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

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28 **Running head:** Dietary supplementation in elite athletes

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1 **Abstract**

2 This study examined the effects of acute supplementation with L-arginine and nitrate on
3 running economy, endurance and sprint performance in endurance-trained athletes. In a
4 randomized cross-over, double-blinded design we compared the effects of combined
5 supplementation with 6 g L-arginine and 614 mg nitrate against 614 mg nitrate alone and
6 placebo in nine male elite cross-country skiers (age 18 ± 0 years, VO_{2max} 69.3 ± 5.8 ml·min⁻¹·
7 kg⁻¹). After a 48-hour standardization of nutrition and exercise the athletes were tested for
8 plasma nitrate and nitrite concentrations, blood pressure, submaximal running economy at 10
9 km·h⁻¹ and 14 km·h⁻¹ at 1% incline and 180 m as well as 5-km time-trial running
10 performances. Plasma nitrite concentration following L-arginine+nitrate supplementation
11 (319 ± 54 nmol·L⁻¹) did not differ from nitrate alone (328 ± 107 nmol·L⁻¹), and both were
12 higher than placebo (149 ± 64 nmol·L⁻¹, $p < 0.01$). There were no differences in physiological
13 responses during submaximal running or in 5-km performance between treatments. The
14 plasma nitrite concentrations indicate greater nitric oxide availability both following acute
15 supplementation of L-arginine+nitrate and with nitrate alone compared to placebo, but no
16 additional effect was revealed when L-arginine was added to nitrate. Still, there were no
17 effects of supplementation on exercise economy or endurance running performance in
18 endurance-trained cross-country skiers.

19

20

21 **Keywords:** endurance athletes, exercise economy, nitric oxide.

1 **Introduction**

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

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

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

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

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

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

1 submaximal work and enhanced high-intensity exercise tolerance (31), later work from the
2 same laboratory (32) presented contrasting findings with acute administration of 6 g of pure
3 L-arginine. In other studies L-arginine supplementation was shown to improve the respiratory
4 response and cause faster O₂ kinetics (33) and in combination with amino acids to improve
5 repeated sprint performance (34) and muscle strength and power (35, 36) in moderately
6 trained subjects. However, no study to date has shown ergogenic effects on L-arginine on
7 elite athletes or examined performance effects of combined L-arginine and nitrate
8 supplementation.

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

18

19 **Methods**

20 *Participants*

21 Nine eighteen year-old male junior-elite cross-country skiers from Norway (height $181.0 \pm$
22 8.5 cm, body mass 74.2 ± 8.6 kg, maximal oxygen uptake (VO_{2max}) 69.3 ± 5.8 mL·kg⁻¹·min⁻¹,
23 heart rate maximum 199 ± 9 beats·min⁻¹) provided written informed consent before
24 volunteering to participate in this study. Ethical approval was provided by the Norwegian

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

4 ***Preliminary Measurements and Standardisations***

5 Preliminary tests were performed to determine each participant's VO_{2max} and maximal heart
6 rate using an incremental running test to exhaustion on a motorised treadmill (Rodby RL
7 2500 E. Rodby Innovation AB, Vänge, Sweden). An individualized treadmill protocol was
8 applied (37). The incline was 10.5% and the speed was increased by $0.5 - 1.0 \text{ km}\cdot\text{h}^{-1}$ every
9 time the participant attained an oxygen uptake (VO_2) that was stable during a 30-s period.
10 VO_{2max} was considered achieved if a plateau in VO_2 was attained despite increased workload,
11 and if the participant attained a respiratory-exchange ratio above 1.10. The VO_2 plateau was
12 defined as an increase in VO_2 of less than 150 mL. Respiratory variables were measured
13 continuously by a computerized metabolic system with mixing chamber (Oxycon Pro, Erich
14 Jaeger GmbH, Hoechberg, Germany). The average of the three highest 10-s consecutive
15 measurements determined VO_{2max} . 20 μl blood samples were taken from the fingertips 1 and
16 3 min after completion of the test to determine the blood lactate concentration (Biosen 5140,
17 EKF Diagnostic GmbH, Magdeburg, Germany). Heart rate was recorded continuously using
18 a Polar RS800 monitor (Polar Electro OY, Kempele, Finland). The highest consecutive 5-s
19 heart rate measurement was recorded during the last minute of the test and defined the
20 maximum heart rate.

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

1 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.

10 ***Experimental Tests***

11 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.

18 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

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

5 Participants were seated in an upright position for 10 min before a venous blood sample was
6 obtained. Samples were centrifuged at room temperature for 10 minutes and at 1000 G within
7 2 min of collection. EDTA plasma was subsequently extracted and immediately frozen at -
8 80° C for later analysis of NO metabolites. Each participant was then supplemented with one
9 opaque gelatine capsule containing either 1 g potassium nitrate (9.9 mmol giving 614 mg
10 nitrate) or 1 g of maltodextrin as a placebo (MaxiNutrition, Hertfordshire, UK) 2.5 h prior to
11 testing. Additionally each subject drank 500 mL of water. Capsules were consumed with a
12 standardised breakfast and were well tolerated. Furthermore, participants were supplemented
13 with additional six opaque gelatine capsules containing in total 6 g L-arginine or 6 g of
14 maltodextrin as a placebo (MaxiNutrition, Hertfordshire, UK) 1 h prior to testing. The
15 participants fasted and avoided strenuous physical activity or exercise in the period prior to
16 testing. On returning to the laboratory, and after 10 min seated rest, additional blood samples
17 were drawn. 2.5 h has been shown to coincide with peak plasma nitrite concentrations via
18 dietary or pharmacological nitrate administration (39). Likewise maximal concentrations of
19 nitrite are reached 60-90 min following ingestion of ~6 g of L-arginine (40).

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

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

14 ***Measurement of nitric oxide blood metabolites***

15 *Ozone-based chemiluminescence set up*

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

1 *Plasma nitrate*

2 Vanadium chloride ($0.05 \text{ mol}\cdot\text{L}^{-1}$) in HCl (30 mL) heated to 80° C was used to reduce NO
3 metabolites to NO. Standards ($0.5\text{-}100 \text{ }\mu\text{mol}\cdot\text{L}^{-1}$) of sodium nitrate (15 μL) were measured in
4 each fresh cleavage reagent. Frozen plasma samples were thawed for 3 min at 37° C and 15
5 μL of each plasma sample injected directly via the injection port. This assay is sensitive to <1
6 $\mu\text{mol}\cdot\text{L}^{-1}$ nitrate with accuracy better than $\pm 7 \%$. The total ozone-based chemiluminescence
7 signal obtained was taken to reflect nitrate plus nitrite in the sample. In order to obtain a true
8 nitrate value the corresponding level from tri-iodide measurement was then subtracted.

9 *Plasma nitrite and protein-bound nitric oxide*

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

19 ***Statistical Analysis***

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

1 mixed model was also employed to identify effects of treatments and time and their
2 interactions on nitrate and nitrite concentrations and for blood pressure. A random intercept
3 was used to model within-subject correlations. To obtain a normal distribution, plasma nitrate
4 concentrations were transformed into log-scale. Where significant overall effects were
5 observed, post hoc tests determined the effects both between treatments at each time point
6 and between time points within each treatment relative to baseline. Bonferroni correction was
7 used to adjust for multiple comparisons. Sample size estimations were based on those
8 reported by Lansley and colleagues (44) who calculated that a sample size of nine provided
9 an 80% power to detect approximately 2% difference in sprint and endurance time-trial
10 performances at an alpha level of 0.05. Statistical tests were conducted using SPSS version
11 21.0 (Chicago, IL).

12

13 **Results**

14 At the two 5-min submaximal exercise bouts (10 and 14 km·h⁻¹), exercise intensity
15 (expressed as percentage of VO_{2max}), steady-state VO₂, respiratory exchange ratio,
16 ventilation, heart rate and blood lactate concentration did not differ between the three
17 experimental conditions (Table 1). There were no significant differences in 5-km time-trial
18 performances (L-arginine and nitrate = 1011 ± 49 s, nitrate = 1016 ± 52 s, placebo = 1005 ±
19 47 s) between supplementations. 180 m time-trial performance after L-arginine and nitrate
20 supplementation (24.4 ± 0.8 s) did not significantly differ from placebo (24.3 ± 0.7 s), but
21 differed from nitrate supplementation (24.1 ± 0.9, p = 0.04). For both time-trials, there were
22 no consistent differences between participants' trials such that no trial-order effects were
23 apparent. The individual running performances achieved by the athletes were close to
24 identical compared to those observed during preliminary repeatability testing. When analysed

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

4 **--- Table 1 around here ---**

5 There were non-significant tendencies towards lower baseline plasma nitrate concentrations
6 prior to the L-arginine and nitrate supplementation ($23 \pm 6 \mu\text{mol}\cdot\text{L}^{-1}$) compared with nitrate
7 ($32 \pm 10 \mu\text{mol}\cdot\text{L}^{-1}$, $p = 0.07$) and placebo ($35 \pm 19 \mu\text{mol}\cdot\text{L}^{-1}$, $p = 0.06$). Nitrate concentrations
8 were elevated after L-arginine and nitrate supplementation ($296 \pm 77 \mu\text{mol}\cdot\text{L}^{-1}$, $p < 0.001$),
9 which did not differ from nitrate alone ($335 \pm 65 \mu\text{mol}\cdot\text{L}^{-1}$), but was significantly higher than
10 with placebo ($26 \pm 16 \mu\text{mol}\cdot\text{L}^{-1}$, $p < 0.001$). Nitrate concentrations for L-arginine and nitrate
11 supplementation remained significantly elevated above placebo concentrations throughout the
12 study (all timepoints: $p < 0.001$), with no difference compared to nitrate alone (Figure 1A).

13 Pre-supplementation baseline concentrations of plasma nitrite did not differ between trials (L-
14 arginine and nitrate = $158 \pm 40 \text{nmol}\cdot\text{L}^{-1}$, nitrate = $124 \pm 68 \text{nmol}\cdot\text{L}^{-1}$, placebo = 114 ± 35
15 $\text{nmol}\cdot\text{L}^{-1}$). Conversion of nitrate to nitrite was apparent from the marked increase in plasma
16 nitrite concentrations after the L-arginine and nitrate supplementation ($319 \pm 54 \text{nmol}\cdot\text{L}^{-1}$, $p <$
17 0.001), which did not differ from nitrate alone ($328 \pm 107 \text{nmol}\cdot\text{L}^{-1}$), but was significantly
18 more elevated than with placebo ($149 \pm 64 \text{nmol}\cdot\text{L}^{-1}$, $p < 0.01$). Nitrite concentrations for L-
19 arginine and nitrate supplementation remained significantly elevated above its respective
20 baseline level and placebo concentrations throughout the study (pre submax until post TT) (p
21 < 0.05), but was normalised throughout recovery. Effects of supplementation with L-arginine
22 and nitrate, and nitrate alone supplementation on nitrite levels were non-significant
23 throughout the study (Figure 1B).

24 **--- Figure 1A-C around here ---**

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

6

7 **Discussion**

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

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

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

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

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

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

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

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

14

15 **Conclusions**

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

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

10

11

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8 **Figure captions**

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

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

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

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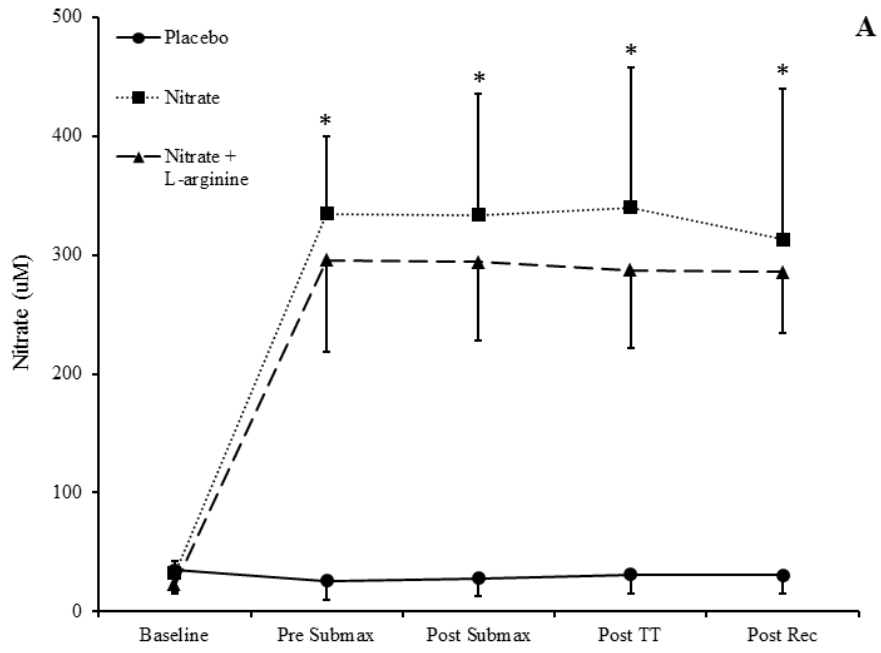
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1 **TABLE 1.** Oxygen uptake (VO_2), respiratory exchange ratio (RER), ventilation, heart rate
 2 (HR), and blood lactate concentration measured during submaximal treadmill running at 10
 3 and 14 $\text{km}\cdot\text{h}^{-1}$ in nine male endurance-trained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

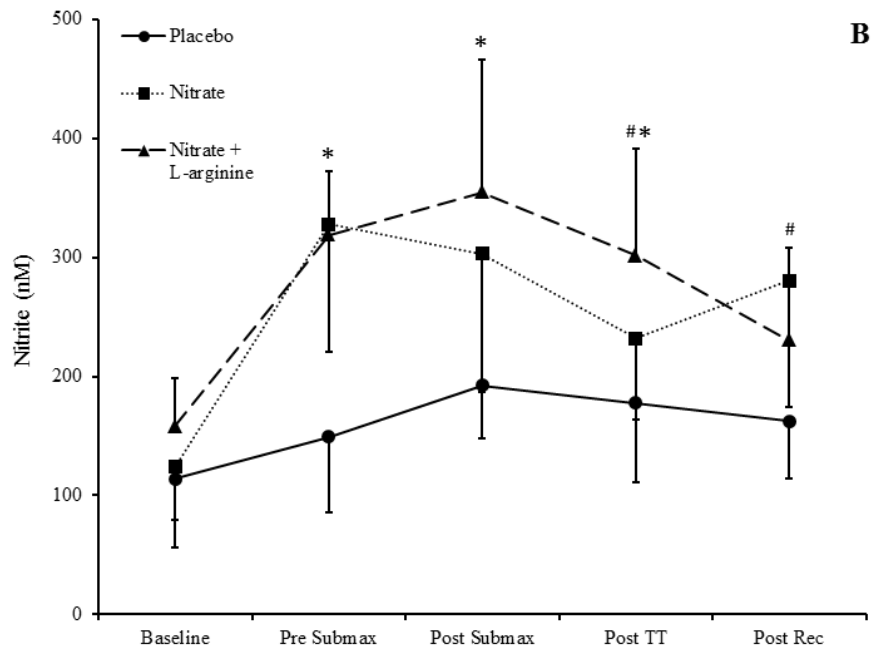
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| | Nitrate + L-arginine | Nitrate | Placebo |
|--|----------------------|-----------------|-----------------|
| 10 $\text{km}\cdot\text{h}^{-1}$ | | | |
| VO_2 ($\text{L}\cdot\text{min}^{-1}$) | 2.84 ± 0.34 | 2.82 ± 0.37 | 2.83 ± 0.39 |
| VO_2 (% of $\text{VO}_{2\text{max}}$) | 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 ($\text{L}\cdot\text{min}^{-1}$) | 66 ± 7 | 66 ± 6 | 65 ± 5 |
| HR (% of HR_{max}) | 74.1 ± 4.3 | 73.6 ± 5.7 | 72.6 ± 4.3 |
| Lactate ($\text{mmol}\cdot\text{L}^{-1}$) | 1.1 ± 0.4 | 1.1 ± 0.5 | 1.2 ± 0.5 |
| 14 $\text{km}\cdot\text{h}^{-1}$ | | | |
| VO_2 ($\text{L}\cdot\text{min}^{-1}$) | 3.90 ± 0.44 | 3.75 ± 0.66 | 3.87 ± 0.46 |
| VO_2 (% of $\text{VO}_{2\text{max}}$) | 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 ($\text{L}\cdot\text{min}^{-1}$) | 103 ± 11 | 104 ± 9 | 103 ± 9 |
| HR (% of HR_{max}) | 89.1 ± 0.9 | 90.2 ± 3.7 | 88.7 ± 2.7 |
| Lactate ($\text{mmol}\cdot\text{L}^{-1}$) | 3.1 ± 1.2 | 3.1 ± 1.4 | 3.1 ± 1.4 |

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