Effects of Acute Supplementation of L-arginine and Nitrate on Endurance and Sprint Performance in Elite Athletes

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Running head: Dietary supplementation in elite athletes

Abstract

This study examined the effects of acute supplementation with L-arginine and nitrate on running economy, endurance and sprint performance in endurance-trained athletes. In a randomized cross-over, double-blinded design we compared the effects of combined supplementation with 6 g L-arginine and 614 mg nitrate against 614 mg nitrate alone and placebo in nine male elite cross-country skiers (age 18±0 years, VO_{2max} 69.3±5.8 ml·min^{-1}·kg^{-1}). After a 48-hour standardization of nutrition and exercise the athletes were tested for plasma nitrate and nitrite concentrations, blood pressure, submaximal running economy at 10 km·h^{-1} and 14 km·h^{-1} at 1% incline and 180 m as well as 5-km time-trial running performances. Plasma nitrite concentration following L-arginine+nitrate supplementation (319±54 nmol·L^{-1}) did not differ from nitrate alone (328 ± 107 nmol·L^{-1}), and both were higher than placebo (149±64 nmol·L^{-1}, $p<0.01$). There were no differences in physiological responses during submaximal running or in 5-km performance between treatments. The plasma nitrite concentrations indicate greater nitric oxide availability both following acute supplementation of L-arginine+nitrate and with nitrate alone compared to placebo, but no additional effect was revealed when L-arginine was added to nitrate. Still, there were no effects of supplementation on exercise economy or endurance running performance in endurance-trained cross-country skiers.

Keywords: endurance athletes, exercise economy, nitric oxide.

Introduction

The signalling molecule nitric oxide (NO) has an important role in the regulation of many body functions including muscle contractility, metabolism, neuronal activity and host defence. NO is produced by NO synthases during the catalysis of L-arginine to L-citrulline (NO synthases dependent) and due to ingestion of nitrate-rich foods via the reduction of nitrate to nitrite (NO synthases independent). Increased NO availability may enhance oxygen and nutrient delivery to active muscles and thereby lower the ATP cost of muscle force production and improve the physiological responses related to endurance performance and recovery (1, 2). The mechanisms responsible for these effects have mainly been linked to improved muscle contractility, mitochondrial respiration and biogenesis, and the regulation of tissue blood flow (3-5).

After ingestion of dietary nitrate, a reduction in the oxygen cost of submaximal exercise and improved tolerance of high-intensity exercise has been consistently reported in recreationally active adults with both acute and chronic supplementation (6-10). A decreased peak oxygen uptake without any changes in exercise performance (11, 12) indicates increased energy efficiency even at maximal aerobic workloads. Although studies in rodents have suggested that 5 to 7 days of nitrate supplementation might improve blood flow and contractile function predominantly in fast-twitch type II muscle fibers (13, 14), factors which might in turn increase performance in short-duration sprint exercise, the effect of nitrate supplementation on repeated sprint performance after nitrate supplementation in humans was not found in a recent study by Martin and colleagues (15).

While most previous studies have examined moderately trained subjects, some recent studies have been carried out in highly trained athletes (16-20). Studies on elite cyclists did not find any changes in physiological responses or endurance performance using acute or chronic

supplementations of nitrate compared to placebo (16-18). In endurance-trained cross-country skiers neither running economy nor endurance running performance were improved (19), whereas well-trained kayakers lowered the submaximal oxygen cost but did not improve performance with acute nitrate supplementation (20). Overall, the available evidence indicates that male athletes with a VO_{2max} of ≥70 $mL \cdot kg^{-1} \cdot min^{-1}$ are unlikely to benefit from nitrate supplementation in their specialist discipline (2).

Elite endurance athletes are well adapted to their specialist sport event and performance effects following dietary nitrate ingestion in most studies have not been found (2). One of the reasons for the lack of performance improvements following dietary nitrate ingestion in endurance-trained athletes may be due to higher baseline levels of NO (21, 22). Higher daily energy expenditure likely leads to higher intakes of nitrate and additionally training itself may elevate plasma nitrite and nitrate through enhanced production of NO via the NOS pathway (2). However, stronger stimulus of NO producing supplementation may be beneficial. Indeed, chronic supplementations of nitrate might be more effective than acute doses (23, 24), and higher acute doses of nitrate have been shown to be beneficial (25, 26). Targeting both pathways for increasing NO availability simultaneously by supplementing with L-arginine in addition to nitrate is a further option, but has not been examined to date.

Although the physiological concentrations of L-arginine are generally sufficient to saturate endothelial nitric oxide synthase (27, 28), there is evidence that increased extracellular and plasma L-arginine levels still enhance endothelial NO production (28-30). This phenomenon is known as the L-arginine paradox. Therefore, many athletes use products with L-arginine to improve performance, but the effect of L-arginine supplementation on sport performance is controversial. While acute supplementation with 6 g of L-arginine (as part of a multi-nutrient supplement that included beetroot) increased plasma nitrite, reduced oxygen cost of

submaximal work and enhanced high-intensity exercise tolerance (31), later work from the same laboratory (32) presented contrasting findings with acute administration of 6 g of pure L-arginine. In other studies L-arginine supplementation was shown to improve the respiratory response and cause faster O_2 kinetics (33) and in combination with amino acids to improve repeated sprint performance (34) and muscle strength and power (35, 36) in moderately trained subjects. However, no study to date has shown ergogenic effects on L-arginine on elite athletes or examined performance effects of combined L-arginine and nitrate supplementation.

Therefore, the purpose of the current study was to examine the effects of acute supplementation with L-arginine and nitrate on NO metabolites, running economy, endurance and sprint performance against in endurance-trained cross-country skiers. Experimentally, supplementation with L-arginine and nitrate was compared to supplementation with nitrate alone to reveal additional or interaction effects, and against placebo treatment. We hypothesized that combined L-arginine and nitrate supplementation would not alter the NO metabolites compared to supplementation of nitrate only and that exercise economy, endurance and sprint performance would remain unaltered between treatments in a group of elite endurance athletes.

Methods

Participants

Nine eighteen year-old male junior-elite cross-country skiers from Norway (height 181.0 ± 8.5 cm, body mass 74.2 ± 8.6 kg, maximal oxygen uptake (VO_{2max}) 69.3 ± 5.8 $mL \cdot kg^{-1} \cdot min^{-1}$, heart rate maximum 199 ± 9 $beats \cdot min^{-1}$) provided written informed consent before volunteering to participate in this study. Ethical approval was provided by the Norwegian

Regional Ethics Committee. Participants were a combination of national and international level junior skiers. All athletes were among the top 20 in the 2010 Norwegian Cup Series and had a training history of 505 ± 50 hours per year.

Preliminary Measurements and Standardisations

Preliminary tests were performed to determine each participant's VO_{2max} and maximal heart rate using an incremental running test to exhaustion on a motorised treadmill (Rodby RL 2500 E. Rodby Innovation AB, Vänge, Sweden). An individualized treadmill protocol was applied (37). The incline was 10.5% and the speed was increased by 0.5 – 1.0 $km \cdot h^{-1}$ every time the participant attained an oxygen uptake (VO_2) that was stable during a 30-s period. VO_{2max} was considered achieved if a plateau in VO_2 was attained despite increased workload, and if the participant attained a respiratory-exchange ratio above 1.10. The VO_2 plateau was defined as an increase in VO_2 of less than 150 mL. Respiratory variables were measured continuously by a computerized metabolic system with mixing chamber (Oxycon Pro, Erich Jaeger GmbH, Hoechberg, Germany). The average of the three highest 10-s consecutive measurements determined VO_{2max}. 20 µl blood samples were taken from the fingertips 1 and 3 min after completion of the test to determine the blood lactate concentration (Biosen 5140, EKF Diagnostic GmbH, Magdeburg, Germany). Heart rate was recorded continuously using a Polar RS800 monitor (Polar Electro OY, Kempele, Finland). The highest consecutive 5-s heart rate measurement was recorded during the last minute of the test and defined the maximum heart rate.

All preliminary testing and subsequent main trials were performed during the cross-country skiers' off-season (in April and May) to ensure standardisation of training and remove the interference of competitions on the results of the study. At this time of year, all participants carried out approximately 50% of their endurance training as running (i.e. 5-8 hours per

week), while the remainder of the endurance training was performed as roller skiing or cycling. Participants were experienced in performing maximal endurance running performances as part of their habitual training. To familiarise the participants with the specific experimental protocol and running time-trial distances, each participant performed two sprint and endurance running time-trial familiarisation sessions that were separated by 5-10 days. Within-subject variation was minimised by testing at the same time of day and following a 2-hour fast. There was no significant difference in the time taken to complete the time-trials between preliminary tests 1 and 2, with a coefficient of variation of 0.8% and 0.6% for the 180 m and the 5-km respectively.

Experimental Tests

Participants performed three main trials in a randomised, counterbalanced order, each separated by 1 week. Conditions were applied in a double-blind manner. Prior to the tests, participants ingested supplementation of either a) L-arginine (6 g) + nitrate (614 mg), b) nitrate (614 mg) + L-arginine-free placebo or c) L-arginine and nitrate-free placebo. Each trial consisted of two 5-min submaximal running tests on a treadmill, followed by 180 m and 5-km running time-trials on an indoor track. Blood pressure and nitrate and nitrite content in the EDTA-plasma were tested before and between the various tests.

Over the 48-h preceding the first experimental trial, each participant recorded their diet and replicated this diet before the second and third trial. Participants self-reported that they did not take any nutritional supplements in the 1-month prior to or during the study. Based on preliminary analyses of the participants' nutrition, there was no requirement for participants to minimise the consumption of nitrate containing foods during the study period. Participants arrived for testing in a rested and hydrated state, at least 2 hours postprandial, and having avoided strenuous exercise, caffeine and alcohol in the 24 h preceding testing sessions. Each

individual performed the same type of training in the week preceding all trials. The use of antibacterial mouthwash products was not permitted during the supplementation period as this has been shown to abolish the reduction of nitrate to nitrite in the oral cavity by commensal bacteria (38).

Participants were seated in an upright position for 10 min before a venous blood sample was obtained. Samples were centrifuged at room temperature for 10 minutes and at 1000 G within 2 min of collection. EDTA plasma was subsequently extracted and immediately frozen at -80^{o} C for later analysis of NO metabolites. Each participant was then supplemented with one opaque gelatine capsule containing either 1 g potassium nitrate (9.9 mmol giving 614 mg nitrate) or 1 g of maltodextrin as a placebo (MaxiNutrition, Hertfordshire, UK) 2.5 h prior to testing. Additionally each subject drank 500 mL of water. Capsules were consumed with a standardised breakfast and were well tolerated. Furthermore, participants were supplemented with additional six opaque gelatine capsules containing in total 6 g L-arginine or 6 g of maltodextrin as a placebo (MaxiNutrition, Hertfordshire, UK) 1 h prior to testing. The participants fasted and avoided strenuous physical activity or exercise in the period prior to testing. On returning to the laboratory, and after 10 min seated rest, additional blood samples were drawn. 2.5 h has been shown to coincide with peak plasma nitrite concentrations via dietary or pharmacological nitrate administration (39). Likewise maximal concentrations of nitrite are reached 60-90 min following ingestion of ~6 g of L-arginine (40).

The blood pressure in the brachial artery was measured with subjects in a rested (10 minutes), seated position prior to each exercise bout via an automated sphygmomanometer (Microlife BP A100 plus, Microlife AG, Espenstrasse 139, 9443 Widnau, Switzerland). Three measurements were taken, and the mean of the two last was used in analysis.

After a standardised low intensity (60-70% of maximal heart rate) 15-min warm-up, two 5-min bouts of submaximal running were performed at 10 $km \cdot h^{-1}$ and 14 $km \cdot h^{-1}$ on a motorised treadmill at a 1% incline. The 1% incline was chosen to simulate flat terrain to compensate for the lack of air drag on the treadmill. The speeds employed here were based on pilot testing that established exercise intensities corresponding to approximately 55% and 75% of the participants' maximal oxygen uptake. Pulmonary gas exchange data were measured during each 5-min stage and a further blood sample was drawn after the completion of submaximal exercise. Following a 15-min period of rest, participants completed a 180-m running time-trial on a 250-m indoor track, before a 5-km running time-trial was performed on the same track 15 min after execution of the 180 m. Performance times were recorded by using two synchronised stop-watches (Regnly RT3, Emit AS, Oslo, Norway). Further blood samples were drawn and blood pressure measured following the performance tests and 30 min post-trial.

Measurement of nitric oxide blood metabolites

Ozone-based chemiluminescence set up

Cleaving reagents were placed in a glass purge vessel with a rubber septum covered injection inlet. Oxygen free nitrogen gas was bubbled through the reagent mix, which was heated in a water bath on a thermostatically controlled hotplate. The reaction vessel, linked to a trap containing 25 mL sodium hydroxide (1N), was further connected to the NO analyser (Sievers NOA 280i, Analytix, UK) as described in detail previously (41-43). Ozone-based chemiluminescence signals obtained were transferred to Origin software (version 7) for smoothing using point-to-point averaging and peak area under the curve analysis. Test sample concentrations were calculated against the area under the curve obtained from a standard curve in each case.

Plasma nitrate

Vanadium chloride (0.05 mol·L^{-1}) in HCl (30 mL) heated to 80° C was used to reduce NO metabolites to NO. Standards (0.5-100 µmol·L^{-1}) of sodium nitrate (15 µL) were measured in each fresh cleavage reagent. Frozen plasma samples were thawed for 3 min at 37º C and 15 µL of each plasma sample injected directly via the injection port. This assay is sensitive to <1 µmol·L^{-1} nitrate with accuracy better than ± 7 %. The total ozone-based chemiluminescence signal obtained was taken to reflect nitrate plus nitrite in the sample. In order to obtain a true nitrate value the corresponding level from tri-iodide measurement was then subtracted.

Plasma nitrite and protein-bound nitric oxide

A stock solution of acidic/tri-iodide cleavage reagent (70 mL) was prepared fresh each day. Frozen plasma samples were thawed for 3 min at 37º C and 200 µL plasma samples injected into 5 mL reagent at 50° C. This assay is sensitive to <10 nmol·L^{-1} nitrite with accuracy better than ± 5 %. The ozone-based chemiluminescence signal obtained was taken to reflect nitrite in the sample. In order to account for protein-bound NO metabolites (RSNO, RNNO), in duplicate plasma samples to the above, we first removed nitrite (using addition acidified sulfanilamide) and recorded the residual ozone-based chemiluminescence signal, which was taken to reflect RSNO+RNNO in the sample. In all samples tested this was <5 % of the nitrite signal.

Statistical Analysis

All data were checked for normality using the Shapiro-Wilk test and are presented as mean ± standard deviation. Statistical significance was set at an alpha level of 0.05. A linear mixed model was used to analyse effects of supplementation and to locate pairwise differences in physiological responses during submaximal exercise and in time-trial performances. A linear

mixed model was also employed to identify effects of treatments and time and their interactions on nitrate and nitrite concentrations and for blood pressure. A random intercept was used to model within-subject correlations. To obtain a normal distribution, plasma nitrate concentrations were transformed into log-scale. Where significant overall effects were observed, post hoc tests determined the effects both between treatments at each time point and between time points within each treatment relative to baseline. Bonferroni correction was used to adjust for multiple comparisons. Sample size estimations were based on those reported by Lansley and colleagues (44) who calculated that a sample size of nine provided an 80% power to detect approximately 2% difference in sprint and endurance time-trial performances at an alpha level of 0.05. Statistical tests were conducted using SPSS version 21.0 (Chicago, IL).

Results

At the two 5-min submaximal exercise bouts (10 and 14 $km \cdot h^{-1}$), exercise intensity (expressed as percentage of VO_{2max}), steady-state VO_2, respiratory exchange ratio, ventilation, heart rate and blood lactate concentration did not differ between the three experimental conditions (Table 1). There were no significant differences in 5-km time-trial performances (L-arginine and nitrate =1011 ± 49 s, nitrate = 1016 ± 52 s, placebo = 1005 ± 47 s) between supplementations. 180 m time-trial performance after L-arginine and nitrate supplementation (24.4 ± 0.8 s) did not significantly differ from placebo (24.3 ± 0.7 s), but differed from nitrate supplementation (24.1 ± 0.9, $p = 0.04$). For both time-trials, there were no consistent differences between participants' trials such that no trial-order effects were apparent. The individual running performances achieved by the athletes were close to identical compared to those observed during preliminary repeatability testing. When analysed

in 250 m running splits for the 5-km, there were no significant differences in performance times between treatments at any time-point, and overall pacing strategy was consistent between trials.

--- Table 1 around here ---

There were non-significant tendencies towards lower baseline plasma nitrate concentrations prior to the L-arginine and nitrate supplementation (23 ± 6 $\mu mol \cdot L^{-1}$) compared with nitrate (32 ± 10 $\mu mol \cdot L^{-1}$, $p = 0.07$) and placebo (35 ± 19 $\mu mol \cdot L^{-1}$, $p = 0.06$). Nitrate concentrations were elevated after L-arginine and nitrate supplementation (296 ± 77 $\mu mol \cdot L^{-1}$, $p < 0.001$), which did not differ from nitrate alone (335 ± 65 $\mu mol \cdot L^{-1}$), but was significantly higher than with placebo (26 ± 16 $\mu mol \cdot L^{-1}$, $p < 0.001$). Nitrate concentrations for L-arginine and nitrate supplementation remained significantly elevated above placebo concentrations throughout the study (all timepoints: $p < 0.001$), with no difference compared to nitrate alone (Figure 1A).

Pre-supplementation baseline concentrations of plasma nitrite did not differ between trials (L-arginine and nitrate = 158 ± 40 $nmol \cdot L^{-1}$, nitrate = 124 ± 68 $nmol \cdot L^{-1}$, placebo = 114 ± 35 $nmol \cdot L^{-1}$). Conversion of nitrate to nitrite was apparent from the marked increase in plasma nitrite concentrations after the L-arginine and nitrate supplementation (319 ± 54 $nmol \cdot L^{-1}$, $p < 0.001$), which did not differ from nitrate alone (328 ± 107 $nmol \cdot L^{-1}$), but was significantly more elevated than with placebo (149 ± 64 $nmol \cdot L^{-1}$, $p < 0.01$). Nitrite concentrations for L-arginine and nitrate supplementation remained significantly elevated above its respective baseline level and placebo concentrations throughout the study (pre submax until post TT) ($p < 0.05$), but was normalised throughout recovery. Effects of supplementation with L-arginine and nitrate, and nitrate alone supplementation on nitrite levels were non-significant throughout the study (Figure 1B).

--- Figure 1A-C around here ---

Pre-supplementation baseline mean arterial blood pressure (MAP) did not differ between trials (L-arginine and nitrate = 96 ± 5 mmHg, nitrate = 98 ± 6 mmHg, placebo = 100 ± 7 mmHg). There was a clear effect of time, but no effect of treatment on MAP, neither after supplementation (L-arginine and nitrate = 100 ± 10 mmHg, nitrate = 98 ± 6 mmHg, placebo 100 ± 5 mmHg), nor throughout the study (time: $p < 0.01$, treatment: $p = 0.80$, Figure 1C).

Discussion

The current study investigated the effects of acute supplementation of L-arginine and nitrate on running economy, endurance and sprint performance in elite cross-country skiers. L-arginine and nitrate supplementation was compared both against nitrate supplementation alone to reveal any additional effects of L-arginine and against placebo treatment. Supplementations with L-arginine and nitrate and with nitrate alone both demonstrated increased plasma nitrate and nitrite concentrations compared to placebo. However, no additional effect of L-arginine compared to nitrate alone was found. There were no differences in exercise economy or 5-km running time-trial performances between treatment conditions, whereas a slightly better sprint performance following nitrate supplementation compared to L-arginine and nitrate was revealed.

There is no previous reported data concerning NO metabolites after acute supplementation of combined L-arginine and nitrate. Here, this supplementation significantly increased plasma nitrate and nitrite concentrations relative to placebo in a similar fashion as nitrate only. Nitrate and nitrite values for both treatment conditions were comparable to previous studies with acute nitrate supplementation (7, 8, 11, 19). Thus, the addition of L-arginine to nitrate supplementation did not have any additional effect on NO metabolites and should theoretically not give any additional ergogenic effect either. In contrast to what was found

here, two previous studies reported higher baseline plasma nitrate and nitrite concentrations in trained populations compared to sedentary controls (21, 22). This indicated that well-trained athletes may have increased baseline levels also without supplementation. However, the current and previous studies on endurance trained cross-country skiers (19) and cyclists (19, 45) contrast these arguments by showing normal baseline levels of plasma nitrate and nitrite in line with concentrations reported for untrained populations. Thus, the lack of ergogenic effects in the current study could not be explained by elevated baseline levels of NO metabolites. After supplementation the plasma nitrite concentration for the treatment conditions remained elevated until the finish of the 5 km time-trial. We therefore regard it unlikely that the lack of ergogenic effect found in the current study was caused by depletion of nitrite following the test battery executed before the time-trials.

Neither steady-state oxygen cost, respiratory exchange ratio, ventilation, heart rate nor blood lactate concentration during low and moderate intensity treadmill running differed between supplementations and placebo. Thus, targeting both pathways for increasing the NO availability simultaneously with L-arginine and nitrate gave no improvements in exercise economy among these cross-country skiers compared to nitrate alone or placebo. Previous research done on endurance-trained cyclists (12) untrained or moderately trained subjects (6-10) reports improved exercise economy following ingestions of nitrate. Although previous studies show positive effects also of L-arginine on exercise economy in untrained and moderately trained individuals, L-arginine was used in combination with other components such as beetroot and amino acids (31, 33-36), and it may be speculated that these other components than L-arginine induced the main effects as shown recently by Vanhatalo et al. (32). The lack of effect with L-arginine supplementation is supported by Abel and colleagues (46) and Colombani and colleagues (47) that reported no modification in endurance performance in a well-trained population with supplementation of combined L-arginine and

L-aspartate. Although the combination L-arginine and nitrate had not been examined previously in elite athletes, no additional effect compared to nitrate could be expected due to the similar nitrate and nitrate responses. Consequently, the lack of supplementation effect on exercise economy found in this study is consistent with previous studies conducted with nitrate supplementation alone in a similar elite athlete population (16-20). Still, since Wylie et al. (26) showed that the effects of nitrate were dose dependent, future studies are needed to examine the effects of higher doses of nitrate on exercise economy.

Also the 5-km endurance running performance was close to identical between treatment conditions. Although this study was the first to examine the performance effects of combined L-arginine and nitrate supplementation in elite athletes, the results correspond with previous studies assessing the effects of acute nitrate supplementation on well-trained populations.(16-20). In an elite population the benefits in pulmonary, cardiovascular and neuromuscular systems induced by long-term training may overcome many of the potential effects from supplements targeting increased NO bioavailability. Repetitive exercise may result in an up-regulation of endothelial NO activity (1), which may also be illustrated by the lack of effects on blood pressure in the current study. Whether exercise modes with more reliance of the upper body, hypoxic environments or chronic supplementation over a longer time periods would induce positive effects on exercise economy or endurance performance also in elite athletes require further examination.

A novelty of the current study was that we examined the effects of acute L-arginine and nitrate supplementation on sprint running performance. Here, we found no differences between L-arginine and nitrate versus placebo, but reduced performance compared to nitrate supplementation alone. Since there was no change in NO metabolites when L-arginine was added to nitrate, explanation of this finding would be speculative, and further research is needed to exclude whether the addition of L-arginine may be harmful for sprint performance

or if supplementation with nitrate alone induce positive effects. Previous studies indicate that up-regulated NO might improve sprint performance, possibly through enhanced blood flow and contractile function of type II muscle fibers (13, 14). However, these previous studies were done with 5 to 7 days of supplementation in rodents, and the effects of enhanced blood flow and muscle contractile function may only be realized following a longer supplementation period than used here. It could be that longer duration of exposure to nitrate supplementation might facilitate changes in mitochondrial and sarcoplasmic reticulum protein expression (4, 14). Still, improvements in repeated sprint performance (34) and muscle strength and power (35, 36) have been observed both for acute and chronic L-arginine supplementation in untrained or moderately trained subjects. Since we examined sprint performance effects in highly endurance-trained athletes in the current study, further elucidation of athletes with more Type II muscle fibers training for short-duration sprint events or more prolonged sprints (200-800 m) are required.

Conclusions

Compared to placebo, greater nitric oxide availability was induced both by acute supplementation of L-arginine and nitrate and with nitrate alone. However, no additional effects on NO biomarkers were revealed with the addition of L-arginine to nitrate supplementation which indicates that L-arginine induce no ergogenic effect in elite endurance athletes. Neither exercise economy nor endurance running performances were altered with supplementation in endurance-trained cross-country skiers. This indicates that groups of elite athletes with a healthy daily nutrition combined with high dosages of endurance training do not alter physiological responses or endurance performance with acute supplementation of NO donors. The reasons for the slightly better sprint performance with nitrate

supplementation compared to the combination with L-arginine and nitrate is unclear and needs further examination. Overall, training status of the subjects is an important factor linked to the ergogenic effect of NO and the absence of ergogenic effects of increased NO availability in endurance-trained athletes may be explained by the physiological and metabolic adaptations derived from chronic physical training. However, potential dietary effects on elite athletes are small and positive individual responses that this study could not detect may still occur. Furthermore, more studies are required to explore whether nutritional supplementations can increase NO availability and enhance performance in other elite athlete groups, exercise modes, hypoxic environments or with chronic supplementation.

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Figure captions

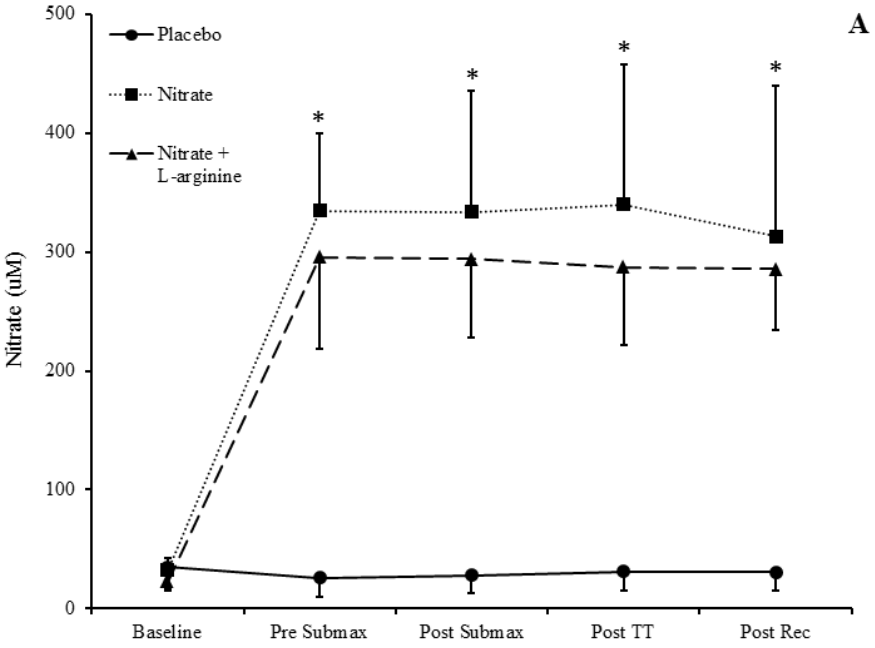
Figure 1. Plasma nitrate (A) and nitrite (B) concentrations and mean arterial pressure (MAP) (C) before (Baseline) and 2.5 h after supplementation with combined L-arginine and nitrate, nitrate alone or placebo (Pre Submax), after submaximal treadmill running at 10 and 14 km h^{-1} and before the 180 m and 5-km running time-trial performances (Pre TT), after the time-trials (Post TT), and 30 min into recovery (Post Rec). Values are means and SD.

* Time points significantly different between L-arginine + nitrate treatment and the placebo condition ($p < 0.05$).

Time points significantly different from baseline in the L-arginine + nitrate treatment ($p < 0.05$).

TABLE 1. Oxygen uptake (VO_2), respiratory exchange ratio (RER), ventilation, heart rate (HR), and blood lactate concentration measured during submaximal treadmill running at 10 and 14 $km \cdot h^{-1}$ in nine male endurance-trained athletes after ingestion of L-arginine and nitrate, nitrate and placebo.

	Nitrate + L-arginine	Nitrate	Placebo
10 $km \cdot h^{-1}$			
VO_2 ($L \cdot min^{-1}$)	2.84 ± 0.34	2.82 ± 0.37	2.83 ± 0.39
VO_2 (% of VO_{2max})	55.6 ± 4.5	55.2 ± 5.3	55.3 ± 4.9
RER	0.88 ± 0.04	0.88 ± 0.03	0.86 ± 0.03
Ventilation ($L \cdot min^{-1}$)	66 ± 7	66 ± 6	65 ± 5
HR (% of HR_{max})	74.1 ± 4.3	73.6 ± 5.7	72.6 ± 4.3
Lactate ($mmol \cdot L^{-1}$)	1.1 ± 0.4	1.1 ± 0.5	1.2 ± 0.5
14 $km \cdot h^{-1}$			
VO_2 ($L \cdot min^{-1}$)	3.90 ± 0.44	3.75 ± 0.66	3.87 ± 0.46
VO_2 (% of VO_{2max})	76. 4 ± 5.6	73.2 ± 9.8	75.6 ± 5.14
RER	0.96 ± 0.05	0.97 ± 0.04	0.96 ± 0.37
Ventilation ($L \cdot min^{-1}$)	103 ± 11	104 ± 9	103 ± 9
HR (% of HR_{max})	89.1 ± 0.9	90.2 ± 3.7	88.7 ± 2.7
Lactate ($mmol \cdot L^{-1}$)	3.1 ± 1.2	3.1 ± 1.4	3.1 ± 1.4


A
Placebo
Nitrate
Nitrate +
L-arginine
Nitrate (uM)
500
400
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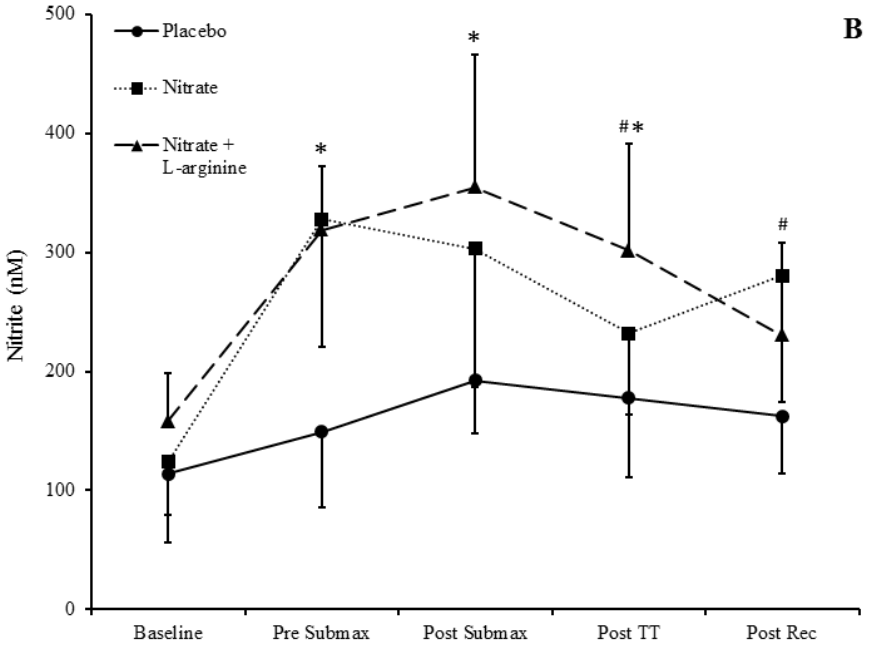
Baseline
Pre Submax
Post Submax
Post TT
Post Rec

1


B
Placebo
Nitrate
Nitrate +
L-arginine
Nitrite (nM)
500
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Baseline
Pre Submax
Post Submax
Post TT
Post Rec

2