A Single Dose of Beetroot Juice Enhances Cycling Performance in Simulated Altitude

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ABSTRACT

MUGGERIDGE, D. J., C. C. F. HOWE, O. SPENDIFF, C. PEDLAR, P. E. JAMES, AND C. EASTON. A Single Dose of Beetroot Juice Enhances Cycling Performance in Simulated Altitude. *Med. Sci. Sports Exerc.*, Vol. 46, No. 1, pp. 143–150, 2014. **Introduction**: Increasing nitric oxide bioavailability via supplementation with nitrate-rich beetroot juice (BR) has been shown to attenuate the negative effect of hypoxia on peripheral oxygen saturation and exercise tolerance. **Purpose**: We investigated the effects of a single dose of concentrated BR on the physiological responses to submaximal exercise and time trial (TT) performance in trained cyclists exposed to moderate simulated altitude (approximately 2500 m). **Methods**: Nine competitive amateur male cyclists (age, 28 ± 8 yr; $\dot{V}O_{2peak}$ at altitude, 51.9 ± 5.8 $mL \cdot kg^{-1} \cdot min^{-1}$) completed four exercise trials consisting of an initial graded test to exhaustion and three performance trials on a cycle ergometer. The performance trials comprised 15 min of submaximal steady-state exercise at 60% maximum work rate and a 16.1-km TT. The second and third trials were preceded by ingestion of either 70 mL of BR or nitrate-depleted BR (PLA) 3 h before exercise. **Results**: Plasma nitrate (PLA, 39.1 ± 3.5 μM; BR, 150.5 ± 9.3 μM) and nitrite (PLA, 289.8 ± 27.9 nM; BR, 678.1 ± 103.5 nM) measured immediately before exercise were higher after ingestion of BR compared with that after PLA ($P < 0.001$, $P = 0.004$). $\dot{V}O_2$ during steady-state exercise was lower in the BR trial (2542 ± 114 $mL \cdot min^{-1}$) than that in the PLA trial (2727 ± 85 $mL \cdot min^{-1}$, $P = 0.049$). TT performance was significantly faster after BR (1664 ± 14 s) than that after PLA (1702 ± 15 s, $P = 0.021$). **Conclusion**: A single dose of BR lowered $\dot{V}O_2$ during submaximal exercise and enhanced TT performance of trained cyclists in normobaric hypoxia. Consequently, ingestion of BR may be a practical and effective ergogenic aid for endurance exercise at altitude. **Key Words:** NITRATE, NITRITE, SUPPLEMENTATION, HYPOXIA, EXERCISE

Dietary nitrate supplementation results in an increase in plasma nitrate and nitrite via a nitric oxide (NO) synthase-independent pathway and has been shown to reduce resting blood pressure (22,39), attenuate the oxygen demand of submaximal exercise (1,2,23,25), and improve cycling, running, and rowing performance (6,8,24). After ingestion of sodium nitrate or nitrate-rich beetroot juice (BR), nitrate is reduced to nitrite, initially by bacteria in the gut and subsequently by commensal bacteria in the oral cavity after reentering the mouth via the enterosalivary system. After this process, the nitrite is further reduced to NO in the acidic conditions of the stomach. However, some nitrite survives this process and is absorbed by the intestines into the systemic circulation. This circulating nitrite is subsequently reduced to bioactive NO when hypoxic (7) and acidic (29) conditions are prevalent within the cell. The consequences of an increased NO concentration may include an increase in muscle blood flow and regulation of muscular contractions, glucose uptake, and cellular respiration (36).

NO also plays an essential role in the physiological response to acute and chronic altitude exposure. For example, when native lowlanders ascend to altitude, they typically experience a reduction in exhaled NO (suggesting reduced NO production), the extent of which may be associated with the prevalence of altitude sickness (10,11). The purported mechanism for this reduced NO production may be an increase in oxidative stress due to hypoxia and/or inactivation of endogenous NO synthase that catalyzes NO from circulating L-arginine (27). The reduction in the partial pressure of arterial oxygen (PO_2) and the consequent tissue hypoxia resulting from altitude exposure also have a profound ergolytic effect on endurance exercise tolerance and physical performance that is due in part to a disturbance in muscle metabolism (29). However, individuals who have adapted to living at high altitude have a higher concentration of NO products, including plasma nitrate and nitrite, than lowland-based controls (14). These individuals appear to maintain

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Submitted for publication January 2013.
Accepted for publication June 2013.

0195-9131/14/4601-0143/0
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DOI: 10.1249/MSS.0b013e3182a1dc51

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basal and maximal oxygen consumption rates that are consistent with sea level residents because of a greater blood flow associated with the increased production of NO (3,20). It is possible that this adaptation occurs because of an increased availability of intracellular L-arginine causing greater endogenous NO synthesis (14), although the precise mechanism is presently unclear. Nevertheless, these adaptations are of limited benefit to individuals who are acutely exposed to hypoxic environments during athletic or sporting competitions. Given that endurance events such as the mountain stages in cycling tours are regularly held at altitude, it is logical to assume that any alternative method to increase the concentration of NO would be of benefit to these athletes.

Intriguingly, increased NO production as a direct consequence of dietary nitrate supplementation may offset the reduction in NO during hypoxia and minimize the negative consequences on exercise performance. For example, Vanhatalo et al. (38) demonstrated that ingestion of BR reduced muscle metabolic perturbation and enhanced exercise tolerance during leg extension exercise when performed under hypoxic conditions (14.5% O_2). Masschelein et al. (28) also investigated exercise tolerance in cyclists during exposure to severe normobaric hypoxia (11% O_2) after a chronic (6-d) supplementation period of dietary nitrate and found an improvement in peripheral oxygen saturation (SpO_2) that was associated with extended time to exhaustion in a maximal incremental exercise test. Despite this, no study has yet determined the effects of an acute dose of BR on the oxygen cost of submaximal exercise and performance at moderate altitude. Therefore, the aim of this study was to investigate the effects of a single dose of BR on the oxygen cost, peripheral oxygen saturation, and time trial (TT) performance of trained cyclists exposed to acute normobaric hypoxia to simulate moderate altitude.

METHODS

Participants

Nine male trained cyclists (age, 28 ± 8 yr; stature, 182 ± 8 cm; body mass, 77.7 ± 14.1 kg; and $\dot{V}O_{2peak}$ (determined at a simulated altitude of approximately 2500 m), 51.9 ± 5.8 $mL \cdot kg^{-1} \cdot min^{-1}$) volunteered and provided written informed consent to participate in the study, which was approved by the Faculty of Science, Engineering and Computing Ethics Committee at Kingston University. Participants were recruited from local cycling and triathlon clubs and were classified as trained or well trained on the basis of cycling training and race status criteria proposed by Jeukendrup et al. (21). The participants were all nonelite cyclists but regularly completed specific cycling training (at least three sessions per week) and took part in competitive races, including road races, TT, and triathlons. All procedures were conducted in accordance with the Declaration of Helsinki.

Experimental Design

Each participant visited the laboratory on four separate occasions. On their first visit, they completed a maximal incremental test to exhaustion for determination of $\dot{V}O_{2peak}$ and maximum work rate (WR_{max}) in a normobaric hypoxic chamber set to a simulated altitude of approximately 2500 m (15% O_2) (Everest Summit II; Hypoxico, New York, NY). The hypoxic chamber was fitted with an alarmed sensor set so that the ambient fraction of inspired oxygen (FiO_2) did not fall below 14.9% or rise above 15.1% throughout the duration of any trial. Participants were not exposed to the hypoxic environment of the chamber at any stage until approximately 5 min before the start of each exercise trial. Each participant then completed three performance trials in the same environmental conditions at the same time of day, at least 5 d apart. The first performance trial consisted of a baseline measurement of performance in hypoxia with no supplementation, and the remaining two trials were preceded by ingestion of either BR (70 mL of concentrated nitrate-rich BR, approximately 5 mmol of nitrate) (Beet IT; James White Drinks Ltd, Ipswich, UK) or a nitrate-depleted placebo of BR (PLA) (approximately 0.01 mmol nitrate) (Beet IT, James White Drinks Ltd.) 3 h before the start of exercise in a double-blind randomized cross-over design. Pharmacokinetic data suggest that plasma nitrite will peak 2.5–3 h after ingestion of a single dose of BR (39), and this method of nitrate delivery has previously been demonstrated to enhance exercise performance (24). Both supplements were identical in taste and packaging, and therefore, neither participants nor lead investigators were able to identify which supplement had been ingested. Participants were asked to follow their normal diet and activity patterns, although they were requested not to exercise or consume alcohol for 24 h before each test, consume caffeine for 6 h before testing, or consume anything other than water for 3 h before testing.

Experimental Procedures

Maximal exercise test. After standard anthropometric measurements, $\dot{V}O_{2peak}$ and WR_{max} were measured using a continuous graded exercise test on an electronically braked cycle ergometer (Velotron cycles; Racermate Inc., Seattle, WA). Participants performed an initial warm-up consisting of cycling at 50 W for 5 min followed by 5 min of static stretching. Subsequently, the exercise test commenced at an initial workload of 50 W, after which, the WR increased by 30 W every minute until volitional exhaustion. Throughout the test, SpO_2 was continuously measured via a pulse oximeter (BCI Autocorr; Smiths Medical, Rockland, MN), WR and cadence were continuously monitored using device software (CS 1.5 software), HR was measured by telemetry (Polar Electro Oy, Kempele, Finland), and respiratory variables were measured via indirect calorimetry. Testing for the initial batch of participants was completed using the Oxycon Pro metabolic cart (Jaeger, Hoechberg, Germany) (n = 7);

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however, because of a malfunction, it was replaced with the K4b^2 portable metabolic analyzer (COSMED, Rome, Italy) (n = 2). The same analyzer was used for the different performance trials of each individual participant. We have previously shown that there is no difference in the measurement accuracy of respiratory variables between the two analyzers in our laboratory (12). Each metabolic analyzer was calibrated immediately before the test. The K4b^2 was calibrated inside the hypoxic chamber after the FiO_2 setting had been altered to 15% using the device software. The Oxycon Pro had the high/low FiO_2 setting enabled on the device software and was calibrated outside of the environmental chamber in accordance with the manufacturer's guidelines.

Performance trials. Approximately 1 wk after the maximal exercise test, each participant completed the first of three separate cycling-specific hypoxic performance trials. These consisted of 15 min of continuous steady-state cycling at 60% of WR_{max} and, after a 5-min passive rest period, a 16.1-km TT (Velotron 3D software, version 1). During the TT, each participant was instructed to cycle at a freely chosen velocity and encouraged to complete the 16.1 km in the shortest time possible. Participants received verbal feedback on the distance they had completed at 1-km intervals and every 100 m for the last kilometer. HR, SpO_2, and respiratory variables were continuously monitored throughout each trial as previously described.

Blood collection and analysis. Before the start of each performance trial, participants were required to remain in the supine position for 10 min, after which, blood pressure of the brachial artery was measured manually using a stethoscope and sphygmomanometer (Accoson, London, UK), and 4 mL of venous blood was collected from the cephalic vein. The blood was collected in a tube containing ethylenediaminetetraacetic acid and immediately centrifuged at 4000 rpm at 4°C for 10 min (2). The plasma was then separated into two cryovials and immediately frozen in liquid nitrogen before being stored at −80°C for a maximum of 4 months for later analysis of nitrate and nitrite via ozone-based chemiluminescence (32). The procedures for the determination of nitrate and nitrite have been previously described by Peacock et al. (31). Briefly, after samples were thawed in a water bath at 37°C for 3 min, nitrate concentration was determined using the reductant vanadium chloride in hydrochloric acid at 80°C. Nitrite was determined in a separate assay using the reductant potassium iodide in acetic acid at 50°C.

Data Analysis

Data are reported as mean ± SEM. Differences in blood pressure, plasma nitrate and nitrite, and TT completion time between PLA and BR conditions were assessed using a paired samples t-test. The remaining data were analyzed using a two-factor, within-subjects, repeated-measures ANOVA to examine the effects of supplement (BR or PLA), time, and the interaction between the two. *Post hoc* analysis was completed using Bonferroni multiple comparisons. The relations between TT performance and plasma nitrate and nitrite concentrations were assessed using Pearson correlation coefficient. The null hypothesis was rejected when $P < 0.05$. Effect size (Cohen d) and 95% confidence intervals (CI) are included, together with P values, where appropriate. All statistical procedures were completed using SPSS for Mac version 19.0.

RESULTS

Plasma nitrate and nitrite and blood pressure. Plasma nitrate concentration in the baseline trial (42.8 ± 3.9 μM) did not change after PLA (39.1 ± 3.5 μM, P = 0.482) but increased significantly after BR ingestion (150.5 ± 9.3 μM, $P < 0.001$; 95% CI, 89.3–133.1 μM) (Fig. 1). Plasma nitrite also increased significantly after BR ingestion compared with baseline (P = 0.004; 95% CI, 165.6–611.1 nM) but was not affected by PLA (P = 0.160) (baseline, 408.5 ± 59.0 nM; PLA, 289.8 ± 27.9; BR, 678.1 ± 103.5 nM) (Fig. 2). Systolic blood pressure was reduced after BR (PLA, 123 ± 7 mm Hg; BR, 120 ± 5 mm Hg, P = 0.041; 95% CI, 0.15–5.85 mm Hg). Diastolic blood pressure and mean arterial pressure also tended to be lower, although there was no statistical difference between trials (diastolic blood pressure PLA, 76 ± 5 mm Hg; BR, 74 ± 5 mm Hg, P = 0.164; mean arterial pressure PLA, 91 ± 5 mm Hg; BR, 90 ± 3 mm Hg, P = 0.089).

Submaximal exercise (15 min). During the 15 min of submaximal steady-state exercise, $\dot{V}O_2$ was significantly lower in the BR trial compared with PLA (P = 0.049; 95% CI, 1.3–369.5 mL·min^{-1}, Fig. 3). *Post hoc* analysis revealed that $\dot{V}O_2$ was significantly reduced at the 12-min interval

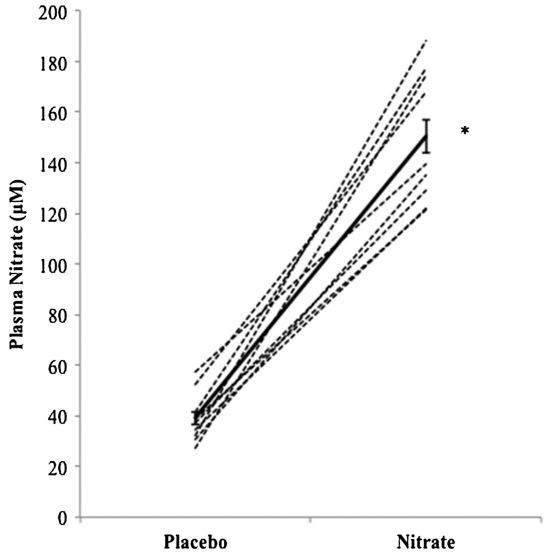

FIGURE 1—Plasma nitrate concentration 3 h postsupplementation during PLA and BR trials. Data are presented as individual responses (*dashed lines*) and mean (*solid line*) ± SEM (*error bars*). *Significant difference from PLA–BR supplementation ($P < 0.001$)

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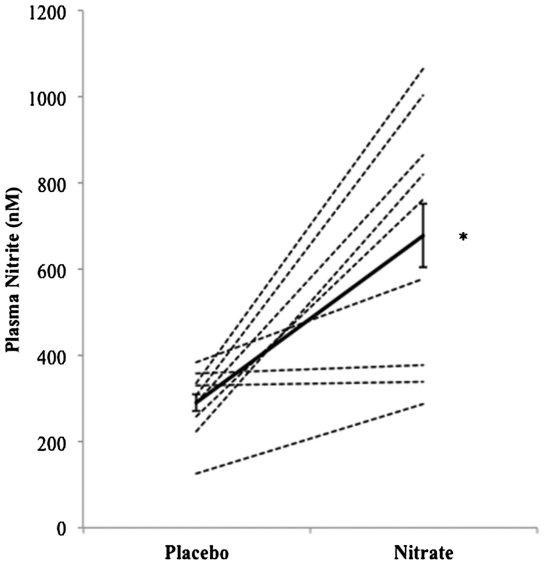

FIGURE 2—Plasma nitrite concentration 3 h postsupplementation during PLA and BR trials. Data are presented as individual responses (*dashed lines*) and mean (*solid line*) ± SEM (*error bars*). *Significant difference from PLA–BR supplementation ($P = 0.004$)

($P = 0.033$; 95% CI, 22.3–393.9 mL·min^{-1}) and 15-min interval ($P = 0.049$; 95% CI, 0.5–316.3 mL·min^{-1}). There was no difference in SpO_2 between trials ($P = 0.137$, Fig. 4).

TT (16.1 km). BR improved performance by 2.9% compared with baseline (baseline, 1716 ± 17 s; BR, 1664 ± 14 s, $P = 0.006$; 95% CI, 15.3–66 s) with a medium effect size ($d = 0.67$), and performance was significantly improved compared with PLA (PLA, 1702 ± 15 s, $P = 0.021$, Fig. 5). Performance was not different between baseline and PLA trials ($P = 0.165$). Eight of the nine participants were quicker during the BR trial than during the PLA trial (Fig. 5). Mean power output during the TT was not different between baseline and PLA trials (baseline, 212 ± 6 W; PLA, 216 ± 6 W, $P = 0.153$); however, it increased significantly after BR supplementation (BR, 224 ± 6 W, $P = 0.021$; 95% CI, 4–19 W). There was no correlation between baseline plasma nitrite concentration and TT performance in either the PLA ($R = 0.030$, $P = 0.940$) or BR trials ($R = 0.523$, $P = 0.149$). There was also no correlation between the change in plasma nitrite, nitrate, and submaximal $\dot{V}O_2$ and the change in TT performance between PLA and BR conditions (nitrite, $R = -0.420$, $P = 0.227$; nitrate, $R = 0.210$, $P = 0.587$; $\dot{V}O_2$, $R = -0.109$, $P = 0.781$).

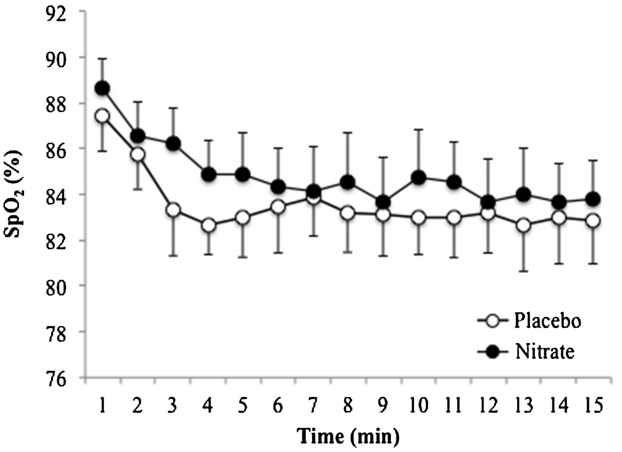

FIGURE 4—Peripheral oxygen saturation during submaximal exercise after PLA (*white circles*) and BR (*black circles*) supplementation. Data are presented as the mean ± SEM (*error bars*).

DISCUSSION

The deleterious effect of a hypoxic environment on endurance exercise performance is a major issue for many athletes. Competitions are regularly held in moderate- and high-altitude environments such as the mountain stages in the Tour de France (up to approximately 2800 m) and the

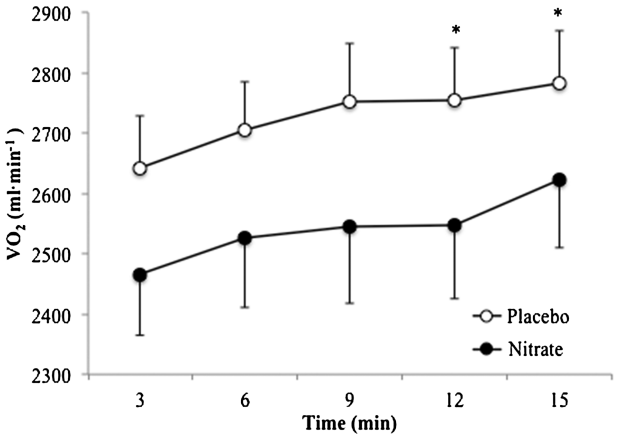

FIGURE 3—Oxygen consumption during submaximal exercise after PLA (*white circles*) and BR (*black circles*) supplementation. Data are presented as the mean ± SEM (*error bars*). *Significant difference between PLA and BR ($P = 0.049$).

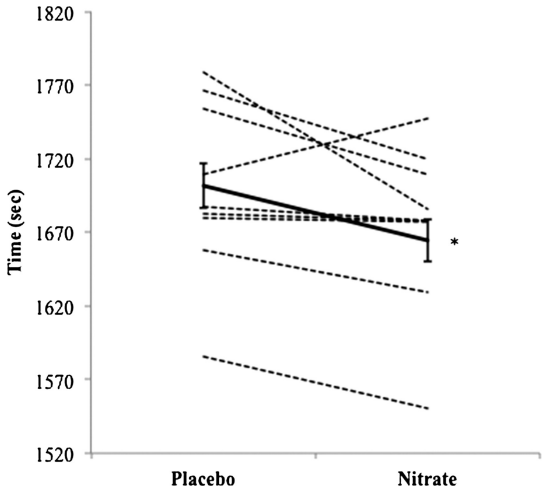

FIGURE 5—Time to complete 16.1-km TT after PLA and BR supplementation. Data are presented as individual times (*dashed line*) and the mean time (solid line) ± SEM (*error bars*). *Significant difference between PLA and BR ($P = 0.006$).

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Everest Marathon (up to approximately 5200 m). The present study investigated the effects of acute BR ingestion on cycling performance in normobaric hypoxic conditions. The principal finding was that a single dose of BR reduced the oxygen cost of steady-state exercise and enhanced 16.1-km TT performance at simulated altitude. Therefore, ingestion of BR before a competition may provide a simple but effective strategy to minimize the ergolytic effects of altitude exposure on endurance exercise performance.

Other recent studies have also investigated the effects of dietary nitrate supplementation on the response to exercise in hypoxia. Simulated altitude has been shown to have a profound ergolytic effect on exercise tolerance, demonstrated by a 36% reduction in cycling time to exhaustion in hypoxic conditions compared with normoxic conditions (28). The authors suggest that this may be due to a reduction in arterial PO_2, resulting in impaired O_2 diffusion to the muscle. However, the same authors demonstrated that supplementation with dietary nitrate, although not affecting cerebral oxygenation and symptoms of acute mountain sickness, reduced $\dot{V}O_2$ and enhanced both muscle oxygenation status and exercise time to exhaustion during cycling exercise at a simulated altitude of 5000 m. Furthermore, Vanhatalo et al. (38) reported that BR reduced muscle metabolic perturbation, demonstrated by a reduction in the rate of phosphocreatine degradation and P_i accumulation, and restored both exercise tolerance and oxidative function during knee extension exercise at a simulated altitude of 3000 m compared with that observed in normoxia. The novel data obtained in the present study suggest that a single bolus of nitrate-rich BR with ensuing reduction in $\dot{V}O_2$ during submaximal exercise also translates to an enhancement in actual exercise performance. This is despite no correlation between the reduction in $\dot{V}O_2$ and the improvement in TT performance between PLA and BR conditions. The enhancement of exercise performance after acute BR ingestion is consistent with some (6,8,24), but not all (4,9,30,31,40), previous research in this area. Nevertheless, the mechanism(s) underpinning this ergogenic effect continue(s) to be debated.

One possible explanation for the ergogenic effects of nitrate supplementation in this and other studies (6,8,24) may be an augmented muscle blood flow during exercise. NO is a potent vasodilator, and nitrate supplementation has been shown to increase estimated local blood volume at the muscle during unloaded cycling and the initial 120 s of moderate-intensity exercise (2). The authors attributed these effects to an enhanced muscle vasodilatation resulting from increased NO production from nitrite. Ferguson et al. (16) demonstrated that blood flow and vascular conductance in the exercising muscle of rats were higher after 5 d of BR supplementation. Intriguingly, the increased blood flow and vascular conductance were observed primarily in fast-twitch Type II muscle fibers, suggesting that the effects of dietary nitrate supplementation may be fiber-type selective. In contrast, Masschelein et al. (28) reported no difference in regional blood volume in humans during submaximal and maximal exercise at simulated altitude between BR and control conditions. Blood volume was estimated by measuring the change in the fraction of total hemoglobin using near-infrared spectroscopy, a measurement that correlates well with changes in tissue blood flow (37). Although blood flow was not measured in the present study, these data suggest that the enhanced exercise performance after nitrate supplementation cannot exclusively be explained by a stimulation of local vasodilation and oxygen delivery to the muscle.

Instead, there is more compelling evidence to suggest that dietary nitrate supplementation may improve the efficiency of mitochondrial respiration (26) and/or reduce the adenosine triphosphate (ATP) cost of muscle force production (1). When ATP is resynthesized via oxidative phosphorylation, there is a leakage of protons back across the inner mitochondrial membrane into the mitochondrial matrix from the intermembrane space. This protein leakage results in a substantial use of oxygen (approximately 25%) that does not contribute to ATP synthesis and accounts for 15% of active and 20% of resting $\dot{V}O_2$ (33). Remarkably, Larsen et al. (26) reported that supplementation with dietary nitrate can reduce proton leakage, and the resulting improvement in mitochondrial efficiency may explain, at least in part, the reduced oxygen cost of exercise. Consistent with this hypothesis was their finding that the *in vitro* mitochondrial phosphate-to-oxygen ratio was reduced after dietary nitrate. This was correlated with the reduction in the *in vivo* power output-to-$\dot{V}O_2$ ratio during exercise, suggesting enhanced efficiency of ATP synthesis. Alternatively, Bailey et al. (1) suggest that the reduced oxygen cost of exercise after nitrate supplementation may be directly related to a reduced ATP cost of cross-bridge cycling and/or calcium handling. Likewise, this hypothesis is underpinned by a sound physiological mechanism because NO has been shown to modulate Ca^{2+} activation and the actin–myosin interaction during submaximal activation of skeletal muscle (19). Indeed, supplementation with dietary nitrate has been shown to increase the myoplasmic free $[Ca^{2+}]$ during tetanic stimulation of isolated mouse fast-twitch muscles, leading to an enhanced contractile force (18). The purported mechanism(s) accounting for the contribution of NO to energy metabolism also indicate(s) that supplementation with dietary nitrate may be particularly pertinent when muscle oxygenation is compromised during hypoxia.

Data from the present study showed that ingestion of BR also resulted in a small increase in SpO_2 compared with the PLA condition, although differences did not reach statistical significance (Fig. 4). Masschelein et al. (28) have recently reported that dietary nitrate supplementation resulted in a significant increase in SpO_2 during exercise in severe hypoxia (11% ambient O_2). It is important to note that the SpO_2 during submaximal exercise in the study of Masschelein et al. (28) (approximately 70%) was substantially lower than that in the present study (approximately 84%), presumably because of the differences in FiO_2 (11% vs 15%,

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respectively). In addition, these authors reported that the muscle tissue oxygenation index, which assesses the fraction of oxygen-saturated tissue hemoglobin and myoglobin, was significantly higher in the *vastus lateralis* muscle after BR supplementation. Taken together, these findings suggest that dietary nitrate supplementation reduces muscle oxygen extraction, which is consistent with the mechanisms proposed by both Bailey et al. (1) and Larsen et al. (26) as discussed previously.

Engan et al. (13) found that BR ingestion increased SpO_2 during a static apnea hold and increased maximal apneic duration by approximately 11%. The authors suggest that the substantial reduction in SpO_2 during a maximal apnea may, as previously described, be partly offset by an increased availability of NO. In direct contrast, a similar study by Schiffer et al. (35) reported that dietary nitrate supplementation actually reduced both SpO_2 and breath hold duration during a static apnea. However, the authors also assessed the effects of BR supplementation on an apnea during light-intensity exercise (50 W). With this experimental protocol, there was a trend toward higher SpO_2 during maximum effort apnea in the BR trial than that in the PLA trial. Comparative analysis is difficult because of the profound differences in methodologies used by the two studies, including the breath hold training status of the participants, the supplementation strategy, the inhalation procedure before apnea, and the placement of the probe to measure SpO_2. Despite this, a reduction in SpO_2 during a static apnea after BR supplementation may not be entirely unexpected because the NO-mediated vasodilation in the microcirculation would enhance peripheral blood perfusion, augmenting arterial oxygen desaturation (35). The contrasting findings during the exercise apnea may be a consequence of the working skeletal muscles becoming the dominant consumer of oxygen during exercise, with nitrate supplementation reducing the rate of oxygen extraction as previously described. Therefore, there is good evidence from our study and others (28,35) that dietary nitrate supplementation results in a small increase in SpO_2 during exercise in hypoxia.

Despite the physiological and ergogenic effects demonstrated here, a single dose of BR has recently been suggested to be less effective than a chronic (approximately 6 d) supplementation protocol (4,9,30,31,40). Recent evidence supporting the use of more prolonged supplementation has been reported by Hernandez et al. (18) who demonstrated that 7 d of BR ingestion increased force production of the fast-twitch muscle fibers in mice, which was associated with an alteration in muscle protein expression. Furthermore, the reported improvement in muscle blood flow (16) and *in vitro* mitochondrial phosphate-to-oxygen ratio (26) occurred after a more prolonged period of nitrate supplementation (5 and 3 d, respectively). It could be argued that the lack of performance effects in these studies may be attributed to the use of highly trained or elite endurance athletes in contrast to the trained or recreationally active participants used in this and other studies. Identifying why this may be the case is problematic because of differences in supplementation protocols and exercise modalities. It should, however, be emphasized that the baseline nitrate/nitrite pool is higher in endurance-trained athletes than that in untrained matched controls, which may partially explain these differences (34).

Recent studies have also shown that individual variability in the response to dietary nitrate supplementation may influence the subsequent effect on exercise performance. Data presented by Wilkerson et al. (40) suggest that there may be a responder versus nonresponder phenomenon with dietary nitrate supplementation. They report a correlation between the change in plasma nitrite and the change in exercise performance after nitrate supplementation and define a "responder" as an increase in plasma nitrite of >30% after nitrate supplementation. When examining the TT and nitrite data from individual participants, the current study would seem to support this hypothesis. Despite a mean increase of 134% in plasma nitrite, similar to Lansley et al. (24) (138%) but substantially greater than others (5,40) (16% and 25% respectively), the change in plasma nitrite levels in two of our nine participants would place them in the nonresponder category (Fig. 2). One of these participants completed the TT slower in the BR condition compared with the PLA condition (the only one of the nine), and the other improved his performance by just 0.2%. Nevertheless, we did not find that the differences in plasma nitrite and TT performance between BR and PLA conditions across the cohort of cyclists were significantly correlated, nor indeed was there a correlation between baseline plasma nitrite concentration and TT performance in either the PLA or BR trials. It is worth noting that one participant appears to be an outlier that may be a consequence of the well-described interindividual variability in the response to normobaric hypoxia (17). When this participant was removed from the analysis, the correlation between the change in plasma nitrite and TT performance between PLA and BR conditions was improved ($R = -0.601$, $P = 0.115$). The individual variability in the response to nitrate supplementation is unquestionably a key issue, and further research investigating the effect of training status, baseline nitrite concentration, and environmental conditions is recommended.

It is acknowledged that a limitation of the current study was that the consumption of nitrate-rich foods in the days preceding each test was not controlled and the use of antibacterial mouthwash was not restricted. Despite this, the increase in plasma nitrite after ingestion of BR in the present study is among the largest in the published literature to date; yet, no changes in nitrite or nitrate were observed after PLA. A further limitation that should be acknowledged is that the normobaric conditions of the exercise trials do not truly represent the hypobaric hypoxia at true altitude (15). However, although further research in this area is clearly warranted, it is likely that our findings would hold true under hypobaric hypoxic conditions because the PO_2 is the critical factor limiting exercise performance at altitude (28).

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CONCLUSION

The principal findings of the present study were that a single dose of BR 3 h before exercise at a simulated altitude of 2500 m resulted in a substantial reduction in $\dot{V}O_2$ and a small increase in SpO_2 during submaximal exercise, which was coupled with an improvement in a 16.1-km TT performance. Although we have reported that BR is a practical yet effective ergogenic aid for exercise at simulated altitude, additional work is required to investigate the mechanism responsible for this effect and the optimum supplementation strategy to maximize performance.

The authors thank all the participants who volunteered for this study. The authors disclose that no funding was received for this work and have no conflicts of interest to declare. The results of the present study do not constitute an endorsement by the American College of Sports Medicine.

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