Nitrate supplementation and high-intensity performance in competitive cyclists¹

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Abstract: Consumption of inorganic nitrate (NO_3^-) is known to enhance endurance exercise performance in recreationally trained subjects. Here we report the effect on a high-intensity performance task in national-level cyclists. The performance test consisted of 2 cycle ergometer time trials of 4 min duration with 75 min between trials. In a randomized crossover design, 26 cyclists performed the test under the following 4 conditions (each separated by a 6-day washout): consumption of 70 mL of nitrate-rich beetroot juice at 150 min or 75 min before the first time trial, addition of a 35 mL “top-up dose” following the first time trial in the 150 min condition, and consumption of a placebo. A linear mixed model with adjustments for learning effects and athlete fitness (peak incremental power) was used to estimate effects on mean power, with probabilistic inferences based on a smallest important effect of 1.0%. Peak plasma nitrite (NO_2^-) concentration was greatest when nitrate was taken 75 min before the first time trial. Relative to placebo, the mean effect of all 3 nitrate treatments was unclear in the first time trial (1.3%, 90% confidence limits: $\pm 1.7\%$), but possibly harmful in the second time trial (-0.3% , $\pm 1.6\%$). Differences between nitrate treatments were unclear, as was the estimate of any consistent individual response to the treatments. Allowing for sampling uncertainty, the effect of nitrate on performance was less than previous studies. Under the conditions of our experiment, nitrate supplementation may be ineffective in facilitating high-intensity exercise in competitive athletes.

Key words: ergogenic aid, athlete, beetroot, nitrite.

Résumé : La consommation de nitrate inorganique améliore, études à l'appui, la performance physique en endurance chez des sujets entraînés à titre récréatif. La présente étude rapporte l'effet du nitrate inorganique sur la performance de cyclistes de niveau national. Le test de performance consiste en deux contre-la-montre de 4 min sur un cycloergomètre intercalés de 75 min. Vingt-six cyclistes effectuent le test selon un devis croisé aléatoire dans quatre conditions, chacune étant séparée par 6 jours d'élimination : consommation de 70 mL de jus de betterave riche en nitrate 150 ou 75 min avant le premier contre-la-montre, puis ajout d'une dose de 35 mL après le premier contre-la-montre dans la condition 150 min et consommation d'un placebo. On utilise un modèle linéaire mixte prenant en compte les effets de l'apprentissage et la condition physique des athlètes (puissance progressive de pointe) pour estimer l'impact sur la puissance moyenne, l'inférence probabiliste établissant le plus petit effet important à 1,0 %. On enregistre une plus forte concentration plasmatique de nitrite quand le nitrate est consommé 75 min avant le premier contre-la-montre. Comparativement au placebo, l'effet moyen des trois apports de nitrate n'est pas net au premier contre-la-montre (1,3 %, IC 90 % : $\pm 1,7$ %) et possiblement nuisible au deuxième contre-la-montre ($-0,3$ %, $\pm 1,6$ %). Les différences entre les traitements au nitrate ne sont pas nettes tout comme les réponses individuelles cohérentes aux traitements. En admettant l'incertitude de l'échantillonnage, l'effet du nitrate sur la performance est inférieur aux résultats des études antérieures. Dans les conditions de la présente étude, la supplémentation en nitrate se révèle probablement inefficace pour l'amélioration de la performance physique de haute intensité chez des athlètes de compétition. [Traduit par la Rédaction]

Mots-clés : agent ergogène, athlète, betterave, nitrite.

Introduction

The consumption of inorganic nitrate (NO_3^-) is currently advocated as a means of improving several facets of cardiovascular health. Recent literature has reported improvements to hypertension (Lundberg et al. 2008), peripheral arterial disease (Pattillo et al. 2010), and metabolic dysfunction (Carlstrom et al. 2010) following doses of nitrate. A currently evolving area of research is now examining the application of nitrate supplementation outside of a clinical setting, focusing on its possible ergogenic effect on exercise performance. Of note, Larsen and colleagues reported

that supplementation with sodium nitrate was able to enhance cycling mechanical efficiency (Larsen et al. 2007). Additional studies investigating supplementation with beetroot juice (a rich source of NO_3^-) have reported similar changes in efficiency (Bailey et al. 2009), as well as improvements in running endurance (Lansley et al. 2011b), power output at ventilatory threshold, and ramp test peak power (Vanhatalo et al. 2010).

The benefits reported in these studies are not attributable to the NO_3^- compound per se, but rather its reduced states: nitrite (NO_2^-) and nitric oxide (NO) (Bailey et al. 2011). Ingested NO_3^- is quickly

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absorbed by the upper gastrointestinal tract before being taken up by the salivary glands and secreted into the mouth, where anaerobic bacteria reduce NO_3^- to bioactive NO_2^- . Nitrite may then be reduced further into NO in the stomach or reabsorbed to increase plasma NO_2^- concentration ($[\text{NO}_2^-]$). Nitric oxide is known to be an important intermediate in numerous physiological pathways, regulating key functions such as blood flow (Umans and Levi 1995), glucose uptake (Merry et al. 2010), muscle contraction (Maréchal and Gailly 1999), and mitochondrial respiration (Moncada and Erusalimsky 2002). The precise mechanism by which nitrogen derivatives enhance exercise efficiency is not yet fully understood, making it difficult to determine the most practical and applicable use for NO_3^- supplementation.

Although several studies have documented improvements in the performance of exercise lasting 7–20 min (Cermak et al. 2012a; Murphy et al. 2012; Lansley et al. 2011b), there is little research focusing on shorter, more intense activities. High-intensity efforts, such as those seen in competitive track cycling, have different physiological characteristics to the activities in the aforementioned research, with a much greater rate of energy demand (Jeukendrup et al. 2000). As a consequence of this energy demand, the body is placed under greater hypoxic and acidic stress, conditions favourable for the reduction of NO_2^- to NO (Lundberg et al. 2008). Recently, nitrate supplementation was reported to improve endurance during a fixed-load, time-to-exhaustion task at severe intensity in healthy males (Kelly et al. 2013). However, it is unclear if this ergogenic effect is apparent in an athletic cohort performing more sport-specific tasks. Furthermore, the competition requirements of typical track cycling events, such as the team pursuit, require multiple efforts over the course of a day, with competitors usually required to perform qualifying races before the finals. Presently, it is not known whether nitrate can be beneficial to performance when maximal bouts are repeated in the same day or what the optimal dosing regimen might be.

Accordingly, the primary purpose of this study was to examine the effect of NO_3^- supplementation on the performance of repeated bouts of high-intensity cycling performance simulating the team pursuit track cycling event. A secondary purpose of the study was to investigate different protocols of supplementation, varying the timing of nitrate intake in relation to the cycling bouts.

Materials and methods

Participants

A total of 28 trained male cyclists (mean $\pm$ SD; age: 20.3 ± 1.4 years; body mass: 72.3 ± 6.4 kg; maximum aerobic power: 356 ± 35 W) volunteered to participate in the study. All participants were involved in a 6 week training camp at the Australian Institute of Sport. Written informed consent was obtained from each individual following explanation of the experimental procedures and associated risks, which were approved by the Australian Institute of Sport Ethics Committee.

Pre-experimental trial procedures

Before undertaking experimental trials, all subjects underwent a graded exercise test on a cycle ergometer (Wattbike Ltd., Nottingham, UK) to determine maximal aerobic power. After a 10 min warm-up at 100 W, the test protocol began at 150 W and increased by 50 W every 5 min until volitional exhaustion. The method used to determine maximal aerobic power is described elsewhere (Quod et al. 2008). All tests were conducted under controlled laboratory conditions (23 ± 2 °C, barometric pressure (P_b) = 702 mm Hg).

Within 1 week of the graded exercise test, subjects performed 2 separate familiarization trials similar to the experimental protocol to acquaint them with the repeated 4 min time trials. Subjects were then assigned a trial order based on their maximal aerobic power

Fig. 1. Trial schedule for each experimental condition. 150-PRE trial consumed 1 bottle (70 mL; 4.1 mmol NO_3^-) nitrate-rich beetroot juice 150 min before the onset of TT1, 75-PRE took beetroot juice 75 min before TT1 and in TOP-UP condition beetroot juice was taken 150 min before TT1 with an additional half dose immediately after. No nitrate-rich beetroot juice was taken in placebo condition (PLA). The placebo beverage contained 0.03 mmol NO_3^- .

and body mass, such that there were minimal differences in these characteristics for each trial order (Hopkins 2010).

Experimental protocol

The study employed a placebo-controlled crossover design which was counterbalanced in a Latin square arrangement. On 4 separate occasions, all subjects completed a trial involving 2 bouts of 4 min cycling time trials (TT1 and TT2) which were separated by 75 min. In all experimental trials, subjects consumed a beverage (70 mL of either nitrate-rich beetroot juice or nitrate-depleted placebo) at 150 min and 75 min before TT1 and an additional half dose 75 min prior to TT2. Briefly, combinations of nitrate-rich and placebo beetroot juice were organised to achieve the following 4 treatments: supplementation to achieve the usual 150 min pretrial ingestion before TT1 (150-PRE); supplementation to achieve 150 min pretrial ingestion before TT2 occurring 75 min before TT1 (75-PRE); supplementation 150 min before TT1 and an additional dose 75 min before TT2 (TOP-UP); and placebo treatments at all time points. The study design is summarized in Fig. 1. The nitrate-rich beverage was 70 mL of commercially available beetroot concentrate (Beet-it, James White Drinks, Ipswich, UK) that contained 4.1 mmol of NO_3^- . The placebo beverage was identical in taste, texture, and packaging, but contained negligible amounts of NO_3^- (0.03 mmol).

All trials were performed at the same time of day (± 1 h) with 6 days washout between each trial. On the morning of experimental trials, subjects reported to the laboratory 3 h before TT1 in an overnight-fasted state (ad libitum water consumption). Following baseline measures of weight, resting blood pressure, and a venous blood sample, subjects were provided with a standardized breakfast (detailed subsequently). Approximately 30 min before TT1, subjects began a controlled warm-up involving cycling for 10 min at 60% HR_{max} , 5 min at 70% HR_{max} , 3 min at 80% HR_{max} , and 1 min at 90% HR_{max} , followed by 5 min of easy pedalling. For TT1, subjects were instructed to achieve the highest average power possible over 4 min. During the time trials, participants received no feedback about their external power output or cadence, but they could view elapsed time. Following TT1, subjects were instructed to cool-down with freely selected pedal cadence for 10 min. Before initiating TT2 45 min later, subjects repeated the same warm-up and were again instructed to achieve the highest average power possible. Mean power and energy expenditure over the 4 min were recorded as the performance measures.

Blood sampling

Blood samples were collected at 0, 75, 150 (just before TT1), and 225 min (just before TT2). Samples were collected via an indwelling cannula inserted into an antecubital vein using a Vacutainer system (Becton, Dickson and Company, Franklin Lakes, N.J., USA). A 5 mL sample was collected into a lithium heparin tube which was immediately centrifuged (4500 r·min⁻¹ (4000 RCF (relative centrifugal force)) at 4 °C for 5 min. Plasma was decanted into 500 µL aliquots and placed in a -80 °C freezer for later analysis of plasma nitrite concentration ([NO₂⁻]) via chemiluminescence as described elsewhere (Bailey et al. 2009). All assays were performed in duplicate with the mean reported.

Dietary control

In view of the large number of subjects participating in the study and their common living environment and on-site food availability, we undertook dietary standardization before each trial using the dietary prescription aided by education tools method (Jeacocke and Burke 2010). Specifically, an accredited sports dietician who was responsible for the dietary standardization section of the study instructed the subjects on a protocol designed to meet a minimum carbohydrate target of 6 g·kg⁻¹ body mass each day for 2 days before each trial. After receiving verbal and written education on carbohydrate content, subjects were free to select the type and quantity of food and fluids to meet this target from the options available in their common living environment. Subjects were instructed to keep a detailed food diary over the 2 days. Food diaries allowed both subjects and researchers to track progress toward meeting target intakes. Subjects were also instructed to avoid using chewing gum and mouthwash for the duration of the study to preserve oral bacteria pertinent to nitrate reduction. They were also requested to refrain from the intake of alcohol and other known ergogenic aids during each period of dietary standardization. The dietician lived in the same environment as the subjects for the duration of the trial and was able to monitor and assist with dietary compliance.

Each morning of testing, subjects arrived at the laboratory after an overnight fast. They provided food diaries to the dietician to check for dietary compliance and were subsequently cleared to undertake the trial. Approximately 2 h before commencement of the first time trial, subjects were provided with a standardized breakfast containing carbohydrate = 2.5 g·kg⁻¹ body mass. Throughout the warm-up and time trial periods, subjects consumed sports products to provide an additional carbohydrate = 2 g·kg⁻¹ body mass. During the warm-up preceding each time trial, subjects consumed a sports gel containing 26 g carbohydrate (PowerBar Gel, PowerBar Oceania, NSW, Australia). Between time trials, subjects were provided with a recovery drink containing 44 g carbohydrate and 15 g protein (PowerBar Protein Plus, PowerBar Oceania, NSW, Australia) and 600–900 mL of sports drink (Gatorade, Cadbury Schweppes, Victoria, Australia) depending on body mass to meet the total carbohydrate target of 2 g·kg⁻¹. Water was consumed ad libitum during testing. No other food or fluids were consumed during the testing period apart from the beetroot juice treatments.

Statistical analysis

Each cyclist's mean power output in each time trial was the dependent variable in a mixed linear model using the Proc Mixed procedure in SAS (version 9.2, SAS Institute, Cary, N.C., USA). The fixed effects were the week of testing (4 levels, first to fourth), to adjust for training and habituation effects and a treatment×trial interaction (8 levels) to estimate mean effects of each of the 4 treatments in the 2 time trials. The random effects were athlete identity, to account for repeated measurement of athletes; the interaction of athlete and treatment identities, to account for repeated measurements on athletes between weeks; the interaction of athlete identity with placebo and nitrate treatments (i.e.,

150-PRE, 75-PRE, and TOP-UP), to estimate individual responses consistent across the 3 nitrate treatments; and the residual, representing test-to-test variation. In a separate mechanisms analysis, a linear fixed effect for [NO₂⁻] was included in the model to estimate and adjust for the mean effects of each treatment on [NO₂⁻] immediately before the 2 time trials.

All data are presented as means ± SD unless otherwise stated. Mean power was log-transformed before analysis, and percent effects were estimated via back transformation. Uncertainty in the estimates of effects on performance are expressed as 90% confidence limits and as probabilities that the true value of the effect was beneficial, trivial, or harmful in relation to threshold values for benefit and harm of ±1.0% (Paton and Hopkins 2006). These probabilities are not presented quantitatively, but were used to make a qualitative probabilistic clinical inference about the effect in preference to a statistical inference based on a null hypothesis test (Hopkins et al. 2009). Briefly, the effect was deemed unclear when the chance of benefit was sufficiently high to warrant use of the treatment but the risk of harm was unacceptable. Such unclear effects were identified as those with an odds ratio of benefit–harm of <66, a ratio that corresponds to an effect that is borderline possibly beneficial (25% chance of benefit) and borderline most unlikely harmful (0.5% risk of harm). All other effects were deemed clinically clear and expressed as the chance of the true effect being trivial, beneficial, or harmful with the following scale: 25%–75%, possibly; 75%–95%, likely; 95%–99.5%, very likely; >99.5%, most likely (Hopkins, 2007). Magnitudes of covariates and effects on measures other than performance were evaluated mechanistically (Hopkins et al. 2009); if the confidence interval overlapped thresholds for substantial positive and negative values (±0.20 standardized units, i.e., ±0.20 of the between-subject SD in the placebo condition), the effect was deemed unclear; all other effects were deemed clear and were evaluated probabilistically as described previously. The relationship between plasma [NO₂⁻] and performance was examined by mechanistically evaluating the effect of a 2 SD change in [NO₂⁻] from baseline (placebo) on mean power.

Results

One participant elected to withdraw from the study because of an intolerance to the beetroot juice and another participant suffered a training injury following the first time trial and could not complete any more testing. Their data was removed from statistical analysis. Two participants elected not to have blood testing performed; therefore, plasma [NO₂⁻] analysis was conducted with an *n* = 24. Analysis of participant food diaries determined that any differences between carbohydrate intake (g·kg⁻¹ body weight) during each trial were likely trivial (99% clear) and, therefore, unlikely to contribute to any performance effects.

Plasma [NO₂⁻]

The plasma nitrite kinetics for each trial are shown in Fig. 2. Baseline [NO₂⁻] was (mean ± SD) 238 ± 87 nmol·L⁻¹, 254 ± 85 nmol·L⁻¹, 229 ± 92 nmol·L⁻¹, and 253 ± 85 nmol·L⁻¹ for placebo, 150-PRE, 75-PRE, and TOP-UP, respectively. From baseline to 150 min, there was a general increase in plasma [NO₂⁻] across all trials. The rise in the 75-PRE (mean, ±90% confidence limits; 70%, ±62%) was likely greater than the rise in TOP-UP (38%, ±36%), which itself was likely greater than the rise in 150-PRE (22%, ±31%) and placebo (13%, ±34%). By 225 min, [NO₂⁻] had increased further in 75-PRE (88%, ±85% of baseline), which was likely greater than the increase in TOP-UP (51%, ±46%), which was likely greater than the rise in 150-PRE (24%, ±26%) and placebo (22%, ±31%).

Cycling performance

The mean power of each 4 min time trial is presented in Table 1, and the estimated effects of supplementation on performance are presented in Table 2. Briefly, there was an unclear effect on per-

Fig. 2. Mean plasma nitrite concentration ($[\text{NO}_2^-]$) for each supplementation condition with SD (error bars). Time trial 1 (TT1) occurs at 150 min and time trial 2 (TT2) occurs at 225 min. *, Likely greater than 150-PRE; **, likely greater than TOP-UP; +, very likely greater than 150-PRE. 75-PRE and 150-PRE time points have been offset (+2 min) for clarity. nM, nmol·L⁻¹.

Table 1. Mean power ($\pm$ SD) for each 4-min time-trial performance in all supplementation conditions.

Condition	Power (W), mean $\pm$ SD	
	Time trial 1	Time trial 2
Placebo	396 $\pm$ 57	397 $\pm$ 56
150-PRE	402 $\pm$ 47	396 $\pm$ 46
75-PRE	403 $\pm$ 52	396 $\pm$ 54
TOP-UP	400 $\pm$ 48	396 $\pm$ 45

Table 2. Effect of nitrate supplementation on 4-min time-trial performance in all cyclists.

Time trial	Difference in mean vs. placebo (%; $\pm$ 90% CL)	Practical inference ^a
Time trial 1		
150-PRE	1.2 $\pm$ 2.1	Unclear
75-PRE	1.7 $\pm$ 2.1	Unclear
TOP-UP	1.1 $\pm$ 2.1	Unclear
ALL	1.3 $\pm$ 1.7	Unclear
Time trial 2		
150-PRE	-0.4 $\pm$ 2.1	Possibly harmful
75-PRE	-0.5 $\pm$ 2.1	Possibly harmful
TOP-UP	-0.1 $\pm$ 2.1	Unlikely harmful
ALL	-0.3 $\pm$ 1.7	Possibly harmful
Both time trials		
150-PRE	0.4 $\pm$ 2.0	Unclear
75-PRE	0.6 $\pm$ 2.0	Unclear
TOP-UP	0.5 $\pm$ 2.0	Unclear
ALL	0.5 $\pm$ 1.6	Unclear

^aBased on a smallest worthwhile change of 1%. Unclear effects are defined by a benefit-harm odds ratio of <66, corresponding to an effect that is borderline possibly beneficial (25% chance of benefit) and borderline most unlikely harmful (0.5% risk of harm). Other effects are deemed clinically clear and expressed as the chance of the effect being trivial, beneficial, or harmful with the following scale: 25%–75%, possibly; 75%–95%, likely; 95%–99.5%, very likely; >99.5%, most likely.

formance of TT1 when each of the nitrate trials (i.e., 150-PRE, 75-PRE, TOP-UP) was compared to placebo, with a combined mean improvement to power of 1.3% (90% confidence limits $\pm$ 1.7%). There were unclear differences between nitrate conditions (data not shown). There was a “possibly harmful” effect on power in TT2

Fig. 3. The relationship between changes in plasma nitrite ($[\text{NO}_2^-]$) concentration (%) and changes in time trial mean power (%). Change scores are calculated by comparing nitrate ingesting trials to the placebo condition (used as baseline results). Results for both time trials are shown.

for each nitrate condition, with an average change of $-0.3, \pm 1.7\%$. There were unclear differences in the magnitude of effect between the nitrate conditions on TT2 (data not shown). With nitrate supplementation, 9 participants improved in at least 4 of 6 time trials, whereas 3 participants did not improve in any time trials.

$[\text{NO}_2^-]$ and performance

Changes in $[\text{NO}_2^-]$ are plotted against changes in performance in Fig. 3. From the mechanistic analysis, the observed effect of $[\text{NO}_2^-]$ on performance was trivial, with a +2 SD change in $[\text{NO}_2^-]$ associated with a +0.1% change in performance. However, because of the large uncertainty (90% confidence limits $\pm 2.5\%$), this effect was unclear.

Random effects

From the analysis of the random effects, time trial-to-time trial SE (expressed as a coefficient of variation) was 2.1% (90% confidence limits $\times/\div 1.13\%$), whereas week-to-week error was 4.0% ($\times/\div 1.29\%$). The CV for individual responses (in performance) to NO_3^- trials was estimated to be $-0.9\% (\pm 3.9\%)$.

Discussion

The principal finding from our investigation was that nitrate supplementation (via a 70–105 mL dose of commercially available concentrated beetroot juice) had an unclear effect on the performance of a maximal 4-min bout of cycling. Performance of a subsequent bout 75 min later appeared to be worse with NO_3^- supplementation compared to a placebo; however, the possibility of the positive (yet unclear) performance boost of NO_3^- in TT1 resulting in residual fatigue during TT2 cannot be excluded. These results are not in agreement with previous research; however, the current study is one of the first to examine the effect of NO_3^- supplementation by higher calibre athletes on repeat performance of high-intensity cycling.

Effect of nitrate dose and timing on performance

No discernible differences in performance of the initial time trial were detected from staggering the timing of nitrate ingestion. It was hypothesized that 150-PRE would result in a greater power output than 75-PRE for TT1, but that the latter would exhibit greater performance during the TT2; however, both had similar outcomes. Both dosing regimens were found to have a possibly negative effect on the second time trial, and an additional half dose of nitrate in the TOP-UP trial was unable to alter this. Although the magnitude of effect on TT2 ($-0.3, \pm 1.7\%$) was

smaller than the unclear effect for TT1 (+1.3, $\pm 1.7\%$), supplementation was still deemed to be possibly negative. This is because of the lower threshold for substantial chance of harm (0.5%) compared to benefit (25%) in the statistical analysis and, given the 90% CL extend into positive effects, it must be considered that nitrate may be beneficial in a secondary time trial for some. Furthermore, we cannot conclude that NO_3^- is harmful to secondary bouts of high-intensity exercise because the interaction between time trials must be considered. It is possible that the small positive effect of NO_3^- on TT1 resulted in greater posttest fatigue causing poorer performance in TT2. Despite the net effect over both time trials leaning slightly in favour of nitrate supplementation (0.5, $\pm 1.6\%$), the effect falls below the smallest meaningful change as described by Paton and Hopkins (Paton and Hopkins 2006). Overall, the reported variation in individual response to NO_3^- (-0.9%) represents no substantial differences among participants; however, in light of the large sampling uncertainty in the present study ($\pm 3.9\%$) we cannot rule out the possibility of a substantial individual response being found in a larger sample.

Although previous studies have reported significant improvements in time-to-exhaustion tasks and related performance measures, the populations under investigation have generally been recreationally active individuals or club-level athletes. In contrast, the cohort of well-trained athletes in the current investigation were training approximately 20 h per week and competing at the highest level of national racing. There has been a recent suggestion that the physiological effects elicited by NO_3^- supplementation is blunted in highly fit populations (Bescós et al. 2012). This notion is supported by other studies of trained individuals which have failed to find an improvement to exercise performance associated with acute nitrate supplementation (Bescós et al. 2012; Cermak et al. 2012b; Peacock et al. 2012). This phenomenon may be attributable to fitter individuals having greater capacity for producing NO through the conventional pathway in which L-arginine is oxidized in a reaction catalysed by nitric oxide synthase (NOS) (Vassalle et al. 2003). A better developed NOS system may result in a diminished need for the NO_3^- - NO_2^- pathway to synthesize NO, thereby moderating the effects of NO_3^- supplementation. Additionally, physiological adaptations to endurance training (i.e., increased capillary density) serve to reduce local muscular hypoxia and acidosis, conditions that would facilitate the reduction of NO_2^- to NO (Lundberg et al. 2008).

The amount of nitrate prescribed in the current study may have been inadequate. There has been a suggestion that endurance trained athletes may require a greater acute NO_3^- dose to elicit performance benefits compared to nontrained counterparts (Hoon et al. 2013a). Indeed, a 8.4 mmol NO_3^- bolus improved 2000 m rowing time in a group of well-trained rowers, whereas a 4.2 mmol dose (similar to the present study) did not (Hoon et al. 2013b). In a similar study, time to completion of multiple bouts of 500 m rowing were improved in well-trained juniors following consumption of 500 mL of beetroot juice per day for 6 days (Bond et al. 2012). Coupled with other results (Vanhatalo et al. 2010), these findings suggest that a moderate to large dose of NO_3^- may be required for performance benefits to be seen in trained athletes, and a chronic supplementation strategy (i.e., supplementing with NO_3^- for several days preceding exercise) may be a practical and effective means of providing these quantities. Although the overall low number of responders in the present study may be attributable to the small dose of NO_3^- , some participants were able to improve most time trials following a relatively small serving of NO_3^- . In contrast, 3 participants were unable to improve regardless of NO_3^- dose or time. This responder and non-responder trait has also been noted in previous literature (Wylie et al. 2013a; Wilkerson et al. 2012) suggesting that some individuals may be more sensitive to the effects of NO_3^- than others. The physiological mechanisms responsible for the differences in inter-

individual response to NO_3^- supplementation remain to be determined and require further investigation.

Nitrate supplementation is hypothesized to be more effective for improving short, high-intensity exercise performance compared to longer, less intense activities (Hoon et al. 2013b; Wilkerson et al. 2012). Higher intensities are more likely to subject working muscles to a hypoxic and acidic environment, facilitating the reduction of NO_2^- to NO (Lundberg et al. 2008). Although the extreme intensity (Jones and Poole 2005) of the 4 min time trials likely elicited these favourable conditions, the present study was unable to show any substantial improvement to performance following NO_3^- supplementation. As discussed previously, this was most likely attributable to the small prescribed dose of NO_3^- relative to the high fitness of subjects. Because of these considerations, we cannot draw any conclusions regarding the effect of exercise intensity on the efficacy of nitrate supplementation from the present study.

Effects of NO_3^- supplementation on plasma NO_2^-

In the present study, all NO_3^- trials successfully elevated plasma $[\text{NO}_2^-]$; however, this was not accompanied by a detectable improvement in performance. The peak concentrations in plasma NO_2^- ($339 \pm 89 \text{ nmol}\cdot\text{L}^{-1}$, $384 \pm 136 \text{ nmol}\cdot\text{L}^{-1}$, and $372 \pm 105 \text{ nmol}\cdot\text{L}^{-1}$ for 150-PRE, 75-PRE, and TOP-UP, respectively) occurred between 75–150 min postingestion and are comparable to findings in investigations employing similar design and analysis (Wylie et al. 2013b). However, despite similar peak concentrations, the relative increase in $[\text{NO}_2^-]$ was considerably smaller in the present investigation, likely because of an elevated baseline $[\text{NO}_2^-]$. Fitter individuals have been noted to have a greater resting $[\text{NO}_2^-]$ compared to lesser trained counterparts (Jungersten et al. 1997). Indeed, the mean $[\text{NO}_2^-]$ baseline value in the present study ($244 \pm 83 \text{ nmol}\cdot\text{L}^{-1}$) was greater than those reported in studies utilizing untrained participants and similar NO_2^- analysis techniques (Wylie et al. 2013b; Lansley et al. 2011b; Bailey et al. 2009, 2010). The relative change in $[\text{NO}_2^-]$ may be a principal determinant of the effectiveness of NO_3^- supplementation and perhaps why performance changes were not detectable in the current study.

Previous investigations have reported a positive correlation between change in $[\text{NO}_2^-]$ and change in exercise capacity (Wylie et al. 2013b; Wilkerson et al. 2012). This relationship was not confirmed in the present study, perhaps because of the large SD of $[\text{NO}_2^-]$ and the small changes in performance. The large variation in $[\text{NO}_2^-]$ (even in this cohort of similarly trained cyclists) is not uncommon and has been observed in other investigations, where up to a $\sim 50\%$ SD in baseline $[\text{NO}_2^-]$ has been reported (Wylie et al. 2013a; Lansley et al. 2011a, 2011b). Discrepancies in $[\text{NO}_2^-]$ are further amplified when the individual response to supplementation is considered, as evident by the increasing SD over time following NO_3^- ingestion. Further research is required to elucidate the particular factors that may influence an individual's ability to reduce NO_3^- to NO_2^- and how this may impact exercise performance.

Limitations and future perspectives

Strengths of this study included the Latin square balanced crossover design and large sample size compared to most other studies; however, a major limitation was the large week-to-week variation in performance (4%) which resulted in greater uncertainty about the ergogenic effect of nitrate. Despite this, the between time trial error of 2% was typical for this cohort and performance test (Driller et al. 2013), indicating that testing was performed to its highest standard. In addition, although dietary records were kept over the duration of the study, subjects were able to eat ad libitum without restriction on food types, and it must be considered that there was a background level of nitrate consumption, estimated to be 100–150 mg in a typical Western diet (Zeegers et al. 2006). However, allowing subjects to freely select food presents a more ecologically valid examination of the

efficacy of nitrate supplementation. Other studies have restricted dietary nitrate consumption for the course of the study (Bescos et al. 2011; Larsen et al. 2007, 2011), which may have amplified the effect of the nitrate supplement given.

Further research should seek to refine guidelines surrounding the use of nitrate supplements, identifying the appropriate populations and situations for its use. Although the present study was unable to confirm its applicability to high-intensity cycling, previous studies have demonstrated that, given the right conditions, nitrate is able to elicit positive improvements in exercise capacity. Conversely, the present study indicates that the potential for a negative effect on performance must also be considered.

Conclusion

The primary finding from the present study was that NO_3^- supplementation administered as beetroot juice concentrate was unable to elicit performance gains to short duration, high-intensity exercise. A subsequent effort appears to be negatively affected; however, this may be attributable to a small improvement in performance of the initial bout (unclear in the present study) leaving residual fatigue. This was found to be true up to an amount of ~ 500 mg of NO_3^- ingested 75–150 min before exercise. These results occurred despite supplementation causing a rise in plasma $[\text{NO}_2^-]$, hypothetically increasing the amount of available NO to support physiological processes during exercise. This finding is contradictory to previous research, but may be explained, in part, by the highly trained nature of the subjects. Under the conditions of our experiment, nitrate supplementation may not be effective in facilitating high-intensity exercise in well-trained athletes.

Conflict of interest statement

All authors declare that there are no conflicts of interest present.

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