Dietary nitrate supplementation: effects on plasma nitrite and pulmonary O_2 uptake dynamics during exercise in hypoxia and normoxia

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Abstract

We investigated the effects of dietary nitrate (NO_3^-) supplementation on the concentration of plasma nitrite ([NO_2^-]), oxygen uptake ($\dot{V}O_2$) kinetics and exercise tolerance in normoxia (N) and hypoxia (H). In a double-blind, crossover study, twelve healthy subjects completed cycle exercise tests, twice in N (20.9% O_2) and twice in H (13.1% O_2). Subjects ingested either 140 $ml \cdot d^{-1}$ of NO_3^--rich beetroot juice (8.4 mmol NO_3; BR) or NO_3^--depleted beetroot juice (PL) for 3-days prior to moderate-intensity and severe-intensity exercise tests in H and N. Pre-exercise plasma [NO_2^-] was significantly elevated in H-BR and N-BR compared to H-PL (P = 0.00) and N-PL (P = 0.00). The rate of decline in plasma [NO_2^-] was greater during severe-intensity exercise in H-BR (-30±22 $nM \cdot min^{-1}$, 95% *CI*; -44, -16) compared to H-PL (-7±10 $nM \cdot min^{-1}$, 95% *CI*; -13, -1; P = 0.00) and in N-BR (-26±19 $nM \cdot min^{-1}$, 95% *CI*; -38, -14) compared to N-PL (-1±6 $nM \cdot min^{-1}$, 95% *CI*; -5, 2; P = 0.00). During moderate-intensity exercise, steady-state pulmonary $\dot{V}O_2$ was lower in H-BR (1.91±0.28 $L \cdot min^{-1}$, 95% *CI*; 1.77, 2.13) compared to H-PL (2.05±0.25 $L \cdot min^{-1}$, 95% *CI*; 1.93, 2.26, P = 0.02) and $\dot{V}O_2$ kinetics was faster in H-BR (τ: 24±13 s, 95% *CI*; 15, 32) compared to H-PL (31±11 s, 95% *CI*; 23, 38; P = 0.04). NO_3^- supplementation had no significant effect on $\dot{V}O_2$ kinetics during severe-intensity exercise in hypoxia, or during moderate-intensity or severe-intensity exercise in normoxia. Tolerance to severe-intensity exercise was improved by NO_3^- in hypoxia (H-PL: 197±28; 95% *CI*; 173, 220 vs. H-BR: 214±43 s, 95% *CI*; 177, 249; P = 0.04) but not normoxia. The metabolism of NO_2^- during exercise is altered by NO_3^- supplementation, exercise and to a lesser extent, hypoxia. In hypoxia, NO_3^- supplementation enhances $\dot{V}O_2$ kinetics during moderate-intensity exercise and improves severe-intensity exercise tolerance. These findings may have important implications for individuals exercising at altitude.

Key Words: hypoxia; beetroot juice; nitric oxide, efficiency, performance.

Introduction

Nitric oxide (NO) is a ubiquitous, water soluble, free radical gas which plays a crucial role in many biological processes. Effective NO production is important in normal physiological functioning, from the regulation of blood flow, muscle contractility and mitochondrial respiration, to host defence, neurotransmission and glucose and calcium homeostasis (11, 17, 60). NO production via the oxidation of L-arginine, in a process catalysed by nitric oxide synthase (NOS), may be blunted in conditions of reduced O_2 availability (52). It is now widely accepted that NO can also be generated via an alternative pathway, whereby inorganic nitrate (NO_3^-) is reduced to nitrite (NO_2^-) and further to NO. This NOS- and O_2- independent NO_3^- -NO_2^- -NO pathway represents a complementary system for NO synthesis spanning a broad range of redox states (49). In addition to being produced endogenously, the body's NO_3^- stores can be increased via the diet, with green leafy vegetables and beetroot being particularly rich in NO_3^-. Upon ingestion, inorganic NO_3^- is absorbed from the gut and passes into the systemic circulation where ~25% of it is concentrated in the saliva (50). Commensal bacteria in the oral cavity then reduce the NO_3^- to NO_2^- (21). Some salivary NO_2^- is converted into NO when swallowed into the acidic environment of the stomach (7), whilst the remainder is absorbed, increasing circulating plasma NO_2^- concentration [NO_2^-]. This NO_2^- may be reduced to NO via a number of enzymatic and non-enzymatic pathways (e.g., xanthine oxidoreductase and deoxyhemoglobin), which are potentiated in hypoxic environments, such as may be evident in contracting skeletal muscle (55).

NO plays a key role in the physiological response and adaptation to hypoxia. A reduced fraction of O_2 in inspired air results in reductions in arterial O_2 concentration and intracellular partial pressure of O_2 (PO_2). The development of muscle hypoxia leads to increased metabolic perturbation (46) and reduced functional capacity at altitude (2) and in several disease conditions (22, 34). In order to restore sufficient O_2 supply, local blood flow is increased via hypoxia-induced vasodilatation with NO being implicated as a major mediator of this process (12). NO_2^- may also promote hypoxic vasodilatation in an NO-independent manner (16).

Dietary NO_3^- supplementation, in the form of nitrate salts and nitrate-rich beetroot juice (BR), represents a practical method of increasing circulating plasma [NO_3^-] (31, 42, 67) and [NO_2^-] (4, 33, 62). NO_3^- supplementation has been shown to reduce resting blood pressure (3, 33, 42) and oxygen uptake ($\dot{V}O_2$) during submaximal exercise (4, 39, 40, 41, 62, 67), and to improve

exercise performance in young, healthy individuals exercising in normoxic conditions (14, 38), but not necessarily in well trained athletes (5-6, 66). These changes may be related to NO-mediated alterations in mitochondrial efficiency (39), muscle contractile function (3, 28) and enhanced muscle blood flow, with preferential distribution to type II fibers (23). These physiological alterations could be particularly beneficial when normal O_2 availability (~21%) is reduced. Indeed, NO_3^- supplementation in the form of BR has recently been shown to reduce muscle metabolic perturbation during exercise in hypoxia and to restore constant-work-rate exercise tolerance and post-exercise indices of oxidative function to values observed in normoxia (64). BR supplementation has also been shown to extend incremental exercise tolerance, improve arterial and skeletal muscle oxygenation (50), and to enhance cycling economy and time-trial performance (51), in hypoxia. However, while these studies suggest that BR can improve physiological responses and exercise performance in hypoxia, it has yet to be determined whether the effects BR are more pronounced in hypoxia relative to normoxia.

The dose-response and pharmacodynamic relationships of BR supplementation have recently been investigated in normoxia (67) and provides a guide to enable optimal timing and dosing of BR intake to elicit peak circulating plasma $[NO_2^-]$ values. However, the kinetics of plasma $[NO_2^-]$ during hypoxic exercise and subsequent recovery, and possible changes elicited by BR supplementation, are presently not known. It was recently reported that during high-intensity, intermittent running exercise, plasma $[NO_2^-]$ declined significantly during exhaustive exercise and showed a tendency to recover back to baseline following 15 min of passive rest (68). Previous research has reported increases (1, 54) but, more commonly, decreases (6, 19, 26, 42, 63) in plasma $[NO_2^-]$ during exercise. In addition to exercise, the metabolism of NO and its derivatives are known to be influenced by intracellular PO_2 and the fraction of inspired oxygen (FIO_2). *In vitro*, endothelial NOS (eNOS) expression and eNOS-derived NO production in human endothelial cells are reduced in hypoxia (25, 53). However, *in vivo*, eNOS expression and activity can be up- or down-regulated by hypoxia, with both decreased (58) and increased (44, 48) NO bioavailability being reported in hypoxia. Characterizing the kinetic changes in $[NO_2^-]$ during exercise and recovery at different FIO_2 may offer insight into NO metabolism during exercise in normoxia and hypoxia. This understanding may have important implications for athletes exercising in hypoxic environments.

Considering that the NO_3^--NO_2^--NO pathway is facilitated in hypoxic conditions (48), we reasoned that BR supplementation may modulate the changes in $[NO_2^-]$ during exercise and recovery and may help to ameliorate the negative effects of hypoxia on exercise tolerance. The primary aim of this study was to investigate the effects of BR supplementation on physiological responses (plasma $[NO_2^-]$ dynamics, pulmonary $\dot{V}_{O_2}$ and muscle oxygenation) and exercise tolerance, in both normoxia and hypoxia. We hypothesized that the reduction of $[NO_2^-]$ during exercise would be greater in hypoxia compared to normoxia but that $[NO_2^-]$ would be higher at the same iso-time during exercise following BR compared to PL supplementation. We also hypothesized that BR supplementation would improve moderate-intensity exercise economy and severe-intensity exercise tolerance in both hypoxia and normoxia, with greater effects being evident in hypoxia.

Methods

Subjects

Twelve physically active male subjects (mean ± SD; age = 22 ± 4 yr, height = 1.80 ± 0.06 m, body mass = 78 ± 6 kg, $\dot{V}_{O_{2peak}}$ = 58.3 ± 6.3 $mL \cdot kg^{-1} \cdot min^{-1}$) volunteered to take part in this study. The protocol and procedures used in this study were approved by the Institutional Research Ethics Committee. All subjects gave written, fully informed consent prior to commencement of the study, once the experimental protocol, associated risks, and potential benefits of participation had been outlined. Subjects were instructed to arrive at the laboratory, at least 3 h postprandial, and to avoid strenuous exercise in the 24 h preceding each testing session. Subjects were 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 h before testing. In addition to this, subjects were asked to abstain from using antibacterial mouthwash and chewing gum for the duration of the study since this has been shown to blunt the conversion of NO_3^- to NO_2^- in the oral cavity (27). Subjects were also instructed to maintain their normal dietary intake for the duration of the study. All exercise tests were performed at the same time of day (± 1 h) for each subject.

Procedures

Subjects were required to attend the laboratory on six occasions over a 4-wk period. All exercise tests were performed using an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands). During *visit 1*, subjects completed a ramp

incremental test to exhaustion for the determination of the maximal O_2 uptake ($\dot{V}O_{2peak}$) and the gas exchange threshold (GET). Subjects performed 3 min of baseline cycling at 20 W and 80 rpm, after which the power output was increased at a rate of 30 $W \cdot min^{-1}$ in a linear fashion until volitional exhaustion. The height and configuration of the saddle and handlebars were recorded and reproduced in subsequent tests. The breath-by-breath pulmonary gas-exchange data were collected continuously during the incremental test and averaged over 10-s periods. $\dot{V}O_{2peak}$ was determined as the highest mean $\dot{V}O_2$ during any 30-s period. The GET was determined from a number of measurements, including: *1)* the first disproportionate increase in CO_2 production ($\dot{V}cO_2$) from visual inspection of individual plots of $\dot{V}cO_2$ and $\dot{V}O_2$; and *2)* an increase in expired ventilation ($\dot{V}_E / \dot{V}O_2$) with no increase in $\dot{V}_E/\dot{V}cO_2$. Power outputs representing moderate- and severe-intensity exercise for each individual were calculated, with account taken of the mean response time for $\dot{V}O_2$ during ramp exercise (i.e., two-thirds of the ramp rate was deducted from the power output at GET).

All subjects were familiar with laboratory exercise testing procedures, having previously participated in studies employing cycle ergometry in our laboratory. *Visit 2* served as a familiarization to exercising in normobaric hypoxia. Following completion of the familiarization session, subjects were randomly assigned to receive 3 days of dietary supplementation with 140 $ml \cdot d^{-1}$ of NO_3^--rich BR or 140 $ml \cdot d^{-1}$ of NO_3^--depleted BR concentrate as a placebo (PL), (see 'Supplementation' below), prior to the subsequent exercise trials.

During *visits 3-6*, the subjects completed step-transition, cycling exercise for the determination of pulmonary $\dot{V}O_2$ and plasma [NO_2^-] kinetics. In total, there were four different experimental conditions: 1) Hypoxia-BR (H-BR); 2) Hypoxia-PL (H-PL); 3) Normoxia-BR (N-BR); and 4) Normoxia-PL (N-PL). Trial order was randomly assigned in a balanced fashion such that three subjects started on H-BR, three started on H-PL, three started on N-BR and three started on the N-PL condition.

Upon arrival at the laboratory, a cannula (Insyte-W ^{TM} Becton-Dickinson, Madrid, Spain) was inserted into the subject's antecubital vein to enable frequent blood sampling before, during and after the exercise protocol. Prior to the exercise protocol, subjects lay in a supine position for 10 min breathing normoxic inspirate. A further 10-min period elapsed with subjects breathing either the hypoxic or normoxic inspirate. The exercise protocol involved two 5-min bouts of moderate-intensity cycling at 80% GET, and one bout of severe-intensity

cycling at 75% Δ (a power output representing GET plus 75% of the difference between the power outputs at GET and $\dot{V}_{O_{2peak}}$) (65) which was continued to volitional exhaustion. Each exercise bout involved an abrupt transition to the target power output initiated from a 20 W baseline, with the three exercise bouts separated by 6 min of passive recovery. The severe-intensity exercise bout was continued until task failure as a measure of exercise tolerance. The time to exhaustion was recorded when the pedal rate fell by > 10 rpm below the 80 rpm pedal rate. In these bouts, the subjects were verbally encouraged to continue for as long as possible. Following exhaustion, a further 10-min recovery period elapsed with subjects continuing to breathe either the hypoxic or normoxic inspirate.

The $\dot{V}_{O_2}$ responses for the two moderate bouts were averaged before analysis to reduce breath-to-breath noise and enhance confidence in the parameters derived from the modelling process (36). Blood was sampled pre-exercise (prior to any exercise and breathing of experimental inspirate), then during the baseline 20 W cycling preceding the first moderate transition (ModBL) and at 1(Mod1), 3 (Mod3) and 5 (Mod5) min of the first moderate-intensity exercise bout. Further samples were drawn during the 20 W baseline preceding the severe transition (SevBL) and after 1 (Sev1) and 3 (Sev3) min of severe-intensity exercise and at exhaustion (Exh). Finally, samples were drawn during recovery from the severe bout at 1.5 (Rec1.5), 3 (Rec3) and 10 (Rec10) min.

Inspirate

The inspirate was generated using a Hypoxico HYP 100 filtration system (Sporting Edge UK Ltd, Basingstoke, UK), with the generator supplying the inspirate via an extension conduit to a 150 L Douglas Bag (Cranlea & Co., Birmingham, UK). This acted as a reservoir and mixing chamber, and had a separate outlet tube feeding into a two-way breathing valve system (Hans Rudolph, Cranlea & Co.). The two-way valve was connected to the mouthpiece which provided a constant, unidirectional flow rate and ensured that no re-breathing of expired air occurred. The O_2 and CO_2 concentration of the inspirate was monitored during each test using a Servomex 5200 High Accuracy Paramagnetic O_2 and CO_2 Analyzer (Servomex, Crowborough, UK). The gas analyzer was calibrated prior to each test with a 16.0% O_2, 8.0% CO_2 and 76.0% N_2 gas mix (BOC Special Gases, Guildford, UK). For the N-PL and N-BR trials, the Hypoxico HYP-100 generator was switched to normoxic mode (i.e. all O_2 filters were turned off so that no O_2 was removed from the ambient air). However,

during the H-PL and H-BR trials, the generator was set to maximum O_2 filtration, which supplied an FIO_2 of 0.131 ± 0.02, and an $FICO_2$ of 0.004 ± 0.00.

Supplementation

After completion of the non-supplemented *visits 1 and 2*, subjects were assigned in a double-blind, randomized, crossover design to receive a course of dietary NO_3^- supplementation before *visits 3-6*. The supplements were either concentrated, NO_3^--rich BR (2 x 70 $mL \cdot d^{-1}$ of BR providing ~8.4 mmol NO_3^- per day; Beet it, James White Drinks, Ipswich, UK) or concentrated, NO_3^--depleted PL (2 x 70 $ml \cdot d^{-1}$ of PL providing ~0.006 mmol NO_3^- per day; Beet it, James White Drinks, Ipswich, UK). The PL beverage was created by passing the juice, before pasteurization, through a column containing Purolite A520E ion exchange resin, which selectively removes nitrate ions. The PL was identical to the BR in appearance, taste and smell. Subjects were instructed to consume the beverages in the morning and afternoon of days 1 and 2 of supplementation, and then in the morning and 2.5 h before the exercise test on day 3. A washout period of at least 72 h separated each supplementation period. Subjects were instructed to follow their normal dietary habits throughout the testing period and to replicate their diet and timing of supplementation across conditions. Subjects were informed that the supplementation may cause beeturia (red urine) and red stools temporarily but that this side effect was harmless.

Measurements

Blood samples were drawn into 5-ml lithium-heparin tubes (Vacutainer, Becton-Dickinson, New Jersey, USA). 200 µl of blood was immediately hemolyzed in 200 µl of cold Triton X-100 buffer solution (Triton X-100, Amresco, Salon, OH) and analyzed to determine blood [lactate] and [glucose] (YSI 2300, Yellow Springs Instruments, Yellow Springs, OH). Blood samples for the determination of plasma [NO_2^-] and [NO_3^-] were collected into lithium-heparin tubes and immediately centrifuged at 4000 rpm and 4 °C for 8 min. Plasma was extracted and immediately frozen at -80 °C for later analysis of [NO_2^-] and [NO_3^-].

Prior to and regularly during analysis, all glassware, utensils, and surfaces were rinsed with deionized water to remove any residual NO_2^-. Plasma [NO_2^-] and [NO_3^-] were analysed using gas phase chemiluminescence. This initially required NO_2^- and NO_3^- to be reduced to NO gas. For reduction of NO_2^-, undiluted plasma was injected into a glass purge vessel containing 5 ml glacial acetic acid and 1ml NaI solution. For NO_3^- reduction, plasma samples were

deproteinized in an aqueous solution of zinc sulphate (10% w/v) and 1 M sodium hydroxide, prior to reduction to NO in a solution of vanadium (III) chloride in 1 M hydrochloric acid (0.8% w/v). Quantification of NO was enabled by the detection of light emitted during the production of nitrogen dioxide formed upon reaction of NO with ozone. Luminescence was detected by a thermoelectrically cooled, red-sensitive photomultiplier tube housed in a Sievers gas-phase chemiluminescence NO analyzer (Sievers NOA 280i, Analytix Ltd, Durham, UK). The concentrations of NO_2^- and NO_3^- were determined by plotting signal area (mV) against a calibration plot of 25nM to 1μM sodium nitrite and 100nM to 10μM sodium nitrate respectively.The rate of change in plasma [NO_2^-] during the severe exercise bout was calculated as the difference between pre-exercise baseline and exercise [NO_2^-] values relative to exercise duration.

During all laboratory 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 (Triple V, Jaeger, Hoechburg, 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, Hoechburg, Germany) via a capillary line connected to the mouthpiece. Pulmonary gas exchange variables were calculated and displayed breath-by-breath. Heart rate (HR) and arterial oxygen saturation (SaO_2) were continuously measured during the test protocol using a pulse oximeter device (Rad-87, Masimo, Irvine, CA), which was attached to the subject's right index finger.

The oxygenation status of the *m. vastus lateralis* of the right leg was monitored via near infrared spectroscopy (NIRS) (NIRO 200, Hamamatsu Photonics KK, Hamamatsu-City, Japan) during the exercise protocol, as described previously (4). Deoxyhemoglobin concentration ([HHb]), oxyhemoglobin concentration ([HbO_2]), total hemoglobin concentration ([Hb_{tot}]) and tissue oxygenation index (TOI) were measured.

Data analysis

The breath-by-breath $\dot{V}_{O_2}$ data from each exercise test were initially examined to exclude errant breaths caused by coughing and swallowing with those values lying more than four SD from the local mean being removed. The breath-by-breath data were subsequently linearly interpolated to provide second-by-second values, and, for each individual, identical moderate-intensity repetitions were time-aligned to the start of exercise and ensemble-averaged. This approach enhances the signal-to-noise ratio and improves confidence in the

parameters derived from the modelling process. The first 20 s of data after the onset of exercise (the phase I response) were deleted, and a non-linear least squares algorithm was used to fit the data thereafter. A single-exponential model was used to characterize the phase II $\dot{V}O_2$ responses to both moderate- and severe- intensity exercise, as described in following equation:

$$\dot{V}O_2(t) = \dot{V}O_{2\ baseline} + A_p\,[1 - e^{-(t-TDp/\tau p)}] \qquad \text{Eqn.1}$$

Where $\dot{V}O_2(t)$ represents the absolute $\dot{V}O_2$ at a given time *t*; $\dot{V}O_{2\ baseline}$ represents the mean $\dot{V}O_2$ over the final 60 s of baseline cycling; A_p, TD_p, and τ_p represent the amplitude, time delay and time constant, respectively, describing the phase II increase in $\dot{V}O_2$ above baseline. An iterative process was used to minimize the sum of the squared errors between the fitted function and the observed values. The end-exercise $\dot{V}O_2$ was defined as the mean $\dot{V}O_2$ measured over the final 30 s of exercise.

The fitting strategy was subsequently used to identify the onset of any 'slow component' in the $\dot{V}O_2$ response to severe-intensity exercise as previously described (56). The fitting window was lengthened iteratively until the exponential model-fit demonstrated a discernible departure from the measured response profile. Identification, via visual inspection, of the flat residual plot profile (signifying a good fit to measured data) systematically differing from zero, gave indication of the delayed slow component onset. The magnitude of the slow component for $\dot{V}O_2$ was measured as the difference between the phase II steady state amplitude and the final $\dot{V}O_2$ value, averaged over the last 30 s of exercise.

To obtain information on muscle oxygenation, the [HHb] response to exercise was also modelled, as described previously (4). The [HHb] kinetics for moderate- and severe-intensity exercise were determined using a single-exponential model similar to that described above (Eqn. 1), with the exception that the fitting window commenced at the time at which the [HHb] signal increased 1 SD above the baseline mean (18). For moderate-intensity exercise, the fitting window was constrained to the point at which mono-exponentiality became distorted, consequent to a gradual fall in [HHb], as determined by visual inspection of the residual plots. For severe-intensity exercise, the [HHb] fast and slow phase responses were determined as described above for the $\dot{V}O_2$. The $[HbO_2]$, $[Hb_{tot}]$ and TOI responses were not modelled as they do not approximate an exponential. Rather, the changes in these variables were assessed by determining the $[HbO_2]$, $[Hb_{tot}]$ and TOI at baseline (60 s preceding step

transition), at 120 s and at end-exercise during moderate exercise and at baseline, 60 s, 120 s and exhaustion for severe exercise.

Statistical analyses

Differences in the cardio-respiratory, NIRS-derived, pulse-oximetry and exercise tolerance variables between conditions were analyzed using two-way (supplement x FIO_2) repeated measures ANOVA. Blood metabolites were analyzed via two-way (condition x time) repeated measures ANOVA, during moderate-, severe-intensity- and in recovery from-exercise (Condition refers to H-BR, H-PL, N-BR or N-PL). Significant effects were further explored using simple contrasts with Fisher's LSD. One-tailed paired *t*-tests were used to compare differences in exercise tolerance between BR and PL treatments in hypoxia and normoxia. Correlations were assessed via Pearson's product-moment correlation coefficient between physiological and performance variables. All data are presented as mean ± SD with statistical significance being accepted when $P < 0.05$.

Results

Self-reported compliance to the supplementation regimen was 100% and subjects' food diaries confirmed that the timing of supplement taken on the morning of the laboratory tests was consistent across the experimental conditions. No deleterious side-effects were reported.

Plasma $[NO_2^-]$ and $[NO_3^-]$

Pre-exercise, plasma $[NO_2^-]$ was significantly elevated in H-BR compared to H-PL (H-BR: 301 ± 89 vs. H-PL: 88 ± 56 nM; $P = 0.02$) and N-BR relative to N-PL (N-BR: 401 ± 276 vs. N-PL: 61 ± 28 nM; $P = 0.01$) but did not differ between H-BR and N-BR ($P = 0.54$) or H-PL and N-PL ($P = 0.66$).

Plasma $[NO_3^-]$ was significantly elevated at all time-points following BR compared to PL in both hypoxia and normoxia although no differences were evident in the kinetic response during exercise and recovery (data not shown).

The group mean kinetic profiles of plasma $[NO_2^-]$ during moderate- and severe- intensity exercise and subsequent recovery are presented in Figure 1.

Moderate exercise. ANOVA revealed there were significant main effects by condition and time on plasma $[NO_2^-]$ during moderate-intensity exercise. BR supplementation significantly

elevated plasma [NO_2^-] across all time points compared to PL in both hypoxic and normoxic conditions (all $P < 0.05$). In N-BR, plasma [NO_2^-] was significantly decreased after 5 min of moderate-intensity exercise (Mod5) compared to ModBL (ModBL: 332 ± 184 vs. Mod5: 290 ± 207 nM, $P = 0.04$). However, the decrease in plasma [NO_2^-] in H-BR only showed a trend towards a reduction (ModBL: 306 ± 109 vs. Mod5: 270 ± 125 nM, $P = 0.10$). The rate of decline in plasma [NO_2^-] from ModBL to Mod5 was not significantly different in H-BR (-7 ± 11.7 nM·min^{-1}) compared to N-BR (-10.6 ± 15.9 nM·min^{-1}), H-PL (-3.9 ± 6.1 nM·min^{-1}) compared to N-PL (-2.1 ± 4 nM·min^{-1}), H-BR (-7 ± 11.7 nM·min^{-1}) compared to N-PL (-2.1 ± 4 nM·min^{-1}) or N-BR (-10.6 ± 15.9 nM·min^{-1}) compared to N-PL (-2.1 ± 4 nM·min^{-1}).

Severe exercise. There were significant main effects by condition and time and an interaction effect for plasma [NO_2^-] during severe-intensity exercise to exhaustion. BR supplementation significantly elevated plasma [NO_2^-] across all time points compared to PL in both hypoxic and normoxic conditions (all $P < 0.05$). In N-BR, plasma [NO_2^-] significantly decreased after 3 min of severe-intensity exercise (Sev3) and at exhaustion, compared to SevBL (SevBL: 271 ± 177; Sev3: 206 ± 129; $P = 0.01$; Exhaustion: 132 ± 117 nM, $P = 0.00$). In H-BR, plasma [NO_2^-] decreased from SevBL (277 ± 142 nM) to Sev1 (229 ± 123 nM, $P = 0.01$), Sev3 (n=10, 164 ± 64 nM, $P = 0.03$) and exhaustion (171 ± 115 nM, $P = 0.00$). The absolute decline in plasma [NO_2^-] from SevBL to exhaustion showed a trend toward being smaller in H-BR (106 ± 60 nM) compared to N-BR (138 ± 79 nM, $P = 0.10$). In N-PL, plasma [NO_2^-] decreased from SevBL (40 ± 23 nM) to exhaustion (22 ± 19 nM, $P = 0.02$). This decrease was not significant in H-PL (SevBL: 53 ± 65 vs. Exhaustion: 37 ± 45 nM, $P = 0.52$). The rate of decline in plasma [NO_2^-] was significantly greater from SevBL to exhaustion in H-BR compared to H-PL (H-BR: -30 ± 22 vs. H-PL: -7 ± 10 nM·min^{-1}, $P = 0.00$) and in N-BR compared to N-PL (N-BR: -26 ± 19 vs. N-PL: -1 ± 6 nM· min^{-1}, $P = 0.00$), but was not different between N-BR and H-BR ($P = 0.66$) or N-PL and H-PL ($P = 0.13$), (Figure 1).

Recovery. During the 10-min recovery from exhaustive exercise, ANOVA revealed significant main effects by condition and time and an interaction effect for plasma [NO_2^-] (Figure 1). BR supplementation significantly elevated plasma [NO_2^-] across all time points compared to PL in both hypoxic and normoxic conditions (all $P < 0.05$). In N-BR, plasma [NO_2^-] was significantly lower at exhaustion compared to 3 min into the recovery period ($P = 0.05$), with a significant difference also evident between Rec1.5 and Rec3 ($P = 0.01$). Plasma [NO_2^-] was significantly higher in H-BR compared to N-BR at Rec1.5 ($P = 0.04$). In N-PL, recovery of plasma [NO_2^-] was evident between exhaustion and Rec10 ($P = 0.04$), with a

significant increase in [NO_2^-] from Rec3 to Rec10 also evident ($P = 0.04$). In H-PL, plasma [NO_2^-] tended to recover between Rec1.5 and Rec3 ($P = 0.06$), with a further increase evident between Rec3 and Rec10 ($P < 0.00$).

Blood [glucose] was significantly reduced in H-BR compared to N-BR at Rec1.5 (H-BR: 4.3 ± 1.0 mmol·L vs. N-BR: 5.5 ± 1.2 mmol·L; P = 0.01), Rec3 (H-BR: 4.5 ± 1.1 mmol·L vs. N-BR: 5.6 ± 1.3 mmol·L; P = 0.02) and Rec10 (H-BR: 4.7 ± 1.0 mmol·L vs. N-BR: 5.3 ± 1.0 mmol·L; P = 0.03). No differences were evident between PL and BR conditions.

Arterial O_2 saturation and heart rate

The SaO_2 data at rest and during moderate- and severe-intensity exercise are reported in Table 1. Resting SaO_2 and HR prior to the administration of inspirate were not significantly different between conditions. However, ANOVA revealed a significant main effect by FIO_2 following 10 min of breathing the hypoxic or normoxic inspirate, with SaO_2 being significantly reduced in H-PL compared to N-PL ($P = 0.00$) and H-BR compared to N-BR ($P = 0.00$). HR was significantly elevated in H-PL compared to N-PL ($P = 0.00$) and H-BR compared to N-BR ($P = 0.02$) in the final 30 s of gas inspiration.

Moderate exercise. During moderate-intensity exercise, SaO_2 was significantly reduced in both hypoxic conditions compared to the normoxic conditions (both $P = 0.00$) (Table 1). HR was significantly elevated in both hypoxic conditions compared to the normoxic conditions in the final 30 s of exercise (both $P = 0.00$), with H-BR being lower than H-PL ($P = 0.05$) over the entire 6-min duration.

Severe exercise. SaO_2 was significantly lower in H-PL compared to N-PL ($P = 0.00$) and in H-BR compared to N-BR ($P = 0.00$) at exhaustion following severe-intensity exercise. There were no differences in SaO_2 between BR and PL in either hypoxia or normoxia. Also, there were no differences in HR between conditions (Table 1).

$\dot{V}O_2$ kinetics

Pulmonary $\dot{V}O_2$ responses across the four experimental conditions are presented in Figures 2 and 3, and the parameters derived from the model fits are summarized in Table 2.

Moderate exercise. ANOVA revealed a significant main effect by supplement and an interaction effect on the $\dot{V}O_2$ response to moderate-intensity exercise. The $\dot{V}O_2$ in the final 30 s

of exercise in H-BR was significantly lower compared to H-PL ($P = 0.02$) and N-PL ($P = 0.01$). BR supplementation also resulted in a reduced $\dot{V}O_2$ during baseline (20 W) exercise in hypoxia compared to PL ($P = 0.02$). The $\dot{V}O_2$ phase II τ tended to be increased (i.e., slower kinetics) in hypoxia ($P = 0.07$). Post-hoc analyses revealed that the $\dot{V}O_2$ phase II τ was smaller (i.e., faster kinetics) in H-BR compared to H-PL ($P = 0.04$).

Severe exercise. During severe-intensity exercise, the $\dot{V}O_2$ slow component amplitude ($P = 0.00$) and $\dot{V}O_2$ at exhaustion ($P = 0.00$) were significantly reduced as a result of the hypoxic inspirate in both PL and BR (Table 2). In hypoxia, BR tended to further reduce the end-exercise $\dot{V}O_2$ compared to H-PL ($P = 0.07$), while BR had no effect upon end-exercise $\dot{V}O_2$ in normoxia.

NIRS

The [HHb], [HbO_2], [Hb_{tot}] and TOI values measured during moderate- and severe-intensity exercise are shown in Table 3.

Moderate exercise. During moderate-intensity exercise, ANOVA revealed a significant main effect by FIO_2. The modelled [HHb] amplitude was significantly greater in hypoxia compared to normoxia in both supplemented conditions across all time points (all $P < 0.05$). The end-exercise [HbO_2] was lower in H-BR compared to N-BR ($P = 0.02$) and H-PL compared to N-PL ($P = 0.01$). TOI at baseline and throughout exercise was also significantly reduced in hypoxia compared to normoxia ($P < 0.05$). Post-hoc analyses revealed that BR tended to offset the negative effects of hypoxia on TOI when compared with PL ($P = 0.08$).

Severe exercise. During severe-intensity exercise, ANOVA revealed a significant main effect by FIO_2. [HHb] was significantly increased in H-BR and H-PL compared to N-BR and N-PL ($P < 0.05$), whereas the [HHb] slow phase amplitude was larger in normoxia compared to hypoxia ($P < 0.05$). [HbO_2] was reduced in hypoxia compared to normoxia ($P < 0.05$) and TOI was lower as a result of hypoxia throughout exercise ($P < 0.05$). No differences in NIRS data between BR and PL were evident during severe-intensity exercise.

Exercise tolerance

ANOVA revealed that hypoxia resulted in a significant reduction in exercise tolerance when compared to normoxia in both PL (H-PL: 197 ± 28 vs. N-PL: 431 ± 124 s, $P = 0.00$) and BR conditions (H-BR: 214 ± 43 vs. N-BR 412 ± 139 s, $P = 0.00$). Although the unspecific *F*-test

for interaction effect across all four conditions did not attain significance at the 95% level, it should be noted that the specific test for a difference between exercise tolerance in H-BR and H-PL was significant (H-BR: 214 ± 43 vs. H-PL: 197 ± 28 s, $P = 0.04$), whereas the comparison between N-BR and N-PL was not (N-BR: 412 ± 139 vs. N-PL: 431 ± 124 s, $P = 0.50$). The change in severe-intensity exercise tolerance was correlated with the change in moderate steady-state $\dot{V}_{O_2}$ following BR supplementation in hypoxia ($r = -0.96$; $P = 0.00$).

Discussion

Consistent with previous findings, the decline of plasma [NO_2^-] during exercise was greater following BR compared to PL supplementation. However, in contrast to our experimental hypothesis, the decline of plasma [NO_2^-] during exercise was similar or slightly smaller in hypoxia compared to normoxia. Nonetheless, 3 days of BR supplementation significantly speeded $\dot{V}_{O_2}$ kinetics and lowered the steady-state $\dot{V}_{O_2}$ during moderate-intensity cycle exercise in hypoxia, but not normoxia. Furthermore, BR supplementation improved severe-intensity exercise tolerance in hypoxia ($P < 0.05$), but not normoxia ($P > 0.05$). These findings suggest that BR is more effective at improving exercise economy and exercise tolerance in hypoxia than normoxia.

Effects of BR supplementation on the kinetic profile of plasma [NO_2^-]

Plasma [NO_2^-] increased significantly following BR supplementation compared with PL, at rest and prior to administration of the inspirate. These findings are consistent with previous research which has consistently reported elevations in plasma [NO_2^-] (3, 4, 33, 34, 51, 62, 67), following BR supplementation.

Previous studies have suggested that baseline plasma [NO_2^-] and/or the change in the concentrations of this metabolite during exercise may be associated with exercise performance (19, 53, 61, 68). This study is the first to characterise [NO_2^-] dynamics during and following exercise of different intensities in hypoxia and normoxia with and without NO_3^- supplementation. The results suggest that the metabolism of NO and its derivatives are altered by exercise and NO_3^- supplementation and, to a lesser extent, FIO_2. The interpretation of these data is not straightforward, however. NO_3^- can be reduced *in vivo* to bioactive NO_2^- and further to NO (47) and this reduction of NO_2^- to NO is expected to be facilitated in hypoxia (13). However, NO_2^- is also an oxidation product of NO generation via the NOS pathway (30) with plasma [NO_2^-] providing a sensitive marker of NO production through

458 NOS (43). Therefore, the dynamics of plasma [NO_2^-] over the exercise bouts is likely
459 reflective of the dynamic balance between NOS-derived NO and NO_2^- reduction to NO. In
460 the present study, plasma [NO_2^-] declined during both moderate- and severe-intensity
461 exercise (Figure 1) with the magnitude and rate of plasma [NO_2^-] decline being significantly
462 greater in the BR trials compared to PL trials, in both normoxia and hypoxia. These findings
463 suggest that the reduction of NO_2^- to NO appeared to outweigh the synthesis of NO through
464 NOS during exercise.

465 The rate of plasma [NO_2^-] decline over the 5-min moderate-intensity bout was not
466 significantly different between N-BR and H-BR, and N-PL and H-PL. However, following
467 5-min of moderate-intensity exercise, plasma [NO_2^-] had fallen significantly below ModBL in
468 N-BR; whereas, there was only a trend for a lower plasma [NO_2^-] in H-BR. Similarly, the rate
469 of plasma [NO_2^-] decline over the severe-intensity exercise bout was not significantly
470 different between N-BR and H-BR or N-PL and H-PL, but the absolute fall in plasma [NO_2^-]
471 tended to be less in H-BR than in N-BR, in spite of a longer exercise duration in N-BR. These
472 results are contrary to our hypothesis and suggest that, in hypoxia, the contribution of NOS to
473 NO production (30), and subsequently to the regulation of muscle perfusion and matching of
474 O_2 supply, may be greater (12).

475 During the 10-min passive recovery from exhaustive exercise, plasma [NO_2^-] increased in a
476 similar fashion in H-PL and N-PL. Specifically, plasma [NO_2^-] increased after 3 min of
477 recovery and plateaued after 10 min. The increases in plasma [NO_2^-] may represent an
478 increase in NO oxidation (as NO is continuing to contribute to muscle perfusion and
479 matching of O_2 supply and demand; 12) during recovery. Following BR supplementation, the
480 recovery profile of plasma [NO_2^-] was slightly different between normoxia and hypoxia.
481 Plasma [NO_2^-] was higher in H-BR than N-BR following 1.5 min of recovery, although the
482 difference between Exh and 1.5Rec was not different between conditions. It is important to
483 note that differences in plasma [NO_2^-] dynamics between hypoxia and normoxia were not
484 substantial either during exercise or in recovery.

485 *Effects of BR supplementation on the physiological response to moderate-intensity exercise*

486 BR supplementation significantly reduced the O_2 cost of sub-maximal cycle exercise in
487 hypoxia. $\dot{V}_{O_2}$ during baseline cycling in H-BR was reduced by 10% compared to H-PL and
488 by 4% compared to N-PL. Furthermore, a 7% reduction in the end-exercise (steady-state) $\dot{V}_{O_2}$
489 was found in H-BR compared to H-PL. These findings are consistent with previous studies

which have reported reductions in submaximal cycling $\dot{V}O_2$ in varying severities of hypoxia. For example, Masschelein et al. (50) reported a 4% reduction in steady state $\dot{V}O_2$ with an FIO_2 of 0.11 during cycle exercise at 45% peak $\dot{V}O_2$ and Muggeridge et al. (51) reported a ~6-8% reduction in steady-state $\dot{V}O_2$ at an FIO_2 of 0.15 during cycle exercise at 60% of maximum work rate, following BR supplementation. A reduction in muscle metabolic perturbation (i.e. slower rates of change of muscle pH and phosphocreatine (PCr) and inorganic phosphate concentrations) during severe-intensity knee-extensor exercise in hypoxia has also been reported following BR supplementation (64).

In the present study, the $\dot{V}O_2$ phase II τ during moderate-intensity exercise was reduced by BR supplementation in hypoxia. This finding is consistent with a recent study in older individuals, where the $\dot{V}O_2$ mean response time was speeded with BR supplementation (32). This may be related to the slower $\dot{V}O_2$ kinetics that is typically found in older individuals and the potential to abate this through enhancing muscle O_2 delivery (57), via increasing NO bioavailability. Similarly, hypoxia tended to slow $\dot{V}O_2$ kinetics in the young healthy participants in the present study. Specifically, the phase II τ tended to be slowed in hypoxia compared to normoxia (from ~22 to ~ 31 s; Table 2). This observation is consistent with previous reports of slower $\dot{V}O_2$ kinetics in hypoxia (29, 59). BR supplementation speeded the phase II τ in hypoxia toward values recorded in normoxia, thereby helping to reverse the detrimental effect of a reduced FIO_2 on $\dot{V}O_2$ kinetics. These findings are consistent with a recent study which showed that muscle PCr recovery kinetics, which reflects the maximal rate of mitochondrial ATP resynthesis and is influenced by O_2 availability, were speeded by BR supplementation in hypoxia (64). These data suggest that, in addition to reducing O_2 demand during exercise (50, 51, present study), BR may enhance skeletal muscle O_2 availability in hypoxia.

In contrast to some (3, 4, 14, 40, 41, 62), but not all (5, 8, 32, 65), previous studies, 3-days of BR supplementation did not significantly reduce $\dot{V}O_2$ during sub-maximal exercise in normoxia. Previous studies have typically reported reductions in steady state $\dot{V}O_2$ of ~3-5% following several days of NO_3^- supplementation (4, 40, 62). The mechanistic bases for this lower O_2 cost of exercise have been suggested to include improved mitochondrial efficiency (39) and/or reductions in the ATP cost of muscle force production (3) which may be linked to enhanced Ca^{2+}-related muscle contractility (28). NO is involved in the regulation of mitochondrial O_2 consumption and it is well established that NO has a strong affinity for cytochrome-*c* oxidase (COX) (9). It has been suggested that competition for the COX binding

site between NO and O_2 may be responsible, in part, for the reduced O_2 cost of exercise following NO_3^- supplementation (4, 41), with this initiating a signalling cascade resulting in mitochondrial protein changes which collectively enhance respiratory chain efficiency (39). Interestingly, hypoxia, *per se*, may also result in an acute, reversible inhibition of COX (10). The combination of hypoxia and BR supplementation may therefore make it more likely for these effects to be manifest. It is also noteworthy that reductions in $\dot{V}O_2$ during moderate-intensity exercise were recently reported to be evident following acute supplementation with 16.8 mmol NO_3^- (4 x 70 ml BR shots), tended to be evident with 8.4 mmol NO_3^- (2 x 70 ml BR shots), but were not evident with 4.2 mmol NO_3^- (1 x 70 ml BR shot) (67). It is therefore possible that an insufficient NO_3^- dose was consumed immediately prior to the tests to significantly influence the $\dot{V}O_2$ response to exercise in normoxia in the present study. Furthermore, the inter-individual differences in the $\dot{V}O_2$ response to exercise in normoxia evident in the current study, may have also contributed to the lack of statistically significant effects. It may be concluded that BR supplementation can (3, 4, 14, 40, 41, 62), but does not always (present study, 5, 8, 32, 66), alter the O_2 cost of exercise in normoxia.

Indices of muscle oxygenation measured with NIRS were altered as a result of the manipulation of FIO_2 during moderate-intensity exercise but BR supplementation did not significantly influence this response. Consistent with a previous study (49), [HHb] was greater in hypoxia indicating that muscle fractional O_2 extraction was increased, while [HbO_2] and TOI were significantly reduced in hypoxia compared to normoxia. Although not significant, BR supplementation tended to ameliorate the negative effects of hypoxia upon TOI during moderate-intensity exercise in the current study (a 3.6% increase in TOI), in a similar fashion to that reported by Masschelein et al. (50) (a 4% increase in TOI). These effects are consistent with observations that the arterial-venous nitrite difference is associated with limb vasodilatation and increased skeletal muscle blood flow during exercise performed in hypoxia (20). The trend for an improved TOI with BR supplementation indicates better muscle oxygenation (24) which may have been responsible for the speeding of the $\dot{V}O_2$ kinetics observed in hypoxia. Consistent with a possible improvement in oxygenation status, the typical compensatory rise in HR in hypoxia was attenuated by BR compared to PL during moderate-intensity exercise. Specifically, HR was 5-6 $b \cdot min^{-1}$ lower in the H-BR compared to the H-PL condition. There were no differences between H-BR and H-PL in indices of muscle oxygenation or HR during severe-intensity exercise.

Whether the reduction in cardiac work (lower HR) and metabolic requirement (lower $\dot{V}_{O_2}$) with BR observed in the present study might translate into enhanced performance during prolonged low-intensity exercise at altitude remains to be determined. Furthermore, older age and a number of disease conditions including peripheral arterial disease, diabetes, COPD and anaemia are associated with tissue hypoxia. A reduced O_2 cost of moderate-intensity exercise (i.e. walking) and reduced muscle metabolic perturbation during physical activity may improve the quality of life in individuals with these diseases (34, 64). However, further research is required to explore the effects of BR supplementation on health and functional capacity in patient populations.

Effects of BR supplementation on the physiological response to severe-intensity exercise

The end-exercise $\dot{V}_{O_2}$ was significantly reduced in hypoxia compared to normoxia. Moreover, [HbO_2] and TOI of the *m. vastus lateralis* were significantly reduced, while [HHb] and HR were significantly increased in hypoxia compared to normoxia, consistent with previous findings (50). There was a trend toward a reduction in end-exercise $\dot{V}_{O_2}$ with BR compared to PL supplementation in hypoxia of ~ 6%. This finding indicates the $\dot{V}_{O_{2peak}}$ may be reduced by NO_3^- supplementation and is consistent with some (6, 42) but not all previous studies (4, 33, 62) conducted in normoxia.

Tolerance to severe-intensity cycle exercise in hypoxia in the present study was significantly improved (9%, $P < 0.05$) following BR supplementation. This finding is consistent with earlier studies which reported that BR supplementation increased exercise tolerance during constant-work-rate (64) and incremental (50) exercise protocols and also enhanced cycling time-trial performance (51) in hypoxia. However, in contrast to previous findings (3, 4, 8, 33, 37), we found no effect of BR supplementation on exercise tolerance in normoxia. An interesting observation in the present study was the significant correlation between the reduction in steady-state $\dot{V}_{O_2}$ and the improvement in exercise tolerance following BR supplementation in hypoxia (r = -0.96). Therefore, the lack of effect on $\dot{V}_{O_2}$ during sub-maximal exercise in normoxia following BR supplementation may explain the lack of effect on exercise tolerance. Further research is required to address the physiological bases for responders and non-responders to dietary nitrate supplementation.

Perspectives

This study provides the first description of the influence of FIO_2 and BR supplementation on plasma [NO_2^-] dynamics during moderate- and severe-intensity exercise and subsequent recovery in humans. The greater rate of decline of plasma [NO_2^-] during exercise following BR compared to PL supplementation suggests that elevating plasma [NO_2^-] prior to exercise may promote NO production through the nitrate-nitrite-NO pathway. In hypoxia, but not normoxia, BR supplementation reduced the O_2 cost of moderate-intensity exercise, speeded $\dot{V}_{O_2}$ kinetics, and improved severe-intensity exercise tolerance. These findings may have important implications for individuals exercising at altitude.

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598 **Figure Legends**

599 **Figure 1.** Plasma [NO_2^-] response during moderate- and severe-intensity exercise and
600 recovery following BR and PL, in normoxia and hypoxia. Error bars indicate SE. H-BR was
601 greater than H-PL at each time point and N-BR was greater than N-PL at each time point. *a* =
602 $P < 0.05$ for N-BR compared to H-BR; *b* = $P < 0.05$ compared to moderate baseline; *c* = $P <$
603 0.05 compared to severe baseline. Where error bars are not visible, the size of the data point
604 exceeds the error.

605 **Figure 2.** Pulmonary O_2 uptake ($\dot{V}O_2$) responses during a step increment to a moderate-
606 intensity work rate, following PL and BR supplementation. Responses following BR are
607 represented as solid circles, with the PL responses being shown as open circles. The dotted
608 vertical line denotes the abrupt 'step' transition from baseline to moderate-intensity cycling
609 exercise. Error bars indicate the SE. *A*: Group mean response to moderate-intensity exercise
610 in normoxia (~21% FIO_2); *B*: Group mean response to moderate-intensity exercise in hypoxia
611 (~13.2 FIO_2); * = $P < 0.05$ compared to H-PL.

612 **Figure 3.** Pulmonary O_2 uptake ($\dot{V}O_2$) responses and time-to exhaustion during a step
613 increment to a severe-intensity work rate, following PL and BR supplementation. Responses
614 following BR are represented as solid circles, with the PL responses being shown as open
615 circles. The dotted vertical line denotes the abrupt 'step' transition from baseline to moderate-
616 intensity cycling exercise. Error bars indicate the SE. *A*: Group mean response to severe-
617 intensity exercise in normoxia (~21% FIO_2); *B*: Group mean response to severe-intensity
618 exercise in hypoxia (~13.2 FIO_2). * = Time to exhaustion greater in H-BR compared to H-PL
619 ($P < 0.05$; one-tailed t-test).

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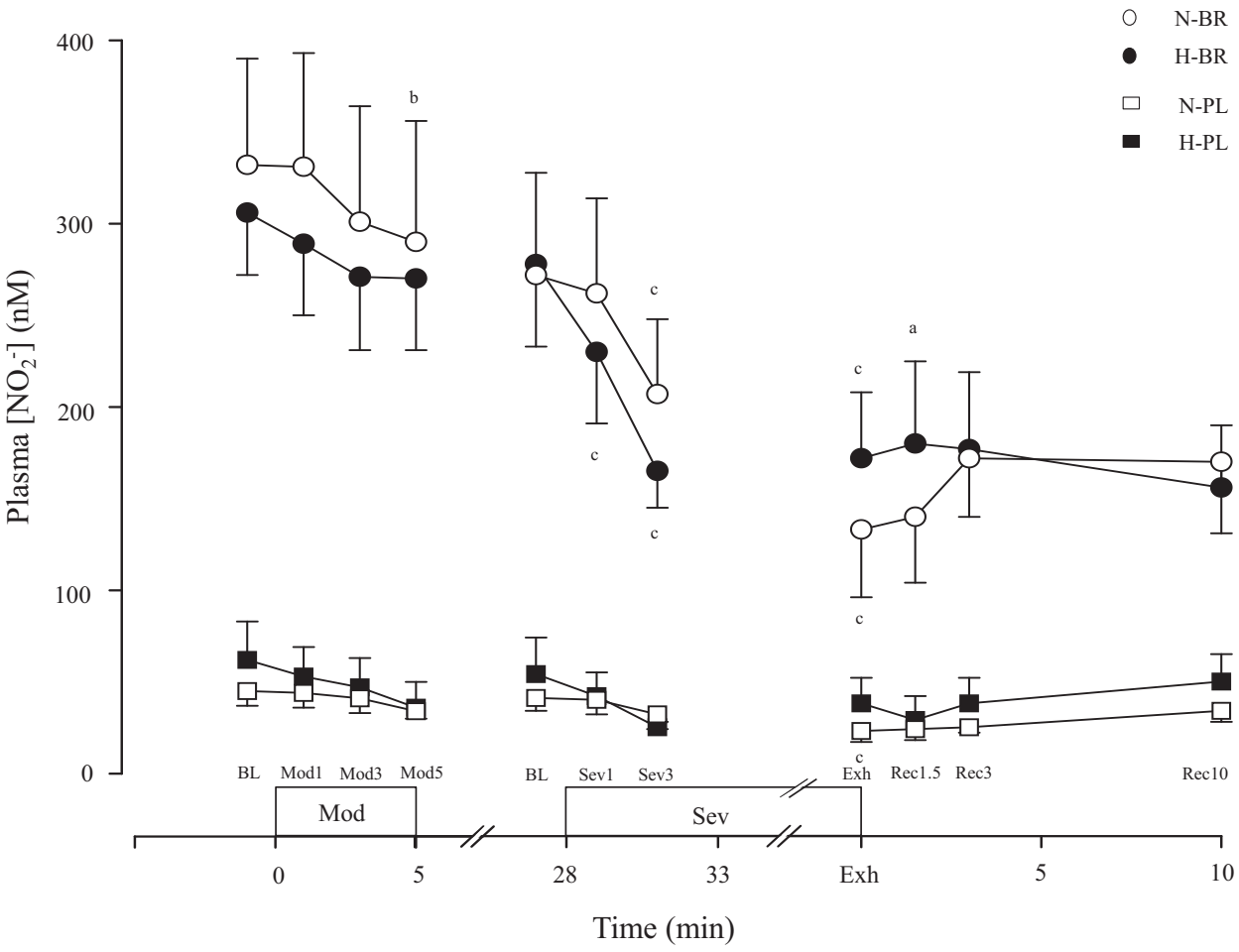
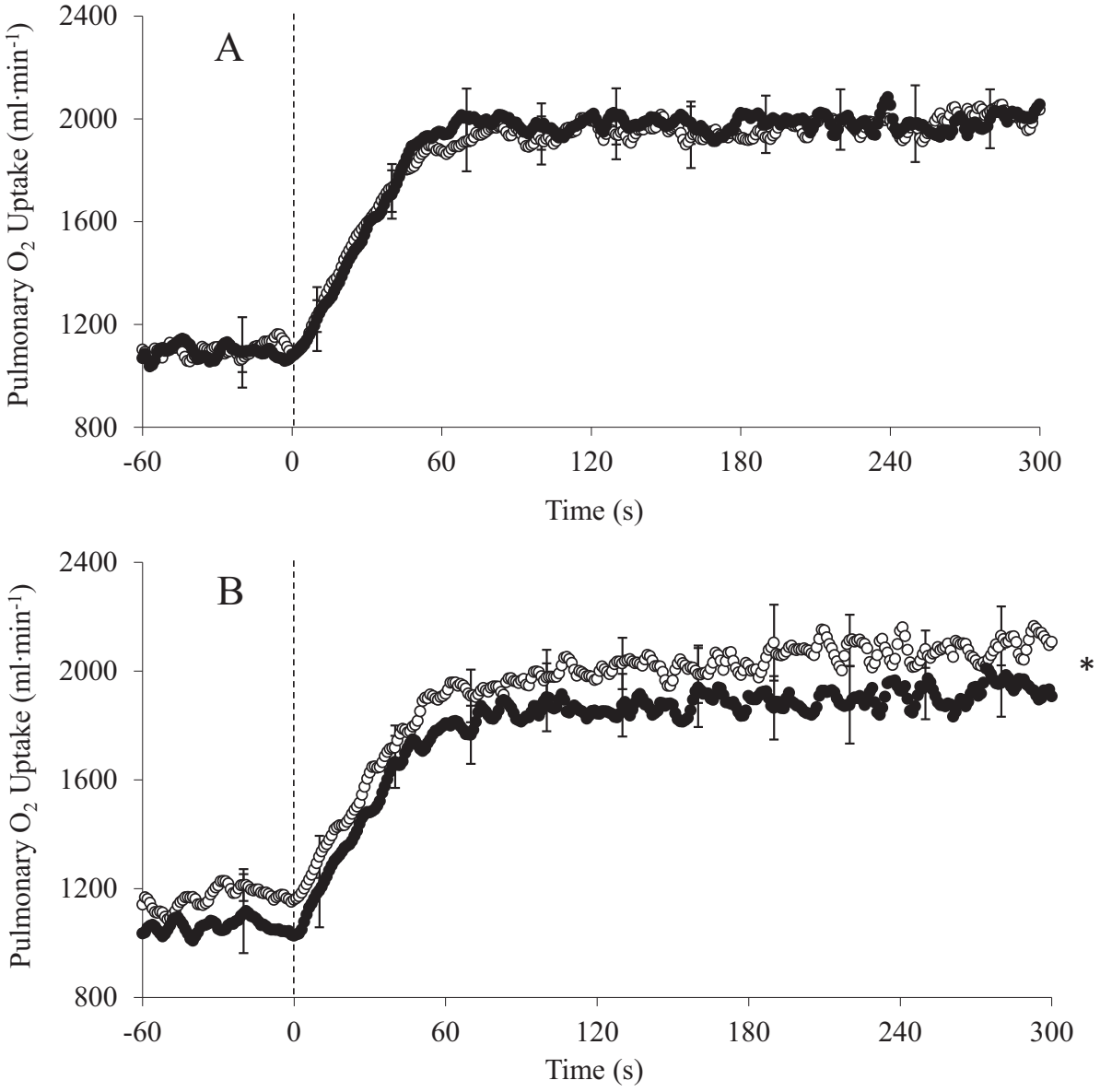
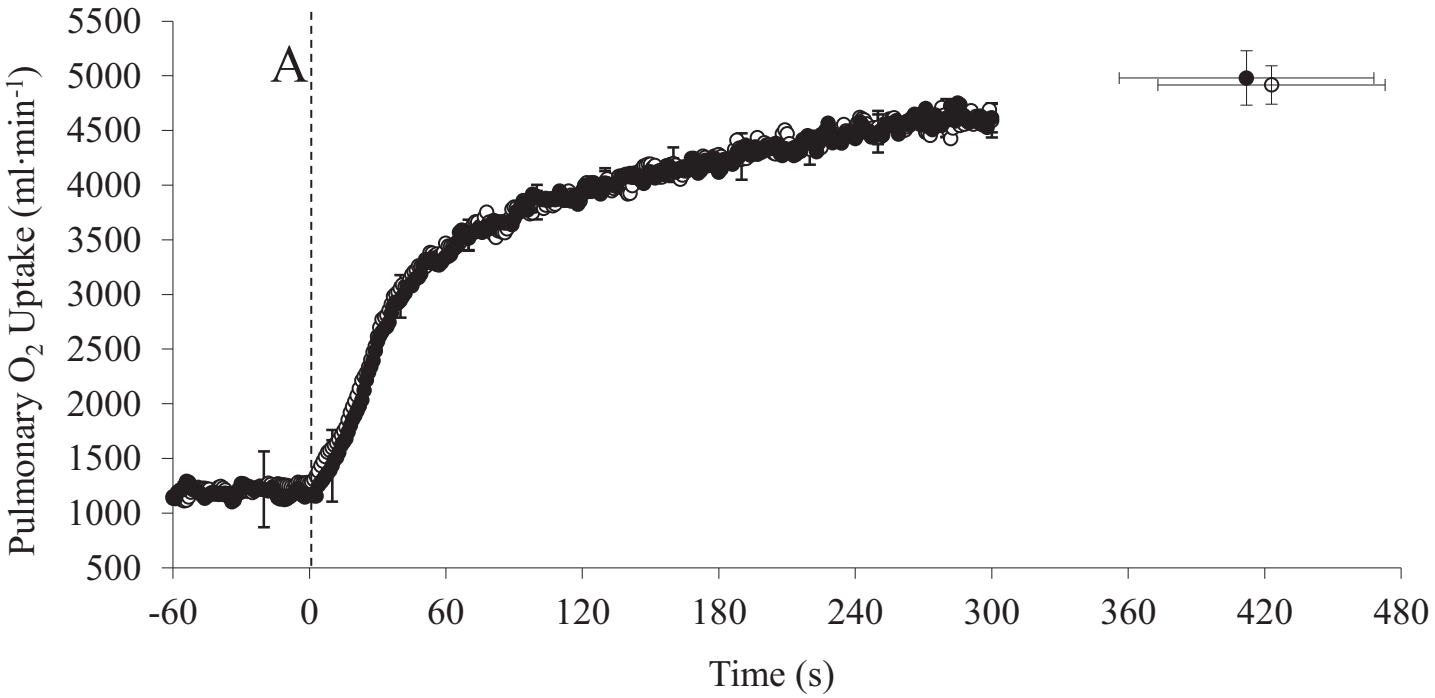

N-BR
H-BR
N-PL
H-PL
Plasma [NO2-] (nM)
400
300
200
100
0
b
c
c
c
a
c
c
c
BL
Mod1
Mod3
Mod5
BL
Sev1
Sev3
Exh
Rec1.5
Rec3
Rec10
Mod
Sev
0
5
28
33
Exh
5
10
Time (min)


A
B
Pulmonary O_2 Uptake (ml·min^{-1})
2400
2000
1600
1200
800
-60
0
60
120
180
240
300
Time (s)
*


A
Pulmonary O_2 Uptake (ml·min^{-1})
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
-60
0
60
120
180
240
300
360
420
480
Time (s)

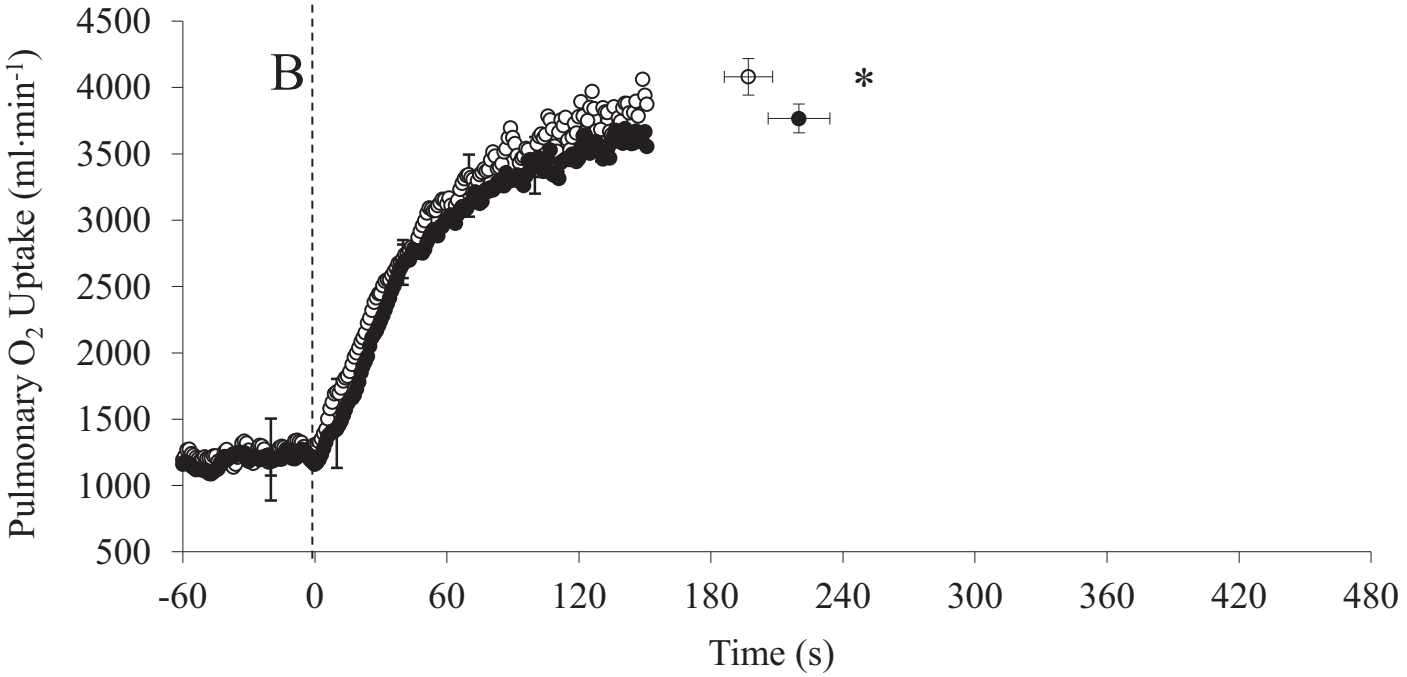

B
*
Pulmonary O_2 Uptake (ml·min^{-1})
4500
4000
3500
3000
2500
2000
1500
1000
500
-60
0
60
120
180
240
300
360
420
480
Time (s)

Table 1. Arterial oxygen saturation and heart rate during rest and in response to moderate- and severe-intensity exercise.

	N-PL	N-BR	H-PL	H-BR
Resting without inspirate				
SaO_2 (%)				
10 min period	99 ± 1	99 ± 1	99 ± 1	99 ± 1
End	99 ± 1	99 ± 1	99 ± 1	99 ± 1
HR (b/min)				
10 min period	59 ± 9	61 ± 10	61 ± 10	61 ± 9
End	60 ± 9	61 ± 9	61 ± 10	61 ± 9
Resting with inspirate				
SaO_2 (%)				
10 min period	99 ± 1	99 ± 1	93 ± 2†	93 ± 2*
End	99 ± 1	99 ± 1	90 ± 3†	91 ± 1*
HR (b/min)				
10 min period	58 ± 9	60 ± 11	68 ± 11	66 ± 10#
End	60 ± 8	60 ± 11	68 ± 11†	66 ± 10*
Moderate-intensity exercise				
SaO_2 (%)				
Baseline	97 ± 3	98 ± 2	87 ± 4	85 ± 4
6 min period	97 ± 3	98 ± 2	83 ± 3†	84 ± 4*
End	97 ± 3	97 ± 3	81 ± 4†	82 ± 5*
HR (b/min)				
Baseline	82 ± 10	86 ± 12	101 ± 16	94 ± 13
6 min period	102 ± 15	107 ± 15	122 ± 15	117 ± 19#
End	105 ± 16	111 ± 17	130 ± 15†	124 ± 19*
Severe-intensity exercise				
SaO_2 (%)				
Baseline	98 ± 2	97 ± 3	86 ± 4	87 ± 4
Exhaustion	94 ± 4	94 ± 4	80 ± 3†	80 ± 4*
HR (b/min)				
Baseline	97 ± 9	103 ± 12	113 ± 9	114 ± 12
Exhaustion	179 ± 4	180 ± 5	172 ± 6	171 ± 6

# $P < 0.05$ compared to H-PL; * $P < 0.05$ compared to N-BR; † $P < 0.05$ compared to N-PL.

Table 2. Oxygen uptake kinetics in response to moderate- and severe-intensity exercise in hypoxic and normoxic conditions.

	N-PL	N-BR	H-PL	H-BR
Moderate-intensity exercise				
$\dot{V}O_2$ *(ml/min)*				
Baseline	1102 ± 156	1010 ± 343	1167 ± 123	1056 ± 133#
End Exercise	1970 ± 251	1908 ± 340	2049 ± 247	1905 ± 275#
Phase II τ, (s)	22 ± 10	17 ± 4#	31 ± 11	24 ± 13#
Primary amplitude	868 ± 210	899 ± 256	882 ± 214	849 ± 208
Severe-intensity exercise				
$\dot{V}O_2$ *(ml/min)*				
Baseline	1212 ± 179	1205 ± 158	1244 ± 175	1193 ± 177
End Exercise	4814 ± 470	4721 ± 434	3986 ± 300†	3751 ± 249*
Phase II τ, (s)	30 ± 6	28 ± 9	35 ± 14	31 ± 11
Primary amplitude	2716 ± 398	2636 ± 486	2450 ± 497	2264 ± 386
Slow Component Amplitude	886 ± 235	881 ± 259	302 ± 290†	301 ± 274*

# $P < 0.05$ compared to H-PL; * $P < 0.05$ compared to N-BR; † $P < 0.05$ compared to N-PL.

Table 3. Near-infrared spectroscopy- derived HHb, HbO_2, Hb_{tot} and TOI dynamics during moderate- and severe-intensity exercise.

	N-PL	N-BR	H-PL	H-BR
Moderate-intensity exercise				
[HHb] (AU)				
Baseline	7 ± 5	6 ± 5	11 ± 5†	10 ± 5*
120 s	11 ± 8	11 ± 7	18 ± 8†	17 ± 10*
End Exercise	12 ± 8	11 ± 7	20 ± 8†	18 ± 10*
Time Constant, (s)	23 ± 7	19 ± 6	22 ± 9	23 ± 7
Amplitude	5 ± 4	6 ± 4	8 ± 5†	7 ± 6*
[HbO_2] (AU)				
Baseline	2 ± 6	3 ± 6	2 ± 5	2 ± 7
120 s	1 ± 6	2 ± 6	-2 ± 4	-2 ± 8
End Exercise	4 ± 5	5 ± 5	0 ± 3†	-2 ± 9*
[Hb_{tot}] (AU)				
Baseline	1 ± 0	1 ± 0	1 ± 0	1 ± 0
120 s	1 ± 0	1 ± 0	1 ± 0	1 ± 0
End Exercise	1 ± 0	1 ± 0	1 ± 0	1 ± 0
TOI (AU)				
Baseline	65 ± 3	65 ± 4	61 ± 4†	63 ± 4*
120 s	61 ± 5	60 ± 6	52 ± 5†	54 ± 6*
End Exercise	62 ± 7	61 ± 7	52 ± 6†	54 ± 6*
Severe-intensity exercise				
[HHb] (AU)				
Baseline	5 ± 5	5 ± 5	10 ± 6†	10 ± 6*
120 s	19 ± 13	18 ± 11	25 ± 12†	24 ± 14*
End Exercise	22 ± 14	21 ± 12	26 ± 12†	26 ± 14*
Time Constant, (s)	13 ± 5	11 ± 5	11 ± 3	12 ± 6
Primary amplitude	14 ± 10	14 ± 8	14 ± 9	14 ± 10
Slow phase amplitude	3 ± 2	3 ± 2	2 ± 2†	2 ± 2*
[HbO_2] (AU)				
Baseline	7 ± 7	8 ± 7	6 ± 5†	5 ± 8*
120 s	-4 ± 7	-3 ± 7	-9 ± 4 †	-10 ± 8*
End Exercise	-7 ± 9	-7 ± 7	-11 ± 5†	-12 ± 7 *
[Hb_{tot}] (AU)				
Baseline	1 ± 0	1 ± 0	1 ± 0	1 ± 0
120 s	1 ± 0	1 ± 0	1 ± 0	1 ± 0
End Exercise	1 ± 0	1 ± 0	1 ± 0	1 ± 0
TOI (AU)				
Baseline	70 ± 5	69 ± 4	64 ± 4	64 ± 4
120 s	52 ± 12	51 ± 10	44 ± 9	44 ± 10
End Exercise	48 ± 11	47 ± 9	41 ± 9†	41 ± 8*

Deoxygenated hemoglobin concentration ([HHb]), oxygenated hemoglobin concentration ([HbO_2]), total hemoglobin concentration ([Hb_{tot}]) and total oxygenation index (TOI) are shown. * $P < 0.05$ compared to N-BR; † $P < 0.05$ compared to N-PL. AU = arbitrary units.