Effects of post-exercise sodium bicarbonate ingestion on acid-base balance recovery and time-to-exhaustion running performance: a randomised crossover trial in recreational athletes

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ABSTRACT

This study investigated the effect of post-exercise sodium bicarbonate ($NaHCO_3$) ingestion on acid-base balance recovery and time-to-exhaustion (TTE) running performance. Eleven male runners (stature, 1.80 ± 0.05 m; body mass, 74.4 ± 6.5 kg; maximal oxygen consumption, 51.7 ± 5.4 $ml.kg^{-1}.min^{-1}$) participated in this randomised, single-blind, counterbalanced and crossover design study. Maximal running velocity (v-VO_{2max}) was identified from a graded exercise test. During experimental trials, participants repeated 100% v-VO_{2max} TTE protocols (TTE1, TTE2) separated by 40 min following the ingestion of either 0.3 $g.kg^{-1}$ BM $NaHCO_3$ (SB) or 0.03 $g.kg^{-1}$ BM sodium chloride (PLA) at the start of TTE1 recovery. Acid-base balance (blood pH and bicarbonate, HCO_3^-) data were studied at baseline, post-TTE1, after 35 min recovery and post-TTE2. Blood pH and [HCO_3^-] were unchanged at 35 min recovery ($p > 0.05$), but [HCO_3^-] was elevated post-TTE2 for SB vs. PLA (+2.6 $mmol.l^{-1}$; $p = 0.005$; $g = 0.99$). No significant differences were observed for TTE2 performance ($p > 0.05$), although a moderate effect size was present for SB vs. PLA (+14.3 s; $g = 0.56$). Post-exercise $NaHCO_3$ ingestion is not an effective strategy for accelerating the restoration of acid-base balance or improving subsequent TTE performance when limited recovery is available.

Key Words: buffering; alkalosis; glycolytic activation; ergogenic aid; exercise capacity; nutrition.

BULLET POINTS

- Post-exercise sodium bicarbonate ingestion did not accelerate the restoration of blood pH or bicarbonate after 35 minutes
- Performance enhancing effects of sodium bicarbonate ingestion may display a high degree of inter-individual variation
- Small-to-moderate changes in performance were likely due to greater up-regulation of glycolytic activation during exercise

INTRODUCTION

Repeated high-intensity training sessions and events require athletes to be able to maximally exert themselves with often only limited recovery between exercise bouts (Barnett, 2006). The considerable reliance on the ATP-PCr and glycolytic energetic systems results in the rapid accumulation of biochemical metabolites such as inorganic phosphate, hydrogen cations (H^+) and lactate within contracting muscle (Allen et al., 2008). Naturally occurring extracellular buffering mechanisms (e.g., blood bicarbonate, HCO_3^-) act to remove these H^+ from the muscle cytosol (Sahlin, 2014), however the production of H^+ overwhelms the rate of neutralisation reactions, contributing towards reduced intramuscular pH (Allen et al., 2008). These metabolically derived disruptions to acid-base balance (i.e., equilibrium between acidity and alkalinity of bodily fluids) have been linked to greater peripheral fatigue via the inhibition of glycolytic flux (Messonnier et al., 2007), disruption of calcium ion cross-bridge formation (Fitts, 2016) and reduced muscle excitability (Sostaric et al., 2006). Extracellular buffering aids such as sodium bicarbonate ($NaHCO_3$) that protect against disturbances to acid-base balance homeostasis could therefore prove beneficial to athletes performing repeated bouts of maximal effort that are likely to exacerbate metabolic perturbation.

Pre-exercise ingestion of 0.3 $g.kg^{-1}$ BM $NaHCO_3$ enhances extracellular buffering capacity by eliciting a metabolic alkalosis (i.e., increased blood [HCO_3^-] of ~5-7 $mmol.l^{-1}$) that allows for greater H^+ efflux from the muscle (Messonnier et al., 2007), which may result in moderate ergogenic benefits during high-intensity exercise (see review, Hadzic et al., 2019). This nutritional strategy also accelerates the restoration of acid-base balance homeostasis within ~40 mins (Pruscino et al., 2008; Gough et al., 2019a), but it is unclear whether the heightened HCO_3^- buffering response extends to subsequent athletic performance (i.e., for athletes competing in multiple exercise events). Authors investigating the effect of pre-exercise $NaHCO_3$ ingestion on repeated bout performance reported minimal ergogenic benefits during wingate protocols (3x30 s; 15 min recovery) and swimming time-trials (2x200 m; 30 min recovery) (Zabala et al., 2011; Pruscino et al., 2008). It is expected that pre-exercise ingestion resulted in the enhanced HCO_3^- buffering capacity being completely utilised within the preliminary bout (i.e., from protecting against severe acid-base balance disturbances), which would explain blunted performance benefits during subsequent exercise bouts.

One relatively novel strategy that accounts for this concern is post-exercise $NaHCO_3$ ingestion, ensuring that a greater alkalotic response is present during recovery and subsequent exercise bouts. Previous studies have demonstrated that $NaHCO_3$ ingested either immediately post-exercise or 30 min after a fatiguing bout elevates blood pH (~0.10 units) and [HCO_3^-] (~7-8 mmol.l^{-1}) following a 75-90 min recovery period (Gough et al., 2017; Gough et al., 2019), which subsequently improved time-to-exhaustion (TTE) running (+70 s; p = 0.084) and cycling (+35 s; p = 0.007). Since the authors opted for relatively long recovery windows, it was noted that blood pH and [HCO_3^-] had not only recovered prior to subsequent exercise, but instead reflected a state of pre-exercise metabolic alkalosis following $NaHCO_3$ ingestion (~7.48 units, ~32 mmol.l^{-1}). Consequently, the ergogenic responses reported may have represented the well-established pre-exercise application of $NaHCO_3$ ingestion. It is therefore considered more appropriate to investigate the potential ergogenic effect of post-exercise $NaHCO_3$ when ingested during a shorter recovery period between two exercise bouts.

Performance enhancing effects following $NaHCO_3$ are not exclusively attributed to substantial changes in blood pH and [HCO_3^-] response (Siegler et al., 2016). This alkalotic state may also increase glycolytic activation by preventing the allosteric inhibition of enzymes such as glycogen phosphorlyase and phosphofructokinase (Messonnier et al., 2007). Pre-exercise $NaHCO_3$ ingestion has been demonstrated to augment glycolytic flux during high-intensity exercise protocols (da Silva et al., 2019; Gurton et al., 2020). The effect of a post-exercise $NaHCO_3$ supplementation strategy on energy system contribution during subsequent exercise remains underexplored, although authors have suggested enhanced glycolytic flux via elevated post-exercise blood lactate (BLa^-) concentration after a 75-90 min recovery (Gough et al., 2017b; Gough et al., 2019b). It is conceivable that some athletes will have less time to recover between high-intensity exercise bouts, therefore research should examine whether $NaHCO_3$ ingested at the start of a shorter recovery period is still able to alter energy demand and contribution from the glycolytic and oxidative systems.

From an applied perspective, athletes might be particularly attracted to nutritional strategies that maximise ergogenic benefits during a second competition bout (i.e., track running/cycling, heat then final; regional tennis, first and second round match). In these instances, ingestion of $NaHCO_3$ during a recovery period may prove more beneficial to subsequent performance. The purpose of this

study was to investigate the effect of post-exercise $NaHCO_3$ ingestion on the recovery of acid-base balance (blood pH, $[HCO_3^-]$, base excess) within 35 mins and subsequent TTE running performance.

MATERIALS AND METHODS

Experimental approach to the problem

A block randomised, across subjects single-blind, placebo-controlled, counterbalanced, crossover experimental design was implemented for this study. Experimental trial order was allocated by the principal investigator using an online randomised number generator (www.randomization.com) and blinded from other members of the research team. Participants attended three separate laboratory visits in a 3-h post-prandial state to perform a graded exercise test and two experimental trials (separated by 5-7 d). All testing was completed at Surrey Human Performance Institute at the same time of day to minimise the confounding effects of circadian rhythms on exercise performance (Reilly, 1990). The final speed completed during the graded exercise test was classified as maximal velocity (100% v-VO_{2max}) and used to prescribe TTE speed. During experimental trials, participants repeated 100% v-VO_{2max} TTE protocols separated by 40 mins following the ingestion of either $NaHCO_3$ (SB) or sodium chloride (NaCl; PLA) at the start of recovery. Vigorous exercise and the consumption of alcohol were prohibited for 24-h prior to visits. Participants were instructed to record nutritional intake for 72-h prior to attending the first laboratory visit using food diaries, which were checked by the principal investigator and returned to participants to replicate before each experimental trial. Online databases were used by the research team to determine the composition and nutritional content of foods.

Participants

A priori power calculation (β = 0.8; α = 0.05) using previous partial eta-squared effect size data (η_p^2; 0.6) for post-exercise blood pH recovery (Gough et al., 2017a) revealed that a sample size of 10 would allow detection of significant changes in blood pH (G*Power 3, Heinrich Heine, Düsseldorf, Germany). Eleven recreationally trained runners (stature: 1.80 ± 0.05 m; body mass: 74.4 ± 6.5 kg; age: 31.0 ± 9.7 years; maximal oxygen consumption: 51.7 ± 5.4 $ml.kg^{-1}.min^{-1}$) were enrolled onto this study from a local running club according to the CONSORT 2010 guidelines (**Figure 1**). All participants completed

at least 60 min vigorous exercise per week, had no history of hypertension (>140/90 mmHg) or renal insufficiencies and had not ingested buffering agents within the previous 6 months. All study procedures were reviewed and approved prior to commencing research by the Faculty of Health and Medical Sciences ethics committee at the University of Surrey (1366-FHMS-18). Participants gave informed consent prior to participating in the study. Procedures were conducted in full accordance with the World Medical Association's Declaration of Helsinki.

[INSERT **Figure 1** NEAR HERE]

Procedures

On the initial visit, baseline physiological measures were recorded (stature, body mass, blood pressure), before participants performed a graded exercise test on a treadmill to determine 100% v-VO_{2max} for TTE protocols. Gaseous exchange was collected using a breath-by-breath metabolic cart (Geratherm Respiratory, Love Medical, Germany). After a 5 min warm-up at 6 $km.h^{-1}$ and 1% gradient, the speed increased by 1 $km.h^{-1}$ every 1 min until volitional exhaustion was achieved (Price and Halabi, 2005). This was deemed as the failure to maintain treadmill speed despite strong verbal encouragement. Heart rate and rating of perceived exertion (RPE) were recorded at the end of each stage and volitional exhaustion. Data were averaged over the peak 30 s for calculating VO_{2max} (Beltz et al., 2016) and 100% v-VO_{2max} was classified as the final completed speed during the graded exercise test.

On arrival to the laboratory during experimental trial visits, participants completed visual analog scales for gastrointestinal (GI) discomfort (0 mm = "no symptom at all"; 100 mm = "severest symptom imaginable") to quantify perceived severity of flatulence, abdominal discomfort (AD), gut fullness (GF), bowel urgency rating (BUR) and diarrhoea (Gough et al., 2017a; Gurton et al., 2020). Resting capillary blood samples (95 µl) were collected into end-to-end sodium-heparised capillary tubes (EKF Diagnostics GmbH, Germany) and analysed for acid-base balance (blood pH, [HCO_3^-], base excess) using a handheld analyser (i-STAT, Abbott Point of Care, New Jersey). The i-STAT analyser demonstrates excellent accuracy and precision for determination of blood pH and [HCO_3^-] after maximal exercise (intraclass correlation coefficients, ICC; r = 0.88, 0.86) (Dascombe et al. 2007).

Additional 20 µl blood samples were analysed for [BLa^-] using an automatic analyser (Biosen C-Line, EKF Diagnostic GmbH, Germany) that displays excellent precision (coefficient of variation, *CV*: <1.5%; EKF Diagnostics, 2017). Participants perceived readiness to exercise (PRE) was measured, before completing the first running TTE protocol (TTE1) at 100% v-VO_{2max} on the treadmill (1% gradient). Breath-by-breath expired gaseous exchange and RPE were recorded throughout TTE protocols. Capillary blood samples and visual analog scales were performed immediately post-TTE1, before participants consumed either 0.3 $g.kg^{-1}$ BM $NaHCO_3$ (SB) or 0.03 $g.kg^{-1}$ BM NaCl (PLA) after 5 min recovery. These doses were chosen to replicate experimental design from previous studies examining the effect of either pre- or post-exercise $NaHCO_3$ ingestion on recovery (Pruscino et al., 2008; Gough et al., 2017b; Gough et al., 2019b). Experimental beverages were administered single-blind as a chilled solution of 400 ml water and 100 ml orange squash to ensure palatability and taste-matching (Lavender and Bird, 1989). Pilot testing revealed it was not possible to taste-match an equimolar 0.21 $g.kg^{-1}$ BM NaCl dose (i.e., equal Na^+ content between beverages). Participants consumed beverages as fast as possible but were allocated a 5 min period if required (i.e., by 10 min post-TTE1), before completing a supplement belief questionnaire to assess the single-blind design and whether bias regarding the effect of $NaHCO_3$ was transferred onto participants (Gough et al. 2017a). Capillary blood samples and visual analogue scales were repeated following 35 min recovery (30 min post-ingestion). Participants were asked for their PRE and performed the second TTE protocol (TTE2) at 100% v-VO_{2max} on the treadmill (40 min after TTE1). Additional blood samples were collected post-TTE2, while visual analogue scales were completed post-exercise and for a 24-h post-laboratory phase. Composite scores for GI discomfort were calculated at each time point from the sum of symptom severity (i.e., scored out of 500 mm). An overview of experimental procedure is displayed in **Figure 2**.

[INSERT **Figure 2** NEAR HERE]

Estimated energy system contribution calculations

Absolute energy demand and contribution from the oxidative and glycolytic energetic systems were estimated during TTE running protocols via non-invasive techniques (Milioni et al., 2017; Brisola et

al., 2015). Oxidative phosphorylation (W_{AER}) was calculated by subtracting resting oxygen consumption (i.e., the average VO_2 value during the final 30 s of baseline) from the area under the oxygen consumption curve for TTE protocols (di Prampero and Ferretti, 1999). Area under the curve was calculated using the trapezoidal method. This approach provides reliable and valid estimations for W_{AER} during high-intensity exercise (da Silva et al., 2019; Milioni et al., 2017). Glycolytic ($W_{[LA]}$) was calculated from delta blood lactate (ΔBLa^-) during TTE protocols, according to the assumption that a difference of 1 $mmol.l^{-1}$ of BLa^- corresponded to 3 $ml.kg^{-1}$ BM of O_2 (Milioni et al., 2017; Brisola et al., 2015; Gurton et al., 2020), by using the following equation: $W_{[LA]} = \Delta BLa^-$ x 3 x body mass (kg). The caloric quotient of 20.92 kJ was used to convert between absolute energy demand (in L of O_2) and energy contribution (in kJ) for both energetic systems.

Statistical analysis

Normality and sphericity were assessed using Shapiro-Wilk and Mauchly tests, respectively. Any violations were corrected via Greenhouse Geisser adjustments. Reproducibility of pre-trial nutritional intake, TTE1 performance, perceived difficulty to exercise and initial acid-base balance perturbation were determined from ICC's and categorised as either poor ($r \leq 0.40$), fair ($r = 0.40 – 0.59$), good ($r = 0.60 – 0.74$) or excellent ($r \geq 0.74$) (Atkinson and Nevill, 1998). Acid-base balance (primary outcome measure) data were analysed using two-way (treatment x time) repeated measures ANOVA's, whereby interaction effects were presented as a *p* value and effect size (η_p^2) (Olejnik and Algina, 2003). Significant interactions were explored further by performing one-way repeated measures ANOVA across treatments at *a* priori defined time points (i.e., post-TTE2) using the bonferroni correction factor. The assumption of normal distribution was violated for GI discomfort therefore Friedman's two-way ANOVA's were conducted as non-parametric alternative. Post-hoc Wilcoxon matched pair signed rank tests were performed when significance was observed (median and Z score reported). Fisher's exact test was used to assess the efficacy of the single-blind design. Paired *t*-tests were conducted on TTE2 performance (secondary outcome measure) and perceived difficulty to exercise. The within-subject *CV* for the TTE1 protocol was calculated as 4.9% and used to interpret individual responses for changes in

TTE2 performance. Between treatment effect sizes were calculated by dividing mean differences by pooled SD, before applying Hedges *g* bias correction (Lakens, 2013). These were interpreted as trivial (< 0.20), small (0.20 – 0.49), moderate (0.50 – 0.79), or large (≥ 0.80) (Cohen, 1988). Data are presented as Mean ± SD (unless otherwise stated) and 95% confidence intervals (CI) reported for between treatment statistical differences. Statistical significance was set at $p < 0.05$ and data were analysed using SPSS v26 (SPSS Inc., IBM, USA).

RESULTS

Pre-trial nutritional intake

Participants demonstrated an excellent degree of repeatability for pre-trial nutritional intake during SB and PLA trials for daily calories ($r = 0.89$, $p = 0.001$), carbohydrate ($r = 0.87$, $p = 0.002$), fat ($r = 0.83$, $p = 0.005$) and protein ($r = 0.83$, $p = 0.014$).

Acid-base balance status

Initial acid-base balance perturbation demonstrated an excellent level of reproducibility during SB and PLA trials for post-TTE1 blood pH ($r = 0.94$; $p < 0.001$), [HCO_3^-] ($r = 0.95$; $p < 0.001$), base excess ($r = 0.92$; $p < 0.001$) and [BLa^-] ($r = 0.93$; $p < 0.001$). Significant two-way interactions (treatment x time) were observed for [HCO_3^-] ($p = 0.002$; $\eta_p^2 = 0.413$) and [BLa^-] ($p = 0.023$; $\eta_p^2 = 0.269$), but not for blood pH ($p = 0.070$; $\eta_p^2 = 0.227$) or base excess ($p = 0.242$; $\eta_p^2 = 0.141$). Acid-base balance status was unchanged at 35 min recovery ($p > 0.05$). Exploratory analysis on a small sub-set ($n = 6$) of blood pH and [HCO_3^-] data at 40 min recovery (i.e., immediately pre-TTE2; not an *a* priori defined time point) revealed a larger magnitude of effect for SB vs. PLA (+0.03 units; +3.9 mmol.l^{-1}). Post-TTE2 [HCO_3^-] was significantly elevated for SB vs. PLA ($p = 0.005$) and displayed a large effect size (+2.6 mmol.l^{-1}; CI: 1.0, 4.2; $g = 0.99$). Peak [BLa^-] following TTE2 was significantly higher for SB vs. PLA ($p = 0.015$) and displayed a large effect size (+1.4 mmol.l^{-1}; CI: 0.3, 2.5; $g = 0.82$). Mean ± SD values for changes in acid-base balance status from baseline to post-TTE2 are displayed in **Figure 3 (A-D)**.

[INSERT **Figure 3 (A-D)** NEAR HERE]

Time-to-exhaustion running performance

Performance during TTE1 demonstrated an excellent degree of repeatability for SB and PLA trials (r = 0.95; p < 0.005; within-subject CV = 4.9%). No significant differences were observed for TTE2 performance (+14.3 s; p = 0.071), although a moderate effect size was present for SB vs. PLA (+9.7%; g = 0.56). There was a large degree of individual variability for TTE2 performance, whereby mean differences for SB vs. PLA ranged from -16.8 s (-14.2%) to +61.2 s (+37.1%). In total, six out of eleven participants reported improvements in TTE2 performance above the 4.9% test-retest variability. Sub-analysis for these six 'responders' revealed a significant increase of +31.2 s for TTE2 performance following SB compared to PLA (+21.1 %; p = 0.007; g = 1.65). Mean values and individual differences for TTE2 performance are displayed in **Figure 4**.

[INSERT **Figure 4** NEAR HERE]

Estimated energy system contribution

Mean ± SD values for energy demand (in L of O_2) and contribution (kJ) from oxidative and glycolytic energetic systems are presented in **Table 1**. Excellent reproducibility was observed between SB and PLA trials for energy contribution from W_{AER} (r = 0.96; p < 0.001) and $W_{[LA]}$ (r = 0.92; p < 0.001) during TTE1. Energy contribution from $W_{[LA]}$ was significantly increased during TTE2 for SB vs. PLA (p = 0.032) and displayed a moderate effect size (+6.84 kJ; CI: 0.7, 13.0; g = 0.69). In total, ten out of eleven participants reported greater $W_{[LA]}$ contribution following SB vs. PLA. No significant differences were observed for energy contribution from W_{AER} during TTE2 (p > 0.05), although a small effect size was present for SB vs. PLA (+11.20 kJ; g = 0.48).

[INSERT **Table 1** NEAR HERE]

Perceived difficulty of exercise

Participants achieved a similar level of volitional exhaustion during TTE1 for SB and PLA trials as good reproducibility was reported for peak RPE (r = 0.66; p = 0.049). No differences were observed between SB and PLA for peak RPE during TTE2 (p > 0.05). No significant differences were reported across treatments for PRE prior to TTE2 (p > 0.05), although a small effect size was present for SB vs. PLA (+0.36 units; g = 0.26).

Perceptual responses to experimental beverages

Four participants identified SB, six were unsure on treatments, whilst one incorrectly identified SB as PLA. Treatments were therefore considered successfully single-blinded and taste-matched (Fisher's exact test, p = 1.000) and only a single participant directly suggested a poor taste.

In total, ten out of eleven participants experienced some GI discomfort for the SB trial (timing and severity of peak symptom for each participant displayed in **Table 2**). Composite scores for GI discomfort revealed no symptoms at baseline, but symptom severity was mildly exacerbated for SB vs. PLA at 35 min recovery (Mdn: 11 mm vs. 10 mm) and post-TTE2 (Mdn: 11 mm vs. 9 mm). No significant treatment effects were present at any time point for AD or GF (p > 0.05). No significant treatment effects were observed at either 35 min recovery or post-TTE2 for flatulence, BUR and diarrhoea (all p > 0.05). Ratings of GI discomfort were significantly exacerbated for SB vs. PLA during the 24-h post-laboratory phase for flatulence (Mdn: 10 mm vs. 0 mm; Z = 2.366; p = 0.018), BUR (20 mm vs. 0 mm; Z = 2.547; p = 0.011) and diarrhoea (17 mm vs. 0 mm; Z = 2.197; p = 0.028). Out of the five participants that failed to improve TTE2 performance for SB vs. PLA, only one directly reported an ergolytic effect of GI discomfort.

[INSERT **Table 2** NEAR HERE]

DISCUSSION

This was the first study to investigate the effect of post-exercise $NaHCO_3$ ingestion on blood pH and [HCO_3^-] recovery after 35 min and subsequent TTE running performance. No changes in acid-base balance status were reported for SB vs. PLA after the 35 min recovery period, however both [HCO_3^-]

and [BLa^-] were elevated post-TTE2. Post-exercise $NaHCO_3$ ingestion did not significantly improve TTE2 performance, although a moderate effect size and a high degree of inter-individual variation was observed. Glycolytic energy system contribution was moderately increased during TTE2 for SB vs. PLA. Lastly, post-exercise $NaHCO_3$ ingestion resulted in moderate-to-severe GI symptoms after TTE2 and for the 24-h post-laboratory phase, therefore athletes should remain cautious when using this strategy to improve performance.

The novel finding from this study was that $NaHCO_3$ ingested after a fatiguing exercise bout failed to accelerate the recovery of blood pH, [HCO_3^-] and base excess to 'neutral' levels within 35 min when compared to PLA. These results contrast findings from pre-exercise ingestion studies suggesting that $NaHCO_3$ accelerated the restoration of acid-base balance homeostasis within 30-40 min (Pruscino et al., 2008; Gough et al., 2019a). This discrepancy is most likely explained by the pre-exercise ingestion strategy previously employed, since this would have resulted in the enhanced buffering response being utilised during initial exercise (i.e., protecting against severe acid-base balance disturbances), in turn allowing for blood pH and [HCO_3^-] to recover to baseline status 'faster'. Indeed, Gough et al. (2017b) reported that $NaHCO_3$ administered following TTE cycling improved acid-base balance within 90 min compared to a placebo (blood pH, ~0.10 units; [HCO_3^-], ~7.0 $mmol.l^{-1}$). Nonetheless, as the authors waited until ~30 min post-exercise to administer $NaHCO_3$, it was observed that blood pH and [HCO_3^-] had already naturally returned close to baseline (~7.42; ~25.0 $mmol.l^{-1}$), therefore their results may represent the widely regarded alkalotic effect of pre-exercise supplementation.

Our findings complement those from Gough et al. (2019b) demonstrating that post-exercise $NaHCO_3$ ingestion from a state of metabolic acidosis resulted in a greater [HCO_3^-] after 75 min recovery (+8.6 $mmol.l^{-1}$). The relatively unchanged acid-base balance status at 35 min recovery herein can be attributed to a blunted extracellular buffering response since orally administered HCO_3^- had likely not been fully absorbed within the duodenum (Gough et al., 2017a). It is expected that substantial changes in enhanced HCO_3^- buffering only commenced during the second TTE protocol, which is demonstrated by the elevated [HCO_3^-] for post-TTE2 following SB, thus offering one possible explanation for the moderate effect size reported for TTE2 performance. These findings suggest however that the ingestion

of $NaHCO_3$ immediately post-exercise is not an effective strategy for accelerating the restoration of acid-base balance homeostasis when only a limited recovery period is available.

The performance enhancing effects of $NaHCO_3$ ingestion on TTE running were considerably blunted when compared to previous post-exercise $NaHCO_3$ studies (Gough et al., 2017b; Gough et al., 2019b), instead more closely representing the equivocal benefits reported during repeated bout exercise protocols (Zabala et al., 2011; Pruscino et al., 2008). The discrepancy between results is attributed to a dampened extracellular buffering response, whereby all participants ended the recovery period in a relatively 'neutral' acid-base balance status (i.e., opposed to a metabolic alkalosis from previous post-exercise studies). Despite the continual rise in $[HCO_3^-]$ during the warm-up prior to TTE2 that was demonstrated by our sub-set of data for 40 min recovery, it is unlikely that differences in $[HCO_3^-]$ for SB vs. PLA consistently achieved the 5.0-6.0 $mmol.l^{-1}$ minimal buffering threshold that has been theorised to maximise potential performance benefits (Carr et al., 2011). These changes in TTE2 performance did however display a large degree of inter-individual variation, whereby a significant 'true' effect of $NaHCO_3$ was observed for the six participants (i.e., 'responders') that improved TTE2 duration above the 4.9% within-subject CV for the TTE protocol. Considering that large variability in performance responses following $NaHCO_3$ ingestion is common (Saunders et al., 2014), likely due to between participant differences in HCO_3^- absorption kinetics (Gough et al. 2017a), it is recommended that athletes first trial the supplement within training regimes, before then applying to a competitive 'real-world' situation if positive results are obtained. Nonetheless, as three of these 'responders' identified SB (from a breaking of study blinding due to GI symptoms) and reported higher PRE (~2 units) prior to TTE2, it is also possible that improvements were partially accounted to an 'expectancy of ergogenicity' following $NaHCO_3$ ingestion (Higgins and Shaabir, 2016). Additional work should now investigate the inter-individual variation of performance enhancing effects following post-exercise $NaHCO_3$ ingestion on more robust time-trial protocols that are less likely to be influenced by psychological bias.

Post-exercise $NaHCO_3$ ingestion elevated $W_{[LA]}$ energy contribution during subsequent exercise, which agrees with previous pre-exercise ingestion findings (Gurton et al., 2020; da Silva et al., 2019) and offers additional mechanistical insight to explain changes in TTE performance. Despite

the limited recovery period, an acute $NaHCO_3$ dose induced a sufficient change in alkalotic state to augment total glycolytic flux during TTE2, which was likely caused by up-regulated lactate-H^+ co-transport from intramuscular environments via the monocarboxylate transporter (Messonnier et al., 2007). The elevated post-TTE2 [BLa^-] indirectly demonstrates increased activation of the glycolytic energetic system following $NaHCO_3$ ingestion, although the magnitude of increase was lower than from previous studies (Gough et al., 2017b; Gough et al., 2019b). One explanation for this discrepancy relates to the diminished extracellular buffering response as [HCO_3^-] was only ~3-4 $mmol.l^{-1}$ higher at the start of TTE2 for SB vs. PLA, whereas Gough et al. (2017b) induced a substantial metabolic alkalosis prior to the second exercise bout ([HCO_3^-]: ~7 $mmol.l^{-1}$ higher), resulting in a greater number of H^+ that could be neutralised. Based on the 1:1 stoichiometry of the HCO_3^- and H^+ reaction, and assuming that total blood volume was ~5 L, then SB from this current study would have only neutralised an additional ~15 $mmol.l^{-1}$ of H^+ compared to PLA (opposed to ~30 $mmol.l^{-1}$ from Gough et al., 2017b), therefore providing justification for our dampened up-regulation of glycolytic contribution. It is important to note though, our results may partially reflect greater lactate efflux from muscles, instead of an increased glycolytic flux (da Silva et al., 2019). Considering that participants also reported small, unexpected increases for W_{AER} during TTE2, which were partly attributed to the greater total work performed for SB vs. PLA (i.e., increased running distance), it is possible that we slightly overestimated the 'true' effect of $NaHCO_3$ on energy system contribution. Future research should investigate whether $NaHCO_3$ ingested within recovery directly up-regulates glycolytic flux (i.e., from invasive muscle biopsies techniques) during subsequent controlled work-rate/duration exercise protocols.

Moderate-to-severe GI symptoms following the dissociation of $NaHCO_3$ within gastric acid have been widely documented (Cameron et al., 2010; Carr et al., 2011). Our results demonstrated mildly exacerbated GI discomfort for SB at 35 min recovery compared to baseline, which is in agreement to one post-exercise ingestion study (Gough et al., 2017b), but not the other (Gough et al., 2019b). These disparities might be attributed to differences in assessment protocols (i.e., visual analogue scales vs. 11-point likert scales), or the established inter-individual variation for GI discomfort, whereby some athletes report greater gastric tolerability to $NaHCO_3$ ingestion than others (Heibel et al., 2018). GI discomfort was moderately exacerbated for SB vs. PLA post-TTE2 (i.e., reflecting worse symptoms

during exercise), however only a single participant suggested these side-effects impacted TTE2 performance, therefore our results reaffirm that GI discomfort might not directly exhibit an ergolytic effect (Higgins et al., 2013; Gough et al., 2018). One novel finding was that post-exercise $NaHCO_3$ ingestion resulted in severe GI discomfort for the 24-h post-laboratory phase (i.e., n = 5, diarrhoea), in theory proving detrimental to athletes during future training and competition. Athletes suffering from severe GI discomfort could opt for delivery strategies that bypass gastric side-effects, such as administration via enteric-coated capsules which adequately reduce severity and occurrence of GI symptoms (Hilton et al., 2020a), whilst still eliciting a sufficient, albeit delayed metabolic alkalosis (>6.0 $mmol.l^{-1}$; peak response, 90-110 min) that improves exercise performance (Hilton et al., 2020b). Additional work is required to elucidate the efficacy of enteric-coated $NaHCO_3$ capsules when only a limited amount of time is available between exercise bouts.

There are some methodological limitations in the present study. Firstly, a single-blind study design is considered sub-optimal for nutritional intervention research, however this was purely due to logistical reasons (i.e., lack of research staff). Additional control measures were put in place such as the standardisation of verbal encouragement during TTE protocols (e.g., "keep going" every 15 s) and the use of a supplement belief questionnaire (Gough et al., 2017a) that for the most part indicated we blinded $NaHCO_3$ from participants. Another limitation was our inability to directly quantify glycolytic flux and contribution from the ATP-PCr energetic system. Due to the importance of measuring blood pH and [HCO_3^-] immediately post-exercise, we were unable to identify a clear excess-post oxygen consumption curve after TTE exercise, therefore ATP-PCr calculations were removed from analysis. Lastly, the 35 min recovery was potentially too short for post-exercise $NaHCO_3$ ingestion to have a substantial effect on acid-base balance recovery, although previously published data (Gough et al., 2017a) suggests that 0.3 $g.kg^{-1}$ BM $NaHCO_3$ increases [HCO_3^-] by ~5.0 $mmol.l^{-1}$ within 40 min. This length of recovery was chosen as it best reflects the time required for blood pH and [HCO_3^-] to naturally return close to baseline (Gough et al., 2017b; Pruscino et al., 2008), which allowed us to investigate the effect of post-exercise $NaHCO_3$ ingestion on the restoration of acid-base balance homeostasis and whether this improved subsequent high-intensity performance.

CONCLUSION

The ingestion of $NaHCO_3$ immediately after an initial fatiguing exercise bout is not an effective strategy for accelerating the restoration of acid-base balance homeostasis (blood pH, HCO_3^-, base excess) when only a limited recovery period is present. This was attributed to a blunted extracellular buffering response, most likely as orally administered HCO_3^- had not been fully absorbed within the duodenum. Post-exercise $NaHCO_3$ ingestion did not significantly improve TTE2 performance, although the moderate effect size and high degree of variability advocate that coaches and practitioners administer $NaHCO_3$ on an individual basis. Small-to-moderate changes in TTE2 performance were likely due to enhanced HCO_3^- buffering and the up-regulation of glycolytic activation specifically during TTE2 exercise. $NaHCO_3$ ingestion resulted in severe post-exercise GI discomfort that may prove detrimental to athletes during subsequent training bouts or competition events. Future research should investigate the application of alternative $NaHCO_3$ administration strategies (e.g., enteric-coated capsules) on the recovery of acid-base balance, GI discomfort and repeated bout exercise performance.

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Competing interests statement

The authors declare that there are no competing interests related to the nutritional supplement or outcomes of the study.

Contributors' statement

WG and DK designed the study. WG, HM and DK collected the data. WG performed data analysis and interpretations. WG prepared the majority of manuscript, HM, LG and DK also contributed. All authors reviewed the manuscript and provided feedback.

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Figure captions

Figure 1 CONSORT 2010 reporting guidelines for research flowchart.

Figure 2 Overview of study design and experimental procedures. Abbreviations: *v-VO2max*, maximal running velocity; *SB*, sodium bicarbonate; *PLA*, placebo; *GI*, gastrointestinal; *BLa^-*, blood lactate; *BG*, blood gas; *PRE*, perceived readiness to exercise; *TTE1/2*, time-to-exhaustion protocol one/two.

Figure 3 (A-D) Mean ± SD changes in acid-base balance status (**A**, blood bicarbonate; **B**, blood pH; **C**, base excess; **D**, blood lactate). Some error bars are removed for clarity. * denotes significantly higher for SB vs. PLA ($p < 0.05$). Dashed horizontal lines represent baseline/normal acid-base balance status.

Figure 4 Mean differences and inter-individual variation (dashed lines between data points) for subsequent time-to-exhausting running protocols.