Nitrate supplementation and high-intensity performance in competitive cyclists

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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: ±1.7%), but possibly harmful in the second time trial (−0.3%, ±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.

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. 2011a), 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
absorbed by the upper gastrointestinal tract before being taken up by the salivary glands and secreted into the mouth, where anaerobic bacteria reduce $\text{NO}_3^{-}$ to bioactive $\text{NO}_2^{-}$. Nitrite may then be reduced further into NOS in the stomach or reabsorbed to increase plasma $\text{NO}_2^{-}$ concentration ([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 $\text{NO}_3^{-}$ supplementation.

Although several studies have documented improvements in the performance of exercise lasting 7–20 min (Cermak et al. 2012a; Murphy et al. 2012; Lamsley 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 $\text{NO}_3^{-}$ 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 $\text{NO}_3^{-}$ 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 ± 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 ($23 ± 2 ^\circ C$, 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 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 randomised to achieve the following 4 treatments: supplementation to achieve the usual 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 beetroot juice was 50 mL of commercially available beetroot concentrate (Beet-it, James White Drinks, Ipswich, UK) that contained 4.1 mmol of $\text{NO}_3^{-}$. The placebo beverage was identical in taste, texture, and packaging, but contained negligible amounts of $\text{NO}_3^{-}$ (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% $HR_{\text{max}}$, 5 min at 70% $HR_{\text{max}}$, 3 min at 80% $HR_{\text{max}}$, and 1 min at 90% $HR_{\text{max}}$, 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 ul 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 to perform in duplicate with the mean reported. The random effects were athlete interaction (8 levels) to estimate mean effects of each of the procedure in SAS (version 9.2, SAS Institute, Cary, N.C., USA). The dependent variable in a mixed linear model using the Proc Mixed section of the study instructed the subjects on a protocol performed in duplicate with the mean reported.

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 performance 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 ([NO₂⁻]) 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.

Fig. 3. The relationship between changes in plasma nitrite (NO₂⁻) 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.

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

<table>
<thead>
<tr>
<th>Condition</th>
<th>Time trial 1</th>
<th>Time trial 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>396±57</td>
<td>397±56</td>
</tr>
<tr>
<td>150-PRE</td>
<td>402±47</td>
<td>396±46</td>
</tr>
<tr>
<td>75-PRE</td>
<td>403±52</td>
<td>396±54</td>
</tr>
<tr>
<td>TOP-UP</td>
<td>400±48</td>
<td>396±45</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Time trial</th>
<th>Difference in mean Power (W), mean ± SD</th>
<th>Practical inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time trial 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150-PRE</td>
<td>1.2±2.1</td>
<td>Unclear</td>
</tr>
<tr>
<td>75-PRE</td>
<td>1.7±2.1</td>
<td>Unclear</td>
</tr>
<tr>
<td>TOP-UP</td>
<td>1.1±2.1</td>
<td>Unclear</td>
</tr>
<tr>
<td>ALL</td>
<td>1.3±1.7</td>
<td>Unclear</td>
</tr>
<tr>
<td>Time trial 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150-PRE</td>
<td>-0.4±2.1</td>
<td>Possibly harmful</td>
</tr>
<tr>
<td>75-PRE</td>
<td>-0.5±2.1</td>
<td>Possibly harmful</td>
</tr>
<tr>
<td>TOP-UP</td>
<td>-0.1±2.1</td>
<td>Unlikely harmful</td>
</tr>
<tr>
<td>ALL</td>
<td>-0.3±1.7</td>
<td>Possibly harmful</td>
</tr>
<tr>
<td>Both time trials</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150-PRE</td>
<td>0.4±2.0</td>
<td>Unclear</td>
</tr>
<tr>
<td>75-PRE</td>
<td>0.6±2.0</td>
<td>Unclear</td>
</tr>
<tr>
<td>TOP-UP</td>
<td>0.5±2.0</td>
<td>Unclear</td>
</tr>
<tr>
<td>ALL</td>
<td>0.5±1.6</td>
<td>Unclear</td>
</tr>
</tbody>
</table>

*Based 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.

Performance 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 ±1.7%). There were unclear differences between nitrate conditions (data not shown). There was a “possibly harmful” effect on power in TT2 for each nitrate condition, with an average change of −0.3, ±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.

[NO₂⁻] and performance
Changes in [NO₂⁻] are plotted against changes in performance in Fig. 3. From the mechanistic analysis, the observed effect of [NO₂⁻] on performance was trivial, with a ±2 SD change in [NO₂⁻] associated with a +0.1% change in performance. However, because of the large uncertainty (90% confidence limits ±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 ±1.13%), whereas week-to-week error was 4.0% (±1.29%). The CV for individual responses (in performance) to NO₃⁻ trials was estimated to be −0.9% (±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 NO₃⁻ supplementation compared to a placebo; however, the possibility of the positive (yet unclear) performance boost of NO₃⁻ 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 NO₃⁻ 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, ±1.7%) was
trained athletes may require a greater acute NO3\(^{-}\) supplementation to show any substantial improvement to performance following NO3\(^{-}\) supplementation. As discussed previously, this was most likely attributable to the small prescribed dose of NO2\(^{-}\), 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 NO3\(^{-}\) supplementation on plasma NO2\(^{-}\)**

In the present study, all NO3\(^{-}\) trials successfully elevated plasma NO2\(^{-}\); however, this was not accompanied by a detectable improvement in performance. The peak concentrations in plasma NO2\(^{-}\) (339 ± 89 nmol·L\(^{-}\)\(^{1}\), 384 ± 136 nmol·L\(^{-}\)\(^{1}\), and 372 ± 105 nmol·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 [NO2\(^{-}\)] was considerably smaller in the present investigation, likely because of an elevated baseline [NO2\(^{-}\)]. Fitter individuals have been noted to have a greater resting [NO2\(^{-}\)] compared to lesser trained counterparts (Jungersten et al. 1997). Indeed, the mean [NO2\(^{-}\)] baseline value in the present study (244 ± 83 nmol·L\(^{-}\)\(^{1}\)) was greater than those reported in studies utilizing untrained participants and similar NO2\(^{-}\) analysis techniques (Wylie et al. 2013a; Lansley et al. 2011b; Bailey et al. 2009, 2010). The relative change in [NO2\(^{-}\)] may be a principal determinant of the effectiveness of NO3\(^{-}\) supplementation and perhaps why performance changes were not detectable in the current study.

Previous investigations have reported a positive correlation between change in [NO2\(^{-}\)] 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 [NO3\(^{-}\)] and the small changes in performance. The large variation in [NO2\(^{-}\)] (even in this cohort of similarly trained cyclists) is not uncommon and has been observed in other investigations, where up to a ~50% SD in baseline [NO2\(^{-}\)] has been reported (Wylie et al. 2013a; Lansley et al. 2011a, 2011b). Discrepancies in [NO2\(^{-}\)] are further amplified when the individual response to supplementation is considered, as evident by the increasing SD over time following NO3\(^{-}\) ingestion. Further research is required to elucidate the particular factors that may influence an individual’s ability to reduce NO3\(^{-}\} to NO2\(^{-}\) 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 effect 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 individual response to NO3\(^{-}\) supplementation remain to be determined and require further investigation.
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 NO₃⁻ 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 NO₃⁻ ingested 75–150 min before exercise. These results occurred despite supplementation causing a rise in plasma [NO₃⁻], 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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