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ABSTRACT

Purpose: The aim of this study was to determine the effect of NO_3^- consumption on measures of perception, thermoregulation and cycling performance in hot conditions. **Methods:** Using a randomised, double-blind, crossover-design, 8 well-trained cyclists (mean ± SD: age: 25 ± 8 y, $\dot{V}O_2$peak: 64 ± 5 $ml \cdot kg^{-1} \cdot min^{-1}$) performed 2 separate trials, in hot (35°C, 60% relative humidity) environments, having ingested either 140 ml NO_3^--rich beetroot juice ~8 mmol NO_3^- (NIT), or placebo (PLA), daily for 3-days with a 7-day washout period separating trials. Trials consisted of 2 x 10 min bouts at 40 and 60% peak power output (PPO) to determine physiological and perceptual responses in the heat, followed by a 4 km cycling time-trial. **Results:** Basal [nitrite] was substantially elevated in NIT (2.70 ± 0.98 µM) vs PLA (1.10 ± 0.61 µM) resulting in a *most likely* (ES = 1.58 ± 0.93) increase after 3-days. There was a *very likely trivial* increase in rectal temperature [T_{re}] in NIT at 40% (PLA;37.4 ± 0.2ºC vs NIT;37.5 ± 0.3ºC, 0.1 ± 0.2°C) and 60% (PLA;37.8 ± 0.2ºC vs NIT;37.9 ± 0.3ºC, 0.1 ± 0.2°C) PPO. Cycling performance was similar between trials (PLA;336 ± 45 W vs NIT;337 ± 50 W, CV±95%CL; 0.2 ± 2.5%). Outcomes for heart rate, and perceptual measures were *unclear* across the majority of time-points. **Conclusions:** Three days of NO_3^- supplementation, resulted in small increases in T_{re} during low- to moderate-intensity exercise, however this did not appear to influence 4 km cycling time-trial performance in hot climates.

Key Words: Nutrition, time-trial, heat-stress, competitive, beetroot juice

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INTRODUCTION

Athletes commonly use dietary supplements in an effort to enhance athletic performance. Recently, consumption of NO_3^- by way of either nitrate salt ($NaNO_3^-$) or beetroot juice has been found to improve both economy and performance[1] potentially due to improved efficiency of mitochondrial coupling[2] and enhanced phosphorylation ratio.[3] Furthermore, NO_3^- has conferred muscular related enhancements in calcium handling, blood flow, and microvascular pressure in type II, but not type I skeletal muscle.[4] However, while performance enhancement has been reported across a range of sports/events and durations/intensity, studies to date have predominantly been conducted in temperate (18-21°C, 20-50% relative humidity (RH)) conditions[1,5,6] with little consideration of how additional heat stress and NO_3^- might interact and influence physiological responses and performance in athletic populations.

Recently, the effects of NO_3^- supplementation in hot environments using either beetroot juice,[7] or L-arginine[8] has been assessed in healthy, non-athletic populations. Specifically, while consumption of beetroot juice over a 6-day period reduced oxygen consumption, an increase in core body temperature in the final third of a 45 min march in a hot (41ºC, 20% RH) environment was observed.[7] The authors projected that NO_3^- would cause participants to reach critical core body temperature (40ºC) earlier, and subsequently reduce distance marched by 10% due to hyperthermic stress.[7] Conversely, Tyler et al[8] reported that an acute dose of L-arginine, which is the precursor of nitric oxide synthase derived production of nitric oxide (NO), had no effect on oxygen consumption or rectal temperature (T_{re}) during moderate intensity exercise in 35ºC, 40% RH environments. Given athletes are often exposed to hot environments during competition, further studies are required to ascertain whether the previously reported favourable effects of NO_3^- consumption are maintained in hot conditions. Therefore, the aims of this study involving well-trained cyclists were to determine 1) the effects of 3-days of NO_3^- supplementation on thermoregulatory and perceptual responses during low-

, moderate- and, maximal-intensity exercise in the heat and 2) the effects of NO_3^- on maximal-intensity exercise performance in the heat.

METHODS

Participants

Eight well-trained, competitive male endurance cyclists (mean ± SD: age: 25 ± 8 y, body mass: 74.9 ± 7.3 kg, height: 180 ± 6 cm, $\dot{V}O_2$peak: 64 ± 5 $ml \cdot kg^{-1} \cdot min^{-1}$) provided written consent to participate in this study which had received ethical approval from the Auckland University of Technology Ethics Committee (10/309). All participants were free of injury and illness for at least six weeks prior to study commencement, and were carrying out regular weekly training sessions amounting to ≥200 $km \cdot wk^{-1}$. Participants were asked to maintain their usual training and racing schedule, but on the day prior to any laboratory assessments, complete no more than a 2 hr low intensity session. Verbal confirmation of this fact was given to the lead investigator the day of the testing and participants received a schedule of dates and times which included instructions for management of training load in the 24 hr prior to assessments. Data collection took place during the competitive phase (November through to January) of each athlete's periodised season, coinciding with the final month of spring and first two months of summer. Participants were fully informed about the possible risks of all experimental procedures prior to informed consent being obtained. Participants were asked to maintain their habitual diet, and record and repeat their dietary intake in the 24 hr leading into the initial experimental trial, with the exception of abstinence from caffeine and alcohol in the 24 and 48 hr prior to testing, respectively. All experimental trials took place in a sea-level altitude laboratory and occurred at the same time of day (± 2 hr) to control for biological variation.

Preliminary Testing – Procedures and Assessments

To ensure the minimum peak oxygen uptake ($\dot{V}O_2$peak >55 $ml \cdot kg^{-1} \cdot min^{-1}$) for study inclusion was attained, prospective participants were required to undertake an incremental ramp assessment on an electromagnetically-braked cycle ergometer (Velotron, Racermate, Seattle, USA), fitted with the participant's own pedals, 10- to 14-days before the main trials began. Measurements of seat and handle bar position were recorded from the participant's own bike and replicated on the ergometer prior to all subsequent assessments. The protocol consisted of 3 min at 50 W, followed by a 20 $W \cdot min^{-1}$ increase until volitional exhaustion or when participants' cadence fell below 70 $rev \cdot min^{-1}$. Gas-exchange and ventilatory measures were assessed using a mixing chamber metabolic system (TrueOne 2400, Parvo Medics, Sandy, UT). Calibration of the gas and turbine sensors took place prior to each test using alpha standard gases (BOC Gases, Auckland, NZ) and known volumes (3 L syringe, Hans Ruldolph, Shawnee, USA) respectively. Peak oxygen uptake was defined as the highest 30 s mean $\dot{V}O_2$ value achieved during the test. Incremental peak power output (PPO) was also determined.

Over the following 7-days participants returned to the lab for familiarisation purposes to complete two 4 km laboratory-based time-trials in a temperate (20°C, 60% RH) environment, located at sea level. Participants were instructed to complete the 4 km distance in the shortest time possible. The time-trial mode used by the Velotron cycle ergometer allowed for the use of self-selected gearing and cadence to best reflect actual individual competition performance. Duration, mean power output (MPO), and average heart rate were recorded during each time-trial in the familiarisation phases.

Experimental Protocol

Participants were randomly assigned to one of two 3-day experimental nitrate (NIT) or placebo (PLA) groups and acted as their own controls in this double-blind, crossover-design study. After each 3-day trial, participants completed a performance trial in hot conditions,

preceded by two sub-maximal exercise bouts. The two 3-day experimental periods were separated by a 7-day washout period. On day 3 of each treatment, participants completed a performance trial in hot conditions, preceded by two x 10 min sub-maximal exercise bouts. Participants were supplied with 3 x 140 ml doses of either NO_3^--rich beetroot juice [~8.0 mmol] or a NO_3^- free placebo of identical taste, smell and appearance, as previously used.[1] The same manufacturer (James White Drinks, Ipswich, UK) produces an identical looking and tasting NO_3^- depleted [~0.003 mmol] placebo juice, and we adopted the supplement and administration procedures of a recent study[1] using organic beetroot juice. Over the course of the study, participants were administered the juice at a rate of 2 x 70 ml daily and asked to consume the juice 90 min prior to their scheduled arrival at the laboratory for trials, whereas on non-trial days, participants were asked to consume it as close to the time on trial days. The tester administering the beverage was unaware of whether the drink was NO_3^--rich or placebo beetroot juice. 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 [NO_2^-].[9]

Blood collection

On arrival at the laboratory, participants were asked to sit motionless in a reclined position for 15 min. 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.

Experimental Exercise Trials, Blood Pressure and Plasma NO_2^-

To begin all trials, and following blood collection procedures and voiding of bladder, participants nude body mass was measured (Seca Ltd, Germany) followed by self-insertion of a disposable rectal thermistor (Monatherm Thermistor, 400 Series, Mallinckrodt Medical, St. Louis, MO) approximately 12 cm past the anal sphincter. Rectal temperature, measured to 0.01ºC, was continuously recorded with a data logger (Squirrel SQ2020, Cambridge, UK) connected to the rectal probe. Afterwards, participants were equipped with a heart rate monitor, which recorded every 5 s, and entered an environmental chamber (Design Environmental Ltd., Gwent, United Kingdom), set to 35ºC and 60% RH, and equal to a Wet Bulb Globe Temperature (WBGT) of 37ºC. To begin each trial, participants were seated on a chair in the heat for a 10 min period in order to achieve core-temperature stabilisation.[10] Following this preliminary phase, participants performed 20 min of cycling at 40% and 60% PPO. Forward facing convective wind movement was simulated with 2 industrial fans (FS-75, FWL, Auckland, New Zealand), generating a wind speed of ~33 $km.h^{-1}$ (3000 Wind Meter, Kestrel, Sylvan Lake, MI); an appropriate wind speed for reducing heat storage and comparable to outdoor cycling as used in previous trials.[10] Thereafter, participants remained in the chamber and were given 5 min of seated rest before they commenced the 4 km time-trial. During this time the Velotron was calibrated and set into time-trial mode. Participants were asked to perform a maximal effort and to complete the trial as quickly as possible. During each trial, the lead researcher provided consistent encouragement to participants while elapsed time, power output, gear selection and distance completed, was provided visually using the Velotron software. Participants were asked to rate their perceived exertion[11] (6 = no exertion at all, to 20 = maximal exertion), modified thermal comfort (1 = comfortable, to 10 = extremely uncomfortable) and modified thermal sensation (1 = unbearably cold, to 13 = unbearably hot),[12] and feeling[13] (5 = very good, to -5 = very bad). These occurred at 4 (ambient conditions,

following 10 min T_{re} stabilisation, prior to, and post time-trial) time-points. Participants then exited the chamber, towelled dry before re-weighing nude body mass with subsequent data used to establish effect of NO_3^- supplementation on sweat rate. Heart rate was measured using a heart rate monitor (Garmin 410, Garmin Ltd., USA). Body mass alterations was calculated as: body mass alterations = Δbody mass + fluid ingestion. During the first trial, participants were provided water ad libitum, and the timing and amount of water consumed was recorded and replicated in the following trial.

Serum NO_2^- Analysis

NO_2^- analysis was carried out using the Griess Method[14] with a commercially available kit (Promega, Wisconsin, USA). 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).[15] 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 manufacturer's instructions. Absorbance was measured at 548 nm. The resulting [NO_2^-] were reported in µM, consistent with previous research using the Griess analysis method.[16]

Statistical Analysis

All data were reported as means ± standard deviations (SD), and mean (95% confidence limits; CL) as appropriate. The differences in performance, physiological and subjective measurements were analysed using a magnitude-based inference approach[17] and were determined using published spreadsheets (xParrallelGroupsTrial.xls) from sportsci.org.[17] Time-trial, heart rate and body mass data were log-transformed prior to statistical analysis. Uncertainties for time-trial performance effects were expressed as probabilities of harm or benefit in relation to the coefficient of variation (±1.0%). This method is used to indicate the possible benefit or harm of each condition. To make inferences for performance measures an

estimate of the smallest worthwhile change (SWC) in power output is required. This is based on published literature on between competition performance variability of top cyclists.[18] Cohen effect sizes (ES) were used to express the magnitude of differences in the changes between trials for heart rate and body mass and were reported as standardised differences. The criteria used for interpreting the magnitude of the ES for these variables were: <0.2, trivial; 0.2-0.5, small; 0.5-0.8, moderate; and >0.8, large.[17] A novel approach for magnitude thresholds was used to determine the SWC for T_{re} and perceptual measures in which the possible range of change was transformed into a full scale of deflection.[19] Briefly, each range was made from 0-100% and magnitude thresholds were defined as 10%, 30%, 50%, 70% and 90% for small, moderate, large, very large and extremely large changes. Quantitative chances of NO_3^- affecting measurement outcomes were assessed qualitatively as follows: <1%, *most unlikely*; 1-5%, *very unlikely*; 5-25%, *unlikely*; 25-75%, *possible*; 75-95%, *likely*; 95-99%, *very likely*; >99% *most likely*.[17] When an effect was >5% for both benefit and harm, the true value of the difference was described as unclear.

RESULTS

Self-reported adherence to supplementation was 100% for both treatment periods for the 8 participants, with four of the cohort consuming NIT in the first treatment round. One participant elected not to have their blood testing performed, therefore serum [NO_2^-] analysis was conducted with n=7. All participants experienced beeturia during the supplementation phases, however no other side effects were reported.

Relative to PLA, there was a *most likely* greater increase (factor ×/÷95%CL: 2.8 ×/÷1.8) in mean [NO_2^-] following 3-days NO_3^- supplementation (Figure 1).

Sweat loss (via Δbody mass) for both PLA (0.66 $L \cdot hr^{-1}$; mean ± SD%; ES ± 95%CL; -0.6 ± 1.1%; 0.04 ± 0.06) and NIT (0.72 $L \cdot hr^{-1}$; -0.8 ± 0.3%; -0.06 ± 0.02) were both *most likely*

trivial, whilst the differences between the two outcomes (-0.2 ± 0.6 kg; -0.23 ± 0.87) were *unclear*.

Measurement outcomes for group mean T_{re} (±95%CL) are shown in Figure 2. Relative to PLA, the NIT trial revealed small changes (range 0.09 to 0.12ºC) in T_{re}, equating to a *very likely trivial* increase (PLA;37.8 ± 0.2ºC vs NIT;37.9 ± 0.3ºC, 0.1 ± 0.2°C) at end 20 min priming, and prior to the 4 km time-trial (PLA;37.9 ± 0.2ºC vs NIT;38.0 ± 0.2ºC, 0.1 ± 0.2°C), however effects for T_{re} were *unclear* (PLA;38.2 ± 0.4ºC vs NIT;38.2 ± 0.2ºC, 0.0 ± 0.3°C) following time-trial performance.

Mean outcomes and standardised effects for comfort, sensation, feeling, and RPE are shown in Figures 3 and 4 respectively. Three days of NO_3^- supplementation had *trivial* or *unclear* effects on the majority of subjective measures of perception (Figure 4). Relative to PLA, a *small possible* increase (ES=0.27 ± 0.42) was reported for NIT in ambient conditions for thermal comfort, whilst a *small likely* decrease, and *small possible* decrease were reported for RPE at end priming (ES=-0.45 ± 0.46), and feeling prior to time-trial (ES=-0.20 ± 0.20), respectively.

Following NIT supplementation, effects for heart rate resulted in a *likely trivial* increase (PLA;69 ± 12 bpm vs NIT;71 ± 12 bpm, ES=0.10 ± 0.12) in ambient conditions, a *likely trivial* decrease (PLA;158 ± 9 bpm vs NIT;157 ± 10 bpm, ES=-0.10±0.19) at the conclusion of priming, and a *likely trivial* increase (PLA;99 ± 10 bpm vs NIT;102 ± 11 bpm, ES=0.26 ± 0.17) prior to time-trial. Post time-trial effects were *unclear* (PLA;186 ± 10 bpm vs NIT;186 ± 12 bpm, ES=-0.06 ± 0.27) following NIT.

Mean power output for the 4 km time-trial is shown in Figure 5. In relation to PLA, NIT showed *unclear* effects for both 4 km performance time (mean ±95%CL: -0.1 ±0.9%) and MPO (0.2 ±2.5%).

Discussion

To our knowledge, this is the first study to report on the effects of NO_3^--rich beetroot juice on thermoregulatory and perceptual responses in cyclists in hot environments and extends the current body of literature regarding the supplementation of NO_3^- supplementation in well-trained endurance athletes. The main findings of this study indicated that although a 3-day NO_3^- supplement period increased T_{re} at various time-points during low- to moderate-intensity exercise in the heat, subsequent time-trial performance in well-trained cyclists was unchanged.

Whilst NO_3^- supplementation has previously been shown to increase T_{re} and reduce estimated exercise tolerance,[7] thus suggesting a potentially negative effect of dietary NO_3^- on walking intensity exercise in hot environments, our results indicate no harm or benefit effects following NO_3^- supplementation in the heat (Figure 5), despite small increases in T_{re} (Figure 2). It is acknowledged that increases in core[20] and muscle[21,22] temperature may serve to improve,[21] or reduce[20,23] performance depending on the magnitude of the temperature rise, exercise type/duration and invoked physiological mechanistic responses.[20,23,24] For instance, relative to cooler (23ºC, 60% RH) environments, Tatterson et al[23] demonstrated increased blood lactate and pH reduction in exercising muscle of elite cyclists during the first third of a 30 min time-trial performance in hot (32ºC, 60% RH) environments.[23] While an increase in muscle acidosis is negatively associated with exercise related fatigue in high-intensity events,[22] acidic conditions appear to enhance the bioavailability and bioactivity of NO[25] and therefore NO_3^- supplementation may serve to maintain or enhance high-intensity performance trials in hot conditions. Conversely, based on the findings of Kuennen et al[7], despite the initial positive effects on walking economy, there is potential for negative outcomes in longer term exercise following NO_3^- supplementation. These initial physiological related improvements, may have resulted due to the larger influence that NO_3^- appears to impart on recreational level cohorts,

relative to those of higher training status.[6] Similarly, the sensitivity to NO_3^- may also, in part, explain the subsequent negative effects witnessed in lesser-trained populations.[7]

We observed *most likely trivial* (range 0.09 to 0.12ºC) increases in T_{re} during the first 20 min of exercise in hot environments as a result of NO_3^- supplementation (Figure 2). Our reported increase is slightly less than that of Kuennen et al[7] who reported a significant (0.14ºC) increase ($p<0.05$) in T_{re} at the conclusion of a longer (45 min), though lower, fixed intensity exercise protocol after consuming NO_3^-. In hyperthermic inducing environments, blood flow is distributed to the periphery of the body in an attempt to maintain thermal equilibrium through evaporative cooling.[26] Under exercising conditions temperature gradient increases of as little as 9ºC has been shown to elevate skin temperature by as much as 22%.[23] As such, blood flow to the skin heavily influences the rate at which the body is able to dissipate stored heat,[27] therefore any diversion of this sympathetic mediated response may have negative consequences on homeostasis. The mechanistic basis for an increase in T_{re} following NO_3^- supplementation appears to be consequent to the step-wise reduction of NO_2^- to NO enabling whole body vasodilation,[25] muscle-fibre specific enhancements,[4] and the over-riding of the standard homeostatic response following exposure to hot environments.[26] Interestingly, our small rise in T_{re} under NO_3^- conditions may have been partially due to the fixed exercise workload, given that previous research has shown participants choose to reduce work output in order to manage increasing core temperatures during longer and more intense exercise than our 20 min sub-maximal exercise protocol.[23]

Whilst the majority of perceptual measures were *unclear* or *trivial*, we reported several negative mean qualitative outcomes following NO_3^- consumption in ambient conditions for thermal comfort, and for feeling, prior to the time-trial (Figures 3 and 4), although exercise exertion (RPE) following end priming equated to a positive outcome under NO_3^- supplemented conditions. In relation to our findings, Kuennen et al[7] reported increased RPE, general

discomfort and thermal sensation from 10 to 45, 25 to 45, and 40 to 45 min respectively, as a result of NO_3^- under hyperthermic conditions. Perceptually, trained populations cope with the effects of exercise in hyperthermic environments to a better extent than less-trained[24] and as such athletes in the present study are likely to have managed the perceptual effects of heat stress better in hot environments as a result of their aerobic (64 $ml \cdot kg^{-1} \cdot min^{-1}$) capabilities, relative to lesser-trained populations.[7] Additionally, it is also important to note that the current study took place in the summer months, whereas Kuennen et al[7] assessed participants during winter months, which may have further predisposed their participants to the negative effects of a hot environment. Clearly further research is required to better understand the balance between desirable increases in core and muscle temperature for optimal performance and athletes' perceived thermal load following NO_3^- supplementation in the heat, especially during trials of varying duration.

Exercise in hot conditions led to a *most likely trivial* reduction in body mass via sweat rate for both NIT (0.72 $L \cdot hr^{-1}$) and PLA (0.66 $L \cdot hr^{-1}$) treatments. The between treatments comparisons were *unclear* suggesting that the consumption of NO_3^- did not lead to elevated sweat rate despite higher T_{re} under NIT conditions. Whilst both we, and Kuennen et al[7] have shown no clear difference in sweat response between NIT and PLA treatments within 45 min total heat exposure, it would be pertinent to explore how maximal intensity exercise over longer durations, e.g. Olympic distance cycling time-trial events in similarly challenging conditions,[28] might influence sweat response, with regards to performance, particularly as sweat-rates of higher-trained athletes appear to be elevated relative to lesser-trained populations.[24]

Finally, it is acknowledged that there are some limitations to the present study that should be considered. Firstly, we did not compare the effectiveness of NO_3^- and placebo in temperate conditions and therefore we are unable to state to what extent heat exposure may have influenced performance. However, previous work in this area suggests that,

in ambient conditions, well-trained male cyclists (63 $ml.kg^{-1}.min^{-1}$) demonstrated a *likely beneficial* improvement in 4 km time-trial MPO (2.4 ±2.5%) following 8 days of NO_3^-.[32] Based on these outcomes, the current findings indicate that either the addition of heat stress impairs the ability of NO_3^- to improve short-term, high-intensity performance, or, that athletes are recommended to supplement for a longer period of time prior to competition in hot environments. Secondly, while the possibility of type II error may exist due to a smaller cohort, the sample size in the present study was consistent with previous studies reporting performance enhancement[29,30] and impairment[31] following NO_3^- supplementation. Furthermore, the reliability of our primary outcome measure (4 km time-trial time) in our laboratory is high (0.7 and 1.2%),[31,32] and participants were well-trained competitive cyclists, who train and race frequently and were familiar with cycle ergometer based exercise. Taken together, the study design, and participant characteristics employed help reduce the chance of type II error. Clearly, given our findings, further research is required to better inform practitioners of the risk to benefit ratio of prescribing NO_3^- supplementation prior to exercise and/or competition in hot environments.

PRACTICAL APPLICATIONS

- Three days of NO_3^- supplementation elevated core body temperature, but not perceptual responses, during fixed intensity sub-maximal exercise in the heat. Practitioners therefore should be aware of the small additional thermal load following NO_3^- consumption, particularly during longer duration exercise bouts.
- In well-trained cyclists, NO_3^- supplementation over a 3-day period has no effect on short-term, high-intensity time-trials performed in hot conditions. However, practitioners are encouraged to investigate the performance effects of NO_3^- supplementation during longer durations, as well as greater dose durations, as these may influence the ergogenic potential of NO_3^- in the heat.

CONCLUSIONS

This is the first investigation to report on the thermoregulatory, perceptual and ergogenic effects of NO_3^- supplementation on maximal-intensity cycling performance in a hot environment in well-trained athletes. Relative to placebo, daily NO_3^-supplementation of 140 ml beetroot juice [~8.0 mmol NO_3^-] over a 3-day period produced small increases in T_{re} during sub-maximal exercise in the heat. However, subsequent 4 km time-trial performance, cardiovascular, and perceptual responses were unaltered following NO_3^- supplementation. Given the tendency for NO_3^- supplementation to elevate T_{re}, future studies should explore the effects of NO_3^- on endurance events of longer duration in the heat.

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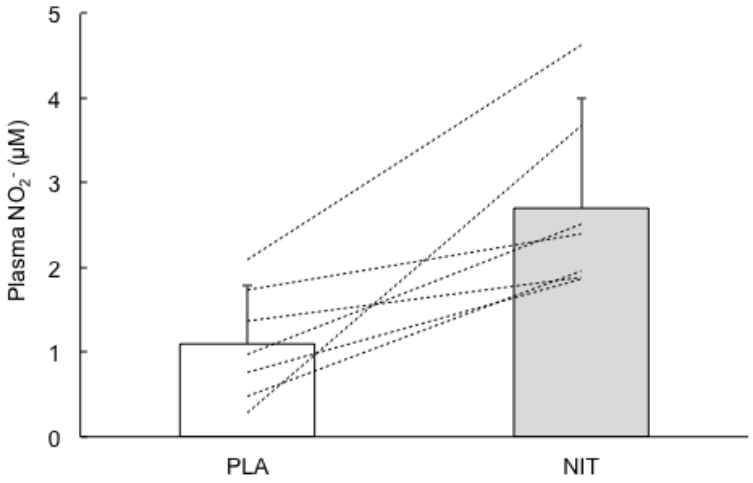

Figure 1. Serum [NO_2^-] levels at baseline, during placebo (PLA; mean + SD; white column), and nitrate (NIT; mean + SD; shaded column) treatments.

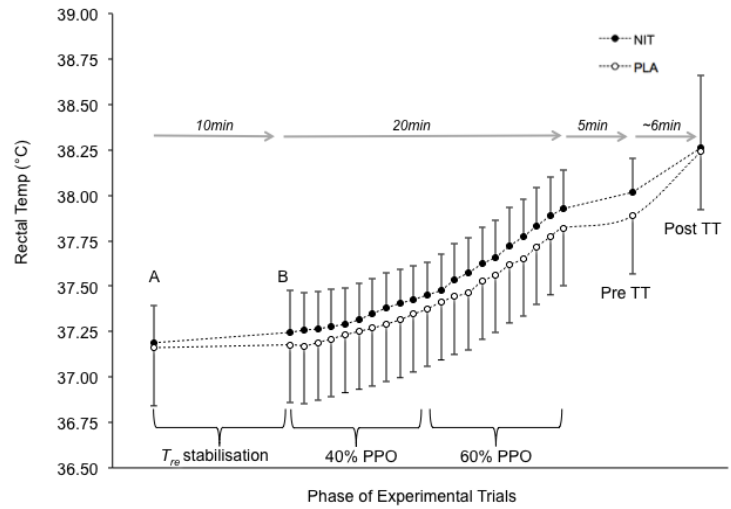

Figure 2. Mean group response during nitrate (NIT; closed circles) + SD, and placebo (PLA; open circles) –SD conditions, for rectal temperature (T_{re}) for A (ambient), B (end 10 min stabilisation), 20 min priming (40 and 60% PPO), prior to, and following 4 km time-trial performance.

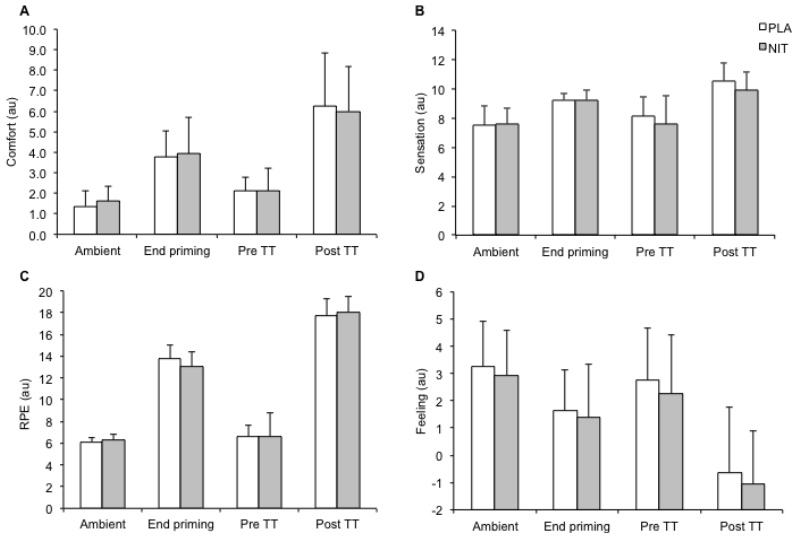

Figure 3. Mean group response (+SD) for perceptual measures of A) Comfort; 1 = comfortable to 10 = extremely uncomfortable, B) Sensation; 1 = unbearably cold, to 13 = unbearably hot, C) RPE (rate of perceived exertion); 6 = no exertion at all, to 20 = maximal exertion, and, D) Feeling; 5 = very good, to -5 = very bad for ambient, end priming, pre time-trial and post time-trial reference points following nitrate (NIT; shaded columns) and placebo (PLA; white columns) treatments. AU = arbitrary units, TT = time-trial.

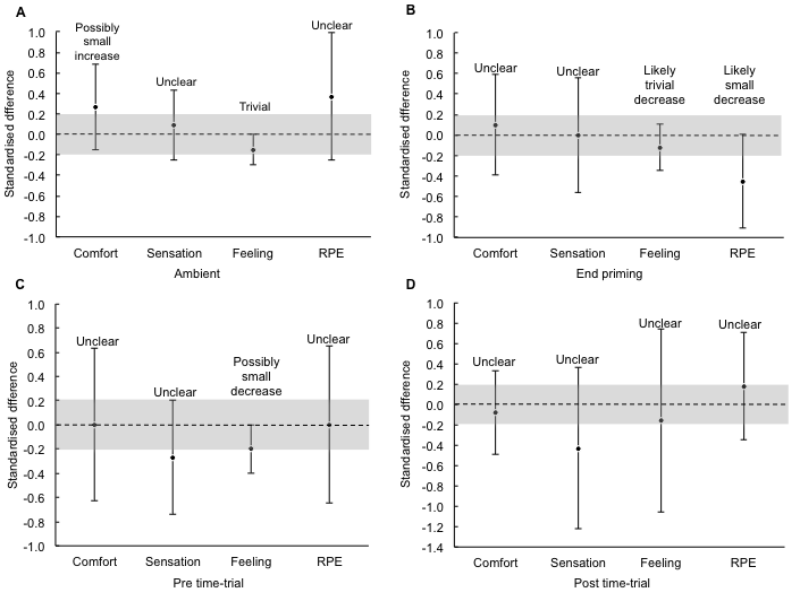

Figure 4. Standardised difference in comfort, sensation, feeling and rate of perceived exertion (RPE) in ambient (A), end-priming (B), pre time-trial (C), and post time-trial (D). Error bars indicate uncertainty in the true mean value with 95% confidence interval; if error bars overlap both the opposing increased and decreased *trivial* (shaded area representing ± 0.2 SD) values, changes are deemed *unclear* (see Methods section). Effects above the black dotted line indicate an increase in measure as a result of nitrate (NIT) vs. placebo (PLA), whereas effects below the line indicate decrease, as a result of NIT. Magnitude and quantitative chances of change are described qualitatively in the text above their respective error bars.

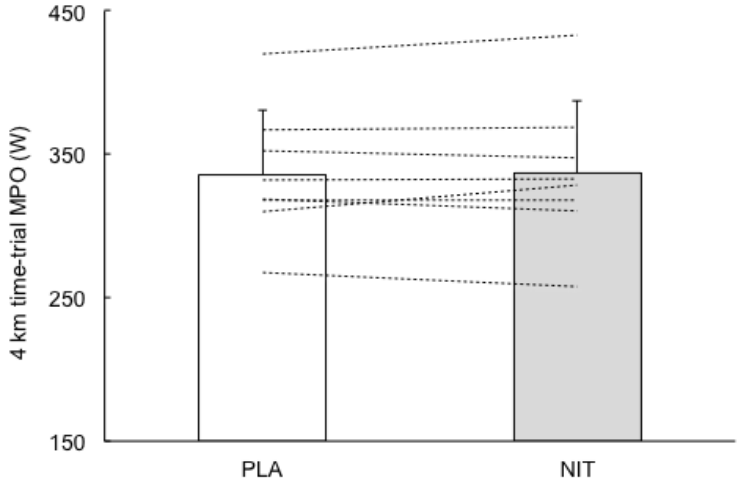

Figure 5. Mean group response for 4 km time-trial mean power output (± SD) and individual responses (dashed lines) for nitrate (NIT; shaded) and placebo (PLA; white) conditions.