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Effect of dietary nitrate supplementation on swimming performance in trained swimmers.

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Title

Effect of dietary nitrate supplementation on swimming performance in trained swimmers

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Running head

Dietary nitrate and swimming performance

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Abstract

Nitrate supplementation appears to be most ergogenic when oxygen availability is restricted and subsequently may be particularly beneficial for swimming performance due to the breath-hold element of this sport. This represents the first investigation of nitrate supplementation and swimming time-trial (TT) performance.

In a randomised double-blind repeated-measures crossover study, ten (5male, 5female) trained swimmers ingested 140ml nitrate-rich (~12.5mmol nitrate) or nitrate-depleted (~0.01mmol nitrate) beetroot juice. Three hours later, subjects completed a maximal effort swim TT comprising 168m (8 x 21m lengths) backstroke.

Pre-exercise fractional exhaled nitric oxide concentration was significantly elevated with nitrate compared to placebo (17±9 vs. 7±3p.p.b., p=0.008). Nitrate supplementation had a likely trivial effect on overall swim TT performance (mean difference 1.22s; 90% CI -0.18–2.6s; 0.93%; p=0.144; d=0.13; unlikely beneficial (22.6%), likely trivial (77.2%), most unlikely negative (0.2%)). The effects of nitrate supplementation during the first half of the TT were trivial (mean difference 0.29s; 90% CI -0.94–1.5s; 0.46%; p=0.678; d=0.05), but there was a possible beneficial effect of nitrate supplementation during the second half of the TT (mean difference 0.93s; 90% CI 0.13–1.70s; 1.36%; p=0.062; d=0.24; possibly beneficial (63.5%), possibly trivial (36.3%), most unlikely negative (0.2%)). The duration and speed of underwater swimming within the performance did not differ between nitrate and placebo (both p>0.30).

Nitrate supplementation increased nitric oxide bioavailability but did not benefit short-distance swimming performance or the underwater phases of the TT. Further investigation into the effects of nitrate supplementation during the second half of performance tests may be warranted.

Introduction

Supplementation with dietary nitrate can elicit myriad potentially beneficial physiological changes. These effects include lower oxygen consumption ($\dot{V}O_2$) during steady-state exercise (Larsen et al., 2007; Bailey et al., 2009; Pawlak-Chaouch et al., 2016), faster $\dot{V}O_2$ and muscle deoxyhaemoglobin kinetics (Breese et al., 2013), attenuated intracellular muscle metabolic perturbations (Bailey et al., 2010), and augmented muscle force generation (Haider & Folland, 2014; Coggan et al., 2015). A number of studies have also demonstrated a beneficial effect of nitrate supplementation on exercise time to exhaustion (TTE) or time-trial (TT) performance (e.g. Bailey et al., 2009, 2010; Lansley et al., 2011; Cermak et al., 2012a; Porcelli et al., 2014; McMahon et al., 2016). However, not all investigations have observed an improvement in exercise performance subsequent to nitrate supplementation (e.g. Cermak et al., 2012b; Peacock et al., 2012; Wilkerson et al., 2012; Muggeridge et al., 2013; Boorsma et al., 2014). The discordance in findings across investigations might be due to different nitrate supplementation strategies, exercise protocols, environmental conditions, and participant aerobic fitness levels (Jones, 2014). The precise conditions under which nitrate supplementation might be ergogenic requires further exploration.

To date, the majority of investigations have employed cycling (e.g. Larsen et al., 2007; Lansley et al., 2011; Cermak et al., 2012a, 2012b; Wilkerson et al., 2012; Wylie et al., 2013) or running (e.g. Peacock et al., 2012; Boorsma et al., 2014; Porcelli et al., 2014; Shannon et al., 2016) protocols to evaluate the performance effects of dietary nitrate supplementation. A small number of studies have also explored the effects of nitrate supplementation on rowing (Bond et al., 2012; Hoon et al., 2013) and kayak (Muggeridge et al., 2013; Peeling et al., 2014) performance. Conversely, the effects of nitrate supplementation on swimming performance –

a sport where, anecdotally, nitrate supplementation appears to be highly prevalent – are poorly understood. Recently, Pinna et al. (2014) reported an increased workload at the anaerobic threshold and reduced aerobic energy cost during incremental tethered swimming consequent to nitrate supplementation. These results suggest a potentially beneficial effect of nitrate supplementation during swimming. Nevertheless, that study did not assess exercise performance, and lacked the desired scientific controls to draw firm conclusions (e.g. no placebo group or assessment of nitric oxide (NO) bioavailability).

As an exercise mode, it is possible that swimming is especially susceptible to the ergogenic effects of nitrate supplementation given that all events require a breath-hold element during the underwater phase of each length which restricts oxygen exchange with the environment. Indeed, recent evidence indicates that nitrate supplementation is more effective at enhancing exercise performance in situations where oxygen availability is decreased (Kelly et al., 2014), possibly due to greater nitrite reduction into NO (Castello et al., 2006). Engan et al. (2012) also recently demonstrated a beneficial effect of nitrate supplementation on apnea performance in trained free divers, which might suggest a possible benefit of nitrate supplementation in improving underwater performance in swimming.

As the effects of nitrate supplementation on swimming TT performance are presently unclear, further exploration is warranted. Therefore, the aim of the present study was to investigate the effects of dietary nitrate supplementation on NO bioavailability and TT performance in a group of competitive swimmers. We hypothesised that nitrate supplementation would elevated NO bioavailability and enhance swimming TT performance.

Methods

Subjects

Ten competitive swimmers (5 male, 5 female) aged 20 ± 1 years, with a stature of 172 ± 10 cm and body mass of 71 ± 9 kg, participated in this study. Twelve participants were initially recruited but two participants withdrew without providing a reason. All subjects were university students who trained a minimum of 3 times per week, and competed locally and for their university. The study was approved by the institutional ethics committee and all subjects provided informed written consent to participate.

Study design

Subjects visited the laboratory on three separate occasions within a four week period. The first visit comprised pre-screening and familiarisation, which replicated the experimental protocol but without the assessment of any physiological parameters. The remaining two visits comprised the performance trials, and were preceded by the consumption of 140 ml (2 x 70 ml) concentrated nitrate-rich (~12.5 mmol) or nitrate-depleted (~0.01 mmol) beetroot juice (Beet It Sport, James White Ltd., UK) administered double blind, 3 hours before the start of exercise. The nitrate content of the supplements was determined via o-zone based chemiluminescence, as previously described (Shannon et al., 2016). Performance trials were conducted in a randomised cross-over design. Four participants completed the nitrate trial first, and 6 participants completed the placebo trial first. There was no effect of trial order on TT performance ($p = 0.825$). Subjects maintained their normal diet and activity levels throughout the testing period, but were asked not to perform strenuous exercise, or consume caffeine and alcohol in the 24 hours preceding each trial. Subjects recorded and replicated their dietary intakes and physical activity levels in the 24 hours before each trial, including their usual pre-

122 competition breakfast (~7 – 8 am), and did not consume anything except for water in the 3
123 hours prior to testing. Subjects avoided antibacterial mouthwash and chewing gum throughout
124 the testing period, given these are known to destroy oral nitrate reducing bacteria (Govoni et
125 al., 2008). All performance trials were conducted at the same time of day (~10 – 12 am) to
126 minimise the influence of circadian variance.

127

128 **Performance trials**

129 Approximately one week after the pre-screening and familiarisation trial, subjects completed
130 the first of two performance trials. All performance trials were conducted over a 2 – 3 week
131 period, with a minimum of 7 days between visits. On arrival at the laboratory (~30 minutes
132 pre-exercise), a blood sample was obtained from the earlobe and analysed for lactate
133 concentration (Lactate Plus, Nova Biomedical, MA). Subsequently, a measure of fractional
134 exhaled NO (FENO) was collected as a marker of NO bioavailability via a hand-held
135 electrochemical analyser (NObreath, Bedfont Scientific Ltd., UK). Three measures of FENO
136 were obtained, and the mean value was used for data analysis.

137

138 Following pre-test measurements, subjects were asked to complete a warm up which replicated
139 their typical pre-race routine, and was identical between trials. After a five minute rest period,
140 subjects then completed a swim TT. The TT consisted of 168 m (8 lengths of a 21 m pool)
141 using the backstroke technique. This stroke was selected because it restricts the breath-hold
142 element of the swim to the start of each length, therefore enabling a more distinct investigation
143 into the ergogenic effects of nitrate supplementation for overall swimming performance and
144 breath-hold (underwater) phases. All tests were conducted as individual TTs (i.e. without
145 competition) in the same lane of the swimming pool. Participants received no feedback during

the test. Water temperature was maintained at 29 ºC across trials. Blood lactate concentration was assessed immediately post-exercise as previously described. The number of strokes, time spent underwater and speed of underwater swimming were monitored every 21 m, while TT performance splits were recorded every 42 m. This data was collected using a commercially available video camera (Panasonic HC-V720, Osaka, Japan), and subsequently analysed using a movement analysis software (Dartfish, Fribourg, Switzerland).

Statistical analysis

Data were analysed using IBM SPSS statistics version 22 for Windows. Three-way repeated measures ANOVA (trial x time x sex) demonstrated no sex-based differences for any of the experimental variables in response to nitrate supplementation ($P > 0.187$ for all trial x sex interactions and trial x time x sex interactions). Subsequently, data for both sexes was pooled and two-way repeated measures ANOVA (trial x time) was used to examine differences between trials over time for performance measures (TT performance splits, number of strokes per 21m, time and speed underwater per 21m) and lactate concentration. Paired t-tests were used to examine differences in FENO between trials. Pearson's correlation coefficient was used to examine the relationship between the change in FENO after nitrate supplementation and changes in TT performance. Null-hypothesis significance testing was performed with an alpha value of 5 % in accordance with current convention.

In addition, magnitude-based inferences were calculated to examine whether the observed differences in TT performance were meaningful. This approach has been supported within exercise science due to reduced inferential error rates compared with null-hypothesis significance testing (Hopkins & Batterham 2016) and to facilitate direct interpretation of the

magnitude of changes and whether these are meaningful (Buchheit 2016). Subsequently, this approach was utilised and prioritised for evaluating TT performance. Using the spreadsheet by Hopkins (2007), the *p* value for TT performance was converted into 90% confidence intervals (CI) for inferences about the true value of the effect statistic (Hopkins 2007). An effect was deemed unclear when the upper and lower confidence limits represented meaningful increases and decreases, respectively. All other effects were deemed clear, and the probabilities that the true effect was a substantial increase, a trivial change, and a substantial decrease were calculated via the sampling t-distribution of the effect in relation to the smallest worthwhile change.

Magnitude-based inferences were also utilised to evaluate performance during the first and second halves of the TT, identified a-priori. This decision was based on evidence that the reduction of nitrite into NO, and thus potentially the ergogenic benefits of nitrate supplementation, increase as oxygen tensions and muscle pH decline (Modin et al., 2001; Castello et al., 2006). We therefore reasoned that nitrate supplementation might be more effective during the later stages of exercise, when muscle oxygen pressure and pH are likely to be lowest.

All results are presented as mean (SD) or 90% confidence intervals where appropriate. Effect sizes are presented as Cohen's *d* and interpreted as ≤ 0.2 trivial, > 0.2 small, > 0.6 moderate, > 1.2 large, > 2 very large and > 4 extremely large (Hopkins 2004). The smallest worthwhile change in TT performance was set as the time difference equating to a standardised effect size of 0.2 based on the between subject SD for the placebo trial (Hopkins, 2004). This approach accounted for the relatively high SD values within the data and provided a conservative

threshold for the smallest worthwhile change. Individual responses are presented within figures to allow further examination of the findings.

Sample size calculation

A sample size of ten participants was determined to be sufficient to detect a significant difference in TT performance based on a small standardised effect size of 0.2 and the variance of the difference between placebo and nitrate trials previously reported (Lansley et al., 2011). Although the study by Lansley and colleagues (2011) employed a cycling TT, the inclusion of this data for the power calculation was deemed preferable to a swimming TT without nitrate supplementation in order to consider the consistency of change between nitrate and placebo trials between participants. Furthermore, a collation of previous competition data suggests that the coefficient of variation for swimming TT events is similar or slightly lower than cycling TT events (Hopkins, 2004). Subsequently, twelve participants were recruited for the study to account for the risk of attrition. This calculation was performed using G*power with an alpha value of 5% and a power of 80% (Faul et al., 2007).

Results

Metabolic markers and FENO

Two-way ANOVA demonstrated no main effect of trial ($p = 0.567$) or trial x time interaction ($p = 0.664$) for blood lactate concentration. A main effect of time demonstrated increased blood lactate concentration after the TT ($p < 0.0005$) (Placebo pre-exercise: 0.9 ± 0.4; Placebo post-exercise: 11.9 ± 2.9; Nitrate pre-exercise: 0.8 ± 0.5; Nitrate post-exercise: 11.2 ± 3.7 $mmol.L^{-1}$).

Student's paired t-tests demonstrated increased FENO (p = 0.008) in nitrate compared with placebo (Figure 1). There was no significant correlation between the change in FENO and change in TT performance after nitrate supplementation (r = -0.184; p = 0.611).

Time trial performance

Two-way ANOVA revealed no significant differences between trials in overall TT performance (main effect of trial, p = 0.144) or across the 42m intermediate splits (trial x time interaction; p = 0.189). A main effect of time demonstrated slower split times as the duration increased (p < 0.0005). The mean (SD) values for TT performance in the placebo and nitrate trials were 131.59 (9.09) s and 130.37 (8.10) s, respectively; therefore a threshold value of 1.82 s was selected for magnitude-based inferences as a standardised effect size of 0.2 based on the between subject standard deviation of the placebo trial. The mean difference in TT performance between nitrate and placebo was 1.22 s (90% CI -0.18 – 2.6 s; 0.93 %; p = 0.144; d = 0.13; unlikely beneficial (22.6%), likely trivial (77.2%), most unlikely negative (0.24%)) (Figure 2).

The mean (SD) for the first and second halves of the TT were as follows: first half 63.14 (5.50) s versus 62.85 (4.03) s; second half 68.45 (3.88) s versus 67.52 (4.33) s for placebo versus nitrate, respectively. Subsequently, threshold values of 1.10 s and 0.78 s were used as the smallest worthwhile change in performance during the first and second half of the TT, respectively, based on a standardised effect size of 0.2. The mean difference in performance between nitrate and placebo during the first half of the TT was 0.29 s (90% CI -0.94 – 1.5 s; 0.46 %; p = 0.678; d = 0.05; unlikely beneficial (12.9%), likely trivial (83.7%), very unlikely negative (3.4%)). During the second half of the TT, the mean difference in performance was

0.93 s (90% CI 0.13 – 1.70 s; 1.36 %; $p = 0.062$; $d = 0.24$; possibly beneficial (63.5%), possibly trivial (36.3%), most unlikely negative (0.2%)) (Figure 3).

Secondary performance variables

Two-way ANOVA demonstrated that there was no main effect of trial or trial x time interaction for the duration or speed of underwater swimming or the number of strokes completed per length (all $p > 0.300$). A significant main effect of time for each of these variables demonstrated reductions in underwater duration and speed as the duration of the swim increased and an increased number of strokes per length as duration increased (all $p < 0.0005$; Figure 4).

Discussion

To the authors' knowledge, this is the first study to investigate the effects of dietary nitrate supplementation on swimming TT performance. The findings demonstrate that nitrate supplementation increased NO bioavailability but had a likely trivial effect on overall swimming TT performance.

The primary aim of this study was to evaluate the effects of dietary nitrate supplementation on swimming TT performance. According to traditional null hypothesis testing, swim TT performance did not differ significantly between nitrate and placebo. This approach to statistical testing has been criticised as unsuitable for assessing the clinical or practical importance of an effect (Hopkins, 2004). Therefore, we also applied an alternative statistical approach which provides a qualitative probabilistic inference as to whether the effect is likely to be of practical benefit to an athlete. This latter approach provided similar interpretations as

the effect of nitrate supplementation on TT performance was deemed 'likely trivial'. Subsequently, the findings from this study do not support the use of nitrate supplementation for short-duration backstroke performance in trained swimmers. However, given nitrate supplementation was also deemed 'most unlikely' harmful for performance, it appears that supplementation will not have any adverse effects on performance. Interestingly, magnitude based inferences suggested that nitrate supplementation might elicit a 'possible beneficial' effect on performance during the second half of the swimming TT. Indeed, the effect of nitrate supplementation during the first half of the TT was deemed 'likely trivial', yet was 64% likely to be beneficial in the second half. It is possible that this effect is accounted for by greater acidosis and hypoxia present in the muscle tissue during the second half of the TT, creating a cellular milieu conducive towards the reduction of nitrite into NO (Modin et al., 2001; Castello et al., 2006), and thus enhancing NO signalling during the later stages of exercise. Whilst it is important to note that this did not translate into a beneficial effect on overall TT performance, a faster finish could offer tactical advantages which warrants investigation during 'real world' competition.

The effects of nitrate on a range of secondary performance variables were also explored. Given previous reports that nitrate supplementation extends maximal apnea duration in trained free-divers (Engan et al., 2012) and elevates arterial oxygen saturation (S_aO_2) following sub-maximal dynamic apnea (i.e. underwater swimming) (Patrician & Schagatay, 2016), we reasoned that nitrate supplementation might allow athletes to improve the duration and speed of underwater swimming each length. However, although a significant effect of time was detected, demonstrating a reduction in these variables as the duration of the swim increased, nitrate supplementation did not influence underwater duration or speed. Nitrate supplementation also had no effect on the number of strokes per length, which also increased

288 with swim duration. This suggests that the ergogenic benefits of nitrate supplementation for
289 swimming do not specifically benefit the breath-hold phases of competition and are not
290 enhanced by the breath-hold component of this activity. These findings also suggest that the
291 reported beneficial effects of nitrate supplementation on muscle contractile function and force
292 generation (Haider & Folland, 2014; Coggan et al., 2015) does not necessarily translate into
293 greater force produced during swimming (which might be indicated as a reduced number of
294 strokes necessary per length), which is possibly due to the technical demands of this sport.

295

296 The sample size calculation for this study was based on data from a previous investigation
297 exploring the effect of nitrate supplementation on cycling TT performance (Lansley et al.,
298 2011). Although this may be criticised due to the investigation of a different exercise mode in
299 the present study, this approach allowed consideration of the consistency of change between
300 participants in response to nitrate compared with placebo supplementation in previous
301 experiments. Furthermore, a collation of previous competition data suggests that the
302 coefficient of variation for swimming TT events is similar or slightly lower than cycling TT
303 events (Hopkins, 2004). This suggests that the absence of a beneficial effect of nitrate
304 supplementation on overall TT performance in the present study is unlikely to be the result of
305 low statistical power, which is further supported by: a) the use of a similar number of
306 participants as other studies that have reported significant performance improvements with
307 nitrate supplementation (e.g. Bailey et al., 2009; Lansley et al., 2011; Breese et al., 2013); b)
308 the trivial effect size observed for the overall TT performance (d = 0.13); and c) the 77.2%
309 likelihood that the effects of nitrate supplementation were trivial for TT performance based on
310 magnitude-based inferences.

311

Several mechanisms have been proposed for the previously observed beneficial effects of nitrate supplementation, including enhanced efficiency of both mitochondrial respiration (Larsen et al., 2011) and muscle contraction (Bailey et al., 2010), augmented muscle tissue blood flow (Ferguson et al., 2013) and elevated calcium handling protein expression (Hernandez et al., 2012). Interestingly, these latter two effects were reported to occur preferentially in type II muscle (Ferguson et al., 2013; Hernandez et al., 2012). As such, it has been suggested that nitrate supplementation may be most beneficial during exercise protocols likely to involve high type II muscle recruitment (Breese et al., 2013; Bailey et al., 2015; Coggan et al., 2015), as may be expected to occur during the short-duration high-intensity TT employed here. Nevertheless, it should be noted that the physiological effects of nitrate supplementation, especially those requiring alterations in protein expression (Larsen et al., 2011; Hernandez et al., 2012), may take several days to fully manifest. It is therefore possible that greater effects might have been observed if a more protracted supplementation strategy was employed (Vanhatalo et al., 2010). However, this study aimed to explore the effects of acute rather than chronic supplementation on swim performance, similar to that demonstrated to be beneficial for cyclists by Lansley et al. (2011), given such a protocol may be more readily adopted by athletes, due to reduced time commitments and financial cost.

Emerging evidence indicates that the beneficial effects of nitrate supplementation are diminished in endurance trained individuals (Porcelli et al., 2014). This may be a consequence of the higher baseline pool of NO metabolites already present in this cohort (Vassalle et al., 2003), the elevated activity and presence of the NOS enzymes (McConell et al., 2007), or greater proportion of type I muscle fibres, which are believed to be less receptive to the effects of nitrate supplementation (Ferguson et al., 2013; Hernandez et al., 2012; Jones et al., 2016). Thus, it is possible that the trivial performance effects of nitrate observed in this investigation

might be magnified in less well-trained swimmers, which remains to be investigated. Further, it should be noted that the TT was conducted in a 21 m swimming pool rather than a traditional 25 m or 50 m pool. Whilst all participants regularly trained in this pool, it is likely that the TT distance (168 m) elicited subtly different physiological demands to a more typical 200 m TT which might have influence the performance response to nitrate supplementation.

Nitrate supplementation elevated FENO by 131 % compared to placebo, reflecting an increase in NO bioavailability. We selected FENO as a marker of NO bioavailability over blood-borne NO markers (e.g. plasma nitrate and nitrite) due to the ease of collection during field-based testing and the non-invasive nature of assessments. Nevertheless, it should be acknowledged that FENO is a less sensitive marker of whole body NO bioavailability compared with plasma nitrate and nitrite, and might not necessarily reflect NO generation and signalling in the muscle (Coggan et al., 2015). The increase in FENO following nitrate supplementation did not significantly correlate with the change in performance ($r = -0.184$), which might be a consequence of the distal (relative to the muscle, where NO is acting) nature of FENO assessments. It is also worth noting that one subject in this study manifested a substantially greater increase in FENO than the group mean (600% vs 131 %). Conversely, three subjects showed a minimal FENO response (0-45 % increase) to nitrate supplementation. This demonstrates considerable variability in the FENO response to nitrate supplementation.

In conclusion, dietary nitrate supplementation increased NO bioavailability but did not benefit short-distance swimming TT performance. Future research may seek to investigate the effects of nitrate supplementation during distinct phases of TT performance (i.e. first and second halves) in different sports, in addition to providing further investigations into the effects of

nitrate supplementation on swimming performance in different populations and in response to longer term supplementation.

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Authorship

The study was designed by SL, OMS, KD, JM and MB. Data were collected, analysed, and subsequently interpreted by SL, OMS, KD, JM and MB. Manuscript preparation, including drafting of the article and manuscript revisions, was undertaken by SL, OMS, KD, JM and MB. All authors approved the final version of the article.

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545 **Figure legends**

546 **Figure 1** Group mean (SD) and individual FENO data following dietary nitrate or placebo
547 supplementation. FENO was significantly elevated following nitrate supplementation (p =
548 0.008).

549

550 **Figure 2** Group mean (SD) and individual performance times during the 168 m backstroke TT
551 following dietary nitrate or placebo supplementation. TT time did not differ significantly
552 between conditions (p = 0.14). Probabilities of a decrease/trivial/increase in performance time
553 with nitrate supplementation; downward arrow represents decreased performance time; dash
554 represents trivial effect of supplementation.

555

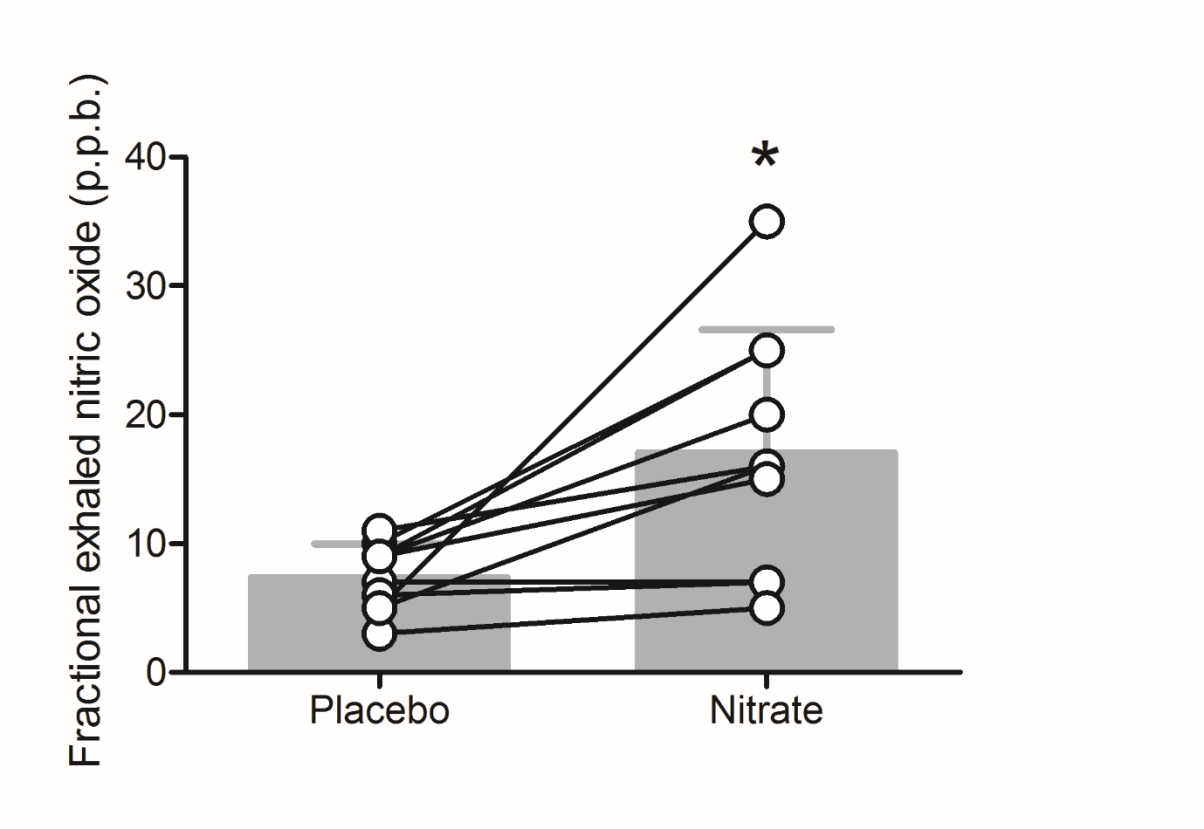
556 **Figure 3** Group mean (SD) and individual performance times during the first and second half
557 of the 168 m backstroke TT following dietary nitrate or placebo supplementation. TT time did
558 not differ significantly between conditions in the first (p = 0.678) or second half (p = 0.062) of
559 the test. Probabilities of a decrease/trivial/increase in performance time with nitrate
560 supplementation; downward arrow represents decreased performance time; dash represents
561 trivial effect of supplementation.

562

563 **Figure 4** Group mean (SD) secondary performance data, including underwater swimming
564 duration (a), underwater swimming speed (b), and number of strokes per 21 m (c) following
565 dietary nitrate or placebo supplementation. None of the variables differed significantly
566 between conditions (all $p > 0.30$).

567

Figures



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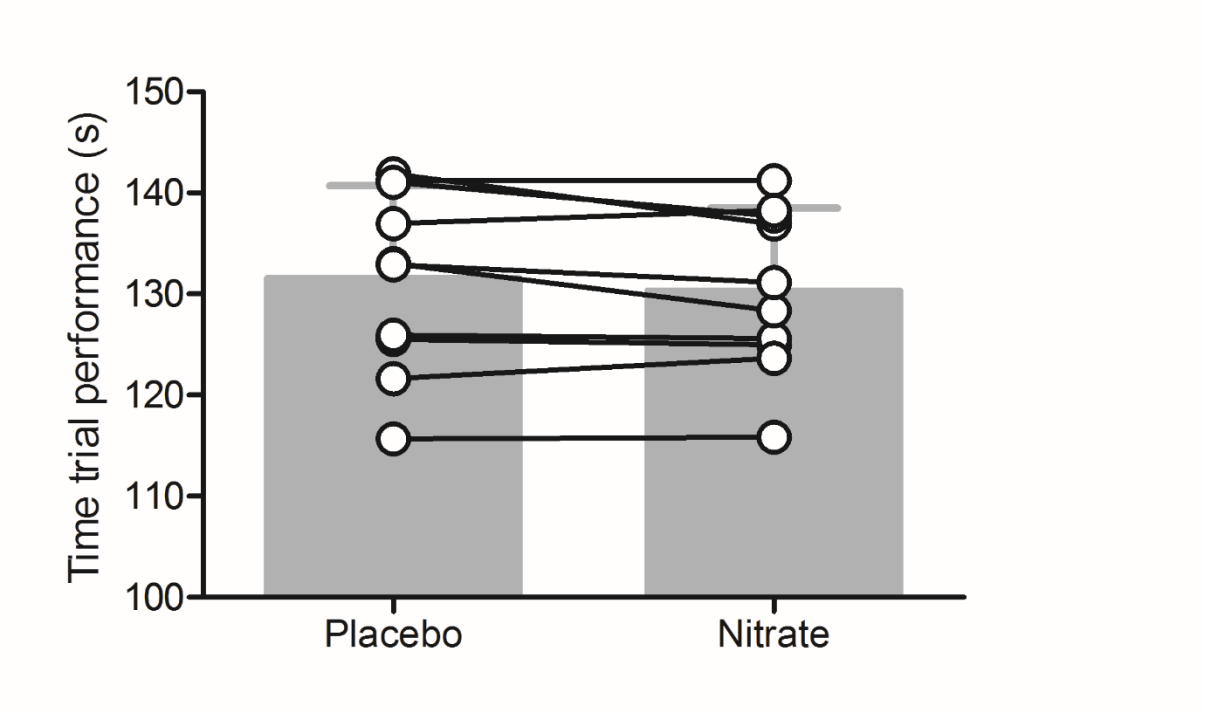

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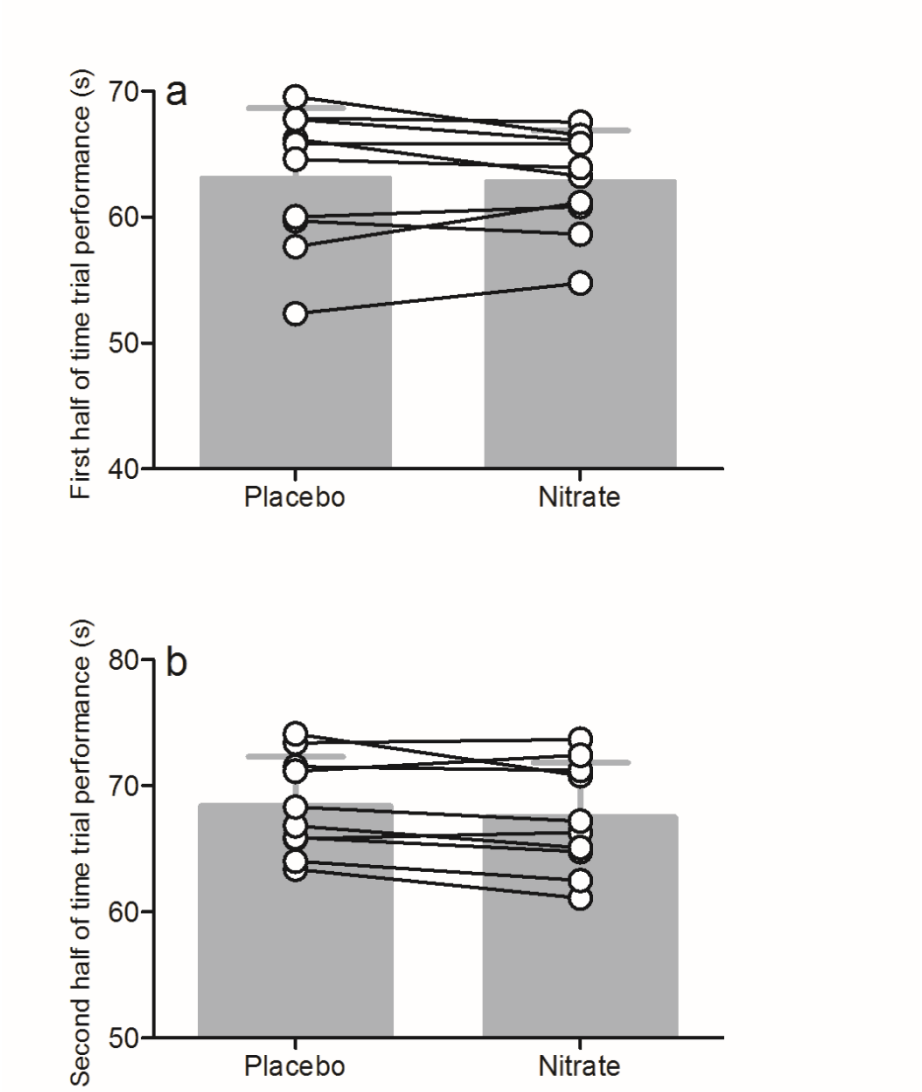

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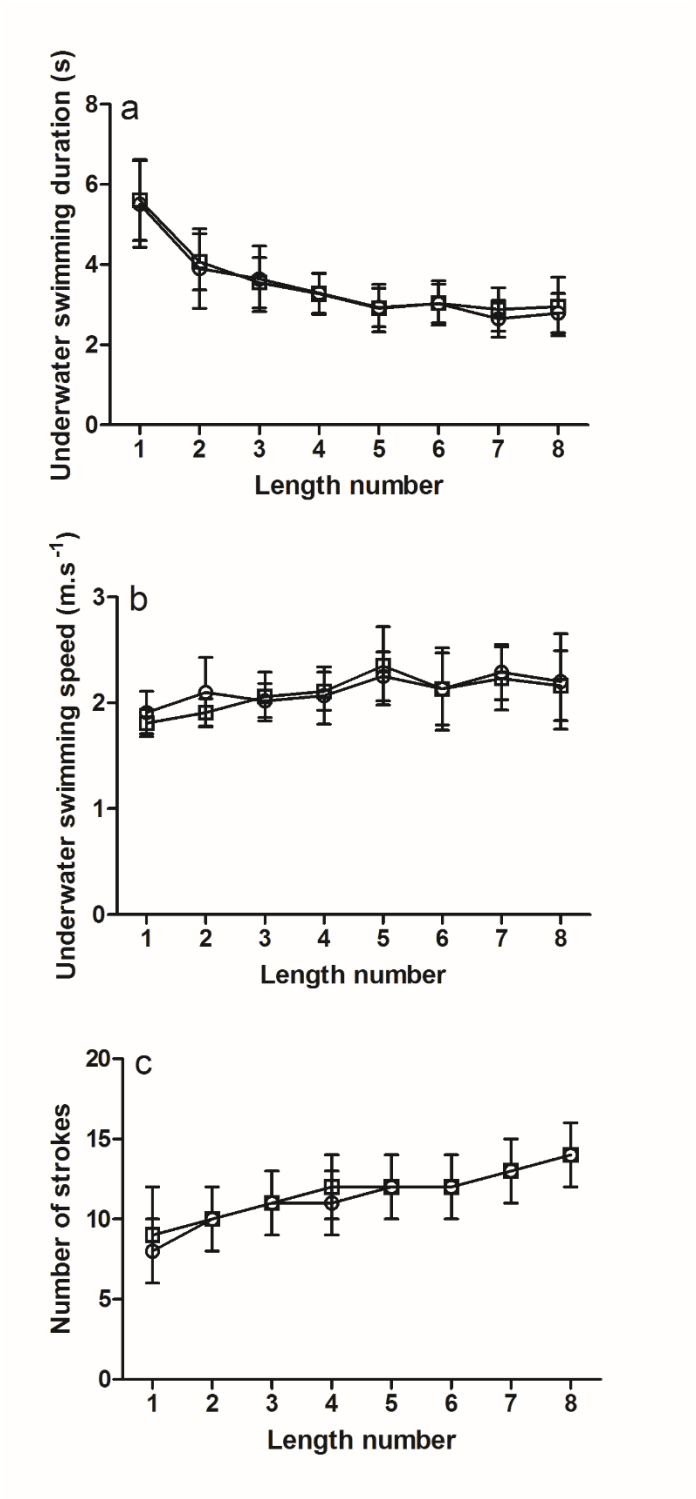

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