High Dose Nitrate Ingestion Does Not Improve 40 km Cycling Time Trial Performance in Trained Cyclists

**Abstract**

This study evaluated the chronic effects of nitrate (NO3-) ingestion over three days, on 40 km TT performance in 11trained cyclists (VO2max: 60.8 ± 7.4 ml.kg-1.min-1; age: 36 ± 9 years; height: 1.80 ± 0.06 m; body mass: 87.2 ± 12.0 kg). Utilising a double blind randomised cross-over design, participants completed three 40 km TT on a Velotron® ergometer following the ingestion of either a 140 ml of “BEET It sport®” NO3- shot containing 12.8 mmol or 800 mg of NO3-, a placebo drink or nothing (control). Performance, oxygen consumption (VO2), blood bicarbonate (HCO3-), pH and lactate (BLa) and ratings of perceived exertion (RPE) were measured every 10 km throughout the TT*.* The present findings show thatNO3- ingestion had no effect on TT performance (NO3-: 4098.0 ± 209.8 vs. Placebo: 4161.9 ± 263.3 s, p = 0.296, ES = 0.11), or VO2 (p = 0.253, ES = 0.13). Similarly, blood lactate and RPE were also unaffected by the experimental conditions (p = 0.522, ES = 0.06; p = 0.085, ES = 0.30) respectively. Therefore, these results suggest that a high dose of NO3- over three days has limited efficacy as an ergogenic aid for 40 km TT cycling performance in trained cyclists.

**Introduction**

Intake of exogenous dietary nitrates (NO3-) through supplementation and whole foods are associated with beneficial cardiovascular health outcomes and are used as an ergogenic aid for athletic performance (Clements et al., 2014). Green leafy vegetables and root vegetables are examples of NO3- rich dietary sources, while commercially available beetroot supplements provide a highly concentrated and practical method to ingest NO3- prior to exercise. The underlying action of NO3- is derived from the enhanced bioavailability of nitric oxide (NO), which can be synthesised via continuous reactions in the L-arginine-NO pathway (Moncada and Higgs, 1993) or reduced from NO3- (Lundberg et al., 2004). The latter is prominent in deriving NO from dietary beetroot supplementation, with NO3- reduced to nitrite (NO2) in the oral cavity (Duncan et al., 1995). Thereafter it is reduced to NO during downstream reactions and absorbed into circulating plasma (Lundberg et al., 2004). Nitric oxide consequently diffuses into the vascular smooth muscle where it acts as a gaseous signalling molecule for numerous physiological processes.

Nitric oxide is predominantly cited as a potent vasodilator (Kelm and Schrader, 1990); but is also central to the processes of muscle contraction (Reid, 2001), mitochondrial respiration (Brown, 1999) and immunoregulation (Bogdan, 2001). Indeed, the effect on these physiological systems has led to NO3- rich supplements demonstrating beneficial effects on the oxygen cost of moderate intensity exercise (Larsen et al., 2010) and gross efficiency (Larsen et al., 2007). Therefore, mediating an improvement in mitochondrial efficiency (Larsen et al., 2011) and reducing the ATP demand of muscular contractions during exercise (Bailey et al., 2010). Cermak et al. (2012a) found a significantly improved 10 km TT performance (after 60 min of submaximal exercise) and power output compared to the placebo condition. Given these promising physiological responses mediated by NO3-, the ergogenicity of concentrated beetrootsupplementation is translated into improved time to exhaustion (TTE) and time trial (TT) performance by some authors (Lansley et al., 2011; Bailey et al., 2009; Larsen et al., 2010; Cermak et al., 2012a). This beneficial effect is most apparent during shorter duration performance bouts, where an intensity equivalent to the severe intensity domain is undertaken (Bailey et al., 2009; Kelly et al., 2014). Time trial events of short durations (4 - 16 km), that require a relatively high exercise intensity, have also reported an ergogenic benefit (Lansley et al., 2011 Cermak et al., 2012a); however, no studies have investigated the effects of this dietary supplement on 40 km TT performance.

Despite the positive outcomes, the overall meta-analytic effect of NO3- on TT performance is considered to be unclear (standardised mean difference ± confidence interval: -0.1 ± 0.06; McMahon et al., 2016). This differential effect could be attributed to the training status of participants and/or the intensity of the bout not eliciting a sufficient physiological demand (Jones, 2014). Indeed, no effects have been observed during prolonged (> 30 min) TT performance from beetroot juice supplementation within highly trained individuals with VO2max of >60 ml·kg-1·min-1 (Cermak et al., 2012b; Wilkerson et al., 2012; Lane et al., 2013). Wilkerson et al., (2012) administered 500 ml beetroot juice containing 6.2 mmol of NO3- prior to an 80 km TT, however the participants exhibited only a small 25% increase in plasma NO2 compared to the 139% increases observed in earlier studies with a similar participant cohort with an identical dose (Lansley et al., 2011). Indeed, a strong negative correlation between plasma NO2 increase and TT performance improvement was reported (r = -0.85; p = 0.01); thus, highlighting the importance of a high bioavailable NO for performance improvements. However, two separate doses 12 hrs apart, increasing total NO3- ingestion to 17.4 mmol prior to exercise, also reported no ergogenic effects during a 43.8 km TT (Lane et al. 2013). These authors, however, utilised a superiorly trained participant cohort (VO2max: 71.6 ± 4.6), which may explain the lack of improvement. Therefore, on the basis of previous work, the aim of this study was to evaluate the chronic effects of nitrate (NO3-) ingestion over three days, on 40 km TT performance in trained cyclists.

**Methods**

Eleven trained cyclists (VO2max 60.8 ± 7.4 ml.kg-1.min-1; age: 36 ± 9 years; height: 1.80 ± 0.06 m; body mass: 87.2 ± 12.0 kg) volunteered to participate in this study. The cyclists had a minimum of one year cycling experience and a minimum weekly training volume of (5 hr.wk-1; and >150 km.wk-1). The research was approved by the Departmental Ethics Committee and participants gave written informed consent prior to any data collection. The participants were categorised as trained competitive cyclists (De Pauw et al., 2013), who were fully familiar with similar cycling TT distances and laboratory testing procedures.

Participants were required to visit the laboratory to complete a preliminary VO2max test which used a ramp incremental exercise test on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). Participants were required to cycle at an initial intensity of 75 W for 1 min, after which the intensity increased at a ramp rate of 30 W.min-1 until volitional fatigue. Breath-by-breath pulmonary gas exchange data was collected continuously using a gas analysis system (K5, Cosmed, Italy) during the incremental test and subsequently analysed using 5 s averaging. The VO2max was calculated as the highest 15 s mean value attained before the participants’ volitional termination of the test. Following completion of this test participants were familiarised with the Velotron® (RacerMate Inc, Velotron, USA) in order to ensure that the specific frame geometry settings were recorded for use in all subsequent TT laboratory visits.

Participants were then required to attend the laboratory on a further three separate occasions at the same time of day. Prior to each of these subsequent visits, participants recorded dietary intake and physical activity, which was replicated for the 24 h preceding each visit. They were also instructed to attend euhydrated, having abstained from caffeine and food ingestion a minimum of 4 h prior to the start of exercise. Participants were also told to avoid the use of antibacterial mouthwash during the ingestion and testing periods. Using a randomised crossover design, the experimental conditions of nitrate, placebo and control were administered. This required participants to ingest either 140 ml of beetroot juice (BEET It sport ® UK) containing 12.8 mmol of NO3- (Larsen et al., 2010) or 140 ml of blackcurrant (P) sugar free cordial (Shannon et al., 2016) once per day for three days. Experimental drinks were consumed 3 h prior to the intended exercise time. In the control condition, participants consumed no experimental supplement. A 48 h period between each ingestion strategy was imposed to ensure adequate washout of supplementary NO3- (Lansley et al., 2011).

The exercise trials required participants to perform a self-selected warm-up for 5 min, which was then replicated prior to all subsequent trials. Exercise performance was then assessed using a 40 km TT at each of final three laboratory visits to assess the effects of the experimental conditions. The TT’s were performed on an electronically braked cycle ergometer (Velotron, Racermate, Seattle, USA) which allowed participants to see an avatar representation of their performance via software (Velotron 3D, Racermate, Seattle, USA), which recorded time, distance, power, and speed. At 10 km intervals, measurements of heart rate (T31, Polar, Finland), ratings of perceived exertion (Borg 1982), felt arousal (Svebak and Murgatroyd, 1985), respiratory gases (VO2) (K5, Cosmed, Italy) and fingertip capillary blood samples were obtained. Blood samples were analysed to determine blood pH and bicarbonate ion (HCO3-) concentrations (ABL800 basic, Radiometer, Denmark) and blood lactate (Lactate Pro2 LT-1730, Arkray, Japan). During the TT all performance data were obscured from view apart from distance completed and remaining. Participants received no verbal or temporal feedback, or any encouragement during the TT.

**Statistical Analysis**

Data were assessed for normality using standard graphical procedures. All respiratory and blood biochemical responses, RPE, felt arousal, heart rate and mean performance indicators (time, speed and power output) were analysed using repeated measures ANOVA. Post-hoc pairwise comparisons were made using a Bonferroni procedure. Effect sizes were calculated using partial eta squared (ƞp2), and were interpreted as: a small (< 0.01), medium (0.01 - 0.06) or a large (≥ 0.14) effect. Statistical significance was set at p < 0.05 and all procedures were conducted using SPSS for Windows (Version 22, IBM®, Chicago, USA).

**Results**

Ingestion of the NO3- drink had no significant effect on overall TT performance time (figure 1 a and 1b), mean power, or mean speed (f = 1.27, p = 0.296, ƞp2 = 0.11, f = 1.14, p = 0.339, ƞp2 = 0.10; and f = 1.15, p = 0.336, ƞp2 = 0.10) respectively. There was also no effect on either mean heart rate (f = 3.51, p = 0.054, ƞp2 = 0.31) or VO2 (f = 1.47, p = 0.253, ƞp2 = 0.13), but there was a significant increase in VO2 (f = 25.68, p < 0.001, ƞp2 = 0.72) during TT performance in all conditions (figure 2). Similarly, lactate and RPE (Table 1) were unaffected by the experimental conditions (f = 0.67, p = 0.522, ƞp2 = 0.06; and f = 2.96, p = 0.085, ƞp2 = 0.30) respectively, but increases in these variables (Table 1) were observed during the course of the TT’s (f = 41.98, p < 0.001, ƞp2 = 0.80; and f = 46.55, p < 0.001, ƞp2 = 0.87) for lactate and RPE respectively. In the case of lactate, concentrations at 40 km where elevated compared to all other distances (p < 0.001). Whereas RPE (Table 1) increased between 10-20 km (p = 0.001), 20-40 km (p = 0.001), between 10-40 km (p < 0.001), and in the last 10 km of the TT (p = 0.014). Felt Arousal (Table 1) was unaffected by either the experimental condition (f = 1.85, p = 0.184, ƞp2 = 0.16) or during the TT (f = 1.66, p = 0.196, ƞp2 = 0.14).

**[Insert Figure 1 Near Here]**

**[Insert Figure 2 Near Here]**

The blood acid-base response variable of pH and HCO3 (Table 1) were also unaffected by the experimental conditions (f = 2.02, p = 0.158, ƞp2 = 0.17) and (f = 1.54, p = 0.239, ƞp2 = 0.13) respectively. There were however some perturbations in these variables during the course of the TT’s (f = 37.60, p < 0.001, ƞp2 = 0.79; f = 48.88, p < 0.001, ƞp2 = 0.83), for pH and HCO3 respectively. The pH response was unchanged between 10 and 20 km (p = 0.060), but it was significantly elevated between 10-30 km (p = 0.006) and then decreased in the final 10 km (p < 0.001). Between 10-30 km there was a significant increase in HCO3 (p = 0.017), but this then decreased rapidly in the final 10 km (p < 0.001).

**[Insert Table 1 Near Here]**

**Discussion**

The aim of this study was to investigate the effects of a 12.8 mmol·day-1 NO3- dose for three days prior the performance of a 40 km TT in trained cyclists. The principle performance findings suggest that NO3-, in the form of beetroot juice, elicited no significant improvements in time to completion or mean power output. These findings support previous research which have reported no change in performance in TT greater than 30 min in superiorly trained cohorts (Cermak et al., 2012b; Wilkerson et al., 2012; Lane et al., 2013). Nevertheless, this study is the first to utilise a chronic 3-day dosing strategy and therefore, suggesting that increasing the concentration of NO3- ingestion does not alter performance during a longer duration 40 km TT in trained cyclists.

The 3-day supplementation protocol utilised in this study administered a total of 38.4 mmol of NO3- prior to exercise, which is substantially greater than the 6 to 17.4 mmol administered during previous investigations (Cermak et al., 2012b; Wilkerson et al., 2012; Lane et al., 2013). These studies reported a plasma NO2 increase by 25% (Wilkerson et al., 2012) and 96% (Cermak et al., 2012b) from baseline to pre-exercise. While we did not measure plasma NO2, Kelly et al., (2014) utilised a similar 3-day supplementation approach providing 25.2 mmol NO3- and reported a substantial 242% and 557% mean increase from baseline, in a similarly trained population (VO2max: 58.3 ± 6.3 ml·kg-1·min-1). Given a greater concentration of NO2 was supplemented in the current study it is fair to postulate a similar increase in plasma NO2, which is substantially larger then that previously reported during prolonged TT investigations (Cermak et al., 2012b; Wilkerson et al., 2012). Consequently, the lack of change in performance is unlikely to be caused by insufficient plasma NO2 bioavailability.

A recent investigation by Shannon et al., (2017) highlighted the ergogenic effects of NO3- may be a manifestation of the exercise intensity and duration. Acute beetroot juice supplementation significantly improved 1500 m TT performance but not 10000 m TT performance in trained runners. The shorter distance requires a relatively higher intensity, thereby prompting an increase in pulmonary VO2 and HR demand, a greater perturbation to muscle metabolic milieu (e.g. Hydrogen ion and phosphate accumulation) and an increased recruitment of type 2 muscle fibres. The underlying mechanism of NO3- ergoencity are vast, but include improved mitochondrial efficiency and reductions in muscle metabolic perturbations (Vanhatalo et al., 2011), particularly within type 2 fibres where blood flow and O2 delivery appears to be enhanced (Ferguson et al., 2015). However, during the current investigation, the 40 km TT was performed at submaximal intensities and thus limiting type 2 fibre recruitment. The differentiation in fibre recruitment may therefore, explain the lack of improvement in the current study, in comparison to the ergogenic effects reported in higher intensity 4 km and 16 km TT’s (Lansley et al., 2011). Interestingly, Wilkerson et al., (2012) observed a significant supplement effect during the final 16 km split of the 80.4 km TT, which was a higher intensity than preceding splits and is characterised by the common increase in power output commonly observed during the final part of TT performances (Jones et al., 2015). While this may suggest NO3- may be beneficial during stochastic activities and the final part of a TT, this effect was not observed in the current study; thereby questioning the application of NO3- in this context.

In the present study there was no effect of NO3- on VO2, but in several studies reduced VO2 has been reported (Bailey et al., 2012; Lansley et al., 2011). Typically, these studies have used shorter duration and therefore, higher intensity exercise protocols; however, Wilkerson et al., (2012) reported a significant increase in the power output: VO2 ratio, suggesting improved efficiency in well trained cyclist with VO2max values of 63 ± 8 ml.kg-1.min-1. This study also reported an increase in plasma NO3-, suggesting that the NO3- ingestion caused the established physiological adaptations of reduced ATP cost of muscle force production and increased mitochondrial functioning. Despite this, along with improvement in the final quintile of the TT, this was still not enough to elicit an overall improvement in TT performance following NO3- ingestion. The present respiratory response data showed no differences between conditions, which is in agreement with the work of Bourdillon et al., (2015) and Christensen et al., (2013) who also reported no change in VO2 and RER during 120 min submaximal cycling or in a subsequent 400 kcal TT in elite cyclists. Christensen et al., (2013), have suggested that training status, determined as a VO2max of 50 mL.kg-1.min-1 in this case, may limit the effectiveness of supplementation and therefore be a factor to explain the lack of difference found in the present study.

**Conclusion**

The aim of this research was to investigate a high dose of NO3- supplementation over three days ingestion on trained cyclists performing a 40km TT performance. This represents a novel ingestion strategy in a highly ecologically valid TT distance. However, the ingestion of dietary NO3- had no significant effect on performance, failed to improve respiratory efficiency, lower blood acidity or affect psychological and physiological fatigue indicators. Therefore, the use of an ergogenic aid containing 12.8 mmol (800 mg) of NO3- each day for three days prior to a 40 km cycling TT, is unlikely to provide a beneficial effect on performance in trained cyclists.

**Acknowledgements**

Conception and design, and analysis and interpretation of data; LM, AS, SM, LG and SD. Data collection: SM, SD, LG. Writing and revising the manuscript: LM, AS, SM, LG and SD. (c) Final approval of the submitted manuscript: LM, AS, SM, LG and SD.

**Funding**

The authors declare that there was no funding provided for any aspects of this study

**Conflict of Interest**

The authors declare that they have no conflicts of interests.

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**Figure Legends**

**Figure 1.** Mean (± SD) overall 40 km performance time for each experimental condition (a) and individual performance responses (b).

**Figure 2.** Mean (± SD) VO2 responses during each experimental condition during the 40 km time trials. (\*) Denotes a significant increase in VO2 from the other distances in all conditions (p < 0.01).

**Table 1.** Mean (±SD) Blood metabolite and subjective rating scale responses to nitrate and placebo ingestion during 40 km cycle time trials.(\*) Represents a significantly increase from all other distances (p < 0.001); (•) represents a significant change over the previous 30 km (p < 0.01); (∇) Denotes a significant decrease over the previous 10 km (p < 0.05); and (Δ) represents a significant increase over the previous 10 km (p < 0.05).

|  |  |
| --- | --- |
|  | Distance (km) |
|  | 10 | 20 | 30 | 40 |
| Lactate (mmol.l-1) |  |  |  |  |
| Control | 5.15 ± 2.04 | 5.04 ± 2.47 | 6.56 ± 3.70 | 9.15 ± 3.47\*• |
| Placebo | 5.05 ± 2.14 | 4.95 ± 2.93 | 5.09 ± 3.03 | 8.75 ± 3.38\*• |
| Nitrate | 4.33 ± 2.05 | 3.97 ± 2.00 | 3.99 ± 2.06 | 7.79 ± 2.00\*• |
|  |  |  |  |  |
| pH |  |  |  |  |
| Control | 7.37 ± 0.05 | 7.39 ± 0.06 | 7.40 ± 0.05 | 7.34 ± 0.06∇ |
| Placebo | 7.40 ± 0.04 | 7.41 ± 0.05 | 7.42 ± 0.05 | 7.34 ± 0.06∇ |
| Nitrate | 7.39 ± 0.05 | 7.40 ± 0.06 | 7.41 ± 0.06 | 7.34 ± 0.07∇ |
|  |  |  |  |  |
| HCO3- (mmol.l-1) |  |  |  |  |
| Control | 20.81 ± 2.86 | 20.79 ± 3.13 | 21.20 ± 2.56 | 18.33 ± 2.43∇ |
| Placebo | 21.43 ± 2.17 | 21.88 ± 2.41 | 22.25 ± 2.52 | 18.58 ± 2.46∇ |
| Nitrate | 21.26 ± 2.37 | 21.43 ± 2.60 | 21.94 ± 2.91 | 17.79 ± 2.20∇ |
|  |  |  |  |  |
| RPE |  |  |  |  |
| Control | 14.0 ± 1.31 | 14.9 ± 2.0Δ | 15.8 ± 2.6 | 19.4 ± 1.1• |
| Placebo | 14.1 ± 1.7 | 15.3 ± 2.0Δ | 16.0 ± 2.2 | 19.0 ± 1.2• |
| Nitrate | 14.5 ± 2.3 | 15.8 ± 1.6Δ | 16.5 ± 1.9 | 19.4 ± 1.2• |
|  |  |  |  |  |
| Felt Arousal |  |  |  |  |
| Control | 4.18 ± 0.75 | 4.45 ± 1.04 | 4.64 ± 0.67 | 4.00 ± 0.77 |
| Placebo | 4.00 ± 0.77 | 4.00 ± 1.10 | 4.00 ± 1.34 | 4.18 ± 1.33 |
| Nitrate | 4.27 ± 1.01 | 4.45 ± 0.82 | 4.45 ± 0.69 | 4.64 ± 0.81 |

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