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Title

Chronic high-dose beetroot juice supplementation improves time trial performance of well-trained cyclists in normoxia and hypoxia

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Abstract

Dietary nitrate (NO$_3^-$) supplementation via beetroot juice (BR) is known to improve endurance performance in untrained and moderately trained individuals. However, conflicting results exist in well-trained individuals. Evidence suggests that the effects of NO$_3^-$ are augmented during conditions of reduced oxygen availability (e.g., hypoxia), thereby increasing the probability of performance improvements for well-trained athletes in hypoxia vs. normoxia. This randomized, double-blinded, counterbalanced-crossover study examined the effects of 7 days of BR supplementation with 12.4 mmol NO$_3^-$ per day on 10-km cycling time trial (TT) performance in 12 well-trained cyclists in normoxia (N) and normobaric hypoxia (H). Linear mixed models for repeated measures revealed increases in plasma NO$_3^-$ and NO$_2^-$ after supplementation with BR (both p<0.001). Further, TT performance increased with BR supplementation (~1.6%, p<0.05), with no difference between normoxia and hypoxia (p=0.92). For respiratory variables there were significant effects of supplementation on VO$_2$ (p<0.05) and VE (p<0.05) such that average VO$_2$ and VE during the TT increased with BR, with no difference between normoxia and hypoxia (p≥0.86). We found no effect of supplementation on heart rate, oxygen saturation or muscle oxygenation during the TT. Our results provide new evidence that chronic high-dose NO$_3^-$ supplementation improves cycling performance of well-trained cyclists in both normoxia and hypoxia.

Keywords:
- Nitrate,
- Nitrite,
- Endurance exercise,
- Cycling performance,
- Hypoxia,
1.1 Introduction

There is general consensus regarding the physiological factors that limit endurance performance [1,2]. These factors include maximal oxygen consumption (VO$_{2\text{max}}$), the fractional utilization of VO$_{2\text{max}}$, and exercise efficiency. Even minor improvements in these factors can enhance performance of endurance athletes. One strategy proposed to improve performance is inorganic nitrate (NO$_3^-$) supplementation, most often in the form of concentrated beetroot juice (BR) [3]. When ingested, nitrate is reduced to nitrite and nitric oxide (NO). This pathway differs from the classical pathway for NO generation which involves specific enzymes, NO-synthases (NOS) that use L-arginine and molecular oxygen to generate NO. Nitric oxide has been demonstrated to alter several physiological processes such as blood flow, mitochondrial function and contractile properties [3-8]. Recently, several studies have provided evidence that dietary intake of NO$_3^-$ can improve exercise efficiency (reduction in VO$_2$ at same work rate) [9-12] and endurance performance [9,10,13-17]. Notably, the majority of studies reporting beneficial effects of NO$_3^-$ has been conducted in untrained and moderately trained individuals (VO$_{2\text{max}}$ < 60 ml/min/kg) [10,15,16,18], whereas studies in highly trained individuals (VO$_{2\text{max}}$ > 60 ml/min/kg) have shown minor [16,19-21] or no improvements [22-27], indicating that NO$_3^-$ may be less effective in this population [28,29]. In addition to this, recent studies in hypoxia have also provided evidence that NO$_3^-$ improves exercise efficiency [17,21,30,31], muscle oxygenation [31] and elevates oxygen saturation (SpO$_2$) [21,30,31]. The lower O$_2$ availability in hypoxia impairs the L-Arginine-NOS pathway, and potentiates the nitrate-nitrite-NO pathway, suggesting that BR may be more effective in hypoxia.
than in normoxia [3,32-34]. Supporting the notion that BR is more effective in hypoxia, Kelly et al. [30] showed that, in healthy individuals, BR improved time to exhaustion during severe intensity exercise in hypoxia but not in normoxia. In addition, BR has been shown to attenuate the decrease in muscle oxygenation and muscle metabolic perturbation in hypoxia in untrained and moderately trained subjects [31,35]. Hence, highly trained athletes may also experience greater performance improvements with BR in hypoxia compared with normoxia.

Recently, few studies have examined this idea with conflicting results. In well-trained athletes NO\textsuperscript{3-} supplementation had no effect on 10-km or 15-km cycling performance, 10-km running performance or roller-skiing treadmill performance in hypoxia [36-39]. Contrary to this, two studies have reported positive effects of BR in hypoxia on 16.1-km cycling performance and 1500m running performance in trained athletes [17,21]. The discrepancy could be due to different supplementation strategies for NO\textsuperscript{3-}. Specifically, the effects of NO\textsuperscript{3-} supplementation seems to be potentiated with BR as source of NO\textsuperscript{3-} [40,41], with chronic loading over several days [42,43], and by using a dose of >8mmol per day [13,20,44]. Optimizing the supplementation strategy of NO\textsuperscript{3-} may be even more important in trained athletes, as this population already exhibit adaptations elicited by endurance training and diet, including higher NO\textsuperscript{3-} plasma levels [45,46], NO release [47], NOS activity[48] and a higher percentage of type I fibers [8,49], that altogether may attenuate the response to NO\textsuperscript{3-} supplementation.

The purpose of the present study was to examine the effects of several days supplementation with a high-dose BR on cycling time trial performance in well-trained cyclists, with continuous measurements of \textit{SpO}\textsubscript{2}, muscle oxygenation and
oxygen uptake in normoxia and normobaric hypoxia. We hypothesized that BR would improve TT cycling performance in hypoxia but not in normoxia.

2.1 Material and Methods

2.1.1 Participants

Twelve healthy male cyclists at the age of 29.1 ± 7.7 yrs (range 22 to 44 yrs) were enrolled in the study. Participants had a VO$_{2\text{max}}$ of 5.09 ± 0.47 L·min$^{-1}$ corresponding to 66.4 ± 5.3 ml·min$^{-1}$·kg$^{-1}$ and a wattmax of 430 ± 35 watt corresponding to 5.6 ± 0.3 watt·kg$^{-1}$ (mean ± SD). Participants were best classified as well-trained in performance level 4 as defined by Jeukendrup et al. [50] and De Pauw et al. [51], respectively. The protocol and test procedures used in the current study were conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Northern Jutland (N-20150049). All participants signed informed consent prior to enrollment.

2.1.2 Study design

Participants reported to the laboratory on five separate occasions. Experimental trials followed a randomized counterbalanced-crossover design and were double-blinded for supplementation and single-blinded for inspiratory conditions. The first visit consisted of a maximal exercise performance test to ensure participants were familiar with testing procedures and to ensure participants met the inclusion criteria (i.e., VO$_{2\text{max}}$ > 60 ml·kg·min$^{-1}$ or wattmax ≥ 5 w/kg). Visits 2-5 involved four experimental trials (Fig 1). Each trial consisted of a 10-km time trial performed in conditions of normoxia or hypoxia, with supplementation of BR or nitrate-depleted BR as placebo (PLA). Specifically, supplementations were ingested in periods of seven days, separated by a wash out period of at least seven
days. During each supplementation period, 10-km time trials were performed on
day four and day seven, in different conditions. The order of condition was
maintained for each individual for the first and second supplementation period
such that visits 1 and 3 (and visit 2 and 4) were performed in the same condition.
The design was counterbalanced for condition and supplementation such that half
of the participants started with normoxia and half of the participants started with
BR. All exercise trials were performed on the Cyclus2 ergometer (RBM Cyclus 2,
Germany) using the participants’ own bike.

Figure 1: Experimental design

2.1.3 Maximal exercise performance

Participants completed a 10-minute warm up at 100 watts and hereafter an
incremental exercise test to exhaustion to determine gas exchange threshold
(GET[30]), VO$_{2max}$ and wattmax (Fig 1). The incremental exercise test
commenced at 100 watts and increased by 30 watts each minute until voluntary
exhaustion. Following a 10-minute rest, participants completed a familiarization
trial for the 10-km TT. While a VO$_{2max}$ validation bout is recommended [52], this
was not performed in this present study as these well-trained cyclists routinely achieve maximal effort during exercise. Respiratory breath-by-breath data were measured throughout the test using a metabolic cart (Jaeger, Vyntus CPX, Carefusion). The metabolic cart was calibrated before each test according to the manufacturer’s recommendations. Maximal oxygen uptake (VO$_{2\text{max}}$) was determined as the highest 30-second average, Wattmax as peak power output from the last minute of the test ((watt) + time in last stage (s)/60 × 30 (W)) and heart rate (HR) as the peak value attained during the test. GET was determined from a number of measurements, including 1) the first disproportionate increase in VCO$_2$ from visual inspection of plotting VCO$_2$ and VO$_2$ and 2) an increase in expired ventilation ($V_E/VO_2$) with no increase in $V_E/VCO_2$ [30]. HR was recorded continuously using a heart rate sensor (Polar Electro, Oy, Finland).

2.1.4 Experimental trials

Participants ingested BR or PLA for seven consecutive days (Fig 1). Specifically, participants consumed 140ml of concentrated BR (~12.4 mmol nitrate) or 140ml of nitrate-depleted BR (PLA; ~0 mmol nitrate) (Beet It Sport, James White Drinks Ltd., Ipswich, UK) per day; one dose (70 ml) in the morning and one dose (70 ml) in the evening. On the days of the experimental trials (i.e., days four and seven), participants were instructed to consume the total dose (i.e., 140 ml) 2-h prior to arriving at the laboratory (approx. 2.75-h. before commencing the time trial). During the 24-h preceding the first experimental trial, each participant recorded their diet and was told to replicate this diet for the remaining three trials. Participants were also instructed to avoid the intake of specific nitrate-rich foods.
The use of antibacterial mouthwash products was not permitted and caffeine intake was prohibited for 12-h preceding each test. For each individual, all experimental trials were performed at the same time of day. Upon arrival at the laboratory, participants rested for 5-minutes before a resting blood sample was drawn into two 4 ml lithium heparin vacutainers (Becton Dickinson, Plymouth, UK). Blood samples were immediately centrifuged for 10 min at 4°C, 3000g after which plasma was extracted and stored at -80°C for later determination of plasma nitrate and nitrite according to the method described by Hezel et al. [53]. A near infrared spectroscopy (NIRS) probe (Oxymon MK III, Artinis Medical Systems, Netherlands) was placed on the belly of the Vastus Lateralis of the right leg in order to measure changes in muscle oxygenation. Probe position was marked with a permanent pen to ensure identical probe placement for subsequent trials, and the NIRS probe was placed with double-sided adhesive tape. Further, elastic bandages were used to ensure a fixed placement of the probe. An earlobe pulse oximeter (Nonin XPod 8000Q2, Nonin Medical, Inc, Plymouth, MN) was used to measure SpO₂ throughout the tests. Participants then rested 5-minutes on the bike while breathing the gas mixture corresponding to the condition for that specific trial. Throughout each trial, participants breathed through a facemask (Hans Rudolph, V-982185) connected to a low resistance y-valve (Hans Rudolph, two way Y-shape non-rebreathing valve, 2730L), with the inspiration valve connected to a closed reservoir. The inspired gas was modified via the closed reservoir using a custom built setup consisting of a mechanical ventilator (SV-300, Maquet, Solna, Sweden) modified such that mixing of gas (pressurized room air and nitrogen) was controlled by manipulating
the inspired oxygen setting on the ventilator. The participants breathed through
the same circuit for all experimental trials. The fraction of inspired oxygen was
adjusted to 15 ± 0.1% in hypoxia (~2500m of altitude) and 20.9 ± 0.1% in
normoxia (sea level). Warm-up consisted of three six-minute exercise bouts at the
power output corresponding to 70% of GET measured in normoxia. A six-minute
rest separated each bout. After the third bout, participants rested for 10 minutes
without the facemask. Prior to the TT, participants sat on the bike for five minutes
while breathing the gas mixture corresponding to the conditions for that specific
trial. Then participants completed a 10-km TT with the instruction of finishing
with the highest average power output and as fast as possible. Participants were
blinded to all information except cadence and remaining distance of the TT, and
were verbally encouraged at each km completed. VO\textsubscript{2} and HR were measured
continuously during the TT. For all physiological variables, average values from
the 10km-TT were calculated and used for further analyses. Further, peak values
for VO\textsubscript{2}, RER (both highest 30-s average) and HR (highest 1-s value) during the
TT were calculated and used for further analyses. The ratio of average power to
average oxygen uptake (PO/VO\textsubscript{2}) during the time trial was used as an index of
exercise efficiency [15]. NIRS variables of oxygenated (HbO\textsubscript{2}), deoxygenated
(HHb) and total (THb) hemoglobin were recorded continuously at 2 Hz and
expressed as relative changes (Δ) from the baseline value measured during the
final 90-seconds pre-exercise rest period.

2.1.5 Statistical analysis

Differences in performance and physiological parameters were analyzed using
linear mixed models for repeated measures. This method of data analysis was
used as it has the advantage of preventing listwise deletion due to missing data (md). For clarification, md for each variable has been noted in table 1. As the dependent variable, the variable of interest was entered (watt, VO₂, VE, VCO₂, SpO₂, etc.) into the model. To investigate the effects of supplementation (BR vs. PLA), condition (hypoxia vs. normoxia) and supplementation-by-condition, these were entered as fixed effects. Subject id was included in the model as a random effect to control for the within-subject nature of the 4 trials. Further, paired t-tests were used to compare differences between the VO₂peak obtained during the normoxic time trials and the VO₂max from the ramp incremental test. Within group effect sizes were calculated as the difference in means (BR vs. PLA) divided by the pooled SD of the change score, using the following definitions: trivial effect d < 0.2, small effect > 0.2, moderate effect > 0.5, large effect > 0.8 [54]. Associations between changes in TT performance and changes in NO₃⁻, NO₂⁻, VO₂, and SpO₂ from PLA to BR were assessed using Pearson correlation coefficient.

All data are presented as means ± SE, unless stated otherwise, with statistical significance being accepted when $P \leq 0.05$. All statistical tests were performed using SPSS 25 (IBM Corp., Armonk, USA) or STATA (Texas, USA) version SE 12.1.

3.1 Results

3.1.1 Plasma nitrate and nitrite

There were significant main effects of supplementation on NO₃⁻ and NO₂⁻ (both p<0.001) such that BR elevated NO₃⁻ and NO₂⁻ (Fig 2). There were no effects of
condition \((\text{NO}_3^-; p=0.858; \text{NO}_2^-; p=0.542)\) or supplementation-by-condition interaction \((\text{NO}_3^-; p<0.907; \text{NO}_2^-; p=0.687)\).

Further, there were no differences in levels of \(\text{NO}_3^-\) \((p=0.234)\) or \(\text{NO}_2^-\) \((p=0.231)\) between 4 and 7 days of supplementation (Fig 3).

Figure 2: Individual and mean plasma levels of \(\text{NO}_3^-\) (A) and \(\text{NO}_2^-\) (B) (mean±SE) prior to time trial tests in normoxia (open bars) and hypoxia (filled bars), after supplementation with beetroot juice (BR) or placebo (PLA). (#, \(p < 0.001\), PLA vs. BR, \(N=11\) in hypoxic conditions).
Figure 3: Individual and mean plasma levels of NO\textsubscript{3}\textsuperscript{−} (A) and NO\textsubscript{2}\textsuperscript{−} (B) (mean±SE) prior to time trial tests at day 4 and day 7 after supplementation with beetroot juice (filled bars) or placebo (open bars). (#, p < 0.001, PLA vs. BR, N=11 in hypoxic conditions).

3.1.2 Time trial performance

All participants completed all four TT’s. However, two tests were discarded due to measurement error (n=1 in N-BR and n=1 in H-PLA). Time trial performance data are presented in Table 1. There was a main effect of condition (p<0.001) on time trial performance such that hypoxia lowered power output by ~15% and ~6%, respectively. Further, there was a main effect of supplementation on time trial power output (p=0.019) and completion time (p=0.024) showing an overall 1.6%
increase in power output and 0.6% reduction in completion time with BR (Fig 4), with no condition-by-supplementation interaction (both p=0.923). Notably, 10 out of 11 participants increased power output in H-BR compared to H-PLA, whereas 6 out of 11 increased power output in N-BR compared to N-PLA (Fig 4). Effect size calculations for within group differences between BR and PLA show moderate (0.703) and small (0.398) effects for hypoxia and normoxia, respectively.

Figure 4. Individual and mean differences in power output (watt) during 10 km TT performance between placebo and beetroot supplementations in normoxic and hypoxic conditions. Bold horizontal lines indicate mean values for each condition. Single dotted line indicates no difference between beetroot and placebo supplementation
Table 1- Average and peak performance, ventilatory and cardiopulmonary data during the TT. md denotes the number of missing data points from each variable (complete number of data points = 48).

3.1.3 TT physiological data

Physiological data obtained during the TT are presented in Table 1. There were significant effects of condition on SpO₂ (p<0.001), VE (p=0.010), RER

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<td>Completion time, sec</td>
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Average values

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Peak values

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NIRS

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(p=0.003), VCO₂ (p=0.001), VO₂ (p<0.001), PO/VO₂(p=0.001) and %VO₂max
(p<0.001) such that hypoxia decreased SpO₂, VCO₂, VO₂, PO/VO₂, VO₂peak,

HR<sub>peak</sub> and %VO₂max while VE,RER and RER<sub>peak</sub> increased. There were significant effects of supplementation on VO₂ (p=0.030) (Fig 5), VE (p=0.019), VCO₂ (p=0.005) and %VO₂max (p=0.038) such that VO₂, VE, VCO₂ and %VO₂max increased with BR. The VO₂peak attained during the time trials in normoxia were significantly lower than the VO₂max measured from the incremental test (N-PLA ~3.3%, p=0.03; N-BR ~3.7%, p=0.02).
Figure 5- Oxygen uptake profiles from an exemplar subject (A) and mean data (B) from all conditions.

3.1.4 Near infrared spectroscopy measures of muscle oxygenation

Data reflecting changes in muscle oxygenation during the TT are presented in Table 1. There was a main effect of condition on $\Delta$HHb ($p=0.042$) and $\Delta$HHb/VO$_2$ ($p=0.017$) such that the increase in $\Delta$HHb and $\Delta$HHb/VO$_2$ during the TT was greater in hypoxia (Table 1). We also found a near-significant main effect
of condition on $\Delta \text{HbO}_2$ (p=0.061) indicating a greater reduction of $\Delta \text{HbO}_2$ during TT in hypoxia.

3.1.5 Correlations

There were no significant correlations between changes in performance and changes in plasma NO$_3^-$ or NO$_2^-$ after BR supplementation in normoxia or hypoxia. Further, there were no significant correlations between changes in performance (BR vs. PLA) and changes in VO$_2$ or SpO$_2$ nor between changes in performance (BR vs. PLA) and VO$_{2\text{max}}$.

4.1 Discussion

This is the first study to examine the effects of chronic supplementation with high-dose NO$_3^-$, in the form of BR, on time trial performance in well-trained athletes in both hypoxia and normoxia. We show a significant main effect of BR on 10-km TT performance, indicating that well-trained cyclists improve power output and completion time with BR in both normoxia and hypoxia. Supplementation with BR also increased VO$_2$ during the TT in hypoxia and normoxia, showing that the participants were able to utilize a higher fraction of VO$_{2\text{max}}$ with BR.

4.1.1 Effects of BR supplementation on TT performance

We found a main effect of BR supplementation on TT performance with no condition-by-supplementation interaction, indicating that BR increased TT performance with no difference between hypoxia and normoxia. However, from a practical perspective, it is worth highlighting that 10 out of 11 participants had higher power output in H-BR vs. H-PLA, while only 6 out of 11 had higher power output in N-BR vs. N-PLA (Figure 3). In support of a small effect of BR, a recent
A meta-analysis, including studies performed in hypoxia and normoxia, reported a non-significant 0.8% improvement in time trial endurance performance following BR supplementation [55]. The improvement in 10-km TT completion time and power output of 0.6% and 1.6%, respectively, in the present study, is of practical relevance for elite and well-trained athletes. Specifically, only 0.9% separated first and fourth position during the 13.8-km TT of stage 1 at the 2015 Tour De France cycling race [56], and only 0.3% separated the first and third position during the 9.7-km TT of stage 1 at the 2018 Giro d’Italia cycling race [57]. Further, 0.6% is the smallest worthwhile change in completion time for road TT cyclists proposed by Paton and Hopkins [58].

Few other studies have examined the effects of NO$_3^-$ on TT performance in well-trained athletes in both normoxia and hypoxia within the same study. None of these studies have reported significant improvements in TT performance after BR supplementation [36,38,39]. Nonetheless, the study by Bourdillion et al. [39] reported statistically non-significant improvements in 15-km TT performance of 16s (~1%) and 151s (~7%) in normoxia and hypoxia, respectively.

In general, studies on TT performance performed in well-trained athletes in hypoxia or in normoxia have reported mixed results. In hypoxia, two studies found statistically significant improvements of 2.2-3.2% (~2.2%) [17,21], while one study reported no effect [37]. In normoxia, numerous studies show no effect [22-27,59-61], while a few studies report a significant effect [15,16,20]. The discrepancy in the literature may partly be due to the use of different NO$_3^-$ supplementation strategies that vary in terms of source, dose, and duration (e.g., chronic vs. acute). Many of the previous TT studies have not used an optimized...
supplementation strategy. Specifically, some studies have used sodium nitrate as the source of NO$_3^-$ [23,39], while there is evidence suggesting that supplementation with NO$_3^-$ in concentrated BR is more effective [40,62]. Several studies have used an acute dose of BR [17,25,26,36-38,59-61], however, a chronic loading protocol consisting of BR supplementation over several days, as used in the present study, has been suggested to be more effective in raising plasma levels of NO$_3^-$ and NO$_2^-$, and improving performance [11,43]. Finally, several studies have used a low-to-moderate dose of NO$_3^-$ [36,37,59-61], while a higher dose (8-16 mmol), as used in the present study, may be more effective in raising plasma levels and improving performance [13,20,44]. The high dose of NO$_3^-$ used in the present study was tolerated without any adverse events or complaints, demonstrating the efficacy of this supplementation strategy for 7 days. However, there is currently no evidence demonstrating additional benefits with doses higher than 8 mmol. In support of the notion that supplementation strategy is important, supplementation of high dose NO$_3^-$ in the source of BR have reported a significant 2.1% [16] and a non-significant 1.7% [24] improvement in TT power output in trained cyclists.

4.1.2 Plasma levels of NO$_3^-$ and NO$_2^-$

In the present study, plasma levels of NO$_3^-$ and NO$_2^-$ after placebo (i.e., nitrate-depleted BR) supplementation, were similar to results from other studies using nitrate-depleted BR [17,21,22,37,38,63]. Four and seven days of BR supplementation increased NO$_3^-$ and NO$_2^-$ to levels reported in studies using a similar supplementation strategy [13,22], with no
differences between 4 and 7 days. Notably, NO\textsuperscript{3−} and NO\textsuperscript{2−} levels, in the present study, were higher than those reported in studies using acute supplementation [17,21,37,38,63] or lower dosage of NO\textsuperscript{3−} [17,37,59,60]. Taken together, markedly elevated levels of NO\textsuperscript{3−} and NO\textsuperscript{2−}, in the present study, indicate that BR supplementation was effective in providing an abundant source of NO via the nitrate-nitrite-NO pathway. Plasma levels of nitrite displayed a higher variability compared to plasma nitrate (Fig 2 and Fig 3). This is a common finding and is most likely due to the shorter half-life of nitrite (less than 1h)[64] compared to nitrate (5-8h)[65]. This may be explained by a much higher reactivity of nitrite being subjected to both enzymatic reduction to NO and oxidation to nitrate [33]. Moreover, due to the markedly lower concentration of nitrite in plasma, measuring techniques display more variable results compared to nitrate.

4.1.3 Physiological effects of beetroot juice supplementation

We found a main effect of supplementation on VO\textsubscript{2}, VE, VCO\textsubscript{2} and %VO\textsubscript{2max} such that BR supplementation resulted in higher VO\textsubscript{2}, VE, VCO\textsubscript{2} and %VO\textsubscript{2max} during the TT in both hypoxia and normoxia. As studies generally show unchanged [10,12,13,30] or reduced [66,67] VO\textsubscript{2max} following BR supplementation, these results indicate that the participants were able to utilize a higher proportion of their maximal aerobic capacity during the TT with BR. Further, in the present study, a proxy of exercise efficiency (PO/VO\textsubscript{2}) during the TT was unaffected by BR supplementation, suggesting that changes in exercise efficiency did not contribute to improved TT performance. In agreement with this, several studies, in well-trained athletes (>60 ml·min\textsuperscript{−1}·kg\textsuperscript{−1}), have shown unchanged exercise efficiency during submaximal exercise following BR.
supplementation [24,37,38,63], while only a single study has reported improved
efficiency (lower VO₂ during submaximal exercise) in well-trained athletes [21].
In club-level cyclists (56.0 ml·min⁻¹·kg⁻¹) [15], BR supplementation improved
power output with unchanged VO₂ (greater PO/VO₂), indicating improved
exercise efficiency. The discrepancy between these results could be due to the
training level of the subjects, as our study included well-trained athletes (66.4
ml·min⁻¹·kg⁻¹). Thus, the increase in %VO₂max with BR was likely the main factor
contributing to increased TT performance. In accordance with these results,
Bourdillion et al. [39] reported greater VO₂ and VE with nitrate supplementation
in trained cyclists during a 15-km TT in normoxia and hypoxia, which was
accompanied by a non-significant increase (1-7%) in performance (discussed
above). Contributing to the increased VO₂ with BR, the increase in VE (~6L/min)
is estimated to account for 10-15 ml/O₂/min (~10-20%) of the increase in VO₂,
due to greater oxygen demands of the respiratory muscles [68-70].
The active skeletal muscles are the primary site for O₂ usage during the TT, and
oxygenation in the vastus lateralis was monitored continuously using NIRS.
During the TT, ∆HHb increased in hypoxia compared with normoxia, indicating
increased O₂ extraction. However, in agreement with Kelly et al. [30] and
Bourdillion et al. [39], ∆HHb was unaffected by BR supplementation, indicating
that fractional O₂ extraction in vastus lateralis was not different between BR and
PLA. Hence, according to the Fick principle, the increased oxygen uptake in the
present study may be a result of increased total O₂ extraction due to increased
blood flow. This interpretation is consistent with results demonstrating that NO₃⁻
supplementation enhances vascular control and muscle blood flow redistribution during exercise [8,49,72].

5.1 Conclusion
In summary, our results provide novel evidence that chronic high-dose BR supplementation improves 10 km time trial performance of well-trained cyclists in both normoxia and hypoxia. Further, BR supplementation resulted in higher VO$_2$ and VE during the TT, suggesting that utilization of a greater proportion of the aerobic capacity contributed to the improved performance. While our results do not identify the underlying mechanisms, enhanced vascular control and muscle blood flow redistribution may contribute to higher VO$_2$ and improved time trial performance with BR supplementation.

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7.1 Conflict of interest statement
The authors declare: no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years; no other relationships or activities that could appear to have influenced the submitted work. EW is a co-applicant on patents related to the therapeutic use of nitrate and nitrite.
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• High-dose NO₃⁻ supplementation improved time trial performance of cyclists
• Oxygen uptake during the time trial was elevated with NO₃⁻ supplementation
• The effects of NO₃⁻ supplementation were not different between hypoxia and normoxia