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Acute Dietary Nitrate Supplementation Does Not Attenuate Oxidative Stress or the Hemodynamic Response During Submaximal Exercise in Hypobaric Hypoxia

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Abstract

Purpose: To investigate changes in oxidative stress, arterial oxygen saturation (SaO₂), blood pressure (BP), and heart rate (HR) while exercising in hypobaric hypoxia following acute dietary nitrate supplementation. Methods: Nine well-trained (60.8±7.8 ml/kg/min) males (29±7 years) visited the lab on 3 occasions, each separated by 1 week. Visit 1 included a maximal aerobic cycling test (VO₂max) and 5 five-minute increasing intensity exercise bouts in a normobaric environment (1600m). A single dose of either a nitrate-depleted placebo (PL) or nitrate-rich (NR; 12.8 mmol nitrate) beverage was consumed 2.5 hours prior to exercise during visit 2 and 3 (3500m) in a double-blind, placebo-controlled, crossover study consisting of a 5-minute cycling warm-up and four bouts, each of 5-minute durations, separated by 4-minute periods of passive rest. Exercise wattages were determined during visit 1 and corresponded to 25, 40, 50, 60, 70% of normobaric VO₂max. Catalase and 8 isoprostane were measured pre-exercise and post-exercise (immediately and 1-hour post-exercise, respectively). Results: Dietary nitrate increased plasma nitrite (1.53±0.83 uM) compared to PL (0.88±0.56 uM) (p<0.05). In both conditions, post-exercise (3500m) 8-isoprostane (23.49±3.38 to 60.90±14.95, PL and 23.23±4.12 to 52.11±19.76 pg/ml, NR) and catalase (63.89±25.69 to 128.15±41.80, PL and 78.89±30.95 to 109.96±35.05 mmol/min/ml, NR) were elevated compared to baseline resting values (p<0.05). However, both 8-isoprostane and catalase were similar (p=0.217 and 0.080, respectively) between groups (PL and NR). Conclusions: An acute, pre-exercise dose of dietary nitrate yielded no beneficial changes in oxidative stress, SaO₂, BP, or HR in healthy, aerobically fit men exercising at 3500m.

Keywords: beetroot juice, hypoxia, nitric oxide, oxidative stress, dietary nitrate
INTRODUCTION

Numerous studies report an increase in reactive oxygen species (ROS) and oxidative stress as a result of exercise and increased oxygen consumption (Radak et al. 2001, Powers and Jackson 2008, Yavari et al. 2015). The imbalance between free radicals and antioxidants can lead to oxidative stress resulting in lipid peroxidation and DNA damage which may further affect the expression of multiple genes in eukaryotic cells. Oxidative stress can also aid in the development of pathologic conditions such as cardiovascular disease, insulin resistance, and metabolic syndrome (Yavari et al. 2015). However, production of low levels of mitochondrial ROS in the muscle is needed for main signaling pathways and normal force production in skeletal muscle (Radak et al. 2001, Powers and Jackson 2008, Yavari et al. 2015). Recently, others have reported nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase) to have a primary role in reactive oxygen and nitrogen species production during muscle contraction (Sakellariou et al. 2013, Pearson et al. 2014). In fact, membrane localized NADPH oxidase produces extracellular superoxide which can interact with local metabolites including NO, or be converted to hydrogen peroxide ($H_2O_2$) by extracellular superoxide dismutase (SOD) (Ferreira and Laitano 2016).

Along with exercise, exposure to high altitudes may increase oxidative stress (Miller et al. 2013, Quindry et al. 2016). Oxidative stress can occur from both long and short durations of exercise, and is worsened in hypoxic conditions in both the blood and muscle (Burton and Jauniaux 2011, Quindry et al. 2016). Oxidative stress is increased during hypoxia due to a lesser availability of oxygen leading to a buildup of unpaired electrons (Burton and Jauniaux 2011). The effect of dietary nitrate on oxidative stress is less conclusive.
Arterial oxygen saturation ($\text{SaO}_2$) decreases during exposure to high altitude, although dietary nitrate has been shown to reduce oxygen cost during exercise and may, therefore, attenuate the decline in $\text{SaO}_2$ following exposure to higher altitudes where a reduced partial pressure of oxygen exists (Carriker et al. 2013). Varied dosing strategies (amount and duration) elicit conflicting results and create difficulty with direct comparison of findings. While dietary nitrate has been shown to attenuate the decline in $\text{SaO}_2$ during exercise in moderate normobaric hypoxia (15% FIO$_2$ or ~2500m equivalent) following a single acute dose of dietary nitrate (~15.2 mmol nitrate) consumed 3 hours prior to exercise (Shannon et al. 2016), this was not observed following a single dose of only 5 mmol of nitrate at the same simulated elevation (Muggeridge et al. 2014). Under more extreme normobaric hypoxic conditions (11% FIO$_2$ or ~5000m equivalent), dietary nitrate consumption yields mixed results as well. Following a 6-day loading phase (0.07 mmol/nitrate/kg/day), $\text{SaO}_2$ was greater in the nitrate group compared to the control group following exercise at 45% of normobaric VO$_{2\text{max}}$ (Masschelein et al. 2012). Alternatively, following a 3-day supplementation protocol (0.1 mmol/kg/day), dietary nitrate had no effect on $\text{SaO}_2$ during a 15 km time trial (11% FIO$_2$ or ~5000m equivalent) (Bourdillon et al. 2015). Dietary nitrate has previously been shown to reduce oxygen consumption during submaximal exercise at altitude (Muggeridge et al. 2014, Kelly et al. 2014) and, therefore, dietary nitrate has potential to slow the decline in saturation of oxygen and reduce the elevation in oxidative stress.

Nitrates have been shown to reduce levels of oxidative stress in rats (Carlström et al. 2011). While a prolonged high-salt diet initiated development of hypertension and oxidative stress, nitrate treatment (0.1 and 1 mmol nitrate/kg/day) restored tissue levels of bioactive nitrogen oxides and reduced the levels of oxidative stress markers, malondialdehyde in plasma as well as Class VI F2-isoprostanes and 8-hydroxy-2-deoxyguanosine in urine (Carlström et al. 2011). In addition, superoxide and production of other reactive oxygen species may increase under hypoxic conditions. Superoxide can be generated in
the muscle mitochondria, as well as the kidney and blood vessels by xanthine oxidoreductase (Suzuki et al. 1998, Harrison 2002, Wilcox 2005). Superoxide and nitric oxide (NO) form peroxynitrite when they are synthesized in close proximity (Huie and Padmaja 1993) and in this manner, may therefore, decrease NO bioavailability (Cai and Harrison 2000, Pacher et al. 2007).

Given the increase in plasma nitrite (and propensity for further reduction to NO) following dietary nitrate consumption, we tested the hypothesis that the consumption of beetroot juice would reduce submaximal oxygen consumption and subsequently decrease oxidative stress while increasing NO bioavailability resulting in reduced exercise blood pressure and attenuated decline in arterial oxygen saturation in response to exercise during exposure to hypobaric hypoxia.

The purpose of this study was to determine the effect of an acute dose of inorganic nitrate on oxidative stress, \( \text{SaO}_2 \), blood pressure (BP), heart rate (HR) and perceived exertion (RPE) while exercising in hypobaric hypoxia (3500 m).

METHODS

Well-trained male cyclists aged 18 – 45 were recruited and provided written consent to participate in the study approved by the University of New Mexico’s institutional review board. Prescreening criteria required participants to have engaged in a minimum of 2 months of moderate intensity cycling for a minimum of 150 minutes per week immediately prior to enrollment in the study. Eligible participants completed a maximal aerobic capacity (\( \text{VO}_{2\text{max}} \)) cycling test in a normobaric environment at 1600 m. Participants were excluded if their measured \( \text{VO}_{2\text{max}} \) was below the 70\(^{th}\) percentile based on their age and sex (Thompson et al. 2010). Participants were healthy and did not undergo travel which resulted in a change in living elevation greater than 400 meters while enrolled in the study. Visit 1 (V1) occurred at
a normobaric elevation of ~1600 m, while visit 2 (V2) and visit 3 (V3) took place in a hypobaric chamber at 3500 m (~500 mmHg barometric pressure).

During V1, a VO$_{2\text{max}}$ test (30 w/min ramp) was followed by a 15-minute rest period and then a series of five 5-minute exercise bouts of increasing intensity. VO$_{2\text{max}}$ was determined using breath-by-breath data (True One, ParvoMedics, Sandy, Utah, USA) to determine the highest 11-breath rolling average during the final 30 seconds preceding volitional fatigue. Participants maintained a constant self-selected pedal cadence between 70 and 90 RPM during the series of 25-minute exercise. Participants were asked to match the cadence from V1 during V2 and V3 cycling bouts. A regression equation allowed for calculation of the watts which corresponded to 25, 40, 50, 60, 70% of their normobaric VO$_{2\text{max}}$ that were to be used during V2 and V3 at altitude. Cycling exercise intensity (watts) was controlled by an electronically braked bike (Velotron, RacerMate, Seattle, WA) with racing saddle, pedals and configuration (seat height, handle bar height and distance to seat) matching their road bike for each visit. Each visit was separated by 7 days, which served as a dietary washout period. To avoid diurnal variations, all visits occurred at the same time of day for each participant.

In a double blind, placebo-controlled crossover design, participants were assigned to either a nitrate-rich beetroot juice beverage (NR; ~12.8 mmol, Beet It, James White Ltd, Ipswich, UK) or nitrate-depleted beetroot juice placebo supplement (PL; negligible nitrate content Beet It, James White Ltd, Ipswich, UK), which was consumed 2.5 hours prior to V2 with the opposite supplement administered prior to V3. The placebo beverage was otherwise identical in taste and appearance. During the 4 day period immediately preceding V2 and V3, participants underwent a 4-day dietary washout period during which they were asked to avoid a list of foods high in dietary nitrate (Hord et al. 2009). Participants were asked to record dietary intake and to avoid strenuous exercise, alcohol, caffeine, chewing gum and mouthwash during
the 24 hours leading to V2. Diet and exercise were replicated prior to V3. Visit 2 and 3 were performed at the same time of day to limit variance due to circadian rhythm.

During V2 and V3, participants exercised at the predetermined watts corresponding to 25, 40, 50, 60, 70% of their normobaric VO_{2max} in a hypobaric chamber with partial pressure maintained at 3500 m. Each intensity was maintained for 5 minutes and was separated by a 4-minute passive recovery while seated on the exercise bike. The first stage at 25% of normobaric VO_{2max} served as a warmup and this period allowed technicians to verify all equipment was operational (watt output, RPM sensor etc.). SaO_{2} (Go2 Achieve, Nonin Medical Inc.), HR (short range radiotelemetry via Polar Electro T31, New York, USA), BP and RPE (Borg 1970) were measured during the final 30 seconds of bouts at 40, 50, 60, 70% of their normobaric VO_{2max}. Blood was drawn from a prominent forearm vein prior to exercise, immediately following the final exercise bout and 1-hour post-exercise to determine changes in oxidative stress.

Blood samples were collected in lithium heparin tubes and were immediately centrifuged at approximately 4,000 rpm at 4°C for 10 minutes. Plasma was then collected and stored at -80°C until analyzed. Pre-exercise plasma nitrite was measured in duplicate using a commercially available microplate-based colorimetric assay kit (Cayman Chemical #780001, Ann Arbor MI, USA). Plasma catalase was measured at baseline and the timepoint immediately post-exercise (Michailidis et al. 2007) using a colorimetric assay kit (Cayman Chemical, protocol #707002). Plasma 8-isoprostane was measured at baseline and 1-hour post-exercise (Mastaloudis et al. 2001, Davison et al. 2012) via an enzyme-linked immunosorbent assay (Cayman Chemical, protocol #516351).

**STATISTICAL ANALYSIS**

Data, presented as mean ± SD, were analyzed using a repeated-measures analysis of variances
ANOVA with two factors: exercise intensity and condition at an alpha level of 0.05 for determination of statistical significance. When an interaction effect was observed, a Bonferroni post hoc analysis was applied. Baseline nitrate concentrations were assessed using nonparametric Wilcoxon signed rank test to account for nonhomogenized variance.

RESULTS

Nine well-trained (60.8±7.8 ml/kg/min) males (29±7 yr) completed all trials (Table 1). Watts for trials 2 and 3 were determined based on regression equations from participants’ visit 1 to correspond to 25, 40, 50, 60 and 70% of their normobaric VO$_{2\text{max}}$ (Table 2). Dietary nitrate increased plasma nitrite from 0.88 ± 0.56 uM at rest before supplementation to 1.53 ± 0.83 uM measured 2.5 hours after supplementation with dietary nitrate (p<0.05) (Figure 1). Resting 8-isoprostane increased after exercise for both the NR (23.23 to 52.11 pg/ml, p<0.05) and PL (23.49 to 60.90 pg/ml, p<0.05) treatments (Figure 2A). However, there were no differences between resting or 60-minute post-exercise plasma 8-isoprostane values between NR and PL conditions (p=0.217). Resting plasma catalase increased after exercise for both the NR (78.88 to 109.96 mmol/min/ml, p<0.05) and PL (63.89 to 128.15 nmol/min/ml, p<0.05) treatments (Figure 2B). However, there were no differences between resting or post-exercise catalase values between NR and PL conditions (p=0.080). No differences were observed between SaO$_2$ at any timepoints between NR and PL conditions (p=0.163) (Figure 3). Additionally, dietary nitrate supplementation did not affect HR or rate pressure product (p=0.894 and p=0.243, respectively) at any of the measured timepoints when compared to the PL control. Resting blood pressure was not different between placebo (101.67/68.33 ± 7.53/6.50 mmHg) and nitrate supplementation (101.17/64.00 ± 8.73/10.20 mmHg). Further, no differences in systolic or diastolic blood pressure were observed at any of the measured timepoints (p=0.645 and 0.827, respectively) (Figure 4). Finally, RPE was not different between conditions at any of the measured timepoints (p=0.844).
DISCUSSION:

Hypoxia combined with increased physical activity has been shown to increase free radical production and when deregulated, this rise could lead to lipid peroxidation and DNA damage (Møller et al. 2001, Bailey et al. 2001). In this manner, decreased tissue oxygenation resulting from a decreased partial pressure may exacerbate oxidative stress (Askew 2002). We hypothesized that dietary nitrate would minimize the rise in oxidative stress following exercise in hypobaric hypoxia. At altitude, when partial pressure is reduced, dietary nitrate may decrease the energy demands of exercise, thereby sparing the use of oxygen at a given exercise intensity. Previous investigations have shown dietary nitrate to be efficacious in improving mitochondrial efficiency via reduced proton leak and enhanced PO ratio (number of adenosine triphosphate, ATP, produced to oxygen consumed) (Larsen et al. 2011). Such changes in mitochondria, combined with enhanced local perfusion following dietary nitrate consumption (Victor et al. 2009) may support other hypotheses that dietary nitrate consumption decreases ATP consumption via enhanced efficiency of muscle contraction (Bailey et al. 2010).

Following exercise at 3500 m, participants experienced a rise in oxidative stress (catalase and 8-isoprostane) compared to pre-exercise values. While exercising at an elevation of 3500 m, where partial pressure is reduced, it is reasonably assumed that oxidative phosphorylation may be impaired as oxygen is the final electron acceptor and is necessary to produce metabolic water at complex IV (cytochrome c oxidase) (Askew 2002). Increased reactive oxygen species may result from both reduced oxygen availability and dysfunction of cytochrome c oxidase (Srinivasan and Avadhani 2012). Moreover, heightened NADPH oxidase activity may also be responsible for increased oxidative stress from skeletal muscle (Ferreira and Laitano 2016). In fact, Nox2 (the predominant source of superoxide within skeletal muscle) produces extracellular superoxide which can be converted to $\text{H}_2\text{O}_2$ by extracellular SOD.
(Ferreira and Laitano 2016). Heightened superoxide production from NADPH oxidase can interact with NO producing peroxynitrite, causing a shift in ROS signaling and reduced NO availability, which is often present during several diseases (Schiffrin 2008). Further, it has been suggested that a crosstalk exists between ROS production from Nox2 and ROS from mitochondrial sources, suggesting a highly complex regulatory process governing muscle ROS (Ferreira and Laitano 2016).

Consumption of dietary nitrate leads to increased plasma nitrate which may be reduced to nitrite, enhancing bioavailability of nitric oxide (possibly counteracting increases in ROS). In hypoxia, the reduction of nitrite to nitric oxide occurs via xanthine oxidase and other proteins including cytochrome c oxidase and endothelial nitric oxide synthase (Lundberg et al. 2008, Kim-Shapiro and Gladwin 2014). While oxidative stress is important in certain cellular signaling processes, increased oxidation via reactive oxygen species (as occurs with reperfusion after ischemia) can cause cell damage (Mylonas and Kouretas 1999). Isolated mitochondria exposed to 30 minutes of anoxia experienced nitrite-mediated protection by inactivating complex I (“dampening” electron transfer and preventing cytochrome c release) and limiting reactive oxygen species production via nitrosation (Shiva et al. 2007b).

In the present study, dietary nitrate had no effect on changes in oxidative stress between treatments (NR and PL). While nitrates have been shown to reduce levels of oxidative stress in male Sprague-Dawley rats (Carlström et al. 2011), the effect may not be as pronounced in humans. Although oxidative stress is consistently elevated during both exercise (Radak et al. 2001, Powers and Jackson 2008, Yavari et al. 2015) and high altitude exposure (Murray 2009, Levett et al. 2011), dietary nitrate supplementation did not affect oxidative stress following exercise at 3500 m in hypobaric hypoxia in our aerobically fit cohort. Others have suggested the muscle fiber type largely affects the reduction of nitrate to nitrite. Specifically, type II fibers may show enhanced reduction capabilities given the low
intramyocellular partial pressure of oxygen (Jones et al. 2016). In part, this hypothesis extends from understanding that nitrate is reduced significantly faster in deoxymyoglobin compared to deoxyhemoglobin (Shiva et al. 2007a). Ferguson et al. (Ferguson et al. 2015) found dietary nitrate improved contracting steady state microvascular partial pressure of oxygen in fast twitch but not slow twitch muscle fibers of adult male Sprague-Dawley rats. As expected, baseline and contracting microvascular partial pressure of oxygen was greater in the slow twitch fibers. The lower partial pressure in the fast twitch fibers responsively increased following dietary nitrate supplementation. Dietary nitrate supplementation for 7 days in a mouse model has demonstrated efficacy as evidenced by increased contractile force of fast-twitch muscles in addition to increased myoplasmic free calcium during tetanic stimulation (Hernández et al. 2012). As dietary nitrate increases NO bioavailability, dietary nitrate may indirectly regulate calcium homeostasis and modulate excitation-contraction coupling as NO has been shown to play a role in such physiologic processes (Stamler and Meissner 2001, Jones et al. 2016).

Arguably, altitude and corresponding decreases in atmospheric partial pressure may also alter muscle partial pressure of slow twitch fibers such that the microvascular partial pressure of oxygen is reduced at baseline and during exercise. In this case, slow twitch muscle fibers may experience benefit (elevation of microvascular partial pressure) following supplementation with dietary nitrate as the partial pressure would be lower prior to supplementation. Ferguson et al. (Ferguson et al. 2015) reports the changes in partial pressure of oxygen in muscles following dietary nitrate supplementation is preferential to fast twitch fibers. However, future investigations should examine slow and fast twitch fibers under hypoxic conditions as we suspect slow twitch fibers may experience benefits similar to normoxic fast twitch fiber types. In this regard, we anticipated dietary nitrate would reduce oxidative stress in our active cohort engaged in submaximal intensity, which may predominately recruit slow twitch fibers.
Emerging research suggests the general benefits of dietary nitrate may be limited to those individuals who are less fit and have not received physiologic adaptations resulting from aerobic training (Porcelli et al. 2014, Jonvik et al. 2015, Carriker et al. 2016). All 9 male subjects were well trained (60.8±7.8 ml/kg/min) and others have also found no effect of dietary nitrate in highly fit participants (Peacock et al. 2012, Boorsma et al. 2014). The dosing amount (~12.8 mmol) would appear to be adequate to observe hypothesized responses (Vanhatalo et al. 2010, Lansley et al. 2011b, Wylie et al. 2016); however, during exercise at all intensities, SaO$_2$, HR and BP were not affected by dietary nitrate. Previously, acute dosing strategies have been used with as little as 5 mmol (Engan et al. 2012, Muggeridge et al. 2013) to 6.2 mmol (Lansley et al. 2011a, Wilkerson et al. 2012) with noted physical changes following supplementation. Perhaps the requirements for reducing ROS-stress is greater than those of exercise performance and other physiologic outcomes. Others have found that dietary nitrate yields a reduction in BP (reducing incidence of hypertension) and improves endothelial function (Gilchrist et al. 2013, Kapil et al. 2015, Kerley et al. 2017) and may also decrease both systolic and diastolic BP (Webb et al. 2008, Kapil et al. 2015, Kerley et al. 2017). In this study, no difference in BP between the PL and NR groups were present at rest or any intensity while exercising. This may be attributed to the fact that all subjects were healthy with normal BP (systolic 103±7 and diastolic 66±7 mmHg). Cohorts with endothelial dysfunction or hypertension may receive greater benefit than a healthy fit group of participants.

Dietary nitrates have also previously been studied to examine their effects on various metabolic and circulatory parameters as well as changes in RPE during exercise in normobaric conditions. In a study using healthy well-trained males performing a submaximal and maximal test on a cycle ergometer under normobaric conditions, no difference was found in RPE between the NR group and PL group (Larsen et
al. 2007). Under normobaric conditions, well-trained male cyclists performing two 50-mile timed trials showed no difference in RPE between the nitrate and placebo groups (Wilkerson et al. 2012). Less is known about the effect of dietary nitrate supplementation on exercise under hypobaric conditions. Ten male runners that completed incremental exercise to exhaustion at 4000 m and a 10-km treadmill trial at 2500 m with placebo or nitrate also had no difference in RPE between the two groups (Arnold et al. 2015). Masschelein et al. (Masschelein et al. 2012) tested 15 healthy volunteers in hypobaric hypoxia during an incremental exercise test and while cycling at 45% of normobaric VO$_{2\text{max}}$ and found no difference in RPE between nitrate and placebo groups.

Similar to the findings of Kelly et al. (Kelly et al. 2014) where the hypoxic conditions were 13.2% FIO$_2$ and Hennis et al. (Hennis et al. 2016) where the hypoxic conditions ranged from 50 m to 5300 m, we found dietary nitrate had little effect on numerous physiologic variables. Hennis et al. reported that their null findings may have been a result of the chronic hypoxic exposure of the climbers. Together, it appears acute dietary nitrate has little effect on performance or markers of oxidative stress in a highly fit cohort exposed to acute hypobaric hypoxia. It remains unclear as to whether dietary nitrate would affect oxidative stress in a lesser fit cohort or following a longer loading period (consecutive days of supplementation) or exposure to a different altitude with less partial pressure of oxygen.

Conflicts of interest: None to disclose

REFERENCES


**FIGURE CAPTIONS**

Figure 1: Mean ± SD plasma nitrite values before and after exercise following the consumption of either a nitrate-depleted (Placebo; striped bars) or 12.8 mmol nitrate-rich (Nitrate; black bars) beverage 2.5 hours prior to exercise. N=9. *Significant difference (p<0.05).

Figure 2: Mean ± SD plasma oxidative stress values before and after exercise following the consumption of either a nitrate-depleted (Placebo; striped bars) or 12.8 mmol nitrate-rich (Nitrate; black bars) beverage 2.5 hours prior to exercise. A) Isoprostane activity (pg/ml) pre- and 60 minutes post-exercise (60 Recovery). B) Catalase activity (nmol/min/ml) pre- and immediately post-exercise. N=9. *Significant difference from respective pre-exercise value (p<0.05).

Figure 3: Mean ± SD arterial oxygen saturation values during exercise following the consumption of either a nitrate-depleted (Placebo; striped bars) or 12.8 mmol nitrate-rich (Nitrate; black bars) beverage 2.5 hours prior to exercise. N=9.

Figure 4: Mean ± SD blood pressure values before and after exercise following the consumption of either a nitrate-depleted (Placebo; striped bars) or 12.8 mmol nitrate-rich (Nitrate; black bars) beverage 2.5 hours prior to exercise. A) Systolic blood pressure (mmHg) (pg/ml) B) Diastolic blood pressure (mmHg) N=9.
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*Height, HT (cm); weight, WT (kg); body surface area, BSA (m²); body mass index, BMI (kg/m²); maximal oxygen consumption, VO\textsubscript{2max} (ml/kg/min)*
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<td>172 ± 25</td>
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Maximal oxygen consumption, VO₂max (ml/kg/min)
Figure 1: Mean ± SD plasma nitrite values before and after exercise following the consumption of either a nitrate-depleted (Placebo; striped bars) or 12.8 mmol nitrate-rich (Nitrate; black bars) beverage 2.5 hours prior to exercise. N=9. *Significant difference (p<0.05).

279x361mm (300 x 300 DPI)
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