High dose Nitrate ingestion does not improve 40 km cycling time trial performance in trained cyclists

Mosher, S., Gough, L., Deb, S., Saunders, B., McNaughton, L., Brown, D. and Sparks, S.A.

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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 (NO_3^-) ingestion over three days, on 40 km TT performance in 11trained cyclists (VO_{2max}: 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®" NO_3^- shot containing 12.8 mmol or 800 mg of NO_3^-, a placebo drink or nothing (control). Performance, oxygen consumption (VO_2), 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 that NO_3^- ingestion had no effect on TT performance (NO_3^-: 4098.0 ± 209.8 vs. Placebo: 4161.9 ± 263.3 s, p = 0.296, ES = 0.11), or VO_2 (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 NO_3^- 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 (NO_3^-) 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 NO_3^- rich dietary sources, while commercially available beetroot supplements provide a highly concentrated and practical method to ingest NO_3^- prior to exercise. The underlying action of NO_3^- 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 NO_3^- (Lundberg et al., 2004). The latter is prominent in deriving NO from dietary beetroot supplementation, with NO_3^- reduced to nitrite (NO_2) 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 NO_3^- 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 NO_3^-, the ergogenicity of concentrated beetroot supplementation 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 NO_3^- 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 VO_{2max} of >60 $ml \cdot kg^{-1} \cdot min^{-1}$ (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 NO_3^- prior to an 80 km TT, however the participants exhibited only a small 25% increase in plasma NO_2 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 NO_2 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 NO_3^- 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 (VO_{2max}: 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 (NO_3^-) ingestion over three days, on 40 km TT performance in trained cyclists.

Methods

Eleven trained cyclists (VO_{2max} 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 VO_{2max} 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 VO_{2max} 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 NO_3^- (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 NO_3^- (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 (VO_2) (K5, Cosmed, Italy) and fingertip capillary blood samples were obtained. Blood samples were analysed to determine blood pH and bicarbonate ion (HCO_3^-) 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 ($\square p^2$), and were interpreted as: a small (< 0.01), medium (0.01 -

129 0.06) or a large (≥ 0.14) effect. Statistical significance was set at $p < 0.05$ and all procedures
130 were conducted using SPSS for Windows (Version 22, IBM®, Chicago, USA).
131
132 **Results**
133 Ingestion of the NO_3^- drink had no significant effect on overall TT performance time (figure 1
134 a and 1b), mean power, or mean speed ($f = 1.27$, $p = 0.296$, $\square p^2 = 0.11$, $f = 1.14$, $p = 0.339$,
135 $\square p^2 = 0.10$; and $f = 1.15$, $p = 0.336$, $\square p^2 = 0.10$) respectively. There was also no effect on
136 either mean heart rate ($f = 3.51$, $p = 0.054$, $\square p^2 = 0.31$) or VO_2 ($f = 1.47$, $p = 0.253$, $\square p^2 =$
137 0.13), but there was a significant increase in VO_2 ($f = 25.68$, $p < 0.001$, $\square p^2 = 0.72$) during TT
138 performance in all conditions (figure 2). Similarly, lactate and RPE (Table 1) were unaffected
139 by the experimental conditions ($f = 0.67$, $p = 0.522$, $\square p^2 = 0.06$; and $f = 2.96$, $p = 0.085$, $\square p^2 =$
140 0.30) respectively, but increases in these variables (Table 1) were observed during the course
141 of the TT's ($f = 41.98$, $p < 0.001$, $\square p^2 = 0.80$; and $f = 46.55$, $p < 0.001$, $\square p^2 = 0.87$) for lactate
142 and RPE respectively. In the case of lactate, concentrations at 40 km where elevated
143 compared to all other distances ($p < 0.001$). Whereas RPE (Table 1) increased between 10-
144 20 km ($p = 0.001$), 20-40 km ($p = 0.001$), between 10-40 km ($p < 0.001$), and in the last 10 km
145 of the TT ($p = 0.014$). Felt Arousal (Table 1) was unaffected by either the experimental
146 condition ($f = 1.85$, $p = 0.184$, $\square p^2 = 0.16$) or during the TT ($f = 1.66$, $p = 0.196$, $\square p^2 = 0.14$).
147
148 **[Insert Figure 1 Near Here]**
149 **[Insert Figure 2 Near Here]**
150
151 The blood acid-base response variable of pH and HCO_3 (Table 1) were also unaffected by the
152 experimental conditions ($f = 2.02$, $p = 0.158$, $\square p^2 = 0.17$) and ($f = 1.54$, $p = 0.239$, $\square p^2 = 0.13$)
153 respectively. There were however some perturbations in these variables during the course of
154 the TT's ($f = 37.60$, $p < 0.001$, $\square p^2 = 0.79$; $f = 48.88$, $p < 0.001$, $\square p^2 = 0.83$), for pH and HCO_3
155 respectively. The pH response was unchanged between 10 and 20 km ($p = 0.060$), but it was
156 significantly elevated between 10-30 km ($p = 0.006$) and then decreased in the final 10 km (p

157 < 0.001). Between 10-30 km there was a significant increase in HCO_3 ($p = 0.017$), but this
158 then decreased rapidly in the final 10 km ($p < 0.001$).
159
160 **[Insert Table 1 Near Here]**
161
162 **Discussion**
163 The aim of this study was to investigate the effects of a 12.8 mmol·day^{-1} NO_3^- dose for three
164 days prior the performance of a 40 km TT in trained cyclists. The principle performance
165 findings suggest that NO_3^-, in the form of beetroot juice, elicited no significant improvements
166 in time to completion or mean power output. These findings support previous research which
167 have reported no change in performance in TT greater than 30 min in superiorly trained
168 cohorts (Cermak et al., 2012b; Wilkerson et al., 2012; Lane et al., 2013). Nevertheless, this
169 study is the first to utilise a chronic 3-day dosing strategy and therefore, suggesting that
170 increasing the concentration of NO_3^- ingestion does not alter performance during a longer
171 duration 40 km TT in trained cyclists.
172
173 The 3-day supplementation protocol utilised in this study administered a total of 38.4 mmol of
174 NO_3^- prior to exercise, which is substantially greater than the 6 to 17.4 mmol administered
175 during previous investigations (Cermak et al., 2012b; Wilkerson et al., 2012; Lane et al., 2013).
176 These studies reported a plasma NO_2 increase by 25% (Wilkerson et al., 2012) and 96%
177 (Cermak et al., 2012b) from baseline to pre-exercise. While we did not measure plasma NO_2,
178 Kelly et al., (2014) utilised a similar 3-day supplementation approach providing 25.2 mmol
179 NO3$^-$ and reported a substantial 242% and 557% mean increase from baseline, in a similarly
180 trained population (VO_{2max}: 58.3 ± 6.3 ml·kg^{-1}·min^{-1}). Given a greater concentration of NO_2 was
181 supplemented in the current study it is fair to postulate a similar increase in plasma NO_2, which
182 is substantially larger then that previously reported during prolonged TT investigations
183 (Cermak et al., 2012b; Wilkerson et al., 2012). Consequently, the lack of change in
184 performance is unlikely to be caused by insufficient plasma NO_2 bioavailability.

A recent investigation by Shannon et al., (2017) highlighted the ergogenic effects of NO_3^- 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 VO_2 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 NO_3^- 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 O_2 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 NO_3^- 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 NO_3^- in this context.

In the present study there was no effect of NO_3^- on VO_2, but in several studies reduced VO_2 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: VO_2 ratio, suggesting improved efficiency in well trained cyclist with VO_{2max} values of 63 ± 8 $ml.kg^{-1}.min^{-1}$. This study also reported an increase in plasma NO_3^-, suggesting that the NO_3^- 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 NO_3^- 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 VO_2 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 VO_{2max} 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 NO_3^- 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 NO_3^- 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 NO_3^- each day for three days prior to a 40 km cycling TT, is unlikely to provide a beneficial effect on performance in trained cyclists.

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Conflict of Interest

The authors declare that they have no conflicts of interests.

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341 **Figure Legends**

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

344

345 **Figure 2.** Mean (± SD) VO_2 responses during each experimental condition during the 40 km
346 time trials. (*) Denotes a significant increase in VO_2 from the other distances in all conditions
347 ($p < 0.01$).

348

349

350

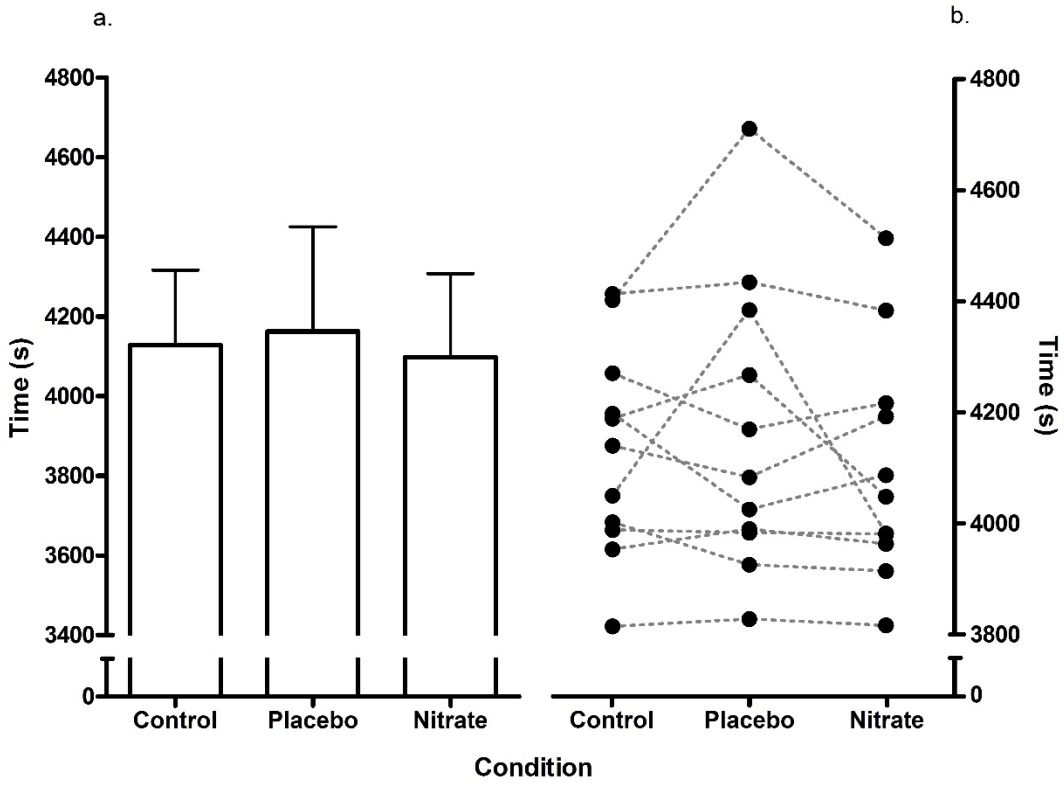
351

352 **Table 1.** Mean (±SD) Blood metabolite and subjective rating scale responses to nitrate and
353 placebo ingestion during 40 km cycle time trials. (*) Represents a significantly increase from
354 all other distances ($p < 0.001$); (•) represents a significant change over the previous 30 km (p
355 < 0.01); (∇) Denotes a significant decrease over the previous 10 km ($p < 0.05$); and (Δ)
356 represents a significant increase over the previous 10 km ($p < 0.05$).

357

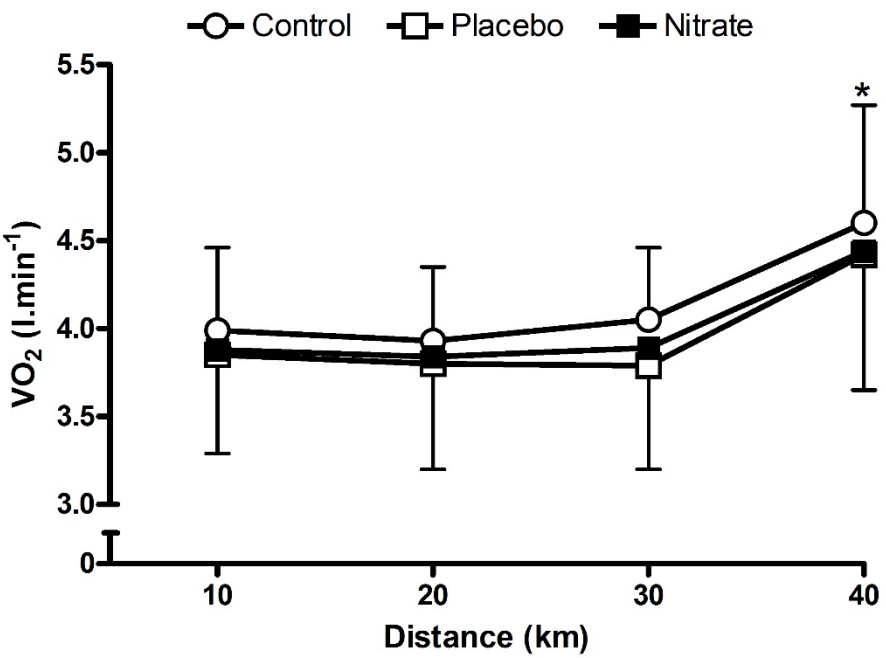
	Distance (km)			
	10	20	30	40
Lactate (mmol.l^{-1})				
Control	5.15 ± 2.04	5.04 ± 2.47	6.56 ± 3.70	9.15 ± 3.47*•
Placebo	5.05 ± 2.14	4.95 ± 2.93	5.09 ± 3.03	8.75 ± 3.38*•
Nitrate	4.33 ± 2.05	3.97 ± 2.00	3.99 ± 2.06	7.79 ± 2.00*•
pH				
Control	7.37 ± 0.05	7.39 ± 0.06	7.40 ± 0.05	7.34 ± 0.06∇
Placebo	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∇
HCO_3- (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

358


a.
b.
Time (s)
4800
4600
4400
4200
4000
3800
3600
3400
0
Control
Placebo
Nitrate
Condition

359

360


Control
Placebo
Nitrate
VO_2 (l.min^{-1})
5.5
5.0
4.5
4.0
3.5
3.0
0
*
10
20
30
40
Distance (km)

361