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Dietary Nitrate Supplementation and Exercise-Related Performance

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Abstract

Over the last decade, there has been a growing interest in the utility of nitrate ($NO3^-$) supplementation to improve exercise-related performance. After consumption, dietary $NO3^-$ can be reduced to nitric oxide, a free radical gas involved in numerous physiological actions including blood vessel vasodilation, mitochondrial respiration, and skeletal muscle contractile function. Emerging evidence indicates that dietary $NO3^-$ supplementation has a small but nevertheless significant beneficial effect on endurance performance through the combined effects of enhanced tissue oxygenation and metabolic efficiency in active skeletal muscle. There is further evidence to suggest that dietary $NO3^-$ exerts a direct influence on contractile mechanisms within the skeletal muscle through alterations in calcium availability and sensitivity. Response heterogeneity and sizeable variability in the nitrate content of beetroot juice products influence the effectiveness of dietary $NO3^-$ for exercise performance, and so dosing and product quality, as well as training history, sex, and individual-specific characteristics, should be considered.

Over the last decade, there has been growing interest in the use of dietary nitrate (NO_3^-) supplementation to improve sport performance in endurance and power-dependent disciplines. Endurance events, characterized by rhythmic contractions of large muscle groups lasting longer than 2 minutes in duration (eg, 800-m run, 200-m swim), necessitate ample distribution and utilization of oxygen to active skeletal muscle tissue.[1] Success in power-dependent events, on the other hand, is primarily contingent upon generating maximal force as quickly as possible (eg, Olympic weightlifting or shot put).

NO_3^- is a precursor to nitric oxide (NO), a free-radical gas involved in numerous physiological processes, including blood vessel vasodilation, mitochondrial respiration, and skeletal muscle contractile function.[2] Endogenous synthesis of NO occurs continuously

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within the vascular endothelium as well as in the tissues themselves via the L-arginine pathway.[3] However, NO can also be produced through the reduction of NO_3^- to nitrite (NO_2^-), especially during situations of high-intensity physical exertion when the need for oxygenated blood to active tissue increases.[4] As exercise intensity rises, a greater amount of oxygen is consumed by contracting skeletal muscles—resulting in an increased number of metabolic by-products (eg, hydrogen ions). With repeated contractions, decreasing oxygen tension and pH level in the skeletal muscle microenvironment further accelerate the transition of NO_2^- (from dietary NO_3^-) to bioavailable NO.[5]

NO_3^- – NO_2^- – NO Pathway and Exercise

The reduction of inorganic NO_3^- found in dietary sources (eg, beetroot, spinach) to NO occurs in parallel with the endogenous synthesis of NO from L-arginine. When consumed, NO_3^- is concentrated in saliva and becomes rapidly reduced to NO_2^- by anaerobic bacteria in the oral cavity.[6] NO_2^- is then reduced to NO as digestion continues by the acidic environment of the stomach and is absorbed along with remaining NO_2^- into the plasma from the upper gastrointestinal tract.[5,7,8] NO_2^- can be further reduced to NO during situations of increased metabolic demand (ie, physical exertion) when contracting skeletal muscles extract a greater amount of oxygen from the peripheral blood per unit time.[4,5] In comparison with resting or sedentary conditions, a heightened level of NO is available to elicit effects on blood vessels and skeletal muscles during exercise.[9] Figure 1 illustrates the pathway by which exercise influences the reduction of NO_3^- to NO.

The diverse role of NO in the regulation of blood flow, oxygen uptake, and skeletal muscle contractions has implications for athletic performance.[2,10,11] Accordingly, dietary NO_3^- supplementation has been shown to result in small but nevertheless significant improvements in the performance of specific endurance and power-dependent disciplines in high-quality, placebo-controlled, double-blinded studies. However, the extent to which dietary NO_3^- ingestion may influence performance is varied and likely dependent on factors involving the dose of NO_3^- consumed, as well as training history, sex, and other individual-specific characteristics.

Dietary NO_3^- Dosing Strategies and Supplementation for Performance Enhancement

Although a variety of leafy green and root vegetables contain NO_3^-, most studies evaluating the influence of dietary NO_3^- supplementation on exercise performance have used bottled beetroot juice beverages.[12] Concentrated forms of dietary NO_3^-, like beetroot juice, deliver more NO_3^- per volume, making it an attractive option for ingestion before athletic pursuits. For example, an athlete would need to consume 12 cups of raw spinach to ingest the equivalent amount of NO_3^- provided by a single 140 mL (≈2/3 cup) serving of commercially available beetroot juice.[13,14]

To date, recommendations for the minimum amount of dietary NO_3^- needed to improve exercise performance are lacking. Doses of dietary NO_3^- ranging from 3.2 to 10.4 mg·kg body mass^{-1} taken either *acutely* (2–3 hours before exercise to coincide with peak plasma NO_2^- and NO_3^- levels) or *chronically* (over several days to weeks) have shown improvements across endurance, power-dependent, and high-intensity intermittent exercise performance (Table). A minimally effective dose of 527 mg—equivalent to 7.5 mg· kg body mass^{-1} for a 70-kg individual—has been suggested.[2] However, it is worth noting that the NO_3^- content of commercially available beetroot juice is highly variable and is rarely independently tested to confirm amounts provided by the manufacturer. For example, Gallardo and Coggan[14] found a ≈50-fold range in NO_3^- content among 24 different beetroot juice products marketed to athletes. Of note, only 2 of the products tested consistently provided an amount of nitrate greater than the minimum dose (527 mg) suggested to enhance exercise performance in most individuals.[2,14] As such, it is recommended that research studies evaluating the effects of beetroot juice supplementation on athletic performance use products of a known quality and dose and investigators should further verify that the NO_3^- content of the product is equivalent to the amount provided by the manufacturer through independent laboratory testing.

Furthermore, most studies evaluating the effects of dietary NO_3^- on athletic performance have not accounted for variations in baseline subject diet. For example, although most interventional studies require that subjects maintain their habitual diet for the duration of the intervention period and ask that they avoid foods containing high amounts of dietary nitrate (eg, leafy green vegetables, rhubarb), often, the amount of additional dietary nitrate consumed is not quantified through dietary recall. As such, it is generally unknown whether the minimally effective dose for performance enhancement is higher than the estimated 527 mg.

Dietary NO_3^- Supplementation and Endurance Performance

Substantial evidence from placebo-controlled double-blinded studies support the use of dietary NO_3^- supplementation for improving aspects related to the performance of endurance events (eg, cycling, running, and rowing).[15,34–43] Because of its role as a potent vasodilator, NO increases blood flow (ie, tissue perfusion), which in turn supports oxygen delivery to metabolically active skeletal muscles.[12] Additionally, it has been suggested that NO increases skeletal muscle contractile function—indicating that more muscular work can be performed per unit time for the same metabolic cost (Figure 2).[2,34,36,37,44] Reductions in the metabolic cost[15,35–38,42,43] and the perception of effort[39] during continuous submaximal and maximal aerobic exercise have occurred with dietary NO_3^- supplementation, presumably as a consequence of improved tissue oxygenation and contractile function. As such, individuals have demonstrated a heightened tolerance to high-intensity aerobic exercise after dietary NO_3^- supplementation.[34,35,39–41] Bailey et al[35] and Lansley et al[40] reported that recreationally active men cycled +15 to +16% longer at an intensity equating to greater than 90% maximal aerobic capacity following a 6-day supplementation protocol with ≈6 to 8 mg·kg body mass^{-1}·day^{-1} of NO_3^- via beetroot juice.

Although the ingestion of dietary NO_3^- seems to elicit favorable effects during high-intensity aerobic exercise, it is less clear whether it improves performance during low- to moderate-intensity exercise. Laboratory-based time trial testing is one of the most reliable measures of submaximal endurance performance.[45] This type of performance-oriented task requires participants to complete a fixed distance as rapidly as possible. In this type of assessment, participants are generally blinded to factors related to their performance, including power, pace, and/or time. Dietary NO_3^- has been found to elicit small, but at least marginally significant, improvements in performance of ≈0.5% to 3% for time trial tasks lasting ≈5 to 30 minutes with supplementation of ≈3 to 10 mg·kg body mass^{-1}·day^{-1} in men and women of varying fitness levels.[15–21,46] Alternatively, no differences in performance have been detected in response to relatively higher dietary NO_3^- doses in the ≈18 to 25 mg·kg body mass^{-1}·day^{-1} range[47,48] or with longer duration time trials lasting ≈30 to 140 minutes in men and women.[20,49–56] Figure 3 provides calculated effect sizes for doses of dietary NO_3^- relative to body mass resulting in endurance performance enhancements among placebo-controlled and double-blinded laboratory-based tests. From this figure, it seems the relationship between the dose of dietary NO_3^- ingested and improvements in endurance performance resemble an "inverted U" function, wherein doses equivalent to approximately 6.8 to 6.9 mg·kg body mass^{-1} may elicit the most positive effects for exercise test–related outcomes.[15,21] Thus, factors related to the amount of NO_3^- consumed and/or the intensity and therefore the duration of the event may influence performance enhancement with dietary NO_3^-.

In practice, the intensity at which a performance trial can be maintained is inversely related to its duration. For example, endurance trained men in the study by Shannon et al[20] completed a 1500 m (≈5 minutes) and 10 000 m (≈45 minutes) running time trial at an intensity equivalent to ≈86% and ≈78% of their maximal aerobic capacity, respectively. After the acute ingestion of beetroot juice, performance improvement was limited to just the 1500 m time trial.[20] Indeed, it is possible that shorter-duration endurance events may benefit more from dietary NO_3^- supplementation compared with longer-duration events. Consistent with the nature of shorter-duration events, higher-intensity physical exertion results in relatively greater cellular perturbations that decrease oxygen tension and pH in the skeletal muscle. Accordingly, short-duration (<30 minutes) endurance events may augment the reduction of NO_2^- to NO after dietary NO_3^- supplementation.[5]

Dietary NO_3^- Supplementation and Power-Dependent Performance

Dietary NO_3^- supplementation may have beneficial effects on explosive, power-dependent pursuits like sprinting[22] or weightlifting.[29] Research again from high-quality, placebo-controlled, and double-blind NO_3^- supplementation interventions indicates maximal power during single-leg knee extension, cycling, and running exercise improves with dietary NO_3^- supplementation administered either acutely within 2 to 3 hours of exercise[23–27,57] or chronically for 5 to 6 days in men and women with varied training histories.[22,28] Although the exact mechanisms accounting for gains in maximal power production are largely unknown, alterations in calcium availability and/or sensitivity in the contracting muscle fibers may be responsible.[58] Dietary NO_3^- doses ranging anywhere from ≈5.5 to 23 mg·kg body mass^{-1} have been reported to favorably enhance muscle contractile function.[24,59]

Such benefits related to increased maximal power with dietary NO_3^- may translate to increased performance during team sport-specific tests. Whether acute or chronic, dietary NO_3^- supplementation has been shown to increase the total amount of work performed[22,29–32,60] and average power maintained[33] during intermittent high-intensity laboratory-based tests with doses equivalent to ≈4.5 to 10.4 mg·kg body mass^{-1}. However, others have shown no improvement in team sport performance tests with nitrate doses of 10.4 mg·kg body mass^{-1} in young male basketball athletes or 10.8 mg·kg body mass^{-1} in elite female water polo players.[61] After 7 days of beetroot juice supplementation to achieve 10.4 mg of $NO_3^- \cdot kg$ body mass^{-1}·day^{-1}, Thompson et al[31] reported a greater amount of total work performed during simulated team-sport matches as well as faster reaction times in male team-sport players. It is possible that dietary NO_3^- ingestion may be more beneficial to certain types of intermittent exercise performance, as Wylie et al[33] showed a 5% increase in mean power output across repeated cycling sprints (6 seconds each) after acute ingestion of beetroot juice in male team-sport players. In contrast, no difference in mean power was observed during repeated 30- or 60-second sprints.[33] However, Dominguez et al[24] found that beetroot juice increased peak power, although the advantage was limited to just the first 5 seconds of a 30-second maximal cycling test in men with a history of power-dependent sports training, whereas no differences were detected during the later stages of the exercise bout.

Possible differences in muscle recruitment patterns may explain the disparities in exercise performance with dietary NO_3^-. Specifically, it has been suggested that dietary NO_3^- may preferentially target fast-twitch skeletal muscle fibers.[62] If so, greater performance gains during shorter-duration, explosive-type movements that require fast-twitch muscle fibers would be expected after dietary NO_3^- supplementation. However, Coggan et al[25] found no relationship between knee extensor muscle contractile properties, that is, maximal shortening velocity or fatigue resistance (surrogate markers of muscle fiber type distribution), at baseline and the magnitude of the increase in muscle power after NO_3^- intake in healthy untrained men and women. Supporting these findings, López-Samanes et al[63] found that the administration of a beetroot juice supplement containing 10.4 mg of $NO_3^- \cdot kg$ body mass^{-1}·day^{-1} had no effect on the performance of movements requiring the recruitment of fast-twitch muscle fibers (ie, countermovement jump height, 10 m/20 m sprint time, agility) in young male basketball players.

Possible Factors Affecting Performance With Beetroot Juice

Endurance Training History

Regular aerobic exercise training increases the expression and activity of enzymes responsible for the endogenous synthesis of NO.[5,64] As such, individuals with a history of endurance training tend to have greater reserves of plasma NO_3^- and NO_2^- and NO release in response to stressors similar to those that occur during the onset of exercise.[65] Perhaps because of a higher potential for generating endogenous NO with exercise, endurance-trained athletes are not "NO limited" and consequently have less to gain from dietary NO_3^- supplementation.[46] In support, Carriker et al[42] observed reductions in oxygen consumption during submaximal exercise in low but not high aerobically fit male runners following 4 days of supplementation with NO_3^-. Yet, others have shown improvements in time trial performance for endurance trained and competitive endurance athletes (ie, cyclists, triathletes) after dietary NO_3^- consumption.[16,17,19,20]

Potential Sex Differences

Due in part to the influence of sex hormones on NO bio-availability, limited evidence suggests that women may be more likely to benefit from dietary NO_3^- supplementation compared with men.[25] Previous work indicates that women have lower plasma NO_3^- compared with men.[66] In addition, there may be differences in NO-dependent vascular function between menstrual cycle phases. When progesterone peaks during the early to mid-luteal phase of the menstrual cycle (20 ± 3 days after onset of menses), NO-dependent vascular function is reduced relative to the follicular phase (12 ± 3 days after onset of menses) when estrogen is elevated.[67] Given these differences, dietary NO_3^- supplementation may increase performance-related outcomes more so in women when compared with men. In addition, a greater performance benefit with NO_3^- supplementation may occur during the mid-luteal phase of the menstrual cycle. Unfortunately, the influence of sex on differences in the exercise response to dietary NO_3^- supplementation remains inconclusive given that women have been largely underrepresented in this domain of research.

NO_3^- Reducing Agent Bioactivity

As previously mentioned, the ergogenic effects of dietary NO_3^- ingestion are predominantly dependent on the reduction of NO_2^- to NO. However, not all individuals respond uniformly to the ingestion of dietary NO_3^-.[18,49] Coggan et al[25] have previously reported that some individuals exhibit a profound increase (≈400%) in plasma NO_2^- after the ingestion a uniform dose of dietary NO_3^-, whereas others failed to show any increase in plasma NO_2^-. It is important to note that the rise in plasma NO_2^- after acute beetroot juice supplementation positively correlates with performance-related improvements.[25,49] As such, individual differences in oral microbiota and/or other reducing agents involved in the $NO_3^- - NO_2^- - NO$ pathway (eg, NO_3^- reductase, stomach pH) may contribute to the between-person difference in response to dietary NO_3^- supplementation.[68] Although genetic determinants may be relatively fixed, strong evidence indicates that habitual use of mouthwash may limit the effects of dietary NO_3^- by destroying the anaerobic bacteria that initiate the reduction of NO_3^- to NO_2^- in the oral cavity.[69] Based on such data, limiting the use of mouthwash may enhance the likelihood of dietary NO_3^- supplementation offering a positive effect on performance-related outcomes.

CONCLUSION

In summary, concentrated forms of dietary NO_3^- particularly in the form of beetroot juice appear to elicit small but nevertheless significant improvements in the performance of endurance, high-power explosive, and high-intensity intermittent exercise. Sufficient evidence from placebo-controlled double-blinded studies suggests that dietary NO_3^- may specifically benefit short-duration (ie, <30 minutes) endurance events or power-dependent, explosive movements. Doses of approximately 6 to 7 mg $NO_3^- \cdot \mathrm{kg}$ body mass^{-1}·d^{-1} administered either acutely within 2 to 3 hours of exercise or chronically over several days to weeks may increase the likelihood of enhanced performance. As a recommendation, additional studies should be performed to determine whether the exercise performance of certain populations (ie, women, endurance trained) benefits more from dietary NO_3^- supplementation. Further NO_3^- intervention studies are warranted that either control subject diet or consider the additional intake of foods containing moderate to high amounts of NO_3^- using dietary recall analysis.

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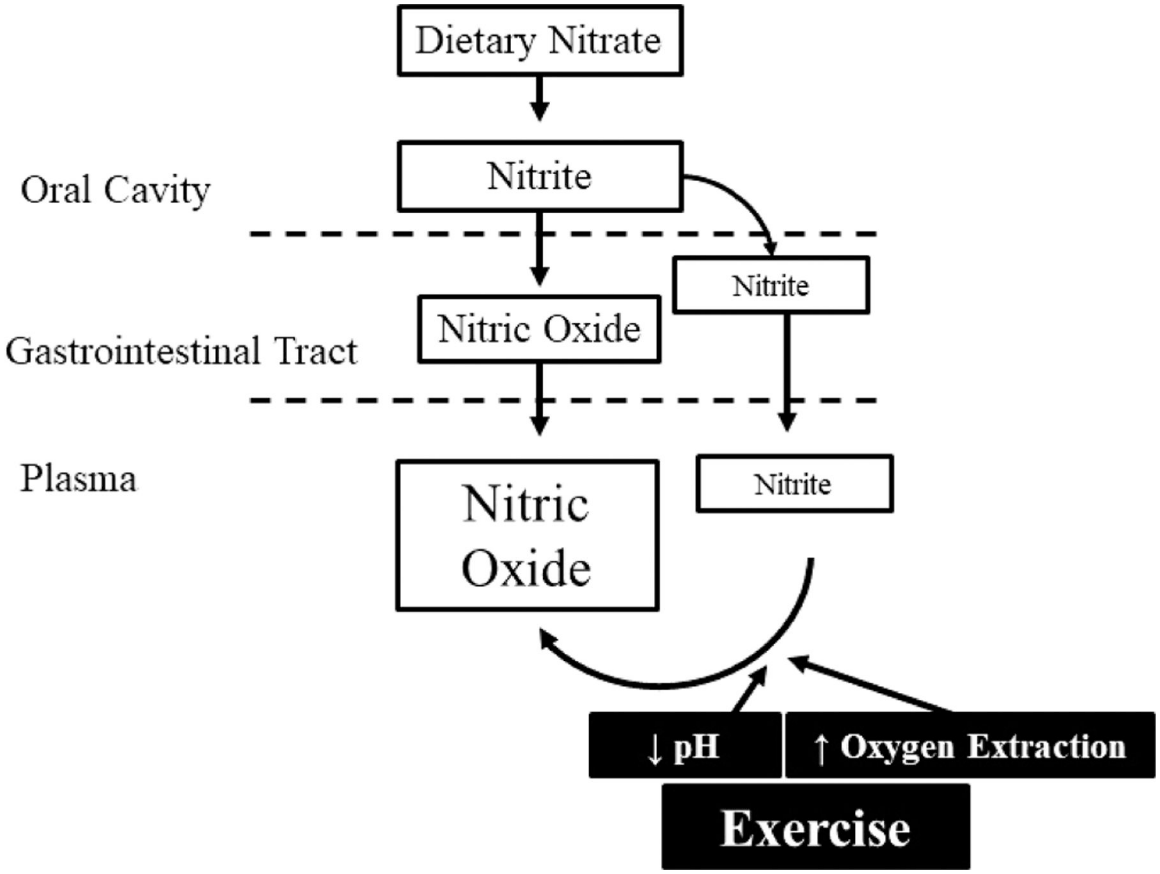

FIGURE 1.

The influence of exercise on the $NO_3^- - NO_2^- - NO$ pathway after the ingestion of dietary NO_3^-. Conditions within exercising skeletal muscle, namely, a relatively lower pH and greater extraction of oxygen from the peripheral blood, promote the reduction of NO_2^- to NO.

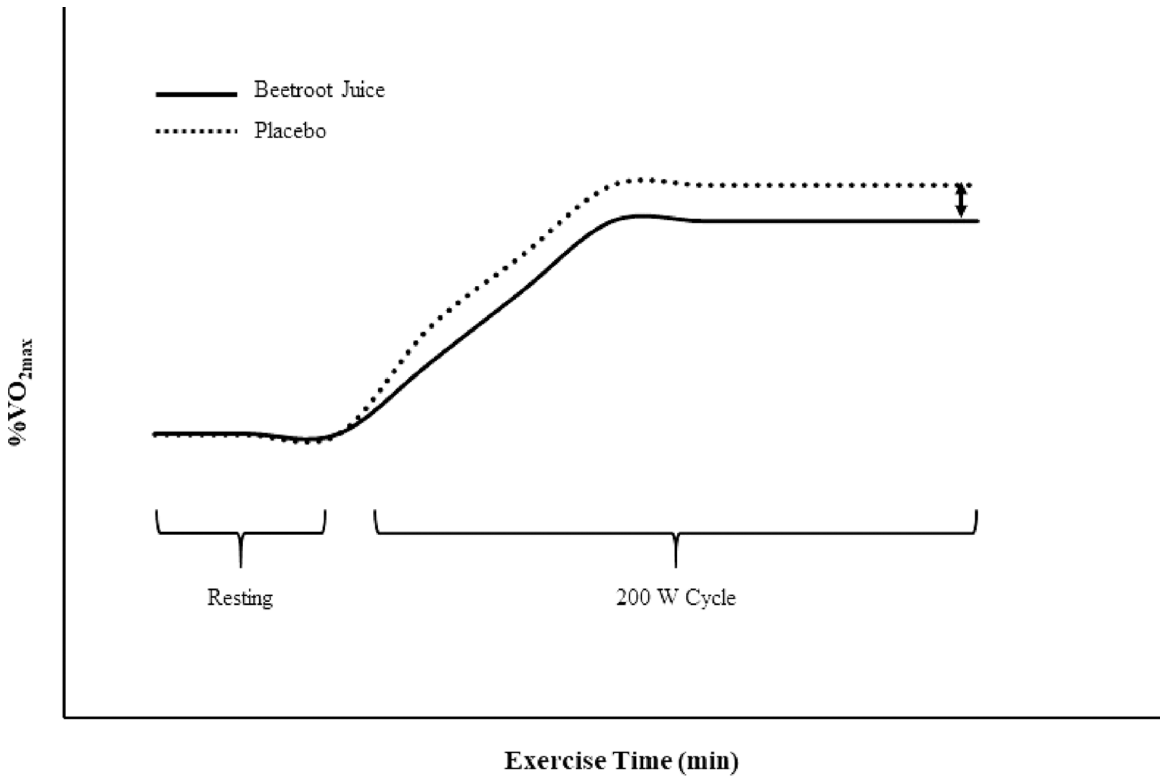

FIGURE 2.

A theoretical response of an individual cycling at a fixed workload of 200 W *with* and *without* dietary NO_3^-. In the absence of preexercise NO_3^- supplementation, the individual is cycling at an intensity corresponding to a higher percentage of their maximal aerobic capacity ($\dot{V}O_{2max}$). After NO_3^- ingestion, the individual performs the same task, though at a lower percentage of $\dot{V}O_{2max}$.

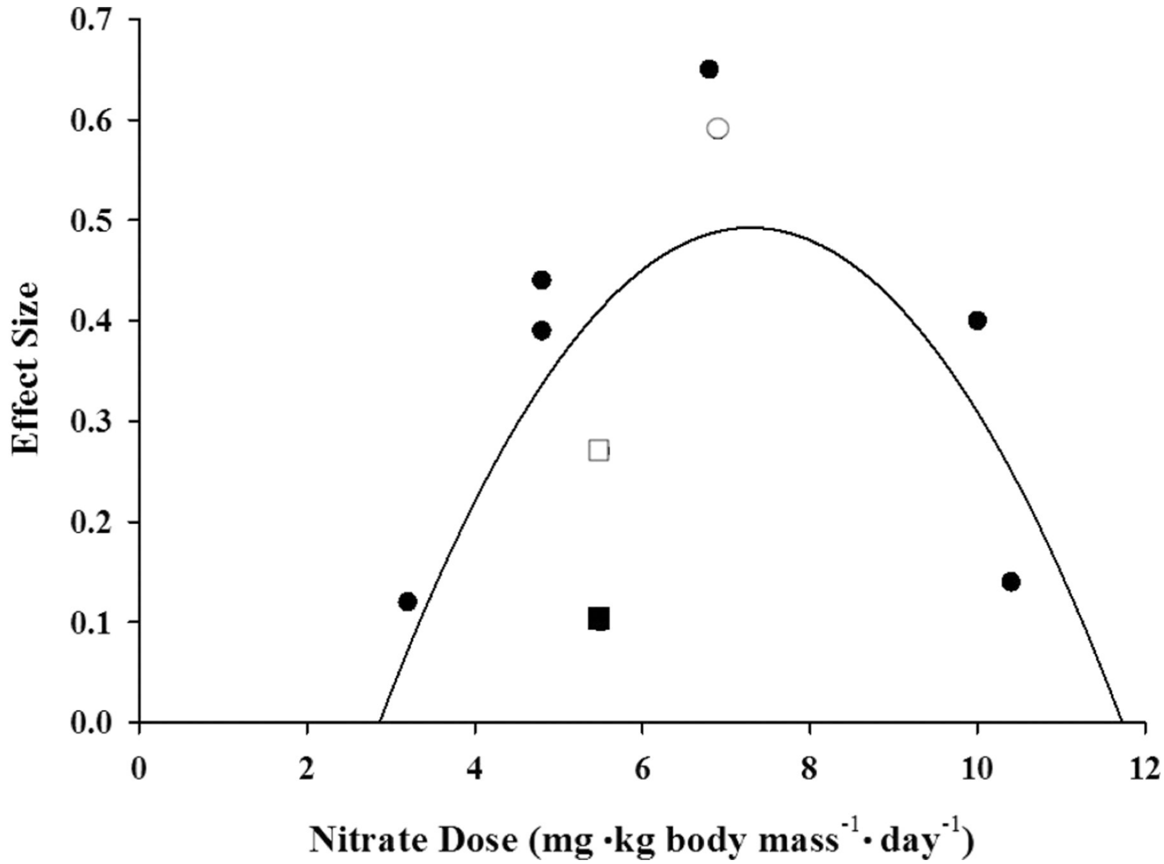

FIGURE 3.

Effect sizes for differences in endurance exercise performance associated with the dose of dietary NO_3^- ingested within a 24-hour window before exercise.[15–17,19–21,46] Subjects included were between the ages of 18 and 44 years and were either untrained men (□),[46] recreationally active men and women (○),[21] recreationally active men (■),[46] and endurance trained (cyclists, runners, or triathletes) men (●).[15–17,19,20] Effect sizes were taken from the corresponding literature, where provided. Otherwise, a Hedges g_{av} effect size statistic was calculated.

TABLE

Doses of Dietary NO_3^- Resulting in Improved Exercise-Related Performance Taken Within 24 Hours Before Exercise

	Subject Characteristics	NO_3^- **Dose (≈mg·kg Body Mass)**
Endurance time-trial performance in events lasting 5–30 min	Combined[15–21]	6.9 (range, 3.2–10.4)
	Endurance trained men[15–20]	6.9 (range, 3.2–10.4)
	Recreationally active men and women[21]	6.9
Maximal power during single leg extension, cycling, and running exercise	Combined[22–28]	6.5 (range, 4.5–9.8)
	Resistance trained men or men with previous experience in power-dependent sports (ie, football, CrossFit)[22,24,26–28]	5.1 (range, 4.5–6)
	Mixed sports (ie, triathlon, tennis) men and women[23]	9.4
	Healthy men and women[25]	9.8
Intermittent high-intensity exercise performance	Combined[22,29–33]	6.7 (range, 4.5–10.4)
	Team-sports players men[22,29–33]	6.7 (range, 4.5–10.4)