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The Effect of Dietary Nitrate Supplementation on Physiology and Performance in Trained Cyclists

Submission Type: Original Investigation

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ABSTRACT

Purpose: To determine the effect of dietary nitrate (NO_3^-) supplementation on physiology and performance in well-trained cyclists following six to eight-days of NO_3^- supplementation. **Methods:** Eight competitive male cyclists (mean ± SD; age = 26 ± 8 y; body mass = 76.7 ± 6.9 kg; VO_2peak = 63 ± 4 ml.kg^{-1}.min^{-1}) participated in a double-blind, placebo-controlled, crossover-design study in which participants ingested 70 ml beetroot juice containing ~4 mmol NO_3^- (NIT) or a NO_3^- depleted placebo (PLA), each for 8-days. Replicating pre-treatment measures, participants undertook an incremental ramp assessment to determine VO_2peak, first (VT_1), and second (VT_2) ventilatory thresholds on day 6 (NIT6 and PLA6), moderate-intensity cycling economy on day 7 (NIT7 and PLA7), and a 4-km time-trial on day 8 (NIT8 and PLA8). **Results:** Relative to PLA, 6 days of NIT supplementation produced *unclear* effects for VO_2peak (mean ±95%CL: 1.8 ±5.5%) and VT_1 (3.7 ±12.3%) and *trivial* effects for both VT_2 (-1.0 ±3.0%) and exercise economy on day 7 (-1.0 ±1.6%). However, effects for time-trial performance time (-0.7 ±0.9%), and power (2.4 ±2.5%), on day 8 were *likely beneficial*. **Conclusions:** Despite mostly *unclear* outcomes for standard physiological determinants of performance, 8-days of NO_3^- supplementation resulted in *likely beneficial* improvements to 4-km time-trial performance in well-trained male endurance cyclists.

Key Words: Beetroot juice, time-trial, economy, cycling

INTRODUCTION

Dietary nitrate (NO_3^-) supplementation, either through ingestion of pharmacological sodium NO_3^{-}[1,2] or naturally occurring beetroot juice[3] has generated significant interest amongst researchers, coaches and sport science practitioners, due to its marked physiological effects.[1] Specifically, NO_3^- ingestion has been shown to reduce oxygen consumption during submaximal exercise,[3,4] improve ventilatory threshold[5] and reduce the VO_2 slow component and mean response time.[3] Individually and collectively, such enhancements are of interest in athletic populations given their relevance to sport performance in endurance events.[6]

The performance enhancing effects of NO_3^- supplementation in endurance events are well documented,[4,7] however, ergogenic effects appear to reduce as performance trial duration increases[2,8] and/or as athlete calibre increases.[9] The range of performance outcomes highlights the need to consider trial duration, athletic calibre and athlete type (sprint vs endurance) when interpreting the effect of NO_3^- supplementation. For example, in cycling, following a single acute dose of NO_3^-, moderately-trained (VO_2peak 56 to 63 ml.kg.$^{-1}$min^{-1}) cyclists improved performance over shorter (~6.45 to 27.30 min)[7] but not longer (40 to 137 min) duration time-trials.[2,8] In contrast to the findings of Lansley et al[7] national level cyclists produced *unclear* and *possibly harmful* effects for 4-min time-trial mean power output (MPO) following acute doses of NO_3^-.[10] Such findings suggest that acute NO_3^- supplementation strategies enhance the performance of moderately trained cyclists within events of shorter - and therefore greater relative intensity - than longer duration trials. Short-duration (1- to 12-min), maximal intensity exercise leads to a rapid increase in blood acidosis[11] and although this may not directly infer a reduction in performance,[12] the stepwise reduction of nitrite (NO_2^-) to nitric oxide (NO) is catalysed in more acidic and hypoxic environments[13] indicating that higher intensity exercise may enhance the effectiveness of NO_3^- supplementation on performance.

Improvements in mitochondrial respiration[14] and a reduction in ATP cost during muscle force production[15] are several mechanisms postulated to positively enhance measures of performance and physiology as a result of longer term (≥ 3 days) NO_3^- supplementation.[14,15] Based on these physiological enhancements, and given the 1.2% improvement over longer (10-km) distance following a 6-day supplementation period,[4] it is of interest to explore whether a similar dose duration augments improvements over and above that of the 2.7% enhancement for 4-km reported by Lansley et al[7] following an acute dose in a similarly trained population. Moreover, this shorter 4-km distance may serve to produce an ideal environment for the reduction of NO_2^- to NO.[13] Therefore, the aim of this study was to determine the effects of six to eight-days of beetroot juice supplementation on 4-km time-trial performance and economy in trained cyclists.

Methods

Subjects

Nine well-trained endurance male cyclists (mean $\pm$ SD; age 26 ± 8 y; body mass 76.7 ± 6.9 kg; sum of 8 skinfold 52 ± 10 mm; VO_2peak 63 ± 4 ml.kg^{-1}.min^{-1}; weekly training duration 9 ± 3 h.wk^{-1}) acted as their own controls in this double-blind, placebo-controlled, crossover-design study. With ethical approval from the Auckland University of Technology research ethics committee, participants were fully informed about the study prior to providing consent. Participants were asked to maintain their usual diet but to abstain from caffeine and alcohol, 24 and 48 h prior to testing, respectively. Participants were requested to refrain from exercise the day of the test and limit exercise the day prior to no more than 2 h at moderate intensity. All experimental trials occurred at the same time of day ($\pm$ 2 h) to control for biological variation.

Design

Over a 5-wk period participants attended 15 separate sessions to perform a range of physiological and performance assessments on an electromagnetically braked cycle ergometer (Velotron, Racermate, Seattle, USA) in a temperature-controlled laboratory (19 °C, 60%rH). The cycle ergometer was fitted with the participant's own pedals with replication of seat and handle bar position from the participant's own bike to the ergometer. On the first visit participants anthropometric characteristics were measured along with VO_2peak and peak power output (PPO) from an incremental cycling assessment. Within the week participants completed two 4-km time-trial familiarisation sessions. Based on MPO from the second 4-km time-trial familiarisation, participants were then randomly assigned according to one of two 11-day experimental periods in which nitrate (NIT) or placebo (PLA) supplements were administered with several physiological and performance measures carried out prior to and following each treatment phase. A 7-day washout period followed the first treatment before participants undertook the second, alternate treatment.

Procedures and Assessments

Incremental Ramp Assessment: Participants carried out the incremental ramp assessment to determine ventilatory thresholds, VO_2peak, and PPO. The protocol started with 3 min at 50 W, followed by a 20 $W^.min^{-1}$ increase until volitional exhaustion on the cycle ergometer. Participants were asked to maintain a cadence of between 70 and 90 $rev^.min^{-1}$ throughout the test, and this pedal rate was recorded and repeated during subsequent assessments. The test was terminated when participants' cadence fell below 70 $rev^.min^{-1}$. During this, and subsequent economy trials, gas-exchange and ventilatory measures were assessed using a breath-by-breath metabolic system (Metamax 3B, Cortex Biophysik, Leipzig, Germany). Calibration of the system took place prior to each test using alpha

standard gases (BOC Gases, Auckland, NZ), while a turbine volume sensor was calibrated using a 3 L syringe (Hans Ruldolph, Shawnee, USA). VO_2peak was established from peak 30 s mean data using raw breath-by-breath measures for oxygen consumption. Ventilatory thresholds were established following the removal of artefacts from raw breath-by-breath data. First (VT_1) and second (VT_2) ventilatory thresholds were established using methods of Whipp et al[16] and Beaver et al[17], respectively.

Economy: Following the incremental assessment and within a 48 to 72 h period, participants completed two x 6-min constant-load square-wave bouts of exercise, separated by a 6-min bout of 'unloaded' cycling, at a prescribed power equivalent to 80% of each individual's VT_1 power during which VO_2 was recorded. The mean VO_2 during the last 60 s of each 6-min bout was averaged and used to reflect the underlying oxygen cost.

Performance Trials: Following the economy assessment and within a 48 to 72 h period, participants returned to the laboratory for the first of two 4-km familiarisation time-trials; the second following another period of 2 to 3 d. A standardised warm-up protocol was employed consisting of 5-min at 100 W, 5-min at 150 W, 3-min at the individual's heavy intensity domain (~90% of PPO group mean) and finally 5-min at 100 W. Participants were instructed to complete the 4-km distance in the shortest time possible. Subsequent trials were performed if the individual variance in performance time was >1% until this was achieved. The time-trial mode of the cycle ergometer allowed for the use of self-selected gearing and cadence to best reflect actual individual competition performance. During each trial, the lead researcher provided consistent encouragement to participants while feedback - including power output, gear selection and distance completed - was provided visually using the Velotron software. Duration, MPO, average heart-rate and cadence were recorded during all time-trials. The 4-km time-trial assessment has previously been used before in NO_3^- research.[7]

Beetroot Juice Supplementation

At the conclusion of the two pre-supplement testing periods, participants were provided with 8 x 70 ml supplements of either unlabelled NO_3^- rich beet-juice [NO_3^- ~4.0 mmol.L^{-1}]; (James White Drinks, Ipswich, UK) or a NO_3^- depleted [NO_3^- ~0.003 mmol.L^{-1}] placebo of identical taste, smell and appearance, supplied by the same manufacturer, consistent with previous research.[4] Participants were asked to consume the juice at a rate of 1 x 70 ml daily and, on the day of a trial, to ingest the juice 2-h before their scheduled lab appointment. The tester administering the beverage was blinded to the supplement condition. Participants were instructed to avoid spitting, chewing gum or using antibacterial mouthwash during the supplementation interventions, as these actions are associated with a lowering of plasma/serum NO_2^- levels.[18,19]

Habitual Training

Quantification of the cyclist's habitual training load (mean for duration, distance, and power) were determined during both experimental and washout phases using a calibrated wireless crank-based power meter (SRM, Julich, Germany), which was installed on each participant's bike.

Blood collection

Participants were asked to sit motionless in a reclined position for 15-min on their arrival at the laboratory. Thereafter, a venous blood sample from an antecubital vein was collected via an evacuated SST monovette tube (Becton Dickinson Biosciences). The 10 ml of extracted blood was then left to clot for 20-min before being centrifuged at 1218 x g for 10-min at 4°C (Heraeus Megafuge 16R, Thermo Scientific) as per the manufacturer's instructions. Serum was then aliquoted into micro containers and stored at -80°C for later analysis.

Serum NO_2^- Analysis

NO_2^- analysis was carried out using the Griess Method[20] with a commercially available kit (Promega, Wisconsin, USA). Following thawing samples were deproteinized by adding 400 µL trichloroacetic acid (TCA) to 400 µL serum, vortexed and centrifuged at 14,500 x rpm for 5-min (Espresso, Thermo Scientific) (Ghasemi et al 2007). Thereafter, 150 µL of supernatant was added to 130 µL of deionized water followed by 20 µL of Griess reagent, prior to an incubation period of 30-min, as per manufacturers instructions. Absorbance was measured at 548 nm. The resulting $[NO_2^-]$ levels were reported in µM, consistent with previous research using the Griess analysis method.[21]

Statistical Analysis

Data are presented as mean ± SD unless otherwise reported. Comparisons were made between each of the four NIT and PLA time-points for 4-km time-trial performance, economy and heart-rate using a customised analysis spread-sheet.[22] Performance data were log-transformed for analysis to reduce bias arising from non-uniformity of error, and subsequently back-transformed to obtain changes in mean and variation as percentages. To make inferences about true (population) values of the effect of NO_3^- supplementation on cycling performance and incremental PPO, the uncertainty in the effect was expressed as 95% confidence limits and as likelihoods that the true value of the effect represents substantial change (harm or benefit). We present these probabilities in quantitative values and qualitative terms in preference to a statistical inference based on a null hypothesis test. An effect was deemed *unclear* if the chance of benefit was sufficiently high to warrant use of the treatment but the associated risk was unacceptable. Such *unclear* effects were identified as those with an odds ratio of benefit-harm <57, a ratio corresponding to an effect that is borderline *possibly beneficial* (12.5% chance of benefit) and borderline *most unlikely harmful*

(0.25% chance of harm).[23] We assumed 1% and 0.4% as smallest worthwhile change for power and time, respectively for 4-km time-trial performance and 1% for incremental PPO.[24] The default values and qualitative terms were set at: <0.5%, *most unlikely*; 0.5-5%, *very unlikely*; 5% - 25%, *unlikely*; 25 to 75%, *possibly*; 75 − 95%, *likely*; 95 − 99.5%, *very likely*; >99.5%, *most likely*. Smallest worthwhile change for remaining (non-clinical) measures were calculated as 0.3 of the coefficient of variation of measurement error arising from their respective reliability trials in agreement with previous studies[25] with effect sizes reported as 0.2 of the between-subject SD.[23] Effect sizes (ES) were calculated using Cohens *d*, with an ES of <0.2 considered *trivial,* >0.2 *small,* >0.6 *moderate,* >1.2 *large* and >2.0 *very large*.

RESULTS

One participant withdrew due to illness during the study. Remaining participants' (n=8) self-reported adherence to supplementation was 100% for both treatment periods. Participants reported similar daily training loads across the two supplement phases of the study (mean ± SD) for duration (PLA: 1.4 ± 1.1 h.day^{-1}; NIT: 1.5 ± 0.9 h.day^{-1}) or intensity (222 ± 18 W; 202 ± 14 W). No significance was detected between trials (P<0.05) for either condition, for training time (0.34) or intensity (0.14). Measurement error (CV) for time-trial to time-trial was 0.7 and 2.1% for 4-km time and power, respectively and 1.7, 4.1, 3.5, 6.5 and 11.8% for incremental peak power, VO_2peak, economy, VT_1 and VT_2, respectively.

Pre- and post-intervention mean group outcomes are summarised in Table 1, whilst between group differences and their subsequent magnitude-based inference (MBI) are reported in Table 2. Relative to PLA, effects of 6 days of NIT supplementation were *unclear* for VO_2peak (mean ±95%CL: 1.8 ±5.5%), and VT_1 (3.7 ±12.3%), whilst VT_2 (-1.0 ±3.0%) was *trivial*. Effects for economy (-1.0 ±1.6%) were *trivial* after 7 days of NIT

supplementation. At day 8, an 88% chance of benefit lead to a *likely beneficial* decrease in 4-km time-trial time (-0.7 ±0.9%; Table 2) under NIT supplemented conditions.

Relative to PLA, there were small to moderate *unclear* increases in mean $[NO_2^-]$ in NIT following NO_3^- supplementation over days 3, 5 and 8 [(factor ×/÷95%CL) 1.19 ×/÷1.37, 1.35 ×/÷1.65 and 1.10 ×/÷1.15, respectively (Figure 1)].

DISCUSSION

The current study aimed to determine whether NO_3^- supplementation over a 6 to 8-day period would improve high-intensity cycling performance and economy in well-trained endurance cyclists. Employing identical statistical methods and terminology as previous investigations[7,10] the major outcomes of this study indicated that relative to PLA, consuming NO_3^- had a *likely beneficial* (-0.7 ±0.9%) effect on 4-km time-trial performance and *unclear* (3.7 ±12.3%) and *trivial* (-1.0 ±3.0 and -1.0 ±1.6%) effects for VT_1, VT_2 and economy respectively, in well-trained cyclists.

Whereas previous findings employing a MBI approach indicated a 98.9% or *very likely* chance of benefit for 4-km performance,[7] we report a smaller (75%) *likely beneficial* outcome, alongside chances of *triviality* and *harm* of 24% and 1%, respectively (Table 2). The smallest worthwhile enhancement for cyclists competing in road time-trials has been reported to be 0.6%,[24] therefore, our 0.7% improvement indicates a small but meaningful performance improvement. However, the magnitude of performance enhancement is substantially lower than the 2.7% performance increase reported by Lansley et al[7] over an identical distance. A potential explanation for such less pronounced effects may relate to differences in athletic ability.[7] Under placebo conditions, the 4-km performance time of 405 s reported by Lansley et al[7] relative to our current investigation of 345 s highlights the difference in athletic calibre of the respective cohorts. The only other cycling related study of

comparable duration reported *unclear* and *possibly harmful* outcomes for two x 4-min all-out trials in national level cyclists.[10] Collectively, the recent findings of Hoon et al[10] and the current study would indicate a reduced effectiveness of NO_3^- supplementation as cycling ability increases. Relative to lesser-trained populations, well-trained athletes appear to have significantly elevated resting levels of circulating $NO_3^{-[9]}$ which may partially explain the attenuated individual effects in highly trained populations.[10] As a result, it may be necessary to elevate resting NO_2^- levels via the provision of larger doses (>4 mmol) of NO_3^-, given that increased availability of NO_3^- will subsequently promote NO_2^- levels and therefore NO availability.[26] Indeed several studies have reported improvements in time to exhaustion in untrained[27] and time-trial performance in trained[28] populations following doses of ~8 but not 4 mmol NO_3^-. Moreover, the significant[27] and near significant relationship[28] between changes in performance and resting plasma $[NO_3^-/NO_2^-]$ following NO_3^- supplementation in the aforementioned studies highlights the influence of basal $[NO_3^-/NO_2^-]$ on performance. Whilst further work is required to elucidate the impact of higher doses of NO_3^- on performance in athletes of varying ability the present findings appear to be consistent with published outcomes in athletic outcomes utilising a 4 mmol dose of NO_3^- as they relate to athletic calibre.[7,10]

In contrast to gains in performance, we report minimal differences in several physiological and performance measures, many of which have been shown to underpin performance in endurance athletes.[6] Firstly, there were *unclear* (mean ±95%CL: 1.8 ±5.5%) improvements in VO_2peak following NO_3^- supplementation whereas previous studies have reported unaltered[9] or reduced[29] VO_2peak in endurance trained athletes. Secondly, there was a *trivial* (-1.0 ±3.0%) tendency for VT_2 to decrease, which is comparable to Bescos et al[2] who reported no change in VT_2, respiratory exchange ratio (NIT 0.96; PLA 0.96) or blood lactate (NIT 7.5; PLA 7.4 mmol) following sodium NO_3^- supplementation in a trained cycling

population.[2] Thirdly, similar trends were observed for measures of submaximal exercise economy (*trivial* and *unclear*) following NO_3^- supplementation. This was surprising since 3 days of NO_3^- supplementation has previously been shown to positively alter mitochondrial efficiency[14] by reducing the cost of ATP force production leading to improved economy.[3-5] In a similarly trained cycling population to that of our current study, Cermak et al[4] noted improvements of 3.5% and 5.1% in economy at 45% and 65% of PPO, respectively following 6 days of NO_3^- supplementation.[4] However, it should be noted that Cermak et al[4] employed a greater NO_3^- concentration (~8 mmol NO_3^-) compared to the current study. Other studies using identical dosages to that of Cermak et al[4] have also demonstrated improvements in economy for population's of low to moderate, but not high fitness levels,[9] again suggesting economy related effects of NO_3^- are constrained to sub-elite athletes. Fourthly, incremental peak power (or speed) is often used as a surrogate marker of endurance performance.[30] A change in PPO would predict that measures of performance requiring substantial aerobic contribution would also change. However, a *possibly harmful* (-1.8 ±2.8%) effect of NO_3^- on PPO opposed the *likely beneficial* effect on 4-km time-trial performance (Table 2). This divergence of effects is consistent with the findings of Porcelli et al[9] where peak run speed reduced, yet 3-km run performance improved in both low- and moderately-trained cohorts following NO_3^- ingestion.[9] Collectively, therefore, the findings of the current and aforementioned studies,[2,9] suggest that despite improved time-trial performance, NO_3^- supplementation has limited effect on several physiological or performance indices synonymous with endurance performance in well-trained athletes (>60 ml.kg^{-1}.min^{-1}).

To date the majority of NO_3^- related studies have analysed blood plasma using the chemiluminescence technique[8,27,28] with significant increases in $[NO_2^-]$ observed in sedentary[3] (96%) and moderately-trained[7] (138%) cohorts. However, like others[9] we employed the Griess technique to measure serum $[NO_2^-]$ and while an initial ~85% increase

in basal serum $[NO_2^-]$ was observed on day one of supplementation, we report small-to-moderate *unclear* effects of NO_3^- supplementation on serum $[NO_2^-]$ (Figure 1). In support, increases in $[NO_2^-]$ appear to be much lower[10] in well-trained cohorts potentially due to high resting levels of $[NO_3^-]$.[9] Furthermore, despite substantial increases in $[NO_2^-]$ in some studies following NO_3^- supplementation, performance effects have been inconsistent[7,10,15] suggesting that factors other than alterations in NO_2^- determine the ergogenic potential of NO_3^- supplementation. Further research is needed to better define the relationship of $[NO_2^-/NO_3^-]$ response relative to any performance changes in well-trained endurance athletes following NO_3^- supplementation.

PRACTICAL APPLICATIONS

- An 8-day period of beetroot juice (~4.0 mmol NO_3^-) supplementation is a beneficial ergogenic strategy for well-trained male cyclists competing within high-intensity short duration (5-6 min) time-trial events

- Athletes wishing to use NO_3^- supplementation should assess its effectiveness using time-trial simulations over the specific distance(s) of interest in order to gauge individual responses

CONCLUSIONS

The results of this current study add to the growing body of knowledge regarding the ergogenic effects of nitrate supplementation on high-intensity endurance performance. Relative to placebo, daily supplementation of 70 ml beetroot juice (~4.0 mmol NO_3^-) over an 8-day period had a *likely beneficial* enhancement on 4-km time-trial performance with *non-clear* outcomes for a range of physiological measures in well-trained competitive cyclists. Future studies should explore the ergogenic effects of NO_3^- on shorter duration trials given

performance improvements appear not to be reliant on enhancements in economy in well-trained cyclists.

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Author Contributions

Conception and design of the experiments: JAM, AEK, PBL.

Data collection and analysis: JAM, DKD.

Manuscript preparation and revision: JAM, AEK, DKD, PBL.

All authors have approved the final version of this manuscript.

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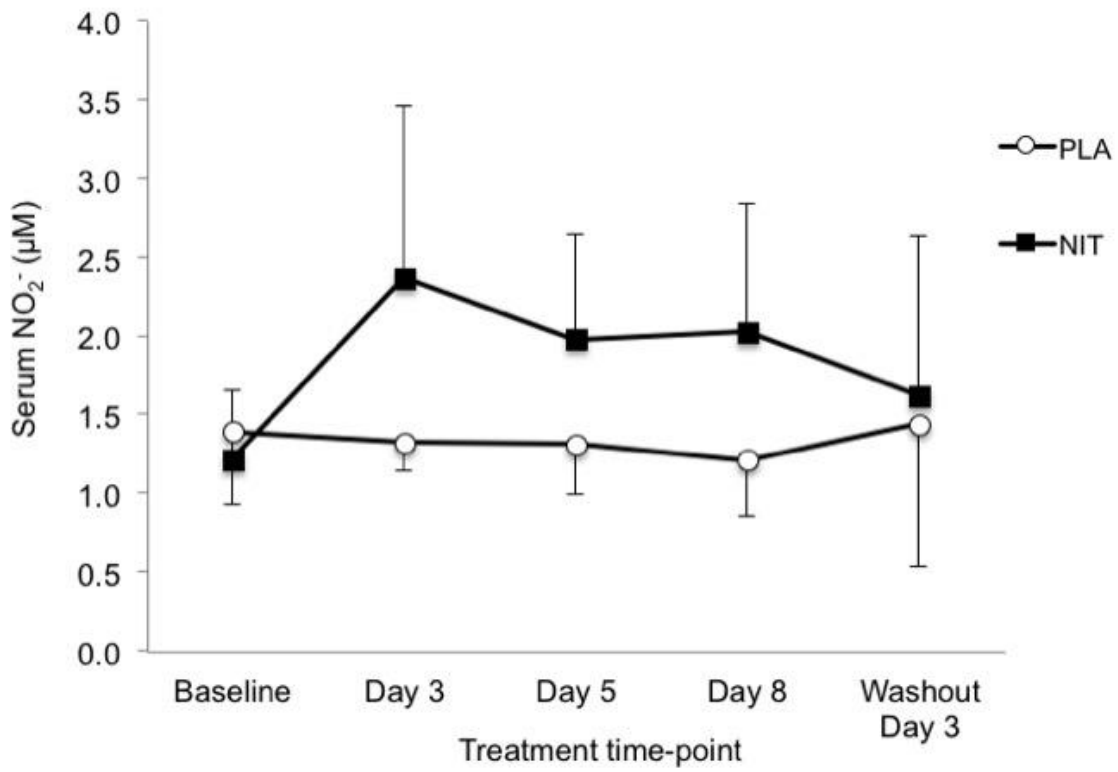
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Figure 1. Serum (NO_2^-) levels at baseline, during placebo (PLA; mean - SD), and nitrate (NIT; mean + SD) treatments.
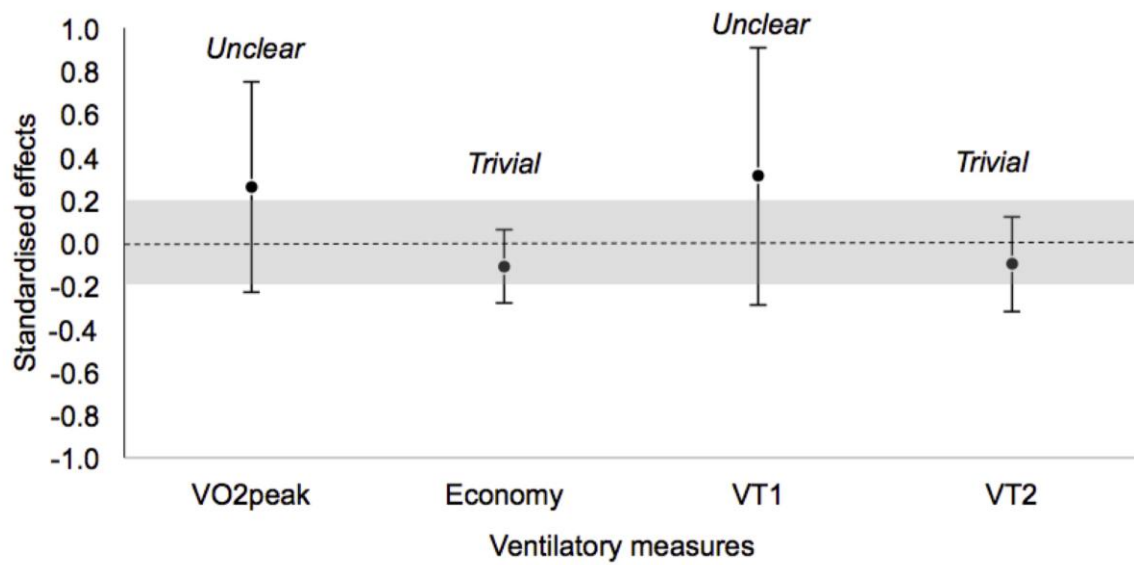
Figure 2. Standardised effects for ventilatory measures. Error bars indicate uncertainty in the true mean with 95% confidence intervals; if error bars overlap both the opposing positive and negative trivial (shaded area representing ± 0.2 SD) value, changes are deemed *unclear*.

Table 1. Pre- and post-measures for performance and physiological measures for placebo (PLA) and nitrate (NIT) treatments. Data are mean ± SD.

	Treatment outcomes			
	PLA Pre	**PLA Post**	**NIT Pre**	**NIT Post**
4-km TT time (s)	344.2 ± 11.3	344.8 ± 14.0	345.4 ± 13.2	343.6 ± 14.3
4-km TT MPO (W)	377 ± 32	375 ± 40	374 ± 40	380 ± 41
VO_2peak (L.min^{-1})	4.73 ± 0.29	4.64 ± 0.34	4.70 ± 0.32	4.70 ± 0.55
Incr. PPO (W)	422 ± 33	429 ± 31	423 ± 31	423 ± 31
Economy (L.min^{-1})	2.73 ± 0.26	2.75 ± 0.26	2.74 ± 0.25	2.73 ± 0.25
VT_1 (L.min^{-1})	3.82 ± 0.32	3.77 ± 0.34	3.60 ± 0.46	3.71 ± 0.46
VT_2 (L.min^{-1})	4.39 ± 0.43	4.41 ± 0.43	4.38 ± 0.43	4.36 ± 0.48
4-km TT HR (BPM)	170 ± 8	171 ± 9	172 ± 6	169 ± 8

TT = time-trial; MPO = mean power output; Incr. PPO = incremental peak power output; VT_1 = first ventilatory threshold; VT_2 = second ventilatory threshold; HR = heart rate.

Table 2. Participant change scores for performance and physiological measures for placebo (PLA) and nitrate (NIT) treatments. Data are mean ±95%CL.

	Between treatment outcomes				
	Mean scores Mean ± SD (%)		**Difference** ±95%CL	**% Chances** **+/trivial/-[a]**	**Practical inference[b]**
	PLA	**NIT**			
4-km TT time (s)	0.2 ± 1.2	-0.5 ± 1.2	-0.7; ±0.9	75/24/1	Likely beneficial
4-km TT MPO (W)	-0.7 ± 3.8	1.7 ± 2.8	2.4; ±2.5	88/11/1	Likely beneficial
VO_2peak (L.min^{-1})	-2.0 ± 4.8	-0.3 ± 6.8	1.8; ±5.5	58/31/11	Unclear
Incr. PPO (W)	1.7 ± 2.4	-0.1 ± 2.7	-1.8; ±2.8	3/25/72	Possibly harmful
Economy (L.min^{-1})	0.7 ± 1.2	-0.3 ± 1.8	-1.0; ±1.6	0.2/88/12	Trivial
VT_1 (L.min^{-1})	-1.4 ± 4.0	2.2 ± 14.3	3.7; ±12.3	62/23/15	Unclear
VT_2 (L.min^{-1})	0.5 ± 2.2	-0.5 ± 3.6	-1.0; ±3.0	2/77/21	Trivial
4-km TT HR (BPM)	1.3 ± 3.5	1.4 ± 1.9	2.6 ± 4.1	4/12/84	Unclear

Data represents comparison of the changes (difference in the means: ±95% confidence limits) between placebo (PLA) and nitrate (NIT). The practical inference is the qualitative assessment of the chances that the true effect is substantially positive (+ive) or negative (-ive)

[a]Percent chances of benefit/triviality/harm as a result of NIT supplementation to outcome

[b]Clinical inference based on odds ratio of benefit/harm >57 where smallest beneficial change of 1% for 4-km time, mean power and incremental peak power output

[b] Non-clinical inference based on 0.3% of CV for SEM for all other measures

%95CL; add and subtract this number to the difference to obtain the 95% confidence limits for the true difference

TT = time-trial; MPO = mean power output; Incr. PPO = incremental peak power output; VT_1 = first ventilatory threshold; VT_2 = second ventilatory threshold; HR = heart rate