Single and Combined Effects of Beetroot Crystals and Sodium Bicarbonate on 4-km Cycling Time Trial Performance

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When ingested alone, beetroot juice and sodium bicarbonate are ergogenic for high-intensity exercise performance. This study sought to determine the independent and combined effects of these supplements. Eight endurance trained (VO_2max 65 mL·kg·min^{-1}) male cyclists completed four × 4-km time trials (TT) in a double-blind Latin square design supplementing with beetroot crystals (BC) for 3 days (15 g·day^{-1} + 15 g 1 h before TT, containing 300 mg nitrate per 15 g), bicarbonate (Bi 0.3 g·kg^{-1} body mass [BM] in 5 doses every 15 min from 2.5 h before TT); BC+Bi or placebo (PLA). Subjects completed TTs on a Velotron cycle ergometer under standardized laboratory conditions. Plasma nitrite concentrations were significantly elevated only in the BC+Bi trial before the TT (1520 ± 786 nmol·L^{-1}) compared with baseline (665 ± 535 nmol·L^{-1}, p = .02) and the Bi and PLA conditions (Bi: 593 ± 203 nmol·L^{-1}, p < .01; PLA: 543 ± 369 nmol·L^{-1}, p < .01). Plasma nitrite concentrations were not elevated in the BC trial before the TT (1102 ± 218 nmol·L^{-1}) compared with baseline (975 ± 607 nmol·L^{-1}, p > .05). Blood bicarbonate concentrations were increased in the BC+Bi and Bi trials before the TT (BC+Bi: 30.9 ± 2.8 mmol·L^{-1}; Bi: 31.7 ± 1.1 mmol·L^{-1}). There were no differences in mean power output (386–394 W) or the time taken to complete the TT (335.8–338.1 s) between any conditions. Under the conditions of this study, supplementation was not ergogenic for 4-km TT performance.

Keywords: nitrate, bicarbonate, exercise performance

Despite plentiful literature on the effects of various nutritional ergogenic aids on sports performance, the real-life scenario in which several supplements are used in combination is relatively unexplored. Two substances that are widely used and have received scientific attention in high-intensity events lasting 4–8 min are inorganic nitrate (NO_3^-) and sodium bicarbonate ($NaHCO_3$). Dietary NO_3^-, often consumed as beetroot juice, increases the bioavailability of the signaling molecule nitric oxide (NO) via the NO_3^--NO_2^--NO pathway, particularly under conditions of hypoxia and metabolic acidosis (De Smet et al., 2016; Kelly et al., 2014; Vanhatalo et al., 2011). NO's effects on physiological functions such as blood flow, muscle oxidative metabolism and mitochondrial biogenesis (Lee et al., 2015; Vaughan et al., 2016) may contribute to observations of improved athletic performance, particularly in shorter, high-intensity activities (De Smet et al., 2016; Lansley et al., 2011). Meanwhile, several studies report a modest benefit of the alkalizing agent $NaHCO_3$ on the performance of high-intensity exercise (Kilding et al., 2012) via an increased capacity to buffer hydrogen ions (H^+) produced by anaerobic glycolysis (Carr et al., 2011a).

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Evidence of independent benefits and mechanisms of action in relation to the performance of high-intensity sports encourage athletes in these events to supplement with both dietary NO_3^- and $NaHCO_3$, for a potential additive effect. However, it is also possible that coingestion of any two supplements might be counter-productive due to side-effects or antagonistic biochemistry (i.e., one blunts the effect of the other). In this case, $NaHCO_3$ supplementation might counteract the acidosis under which the NO_3^--NO_2^--NO pathway provides an important source of NO production. This study investigated the single and combined effects of NO_3^- rich beetroot crystals and $NaHCO_3$ supplementation on a 4-km cycling time-trial (TT).

Methods

Eight well-trained male cyclists (34 ± 7 y, 73.8 ± 10.1 kg, VO_{2max}: 65.2 ± 4.2 mL·kg^{-1}·min^{-1}, BMI: 22.3 ± 2.0 kg·m^{-2}) provided informed consent to participate in this study, approved by the Australian Catholic University Human Research Ethics Committee and registered on the Australian New Zealand Clinical Trials Registry (ACTRN12614000670673). We implemented a double-blind, placebo-controlled, Latin square application of four treatments separated by a 7-day wash out period between each trial: bicarbonate + beetroot crystals (BC+Bi), bicarbonate + placebo beetroot crystals (Bi), beetroot crystals

+ placebo bicarbonate (BC) and placebo beetroot crystals + placebo bicarbonate (PLA). The manufacturer's recommended BC treatment (packed into gelatin capsules) and color-matched placebo (90% BeetEssence, Green Foods Corp., CA, USA; 10% Black Cherry Kool-Aid, Kraft Foods Group Inc., IL, USA, packed into gelatin capsules) involved subjects completing a three-day loading protocol (15 g·d^{-1} containing 300 mg or ~5 mmol NO_3^- ingested at ~2000 hr), plus an acute dose (15 g) on trial morning, mixed with 400 mL water and consumed 1 hr pre time-trial (TT). Subjects were instructed not to spit or chew gum during supplementation and to avoid antibacterial mouth rinses and routine oral hygiene practices for 90 min after ingesting the capsules. These restrictions were implemented so that commensal bacteria present in the oral cavity important for the reduction of NO_3^- to NO_2^- were not completely eradicated (Govoni et al., 2008). Bicarbonate (HCO_3^-) (sodium bicarbonate, McKenzie Pty. Ltd, Australia) or placebo (75% maltodextrin, 25% sodium chloride) supplementation occurred on trial day, with the encapsulated dose (0.3 g·kg BM $^{-1}$) ingested in five equal amounts every 15 min, commencing 150 min before the TT.

Subjects visited the laboratory (46 m above sea level) on nine occasions. Preliminary testing and familiarization consisted of three phases. First, subjects completed an incremental cycling test commenced at a power output of 150 W with 50 W increases each subsequent 5 min to determine the power output to be used in experimental trials. Capillary blood samples were taken from the earlobe during the final 30 s of each work rate for the determination of lactate concentration. The test was stopped once a lactate concentration of >4–7 mmol·L^{-1} was attained. Subjects then completed 10 min of cycling at 150 W then dismounted the ergometer (Velotron, Racermate Inc, Seattle, USA) and rested in the laboratory for a further 10 min. Phase two of preliminary testing commenced once a blood lactate concentration <2.5 mmol·L^{-1} had been measured, and consisted of a maximal 4-min cycling TT for the determination of VO_{2peak}. An earlobe blood lactate sample was taken immediately after exercise followed by a rest period identical to the first. Lastly, subjects completed a familiarization 4-km TT identical to those performed during the subsequent experimental trials. For all three phases of preliminary testing and familiarization, subjects wore a mouthpiece (Hans Rudolph Inc., USA) connected to an automated online gas analyzer (True One 2400 Metabolic Measurement System, Parvo Medics, USA) for collection of respiratory gases.

Subjects visited the laboratory 72 hr pretrial for blood collection. Strict diet-exercise control was then implemented until trial day, including avoidance of oral mouthwashes and consumption of a standardized prepackaged diet (210 kJ·kg BM^{-1}, 8 g·kg BM^{-1} carbohydrate (CHO); 2.0 g·kg BM^{-1} protein; 1.0 g·kg BM^{-1} fat) for the last 24 hr (Jeacocke & Burke, 2010). On trial morning (**Figure 1**), subjects reported overnight-fasted, completed a compliance check, and were cannulated via a forearm vein. All subjects completed each trial at the same time (± two h). Subjects completed a gastrointestinal (GI) distress questionnaire then consumed a standardized meal (2 g·kg BM^{-1} CHO) with the first $NaHCO_3$/placebo dose (150 min pre-TT). The same questionnaire was completed 65-min prior, 5-min prior, 6-min post and 81-min post TT and has been reported in another study to discriminate GI problems (Carr et al., 2011b). Sixty minutes before the TT, subjects ingested the BC/placebo supplement, then after a further 15 min mounted the cycle ergometer and commenced a warm-up (50, 70, 85 and 100% of individual anaerobic threshold for 8, 5, 3 and 3 min + 5 min at 100 W + 1 min build-up to average TT power, sustained for 30 s). After a 15 min rest and consumption of a sports gel (27 g CHO; Powerbar Inc., Florham Park, USA), subjects then undertook the TT; a 4-km all-out effort to which they had been familiarized.

Six venous blood samples (6 mL) were collected in EDTA tubes per trial: baseline (72 hr prior) and five trial-day samples (see **Figure 1**). Blood [glucose], [lactate] (YSI 2300, Life Sciences, OH, USA), [HCO_3^-] and pH (i-STAT, Princeton, NJ, USA) were determined immediately, while plasma was collected (centrifuged at 3000 rpm for 10 min), aliquoted and stored at -80 °C for analysis of [NO_3^-] and [NO_2^-]. Complete analytical procedures for NO_3^-/NO_2^- via the ENO-20 HPLC System (EiCom Corporation, USA) have been previously described (Bryan & Grisham, 2007).

Statistical analyses were undertaken using SPSS (Version 22) software. Comparisons between trials, performance and physiological measures involved a linear mixed model, while within-condition comparisons involved repeated measures analysis of variance with Bonferroni adjustment. Significance was set at $p < .05$. Data are presented as mean ± *SD*. Data for time to completion were analyzed between conditions using magnitude based inferences by calculating the percentage difference ±90% confidence limits (Hopkins, 2006). Chances that the true value of the statistic was positive, trivial or negative relative to the smallest important value (1%; (Paton & Hopkins, 2006) was based on the following scale: <0.5%, almost certainly not; <5%, very unlikely; <25%, unlikely, probably not; 25–75%, possibly, possibly not; >75%, likely, probably; >95%, very likely; >99.5%, almost certainly (Hopkins, 2006). When there was a >5% chance of the statistic being both positive and negative, the effect was deemed *unclear.*

Results

There was no effect of trial treatment or order on performance parameters (**Table 1; Figure 2**). The coefficient of variation (CV) for time (m:s) for each subject across all four trials was calculated and resulted in a group mean of 0.7 ± 0.4%. Percentage changes between group means revealed trivial differences between each of the conditions (**Tables 2 and 3**). Plasma [NO_3^-] and [NO_2^-] (**Figure 3**), blood [HCO_3^-] and pH (**Figure 4**) all returned to baseline

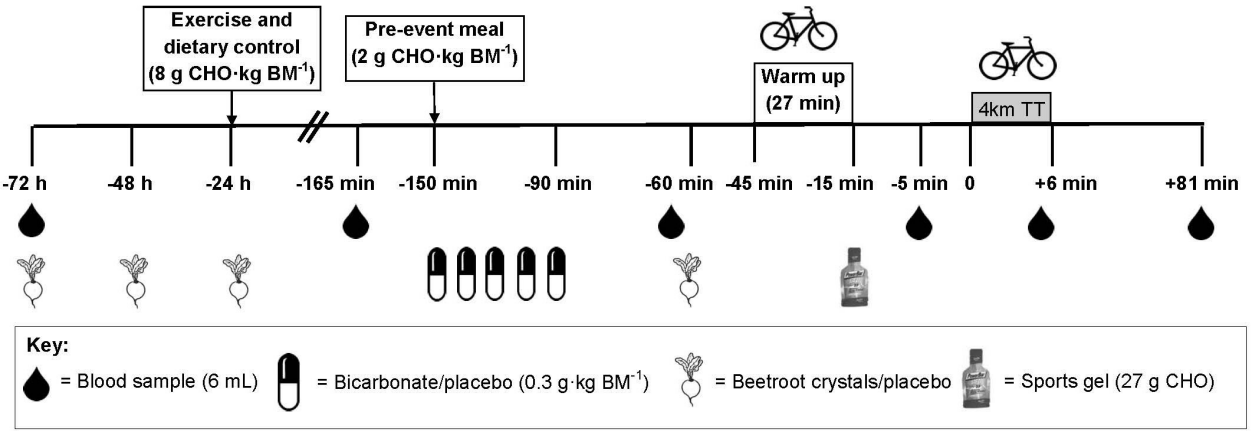

Figure 1 — Schematic figure of experimental design. After 24 h of exercise and dietary control, each subject undertook four trials of simulated 4 km all-out cycling time-trials (TTs). Subjects completed these in a double-blind, placebo-controlled, Latin square design, one week apart: bicarbonate + beetroot crystals (BC+Bi), bicarbonate + placebo beetroot crystals (Bi), beetroot crystals + placebo bicarbonate (BC) and placebo beetroot crystals + placebo bicarbonate (PLA). Following a standardized meal (2 g·kg BM^{-1} CHO) the first HCO_3^-/placebo dose (150 min pre-TT) was consumed, 60 min pre TT, subjects ingested the BC/placebo supplement, then a further 15 min after commenced a warm-up (50, 70, 85 and 100% of individual anaerobic threshold for 8, 5, 3 and 3 min + 5 min at 100 W + 1 min build-up to average TT power, sustained for 30 s), and 15 min before the TT subjects consumed a sports gel (27 g CHO). Blood samples were obtained 72 h prior and five times on trial day.

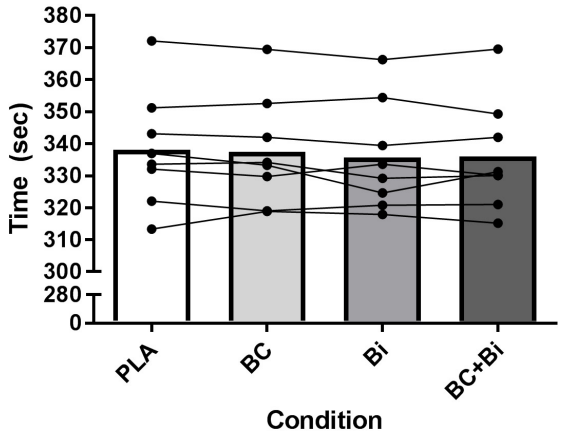

Figure 2 — Mean and individual time to completion data for 4-km cycling time trial.

values at the commencement of each pretrial period. The 3-day NO_3^- preload tended to increase plasma [NO_3^-] at rest compared with baseline (BC+Bi: +54 ± 65 mmol·L^{-1}; BC: +77 ± 52 mmol·L^{-1}, **Figure 3A**). The acute trial day dose increased plasma [NO_3^-] from rest to pre-TT for the BC trials (BC+Bi: +198 ± 40 mmol·L^{-1}; BC: +179 ± 55 mmol·L^{-1}) compared with the nonsupplemented trials (Bi: +24 ± 42; PLA: +9 ± 12 mmol·L^{-1}) such that plasma [NO_3^-] in the BC trials was significantly greater than baseline ($p < .01$, **Figure 3A**). NO_3^- supplementation increased plasma [NO_2^-] at pre-TT only in BC+Bi compared with baseline ($p = .02$) and nonsupplemented trials (Bi: +928 ± 879 nmol·L^{-1}, $p < .01$; vs. PLA: +978 ± 748 nmol·L^{-1}, $p < .01$, **Figure 3B**).

Blood [HCO_3^-] increased with $NaHCO_3$ supplementation at pre-TT (**Figure 4C**) and decreased in all trials post-TT (BC+Bi: -9.4 ± 2.6 mmol·L^{-1}; Bi: -11.4 ± 3.7 mmol·L^{-1}; BC: -8.9 ± 2.3 mmol·L^{-1}; PLA: -6.6 ± 1.5 mmol·L^{-1}, $p < .01$). Blood [HCO_3^-] remained elevated above resting values at 75 min post-TT in the $NaHCO_3$ trials (BC+Bi: +4.3 ± 0.2 mmol·L^{-1}; Bi: +4.4 ± 0.6 mmol·L^{-1}, $p < .01$). Blood pH values mirrored these results (**Figure 4D**). BC+Bi and Bi trials resulted in higher blood [lactate] post-TT compared with BC and PLA ($p < .01$; **Figure 4A**). Blood [glucose] did not differ between trials (**Figure 4B**). A questionnaire on gastrointestinal distress on trial days revealed no cases of gastrointestinal disturbance (data not shown).

Discussion

The results of the current study are the first to demonstrate the single and combined effects of supplementation with NO_3^- and $NaHCO_3$ on a simulated track cycling event. We used protocols designed to achieve physiological changes favorable for high-intensity exercise lasting ~4–6 min (Hoon et al., 2014; Kilding et al., 2012). Specifically, our cyclists consumed chronic plus acute doses of NO_3^- rich beetroot crystals (300 mg NO_3^- per 15 g for three days and 15 g 1 hr preexercise) and a well-tolerated alkalizing protocol (0.3 g·kg BM^{-1} $NaHCO_3$ in five doses, commencing 2.5 hr preexercise) (Carr et al., 2011b). Despite changes in plasma [NO_3^-] and blood [HCO_3^-], we failed to find enhancement of a laboratory-based 4-km cycling TT with either supplement or their combination. Careful experimental design (well-trained subjects, performance familiarization, control of pretrial training, standardized

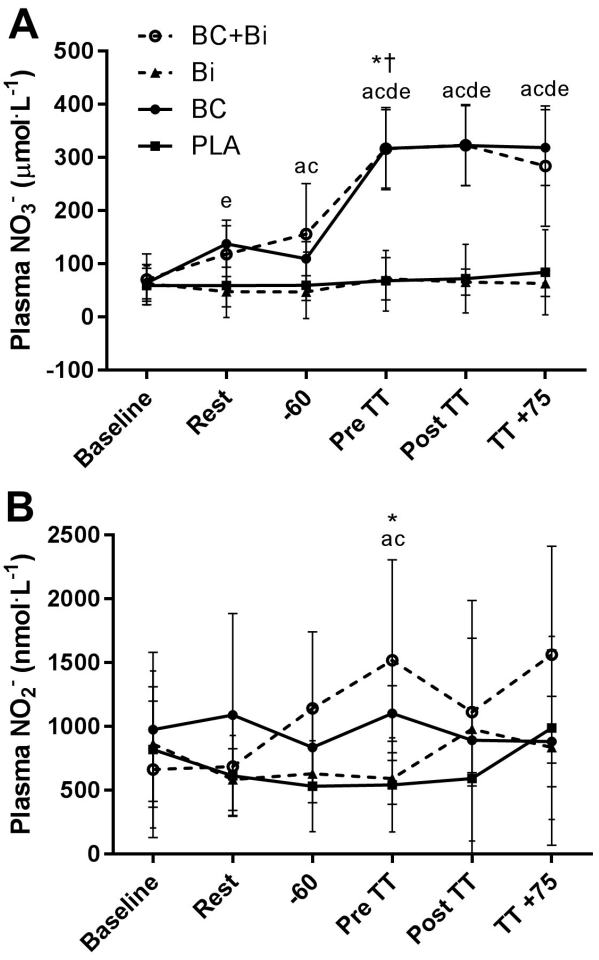

Figure 3 — Plasma NO_3^- (A) and NO_2^- (B) concentrations measured in well-trained male cyclists ($n = 8$) during 4-km cycling time trials. Significantly different: a, BC+Bi vs. PLA; c, BC+Bi vs. Bi; d, BC vs. PLA; e, BC vs. Bi; *, BC+Bi different from baseline; †, BC different from baseline. Data are mean ± *SD*.

pretrial diets, supervised supplementation) provides confidence in the reliability of our performance data.

$NaHCO_3$ loading can enhance the performance of laboratory-based cycling (4-min TT; Bellinger et al., 2012; 3-km TT; Kilding et al., 2012), and other laboratory or field protocols of similar duration and intensity (e.g., 2000-m rowing ergometer TT (Hobson et al., 2014); 1500-m running (Bird et al., 1995)). Indeed, a meta-analysis of $NaHCO_3$ loading and sports performance found a possibly moderate enhancement of 1.7% (90% CL ± 2.0%) in a 1-min sprint following blinded consumption of 0.3 g·kg^{-1} BM $NaHCO_3$ by male athletes that is reduced with each 10-fold increase in test duration by 0.6% (±0.9%) (Carr et al., 2011a). Increases in preexercise blood [HCO_3^-] were identified as a determinant of performance benefits. Similarly, NO_3^-supplementation was shown by meta-analysis to provide a trivial but non-significant benefit for TT performance (ES = -0.10, 95% CI: -0.27–0.06), with NO_3^- dose (+8 mmol) and type of exercise (higher vs. lower intensity) being factors likely to increase the benefit (Jones, 2014b; McMahon et al., 2016; Wylie et al., 2013). Of specific interest to our study, Lansley and colleagues (2011) reported a 2.8% enhancement of a 4-km TT in club-level cyclists following acute supplementation with 0.5 L beetroot juice containing ~6.2 mmol of NO_3^-.

We are not alone in failing to find performance benefits from either of the supplements examined in the current investigation. Others have failed to find ergogenic outcomes associated with NO_3^- supplementation in a similar cycling protocol in competitive cyclists (Hoon et al., 2014), or other exercise protocols of similar duration, such as a 1500-m run (Boorsma et al., 2014). The magnitude of plasma [NO_3^-]/[NO_2^-] increases (Hoon et al., 2014; Wilkerson et al., 2012; Wylie et al., 2013) and apparently different responses in highly-trained vs. lower-caliber athletes (Porcelli et al., 2015) may underpin these observations. Specifically, a lower proportion of Type II muscle fibers which are more responsive to NO_3^- supplementation, higher baseline NO_2^- status due to endogenous production via the NOS pathway, and better oxygenation of muscles in highly trained individuals may reduce the importance of an enhanced NO_3^-/NO_2^-/NO pathway (Jones, 2014b). Analysis of plasma [NO_2^-] provide direct evidence of several important methodological aspects of our work: NO_2^- status returned to baseline in the 3-day washout between trials, and the combination of preload and acute supplementation before the TT achieved large increases in plasma [NO_3^-], at greater levels than achieved previously where NO_3^- supplementation was shown to enhance 4-km TT performance (Lansley et al., 2011). Furthermore, plasma [NO_2^-] reported in the current study were greater than those seen in another investigation also using a three-day loading protocol (BC+Bi, 1521 nmol·L^{-1}; BC, 1102 vs. 334 nmol·L^{-1}) (Bescos et al., 2012). However, we do acknowledge some anomalies with our data: the baseline levels of plasma [NO_2^-] were higher than that reported in other studies (~200–300 nM, (Hoon et al., 2014; Lansley et al., 2011)) and were only elevated for the TT in the case of the BC+Bi trial. Therefore, the difficulty in achieving a performance enhancement with the BC may reside with the inability of this dose to further raise the already high NO_2^- levels in our well-trained population. Indeed, we acknowledge that the acute dose used on the day of the TT is lower than the amounts others have shown to be optimal (Wylie et al., 2013), that acute doses are typically taken 2–2.5 hr preevent rather than 60 min, and that greater doses may be needed in highly trained subjects compared with recreational athletes (Jones, 2014b). However, the protocol chosen for this study followed the dose recommended by the manufacturer and provided a three-day preload to try to increase background NO availability before the final dose. Whether there was interaction with HCO_3^- supplementation to decrease the importance of enhanced NO_3^-/NO_2^- concentrations in the face of reduced metabolic acidosis cannot be detected in this study but presents

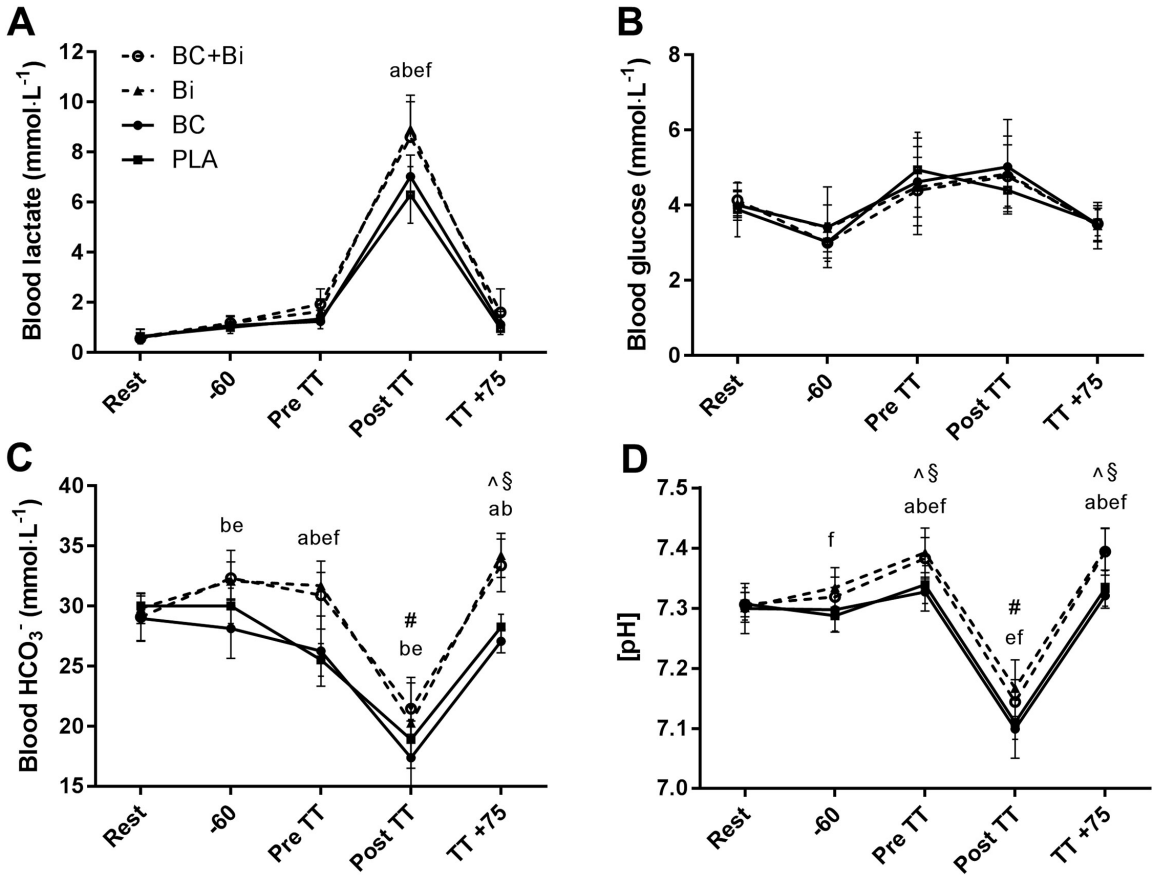

Figure 4 — Blood lactate concentrations (A), glucose concentrations (B), HCO_3^- concentrations (C), and pH (D) measured in well-trained male cyclists (n = 8) during 4-km cycling time trials. Significantly different: a, BC+Bi vs. PLA; b, BC+Bi vs. BC; e, BC vs. Bi; f, Bi vs. PLA; #, all trials different from pre TT, ^, BC+Bi different from rest; §, Bi different from rest. Data are mean ± *SD*.

Table 1 Mean ± *SD* for Mean Power Output and Heart Rate During 4-km Cycling TT

	Mean Power Output		Mean time to	Mean Heart Rate
Condition	(W)	(W·kg^{-1})	Completion (s)	(beats·min^{-1})
PLA	386 ± 55	5.25 ± 0.51	338.10 ± 18.04	171 ± 11
BC	388 ± 54	5.28 ± 0.47	337.41 ± 17.11	169 ± 13
Bi	394 ± 52	5.37 ± 0.52	335.78 ± 16.94	172 ± 11
BC+Bi	393 ± 54	5.33 ± 0.53	336.10 ± 17.28	175 ± 7

Table 2 Effect of Nitrate and Bicarbonate Supplementation on 4-km Cycling Time Trial Time to Completion Compared with Placebo

Condition	Change in Mean Compared with Placebo (%, ±90% CL)	Nonclinical Inference*a*
BC	-0.2 ± 0.6	very likely trivial
Bi	-0.7 ± 1.1	possibly trivial
BC+Bi	-0.6 ± 0.4	likely trivial

[a]Chances that the true value of the statistic was positive, trivial or negative relative to the smallest important value of 1% was based on the following scale: < 0.5%, almost certainly not; < 5%, very unlikely; < 25%, unlikely, probably not; 25–75%, possibly, possibly not; > 75%, likely, probably; > 95%, very likely; > 99.5%, almost certainly. When there was a > 5% chance of the statistic being both positive and negative, the effect was deemed *unclear*.

Table 3 Effect of NO_3^- and $NaHCO_3^-$ Supplementation on 4-km Cycling Time Trial Time to Completion

Condition	Change in Mean (%, ±90% CL)	Nonclinical Inference*a*
BC+Bi vs. Bi	0.1 ± 0.8	Very likely trivial
BC+Bi vs. BC	0.7 ± 1.1	Possibly trivial
BC vs. Bi	0.5 ± 0.8	Likely trivial

[a]Chances that the true value of the statistic was positive, trivial or negative relative to the smallest important value of 1% was based on the following scale: < 0.5%, almost certainly not; < 5%, very unlikely; < 25%, unlikely, probably not; 25–75%, possibly, possibly not; > 75%, likely, probably; > 95%, very likely; > 99.5%, almost certainly. When there was a > 5% chance of the statistic being both positive and negative, the effect was deemed *unclear.*

another contributing factor to the lack of efficacy of the combined supplementation protocol.

Despite significant elevation of blood [HCO_3^-] and pH before exercise and blood lactate immediately following exercise, we failed to detect a clear performance benefit of $NaHCO_3$ loading on a 4-km cycling TT (~5.5 min). Indeed, this is similar to findings from other studies involving exercise protocols of similar duration (Carr et al., 2011c; Christensen et al., 2014; Kupcis et al., 2012) and again demonstrates that certain conditions such as high intensity short-duration (~1-min) events involving repeated efforts may be needed before clear benefits are seen (Sutton et al., 1981). Although benefits provided by $NaHCO_3$ loading may be counteracted by the potentially associated gastrointestinal distress, this risk can be reduced by the use of specific protocols involving encapsulated supplements, a spread intake, and coingestion with a light carbohydrate meal (Carr et al., 2011b). These conditions were implemented in the current study; indeed, no gut problems were reported by our subjects. Blood lactate concentrations were shown to be higher in the $NaHCO_3$ conditions following the 4-km TT (**Figure 4C**) indicating increased energy production by anaerobic glycolysis however no performance benefits were observed. The lack of efficacy of $NaHCO_3$ supplementation may be due to insufficient buffering capacity despite induced muscle alkalosis (Sutton et al., 1981), fatigue unrelated to acidosis (Cairns, 2006) or improved anaerobic capacity in well-trained subjects (Medbo & Burgers, 1990). A novel finding of our study was the maintenance of elevated blood [HCO_3^-], 75 min after the performance bout. This may be meaningful for athletes who compete in multiple events in a single session as our results suggest no need to redose with $NaHCO_3$ for events 75 min apart.

In conclusion, ingestion of a validated $NaHCO_3$ protocol or the manufacturer's recommended protocol of intake of an inorganic NO_3^- product failed to enhance the performance of 4-km cycling TT by well-trained cyclists, either alone or in combination. Although it is possible that the combined use of two supplements, each of which is known to have ergogenic effects when used individually, could have an additive effect, the working hypothesis of this study was that there would be an interference effect, with $NaHCO_3$ removing one of the physiological conditions under which dietary NO_3^- might be efficacious. The findings of this investigation do not exclude the possibility that either or both supplements are ergogenic under different conditions, or that alkalosis achieved by $NaHCO_3$ loading might reduce the efficacy of NO_3^- supplementation Future research should continue to investigate supplementation of dietary NO_3^- in combination with other nutritional aids in well-trained populations, since the real life practices of athletes commonly include the parallel use of several different supplements. Specifically, exercise scenarios that induce metabolic acidosis or hypoxia may favor the physiological effects of the NO_3^--NO_2^--NO pathway and should be targeted for further research with dietary NO_3^-supplementation. When these are better identified, there will be opportunity to investigate the potential interactive effects of achieving alkalosis via $NaHCO_3$ supplementation protocols.

Acknowledgments

The study was designed by MJC, EBP, JAH and LMB; data were collected and analyzed by MJC and EBP, data interpretation and manuscript preparation were undertaken by MJC, EBP, JAH and LMB. All authors approved the final version of the paper. The authors declare no conflict of interest. The authors would like to thank Liv Warnes, Tim Dekker and Jason Bartram of Cycling Australia for their input into the study design. This study was funded by research grants from the Sports Nutrition Discipline at the Australian Institute of Sport (AIS) and Sports Dieticians Australia (SDA). This study was prospectively registered on the Australian New Zealand Clinical Trials Registry (ACTRN12614000670673).

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