Effects of Nitrate on the Power-Duration Relationship for Severe-Intensity Exercise

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Running Head: Nitrate supplementation and critical power

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Abstract

Purpose: The power asymptote (critical power, CP) and curvature constant (W’) of the power-duration relationship dictate the tolerance to severe-intensity exercise. We tested the hypothesis that dietary nitrate supplementation would increase the CP and/or W’ during cycling exercise.

Methods: In a double-blind, randomized, crossover study, nine recreationally-active male subjects supplemented their diet with either nitrate-rich concentrated beetroot juice (BR; 2 x 250 ml/day, ~8.2 mmol/day nitrate) or a nitrate-depleted beetroot juice placebo (PL; 2 x 250 ml/day, ~0.006 mmol/day nitrate). In each condition, the subjects completed four separate severe-intensity exercise bouts to exhaustion at 60% of the difference between the gas exchange threshold and peak power attained during incremental exercise (60%Δ), 70%Δ, 80%Δ and 100% peak power, and the results were used to establish CP and W’. Results: Nitrate supplementation improved exercise tolerance during exercise at 60%Δ (BR: 696 ± 120 vs. 593 ± 68 s, P<0.05), 70%Δ (BR: 452 ± 106 vs. PL: 390 ± 86 s, P<0.05), and 80%Δ (BR: 294 ± 50 vs. PL: 263 ± 50 s, P<0.05) but not 100% peak power (BR: 182 ± 37 vs. PL: 166 ± 26 s, P=0.10). Neither CP (BR: 221 ± 27 vs. PL: 218 ± 26 W) nor W’ (BR: 19.3 ± 4.6 vs. PL: 17.8 ± 3 kJ) were significantly altered by BR. Conclusion: Dietary nitrate supplementation improved endurance during severe-intensity exercise in recreationally-active subjects without significantly increasing either the CP or W’.

Key Words: maximal exercise; endurance; beetroot juice; critical power
**Introduction**

The critical power (CP) and the W’ are the two parameters which characterize the hyperbolic power-duration relationship that is evident during high-intensity exercise (30, 33). The CP is the power-asymptote of the relationship and demarcates the boundary between ‘heavy’ intensity exercise, within which a physiological steady state is attained, and ‘severe’ intensity exercise, which by definition does not permit steady-state behaviour (20). Thus, the CP theoretically represents the highest power output that can be maintained via predominantly aerobic metabolism, where pulmonary oxygen uptake ($\dot{V}o_2$), blood lactate and concentrations of intramuscular metabolites such as phosphocreatine ([PCr]), [H+] and inorganic phosphate ([P_i]) can be stabilized (21, 33). The W’ represents the curvature constant of the relationship and can be considered as the finite work capacity available above the CP before the limit of tolerance ($T_{lim}$) is reached (30, 33). The physiological determinants of the W’ are debated (12, 29, 38).

The hyperbolic power-duration relationship is given by:

$$T_{lim} = \frac{W'}{P - CP}$$  \hspace{1cm} (Eqn. 1)

where P is a given severe-intensity power output (17, 20, 40). The linear transformations of this relationship are the power-1/time equation:

$$P = \frac{W'}{T_{lim}} + CP$$  \hspace{1cm} (Eqn. 2)

and the work-time equation, where P is replaced with work done (W) per unit time:

$$W = CP \cdot T_{lim} + W'$$  \hspace{1cm} (Eqn. 3)
It is evident that when the CP and W' are known, performance time within the severe domain (indicated by $T_{lim}$) can be accurately predicted by rearranging Eqn. 3 (17, 20, 40):

$$T_{lim} = (W-W')/CP$$  
(Eqn. 4)

The CP and W' are important determinants of sport and exercise performance (20, 40).

Importantly, Eqn. 1-4 indicate that performance in the severe domain is a function of both the CP and the W', which act in concert to determine the shortest possible time required to complete a given target total work done.

There is a growing body of evidence to suggest that supplementing an individual’s diet with inorganic nitrate (NO$_3^-$) can have beneficial effects on cardiovascular health and exercise performance. The NO$_3^-$ anion itself is inert and its in vivo conversion to bioactive nitrite (NO$_2^-$) and nitric oxide (NO) is likely responsible for the biological effects observed. Upon ingestion, up to 25% of the inorganic NO$_3^-$ enters the enterosalivary circulation and is concentrated in the saliva (28). Facultative anaerobic bacteria in the oral cavity then reduce the NO$_3^-$ to NO$_2^-$ (10). When swallowed into the acidic environment of the stomach, some of the NO$_2^-$ is further converted into NO, whilst the remainder is absorbed to increase circulating plasma NO$_2^-$ concentration [NO$_2^-$]. This NO$_2^-$ may be reduced further to NO, particularly in tissues which may be relatively hypoxic, such as contracting skeletal muscle (34). NO is a physiological signaling molecule with various functions in the body including the regulation of vascular tone, blood flow, muscle contractility and mitochondrial respiration (8, 15, 35).

It is now widely accepted that dietary nitrate supplementation via nitrate salts or nitrate-rich beetroot juice (BR) can significantly reduce resting blood pressure in young, normotensive adults (24, 41). Moreover, dietary nitrate supplementation has been shown to reduce the O$_2$ cost of
moderate-intensity exercise (1, 25, 36). This improved muscle efficiency may potentially result from NO-mediated enhanced mitochondrial efficiency (26) and/or a reduced ATP cost of muscle force production (2). It is not known to what extent improved mitochondrial efficiency following nitrate supplementation (26) might influence skeletal muscle energy metabolism at rest. If the resting metabolic rate (RMR) is significantly reduced following nitrate intake this could have implications for daily energy expenditure and weight management. The influence of nitrate supplementation on resting metabolic rate (RMR) is yet to be examined.

In recreationally-active subjects or sub-elite athletes, dietary nitrate supplementation has been reported to improve tolerance to constant power output, high-intensity cycling (1), knee-extensor exercise (2) and running (23). Typically, enhanced exercise tolerance has been reported in exercise trials of 6-15 min in duration. However, it remains unknown whether nitrate supplementation may be ergogenic during shorter-duration, higher-intensity exercise. Improvements in cycling time trial (TT) performance over 4 km and 16.1 km (22) and 10 km (6), with a range of maximal exercise durations of 6-30 min, have also been reported following nitrate supplementation. Since exercise performance in the severe domain is a function of CP and W’, collectively these results suggest a beneficial shift in the power-duration relationship (rightwards and/or upwards) for severe-intensity exercise as a result of nitrate supplementation. Because the CP is associated with a particular metabolic rate (3), the increased ratio of power output to $\dot{V}O_2$ with nitrate (1, 6, 22, 25) indicates that nitrate supplementation might increase the CP. However, recent studies have suggested that nitrate supplementation might impact specifically on blood flow and contractile function in type II muscle muscle fibers (11, 16), factors which might, in turn, be expected to increase the W’.
Therefore the purpose of this study was to investigate the effects of dietary nitrate supplementation on 1) the power-duration relationship for severe-intensity exercise (CP and W’) and 2) the RMR. We hypothesized that nitrate supplementation would improve exercise tolerance across a range of severe-intensity exercise bouts by increasing the CP and/or W’. We also hypothesized that nitrate supplementation would reduce the RMR.

**Methods**

**Subjects**

Nine habitually active, male subjects (mean ± SD: age 22 ± 3 yrs, height 180 ± 7 cm, body mass 77 ± 9 kg; $\dot{V}O_{2peak}$ 54.5 ± 7.5 ml/kg/min) volunteered to take part in this study. All procedures employed in this study were approved by the Institutional Ethics Committee. The subjects gave their written, informed consent before the commencement of the study, once the experimental procedures, associated risks, and potential benefits of participation had been described. Subjects were instructed to arrive at the laboratory in a rested and fully hydrated state, at least 4 h postprandial, and to avoid strenuous exercise in the 24 h preceding each testing session. Subjects were also asked to refrain from caffeine and alcohol intake 6 and 24 h before each test, respectively and to consume the same light pre-exercise meal of their choice 4-5 hours before testing (see ‘Supplementation’). In addition to this, subjects abstained from using antibacterial mouthwash and chewing gum throughout the study (14). For each subject, all exercise tests were performed at the same time of day (± 2 h).
**Experimental Design**

The protocol involved twelve separate visits to the laboratory and consisted of: a ramp incremental test at the beginning and end of the study; and, for each of the two conditions (experimental and placebo), a RMR test and four separate constant power output trials at different severe-intensity work rates which were presented in random order. Subjects were given a minimum of 24 h rest between each visit, with all tests being completed within a 4 week period. During Visit 1, subjects performed a ramp incremental test to exhaustion in order to assess \( V_{O_2}\text{peak} \) and gas exchange threshold (GET). Following this, the subjects were assigned in a double-blind, randomized, crossover design to consume 500 ml/day of nitrate-rich beetroot juice (BR) or nitrate-depleted beetroot juice (PL). During Visits 2-6, subjects performed four, severe-intensity, constant power output trials to exhaustion in order to determine CP and \( W' \) and one RMR test. These tests were repeated (Visits 7-11) once a washout period of at least 72 h had elapsed. Finally, following a further 72 h washout period, a follow up ramp incremental test was performed (Visit 12), in order to assess whether the prediction trials had resulted in a training effect.

**Ramp Incremental Tests**

All exercise testing was performed using an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands). During visit 1 subjects completed 3 min of baseline cycling at 20 W and 80 rpm, after which the power output was increased at a rate of 30 W·min\(^{-1}\) in a linear fashion until volitional exhaustion was achieved or until the subject was unable to maintain the 80 rpm pedal rate. The height and configuration of the saddle and
handlebars were recorded and reproduced in subsequent tests. Breath-by-breath pulmonary gas-exchange data were collected continuously during the incremental test and averaged over consecutive 10 s periods. $\dot{V}O_2\text{peak}$ was determined as the highest mean $\dot{V}O_2$ during any 30 s period. The GET was determined from a cluster of measurements, including 1) the first disproportionate increase in $CO_2$ production ($\dot{V}CO_2$) from visual inspection of individual plots of $\dot{V}CO_2$ vs. $\dot{V}O_2$; 2) an increase in expired ventilation ($\dot{V}_E$/ $\dot{V}O_2$ with no increase in $\dot{V}_E$/ $\dot{V}CO_2$; and 3) an increase in end-tidal O$_2$ tension with no fall in end-tidal CO$_2$ tension.

Supplementation

After completion of the non-supplemented Visit 1, subjects were assigned in a double-blind, randomized, crossover design to receive a course of dietary supplementation prior to Visits 2-6 and Visits 7-11. The supplements were either concentrated nitrate-rich BR (2 x 250 ml/day of beetroot juice providing a total of 8.2 mmol nitrate per day; Beet it, James White Drinks, Ipswich, UK) or nitrate-depleted PL (2 x 250 ml/day of beetroot juice providing a total of 0.006 mmol nitrate per day; Beet it, James White Drinks, Ipswich, UK). The PL beverage was created by passage of the juice, before pasteurisation, through a column containing Purolite A520E ion exchange resin, which selectively removes nitrate ions (23). The PL was similar to the BR in appearance, taste and smell. Subjects were instructed to consume the beverages in the morning and afternoon of day 1 and 2 of supplementation, then in the morning and 2.5 hours prior to their first laboratory visit on day 3. Subjects continued to consume two 250 ml beverages each day, and two on the day of testing (one on waking and the other 2.5 hours prior to commencement of the test) until the 4 exercise tests and the RMR test were complete. In total, the subjects
consumed BR and PL for a minimum of 7 and a maximum of 12 days. A washout period of at least 72 hours separated each supplementation period. Subjects were instructed to follow their normal dietary habits throughout the testing period and to replicate their diet between conditions during the supplementation periods. Subjects were told that supplementation may cause beeturia (red urine) and red stools temporarily but that this side effect was harmless.

**Determination of Power-Duration Relationship**

In order to estimate CP and W’, four prediction trials were completed during each supplementation period. The power outputs for the trials were equal to 70%Δ (a power output representing GET plus 70% of the interval between the power outputs at GET and $\dot{V}O_{2peak}$), 80%Δ and 100% $\dot{V}O_{2peak}$, with the power output for the final trial being calculated in order to obtain a range of times to exhaustion between 2 and 15 min as has been recommended (17, 20). This calculated intensity typically approximated 60%Δ. Each prediction trial began with a 3-min baseline period at 20 W. This was followed by an abrupt transition to the appropriate power output. Subjects maintained a cadence of 80 rpm for as long as possible, with tests being terminated when cadence fell below 70 rpm for more than 5 s. Strong verbal encouragement was provided during each test and the time to exhaustion was recorded to the nearest second. Breath-by-breath pulmonary gas-exchange data were collected continuously, while blood [lactate] was measured at rest and as soon as possible following the termination of exercise in each trial. Subjects were not informed of their power outputs or performance on any of the tests until the entire experiment had been completed.
RMR Assessment

Upon arrival at the laboratory, subjects were seated and asked to rest for 10 min prior to the start of the test. Measurements were made in a well ventilated, quiet laboratory setting at a temperature of 22°C to 25°C, with mild ambient lighting throughout all tests. RMR was measured using indirect calorimetry. Breath-by-breath pulmonary gas exchange and ventilation were measured by an open circuit ventilated hood system (Oxycon beta, Mijnhardt, Bunnik, The Netherlands). Data were collected over a 15 min period. The first 5 min of data were discarded and the remaining 10 min of data were used in subsequent analyses.

Measurements

Prior to each testing session, blood pressure (BP) of the brachial artery was measured using an automated sphygmomanometer (Dinamap Pro, GE Medical Systems, Tampa, FL), while subjects were seated at rest. Subjects were seated in a resting state for 10 minutes prior to the measurements. A total of four measurements were recorded, with the mean of the final three measurements being calculated. Mean arterial pressure (MAP) was calculated as $1/3 \cdot$ systolic pressure $+ 2/3 \cdot$ diastolic pressure. The mean systolic, diastolic and MAP for all sessions (four prediction trials and RMR session) was calculated, for both the BR and PL conditions.

Also, at each test session, following the measurement of blood pressure, a venous blood sample was taken for the determination of plasma $[NO_2^-]$, a biomarker for NO availability. Venous blood samples (~4 ml) were drawn into lithium-heparin tubes (Vacutainer, Becton Dickinson, New Jersey, USA). Within 3 min of collection, samples were centrifuged at 4,000 rpm and 4°C for 10 min. Plasma was extracted and immediately frozen at -80°C for later analysis of $[NO_2^-]$. Prior to,
and regularly during analysis, all glassware, utensils and surfaces were rinsed with deionised water in order to remove any residual NO$_3^-$ . After plasma samples were thawed at room temperature the [NO$_2^-$] of the samples was determined using a modification of the chemiluminescence technique as described previously by Bailey et al. (1).

During all exercise tests, pulmonary gas exchange and ventilation were measured continuously with subjects wearing a nose clip and breathing through a mouthpiece and impeller turbine assembly (Jaeger Triple V, Hoechberg, Germany). The inspired and expired gas volume and gas concentration signals were continuously sampled at 100 Hz, the latter using paramagnetic (O$_2$) and infrared (CO$_2$) analyzers (Oxycon Pro, Jaeger, Hoechberg, Germany) via a capillary line connected to the mouthpiece. The gas analyzers were calibrated before each test with gases of known concentration, and the turbine volume transducer was calibrated using a 3-liter syringe (Hans Rudolph, Kansas City, MO). Pulmonary gas exchange variables were calculated and displayed breath-by-breath. The same gas analysis equipment was employed during the RMR tests, although the subjects had a clear, ventilated hood system (Oxycon beta, Mijnhardt, Bunnik, The Netherlands) placed over their head, instead of the aforementioned mouthpiece and nose clip. Heart rate (HR) was measured using short-range radiotelemetry (model 610; Polar Electro Oy, Kempele, Finland). At rest and following the termination of exercise, fingertip blood samples were collected into a capillary tube and analysed for [lactate] (YSI 2300 STAT Plus, Yellow Springs Instruments, Yellow Springs, OH, US).
Data Analysis

Critical Power and $W'$

Estimates of CP and $W'$ from the prediction trials were calculated using three different models (using Eqns. 1-3) as described previously (17, 20). The model producing the lowest standard error (SE) was used in subsequent analysis (17, 18). Estimates of CP and $W'$ were subsequently used to predict the time taken to complete a range of total work done (W) targets (50, 75, 100, 125, 150, 200, 225, 250 kJ). These work done targets were chosen to represent the applicable range of the power-duration relationship within the severe intensity domain, where performance times would range from approximately 2 min to 15 min.

Oxygen Uptake Analysis

The breath-by-breath $\dot{V}O_2$ data from each exercise test were initially examined to exclude errant breaths caused by coughing, swallowing, etc., with those values lying more than four SDs from the local mean being removed. The first 20 s of data after the onset of exercise (the phase I response) were deleted and a nonlinear least-square algorithm was used to fit the data. A bi-exponential model was employed to characterise the $\dot{V}O_2$ responses to the severe-intensity exercise bouts as described in the following equation:

$$\dot{V}O_2(t) = \dot{V}O_{2\text{baseline}} + A_p [1 - e^{-((t-TD_p)/\tau_p)}] + A_s [1 - e^{-((t-TD_s)/\tau_s)}]$$ (Eqn. 5)

Where $\dot{V}O_2(t)$ represents the absolute $\dot{V}O_2$ at a given time $t$; $\dot{V}O_{2\text{baseline}}$ represents the mean $\dot{V}O_2$ during the final 90 s of the baseline period; $A_p$, $TD_p$, and $\tau_p$ represent the amplitude, time delay, and time constant, respectively, describing the phase II increase in $\dot{V}O_2$ above baseline; and $A_s$,
TD<sub>s</sub>, and τ<sub>s</sub> represent the amplitude of, time delay before the onset of, and time constant describing the development of the \( \dot{V}o_2 \) slow component, respectively.

An iterative process was used to minimize the sum of the squared errors between the fitted function and the observed values. The end-exercise \( \dot{V}o_2 \) for all four work rates was defined as the mean \( \dot{V}o_2 \) measured over the final 15 s of exercise. The same ‘filtering’ technique was used for the breath-by-breath \( \dot{V}o_2 \) data collected during the RMR tests. A mean value over the 10 min collection period was calculated.

**Statistical Analyses**

Differences in plasma [NO<sub>2</sub>], BP, time to exhaustion, CP, W' and cardio-respiratory responses, between the conditions, were analyzed with paired-samples \( t \)-tests. Additional paired samples \( t \)-tests were performed on the phase II \( \dot{V}o_2 \) time constants irrespective of exercise intensity. A one-way repeated measures ANOVA was employed to identify differences in CP and W' estimates between the three models, and plasma [NO<sub>2</sub>] across visits 1-5 as well as across work rates. A two-way repeated measures ANOVA (condition by work rate) was used to assess differences in end exercise \( \dot{V}o_2 \) and predicted performance times. Significant main effects were further analysed using simple contrasts with Fisher’s LSD. All data are presented as mean ± SD unless stated otherwise with statistical significance being accepted when \( P<0.05 \).
Results

Self-reported compliance to the supplementation regimen was 100% and no deleterious effects were reported.

Plasma \([\text{NO}_2^-]\) and Blood Pressure

ANOVA revealed that plasma \([\text{NO}_2^-]\) was significantly elevated for BR compared to PL \((P<0.01)\) but there was no difference across time \((P>0.05)\) or exercise intensity \((P>0.05)\). For PL visits 1-5, the plasma \([\text{NO}_2^-]\) was 98 ± 41, 96 ± 29, 75 ± 16, 86 ± 26, and 73 ± 31 nM, respectively, whereas for BR visits 1-5, the plasma \([\text{NO}_2^-]\) was 297 ± 98, 262 ± 107, 295 ± 108, 209 ± 86, and 228 ± 135 nM, respectively. The plasma \([\text{NO}_2^-]\) was higher \((P<0.05)\) in BR than PL for the RMR visit (BR: 286 ± 113 vs. PL: 89 ± 32 nM), 60\%Δ (BR: 213 ± 146 vs. PL: 69 ± 29 nM), 70\%Δ (BR: 223 ± 93 vs. PL: 87 ± 28 nM), 80\%Δ (BR: 270 ± 131 vs. PL: 96 ± 46 nM) and 100\%peak (BR: 285 ± 97 vs. PL: 87 ± 19 nM). On average, the subjects consumed the BR or PL supplement for 5 ± 2 days prior to each of the experimental trials. Across all testing conditions, BR significantly increased plasma \([\text{NO}_2^-]\) by 197\% when compared to PL (BR: 255 ± 70 vs. PL 86 ± 21 nM, \(P<0.01\)). BR also reduced systolic BP compared to PL (BR: 118 ± 5 vs. 122 ± 5 mmHg, \(P<0.01\)), while diastolic BP (BR: 65 ± 5 vs. PL: 65 ± 5 mmHg, \(P>0.05\)) and MAP (BR: 86 ± 7 vs. PL: 84 ± 4 mmHg, \(P>0.05\)) were not significantly different between conditions.
**Ramp Incremental Test**

The main experiment resulted in no training effect ($P>0.05$) upon $V_{o2peak}$ (initial test: $4.13 \pm 0.44$ vs. final test: $4.09 \pm 0.56$ ml·min$^{-1}$), peak power output (initial test: $344 \pm 34$ vs. final test: $338 \pm 32$ W) or power output at GET (initial test: $129 \pm 30$ vs. final test: $125 \pm 20$ W).

**Exercise Tolerance, CP and W’**

Exercise tolerance (expressed as time to exhaustion) across the four severe-intensity prediction trials following PL and BR is displayed in Figure 1. BR significantly improved time to exhaustion for three of the four intensities when compared to PL: at 60%Δ (BR: $696 \pm 120$ vs. $593 \pm 68$ s, $P<0.05$), 70%Δ (BR: $452 \pm 106$ vs. PL: $390 \pm 86$ s, $P<0.05$), and 80%Δ (BR: $294 \pm 50$ vs. PL: $263 \pm 50$ s, $P<0.05$) but not at 100%peak (BR: $182 \pm 37$ vs. PL: $166 \pm 26$ s, $P=0.10$). Baseline and end-exercise HR and blood [lactate] were not significantly different between conditions (Table 1).

ANOVA revealed that the estimates of CP and W’ derived from the three different models (hyperbolic power-time, power-1/time model, and work-time; Eqns. 1-3) were not significantly different from one another ($P>0.05$). For the power-1/time model, the values for CP were $218 \pm 26$ and $221 \pm 27$ W for PL and BR, respectively, and the values for W’ were $17.8 \pm 3.0$ and $19.3 \pm 4.6$ kJ for PL and BR, respectively. For the work-time model, the values for CP were $218 \pm 26$ and $217 \pm 28$ W for PL and BR, respectively, and the values for W’ were $17.7 \pm 3.0$ and $19.7 \pm 4.1$ kJ for PL and BR, respectively. For the power-time model, the values for CP were $216 \pm 26$ and $217 \pm 27$ W for PL and BR, respectively, and the values for W’ were $17.8 \pm 3.0$ and $19.3 \pm 4.6$ kJ for PL and BR, respectively.
and 214 ± 27 W for PL and BR, respectively, and the values for W’ were 18.5 ± 3.3 and 21.2 ± 4.4 kJ for PL and BR, respectively. The coefficients of variation (CV; SE expressed as a percentage of the parameter estimate) associated with the CP estimates from all models were < 5%. However, the CV associated with the W’ estimates for the power-time (PL: 10.5 ± 8.1%, BR: 10.8 ± 7.3%), work-time (PL: 9.9 ± 7.7%, BR: 10.5 ± 8.0 %), and power-1/time (PL: 8.3 ± 5.9 %, BR: 8.3 ± 6.1 %) models were typically slightly larger than the arbitrary cut-off points that have been proposed as acceptable (17, 18). Overall, the power-1/time model elicited the lowest SE (CP, PL: 6 ± 2 W; BR: 5 ± 4 W; W’, PL: 1.4 ± 1.0 kJ; BR: 2.3 ± 2.1 kJ). The CV averaged across CP and W’ in both conditions was lowest in the power-1/time model (5.5%) compared to the power-time (6.4%) and work-time models (6.2%), and so, as is the convention (17, 18), the estimates from the model associated with the lowest error were used in further analysis.

A representation of the power-duration and power-1/time relationships in BR and PL is presented in Figure 2. For the power-1/time model, BR resulted in small but non-significant changes in both CP (BR: 221 ± 27 vs. PL: 218 ± 26 W) and W’ (BR: 19.3 ± 4.6 vs. PL: 17.8 ± 3.0 kJ). The group mean difference in CP between BR and PL (+1.4%) was smaller than the mean CV associated with the parameter estimates. The CP increased in 8 out of 9 subjects and in 4 of these subjects the increase in CP was greater than the CV associated with the individual CP estimates in BR and PL. The group mean difference in W’ between BR and PL (+8.4%) was similar to the mean CV associated with the parameter estimates. The W’ increased in 6 out of 9 subjects and in 4 of these subjects the increase in W’ was greater than the CV associated with the individual W’ estimates. When the CP and W’ were combined to predict performance in a time-trial scenario (using Eqn. 4) and work-done targets of 50, 75, 100, 125, 150, 175, 200 and 225...
kJ), the ANOVA revealed a significant main effect by supplement (P<0.05) and an interaction effect (P<0.05). Specifically, the performance times were significantly lower in BR compared to PL for all ‘time-trials’ except the shortest one (50 kJ) (Figure 3).

Oxygen Uptake Kinetics

The pulmonary \( \dot{V}O_2 \) parameters derived from the model fit, during each of the prediction trials, are presented in Table 2. \( \dot{V}O_2 \) values during the baseline period and at the end of exercise were unchanged with BR compared to PL across all prediction trials. Likewise, compared to PL, BR had no effect upon the Phase II time constant, the \( \dot{V}O_2 \) primary amplitude or the \( \dot{V}O_2 \) slow component amplitude, for any of the individual prediction trials. However, when the phase II time constant was compared between conditions irrespective of exercise intensity, it was significantly shorter in BR (BR: 22.8 ± 7.4 vs. PL: 25.4 ± 7.2 s, P<0.05). The end-exercise \( \dot{V}O_2 \) values across all trials were not significantly different from the \( \dot{V}O_2\text{peak} \) attained during the ramp incremental tests.

Resting Metabolic Rate

Resting metabolic rate was not altered by BR (BR: 0.27 ± 0.06 vs. PL: 0.27 ± 0.06 L·min⁻¹).
Discussion

The principal original finding of this investigation was that dietary supplementation with nitrate-rich BR significantly improved exercise tolerance in three out of four severe-intensity constant power output exercise bouts (ranging between ~4 and 12 min duration), with a trend for improved performance also in the shortest bout (~3 min duration). In contrast to our hypothesis, neither the CP nor the W’ were significantly improved by BR supplementation. Nevertheless, the improved exercise tolerance across the severe exercise intensity domain would be expected to result in a significant improvement in performance as predicted by the 2-parameter critical power model. Another original finding of this study was that BR did not significantly alter RMR.

Effects of Nitrate Supplementation on Plasma [NO\textsubscript{2}\textsuperscript{-}] and Blood Pressure

Plasma [NO\textsubscript{2}\textsuperscript{-}] was significantly increased following nitrate-rich BR supplementation compared to PL. These findings are consistent with previous research which has consistently reported elevations in plasma [NO\textsubscript{2}\textsuperscript{-}] following dietary nitrate supplementation (1, 14, 25, 36). Importantly, plasma [NO\textsubscript{2}\textsuperscript{-}] was not significantly higher prior to the later laboratory visits compared to the earlier ones and the elevation in plasma [NO\textsubscript{2}\textsuperscript{-}] in BR compared to PL was similar for all exercise intensities. Also consistent with previous literature (24, 41), systolic BP was significantly reduced (-4 mmHg) with BR compared to PL. Increased NO bioavailability stimulates smooth muscle relaxation via the synthesis of cyclic guanosine monophosphate (cGMP). It is this NO-mediated smooth muscle relaxation that is considered to be responsible for reductions in BP following nitrate supplementation (24, 41).
Effect of Nitrate Supplementation on Exercise Tolerance, CP & W’

A novel finding of the present study was that dietary nitrate supplementation significantly improved exercise tolerance during several severe-intensity exercise bouts. Previous studies (1, 23) have reported that nitrate supplementation can enhance exercise tolerance but these have focused on just one exercise intensity (70-75%Δ). Interestingly, in these studies it was reported that, compared to PL, BR increased exercise tolerance by 14-16%, which is very similar to the 17% improvement at 60%Δ (exercise duration of ~10-11 min) and 16% improvement at 70%Δ (exercise duration of ~7 min) recorded in the present study. Compared to PL, BR enhanced exercise tolerance at higher exercise intensities too: there was a significant 12% improvement at 80%Δ (exercise duration of ~4-5 min) and a non-significant (P=0.10) 10% improvement at 100%peak (exercise duration of ~3 min). This suggests that nitrate supplementation may benefit performance in shorter, higher-intensity sports events than have been considered previously.

While the increased exercise tolerance during constant power output exercise bouts indicates a physiological benefit of nitrate supplementation, it has been proposed that the magnitude of the changes elicited following an intervention can be difficult to interpret due to the shape of the power-duration relationship (42). We therefore used the four constant power output exercise bouts in the BR and PL conditions to calculate the CP and W’ using the power-1/time model since this produced the lowest error associated with the parameter estimates. Nitrate supplementation resulted in a 1.4% (3 W) increase in CP (which approached statistical significance, P=0.07) and an 8.4% (1.5 kJ) increase in W’ (not significant).

While the modest 1.4% improvement in CP and 8.4% increase in W’ do not appear to be substantial and were not statistically significant, it is only when these values are applied to an
exercise performance scenario that their potential importance becomes clear. It is important to note that severe-intensity exercise performance is determined by the interplay of the CP and W’, and not by either parameter alone (20). When the two parameters were combined to predict performance according to the 2-parameter critical power model (Eqn. 4), the time to complete a fixed amount of work was significantly less in BR compared to PL across the applicable range of the power-duration relationship, except for the shortest target work done (50 kJ) where the predicted completion time was approximately 2.5 min (Figure 3). The improvement in predicted performance is consistent with the experimental data which showed an increased $T_{lim}$ at the three lowest work rates, but no significant improvement in the shortest trial (100%peak) after BR (Figure 1). These analyses demonstrate that the apparently small, non-significant changes in CP and W’ together result in a significant alteration in predicted endurance performance. The potential benefits highlighted for performance (approximately 2-3%) are much greater than the 0.6% value suggested to be the smallest ‘worthwhile’ improvement for road TT cyclists (31). Interestingly, the differences between PL and BR in predicted performance (Figure 3) are very similar to the beneficial effects of nitrate supplementation reported for cycling TT performance previously (4 km TT improved by 2.8% (22); 10 km TT improved by 1.2% (6); and 16.1 km TT improved by 2.7% (22).
Effect of Nitrate Supplementation on Oxygen Uptake Kinetics

The improvements in exercise tolerance at any given power output in the present study were evident without any significant changes in the dynamic \( \dot{V}o_2 \) response to exercise. Previous studies have suggested that the improvements in exercise tolerance and/or performance following nitrate supplementation might be linked to changes in \( \dot{V}o_2 \) kinetics. For example, Bailey et al. (1) reported that BR resulted in a 23% reduction in the \( \dot{V}o_2 \) slow component and a 16% improvement in exercise tolerance during constant power output cycle exercise at 70%Δ. As discussed by Burnley and Jones (4), a reduction in the amplitude of the \( \dot{V}o_2 \) slow component would delay the time before \( \dot{V}o_{2\text{max}} \) is attained and could therefore contribute to enhanced exercise tolerance. Also, Lansley et al. (22) found that BR increased power output for the same \( \dot{V}o_2 \) during cycling TT performances, implying improved muscle efficiency. An improved muscle efficiency following nitrate supplementation may be observed either as a lower \( \dot{V}o_2 \) for the same power output or, conversely, a higher power output for the same \( \dot{V}o_2 \). In the present study, the amplitudes of the \( \dot{V}o_2 \) primary and slow components were not different between BR and PL. Moreover, ANOVA revealed that the end-exercise \( \dot{V}o_2 \) was neither different between conditions nor different from the \( \dot{V}o_{2\text{peak}} \) measured during ramp incremental exercise. This latter result indicates that \( \dot{V}o_{2\text{peak}} \) is not reduced by nitrate supplementation which is consistent with some (1, 36), but not all (4, 27), previous studies.

Interestingly, the \( \dot{V}o_2 \) phase II time constant was slightly but significantly shorter in BR compared to PL when all data were considered together, irrespective of exercise intensity. A possible mechanism for faster \( \dot{V}o_2 \) kinetics in BR compared to PL is a preferential distribution of O\(_2\) delivery to type II muscle fibres (11) and/or to muscle loci that may be relatively more
hypoxic (39). Theoretically, faster $\dot{V}O_2$ kinetics would reduce the contribution of substrate-level phosphorylation to energy turnover in the first 1-2 min following the transition to high-intensity exercise and may lead to improved exercise tolerance (5, 31). It is not clear, however, whether this small improvement in $\dot{V}O_2$ kinetics (~2.6 s) might have contributed to the slightly higher CP (~3 W) observed with BR compared to PL in the present study.

It is noteworthy that training and other interventions often result in opposite effects on CP (i.e., increased) and $W'$ (i.e., decreased), (19, 37, 38). In the present study, we observed small, albeit non-significant, increases in both CP and $W'$ which may be a consequence of a multiplicity of effects of nitrate supplementation on muscle and vascular function. It is known that NO can modulate key processes involved in muscle force production including the ATP cost of actin-myosin interaction and $Ca^{2+}$ handling (13), mitochondrial efficiency (26), and vascular tone and blood flow regulation (15). Exercise above CP elicits a disproportionate recruitment of type II fibres (9). It has recently been reported that BR supplementation results in a marked increase in hind limb blood flow during exercise in rats following dietary supplementation with BR, with the increased blood flow being preferentially distributed to muscle groups that principally contain type II fibres (11). It has also been reported that nitrate supplementation increases muscle force production in mice via modulations to intracellular $Ca^{2+}$ handling in fast-twitch fibres (16).

Collectively, these modifications may account for the small improvements in CP and/or $W'$ observed in the present study which, in turn, resulted in improved exercise tolerance during severe-intensity exercise.

It should be noted that recent studies indicate that nitrate supplementation may be less effective as an ergogenic aid in highly-trained endurance athletes, at least when nitrate is ingested acutely and/or longer duration, lower-intensity endurance performance is assessed (4, 7, 43). Therefore,
it is not clear whether the results of the present study can be applied to highly-trained endurance athletes. Compared to less well-trained subjects, endurance athletes have higher baseline plasma [NO$_2^-$], greater training-related NOS activity, a higher proportion of type I fibres, and greater mitochondrial and capillary density, all of which may reduce the potential benefits of nitrate supplementation (43). The dose-response relationship, including the optimal amount, duration and timing of nitrate supplementation, and the interaction of nitrate supplementation with subject training status and exercise intensity/duration are presently not known and are an important focus of ongoing research.

*Effect of Dietary Nitrate on Resting Metabolic Rate*

In contrast to our hypothesis, dietary nitrate supplementation did not change RMR. Previous research has shown that nitrate supplementation can increase the efficiency of mitochondrial respiration (26). Specifically, these authors reported a 19% improvement in oxidative phosphorylation efficiency in skeletal muscle mitochondria harvested after nitrate supplementation. The explanation for these improvements included a reduced proton slippage across the inner mitochondrial membrane which is believed to account for a substantial amount of resting energy expenditure (26). In the present study, however, nitrate supplementation did not alter RMR. The lack of change in resting $\dot{V}O_2$ in the present study suggests either that nitrate supplementation did not alter oxidative phosphorylation efficiency or that the method used (whole-body indirect calorimetry) was not sufficiently sensitive to detect such changes.
In conclusion, short-term dietary supplementation with nitrate-rich BR increases exercise tolerance within the severe-intensity exercise domain. Although statistically non-significant, in concert, the small improvements in CP and W’ would be expected to conflate into a meaningful improvement in cycling TT performance in sub-elite cyclists.

**Disclosure:** The authors thank Beet It Ltd. for providing the beverages used in this study *gratis*. The authors have no conflicts of interest to declare. The results of this study do not constitute endorsement by the American College of Sports Medicine.
References


**Figure Legends**

Figure 1: Group mean ± SD times to exhaustion across four severe-intensity power outputs, following PL (grey bars) and BR (black bars) supplementation. * = $P<0.05$.

Figure 2: Effects of BR on the power-duration relationship using parameters derived from four severe-intensity prediction trials. The group mean ± SE power-duration profiles are shown in Panel A and the group mean ± SE power-1/time relationships are shown in Panel B. Responses following PL are represented by the dashed line and open symbols and BR by the black curve and closed symbols. Note the rightward-shifted power-duration curve following BR.

Figure 3: Mean ± SD predicted time to complete given amounts of work as calculated using the CP and $W'$ estimates from the power-1/time model following PL (grey bars) and BR (black bars) supplementation. BR is expected to improve performance compared to PL by 2.1-4.1% for target word done trials between 75 and 225 kJ. * = $P<0.05$. 
Figure 1

![Bar chart showing time to exhaustion for different exercise intensities.](image)
Table 1: Mean ± SD heart rate and blood [lactate], during four different severe intensity exercise bouts

<table>
<thead>
<tr>
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<th>PL</th>
<th>BR</th>
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<tbody>
<tr>
<td><strong>60%Δ</strong></td>
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</tr>
<tr>
<td>Baseline heart rate, bpm</td>
<td>101 ± 11</td>
<td>101 ± 7</td>
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<tr>
<td>End exercise heart rate, bpm</td>
<td>188 ± 8</td>
<td>189 ± 10</td>
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<tr>
<td>Baseline blood [lactate], mM</td>
<td>0.9 ± 0.2</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>End exercise blood [lactate], mM</td>
<td>8.1 ± 2.3</td>
<td>7.9 ± 1.9</td>
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|               |        |        |
| **70%Δ**      |        |        |
| Baseline heart rate, bpm | 100 ± 9  | 97 ± 9  |
| End exercise heart rate, bpm | 189 ± 9  | 190 ± 9 |
| Baseline blood [lactate], mM   | 0.9 ± 0.2 | 0.8 ± 0.3 |
| End exercise blood [lactate], mM | 8.7 ± 1.6 | 8.4 ± 1.8 |

|               |        |        |
| **80%Δ**      |        |        |
| Baseline heart rate, bpm | 97 ± 12  | 98 ± 11 |
| End exercise heart rate, bpm | 184 ± 9  | 184 ± 9 |
| Baseline blood [lactate], mM   | 0.9 ± 0.2 | 0.9 ± 0.2 |
| End exercise blood [lactate], mM | 8 ± 1.6  | 8.6 ± 1.2 |

|               |        |        |
| **100% Peak** |        |        |
| Baseline heart rate, bpm | 99 ± 11  | 101 ± 10 |
| End exercise heart rate, bpm | 180 ± 9  | 182 ± 9 |
| Baseline blood [lactate], mM   | 1 ± 0.1  | 0.9 ± 0.2 |
| End exercise blood [lactate], mM | 8.4 ± 1.1 | 8.8 ± 1.4 |
Table 2: Mean ± SD oxygen uptake dynamics during four different severe intensity exercise bouts

<table>
<thead>
<tr>
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<th>PL</th>
<th>BR</th>
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<tbody>
<tr>
<td><strong>60%ΔVO₂</strong></td>
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<tr>
<td>Baseline, ml·min⁻¹</td>
<td>1125 ± 158</td>
<td>1072 ± 85</td>
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<tr>
<td>End exercise, ml·min⁻¹</td>
<td>4382 ± 270</td>
<td>4369 ± 329</td>
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<tr>
<td>Phase II time constant, s</td>
<td>23 ± 5</td>
<td>20 ± 4</td>
</tr>
<tr>
<td>Primary amplitude, ml·min⁻¹</td>
<td>2081 ± 381</td>
<td>2060 ± 313</td>
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<tr>
<td>Slow component amplitude, ml·min⁻¹</td>
<td>1180 ± 246</td>
<td>1208 ± 52</td>
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<tr>
<td><strong>70% ΔVO₂</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline, ml·min⁻¹</td>
<td>1151 ± 183</td>
<td>1142 ± 118</td>
</tr>
<tr>
<td>End exercise, ml·min⁻¹</td>
<td>4405 ± 476</td>
<td>4499 ± 371</td>
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<tr>
<td>Phase II time constant, s</td>
<td>30 ± 8</td>
<td>28 ± 8</td>
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<tr>
<td>Primary amplitude, ml·min⁻¹</td>
<td>2357 ± 458</td>
<td>2345 ± 329</td>
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<tr>
<td>Slow component amplitude, ml·min⁻¹</td>
<td>896 ± 217</td>
<td>1027 ± 116</td>
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<td><strong>80 ΔVO₂</strong></td>
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<tr>
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<td>1080 ± 144</td>
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<tr>
<td>End exercise, ml·min⁻¹</td>
<td>4115 ± 425</td>
<td>4222 ± 320</td>
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<td>25 ± 5</td>
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<tr>
<td>Primary amplitude, ml·min⁻¹</td>
<td>2272 ± 252</td>
<td>2261 ± 252</td>
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<tr>
<td>Slow Component amplitude, ml·min⁻¹</td>
<td>721 ± 139</td>
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<td><strong>100% Peak</strong></td>
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<tr>
<td>Baseline, ml·min⁻¹</td>
<td>1151 ± 172</td>
<td>1125 ± 118</td>
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<tr>
<td>End exercise, ml·min⁻¹</td>
<td>4119 ± 420</td>
<td>4180 ± 390</td>
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<td>24 ± 7</td>
<td>21 ± 5</td>
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<tr>
<td>Primary amplitude, ml·min⁻¹</td>
<td>2232 ± 397</td>
<td>2371 ± 330</td>
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<tr>
<td>Slow Component amplitude, ml·min⁻¹</td>
<td>695 ± 215</td>
<td>686 ± 219</td>
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