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Nitric Oxide

Implications of a potential ergogenic aid

Nitric Oxide (NO) is an endogenous free radical and a potent vasodilator in the human body. While it has many clinical applications, interest in NO use as a potential ergogenic aid has increased greatly in recent years. There are now many different types of NO-producing supplements, split into three major categories: arginine, citrulline, and nitrate-based supplementation. Recent literature has yielded mixed results for all three. Arginine-based supplements work in some cases, but have several recurring limitations that question the validity of their conclusions. There is currently no conclusive or decisive evidence to support the claims made regarding arginine or citrulline-based supplements. Nitrate-based supplements taken 2.5 hours prior to aerobic exercise produce positive ergogenic effects such as decreased oxygen consumption and increased exercise tolerance at submaximal and moderate intensities; however, these supplements have no ergogenic effect on highly trained subjects. The amount of nitrate that needs to be consumed to obtain ergogenic effects can be obtained through a meal of 100g of nitrate-rich vegetables such as beetroot, spinach, and lettuce. Considering the unstable nature of nitric oxide, there is also a lack of studies observing the magnitude of protein damage over chronic supplementation. There is also a lack of studies that observed elderly and female populations. Future studies should investigate the effects of chronic supplementation on 3NT levels—a marker of protein damage.

Keywords: nitric oxide, arginine, nitrate, performance, beetroot, ergogenic

INTRODUCTION

Vasodilation is the process by which blood vessels increase in diameter, allowing for an increase in blood flow. Nitric Oxide (NO) is a potent vasodilator which is actively produced by the human body to increase blood flow and decrease blood pressure (Bescos, Sureda, Tur, & Pons, 2012; Larsen et al., 2011; Lundberg et al., 2011). However, NO is an unstable free radical, meaning that it is a compound that has potential to cause cellular damage if it is in high concentrations. This is avoided

because NO is stored in the body as its more stable forms: nitrate (NO_3) and nitrite (NO_2) (Hord, Tang, & Bryan, 2009). NO can be safely produced via oral bacterial enzymes that can convert NO_3 to NO_2, which can then be converted to NO by a number of other enzymes in the body (Lundberg et al., 2011). The primary method of increasing NO and inducing vasodilation, however, is through the activation of Nitric Oxide Synthases (NOS) located in endothelial cells. With the help of oxygen, NOS convert arginine (Arg), a conditionally essential amino acid (i.e. an amino acid which is sufficiently produced by the body except during times of metabolic stress or illness), to NO and its by-product Citrulline (Cit). NO then diffuses into smooth muscle cells causing changes that lead to smooth muscle vasodilation (Lundberg et al., 2011).

Historically, NO has been widely used in clinical settings because of its vasodilatory effects. NO-induced vasodilation has been shown to help patients with cardiovascular diseases such as coronary atherosclerosis, hypertension, and asthmatic bronchoconstriction (Bryan & Loscalzo, 2009). Interest and research in the field of NO-producing supplementation for sport performance has grown immensely in the past 30 years. Indeed, studies show that people with impaired NO synthesis have poor exercise tolerance (Lauer et al., 2009). The three major forms of NO-producing supplementation include arginine, citrulline, and NO_3-based supplementation.

ARGININE, CITRULLINE, AND NO_3 SUPPLEMENTATION

Due to its short half-life (1-2 ms) and its nature as a free radical, simply ingesting or injecting NO is neither a safe nor effective option (Hord, Tang, & Bryan, 2009). As such, to use NO as an ergogenic aid one must find a safe way to increase the bioavailability of NO.

Arginine and citrulline-based supplements work by increasing the amount of substrate (arginine) for NOS, leading to an increase in NO production. As mentioned above, arginine (Arg) is a conditionally essential amino acid and can easily be obtained through diet (Hord, Tang, & Bryan, 2009). Citrulline (Cit) is a non-standard amino acid that can be converted to arginine in the body with the help of several enzymes (Toda, 2008).

Unlike Arg and Cit supplements, NO_3-based supplements operate independent of NOS. Under exercising conditions, the NO_3 and NO_2 in one's body are naturally converted to NO for use (Bailey, Vanhatalo, Winyard, & Jones, 2012; Bescos, Sureda, Tur, & Pons, 2012; Lundberg et al., 2011). Additional NO_3 can be naturally found in the diet through dark leafy vegetables and has a half-life of 5-8 hours (Hord, Tang, & Bryan, 2009). About 60% of ingested nitrate is excreted in urine and about 25% gets concentrated in saliva (Lundberg et al. 2011). Spitting out saliva or using antibacterial mouthwash after taking an NO_3 supplement abolishes the effects of nitrate (Govoni, Jansson, Weitzberg, & Lundberg, 2008; Webb et al., 2008).

This paper will explore whether or not these common forms of NO supplementation work, through which mechanisms they might act, and under what conditions.

Arginine-based supplementation

We reviewed 20 studies that used Arg-based supplements and found mixed results. Out of 20 studies, nine claimed the supplement worked while 11 claimed it did not (Appendix, Table 1). However, when we examined these studies, we came across several recurring limitations that must be addressed.

First, many of the Arg supplements reviewed were mixed with other compounds, most of which had their own ergogenic effects. For example, Chen et al. (2010) set out to investigate the effect of chronic L-arg supplementation on moderately trained elderly men (>50yrs) performing a max incremental exercise test. They found no difference in baseline exercise parameters (VO_2 or power output), but did find a sustained 16% increase in anaerobic threshold. However, the supplement was mixed with several other compounds including citrulline, vitamin E, and alpha lipoic acid; therefore, the authors could not conclude that the increase in anaerobic threshold was solely due to L-arg. We found that 12 of the 20 Arg studies we reviewed included some form of mixed supplement (Appendix, Table 1). Seven of those 12 studies concluded that Arg supplementation worked as an ergogenic aid. The mixed supplementation casts doubt on the validity of these conclusions.

The second major limitation was that only five out of the 20 studies we reviewed measured NO metabolite levels (NO_X, referring to NO_3 or NO_2 in the body), and only one of those five reported a significant difference in NO_X levels (Bailey et al., 2010). This makes it difficult to know if the results of these studies can be attributed to NO supplementation.

The third limitation is that arginine is involved in several other metabolic pathways. This means it may not always lead to an increase in NO production. This was well illustrated in a prior study conducted by Fricke et al. (2008), which investigated the effect of 18g L-arg on muscle force and power in postmenopausal women. The authors found no increase in maximum grip force, or peak jump force, but did find a significant increase in maximum power in relation to body mass (measured as peak jump force divided by body weight). They concluded that the supplement may have increased maximum force and prevented muscle force decline in postmenopausal women. However, while these authors concluded that Arg supplements can have a positive benefit, they also note NO was likely not the cause of the observed result and stated that increased Arg may not necessarily lead to an increase in NO synthesis. Arg is known to actively participate in the synthesis of creatine (Buford et al., 2007) and L-Arg infusion at rest is known to increase plasma insulin, glucagon, growth hormone, IGF-1, prolactin, and catecholamine

concentrations (McConell, 2007), all compounds that are ergogenic aids in their own right.

It is difficult to isolate the ergogenic effects of Arg-based supplementation to arginine itself. Arg is active in many other pathways and may not always stimulate NO production. Further concerns regarding arginine supplementation include the fact that NOS must compete with arginase enzymes, which use Arg in the urea cycle (Bescos, Sureda, Tur, & Pons, 2012). Arginase activity seems to increase with exercise, which suggests additional arginine will not be converted to NO (Sureda et al., 2006).

Citrulline-based supplementation

Like Arg-based supplements, Cit-based supplements are also NOS dependent; however, unlike Arg, Cit is not a substrate for arginase enzymes. We came across only one Cit-based study that did not use a mixed supplement. Subjects were given an oral L-Cit supplement, and then completed an incremental test to exhaustion on a treadmill (Hickner et al., 2006). Contrary to the author's hypotheses, treadmill time to exhaustion was 1.5% lower and rate of perceived exertion was found to be higher compared to placebo. In addition, NO_X levels were observed to be 7% lower following supplementation, suggesting Cit actually decreased levels of NO production. As a by-product of NO production, it is possible that higher levels of Cit may have suppressed NOS activity.

In light of the findings outlined above and the reported side effects of Arg and Cit-based supplementation (e.g. nausea, vomiting, and diarrhea [Grimble, 2006]), we cannot recommend either as an effective form of NO supplementation.

Nitrate-based supplementation

The three main forms of NO_3 supplementation are two pharmaceutical nitrates ($NaNO_3$ and KNO_3) and Beetroot Juice (BRJ). We reviewed 27 studies that used one of these forms of NO_3-based supplementation. Appendix, Table 2 summarizes each study and Table 3 provides an overall summary of the findings. Like the Arg-based studies, the NO_3 studies produced varying results, though 22 out of 27 showed a performance benefit. For example, Wilkerson et al. (2012) revealed that there was a strong negative correlation (r= -0.81) between the change in plasma NO_2 levels and the change in performance. This finding provides strong evidence that increased NO in one's system is related to better performance (lower times) on an aerobic time trial.

Interestingly, recent literature suggests that NO_3 can have ergogenic effects in dosage amounts that are comparable to what one may obtain from a meal including 100g of NO_3-rich vegetables (Hord, Tang, & Bryan., 2009). Studies also show that the optimal time to take NO_3 supplements is 2.5-3 hours prior to exercise in order to obtain the greatest benefit (Webb et al., 2008).

Training status

Unlike the Arg-based studies, all NO_3 studies reported an increase in NO_X levels, regardless of whether or not there was a positive performance effect reported. Interestingly, the studies with the lowest percent increases in NO_X were among the five studies that did not report any significant ergogenic effect (Bescós et al., 2011; Wilkerson et al., 2012; Peacock et al., 2012). This suggests that the subjects in these studies had a lower response to NO supplementation compared to those in other studies. Further investigation revealed that the subjects of these studies had one trait in common: their training status. VO_2max is a measure that reflects maximal oxygen uptake. A higher VO_2max means that more oxygen can be used during exercise. All subjects in these five studies were classified as highly trained aerobic athletes with VO_2max greater than 60 mL/kg/min. With all other variables being controlled, these athletes did not show any performance enhancement through NO supplementation. This is a previously unreported finding, and we believe this is the single-most-important factor in determining whether or not NO_3 supplementation will have an ergogenic effect. Illustrating this point, a recent study investigated the effect of 6.2mmol of NO_3, consumed 2.5 hours prior to exercise by highly trained athletes, on an 80 km time trial and reported no significant performance benefit (Wilkerson et al., 2012). This is despite having similar experimental protocols as two other studies that reported a benefit from NO_3 supplementation (Lansley et al. 2011; Murphy, Eliot, Heuertz, & Weiss, 2012).

Unlike the mixed supplementation used in Arg-based supplementation studies, NO_3 was shown to be the active ingredient in the three different forms of NO_3 supplementation used in the NO_3 studies that we reviewed. By using KCl and NaCl as placebos, several studies have proved that the observed effects of supplementation were the result of NO_3 alone (Bescós et al., 2012; Bescós et al., 2011; Larsen et al., 2006; Larsen, Weitzberg, Lundberg, & Ekblom, 2010; Larsen, Weitzberg, Lundberg, & Ekblom, 2007). Another recent study was able to isolate the effects of BRJ supplementation to its high NO_3 content and not any other substance (Lansley et al., 2011). BRJ was used as an alternative form of NO_3 supplementation in many studies because of its high NO_3 content (Hord, Tang, & Bryan, 2009) and because of fears surrounding the safety of pharmaceutical NO_3 supplementation (Lundberg, Larsen, & Weitzberg, 2011; Rogers, Vaughan, Davis, & Thomas, 1995). Together, these studies show that NO_3 is the active ingredient in pharmaceutical and dietary nitrate supplementation.

NO_3 limitations

Performance benefits were not consistent across the different nitrate studies reviewed (i.e: some studies reported larger decreases in blood pressure than others). We believe the reason for this is the vastly different methodology used in each study. It is also important to note that a few studies had experimented with NO_3 supplements

that had been mixed with other compounds. We did not review these extensively because, like the mixed arginine supplements, it is difficult to attribute mixed supplement effects to NO alone. These mixed compounds include 2-ethyl, GPLC (a carnitine-based supplement), and store-bought NO_3 supplements that were reported to be mixed with over 30 other compounds (Bloomer et al., 2010).

NO_3 controversies

There have been several controversies surrounding the use of NO_3 supplements. Of minor concern is that subjects who supplemented with BRJ also reported minor side effects such as Beeturia and red stools (Bailey et al., 2010a; Bailey et al., 2010b; Vanhatalo et al., 2010; Webb et al., 2008). The most significant controversy is concerned with the use of pharmaceutical NO_3. Due to health and ethical concerns, human supplementation with pharmaceutical NO_3 was not allowed in the United Kingdom (Jones et al., 2011). As such, UK-based studies used BRJ as an NO_3 supplement (Bailey, Vanhatalo, Winyard, & Jones, 2012). However, it has been observed that the lethal oral dose of NO_3 in humans is around 330 mg/kg body weight (European Food Safety Authority, 2008). Thus, while the dosages used in the studies reviewed were well above the WHO recommended Adequate Daily Intake (ADI) of 0–3.7 mg/kg or about 0-0.06mmol/kg (Hord, Tang, & Bryan, 2009), they are also significantly below what may be considered a lethal dosage. Some researchers have claimed, however, that even at low levels NO_3 could be dangerous, and they have warned against its uncontrolled use (e.g. Lundberg, Larsen, & Weitzberg, 2011). This claim was tested in a 2012 study that examined cell damage after NO_3 supplementation in highly trained athletes and found no significant changes over three days (Bescós et al., 2012). This study concluded that acute supplementation of $NaNO_3$ was safe for humans if consumed alongside dietary nitrate. Therefore, the concerns surrounding NO_3 use as an ergogenic may not be applicable in all situations.

SUMMARY OF FINDINGS AND DISCUSSION

NO supplements are increasingly being used by recreational athletes as an ergogenic aid, but little is currently known about the nature of these supplements. After reviewing recent literature, several conclusions and inferences may be made. Arg and Cit supplements that use endogenous NOS to convert Arg to NO have yielded inconsistent results and there are no consistent data from which to make any reliable conclusions.

NO_3-based supplements show the most promise. There is a strong correlation between the change in plasma NO_2 levels and a change in performance. These supplements have been shown to work across a large range of aerobic exercise modalities.

Importantly for experimental control, NO_3 is the only active ingredient in $NaNO_3$, KNO_3, and BRJ, the three most common forms of NO_3-based supplementation. While all NO_3 supplements are shown to exert their effect by increasing NO, this increase is dependent on the training status of the individual. Highly trained athletes have the lowest-percent increases post-ingestion and are not likely to gain any performance benefit from the additional NO_3.

There have been warnings that ingesting pharmaceutical NO_3 can lead to protein damage or cancer (Rogers, Vaughan, Davis, & Thomas, 1995). Despite such fears, $NaNO_3$ supplements, if taken safely with dietary nitrate, do not cause any significant protein damage over an acute dosage period.

SUGGESTIONS FOR FUTURE RESEARCH

Chronic exercise has also been shown to increase NOS expression in dogs (Sessa et al., 1994) and to increase NO production in hypercholesterolemic patients (Lewis, Dart, Chin-Dusting, & Kingwell, 1999). It is possible that chronic exercise training over a lifetime may increase NOS expression in human subjects to the point where NO_3 supplementation is no longer effective, which may be the case with highly trained athletes. This has potential implications for elderly populations, who are known to have decreased levels of NO production (Goubareva et al., 2007).

In addition, excessive NO production is dangerous because of its capacity for protein damage. Indeed, the dosages used in the studies reviewed were far in excess of those recommended by the WHO (Hord, Tang, & Bryan, 2009). A recent study proved that acute supplementation of $NaNO_3$ with dietary nitrate does not result in protein damage, reflected in 3NT levels (Bescós et al., 2012); there are, however, no studies that have examined 3NT levels with chronic (>5 days) supplementation. Therefore, future studies should examine the effects of chronic exercise on NOS expression, the effects of NO_3 supplementation in elderly populations, and 3NT levels over chronic supplementation periods.

CONCLUSION

After reviewing all the pertinent literature, the claim can be made that NO_3 supplements can help to improve aerobic exercise tolerance and performance in young, moderately trained men and are not suitable for highly trained endurance athletes. Arg and Cit-based supplements are not recommended. Rather than buying a supplement, however, it is recommended that individuals interested in NO_3 supplementation should consume about 100g worth of NO_3-rich vegetables 2.5-3 hours before exercise. One would receive the same amount of NO_3 as the subjects in most of the studies reviewed and save a considerable amount of money.

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APPENDIX

Table 1. Side by Side Comparison and Summary of Studies Using Arginine or Citrulline Supplementation.

Study Author & year	Study design	Subjects (number, gender, (wt) VO_2max)	Supplement	Dose & duration	Signigicant Physio-logical Results	Significant Performance Results (%)	Worked as an Aid	Measured NO_X
Stevens et al., 2000	r, db, co	13 m	L-Arg + GaKic	11.2g for 23 d	none reported	↑FRI (28%), ↑Muscle work (0.8%)	Yes	No
Buford et al., 2004	r, db, pl	10 m	L-Arg + GaKic	11.2g for 1 d	↑ [L-Arg]	↓ change in peak mucle output	Yes	No
Campbell et al., 2006	1) r, db, c 2) r db cl	1) 10 m 2) 35 m	L-Arg + AAKG	1) 4g 2) 12g for 1 d	none reported	2) ↑1RM bench press, ↑ peak power output	Yes	Yes
Matsumoto et al., 2007	r, db, pl. co	8 m (72.6 ± 3.9kg)	L-Arg + BCAA	2.5 g for 1 d	↑[plasma BCAA], ↑ Phenylalanine release from the leg	none reported	Yes	No
Little et al., 2008	r, db	35 m	L-Arg + AAKG + Cr	0.175g for 10 d	none reported	↑ Bench-press repetitions (12.4%), ↑ Peak power (7.1%)	Yes	No
Fricke et al., 2008	r, db	23 f (>50y)	L-Arg+HCL	18g for 180 d	none reported	↑peak jump force	Yes	No
Bailey et al. 2010	r, db, co	9 m	L-Arg + Vitamins + Amino acids	6 g for 3d	↑ NO_X, ↓ 7% SBP	↑ TTE, ↓ VO_2	Yes	Yes
Camic et al., 2010	r, db, parallel	50 m	L-arg + GSA	1.5g or 3g for 21 d	none reported	↓GET (4.1%)	Yes	No
Chen et al., 2010	r, db, pl, ce, MI	21 m(>50y), VO_2max=3.71 ± 0.34 L/min	L-Arg + L-Cit + antioxidants + VitE + folic acid	5.2g for 21 d	none reported	↑ anaerobic threshold (16.7%)	Yes	No
Denis et al., 1991	r, db, co, ce	15 m/f (61 kg)	L-Arg + L-asp	5 g for 10 d	↓ [plasma NH4]	none reported	No	No
Eto et al., 1994	ce	3 m	L-Arg + L-asp	24 g for 1 d	↓ [plasma NH3]	none reported	No	No
Colombani et al., 1999	r, db	14 m	L-Arg + L-asp	15g for 28 d	↑ [glucagon], ↑ urea, ↑ [L-Arg]	none reported	No	No
Schaefer et al., 2002	r, db, cl, co	8 m	L-Arg	3 g for 1 d	↓ [plasma NH3], ↓ [bLac], ↑ [L-Cit]	none reported	No	No

Abel et al., 2005	r,pl, ce, MI	30 m (74kg), VO_2peak= 56±7.8 ml/kg/m	L-Arg + L-asp	5.7+8.7g for 28 d	none reported	none reported	No	No
Burtscher et al., 2005	r, db, pl, ce, MI	16 m (72.5 ± 6.5kg)	L-Arg + L-asp	3g for 21 d	↓ [bLac]	↓ VO_2, ↓ VCO_2	No	No
McConnell et al., 2006	r, db, co	9 m	L-Arg + HCL	30g for 1 day	↓ [blood glucose]	none reported	No	No
Liu et al., 2008	r, db, co	10 m	L-Arg	6g for 3 d	none reported	none reported	No	Yes
Bescós et al., 2009	r	9 m (67.7 ± 8.7kg)	L-Arg	5.5 ± 0.3g for 3 d	↓ [bLac]	none reported	No	Yes
Tsai et al., 2009	r, pl	12 m (75.75 kg)	L-Arg	7.5 g for 1 d	↑ [BG], ↑ [insulin]+ ↓ [blood FFA]	none reported	No	Yes
Koppo et al., 2009	r, db, co, ce	7, VO_2peak=52.0 ± 4.8 ml/kgm	L-Arg	6 g for 14 d	↑ [serum L-Arg]	↑ phase 2 VO_2 (12%)	No	No
Hickner et al., 2006	r, pl, db, cb	17 m/f, VO_2max = 52.1 ± 1.9	L-Cit	3g or 9g (3x3) for 1 d	↓ NO_X (7%)	↓time to exhausion (1.5%), ↑ RPE	No	yes

Legend. r=randomized, pl=Placebo-Controlled, co=crossover, db=double blind, ol=open-label, rm=repeated measures, cb=counterbalanced, GaKic=glycine-arginine-alpha ketoisocaarproic acid, AAKG=alpha ketogluterate, L-asp=L-aspartate, L-glut=L-glutamate, ce=cycle ergometer used, T=treadmill used, TT=Time trial, DT=Distance Trial, LI=low intensity, MI=Moderate intensity, SI=Severe Intensity, GE=gross efficiency, GET=gas exchange threshold, L-Arg=L-Arginine, NO_X=nitric oxide metabolites (NO_2 and NO_3), VO_2=Oxygen uptake, PO=power output, TTE=Time to exhaustion, TTC=Time to completion, BG=Blood Glucose, BI=Blood Insulin

Table 2. Side by Side Comparison and Summary of Studies Using Nitrate Supplementation.

Study Author & year	Study design	Exercise Modality	Subjects (number, gender, (wt) VO_2max)	Dosage & Dosage duration	Dose Timing	Physiological Results	Performance Results (%)	Worked as an Aid
Bescós et al., 2012	r, db, co,	40min cycling DT (91% Hrmax)	13 m, (72.4 ± 9.7 kg),VO_2max= 60 ± 7 mL/kgmin	11.6 mmol $NaNO_3$ for 3 d	3 h	↑ NO_2 (78%), ↑ET-1	DNI	N
Bescós et al., 2011	R, db, co	Four 6-min submax cycling (2-3.5W/kg) and one IT to exhaustion	11 m (73.3 ± 5.6 kg), VO_2peak= 65.1 ± 6.2 mL/kg/min	11.8 mmol $NaNO_3$ for 1 d	3 h	↑ NO_2, (16%)	↓ VO_2 2.9% at RCP SI	N
Wilkerson et al., 2012	R, sb, co	80km cycling TT at 75% VO_2max	8 m (79 ± 9 kg), VO_2peak= 63 ± 8 mL/kg/min	6.2 mmol BRJ for 7d	2.5 h	↑ NO_2 (25%), ↓BP	↓ TTC (0.8%) but was NS	N
Peacock et al., 2012	r, db	LI cycle exercise (55-75% Vomax)	10 m (74 ± 8 kg), VO_2peak= 69.6 ± 5.1 mL/kg/min	9.9 mmol KNO_3 for 1 d	2.5 h	↑ NO_2 (127%),	DNI oxygen cost	N
Christensen et al., 2013	R, sb, co	O_2 kinetics (3 x 6min at 298W), 400 kcal TT and repeated sprint capacity (6x20 s sprints)	10 m (69 ± 8 kg), VO_2peak= 72.1 ± 4.5 mL/kg/min	8 mmol BRJ for 6d	3 h	↑ NO_X (Day 4 = 258%, Day 6 = 298%)	DNI VO_2 kinetics	N
Larsen et al., 2006	R, db, co	no exercise	17 m/f	0.1 mmol/kg $NaNO_3$ for 1 d	n/a	↑ NO_2 (59%), ↓DBP	No exercise	Y
Larsen et al., 2007	R, db, co	5 minutes cycling at work rates equivalent to 45 - 100% VO_2peak	9 m, VO_2peak= 55 ± 3.7 mL/kg/min	0.1 mmol/kg $NaNO_3$ for 3 d	1 h	↑ NO_2 (82%), ↓SBP (6.7%)	↓ submax VO_2,↑GE (6.6%),	Y
Webb et al., 2008	ol, co	no exercise	14 m/f	6.2 mmol BRJ for 1 d	0.5 h	↑ NO_2 (100%), ↓SBP(8%), ↓DBP(10%)	No exercise	Y
Bailey et al., 2009	R, pc, co	4 MI (80%GET) and 2 SI (70%D) ce tests	8 m, (82 ± 6 kg) VO_2peak= 49 ± 5 mL/kg/min	5.5 mmol BRJ for 6 d	sipped throughout the day	↑ NO_2 (96%), ↓SBP, ↑ [Hbtot], ↑ [HbO_2], ↓ [HHb]	↓ O_2 amplitude during MI, ↓VO_2 slow component during SI, ↑TTF (16%) during SI,	Y

Larsen et al., 2010	R, db, co	LI cycle exercise, combined arm and leg cycle IT, 80rpm	7m, 2f, VO_2max= 3.72 ± 0.33 L/min	0.1 mmol/kg $NaNO_3$ for 3 d	40 mins	↑ NO_2 (133%), ↑ in plasma cGMP	↓TTE during SI, ↓ VO_2 (5.8%) during LI, ↓VO_2Max (2.8%)	Y
Bailey et al., 2010	R, db, co	6 LI (15%MVC)and 3 HI (30% MVC) two-legged knee extensor exercise	7 m, (81 ± 7 kg)	5.1 mmol BRJ for 6 d	n/a	↑ NO_2 137%, ↓SBP 4%, ↓DBP, ↓MAP 2%, ↓ muscle ATP turnover, ↓ muscle ADP accumulation, ↓ muscle Pi accumulation, ↓ muscle PCr depletion	↓VO_2 10.6% during LI, ↓ VO_2 13.7% during HI, ↑ TTF25%, ↓ muscle ATP turnover rate, ↓ muscle ADP accumulation, ↓ muscle Pi accumulation, ↓ muscle PCr depletion	Y
Kapil et al., 2010	1) db, co 2) ol, co 3) ol, co	no exercise	1) 6 2) 20 3) 9	1) 4, 12 2) 24 mmol KNO_3 3) 5.5 mmol of BRJ for 1 d	n/a	↑ NO_2 (30-300%),	No exercise	Y
Vanhatalo et al., 2010	R, b, co	2 bouts of MI (90%GET)and 1 IT to exhausion	8 m/f (71.8 ± 11.5 kg), VO_2: 47 ± 8 ml/kg/min	5.2 mmol BRJ for 15 d	2.5 h	↑ NO_2 (Day 1 = 39%, Day 5 = 25%, Day 15 = 46%), ↑ SBP, ↑MAP	↓VO_2 3.6% on d1, 4.8% on d 5, 4.2% on d15.,↓VO_2 amplitude during MI, After 15 days:↑W 2.5%, ↑Peak Work Rate in IT, ↑GET Work rate	Y
Larsen et al., 2011	r, db, co	LI cycle exercise, 60-70rpm	14 m (70 ± 2 kg), VO_2: 56 ± 3 ml/kg/min	7mmol $NaNO_3$ for 3 d	1.5 h	↑ NO_2 (526%)	↓ O_2 consumption (3%) during LI exercise, ↑ mitochondrial P/O ratio (19%)	Y
Lansley et al., 2011a	R, db, co	4- and 16.1-km cycling TT	9 m (69.3 ± 7.2 kg), VO_2: 56 ± 6 ml/kg/min	6.2 mmol BRJ for 1 d	2.75 h	↑ NO_2 (138%), ↓SBP (5%)	↑ PO/VO_2 7%, ↓ TTC (2.7-2.8%)	Y

Lansley et al., 2011b	R, db, co	4 MI (80%GET) and 2 SI (70%D) tests	9 m (79.6 ± 9.7 kg), VO_2: 55 ± 7 ml/kg/min	6.2 mmol BRJ for 6 d	3 h	↑ NO_2 (105%), ↓SBP (4%)	↓ VO_2 during walking (12%), ↓ VO_2 during MI & SI (7%), ↓VO_2 (14%) during SI walkin, ↑ TTF (15%)	Y
Kenjale et al., 2011	R, ol, co	CPX incremental test to exhausion	8 m/f (84.5 ± 16.5 kg)	6.2 mmol $NaNO_3$ for 1 d	1.75 h	↑ NO_2 (520%), ↓DBP, ↑ [Hbtot], ↑ [HbO_2], ↓ [HHb]	↓VO_2 , ↑TTE (17%)	Y
Bahra et al., 2012	R, db, co	no exercise	14 m/f	8 mmol KNO_3 for 1d	3 h	↑ NO_2 (75%), ↓SBP 3.6%	No exercise	Y
Cermak et al., 2012	R, rm, co	10km TT at LI and MI (45% and 65% Wmax)	12 m (73 ± 2 kg), VO_2: 58 ± 2 ml/kg/min	8 mmol BRJ for 6 d	2.5 h	↑ NO_3 (1900%),	↓ TT completion time (1.2%), ↑ PO (2.1%), ↓ VO_2 (3.5-5.1%)	Y
Murphy et al., 2012	db, co	5km TT	11 m/f (23.7 kg)	8 mmol BR for 1 d	1.15 h	DNM NO_X,	↑ running velocity (5%)	Y
Wylie et al., 2013	R, db, co,	YoYo HI intermittent cycling test	14 m (83 ± 10 kg), VO_2: 52 ± 7 ml/kg/min	28.7 mmol for 1 d	1.5 h	↑ NO_2 (395%), ↓ blood [glucose] ↓rise in plasma K+	↑ Performance in the Yo–Yo IR1 by 4.2 %	Y

Legend. r=randomized, pl=Placebo-Controlled, co=crossover, db=double blind, sb=single blind, ol=open-label, rm=repeated measures, ce=cycle ergometer used, TT=Time trial, DT=Distance Trial, LI=low intensity, MI=Moderate intensity, SI=Severe Intensity, IT=incremental exercise, GE=gross efficiency, GET=gas exchange threshold, $NaNO_3$=Sodium nitrate, KNO_3=Potassium Nitrate, BRJ=Beetroot juice, BR=Beetroot, RCP=respiratory compensation point, NO_X=nitric oxide metabolites (NO_2 and NO_3), NO_3=nitrate, NO_2=nitrite, ET-1=Endothelin-1, VO_2=Oxygen uptake, PO=power output, TTE=Time to exhaustion, TTC=Time to completion, DNI=did not improve, DNM=Did not Measure, Dosage: BRJ NO_3 dosage assumed to be 6.2mmol per 0.5L unless stated otherwise 10mg/kg NO_3 ~ 0.161mmol/kg

Table 3. Summary of Results for Studies on the Ergogenic Effects of Nitrate Supplementation.

Physiological results	Performance-related results
2.9-14% decrease in VO_2	1.2% decrease in TTC
22-526% increase in NO_X	15-25% increase in TTF and TTE
3.6-7.8% decrease in SBP	2.1-2.5%increase in W and PO
10% decrease in DBP	5% increase in running velocity
2% decrease in MAP	