include improvement of 4 km cycle time trial (Lansley et al., 2011a) and repeated 500 m time trials on a rowing ergometer (Bond et al., 2012) in trained participants. These high intensity events are likely to induce local (intracellular) acidosis and hypoxia, during which the reduction of nitrate to nitric oxide is greatest, having its strongest physiological effect (Lundberg et al., 2008). However there is currently a lack of research on the effect of nitrate on high intensity exercise and these purported physiological mechanisms remain hypothetical.

Two studies were excluded from pooled effect estimates as the exercise tests were undertaken under environmental hypoxia, presenting a distinctly different scenario to the majority of studies. A reduction in atmospheric oxygen is known to have an ergolytic effect on endurance performance (Masschelein et al., 2012). The nitrate-nitrite-nitric oxide pathway is more active under hypoxia (Lundberg et al., 2008) serving to offset the its detrimental effects, to the extent that time to exhaustion in hypoxia following beetroot consumption was similar to a control trial conducted in normoxia (Vanhatalo et al., 2011). It is postulated that the reduction of nitrate to nitric oxide serves to improve the transport and utilization of oxygen, a key limiting factor of endurance in hypoxic conditions. Further investigation has demonstrated an increase in arterial and muscle oxygenation (as measured by near-infrared spectroscopy) during exercise at a simulated 5,000 m following nitrate supplementation. This was accompanied by a 36% greater incremental time to exhaustion compared with a hypoxic control (Masschelein et al., 2012). To date, the two aforementioned studies are the only investigations of nitrate use in hypoxia, however positive findings from research into other vasodilative agents (Hsu et al., 2006) suggest this area to be of potential.

Therapeutic nitrates have traditionally been used for their vasodilative properties, relieving ischemic-based complications in morbidities such as peripheral artery and cardiovascular disease. In addition, nitrate has been found to improve the efficiency (i.e., reduce the energy cost) of exercise as indicated by a 4–5% reduction in VO_2 at steady state (Bailey et al., 2009, 2010; Lansley et al., 2011b; Larsen et al., 2007). In a review by Joyner and Coyle, exercise efficiency was identified as one of three key physiological components predicting endurance exercise performance, as it directly determines the speed or power that may be maintained at a particular rate of oxygen consumption (Joyner & Coyle, 2008). The mechanism by which nitrates act to alter exercise efficiency/economy remains contentious. It is hypothesized that increased levels of nitric oxide following supplementation may reduce the ATP cost of force production (Bailey et al., 2010). In vivo analysis of exercising muscle by 31-phosphorus magnetic resonance spectroscopy (^{31}P-MRS) showed a decline in muscular [Pi] and [ADP] accumulation and a reduced utilization of PCr stores following supplementation with beetroot juice (Bailey et al., 2010), suggesting a reduction in ATP turnover at the same work rate. Based on biochemical analysis of animal muscle, researchers have suggested that the reduction of ATP use is a consequence of nitric oxide's regulatory effect on the ATP consuming processes of sarcoplasmic reticulum calcium pumping (Viner et al., 2000) or myofibrillar actin-myosin interaction (Galler et al., 1997) in force production. A recent investigation found increases in myoplasmic [Ca^{2+}] and Ca^{2+} handling proteins (accompanied by an increase in contractile force of fast-twitch muscle fibers) following seven days of nitrate treatment in mice (Hernandez et al., 2012), supporting the idea nitrate may elicit its effect in this manner. Alternatively, the nitrate-nitrite-nitric oxide pathway may directly influence mitochondrial efficiency. Three days of nitrate supplementation reduced the P/O ratio (i.e., the amount of oxygen consumed per ATP molecule produced) of isolated mitochondria from the vastus lateralis, which explained much of the variance in the reduced oxygen cost of exercise (Larsen et al., 2011). The authors suggested that this effect is attributable to the reduction in proton leakage through the mitochondrial membrane (i.e., mitochondrial coupling) possibly due to reduced expression of adenine nucleotide translocase.

The literature suggests an interaction between training status and the ergogenic effect of nitrate supplementation. All of the eleven trials conducted in untrained individuals reported a favorable result with nitrate supplementation, compared with seven of ten trials in trained subjects. The reason for this phenomenon has not been established; however, adaptations to endurance training may play a role (Bescós et al., 2012b). Fitter individuals have been found to possess superior vascular control as characterized by a greater activity and presence of eNOS (endothelial nitric oxide synthase), the enzyme responsible for endogenous generation of nitric oxide (Green et al., 2004). An increase in eNOS activity may diminish the reliance on nitrate-derived nitric oxide thereby reducing the potency of nitrate supplementation. Similarly, when compared with an untrained individual, a trained athlete may be less likely to experience the physiological stimuli (low muscle oxygenation and muscle acidosis) favorable for nitrate reduction at a given work rate (Wilkerson et al., 2012). Six of the nine studies which employed a trained subject cohort reported a favorable result for nitrate supplementation; however, none reached significance. Further research is required to elucidate the factors influencing individual response to nitrate supplementation.

Limitations

The heterogeneity in study design across included studies restricted pooled effect estimate of the combined total data set and subanalyses of parameters likely to influence the potency of nitrate supplementation (i.e., training status and dosage strategy). We chose a priori to differentiate studies only on the basis of exercise test protocol as we have justified previously (Temesi et al., 2011). We acknowledge that differences existed in subject characteristics and nitrate supplementation regimens within these subanalyses, but the heterogeneity of these was low ($I^2 < 25\%$) supporting the use of our pooled approach. Studies were generally characterized by small sample sizes, further warranting pooling of data to increase statistical power. A limitation of our analyses, which were based on effect size calculations, is that small but meaningful performance effects may have been undetected. The expressions of *small*, *moderate*, and *large* used by Cohen to describe effect size measures have been noted to be rather arbitrary in nature and should rather be viewed as relative terms (Cohen, 1988). As discussed by Hopkins, traditional statistical methods may not be sensitive enough to detect the small changes in performance considered practically useful to an athlete (Hopkins & Batterham, 2005).

Future Directions

At present, the research of nitrate as an ergogenic aid for exercise performance is in its infancy. The bulk of evidence shows promise, with a majority of studies reporting a favorable result for nitrate supplementation. However, as is often the case with nutritional supplements, protocols of best practice need to be developed for optimal usage. This includes elucidating the sports and environments where nitrate supplements may be most useful and refining the procedure on how it is applied, with particular focus on the quantity, timing, and quality (i.e., nitrate source) of supplementation.

Conclusion

The current meta-analysis of available research suggests a small benefit to performance may be afforded by taking nitrate-based supplements. Despite the small effect size, these gains may be considered extremely meaningful in a sports performance context. Across studies measuring time trial performance in trained cohorts, there was an approximately 0.9% improvement following nitrate supplementation. To put this in context, the measured difference between first and fourth place for elite swimming performance has been calculated to be 0.6% (Trewin et al., 2004), and improvements as little as 0.3% have been noted to be valuable to elite track and field athletes (Hopkins, 2005). At the recent UCI world track cycling championships, first and third place in both the men's individual sprint and pursuit events were separated by <0.5% (www.cyclingnews.com). As is the nature with all sports supplements, the risk to reward ratio should be considered. Given nitrate may be ingested through natural forms such as beetroot juice and vegetables, this may limit the risk of prohibited substance contamination (Maughan et al., 2004) and carry complementary health benefits associated with increased dietary nitrate intake (Lidder & Webb, 2012). In conjunction with the low number of studies reporting negative effects, nitrate supplements present as a low risk intervention that may aid endurance exercise performance.

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