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.

56   One relatively novel strategy that accounts for this concern is post-exercise $NaHCO_3$ ingestion,

57   ensuring that a greater alkalotic response is present during recovery and subsequent exercise bouts.

58   Previous studies have demonstrated that $NaHCO_3$ ingested either immediately post-exercise or 30 min

59   after a fatiguing bout elevates blood pH (~0.10 units) and $[HCO_3^-]$ (~7-8 $mmol.l^{-1}$) following a 75-90

60   min recovery period (Gough et al., 2017; Gough et al., 2019), which subsequently improved time-to-

61   exhaustion (TTE) running (+70 s; $p = 0.084$) and cycling (+35 s; $p = 0.007$). Since the authors opted

62   for relatively long recovery windows, it was noted that blood pH and $[HCO_3^-]$ had not only recovered

63   prior to subsequent exercise, but instead reflected a state of pre-exercise metabolic alkalosis following

64    $NaHCO_3$ ingestion (~7.48 units, ~32 $mmol.l^{-1}$). Consequently, the ergogenic responses reported may

65   have represented the well-established pre-exercise application of $NaHCO_3$ ingestion. It is therefore

66   considered more appropriate to investigate the potential ergogenic effect of post-exercise $NaHCO_3$

67   when ingested during a shorter recovery period between two exercise bouts.

68   Performance enhancing effects following $NaHCO_3$ are not exclusively attributed to substantial

69   changes in blood pH and $[HCO_3^-]$ response (Siegler et al., 2016). This alkalotic state may also increase

70   glycolytic activation by preventing the allosteric inhibition of enzymes such as glycogen phosphorlyase

71   and phosphofructokinase (Messonnier et al., 2007). Pre-exercise $NaHCO_3$ ingestion has been

72   demonstrated to augment glycolytic flux during high-intensity exercise protocols (da Silva et al., 2019;

73   Gurton et al., 2020). The effect of a post-exercise $NaHCO_3$ supplementation strategy on energy system

74   contribution during subsequent exercise remains underexplored, although authors have suggested

75   enhanced glycolytic flux via elevated post-exercise blood lactate (BLa^-) concentration after a 75-90 min

76   recovery (Gough et al., 2017b; Gough et al., 2019b). It is conceivable that some athletes will have less

77   time to recover between high-intensity exercise bouts, therefore research should examine whether

78    $NaHCO_3$ ingested at the start of a shorter recovery period is still able to alter energy demand and

79   contribution from the glycolytic and oxidative systems.

80   From an applied perspective, athletes might be particularly attracted to nutritional strategies

81   that maximise ergogenic benefits during a second competition bout (i.e., track running/cycling, heat

82   then final; regional tennis, first and second round match). In these instances, ingestion of $NaHCO_3$

83   during a recovery period may prove more beneficial to subsequent performance. The purpose of this

84   study was to investigate the effect of post-exercise NaHCO$_3$ ingestion on the recovery of acid-base

85   balance (blood pH, [HCO$_3^-$], base excess) within 35 mins and subsequent TTE running performance.

86

87   **MATERIALS AND METHODS**

88   **Experimental approach to the problem**

89   A block randomised, across subjects single-blind, placebo-controlled, counterbalanced, crossover

90   experimental design was implemented for this study. Experimental trial order was allocated by the

91   principal investigator using an online randomised number generator (www.randomization.com) and

92   blinded from other members of the research team. Participants attended three separate laboratory visits

93   in a 3-h post-prandial state to perform a graded exercise test and two experimental trials (separated by

94   5-7 d). All testing was completed at Surrey Human Performance Institute at the same time of day to

95   minimise the confounding effects of circadian rhythms on exercise performance (Reilly, 1990). The

96   final speed completed during the graded exercise test was classified as maximal velocity (100% v-

97   VO$_{2max}$) and used to prescribe TTE speed. During experimental trials, participants repeated 100% v-

98   VO$_{2max}$ TTE protocols separated by 40 mins following the ingestion of either NaHCO$_3$ (SB) or sodium

99   chloride (NaCl; PLA) at the start of recovery. Vigorous exercise and the consumption of alcohol were

100 prohibited for 24-h prior to visits. Participants were instructed to record nutritional intake for 72-h prior

101 to attending the first laboratory visit using food diaries, which were checked by the principal

102 investigator and returned to participants to replicate before each experimental trial. Online databases

103 were used by the research team to determine the composition and nutritional content of foods.

104

105 **Participants**

106 A priori power calculation ($\beta = 0.8$; $\alpha = 0.05$) using previous partial eta-squared effect size data (η_p^2;

107 0.6) for post-exercise blood pH recovery (Gough et al., 2017a) revealed that a sample size of 10 would

108 allow detection of significant changes in blood pH (G*Power 3, Heinrich Heine, Düsseldorf, Germany).

109 Eleven recreationally trained runners (stature: 1.80 ± 0.05 m; body mass: 74.4 ± 6.5 kg; age: 31.0 ± 9.7

110 years; maximal oxygen consumption: 51.7 ± 5.4 ml.kg^{-1}.min^{-1}) were enrolled onto this study from a

111 local running club according to the CONSORT 2010 guidelines (**Figure 1**). All participants completed

112   at least 60 min vigorous exercise per week, had no history of hypertension (>140/90 mmHg) or renal

113   insufficiencies and had not ingested buffering agents within the previous 6 months. All study procedures

114   were reviewed and approved prior to commencing research by the Faculty of Health and Medical

115   Sciences ethics committee at the University of Surrey (1366-FHMS-18). Participants gave informed

116   consent prior to participating in the study. Procedures were conducted in full accordance with the World

117   Medical Association's Declaration of Helsinki.

118

119   [INSERT **Figure 1** NEAR HERE]

120

121   **Procedures**

122   On the initial visit, baseline physiological measures were recorded (stature, body mass, blood pressure),

123   before participants performed a graded exercise test on a treadmill to determine 100% v-VO_{2max} for

124   TTE protocols. Gaseous exchange was collected using a breath-by-breath metabolic cart (Geratherm

125   Respiratory, Love Medical, Germany). After a 5 min warm-up at 6 km.h^{-1} and 1% gradient, the speed

126   increased by 1 km.h^{-1} every 1 min until volitional exhaustion was achieved (Price and Halabi, 2005).

127   This was deemed as the failure to maintain treadmill speed despite strong verbal encouragement. Heart

128   rate and rating of perceived exertion (RPE) were recorded at the end of each stage and volitional

129   exhaustion. Data were averaged over the peak 30 s for calculating VO_{2max} (Beltz et al., 2016) and 100%

130   v-VO_{2max} was classified as the final completed speed during the graded exercise test.

131   On arrival to the laboratory during experimental trial visits, participants completed visual

132   analog scales for gastrointestinal (GI) discomfort (0 mm = "no symptom at all"; 100 mm = "severest

133   symptom imaginable") to quantify perceived severity of flatulence, abdominal discomfort (AD), gut

134   fullness (GF), bowel urgency rating (BUR) and diarrhoea (Gough et al., 2017a; Gurton et al., 2020).

135   Resting capillary blood samples (95 μl) were collected into end-to-end sodium-heparised capillary tubes

136   (EKF Diagnostics GmbH, Germany) and analysed for acid-base balance (blood pH, [HCO_3^-], base

137   excess) using a handheld analyser (i-STAT, Abbott Point of Care, New Jersey). The i-STAT analyser

138   demonstrates excellent accuracy and precision for determination of blood pH and [HCO_3^-] after

139   maximal exercise (intraclass correlation coefficients, ICC; r = 0.88, 0.86) (Dascombe et al. 2007).

140   Additional 20 μl blood samples were analysed for [BLa⁻] using an automatic analyser (Biosen C-Line,

141   EKF Diagnostic GmbH, Germany) that displays excellent precision (coefficient of variation, *CV*:

142   <1.5%; EKF Diagnostics, 2017). Participants perceived readiness to exercise (PRE) was measured,

143   before completing the first running TTE protocol (TTE1) at 100% v-VO$_{2max}$ on the treadmill (1%

144   gradient). Breath-by-breath expired gaseous exchange and RPE were recorded throughout TTE

145   protocols. Capillary blood samples and visual analog scales were performed immediately post-TTE1,

146   before participants consumed either 0.3 g.kg⁻¹ BM NaHCO$_3$ (SB) or 0.03 g.kg⁻¹ BM NaCl (PLA) after

147   5 min recovery. These doses were chosen to replicate experimental design from previous studies

148   examining the effect of either pre- or post-exercise NaHCO$_3$ ingestion on recovery (Pruscino et al.,

149   2008; Gough et al., 2017b; Gough et al., 2019b). Experimental beverages were administered single-

150   blind as a chilled solution of 400 ml water and 100 ml orange squash to ensure palatability and taste-

151   matching (Lavender and Bird, 1989). Pilot testing revealed it was not possible to taste-match an

152   equimolar 0.21 g.kg⁻¹ BM NaCl dose (i.e., equal Na⁺ content between beverages). Participants

153   consumed beverages as fast as possible but were allocated a 5 min period if required (i.e., by 10 min

154   post-TTE1), before completing a supplement belief questionnaire to assess the single-blind design and

155   whether bias regarding the effect of NaHCO$_3$ was transferred onto participants (Gough et al. 2017a).

156   Capillary blood samples and visual analogue scales were repeated following 35 min recovery (30 min

157   post-ingestion). Participants were asked for their PRE and performed the second TTE protocol (TTE2)

158   at 100% v-VO$_{2max}$ on the treadmill (40 min after TTE1). Additional blood samples were collected post-

159   TTE2, while visual analogue scales were completed post-exercise and for a 24-h post-laboratory phase.

160   Composite scores for GI discomfort were calculated at each time point from the sum of symptom

161   severity (i.e., scored out of 500 mm). An overview of experimental procedure is displayed in **Figure 2**.

162

163   [INSERT **Figure 2** NEAR HERE]

164

165   **Estimated energy system contribution calculations**

166   Absolute energy demand and contribution from the oxidative and glycolytic energetic systems were

167   estimated during TTE running protocols via non-invasive techniques (Milioni et al., 2017; Brisola et

168   al., 2015). Oxidative phosphorylation (W_{AER}) was calculated by subtracting resting oxygen

169   consumption (i.e., the average VO_2 value during the final 30 s of baseline) from the area under the

170   oxygen consumption curve for TTE protocols (di Prampero and Ferretti, 1999). Area under the curve

171   was calculated using the trapezoidal method. This approach provides reliable and valid estimations for

172   W_{AER} during high-intensity exercise (da Silva et al., 2019; Milioni et al., 2017). Glycolytic ($W_{[LA]}$) was

173   calculated from delta blood lactate (ΔBLa^-) during TTE protocols, according to the assumption that a

174   difference of 1 $mmol.l^{-1}$ of BLa^- corresponded to 3 $ml.kg^{-1}$ BM of O_2 (Milioni et al., 2017; Brisola et

175   al., 2015; Gurton et al., 2020), by using the following equation: $W_{[LA]} = \Delta BLa^- \times 3 \times$ body mass (kg).

176   The caloric quotient of 20.92 kJ was used to convert between absolute energy demand (in L of O_2) and

177   energy contribution (in kJ) for both energetic systems.

178

179   **Statistical analysis**

180   Normality and sphericity were assessed using Shapiro-Wilk and Mauchly tests, respectively. Any

181   violations were corrected via Greenhouse Geisser adjustments. Reproducibility of pre-trial nutritional

182   intake, TTE1 performance, perceived difficulty to exercise and initial acid-base balance perturbation

183   were determined from ICC's and categorised as either poor ($r \leq 0.40$), fair ($r = 0.40 - 0.59$), good ($r =$

184    $0.60 - 0.74$) or excellent ($r \geq 0.74$) (Atkinson and Nevill, 1998). Acid-base balance (primary outcome

185   measure) data were analysed using two-way (treatment x time) repeated measures ANOVA's, whereby

186   interaction effects were presented as a p value and effect size (η_p^2) (Olejnik and Algina, 2003).

187   Significant interactions were explored further by performing one-way repeated measures ANOVA

188   across treatments at a priori defined time points (i.e., post-TTE2) using the bonferroni correction factor.

189   The assumption of normal distribution was violated for GI discomfort therefore Friedman's two-way

190   ANOVA's were conducted as non-parametric alternative. Post-hoc Wilcoxon matched pair signed rank

191   tests were performed when significance was observed (median and Z score reported). Fisher's exact

192   test was used to assess the efficacy of the single-blind design. Paired t-tests were conducted on TTE2

193   performance (secondary outcome measure) and perceived difficulty to exercise. The within-subject CV

194   for the TTE1 protocol was calculated as 4.9% and used to interpret individual responses for changes in

195 TTE2 performance. Between treatment effect sizes were calculated by dividing mean differences by

196 pooled SD, before applying Hedges g bias correction (Lakens, 2013). These were interpreted as trivial

197 (< 0.20), small ($0.20 - 0.49$), moderate ($0.50 - 0.79$), or large (≥ 0.80) (Cohen, 1988). Data are presented

198 as Mean ± SD (unless otherwise stated) and 95% confidence intervals (CI) reported for between

199 treatment statistical differences. Statistical significance was set at $p < 0.05$ and data were analysed using

200 SPSS v26 (SPSS Inc., IBM, USA).

201

202 **RESULTS**

203 **Pre-trial nutritional intake**

204 Participants demonstrated an excellent degree of repeatability for pre-trial nutritional intake during SB

205 and PLA trials for daily calories ($r = 0.89$, $p = 0.001$), carbohydrate ($r = 0.87$, $p = 0.002$), fat ($r = 0.83$,

206 $p = 0.005$) and protein ($r = 0.83$, $p = 0.014$).

207

208 **Acid-base balance status**

209 Initial acid-base balance perturbation demonstrated an excellent level of reproducibility during SB and

210 PLA trials for post-TTE1 blood pH ($r = 0.94$; $p < 0.001$), [HCO_3^-] ($r = 0.95$; $p < 0.001$), base excess (r

211 $= 0.92$; $p < 0.001$) and [BLa^-] ($r = 0.93$; $p < 0.001$). Significant two-way interactions (treatment x time)

212 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

213 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

214 unchanged at 35 min recovery ($p > 0.05$). Exploratory analysis on a small sub-set ($n = 6$) of blood pH

215 and [HCO_3^-] data at 40 min recovery (i.e., immediately pre-TTE2; not an *a* priori defined time point)

216 revealed a larger magnitude of effect for SB vs. PLA ($+0.03$ units; $+3.9$ mmol.l^{-1}). Post-TTE2 [HCO_3^-]

217 was significantly elevated for SB vs. PLA ($p = 0.005$) and displayed a large effect size ($+2.6$ mmol.l^{-1};

218 CI: 1.0, 4.2; $g = 0.99$). Peak [BLa^-] following TTE2 was significantly higher for SB vs. PLA ($p = 0.015$)

219 and displayed a large effect size ($+1.4$ mmol.l^{-1}; CI: 0.3, 2.5; $g = 0.82$). Mean ± SD values for changes

220 in acid-base balance status from baseline to post-TTE2 are displayed in **Figure 3 (A-D)**.

221

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

223

224   **Time-to-exhaustion running performance**

225   Performance during TTE1 demonstrated an excellent degree of repeatability for SB and PLA trials ($r =$

226   0.95; $p < 0.005$; within-subject $CV = 4.9\%$). No significant differences were observed for TTE2

227   performance (+14.3 s; $p = 0.071$), although a moderate effect size was present for SB vs. PLA (+9.7%;

228    $g = 0.56$). There was a large degree of individual variability for TTE2 performance, whereby mean

229   differences for SB vs. PLA ranged from -16.8 s (-14.2%) to +61.2 s (+37.1%). In total, six out of eleven

230   participants reported improvements in TTE2 performance above the 4.9% test-retest variability. Sub-

231   analysis for these six 'responders' revealed a significant increase of +31.2 s for TTE2 performance

232   following SB compared to PLA (+21.1 %; $p = 0.007$; $g = 1.65$). Mean values and individual differences

233   for TTE2 performance are displayed in **Figure 4**.

234

235   [INSERT **Figure 4** NEAR HERE]

236

237   **Estimated energy system contribution**

238   Mean ± SD values for energy demand (in L of O_2) and contribution (kJ) from oxidative and glycolytic

239   energetic systems are presented in **Table 1**. Excellent reproducibility was observed between SB and

240   PLA trials for energy contribution from W_{AER} ($r = 0.96$; $p < 0.001$) and $W_{[LA]}$ ($r = 0.92$; $p < 0.001$)

241   during TTE1. Energy contribution from $W_{[LA]}$ was significantly increased during TTE2 for SB vs. PLA

242   ($p = 0.032$) and displayed a moderate effect size (+6.84 kJ; CI: 0.7, 13.0; $g = 0.69$). In total, ten out of

243   eleven participants reported greater $W_{[LA]}$ contribution following SB vs. PLA. No significant differences

244   were observed for energy contribution from W_{AER} during TTE2 ($p > 0.05$), although a small effect size

245   was present for SB vs. PLA (+11.20 kJ; $g = 0.48$).

246

247   [INSERT **Table 1** NEAR HERE]

248

249   **Perceived difficulty of exercise**

250 Participants achieved a similar level of volitional exhaustion during TTE1 for SB and PLA trials as

251 good reproducibility was reported for peak RPE ($r = 0.66$; $p = 0.049$). No differences were observed

252 between SB and PLA for peak RPE during TTE2 ($p > 0.05$). No significant differences were reported

253 across treatments for PRE prior to TTE2 ($p > 0.05$), although a small effect size was present for SB vs.

254 PLA (+0.36 units; $g = 0.26$).

255

256 **Perceptual responses to experimental beverages**

257 Four participants identified SB, six were unsure on treatments, whilst one incorrectly identified SB as

258 PLA. Treatments were therefore considered successfully single-blinded and taste-matched (Fisher's

259 exact test, $p = 1.000$) and only a single participant directly suggested a poor taste.

260 In total, ten out of eleven participants experienced some GI discomfort for the SB trial (timing

261 and severity of peak symptom for each participant displayed in **Table 2**). Composite scores for GI

262 discomfort revealed no symptoms at baseline, but symptom severity was mildly exacerbated for SB vs.

263 PLA at 35 min recovery (Mdn: 11 mm vs. 10 mm) and post-TTE2 (Mdn: 11 mm vs. 9 mm). No

264 significant treatment effects were present at any time point for AD or GF ($p > 0.05$). No significant

265 treatment effects were observed at either 35 min recovery or post-TTE2 for flatulence, BUR and

266 diarrhoea (all $p > 0.05$). Ratings of GI discomfort were significantly exacerbated for SB vs. PLA during

267 the 24-h post-laboratory phase for flatulence (Mdn: 10 mm vs. 0 mm; $Z = 2.366$; $p = 0.018$), BUR (20

268 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

269 five participants that failed to improve TTE2 performance for SB vs. PLA, only one directly reported

270 an ergolytic effect of GI discomfort.

271

272 [INSERT **Table 2** NEAR HERE]

273

274 **DISCUSSION**

275 This was the first study to investigate the effect of post-exercise $NaHCO_3$ ingestion on blood pH and

276 [HCO_3^-] recovery after 35 min and subsequent TTE running performance. No changes in acid-base

277 balance status were reported for SB vs. PLA after the 35 min recovery period, however both [HCO_3^-]

278 and [BLa$^-$] were elevated post-TTE2. Post-exercise NaHCO$_3$ ingestion did not significantly improve

279 TTE2 performance, although a moderate effect size and a high degree of inter-individual variation was

280 observed. Glycolytic energy system contribution was moderately increased during TTE2 for SB vs.

281 PLA. Lastly, post-exercise NaHCO$_3$ ingestion resulted in moderate-to-severe GI symptoms after TTE2

282 and for the 24-h post-laboratory phase, therefore athletes should remain cautious when using this

283 strategy to improve performance.

284 The novel finding from this study was that NaHCO$_3$ ingested after a fatiguing exercise bout

285 failed to accelerate the recovery of blood pH, [HCO$_3^-$] and base excess to 'neutral' levels within 35 min

286 when compared to PLA. These results contrast findings from pre-exercise ingestion studies suggesting

287 that NaHCO$_3$ accelerated the restoration of acid-base balance homeostasis within 30-40 min (Pruscino

288 et al., 2008; Gough et al., 2019a). This discrepancy is most likely explained by the pre-exercise ingestion

289 strategy previously employed, since this would have resulted in the enhanced buffering response being

290 utilised during initial exercise (i.e., protecting against severe acid-base balance disturbances), in turn

291 allowing for blood pH and [HCO$_3^-$] to recover to baseline status 'faster'. Indeed, Gough et al. (2017b)

292 reported that NaHCO$_3$ administered following TTE cycling improved acid-base balance within 90 min

293 compared to a placebo (blood pH, ~0.10 units; [HCO$_3^-$], ~7.0 mmol.l^{-1}). Nonetheless, as the authors

294 waited until ~30 min post-exercise to administer NaHCO$_3$, it was observed that blood pH and [HCO$_3^-$]

295 had already naturally returned close to baseline (~7.42; ~25.0 mmol.l^{-1}), therefore their results may

296 represent the widely regarded alkalotic effect of pre-exercise supplementation.

297 Our findings complement those from Gough et al. (2019b) demonstrating that post-exercise

298 NaHCO$_3$ ingestion from a state of metabolic acidosis resulted in a greater [HCO$_3^-$] after 75 min recovery

299 (+8.6 mmol.l^{-1}). The relatively unchanged acid-base balance status at 35 min recovery herein can be

300 attributed to a blunted extracellular buffering response since orally administered HCO$_3^-$ had likely not

301 been fully absorbed within the duodenum (Gough et al., 2017a). It is expected that substantial changes

302 in enhanced HCO$_3^-$ buffering only commenced during the second TTE protocol, which is demonstrated

303 by the elevated [HCO$_3^-$] for post-TTE2 following SB, thus offering one possible explanation for the

304 moderate effect size reported for TTE2 performance. These findings suggest however that the ingestion

305   of NaHCO$_3$ immediately post-exercise is not an effective strategy for accelerating the restoration of

306   acid-base balance homeostasis when only a limited recovery period is available.

307   The performance enhancing effects of NaHCO$_3$ ingestion on TTE running were considerably

308   blunted when compared to previous post-exercise NaHCO$_3$ studies (Gough et al., 2017b; Gough et al.,

309   2019b), instead more closely representing the equivocal benefits reported during repeated bout exercise

310   protocols (Zabala et al., 2011; Pruscino et al., 2008). The discrepancy between results is attributed to a

311   dampened extracellular buffering response, whereby all participants ended the recovery period in a

312   relatively 'neutral' acid-base balance status (i.e., opposed to a metabolic alkalosis from previous post-

313   exercise studies). Despite the continual rise in [HCO$_3^-$] during the warm-up prior to TTE2 that was

314   demonstrated by our sub-set of data for 40 min recovery, it is unlikely that differences in [HCO$_3^-$] for

315   SB vs. PLA consistently achieved the 5.0-6.0 mmol.l^{-1} minimal buffering threshold that has been

316   theorised to maximise potential performance benefits (Carr et al., 2011). These changes in TTE2

317   performance did however display a large degree of inter-individual variation, whereby a significant

318   'true' effect of NaHCO$_3$ was observed for the six participants (i.e., 'responders') that improved TTE2

319   duration above the 4.9% within-subject CV for the TTE protocol. Considering that large variability in

320   performance responses following NaHCO$_3$ ingestion is common (Saunders et al., 2014), likely due to

321   between participant differences in HCO$_3^-$ absorption kinetics (Gough et al. 2017a), it is recommended

322   that athletes first trial the supplement within training regimes, before then applying to a competitive

323   'real-world' situation if positive results are obtained. Nonetheless, as three of these 'responders'

324   identified SB (from a breaking of study blinding due to GI symptoms) and reported higher PRE (~2

325   units) prior to TTE2, it is also possible that improvements were partially accounted to an 'expectancy

326   of ergogenicity' following NaHCO$_3$ ingestion (Higgins and Shaabir, 2016). Additional work should

327   now investigate the inter-individual variation of performance enhancing effects following post-exercise

328   NaHCO$_3$ ingestion on more robust time-trial protocols that are less likely to be influenced by

329   psychological bias.

330   Post-exercise NaHCO$_3$ ingestion elevated W$_{[LA]}$ energy contribution during subsequent

331   exercise, which agrees with previous pre-exercise ingestion findings (Gurton et al., 2020; da Silva et

332   al., 2019) and offers additional mechanistical insight to explain changes in TTE performance. Despite

333 the limited recovery period, an acute $NaHCO_3$ dose induced a sufficient change in alkalotic state to

334 augment total glycolytic flux during TTE2, which was likely caused by up-regulated lactate-H^+ co-

335 transport from intramuscular environments via the monocarboxylate transporter (Messonnier et al.,

336 2007). The elevated post-TTE2 [BLa^-] indirectly demonstrates increased activation of the glycolytic

337 energetic system following $NaHCO_3$ ingestion, although the magnitude of increase was lower than from

338 previous studies (Gough et al., 2017b; Gough et al., 2019b). One explanation for this discrepancy relates

339 to the diminished extracellular buffering response as [HCO_3^-] was only ~3-4 $mmol.l^{-1}$ higher at the start

340 of TTE2 for SB vs. PLA, whereas Gough et al. (2017b) induced a substantial metabolic alkalosis prior

341 to the second exercise bout ([HCO_3^-]: ~7 $mmol.l^{-1}$ higher), resulting in a greater number of H^+ that could

342 be neutralised. Based on the 1:1 stoichiometry of the HCO_3^- and H^+ reaction, and assuming that total

343 blood volume was ~5 L, then SB from this current study would have only neutralised an additional ~15

344 $mmol.l^{-1}$ of H^+ compared to PLA (opposed to ~30 $mmol.l^{-1}$ from Gough et al., 2017b), therefore

345 providing justification for our dampened up-regulation of glycolytic contribution. It is important to note

346 though, our results may partially reflect greater lactate efflux from muscles, instead of an increased

347 glycolytic flux (da Silva et al., 2019). Considering that participants also reported small, unexpected

348 increases for W_{AER} during TTE2, which were partly attributed to the greater total work performed for

349 SB vs. PLA (i.e., increased running distance), it is possible that we slightly overestimated the 'true'

350 effect of $NaHCO_3$ on energy system contribution. Future research should investigate whether $NaHCO_3$

351 ingested within recovery directly up-regulates glycolytic flux (i.e., from invasive muscle biopsies

352 techniques) during subsequent controlled work-rate/duration exercise protocols.

353   Moderate-to-severe GI symptoms following the dissociation of $NaHCO_3$ within gastric acid

354 have been widely documented (Cameron et al., 2010; Carr et al., 2011). Our results demonstrated mildly

355 exacerbated GI discomfort for SB at 35 min recovery compared to baseline, which is in agreement to

356 one post-exercise ingestion study (Gough et al., 2017b), but not the other (Gough et al., 2019b). These

357 disparities might be attributed to differences in assessment protocols (i.e., visual analogue scales vs. 11-

358 point likert scales), or the established inter-individual variation for GI discomfort, whereby some

359 athletes report greater gastric tolerability to $NaHCO_3$ ingestion than others (Heibel et al., 2018). GI

360 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.

389 **CONCLUSION**

390 The ingestion of $NaHCO_3$ immediately after an initial fatiguing exercise bout is not an effective strategy

391 for accelerating the restoration of acid-base balance homeostasis (blood pH, HCO_3^-, base excess) when

392 only a limited recovery period is present. This was attributed to a blunted extracellular buffering

393 response, most likely as orally administered HCO_3^- had not been fully absorbed within the duodenum.

394 Post-exercise $NaHCO_3$ ingestion did not significantly improve TTE2 performance, although the

395 moderate effect size and high degree of variability advocate that coaches and practitioners administer

396 $NaHCO_3$ on an individual basis. Small-to-moderate changes in TTE2 performance were likely due to

397 enhanced HCO_3^- buffering and the up-regulation of glycolytic activation specifically during TTE2

398 exercise. $NaHCO_3$ ingestion resulted in severe post-exercise GI discomfort that may prove detrimental

399 to athletes during subsequent training bouts or competition events. Future research should investigate

400 the application of alternative $NaHCO_3$ administration strategies (e.g., enteric-coated capsules) on the

401 recovery of acid-base balance, GI discomfort and repeated bout exercise performance.

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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.