Dietary nitrate supplementation reduces the O$_2$ cost of walking and running: a placebo-controlled study

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Abstract

Dietary supplementation with beetroot juice (BR) has been shown to reduce resting blood pressure and the O₂ cost of sub-maximal exercise and to increase the tolerance to high-intensity cycling. We tested the hypothesis that the physiological effects of BR were consequent to its high nitrate content, per se, and not to the presence of other potentially bioactive compounds. We investigated changes in blood pressure, mitochondrial oxidative capacity (Q_max), and the physiological responses to walking, moderate-intensity running and severe-intensity running following dietary supplementation with BR and nitrate-depleted beetroot juice (PL). Following control (non-supplemented) tests, nine healthy, physically-active male subjects were assigned in a randomized, double-blind, cross-over design to receive BR (0.5 L•d⁻¹; containing ~6.2 mmol of nitrate) and PL (0.5 L•d⁻¹; containing ~0.003 mmol of nitrate) for six days. Subjects completed treadmill exercise tests on days four and five, and knee-extension exercise tests for the estimation of Q_max (using ³¹P-MRS) on day six of the supplementation periods. Relative to PL, BR elevated plasma [nitrite] (PL: 183±119 vs. BR: 373±211 nM, P<0.05) and reduced systolic blood pressure (PL: 129±9 vs. BR: 124±10 mmHg; P<0.01). Q_max was not different between PL and BR (PL: 0.93±0.05 vs. BR: 1.05±0.22 mM•s⁻¹). The O₂ cost of walking (PL: 0.87±0.12 vs. BR: 0.70±0.10 L•min⁻¹; P<0.01), moderate-intensity running (PL: 2.26±0.27 vs. BR: 2.10±0.28 L•min⁻¹; P<0.01), and severe-intensity running (End-exercise VO₂; PL: 3.77±0.57 vs. BR: 3.50±0.62 L•min⁻¹; P<0.01) was reduced by BR, and time-to-exhaustion during severe-intensity running was increased by 15% (PL: 7.6±1.5 vs. BR: 8.7±1.8 min; P<0.01). In contrast, relative to control, nitrate-depleted beetroot juice did not alter plasma [nitrite], blood pressure or the physiological responses to exercise. These results indicate that the positive effects of 6 days of BR supplementation on the physiological responses to exercise can be ascribed to the high nitrate content per se.
Introduction

A diet rich in vegetables is well documented to have cardiovascular benefits and to be associated with a longer lifespan (1). It has been suggested that these effects might be attributable, at least in part, to the high nitrate ($\text{NO}_3^-$) content of vegetables, particularly leafy greens and beetroot (25). That dietary $\text{NO}_3^-$ can reduce blood pressure and thus potentially be cardio-protective is well established (20, 62). However, evidence is emerging that dietary $\text{NO}_3^-$ supplementation may also positively impact on the physiological responses to exercise. It was originally reported that dietary supplementation with pharmacological sodium nitrate (0.1 mmol•kg$^{-1}$•d$^{-1}$) resulted in a significant reduction in the $\text{O}_2$ cost of sub-maximal cycling (40). A similar improvement in cycling economy and an improved tolerance to high-intensity cycling was reported when nitrate was administered in the form of beetroot juice (BR; 0.5 L•d$^{-1}$; ~5.5 mmol•d$^{-1}$ of $\text{NO}_3^-$, 3). We have subsequently demonstrated that the reduced $\text{O}_2$ cost of sub-maximal exercise following BR consumption is consequent to a reduced ATP cost of muscle force production (2), and that the enhanced efficiency is evident acutely (2.5 h following a single 0.5 L BR dose) and for at least 15 days when supplementation is continued (60).

The improvement in exercise efficiency with BR supplementation (2, 3, 60) has led to the assumption that BR exerts its effects via the metabolic conversion of inorganic nitrate ($\text{NO}_3^-$) to bioactive nitrite ($\text{NO}_2^-$) and nitric oxide (NO; 6, 44). However, given that beetroot is rich in several potentially metabolically-active compounds, it is presently unclear whether the cardiovascular and physiological changes observed following BR supplementation can be ascribed exclusively to its high $\text{NO}_3^-$ content. For example, the amino acid, betaine, which is present in beetroot, has been used in the treatment of cardiovascular disease (9, 61) and betaine supplementation has elicited improvements in muscular endurance, strength and power (23, 45). In addition, beetroot is rich in several polyphenols, of which quercetin and resveratrol have been linked with mitochondrial biogenesis and an associated increase in aerobic capacity (17, 38; cf. 16, 19). The high antioxidant content of beetroot may also provide protection against exercise-induced oxidative stress (31). Consequently, BR supplementation has the potential to affect exercise efficiency and performance via numerous pathways.
The purpose of the present study was three-fold. Firstly, we wished to determine whether the physiological effects of BR supplementation (i.e., reduced blood pressure, lower O$_2$ cost of sub-maximal exercise, and enhanced tolerance to high-intensity exercise) were consequent to the high NO$_3^-$ content of BR. We have recently developed a process to selectively remove the NO$_3^-$ from BR using a commercially available resin (M. Gilchrist, N. Benjamin and P.G. Winyard), whilst leaving it identical in colour, taste, smell and texture. This important advance enables us to isolate the effects of dietary NO$_3^-$ from the other potential ‘active ingredients’ found in BR using a genuinely double-blind experimental design. The second purpose of the study was to investigate the extent to which elevating NO bioavailability through BR consumption might increase mitochondrial biogenesis (14, 48) and thus contribute to the reported improvements in aerobic exercise performance (2, 3, 60). We therefore estimated muscle oxidative (mitochondrial) capacity following BR supplementation from the maximum rate of PCr resynthesis following high-intensity exercise ($Q_{\text{max}}$) using $^{31}$-phosphorus magnetic resonance spectroscopy ($^{31}$P-MRS). The third purpose of the present study was to extend our previous findings in knee-extension (2) and cycling exercise (3, 60) to walking and running, thereby broadening the potential application of this intervention.

We investigated the influence of dietary nitrate-rich (BR), and nitrate-depleted beetroot juice (placebo; PL), supplementation on plasma [nitrite] (a biomarker of NO production; 37, 42), blood pressure, and $V_O_2$ dynamics during step transitions from walking to moderate-intensity and severe-intensity running. We hypothesised that, relative to PL, dietary BR supplementation would: 1) increase plasma [nitrite] and reduce blood pressure; 2) reduce the O$_2$ cost of walking and running and increase exercise tolerance, measured as the time to task failure; and 3) increase muscle oxidative capacity as assessed by $Q_{\text{max}}$. We also hypothesised that the nitrate-depleted PL juice would not alter these physiological indices relative to the pre-intervention control measurements, indicating that the physiological effects of short term (4-6 days) BR supplementation are related to the NO$_3^-$ content.
 Methods

 Subjects

 Nine healthy, physically active males volunteered to participate in this study (mean ± SD: age 22 ± 4 years, body mass 69.3 ± 7.2 kg, height 1.77 ± 0.06 m, maximal O₂ uptake (\(\dot{V}O_{2\max}\)) 55 ± 7 mL·kg\(^{-1}\)·min\(^{-1}\)). Subjects gave their written informed consent to participate after the experimental procedures, associated risks, and potential benefits of participation had been explained. All procedures employed were approved by the Institutional Research Ethics Committee. In the 24 h preceding the first exercise test, subjects recorded their food intake and this was replicated in the 24 h preceding subsequent tests. Subjects were instructed to arrive at the laboratory in a rested and fully hydrated state, at least 3 h post-prandial, and to avoid strenuous exercise in the 24 h preceding each testing session. Participants were asked to refrain from caffeine and alcohol 6 and 24 h before each test, respectively. The subjects also abstained from using antibacterial mouthwash and chewing gum throughout the study (21).

 Experimental Overview

 The subjects reported to the laboratory on ten occasions over a 4-5 week period. Subjects initially completed a preliminary incremental treadmill test for the determination of \(\dot{V}O_{2\max}\) followed by ‘step’ running tests over two consecutive days with no dietary supplementation of any kind (pre-intervention control). During these tests plasma [nitrite], blood pressure, blood [lactate], heart rate, pulmonary \(\dot{V}O_2\) dynamics and time to task failure were measured. Subjects were then assigned in a double-blind, randomized, cross-over design to consume 0.5 L·d\(^{-1}\) of nitrate-rich beetroot juice (BR) or nitrate-depleted beetroot juice (as placebo, PL) for six days. Subjects repeated the ‘step’ running tests on days four and five of supplementation. On the last day of each supplementation period, subjects performed knee-extension exercise in the bore of a 1.5 T superconducting magnet (see below) for the estimation of mitochondrial oxidative capacity (using \(^{31}\)P-MRS).
Exercise Tests

All treadmill exercise testing sessions were carried out in a well-ventilated laboratory at a temperature of 20-22°C and were performed on a Woodway PPS-55 Sport slat-belt treadmill (Woodway GmbH, Weil am Rhein, Germany) set at a 1% gradient (28). Subjects initially performed a ramp incremental exercise test for the determination of \( \dot{V}O_{2\text{max}} \) and the gas exchange threshold (GET). The protocol began with subjects walking at 7 km·h\(^{-1}\) for 6 min, after which the belt speed was increased by 1 km·h\(^{-1}\) every minute until volitional exhaustion. The breath-by-breath pulmonary gas exchange data were collected continuously during the incremental test and averaged over consecutive 10 s periods. The \( \dot{V}O_{2\text{max}} \) was taken as the highest 30 s mean value attained prior to the subject's volitional exhaustion. The GET was determined as described previously (3, 5). Subsequently, the treadmill speeds that would require 80% of the GET (moderate-intensity exercise) and 75% \( \Delta \) (75% of the difference between the speed at the GET and \( \dot{V}O_{2\text{max}} \) + the speed at GET; severe-intensity exercise) were calculated, with account taken of the mean response time (MRT) for \( \dot{V}O_2 \) during ramp exercise (i.e., two-thirds of the ramp rate was deducted from the running speeds at GET and \( \dot{V}O_{2\text{max}} \); 63).

Subjects completed 'step' tests during the non-supplemented control condition and on days 4 and 5 of both supplementation periods for the determination of pulmonary \( \dot{V}O_2 \) dynamics (Figure 1). The protocol on day 4 of supplementation consisted of two six-minute bouts of moderate-intensity running (80% GET) and one exhaustive bout of severe-intensity running (75% \( \Delta \)) as a measure of exercise tolerance. During the exhaustive bout, the subjects were verbally encouraged to continue for as long as possible. On day 5 of supplementation, the participants returned to the laboratory and completed two six-minute bouts of moderate-intensity running (80% GET) and one six-minute bout of severe-intensity (75% \( \Delta \)) running. All exercise bouts involved an abrupt transition to the target speed initiated from a walking baseline (4 km·h\(^{-1}\)) and exercise bouts were separated by 10 min of walking at 4 km·h\(^{-1}\). The \( \dot{V}O_2 \) responses from like-transitions were averaged before analysis to enhance the signal-to-noise ratio and improve confidence in the parameters derived from the modeling process (39, 64).
On the sixth day of each supplementation period, subjects were required to complete
an incremental, single-legged, knee-extension exercise test whilst lying prone inside a
1.5 T superconducting magnetic resonance scanner (Philips Gyroscan Clinical Intera).
Subjects were familiarized with the knee-extension exercise prior to testing. The
exercise protocol consisted of unilateral knee extensions with the right leg using a
custom built non-ferrous ergometer. The foot was fastened securely to a padded foot
brace using Velcro straps, and this was connected to the ergometer load basket via a
simple rope and pulley system. Knee-extensions over a distance of 0.22 m were
performed continuously at a constant frequency, set in unison with the magnetic pulse
sequence (40 pulses min⁻¹) to ensure the quadriceps muscles were positioned in
approximately the same phase of contraction during each MR pulse acquisition. As
the MRS sequence during the exercise protocol is pulse-acquired rather than spatially
localized, the signal originates from the muscle lying within the sensitive region of the
coil and is thus relatively insensitive to subject motion. However, to prevent
displacement of the quadriceps relative to the MRS coil during the exercise, leading to
a variable muscle volume being sampled, Velcro straps were fastened over the
subject’s legs, hips and lower back. Following a 2 minute rest period, subjects
performed a 42 s exercise bout at 81.2 ± 4.3 % of individual maximum (21.7 ± 1.8
W) designed to reduce muscle phosphocreatine concentration ([PCr]) without
substantially altering muscle pH, for the estimation of Q_max. Then, following a further
5 minute rest period, subjects completed an incremental test to volitional exhaustion.
The basket load was increased in steps of 0.5 kg every 30 s until the subject was
unable to continue. Knee extensor displacement was measured using a calibrated
optical shaft encoder (Type BDK.06.05A 100-5-4; Baumer Electric, Swindon, UK.)
connected to the weight basket pulley, and load was measured using an aluminium
load cell (Type F250EBR0HN, Novatech Measurements Ltd., St. Leonards-on-Sea,
East Sussex, UK.). Work done was calculated continuously during the knee extensor
exercise using the product of force and distance.

Supplementation protocol

Following the completion of the non-supplemented control condition, subjects were
assigned in a double-blind, randomised, cross-over design to receive six days of
dietary supplementation with either nitrate-rich BR (0.5 L•d⁻¹ organic beetroot juice containing ~6.2 mmol of NO₃⁻; Beet it, James White Drinks Ltd., Ipswich) or placebo (PL; 0.5 L•d⁻¹ organic nitrate-depleted beetroot juice containing ~0.0034 mmol of NO₃⁻; Beet it, James White Drinks Ltd., Ipswich). For the PL beverage, the nitrate was removed by passing the juice, before pasteurization, through a column containing Purolite A520E ion-exchange resin which selectively removes nitrate ions. Five subjects began with the nitrate-rich beetroot juice condition and the other four subjects began with the nitrate-depleted beetroot juice condition. The subjects were instructed to consume the beverages slowly 3 hours prior to each exercise test. A 10 day wash-out period separated each supplementation period. Throughout the study, subjects were instructed to maintain their normal daily activities and food intake, i.e. unlike in previous studies (2, 3, 40, 41), in which subjects were instructed to minimize the consumption of nitrate-rich foods throughout the study period.

241 Measurements

242 Prior to each testing session, blood pressure of the brachial artery was measured with subjects in a rested, seated position using an automated sphygmomanometer (Dinamap Pro, GE Medical systems, Tampa USA). Four measurements were recorded and the mean of the final three measurements was used in subsequent analysis. Plasma [NO₂⁻] was used as a biomarker for NO availability (37, 42). Venous blood samples (~4 ml) were drawn into lithium-heparin tubes (7.5mL Monovette Lithium Heparin, Sarstedt Ltd., Leicester, UK) which have very low levels of nitrate (0.89 ± 0.35 µM) and nitrite (0.05 ± 0.01 µM). Samples were centrifuged at 4000 rpm and 4°C for 10 min, within 3 min of collection. Plasma was extracted and immediately frozen at -80°C, for later analysis of [NO₂⁻]. All glass wear, utensils and surfaces were rinsed with deionised water to remove residual NO₂⁻ prior to analysis. After thawing at room temperature, plasma samples were initially deproteinized using cold ethanol precipitation. The ethanol was chilled to 0°C and 1 ml of cold ethanol was added to 0.5 ml of plasma sample, after which the sample was vortexed and left to stand at 0°C for 30 min. Thereafter, samples were centrifuged at 14000 rpm for 5 min and the supernatant was removed. The [NO₂⁻] of the deproteinized plasma samples was determined using a modification (3) of the chemiluminescence technique (4).
During all exercise tests, pulmonary gas exchange and ventilation were measured continuously using a portable metabolic measurement system (MetaMax 3B, Cortex Biophysik, Leipzig, Germany). A digital volume transducer turbine measured inspired and expired airflow while an electro-chemical cell O₂ analyzer and an infrared CO₂ analyzer simultaneously measured expired gases. Subjects wore a nose clip and breathed through a low dead-space, low-resistance mouthpiece that was securely attached to the volume transducer. The inspired and expired gas volume and gas concentration signals were continuously sampled via a capillary line connected to the mouthpiece. The gas analyzers were calibrated before each test with gases of a known concentration, and the turbine volume transducer was calibrated using a 3-liter syringe (Hans Rudolph, Kansas City, MO). Pulmonary gas exchange data were calculated and displayed breath by breath. Heart rate (HR) was measured during all tests using short-range radiotelemetry (Polar s610, Polar Electro Oy, Kempele, Finland).

During the transitions to moderate- and severe-intensity running, a fingertip blood sample was collected into a capillary tube over the 20 s preceding each step transition and within the last 20 s of exercise. These whole blood samples were analyzed within 30 s of collection to determine blood lactate concentration (YSI Stat 2300, Yellow Springs, OH). Blood lactate accumulation (Δ blood [lactate]) was calculated as the difference between blood [lactate] at end-exercise and blood [lactate] at baseline.

For the MRS measurements, subjects were positioned inside a whole-body scanner with a 6-cm 31P transmit/receive surface coil placed within the subject bed such that it was centered over the quadriceps muscle of the right leg. Cod liver oil capsules that yield high-intensity signal points within the image were placed adjacent to the coil and gradient echo images were produced to ensure that the muscle was positioned correctly. A number of pre-acquisition steps were carried out to optimize the signal from the muscle. Tuning and matching of the coil were performed to maximize energy transfer between the coil and the muscle followed by an automatic shimming protocol undertaken within a volume that defines the quadriceps muscle to optimize the homogeneity of the local magnetic field. Before exercise, during exercise and during recovery, data were acquired every 1.5 s, with a spectral width of 1,500 Hz and...
1,000 data points. Phase cycling with four phase cycles was employed, which lead to a spectrum being acquired every 6 s. The subsequent spectra were quantified via peak fitting, with the assumption of prior knowledge, using the jMRUI (version 3) software package (47) and the AMARES fitting algorithm (58, 59). Spectra were fitted according to the assumption that Pi, PCr, α-ATP (two peaks, amplitude ratio 1:1), γ-ATP (two peaks, amplitude ratio 1:1), β-ATP (three peaks, amplitude ratio 1:2:1) and phosphodiester peaks were present.

Absolute metabolite values were established via a technique similar to that described by Kemp et al. (32). Prior to the exercise studies, spatially localized spectroscopy was undertaken to determine the relative signal intensities obtained from a phosphoric acid source within the scanner bed and an external inorganic phosphate solution. A subsequent unsaturated scan was obtained comparing the signals obtained from the phosphoric acid standard and inorganic phosphate muscle tissue, where the localized volume sampled within the muscle was of the same dimensions and distance from the coil as the inorganic phosphate solution, allowing the calculation of muscle P_i concentration, following corrections for relative coil loading. Absolute values of PCr and ATP concentrations were subsequently calculated via the ratio of P_i:PCr and P_i:ATP. Intracellular pH was calculated using the chemical shift of the P_i spectral peak relative to the PCr peak (57). ADP concentration was calculated as described by Kemp et al. (34). Baseline and end-exercise values of [PCr], [P_i], and ADP were calculated over the last 30 s of the rest or exercise period.

Data Analysis Procedures

The Q_max was calculated as described by Layec et al. (43). Briefly, a time constant (k) was determined to describe the rate of PCr recovery by fitting a single exponential function to the [PCr] recorded following the 42 s exercise bout, where the intensity was sufficiently low to ensure no substantial reduction in pH relative to baseline. From this, the Q_max was given by:

$$Q_{\text{max}} = k[\text{PCr}]_c \left(1+K_m/[\text{ADP}]_{\text{end}}\right)$$  \hspace{1cm} (Eqn. 1)
Where $[\text{PCr}]_c$ is the depletion in PCr concentration, $[\text{ADP}]_{\text{end}}$ is the ADP concentration at the end of exercise and $K_m$ the ADP concentration at half-maximal oxidation rate which is assumed to be 30 μM in skeletal muscle (33).

The breath-by-breath $\dot{V}O_2$ data from each test were initially examined to exclude errant breaths and those values lying more than four standard deviations from the local mean were removed. The breath-by-breath data were subsequently linearly interpolated to provide second-by-second values and, for each individual, identical repetitions were time-aligned to the start of exercise and ensemble-averaged. The two severe-intensity running bouts were of different durations (until volitional exhaustion in the first bout and 6 min for the second bout) and so, at this intensity, only the first 6 min of data were averaged together and modeled. The first ~20 s of data after the onset of exercise (i.e., the phase I response) were deleted and a nonlinear least-square algorithm was used to fit the data thereafter. A single-exponential model was used to characterize the $\dot{V}O_2$ responses to moderate exercise, and a biexponential model was used for severe exercise, as described in the following equations:

\[
\dot{V}O_2(t) = \dot{V}O_2_{\text{baseline}} + A_p (1-e^{-(t-TDp/\tau_p)}) \quad \text{(moderate)} \quad \text{(Eqn. 2)} \\
\dot{V}O_2(t) = \dot{V}O_2_{\text{baseline}} + A_p (1-e^{-(t-TDp/\tau_p)}) + A_s (1-e^{-(t-TDs/\tau_s)}) \quad \text{(severe)} \quad \text{(Eqn. 3)}
\]

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 $\dot{V}O_2$ in the baseline period; $A_p$, $TDp$, 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 $A_s$, $TDs$, and $\tau_s$ 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. $\dot{V}O_2_{\text{baseline}}$ was defined as the mean $\dot{V}O_2$ measured over the final 90 s of baseline walking. The end-exercise $\dot{V}O_2$ was defined as the mean $\dot{V}O_2$ measured over the final 30 s of exercise. Because the asymptotic value ($A_s'$) of the exponential term describing the $\dot{V}O_2$ slow component may represent a higher value than is actually reached at the end of exercise, the actual amplitude of the $\dot{V}O_2$ slow component was defined as $A_s'$. The $A_s'$ parameter was compared to the
same iso-time (360 s) under the three experimental conditions. The amplitude of the
\( \dot{V}o_2 \) slow component was also described relative to the entire \( \dot{V}o_2 \) response. To
determine the overall kinetics of the \( \dot{V}o_2 \) response both for moderate-intensity and
severe-intensity exercise, data were fit with a mono-exponential model from 0 s to
end-exercise, without time delay.

The baseline \( \dot{V}co_2 \), RER and \( \dot{V}E \) were calculated as mean values over the final 60 s
preceding the start of exercise and end-exercise values were calculated as mean values
over the final 30 s. We also modeled the HR response to exercise in each condition.

For this analysis, HR data were linearly interpolated to provide second-by-second
values, and, for each individual, identical repetitions from like-transitions were time-
aligned to the start of exercise and ensemble-averaged. Nonlinear least squares
mono-exponential and biexponential models without TD were used to fit the data to
moderate-intensity and severe-intensity exercise respectively, with the fitting window
commencing at \( t = 0 \) s. The HR \( \tau \) so derived provides information on the overall HR
response dynamics.

Statistical analyses

Effects on the relevant physiological variables were assessed using one-way repeated-
measures ANOVAs across the control, BR and PL supplementation periods. Two-
way (treatment by time) ANOVAs were used to identify differences in plasma \([NO_2^-]\)
and blood pressure across days 4 - 6 of the control and supplementation conditions.
Where significant main effects were detected, Bonferroni-adjusted paired \( t \)-tests were
used to identify specific differences. All data are presented as means ± SD unless
stated otherwise. Statistical significance was accepted at the \( P < 0.05 \) level.

Results

Plasma \([NO_2^-]\) and blood pressure

The group mean plasma \([NO_2^-]\) value in the control condition was 197 ± 184 nM. No
significant change was observed following PL supplementation (PL: 183 ± 119 nM;
\( P > 0.05 \)). However, relative to PL, BR ingestion increased plasma \([NO_2^-]\) by 105%
A non-significant main effect for time indicated that the BR-induced elevations in plasma $[NO_2^-]$ were not different between days 4, 5 and 6 ($F=1.08$, $P=0.315$).

Compared to control, PL supplementation had no effect upon systolic, diastolic or mean arterial blood pressure. The ingestion of BR significantly reduced systolic BP by 4% relative to placebo (PL: $129 \pm 9$ vs. BR: $124 \pm 10$ mmHg; $P<0.01$). The BR-induced reductions in systolic blood pressure were not significantly different between days 4, 5 and 6 ($F=1.77$, $P=0.221$). Diastolic blood pressure ($\sim 66 \pm 5$ mmHg) and mean arterial pressure ($\sim 89 \pm 5$ mmHg) were not significantly affected by BR ingestion ($P>0.05$).

**Moderate-intensity exercise**

The pulmonary $\dot{V}_O_2$ responses across the control, PL and BR supplementation periods are presented in Figure 2 and the parameters derived from the model fit are summarised in Table 1. One-way repeated-measures ANOVAs revealed significant main effects for treatment across the $\dot{V}_O_2$ response, and Bonferroni-adjusted paired t-tests revealed no significant differences between control and PL supplementation for any $\dot{V}_O_2$ variables (Fig 2A). Relative to PL, BR supplementation reduced $\dot{V}_O_2$ during the baseline walking period by $\sim 12\%$ (PL: $0.87 \pm 0.12$ vs. BR: $0.77 \pm 0.10$ L min$^{-1}$; $P<0.01$). The absolute $\dot{V}_O_2$ values over the final 30 s of moderate-intensity running were also significantly lower ($\sim 7\%$) following BR supplementation (PL: $2.26 \pm 0.27$ vs. BR: $2.10 \pm 0.28$ L min$^{-1}$; $P<0.01$). In addition to these changes in the baseline and end-exercise $\dot{V}_O_2$ values, there was a reduction in the amplitude of the pulmonary $\dot{V}_O_2$ response ($\sim 4\%$; PL: $1.37 \pm 0.19$ vs. BR: $1.31 \pm 0.23$ mL min$^{-1}$; $P<0.05$) and in the $O_2$ cost of running 1 km ($\sim 6\%$; PL: $244 \pm 16$ vs. BR: $229 \pm 17$ mL kg$^{-1}$ km$^{-1}$; $P<0.01$).

The phase II time constant was not significantly altered by BR supplementation (PL: $26 \pm 5$ vs. BR: $25 \pm 5$ s; $P>0.05$; Table 1). The baseline and end-exercise values of $\dot{V}CO_2$, $\dot{V}E$, RER, HR and blood [lactate] were not significantly different between conditions (Tables 1 and 2), although there was a trend for a reduced end-exercise $\dot{V}CO_2$ ($\sim 4\%$, $P=0.16$), end-exercise $\dot{V}E$ ($\sim 4\%$, $P=0.08$) and end-exercise RER (PL: $0.83 \pm 0.05$ vs. BR: $0.86 \pm 0.03$; $P=0.08$) following BR supplementation.
Severe-intensity exercise

The pulmonary \( \dot{V}_{O_2} \) responses during severe-intensity exercise in the non-supplemented control condition and following PL and BR supplementation are shown in Figure 3 and the \( \dot{V}_{O_2} \) parameters derived from the bi-exponential model fit are presented in Table 1. Similar to the effects observed for moderate-intensity exercise, Bonferroni-adjusted paired t-tests revealed no differences in \( \dot{V}_{O_2} \) variables between control and PL supplementation (Fig 3A). BR supplementation resulted in a ~14 % reduction in \( \dot{V}_{O_2} \) during walking relative to placebo (PL: 0.91 ± 0.13 vs. BR: 0.78 ± 0.09 mL•min\(^{-1} \); \( P<0.05 \)), and a ~7 % reduction in the end-exercise \( \dot{V}_{O_2} \) during the 6-min severe-intensity running bouts (PL: 3.77 ± 0.57 vs. BR: 3.50 ± 0.62 L•min\(^{-1} \); \( P<0.01 \)). The \( \dot{V}_{O_2} \) obtained at task failure was ~6 % lower following BR supplementation (PL: 3.89 ± 0.57 vs. BR: 3.67 ± 0.65 L•min\(^{-1} \); \( P<0.01 \)). The phase II time constant was not significantly altered following BR supplementation (PL: 21 ± 3 vs. BR: 22 ± 3 s; \( P>0.05 \)). The reduction in the amplitude of the primary \( \dot{V}_{O_2} \) response with BR supplementation (~3 %) was not significant (PL: 2.56 ± 0.36 vs. BR: 2.50 ± 0.40 L•min\(^{-1} \); \( P=0.38 \)) and there was no significant change in the amplitude of the subsequent \( \dot{V}_{O_2} \) slow component (PL: 0.38 ± 0.16 vs. BR: 0.37 ± 0.19 L•min\(^{-1} \); \( P=0.98 \)). BR supplementation resulted in an enhanced exercise tolerance as demonstrated by an increased time to task failure of ~15 % (Con: 7.5 ± 1.7; PL: 7.6 ± 1.5 vs. BR: 8.7 ± 1.8 min; \( P<0.01 \)). Importantly, all nine subjects were able to exercise for longer following BR supplementation compared to PL. The baseline and end-exercise values of \( \dot{V}_{CO_2} \), \( \dot{V}_E \), RER, HR and blood [lactate] were not significantly different between conditions (Tables 1 and 2).

Muscle metabolic measurements

The 42 s bout of intense exercise resulted in a ~9mM reduction in muscle [PCr] with a negligible fall in pH. There was no significant difference in the time constant for the [PCr] recovery kinetics following this bout of exercise (Con: 26 ± 6; PL: 24 ± 3; BR: 24 ± 4 s; \( P>0.05 \); Figure 4). Moreover, there was no difference in the estimated \( Q_{max} \) between the conditions (Con: 1.08 ± 0.22; PL: 0.93 ± 0.05; BR: 1.05 ± 0.22 mM.s\(^{-1} \); \( P>0.05 \)). Muscle metabolite concentrations at rest, following 42 s of intense exercise and subsequent recovery, and at the completion of the incremental exercise
test are reported in Table 3 for the control condition and following PL and BR supplementation. The time to task failure during incremental knee-extension exercise was significantly longer following six days of BR compared to control and PL (Con: 8.2 ± 0.9; PL: 8.2 ± 0.9 vs. BR: 8.5 ± 0.8 min; F=3.71, P<0.05). There was no significant difference in the metabolite concentrations measured at end-exercise (Table 3).

Discussion

The principal findings of this study are that short-term (4-6 days) dietary supplementation with nitrate-rich beetroot juice: increased plasma [nitrite] and reduced systolic blood pressure; reduced the O2 cost of walking, moderate-intensity and severe-intensity running; and increased the time to task failure during both constant-speed severe-intensity running and incremental knee-extension exercise. These results confirm our earlier reports (2, 3, 60) and extend them by demonstrating improved exercise economy and performance during treadmill walking and running. An important advance in the present study was the use of a nitrate-depleted placebo beverage which ensured that the study was conducted in a genuinely double-blind fashion. We found that dietary supplementation with nitrate-depleted beetroot juice did not alter plasma [nitrite], systolic blood pressure, exercise $\dot{V}_{O2}$ or the time to task failure, relative to the non-supplemented control condition. These data therefore indicate that the physiological effects of BR supplementation reported herein and previously (2, 3, 60) are consequent to the high NO3$^-$ content of the beverage. In the present study, we also measured $Q_{max}$ using $^{31}$P-MRS to estimate muscle oxidative capacity. Although there are several assumptions and limitations to this approach (see Effects of dietary nitrate supplementation on mitochondrial capacity), $Q_{max}$ was not significantly different between the control, PL and BR conditions. These data therefore suggest that the enhanced aerobic exercise performance (i.e., increased time to task failure) observed following short-term BR supplementation is unlikely to be related to possible effects of increased NO bioavailability on mitochondrial biogenesis (14, 48).
Effects of dietary nitrate on plasma [nitrite] and blood pressure

The nitrate-rich beetroot supplementation period increased plasma [NO$_2^-$] by +105 % and resulted in a 5 mmHg (4 %) reduction in systolic BP in the normotensive young adults who participated in this study. This change occurred without any alterations in diastolic BP or mean arterial pressure, consistent with another recent study which used a similar intervention (3). Other studies have reported a reduction in both systolic and diastolic pressure after 2.5 h (62), 3 days (40), and 15 days of dietary nitrate supplementation (60). Collectively, these data suggest that the consumption of nitrate-rich vegetables might confer benefits to cardiovascular health (1, 20, 53). Systolic and diastolic blood pressure were unchanged during placebo supplementation suggesting that the reduction in systolic blood pressure with nitrate-rich beetroot juice was mediated through the systemic reduction of nitrate-derived nitrite to NO (6, 11, 18).

Effects of dietary nitrate supplementation on the physiological responses to moderate-intensity exercise

Consistent with previous studies employing other exercise modalities (2, 3, 40, 41, 60), dietary NO$_3^-$ supplementation reduced the O$_2$ cost of sub-maximal running. However, a novel finding in the present study was that dietary NO$_3^-$ intake resulted in a 12% reduction in the O$_2$ cost of walking. This is in contrast to previous studies which have reported no effect of NO$_3^-$ supplementation on $\dot{V}$O$_2$ during very low-intensity exercise, for example, cycling at 20 W (3, 60). The explanation for these differing results is unclear. However, a reduced O$_2$ cost of walking potentially has important implications for enhancing the ability to complete tasks of daily living within elderly and patient populations. These groups can have a significantly reduced $\dot{V}$O$_2$peak (46, 52) and, consequently, the activities of daily living often require them to work towards the higher end of their exercise capacity, resulting in severe metabolic stress. If dietary NO$_3^-$ supplementation were to reduce the O$_2$ cost of these activities, it would have the potential to enhance functional capacity and the quality of life. Further research is required to investigate the effects of dietary NO$_3^-$ supplementation on the O$_2$ cost of walking and functional performance within clinical populations.
The steady-state $\dot{V}O_2$ during moderate-intensity running was reduced by $\sim$7\% following dietary $NO_3^-$ supplementation, whilst the amplitude of the $\dot{V}O_2$ response from baseline to steady-state was reduced by $\sim$4\%. These changes in the $O_2$ cost of moderate exercise are less pronounced than in our previous study where dietary $NO_3^-$ supplementation reduced the steady-state $\dot{V}O_2$ by 10\% and the amplitude of the $\dot{V}O_2$ response by $\sim$20\% during cycle ergometer exercise (3). The reduced effect in the present study is likely to be due, at least in part, to the fact that habitual dietary $NO_3^-$ intake was not restricted during the period of experimentation (60). In previous studies (2, 3, 40, 41), subjects have been instructed to exclude nitrate-rich foods from their diet (such as certain vegetables and cured meats). The present results therefore indicate that the positive effects of $NO_3^-$ supplementation on blood pressure and exercise economy are still present when habitual $NO_3^-$ intake is not restricted.

The reduced $\dot{V}O_2$ during moderate exercise in the present data set was reflected in a significant reduction ($\sim$6\%) in the energy cost required to run 1 km. This result is remarkable as just four days of dietary $NO_3^-$ supplementation elicited improvements in running economy comparable to those observed following 6-9 weeks of physical training (e.g., 27, 49). Superior running economy is associated with enhanced endurance running performance (15, 26, 27). Although not directly tested in the present study, the results suggest that increased dietary $NO_3^-$ intake has the potential to enhance exercise tolerance during longer-term endurance exercise.

In the present investigation, we employed a double-blind experimental design using a placebo beverage with negligible $NO_3^-$ content. The placebo beverage, which was created by passing the juice through an ion-exchange resin which is selective for nitrate ions, was otherwise similar to the experimental beverage in appearance, odour and taste. While we cannot exclude the possibility that this treatment had other effects in addition to the removal of nitrate, there were no appreciable differences in sodium, potassium, calcium, or magnesium concentrations, and the proton NMR spectra of the BR and PL beverages were similar (Gilchrist et al., unpublished). In previous studies, it could only be speculated that the physiological effects of BR consumption were mediated through the reduction of $NO_3^-$ to bioactive $NO_2^-$ and NO. However, beetroot is also rich in several other compounds which might influence human physiology either at rest or during exercise, including betaine, antioxidants,
and polyphenols. It has been reported that betaine supplementation may enhance muscular endurance, strength and power (23, 45). Moreover, two polyphenols found in beets, quercetin and resveratrol, have the potential to increase aerobic capacity through stimulation of mitochondrial biogenesis (17, 38) although this is by no means a consistent finding (16, 19). While we cannot rule out the possibility that NO₃⁻ operates synergistically with one or more of these potentially active compounds, the unchanged plasma [nitrite], blood pressure and $\bar{VO}_2$ response with nitrate-depleted BR supplementation suggests that the physiological effects of BR consumption can be attributed, in large part, to its high NO₃⁻ content. The significant increase in plasma [nitrite] following dietary NO₃⁻ intake combined with a reduced blood pressure suggests that inorganic NO₃⁻ was metabolised in vivo to form NO₂⁻ and to the physiological signalling molecule and potent vasodilator, NO (6, 44). In a recent study, we reported that the reduced $O_2$ cost of moderate-intensity knee-extensor exercise following NO₃⁻ supplementation was linked to a reduction in the ATP cost of muscle force production (2). However, the precise manner by which this occurs, i.e. through an NO-mediated reduction in the activity of actomyosin-ATPase, Ca²⁺-ATPase, and/or Na⁺-K⁺, is presently unclear.

Effects of dietary nitrate supplementation on the physiological responses to severe-intensity exercise

Constant-work-rate exercise performed above the GET results in a progressive loss of muscle efficiency as reflected in the development of a ‘slow component’ of $\bar{VO}_2$ (30) which has been suggested to be related to the process of muscle fatigue (12). An interesting observation in the present study was that there was no change in the amplitude of the $\bar{VO}_2$ slow component following NO₃⁻ supplementation. This is in contrast with previous studies from our laboratory showing a significant reduction of the $\bar{VO}_2$ slow component following dietary nitrate intake in other exercise modalities (2, 3). The $\bar{VO}_2$ slow component amplitude is smaller during running compared to cycling (7, 29), which might make it more difficult to observe changes in this parameter of the $\bar{VO}_2$ kinetics following an intervention. However, the more likely explanation for the unchanged $\bar{VO}_2$ slow component following NO₃⁻ supplementation in the present study is the 6% reduction in $\bar{VO}_2$ at the point of task failure. This reduction in peak $\bar{VO}_2$ is consistent with the observations of Larsen et al. (41) for
combined incremental cycle and arm-crank exercise but contrasts with our previous
findings for cycle (3, 60) and knee-extension exercise (2). The explanation for this
small reduction in peak $\dot{V}_O_2$ in the present study is unclear. However, it is evident that
this effect is only evident during ‘whole-body’ exercise such as running (present
study) or combined cycle/arm-cranking (41) and not during exercise involving a
smaller muscle mass which result in lower, i.e., non-‘maximal’, $\dot{V}_O_2$ values (56).
During whole-body exercise, it is widely accepted that the highest attainable $\dot{V}_O_2$ is
linked to the maximal cardiac output and thus muscle blood flow and $O_2$ delivery (54,
55, 66). Exactly how increased NO availability following dietary NO$_3$–
supplementation might impact on the determinants of $\dot{V}_O_2$ max is unclear. However, it
has been reported that the addition of NO$_3$– to the perfusate in a rat heart model
reduced left ventricular pressure, contractile function and $\dot{V}_O_2$ (51). The reduced $\dot{V}_O_2$
max values reported herein and also by Larsen et al. (41) were not related to a
significant reduction in maximal heart rate, suggesting that stroke volume or arterio-
venous $O_2$ content difference might have been reduced following NO$_3$–
supplementation. Further studies are required to investigate this possibility.

Despite the reduction in $\dot{V}_O_2$ at task failure, NO$_3$– supplementation resulted in a ~15% improvement in the time to task failure during severe-intensity running. This indicates that the enhanced exercise economy following dietary NO$_3$– supplementation was more than sufficient to offset the reduced $\dot{V}_O_2$ max. A ~15% improvement in the time to task failure would be expected to be equivalent to a ~ 1% reduction in the time taken to cover a set distance (24), an effect that is certainly meaningful in elite sport. Importantly, this improvement in time to task failure was evident in all nine subjects.

An enhanced exercise tolerance was also observed during the incremental knee-
extension exercise test. Although the effect was smaller (5% compared to 15%), this is the expected consequence of the different exercise protocols employed (i.e.
incremental vs. constant-work-rate; 60, 65).

Effects of dietary nitrate supplementation on mitochondrial capacity

An elevated NO bioavailability has the potential to increase mitochondrial biogenesis through the activation of the cyclic GMP mediated pathway (14, 48). In the present study, six days of dietary NO$_3$– supplementation resulted in no significant change in
muscle PCr recovery kinetics or $Q_{\text{max}}$. This suggests that the estimated maximal rate of mitochondrial ATP synthesis was unchanged (35) and therefore no substantial mitochondrial biogenesis occurred during the supplementation period. We cannot rule out the possibility that a more prolonged NO$_3^-$ supplementation period might induce mitochondrial biogenesis and thereby enhance aerobic function and exercise performance independently of the more acute effects on muscle efficiency (see ref. 60). It should also be acknowledged that NO has the potential to inhibit mitochondrial respiration (10, 13) which may consequently affect certain assumptions inherent in the calculation of $Q_{\text{max}}$, such as the Km for ADP and the form of the hyperbolic relationship between the oxidative ATP resynthesis rate and ADP concentration (36). Moreover, changes in NO availability might modulate blood flow especially during recovery from exercise (50) and thus impact on the muscle PCr recovery profile (22). If present, these effects would complicate the comparison and interpretation of the estimated $Q_{\text{max}}$ in the BR and PL conditions. However, it is important to note that the initial PCr recovery kinetics, which is considered to be due entirely to oxidative ATP synthesis (8), and the estimated $Q_{\text{max}}$ values were not significantly different from the non-supplemented control condition following either BR or PL despite the expected differences in the potential for NO production between these latter conditions. Additional longer-term studies are needed to explore the possible effects of NO$_3^-$ supplementation on mitochondrial biogenesis and muscle oxidative capacity. However, the present data suggest that the reduced $O_2$ cost of exercise and increased time to task failure during exercise observed after short-term NO$_3^-$ supplementation are related to nitrite- or NO-mediated effects on muscle contractile function (2) rather than to changes in mitochondrial volume.

In conclusion, short-term (4-6 days) dietary NO$_3^-$ supplementation (at ~0.09 mmoL·kg$^{-1}$) significantly increased plasma [nitrite] and reduced systolic blood pressure in normotensive young males consuming a normal, balanced diet. The $\dot{V}O_2$ required for constant-work-rate moderate-intensity and severe-intensity running was reduced (by ~7%), and the time to task failure was increased during both severe-intensity running (by ~15%) and incremental knee-extension exercise (by ~5%). These results might have important implications for athletic performance enhancement. However, a striking finding of the present study was the appreciable (12-14%) reduction in the $O_2$ cost of walking following NO$_3^-$ supplementation. For
senescent populations or individuals with pulmonary, cardiovascular or metabolic disorders, a reduction in the O$_2$ cost of daily activities might significantly improve functional capacity. Relative to the control (non-supplemented) condition, nitrate-depleted BR did not alter the physiological variables of interest either at rest or during exercise. These data indicate that the positive physiological effects of BR ingestion on blood pressure and exercise performance are consequent to the high NO$_3^-$ content.
References


28. Jones AM, Doust JH. A 1% treadmill grade most accurately reflects the


**Figure 1.** Schematic illustration of exercise test protocol.

**Figure 2.** Pulmonary oxygen uptake ($\dot{V}O_2$) responses during step increments to a moderate-intensity running speed. The upper panel (A) shows the group mean responses following the non-supplemented control (grey rhombus) and placebo supplementation (open circles) with error bars shown every 30 s for clarity. The lower panel (B) shows the group mean $\dot{V}O_2$ response following nitrate-rich beetroot (filled circles) and placebo (open circles) supplementation. The dashed vertical line represents the abrupt transition to the moderate work rate from a baseline walking pace. Notice that the $O_2$ cost of walking and running is reduced following nitrate-rich beetroot juice supplementation but not following nitrate-depleted beetroot juice supplementation.

**Figure 3.** Pulmonary oxygen uptake ($\dot{V}O_2$) responses during step increments to a severe-intensity running speed. The upper panel (A) shows the group mean $\dot{V}O_2$ responses following the non-supplemented control (grey rhombus) and placebo supplementation (open circles) with error bars shown every 30 s for clarity. The lower panel (B) shows the group mean $\dot{V}O_2$ response following nitrate-rich beetroot (filled circles) and placebo (open circles) supplementation. The dashed vertical line represents the abrupt transition from baseline walking to the severe work rate. The group mean ± SEM $\dot{V}O_2$ at task failure is also shown (* = Dietary nitrate supplementation resulted in an increased time to task failure). Notice that the $O_2$ cost of walking and running is reduced following nitrate-rich beetroot juice supplementation but not following nitrate-depleted beetroot juice supplementation.

**Figure 4.** The intramuscular [PCr] during the 42-s bout of exercise and subsequent recovery in a representative subject (#6). Notice that the [PCr] responses in control (closed circles), placebo (open circles) and beetroot conditions (open triangles) are superimposed during the exercise bout and the following recovery. The time constant for the [PCr] recovery kinetics was 22, 23, and 23 s, for the control, placebo and beetroot conditions, respectively.
Table 1. Mean ± SD oxygen uptake ($\dot{V}_{O_2}$) dynamics during moderate-intensity and severe-intensity exercise following the non-supplemented control condition and supplementation with nitrate-rich beetroot juice and placebo

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Placebo</th>
<th>Beetroot</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Moderate-intensity exercise</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Oxygen uptake ($\dot{V}_{O_2}$)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking baseline (L·min$^{-1}$)</td>
<td>0.86 ± 0.14</td>
<td>0.87 ± 0.12</td>
<td>0.77 ± 0.10*</td>
</tr>
<tr>
<td>End-exercise (L·min$^{-1}$)</td>
<td>2.20 ± 0.28</td>
<td>2.26 ± 0.27</td>
<td>2.10 ± 0.28*</td>
</tr>
<tr>
<td>Phase II Time Constant (s)</td>
<td>24 ± 5</td>
<td>26 ± 5</td>
<td>25 ± 5</td>
</tr>
<tr>
<td>Mean Response Time (s)</td>
<td>36 ± 6</td>
<td>36 ± 6</td>
<td>36 ± 4</td>
</tr>
<tr>
<td>Primary Amplitude (L·min$^{-1}$)</td>
<td>1.33 ± 0.17</td>
<td>1.37 ± 0.19</td>
<td>1.32 ± 0.23 #</td>
</tr>
<tr>
<td><strong>Expired Carbon Dioxide ($\dot{V}_{CO_2}$)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Walking baseline (L·min$^{-1}$)</td>
<td>0.71 ± 0.16</td>
<td>0.67 ± 0.13</td>
<td>0.67 ± 0.10</td>
</tr>
<tr>
<td>End-exercise (L·min$^{-1}$)</td>
<td>1.82 ± 0.37</td>
<td>1.95 ± 0.32</td>
<td>1.87 ± 0.24</td>
</tr>
<tr>
<td><strong>Minute Ventilation ($\dot{V}_E$)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking baseline (L·min$^{-1}$)</td>
<td>22.0 ± 4.2</td>
<td>21.0 ± 4.2</td>
<td>20.0 ± 2.4</td>
</tr>
<tr>
<td>End-exercise (L·min$^{-1}$)</td>
<td>48.4 ± 8.8</td>
<td>50.3 ± 7.5</td>
<td>48.0 ± 6.7</td>
</tr>
<tr>
<td><strong>Respiratory Exchange Ratio</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking baseline</td>
<td>0.80 ± 0.11</td>
<td>0.75 ± 0.08</td>
<td>0.78 ± 0.05</td>
</tr>
<tr>
<td>End-exercise</td>
<td>0.86 ± 0.04</td>
<td>0.83 ± 0.05</td>
<td>0.86 ± 0.03</td>
</tr>
<tr>
<td><strong>Severe-intensity exercise</strong></td>
<td></td>
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</tr>
<tr>
<td><strong>Oxygen uptake ($\dot{V}_{O_2}$)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking baseline (L·min$^{-1}$)</td>
<td>0.86 ± 0.13</td>
<td>0.91 ± 0.13</td>
<td>0.78 ± 0.09 #</td>
</tr>
<tr>
<td>End-exercise (L·min$^{-1}$)</td>
<td>3.64 ± 0.62</td>
<td>3.77 ± 0.57</td>
<td>3.50 ± 0.62*</td>
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<tr>
<td>Exhaustion (L·min$^{-1}$)</td>
<td>3.82 ± 0.53</td>
<td>3.89 ± 0.57</td>
<td>3.67 ± 0.65*</td>
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<tr>
<td>Phase II Time Constant (s)</td>
<td>22 ± 4</td>
<td>21 ± 3</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>Primary Amplitude (L·min$^{-1}$)</td>
<td>2.53 ± 0.32</td>
<td>2.58 ± 0.36</td>
<td>2.50 ± 0.40</td>
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<td>Slow phase Amplitude (L·min$^{-1}$)</td>
<td>0.36 ± 0.14</td>
<td>0.38 ± 0.16</td>
<td>0.37 ± 0.19</td>
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<tr>
<td>Slow phase Amplitude (%)</td>
<td>12 ± 4</td>
<td>13 ± 4</td>
<td>12 ± 5</td>
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<tr>
<td>Overall Mean Response Time (s)</td>
<td>47 ± 6</td>
<td>49 ± 4</td>
<td>48 ± 4</td>
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<tr>
<td><strong>Expired Carbon Dioxide ($\dot{V}_{CO_2}$)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking baseline (L·min$^{-1}$)</td>
<td>0.74 ± 0.14</td>
<td>0.81 ± 0.14</td>
<td>0.84 ± 0.14</td>
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<tr>
<td>End-exercise (L·min$^{-1}$)</td>
<td>3.98 ± 0.55</td>
<td>3.92 ± 0.56</td>
<td>3.93 ± 0.49</td>
</tr>
<tr>
<td><strong>Minute Ventilation ($\dot{V}_E$)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Walking baseline (L·min$^{-1}$)</td>
<td>23.5 ± 4.1</td>
<td>24.9 ± 4.1</td>
<td>25.4 ± 4.4</td>
</tr>
<tr>
<td>End-exercise (L·min$^{-1}$)</td>
<td>129.1 ± 23.8</td>
<td>127.6 ± 30.8</td>
<td>124.6 ± 22.0</td>
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<tr>
<td><strong>Respiratory Exchange Ratio</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking baseline</td>
<td>0.85 ± 0.08</td>
<td>0.88 ± 0.09</td>
<td>0.88 ± 0.07</td>
</tr>
<tr>
<td>End-exercise</td>
<td>1.05 ± 0.06</td>
<td>1.02 ± 0.04</td>
<td>1.05 ± 0.06</td>
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</table>

# = significantly different from control and placebo (P<0.05); * = significantly different from control and placebo (P<0.01)
Table 2. Mean ± SD heart rate and blood lactate responses to moderate-intensity and severe-intensity exercise following the control condition (no supplementation) and supplementation with nitrate-rich beetroot juice and placebo

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Placebo</th>
<th>Beetroot</th>
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<tbody>
<tr>
<td><strong>Moderate-intensity exercise</strong></td>
<td></td>
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</tr>
<tr>
<td><strong>Heart rate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking baseline (b·min⁻¹)</td>
<td>89 ± 13</td>
<td>93 ± 10</td>
<td>88 ± 12</td>
</tr>
<tr>
<td>End (b·min⁻¹)</td>
<td>136 ± 16</td>
<td>139 ± 13</td>
<td>133 ± 15</td>
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<tr>
<td>Time Constant (s)</td>
<td>56 ± 55</td>
<td>33 ± 18</td>
<td>40 ± 23</td>
</tr>
<tr>
<td>Amplitude (b·min⁻¹)</td>
<td>47 ± 8</td>
<td>44 ± 8</td>
<td>44 ± 6</td>
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<td><strong>Blood [Lactate]</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Walking baseline (mM)</td>
<td>1.1 ± 0.4</td>
<td>1.2 ± 0.5</td>
<td>1.2 ± 0.4</td>
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<tr>
<td>End (mM)</td>
<td>1.6 ± 0.5</td>
<td>1.6 ± 0.6</td>
<td>1.7 ± 0.9</td>
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<tr>
<td>∆ (mM)</td>
<td>0.5 ± 0.5</td>
<td>0.3 ± 0.5</td>
<td>0.5 ± 0.7</td>
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<tr>
<td><strong>Severe-intensity exercise</strong></td>
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<td></td>
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</tr>
<tr>
<td><strong>Heart rate</strong></td>
<td></td>
<td></td>
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<tr>
<td>Walking baseline (b·min⁻¹)</td>
<td>104 ± 15</td>
<td>111 ± 11</td>
<td>105 ± 14</td>
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<td>End (b·min⁻¹)</td>
<td>192 ± 6</td>
<td>191 ± 4</td>
<td>188 ± 4</td>
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<tr>
<td>Exhaustion (b·min⁻¹)</td>
<td>197 ± 8</td>
<td>195 ± 4</td>
<td>196 ± 4</td>
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<td>Time Constant (s)</td>
<td>26 ± 19</td>
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<td>28 ± 13</td>
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<td><strong>Blood [Lactate]</strong></td>
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<tr>
<td>Walking baseline (mM)</td>
<td>1.0 ± 0.6</td>
<td>1.3 ± 0.6</td>
<td>1.1 ± 0.6</td>
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<tr>
<td>End (mM)</td>
<td>8.1 ± 1.7</td>
<td>7.1 ± 0.9</td>
<td>8.0 ± 2.2</td>
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<tr>
<td>∆ (mM)</td>
<td>7.0 ± 1.9</td>
<td>5.8 ± 0.9</td>
<td>6.1 ± 3.1</td>
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<td>Exhaustion (mM)</td>
<td>8.6 ± 1.4</td>
<td>8.6 ± 0.8</td>
<td>9.0 ± 1.1</td>
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Table 3. Mean ± SD muscle metabolite concentrations and pH at resting baseline and during the exercise protocol following the control condition (no supplementation), placebo and nitrate-rich beetroot juice supplementation. See text for details.

<table>
<thead>
<tr>
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<tr>
<td><strong>Baseline</strong></td>
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<tr>
<td>PCr (mM)</td>
<td>33.0 ± 2.7</td>
<td>32.4 ± 2.8</td>
<td>32.4 ± 3.0</td>
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<tr>
<td>P_i (mM)</td>
<td>3.7 ± 0.5</td>
<td>3.8 ± 0.4</td>
<td>3.9 ± 0.5</td>
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<tr>
<td>ADP (µM)</td>
<td>5.8 ± 0.9</td>
<td>6.6 ± 1.2</td>
<td>6.2 ± 1.6</td>
</tr>
<tr>
<td>pH</td>
<td>7.0 ± 0.0</td>
<td>7.1 ± 0.0</td>
<td>7.1 ± 0.0</td>
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<tr>
<td><strong>End-exercise (42 s bout)</strong></td>
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<td></td>
</tr>
<tr>
<td>PCr (mM)</td>
<td>23.5 ± 1.7</td>
<td>23.5 ± 1.2</td>
<td>23.1 ± 1.4</td>
</tr>
<tr>
<td>P_i (mM)</td>
<td>10.2 ± 1.6</td>
<td>10.5 ± 1.7</td>
<td>10.8 ± 1.8</td>
</tr>
<tr>
<td>ADP (µM)</td>
<td>32.5 ± 7.8</td>
<td>36.8 ± 5.6</td>
<td>38.6 ± 9.1</td>
</tr>
<tr>
<td>pH</td>
<td>7.0 ± 0.0</td>
<td>7.0 ± 0.0</td>
<td>7.0 ± 0.0</td>
</tr>
<tr>
<td><strong>End of recovery</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCr (mM)</td>
<td>33.0 ± 2.7</td>
<td>32.3 ± 2.8</td>
<td>32.4 ± 3.0</td>
</tr>
<tr>
<td>P_i (mM)</td>
<td>3.7 ± 0.6</td>
<td>3.9 ± 0.4</td>
<td>4.0 ± 0.5</td>
</tr>
<tr>
<td>ADP (µM)</td>
<td>6.0 ± 0.8</td>
<td>6.5 ± 0.7</td>
<td>6.2 ± 1.3</td>
</tr>
<tr>
<td>pH</td>
<td>7.0 ± 0.0</td>
<td>7.1 ± 0.0</td>
<td>7.0 ± 0.0</td>
</tr>
<tr>
<td><strong>End-exercise (ramp test)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCr (mM)</td>
<td>14.5 ± 4.6</td>
<td>14.0 ± 2.7</td>
<td>12.7 ± 4.9</td>
</tr>
<tr>
<td>P_i (mM)</td>
<td>15.8 ± 7.1</td>
<td>15.4 ± 4.8</td>
<td>15.4 ± 4.3</td>
</tr>
<tr>
<td>ADP (µM)</td>
<td>57.6 ± 15.6</td>
<td>91.3 ± 57.5</td>
<td>82.1 ± 50.0</td>
</tr>
<tr>
<td>pH</td>
<td>6.7 ± 0.2</td>
<td>6.8 ± 0.2</td>
<td>6.8 ± 0.1</td>
</tr>
</tbody>
</table>
Figure 1

Key:

- Venous blood sample and blood pressure measurement
- Capillary blood sample

6 min walk at 4 km.h⁻¹
6 min 80% GET
10 min walk at 4 km.h⁻¹
6 min 80% GET
10 min walk at 4 km.h⁻¹
6 min 75% Δ

Continued until volitional exhaustion on day 4 of supplementation
Figure 3