The Reproducibility of Blood Acid Base Responses in Male Collegiate Athletes Following Individualised Doses of Sodium Bicarbonate: A Randomised Controlled Crossover Study

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Title: The reproducibility of blood acid base responses in male collegiate athletes following individualised doses of $NaHCO_3$: a randomised controlled crossover study.

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Abstract

Background: Current evidence suggests sodium bicarbonate ($NaHCO_3$) should be ingested based upon the individualised alkalotic peak of either blood pH or bicarbonate (HCO_3^-), as a result of a large inter-individual variation reported (10-180 min). If such a strategy is to be practically applied, the blood analyte response needs to be reproducible and therefore, this study aimed to evaluate the degree of reproducibility of both time to peak (TTP) and absolute change in blood pH, HCO_3^- and sodium (Na^+) following acute $NaHCO_3$ ingestion. ***Methods:*** Fifteen male participants with backgrounds in rugby, football and sprinting completed six randomised treatments entailing ingestion of 0.2 $g.kg^{-1}$ body mass (BM) $NaHCO_3$ (SBC2a and b) twice, 0.3 $g.kg^{-1}$ BM $NaHCO_3$ (SBC3a and b) twice, or two control treatments (CON1a and b) on separate days. Blood analysis included pH, HCO_3^- and Na^+ prior to and at regular time points following $NaHCO_3$ ingestion over a three hour period. ***Results:*** Compared to pH, HCO_3^- displayed greater reproducibility in intraclass correlation coefficient (ICC) analysis for both TTP (HCO_3^- SBC2 $r = 0.77$, $P = 0.003$, SBC3 $r = 0.94$, $P < 0.001$; pH SBC2 $r = 0.62$, $P = 0.044$ SBC3 $r = 0.71$, $P = 0.016$) and absolute change (HCO_3^- SBC2 $r = 0.89$, $P < 0.001$, SBC3 $r = 0.76$, $P = 0.008$; pH SBC2 $r = 0.84$, $P = 0.001$, SBC3 $r = 0.62$, $P = 0.041$). ***Conclusion:*** Our results indicate both the TTP and absolute change in HCO_3^- is more reliable compared to pH, and as such, these data provide support for an individualised $NaHCO_3$ ingestion strategy to be used to elicit peak alkalosis consistently prior to exercise. Future work should utilise an individualised $NaHCO_3$ ingestion strategy based on HCO_3^- responses and evaluate the effects on exercise performance.

Key Points

- Although both the blood pH and HCO_3^- response following $NaHCO_3$ displays good test-retest reliability, the HCO_3^- response is more reproducible. Therefore the individualised $NaHCO_3$ ingestion strategy should be based on time to peak HCO_3^-.
- The large inter-individual variability to achieve both peak pH and HCO_3^- suggests an individualised $NaHCO_3$ ingestion strategy based on time to peak HCO_3^- is the most appropriate to heighten the potential ergogenic effects on performance.
- Within the first 60 mins following both 0.2 and 0.3 $g.kg^{-1}$ BM $NaHCO_3$, the acid-base balance kinetics are similar, meaning smaller doses of $NaHCO_3$ may be appropriate when <60 min is available, particularly for those individuals who suffer from gastrointestinal discomfort (GI).

1.0 Introduction

Research investigating nutritional ergogenic aid strategies that delay the occurrence of metabolic acidosis during high intensity exercise have been widely investigated [4, 16, 41]. In particular, exogenous enhancement of the bicarbonate buffering systems is thought to have an important role in offsetting the metabolite fatigue process, by dampening critical rises in hydrogen cations (H^+) [17]. Ingestion of a known alkalotic buffer, namely sodium bicarbonate ($NaHCO_3$), can achieve such ergogenic effects through increasing blood bicarbonate [HCO_3^-] concentration within the extracellular fluid of between 4-8 mmol.L^{-1} [36], which typically relates to the point of peak alkalosis [34]. Most common ingestion practices include doses of between 0.2 and 0.3 g.kg^{-1} BM $NaHCO_3$, as amounts lower than this are not considered sufficient to induce a level of peak alkalosis to improve performance [36]. Doses above this concentration exacerbate the incidence and severity of gastrointestinal (GI) discomfort [16].

Multiple studies using group mean data have reported a high variation in time to peak (TTP) alkalosis (i.e. HCO_3^-) following various doses of $NaHCO_3$ [6, 29, 33, 34]. Peak HCO_3^- has previously been observed at 40 min and 60 min following 0.2 g.kg^{-1} BM and 0.3 g.kg^{-1} BM $NaHCO_3$ respectively [33], whereas others have reported 90 [29], 120 [6] and 180 min [34]. Differences may be evident either as a result of sampling range (20-60 min), or inter-individual variation within participants, since individual absorption characteristics of blood pH and HCO_3^- have potentially been overlooked in previous studies [6, 29, 33, 34]. Consequently this generic approach has led to a potential reduction in the ergogenic effect on exercise performance, or caused variation in performance benefits [7, 31]. More specifically, Dias et al [7] reported a lack of consistency in performance response following $NaHCO_3$ during a 110% peak

power output cycling time to exhaustion (TTE). Fifteen recreationally active participants consumed 0.3 $g.kg^{-1}$ BM $NaHCO_3$ on four occasions, or a placebo on two occasions. Only one participant produced ergogenic effects in all $NaHCO_3$ treatments, with five failing to improve in any treatment. This suggests some degree of intra-individual variation is evidence, which may be as a result of intra-individual blood responses, although this is difficult to define as only group mean blood responses were reported.

A contemporary approach involves individualising the ingestion strategy, and Stannard et al. [36] reported TTP HCO_3^- displayed a large inter-individual variation (0.2 $g.kg^{-1}$ BM = 40-165 min, 0.3 $g.kg^{-1}$ BM = 75-180 min). These findings challenge the aforementioned studies who reported group level analysis following $NaHCO_3$ supplementation at a fixed time frame [17, 31, 33]. Furthermore, variations in TTP arguably provides insight to the commonly reported inter and intra-individual variations following $NaHCO_3$ ingestion on performance [7, 31], as participants may not have elicited peak alkalosis at the commencement of exercise [17]. Recent work by Miller et al. [19] supports this claim, demonstrating during repeated sprint cycling (10 x 6 s) total work done (TWD) improved by 11% with an individualised ingestion strategy, a response greater than the 5% change in a similar study employing a standardised ingestion strategy [3].

Further research to identify individualised $NaHCO_3$ ergogenic strategies that elicit peak alkalosis are necessary. Equally for practical application in the field, a greater understanding of the reproducibility of blood analytes (pH and HCO_3^-) following acute $NaHCO_3$ is required. Daily biological variation, either short term or long term, may

occur in response to changes in nutritional practices and therefore effect daily acid load fluxes (potential renal acid load; PRAL) [23, 27, 28] with the potential to affect the reproducibility of TTP alkalosis. As a result, this may negatively affect the efficacy of employing an individualised $NaHCO_3$ ingestion strategy to improve exercise performance consistently. Therefore, the aim of this study was to assess the reproducibility of the individual blood pH, HCO_3^- and Na^+ response following acute $NaHCO_3$ ingestion in both 0.2 and 0.3 $g.kg^{-1}$ BM doses.

2.0 Materials and Methods

2.1 Participants

Participants were recruited on the basis they may gain a performance benefit from enhancing their buffering capacity (McNaughton et al., 2016). As a result, sixteen team and individual sports participants with backgrounds in rugby, football and running volunteered for this single blind, randomised, crossover designed study. One participant withdrew from the study due to GI upset (vomiting) from $NaHCO_3$ (0.3$g.kg^{-1}$ BM dose; first session), therefore 15 male participants (n=5 rugby, n=7 football, n=3 sprinting) completed the study (height 1.81 ± 0.06 m, body mass 84 ± 8 kg, age 21 ± 2 years, VO_{2MAX} 52.1 ± 2.2 $ml.kg^{-1}.min^{-1}$). Participants habitually completed four exercise bouts per week (4 ± 1 $p.wk^{-1}$), lasting two hours per session (2 ± 0 hr) and had ten years training experience (10 ± 3 years) within their respective sports. Ethical approval was obtained from Departmental Research Ethics Committee (SPA-REC-2015-325) and each participant provided written informed consent and completed a health screening procedure prior to data collection. The research was conducted in accordance with the Helsinki declaration. Participants were verbally screened to

150 ensure $NaHCO_3$ or similar intracellular or extracellular buffers such as beta alanine
151 were not ingested for six months prior to, or outside of the experimental conditions.
152
153 *2.2 Pre-experiment procedures*
154 Participants visited the laboratory on seven occasions at the same time of day to
155 minimise the effects of circadian rhythms [26] and 4 hr postprandial. Avoidance of
156 alcohol and any strenuous/unaccustomed exercise was requested 24 hr period prior
157 to experimental treatment arm [30]. Caffeine and spicy foods were also prohibited 12
158 hr prior to experimental treatments, as they may influence metabolic regulation [14,
159 42]. Compliance to the above procedures was checked via a written log of nutritional
160 intake 24 hr prior to each experimental treatment, which was replicated for each visit
161 (adherence = 100%) and was later analysed for reproducibility. Each treatment was
162 conducted at least seven days apart to allow for washout of residual $NaHCO_3$ [3]. The
163 $NaHCO_3$ used in this study was purchased from the manufacturer and stored safely
164 accordingly to laboratory guidelines to avoid contamination of other stimulants.
165
166 *2.3 Maximal oxygen uptake protocol*
167 Initially, an incremental ramp maximal oxygen uptake (VO_{2max}) test on an
168 electromagnetically braked cycle ergometer was conducted (Lode Excalibur,
169 Germany). After a 5 min warm up (70 W), participants began cycling at their respective
170 self-selected cadence (n = 10, 80 $r.min^{-1}$; n = 5, 90 $r.min^{-1}$) at a power output of 75 W.
171 This then increased by 1 W every 2 s (30 $W.min^{-1}$) until volitional exhaustion. Using a
172 gas analyser (Cosmed, K5, Italy), samples were continuously analysed for oxygen
173 consumption (VO_2), carbon dioxide expired (VCO_2) and respiratory exchange ratio

(RER). Data was averaged over the last thirty seconds of exercise to determine the VO_{2MAX}.

2.4 Main treatment arms

Administered in a block randomised method, the subsequent six treatments involved two treatment arms of no treatment (CON1a, CON1b) to assess daily variation of blood analytes, two treatment arms requiring ingestion of 0.2 $g.kg^{-1}$ (SBC2a, SBC2b), and two with 0.3 $g.kg^{-1}$ BM $NaHCO_3$ (SBC3a, SBC3b). Solutions were mixed by a laboratory technician not involved with the research by mixing 400 ml of water with 50 ml of flavoured sugar free squash and placed within a refrigerator to enhance palatability [19]. Treatments were administered single blind and participants ingested within 10 min for all treatments [36].

An arterialised finger prick capillary blood sample was obtained from the finger whilst in a rested and seated state, prior to $NaHCO_3$ ingestion. Arterialisation was achieved by warming the hand with a heated blanket (45^{o}C) for 5 min prior to each individual sample [12]. After $NaHCO_3$ ingestion, a further 15 blood samples were obtained over a 180 min period in each treatment (Table 1). At multiple time points, a GI questionnaire (VAS scale; 0 = *no instance*, 10 = *most severe*) was completed as per previous research within a range of symptoms [19] (Table 3). Participants remained seated throughout, with only toilet breaks permitted. No food was allowed to be consumed during this period, and water was consumed *ab libitum*, with total volume replicated in subsequent treatment arms. Blood samples were collected in 100 µl heparin-coated clinitubes (Radiometer Medical Ltd, Denmark) and subsequently analysed for blood pH, HCO_3^- and Na^+ (ABL800 BASIC, Radiometer Medical Ltd.

199 Denmark). This radiometer has demonstrated a low bias in pH, PCO_2 and Na^+
200 (ABL800 reference manual; [25]) and reported a correlation coefficient of r >0.98 for
201 both HCO_3^- and pH against other commercially available blood gas analysers [37].
202 Moreover, a small pilot study (n = 8) also revealed high test-retest reliability for both
203 HCO_3^- (16 samples: CV: 3.0 to 4.9%) and pH (16 samples: CV: 0.17% to 0.20%) at
204 both resting levels and following $NaHCO_3$ ingestion.
205
206 *2.5 Statistical analysis*
207 A *priori* power calculation was conducted using a statistical software package (SPSS
208 Sample Power 3, IBM, Chicago, USA). Based upon the expected population
209 correlation of r = 0.80 between both $NaHCO_3$ conditions (SBC2 and SBC3), a
210 minimum of 11 participants were required to achieve 80% power (P <0.05).
211
212 Assessed variables were initially analysed for normality (Shapiro-Wilks and Q-Q plots)
213 and homogeneity of variance/sphericity (Mauchly) respectively. To assess the
214 differences between conditions, T-Tests were used. For non-normally distributed data,
215 a Mann-Whitney U test was used with Z score and significance reported (e.g. GI data).
216 Likewise for violations of sphericity the appropriate correction was applied
217 (Greenhouse Geisser). Both one (Treatment) and two (Treatment * Time) way
218 repeated measured ANOVA was used to analyse differences in blood parameters with
219 Bonferroni-corrections applied. Tukeys honestly significance difference (HSD) post-
220 hoc analysis was carried out to assess interactions, by calculating the minimal
221 difference required between means to identify significance had been achieved [40].
222 Statistical significance was set a P >0.05.
223

Limits of agreement (LOA) with 95% percent limits and Bland-Altman plots were utilised for within-subject variance and to determine if data was heteroscedastic (Bland and Altman, 1986). This method is widely used [20, 35] and accounts for bias between the mean differences [8]. Intraclass correlation coefficient (ICC) were displayed with *r* value and significance level, as per previous recommendations [1]. Coefficient of variation (CV) is reported using SD/mean*100. Correlation between HCO_3^- and pH TTP was calculated using Pearson correlation, from Hopkins spreadsheet [11]. Statistical procedures were completed using SPSS version 22 (IBM, Chicago, USA) and calculations were carried out using Microsoft Excel 2013 (Microsoft Inc., USA).

3.0 Results

3.1 Nutritional intake

Total daily calorie intake was highly reproducible for all treatments (r = 0.78, P <0.001; Mean ± SD = 2283 ± 75), as was carbohydrate (r = 0.97 P <0.001; 253 ± 4 g), protein (r = 0.98, P <0.001; 85 ± 2 g) and fat (r = 0.97, P <0.001; 126 ± 3 g) intake.

3.2 Gastrointestinal upset

Both the severity, and TTP GI displayed excellent reproducibility in SBC2 and SBC3 (severity SBC2 r = 0.92, P <0.001; LOA: B -0.5, -3.1, +2.2; TTP SBC2 r = 0.91, P <0.001; LOA: B 5, -38, +47 vs. severity SBC3 r = 0.90, P <0.001; LOA: B -0.4, -4.7, +3.8; TTP SBC3 r = 0.78, P = 0.005; LOA: B 7, -64, 77). In total 8/15 of the participants reported symptoms of GI in both SBC2 and SBC3, and the specific symptoms are depicted in Table 3. The severity of GI was decreased in SBC2 compared to SBC3 (mean = 2.0 vs. 3.6), however not significantly (Z = 0.922, P = 0.356). TTP GI in SBC2

248 was established earlier in SBC2 compared to SBC3 (mean = 29 vs. 36 min), however
249 not significantly (Z = 0.439, P = 0.661).
250
251 *3.2 Reproducibility of blood pH, HCO_3^- and Na^+*
252 Baseline measures for both HCO_3^- (r = 0.83, P <0.001) and Na^+ (Na^+ r = 0.86, P
253 <0.001) displayed excellent reproducibility, whereas pH displayed good reproducibility
254 (r = 0.66, P = 0.002). Values for ICC across the three hour sampling period ranged
255 from fair to excellent (r = 0.530-0.914) for pH in SBC2 and good to excellent (r = 0.76-
256 0.92) in SBC3 upon excluding two poor values at 80 (r = 0.05) and 85 min (r = 0.01).
257 Reproducibility for HCO_3^- in SBC2 demonstrated excellent reproducibility (r = 0.76-
258 0.87), whereas SBC3 displayed good to excellent (r = 0.65-0.87) reproducibility across
259 all time points (Table 1).
260
261 TTP HCO_3^- demonstrated greater reproducibility for SBC3 compared to SBC2 (SBC3
262 ICC: r = 0.94, P <0.001; LOA: B 2.3, -15.9, +20.5 vs. SBC2 ICC: r = 0.77, P = 0.003;
263 LOA: B -6, -36, +24). Likewise, TTP pH demonstrated a greater reproducibility for
264 SBC3 compared to SBC2 (SBC3 ICC: r = 0.71, P = 0.016; LOA: B 2.3, -37.3, +42;
265 SBC2 ICC: r = 0.62, P = 0.044; LOA: B 2.3, -39.3, +42). The correlation between TTP
266 pH and TTP HCO_3^- was greater in SBC2 compared to SBC3 (SBC2 r = 0.61 and r =
267 0.66; SBC3 r = 0.26 and r = 0.17). The relationship between TTP Na^+ was greater for
268 SBC2 compared to SBC3, however neither were significant in ICC and displayed large
269 bias in LOA analysis (SBC2 ICC: r = 0.75, P = 0.838; LOA: B 8.7, +41.8, -73.2; SBC3
270 ICC: r = 0.56, P = 0.061; LOA: B 15, +44.4, -71.9).
271

Absolute change (peak change from baseline) for HCO_3^- displayed high reproducibility for SBC2 compared to SBC3 (SBC2 ICC: *r* = 0.90, P <0.001; LOA: B 0.1, -0.9, +1.1 vs. SBC3 ICC: *r* = 0.76, P = 0.008; LOA: B 0.1, -1.9, +2.0). The absolute change in pH was highly reproducible in SBC2 compared to SBC3 (SBC2 ICC: *r* = 0.84, P = 0.001; LOA: B -0.1, -0.04, +0.03 vs. SBC3 ICC: *r* = 0.62, P = 0.041; LOA: B 0.01, -0.04, +0.05). In contrast, the absolute change in Na^+ displayed no relationship in both SBC2 (ICC: *r* = 0.10, P = 0.562; LOA: B 0.1, -4.9, +5.1) or SBC3 (ICC: *r* = 0.10, P = 0.425; LOA: B 1.3, -6.2, +8.7).

3.3 Differences between treatments

TTP HCO_3^- was not significantly different between SBC2 and SBC3 (all P >0.05) (Table 2). Whereas, TTP pH occurred significantly later in SBC3a compared to SBC2a (+17 min; P <0.026), however non-significantly later in SBC3b compared to SBC2b (+8 min; P = 0.392) (Table 2). TTP Na^+ occurred significantly later in SBC3a compared to SBC2a (+32 min; P = 0.027) and 25 min later for SBC3b compared to SBC2b (P = 0.061). A large inter-individual variation in TTP pH, HCO_3^- and Na^+ in both SBC treatments was observed (Table 2).

The absolute change in blood analytes HCO_3^- and pH can be observed in Table 2. Absolute change in HCO_3^- was greater in SBC3 compared to SBC2 (P <0.001; Table 2). Absolute pH change was significantly greater for SBC3a compared to SBC2a (+0.2; P = 0.018), however not in SBC2b and SBC3b (+0.1; P = 0.242). Absolute change in Na^+ was significantly greater in SBC3 compared to SBC2 (P >0.05; Figure 1). A large inter-individual variation in absolute change of pH, HCO_3^- and Na^+ in both SBC2 and SBC3 was observed (Table 2). Lastly, up to 60 min post $NaHCO_3$ ingestion both HCO_3^-

297 and pH was not significantly different between SBC2 and SBC3 (all P >0.05; Figure
298 1).
299
300 **4.0 Discussion**
301 This is the first study to investigate the reproducibility of individual blood analytes pH,
302 HCO_3^- and Na^+ following acute induced metabolic alkalosis. Our findings suggest
303 blood pH and HCO_3^- are highly reproducible in most participants (13 out of 15),
304 whereas in contrast, Na^+ displays poor reproducibility. In light of both the TTP and
305 absolute change reflecting greater reproducibility for HCO_3^-, combined with the lack of
306 correlation between pH and HCO_3^- (no to moderate correlation; section 3.2), it is
307 essential a prior knowledge of HCO_3^- absorptions characteristics following $NaHCO_3$
308 ingestion is obtained. As such, practitioners and athletes should develop their
309 respective $NaHCO_3$ dosing strategies based on TTP HCO_3^-.
310
311 The present studies data challenges the common ingestion strategy of 0.3 $g.kg^{-1}$ BM
312 $NaHCO_3$ 1 to 4 hours prior to exercise [16, 29, 34], displaying a large inter-individual
313 variation to obtain peak alkalosis (Table 2). For instance the absolute changes in
314 HCO_3^- observed in this study for SBC2 (~5.7 $mmol.L^{-1}$) and SBC3 (~7.1 $mmol.L^{-1}$)
315 (Table 2) were greater than the typical change with standardised ingestion strategies
316 [33]. This is also within the range of absolute change that is suggested to be required
317 to potentially produce ergogenic effects (>5 $mmol.L^{-1}$; [5]). Moreover, in light of similar
318 reports of inter-individual variation [19, 36, 34] a standardised ingestion strategy is not
319 suitable to heighten the potential ergogenic effects from alkalotic substances (i.e.
320 $NaHCO_3$ and sodium citrate). Rather, an individualised ingestion strategy is more

relevant to optimise peak alkalosis and therefore, individuals should identify their respective alkalotic peak.

TTP HCO_3^- was achieved considerably earlier in the present study (<90 min), compared to previous work (>95 min) who adopted the same ingestion window (10 min) [36]. Both studies controlled nutritional intake and employed the same 4 hr post prandial strategy, however, as 10% of food is suggested to be present in the stomach even after a 4 hr fast [36], small contributions from meal volume, composition and texture may have produced equivocal time frames. It is more plausible however, the differences in $NaHCO_3$ administration (solution vs. capsule) between studies explains the discrepancies in TTP, due to the differential rapid emptying of liquids vs. the slower emptying of solids [10]. In support, TTP HCO_3^- has occurred earlier in other studies employing solution [5, 19, 24, 29, 31] compared to capsule $NaHCO_3$ administration [5, 31, 36]. In future, individuals should consider the time until competition/exercise and the palatability of $NaHCO_3$ in solution; or the high amount of capsules (~20) required within their respective ingestion strategies.

In some participants, the absolute HCO_3^- change lacked reproducibility (SBC3 n = 6; SBC2 n = 2), with differences >1 $mmol.L^{-1}$ observed (Table 2). Participant 1 for instance, elicited a 6.9 $mmol.L^{-1}$ change in HCO_3^- in SCB3a compared to a 5.6 $mmol.L^{-1}$ change in SBC3b. Additionally, there were two participants who failed to reproduce a similar TTP HCO_3^-, with over 15 mins difference in both SBC2 and SBC3 (Table 2). It is unclear why this was observed in our study considering participants replicated nutritional intake. Nonetheless, some individuals may require a test-retest to evaluate the reproducibility of the absolute change in HCO_3^-, which presents a logistical

limitation to the practitioner/athlete. Whether such discrepancies would translate to a lack of consistency in the performance response is unknown, however, research by McNaughton [16] has demonstrated that with HCO_3^- differences of around 1 $mmol.L^{-1}$ different performance responses occur. Future work should assess if discrepancies in either TTP or absolute change within such individuals effects performance responses.

For four of the participants, the absolute change in HCO_3^- following SBC2 was not enhanced further following SBC3. For instance, participant 1 displayed a minimal improvement of 0.1 $mmol.L^{-1}$ between SBC2 and SBC3. In comparison, participant 13 increased nearly two fold between SBC2 (+4.8 $mmol.L^{-1}$) and SBC3 (+8.8 $mmol.L^{-1}$). This suggests identification of the absolute HCO_3^- change between different doses of $NaHCO_3$ is required, as some fail to display any further increase in HCO_3^- from doses above 0.2 $g.kg^{-1}$ BM $NaHCO_3$. Meaning for those individuals who display small changes between $NaHCO_3$ doses, ingestion of >0.2 $g.kg^{-1}$ BM $NaHCO_3$ may not be warranted. This finding is of practical significance to individuals who suffer from GI upset from a 0.3 $g.kg^{-1}$ BM dose, considering the same acid-base response can be elicited from a smaller dose. Further research may wish to evaluate if both doses improve performance to a similar extent in individuals who respond this way.

This study reports HCO_3^- and pH between SBC2 and SBC3 were not significantly different up to 60 min, supporting previous findings [36]. This suggests that if a limited time is available prior to exercise (<60 min), it may be plausible for individuals to ingest a smaller dose. This may be of significance to individuals who participate in two bouts of exercise with a small amount of recovery (e.g. track and field athletes) or those who

371 suffer from GI upset, as lower doses have been shown to reduce the severity and
372 incidence of such occurrences [16].
373
374 Inconsistencies in pH reproducibility observed in this study could be explained by the
375 breadth of factors that affect pH, including contributions from intracellular buffering
376 such as carnosine, phosphocreatine and phosphates [9, 13]. Moreover, as ingestion
377 of a $NaHCO_3$ bolus will initially and directly increase HCO_3^- concentration, the effect
378 on pH is secondary and therefore may lead to increased variability [9]. A variability in
379 pH has also been observed in a recent study, even when HCO_3^- was similar [7]. For
380 instance, following $NaHCO_3$ ingestion, pH increased by 0.045 ± 0.029 in one treatment
381 compared to only 0.027 ± 0.054 in another. Conversely, in the same treatments, HCO_3^-
382 increased by 6.1 ± 2.3 and 5.9 ± 2.7 $mmol.L^{-1}$ respectively, but one of the limitations
383 in this study was that data were analysed on a group level, and only at two time points.
384 Alternatively, the effect on nutritional intake may have caused pH variability. It is well
385 known that the level of acid/alkaline (PRAL) within nutritional intake may affect the
386 acid base balance [27, 28]. Therefore, a limitation of this study is that only a 24 hr
387 nutrition log was completed. Further research may wish to investigate the effects of
388 PRAL and longitudinal nutritional practices on $NaHCO_3$ absorption characteristics.
389
390 The Na^+ response displayed a high intra-individual variability following $NaHCO_3$
391 (section 3.2; Figure 1). This study requested participants to replicate nutritional
392 practices prior to experiments and analysis revealed this was highly reproducible
393 (section 3.1), however not specifically Na^+ ingestion, therefore small changes in total
394 Na^+ ingested may explain these findings. Moreover, whilst the volume of water was
395 controlled for during experimental treatments, a limitation of this study is the frequency

396 of ingestion was not measured, which may have also effected Na^+ concentrations [21].
397 Nonetheless it is unclear whether small differences in total Na^+ ingested, or frequency
398 of water consumption would account for a meaningful change. An alternative factor,
399 although speculative, may be gastric emptying which has displayed intra-individual
400 variability in previous work [2, 22, 38]. In view of our analysis being focused on blood
401 Na^+, different quantities may/or may not have reached the bloodstream on the second
402 time of ingesting the same $NaHCO_3$ dose and consequently produced equivocal
403 responses.
404
405 It is proposed that disturbances to the acid base balance of the stomach, from high
406 Na^+ load accompanying $NaHCO_3$ ingestion, can cause the onset of GI upset [36].
407 Considering participants who suffered from GI upset in this study, TTP GI broadly
408 corresponded with peak Na^+ in SBC2 (peak GI = ~30 min, peak Na^+ = 41 min),
409 however not as strongly in SBC3 (peak GI = ~35 min, peak Na^+ = ~70min). The
410 absolute change in Na^+ was significantly higher in SBC3 compared to SBC2 (~2 vs.
411 ~6 $mmol.L^{-1}$), however the incidence and severity of GI upset was not significantly
412 different. Therefore, it is unclear if the magnitude of change in Na^+ is useful to predict
413 the onset of GI upset. Interestingly, the same severity from nausea in SBC2 and
414 diarrhoea in SBC3 was observed in participant 8 (Table 3), with this theme apparent
415 for seven participants in total. As such, these differences between doses will plausibly
416 effect the ability to perform exercise variably. It is therefore important to evaluate the
417 severity of the specific symptom suffered from GI upset and make judgement on the
418 cost:benefit of $NaHCO_3$ ingestion.
419
420 **5.0 Conclusion**

In summary, the blood analyte response following acute $NaHCO_3$ ingestion is highly reproducible. The practitioner/athlete should identify both the TTP and absolute change in HCO_3^- to determine both the time, and amount to ingest prior to usage in training or competition. Caution should be taken however with participants who displayed intra-individual variation in both TTP and absolute change in HCO_3^-, with these individuals potentially not suitable for $NaHCO_3$ ingestion. Future work should investigate why some participants fail to reproduce the blood analyte response from $NaHCO_3$ ingestion, including investigation into the role of PRAL and longitudinal nutritional practices. Lastly, based on both SBC2 and SBC3 eliciting a change in HCO_3^- that may improve performance, establishing the performance response utilising an individualised $NaHCO_3$ strategy is required.

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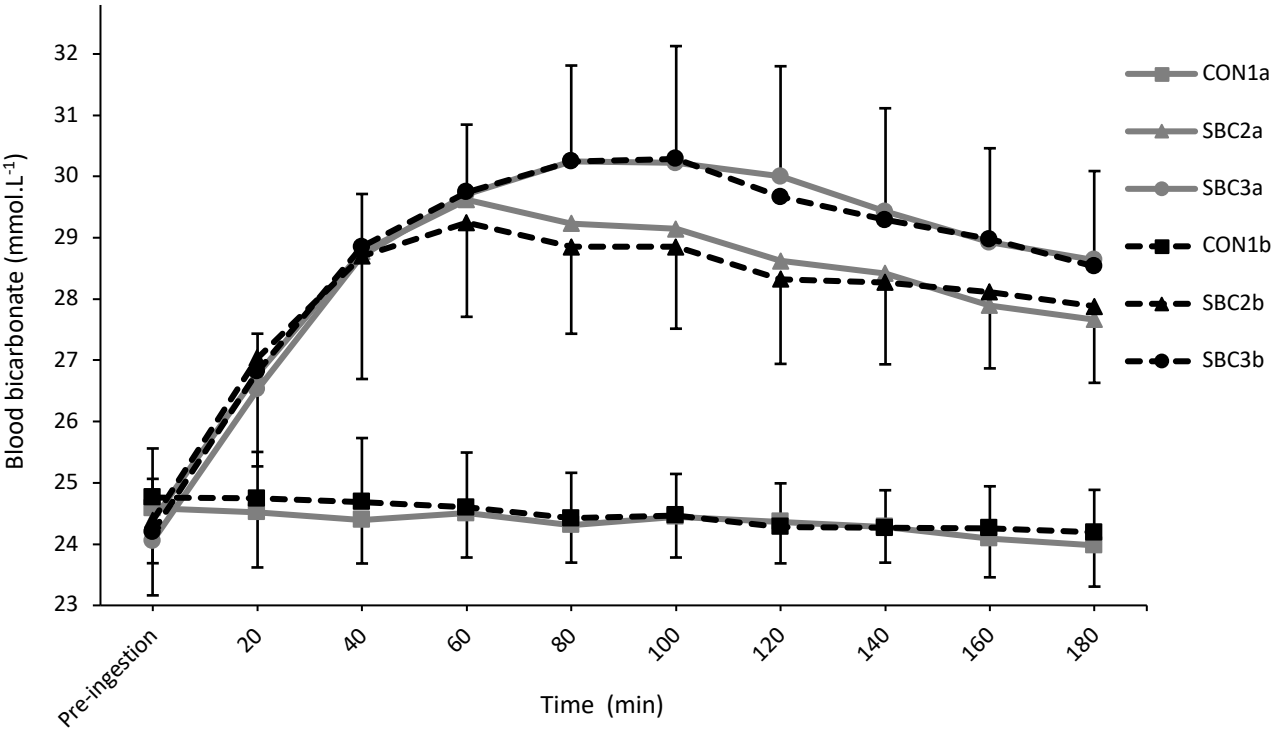
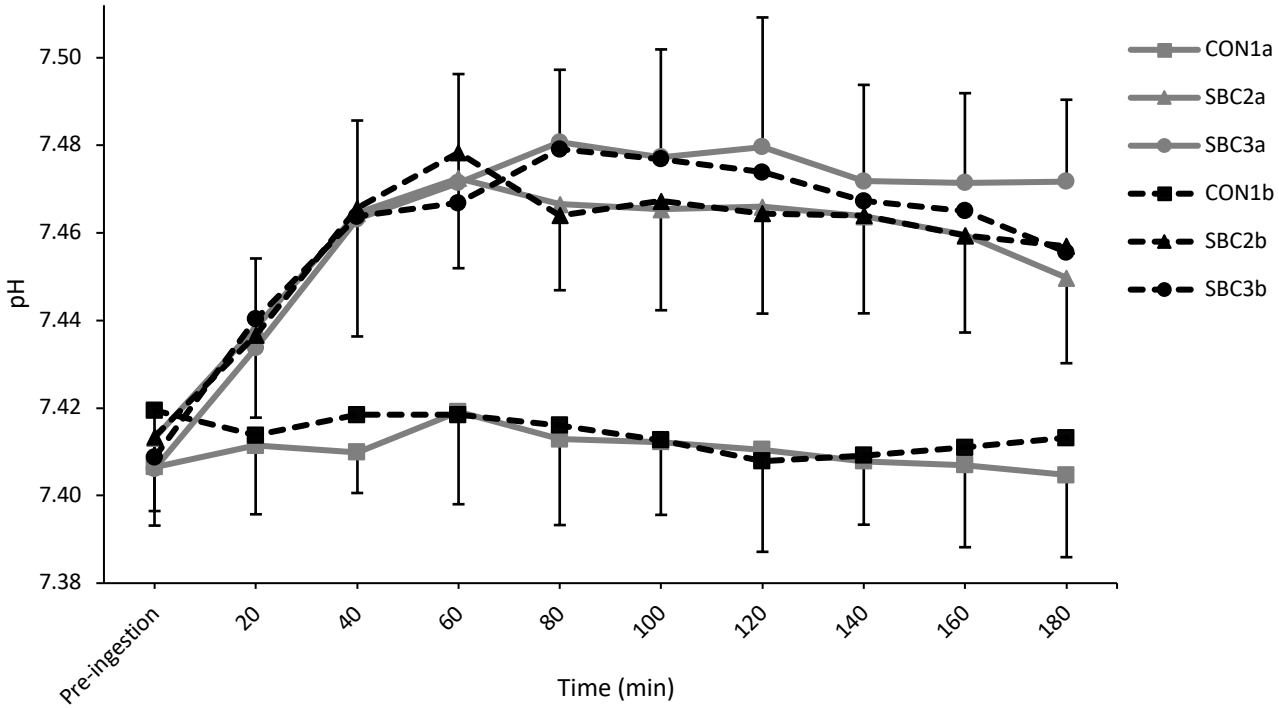
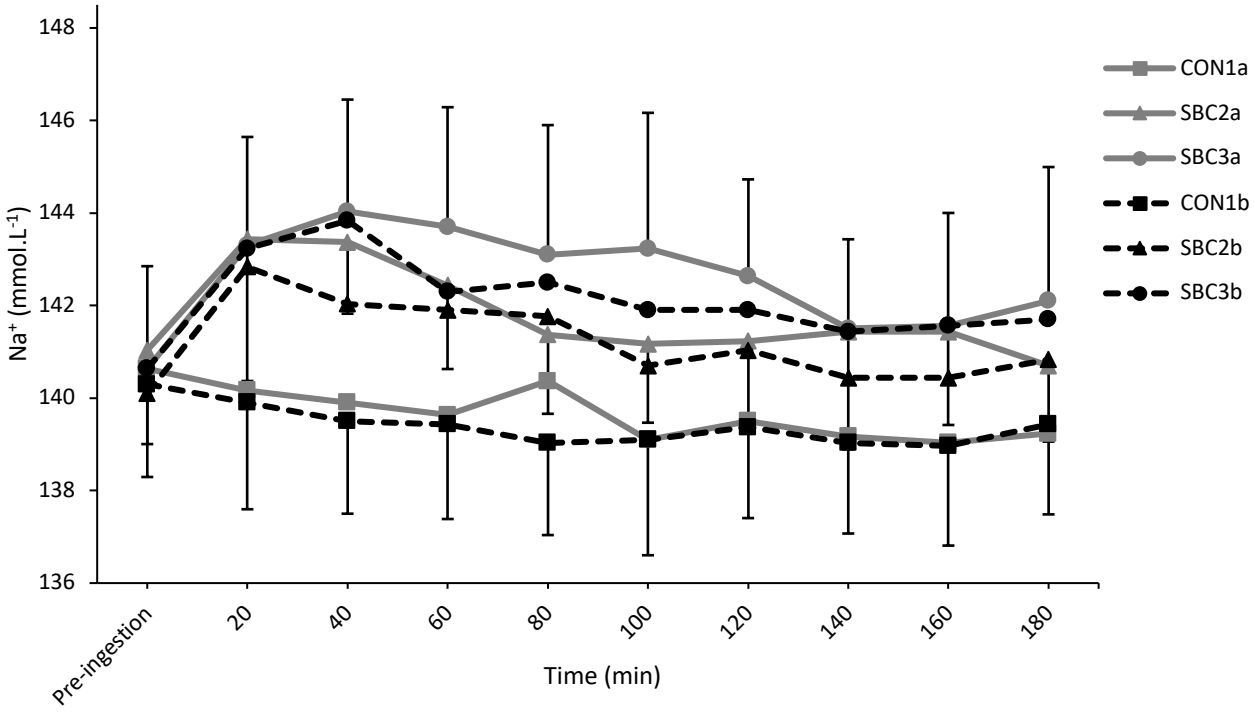
Figure Legends

Figure 1: Mean blood analyte responses for blood bicarbonate (HCO_3^-), pH and sodium (Na^+) following CON (solid square), SBC2 (solid triangle) and SBC3 (solid circle). Some error bars and time points (5 min interval samples) are omitted for clarity.


Blood bicarbonate (mmol.L-1)
32
31
30
29
28
27
26
25
24
23
Pre-ingestion
20
40
60
80
100
120
140
160
180
Time (min)
CON1a
SBC2a
SBC3a
CON1b
SBC2b
SBC3b


pH
7.50
7.48
7.46
7.44
7.42
7.40
7.38
Pre-ingestion
20
40
60
80
100
120
140
160
180
Time (min)
CON1a
SBC2a
SBC3a
CON1b
SBC2b
SBC3b


148
146
144
142
140
138
136
Na⁺ (mmol.L⁻¹)
Pre-ingestion
20
40
60
80
100
120
140
160
180
Time (min)
CON1a
SBC2a
SBC3a
CON1b
SBC2b
SBC3b

Tables

Table 1: Statistical summary table of limit of agreement analysis (LOA) and coefficient of variation (CV) of both blood pH and bicarbonate (HCO_3^-) following SBC2 and SBC3. Time points included cover the respective time taken to achieve peak (TTP) pH or HCO_3^-.

Table 2: Individual data displaying time to peak (TTP) (in mins) and absolute change (peak change from baseline) in both pH and blood bicarbonate (HCO_3^-) ($mmol.L^{-1}$) following SBC2a, SBC2b, SBC3a and SBC3b. CV = coefficient of variation, SEM = standard error of measure.

Table 3: The most severe individual symptom of GI upset suffered following SBC2a, SBC2b, SBC3a and SBC3b.

Table 1

A (pH)

SBC2												
Time Point	**40**	**60**	**80**	**85**	**90**	**95**	**100**	**120**	**125**	**130**	**135**	**140**
LOA												
Bias	-0.001	-0.007	0.001	0.004	-0.002	-0.001	-0.001	0.000	-0.008	-0.007	-0.004	0.001
SD	0.02	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.01
-	-0.04	-0.05	-0.02	-0.04	-0.03	-0.03	-0.03	-0.03	-0.05	-0.06	-0.03	-0.03
+	0.04	0.04	0.03	0.05	0.03	0.03	0.03	0.03	0.03	0.04	0.02	0.03
CV	0.4	0.3	0.3	0.2	0.2	0.3	0.3	0.30	0.3	0.3	0.4	0.3
Interpretation	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent

SBC3												
Time Point	**40**	**60**	**80**	**85**	**90**	**95**	**100**	**120**	**125**	**130**	**135**	**140**
LOA												
Bias	-0.001	0.005	0.003	0.002	0.007	-0.002	0.002	0.006	0.005	0.001	0.005	0.005
SD	0.01	0.02	0.03	0.02	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02
-	-0.03	-0.02	-0.05	-0.03	-0.02	-0.04	-0.04	-0.03	-0.03	-0.03	-0.03	-0.03
+	0.03	0.04	0.06	0.03	0.03	0.04	0.04	0.04	0.04	0.03	0.04	0.04
CV	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3
Interpretation	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent	Excellent

B (HCO_3^-)

SBC2									
Time Point	**40**	**60**	**80**	**85**	**90**	**95**	**100**	**120**	**125**
LOA									
Bias	0.1	0.4	0.4	0.3	0.5	0.2	0.3	0.3	0.0
SD	1.4	1.2	1.1	1.2	1.1	1.2	1.0	1.1	0.9
-	-2.7	-2.0	-1.9	-2.0	-1.7	-2.1	-1.7	-1.8	-1.8
+	2.8	2.7	2.6	2.6	2.7	2.5	2.2	2.4	1.8
CV	6.2	5.4	5.2	4.2	4.6	5.1	4.5	4.8	4.6
Interpretation	Good	Good	Good	Excellent	Excellent	Good	Excellent	Excellent	Excellent

SBC3									
Time Point	**40**	**60**	**80**	**85**	**90**	**95**	**100**	**120**	**125**
LOA									
Bias	-0.1	0.0	0.0	0.1	0.0	0.1	-0.1	0.3	0.3
SD	1.0	1.1	1.2	1.2	1.2	1.2	1.2	1.5	1.1
-	-2.2	-2.3	-2.4	-2.3	-2.4	-2.3	-2.4	-2.6	-1.7
+	1.9	2.2	2.4	2.4	2.4	2.4	2.2	3.2	2.4
CV	3.6	3.8	4.6	4.7	5.1	5.5	5.5	5.6	4.7
Interpretation	Excellent	Excellent	Excellent	Excellent	Good	Good	Good	Good	Excellent

* LOA = limits of agreement, SD = standard deviation, + = upper bound, - = lower bound. CV = coefficient of Variation.

Table 2

pH (TTP)				HCO_3^- (TTP)				pH (Abs. Δ)				HCO_3^- (Abs. Δ)			
P.no	**SBC2a**	**SBC2b**	**SBC3a**	**SBC3b**	**SBC2a**	**SBC2b**	**SBC3a**	**SBC3b**	**SBC2a**	**SBC2b**	**SBC3a**	**SBC3b**	**SBC2a**	**SBC2b**	**SBC3a**	**SBC3b**
1	80	85	125	95	80	85	125	100	0.08	0.05	0.08	0.07	6.8	5.6	6.9	5.6
2	85	120	80	85	85	80	80	80	0.03	0.06	0.07	0.08	5	4.8	6	5.4
3	80	40	125	100	60	60	90	90	0.07	0.08	0.13	0.08	6	7.2	6.5	6.3
4	40	40	60	60	60	60	95	95	0.07	0.07	0.14	0.13	4.8	4.9	7.9	8
5	60	60	90	90	60	60	85	85	0.06	0.10	0.14	0.07	7.1	7.2	9.3	7.1
6	60	125	80	140	80	125	100	120	0.10	0.12	0.09	0.09	7.1	7.3	8.3	8.4
7	140	135	130	130	85	85	60	60	0.10	0.10	0.08	0.07	5.3	5	6.5	6.6
8	100	130	100	90	85	95	100	90	0.11	0.10	0.10	0.09	7.2	7.2	7.5	9.3
9	40	60	100	100	60	85	95	95	0.11	0.14	0.12	0.12	5.2	5.4	7.3	7
10	40	130	80	80	95	85	80	80	0.06	0.06	0.08	0.10	5.2	5	6.2	6.2
11	120	135	135	120	85	85	120	120	0.10	0.10	0.10	0.09	4.8	4.3	4.9	6.1
12	60	40	90	100	60	40	40	40	0.05	0.04	0.05	0.08	5	4.9	5.9	6.2
13	140	95	125	120	100	125	95	80	0.07	0.06	0.10	0.10	4.8	4.6	8.8	7.7
14	95	100	120	95	85	95	85	80	0.07	0.07	0.10	0.11	5.4	4.7	6.6	8.1
15	130	85	90	90	80	85	90	90	0.07	0.10	0.09	0.10	6.1	6.1	7.8	7.6
Mean	85	92	102	100	77	83	89	87	0.08	0.08	0.10	0.09	5.7	5.6	7.1	7.0
SD	35	37	23	20	14	23	21	20	0.02	0.03	0.02	0.02	0.9	1.1	1.2	1.1
CV	40.5	38.5	21.8	19.9	17.2	26.4	22.5	22.3	29.1	31.4	25.2	23.2	15.6	17.7	13.6	17.0
SEM	9.2	9.5	6.0	5.3	3.6	5.9	5.4	5.2	0.01	0.01	0.01	0.00	0.2	0.3	0.3	0.3

* TTP = time to peak, CV = coefficient of variation, SEM = standard error of mean.

Table 3

P.no	SBC2a	SBC2b	SBC3a	SBC3b
1	None	None	None	None
2	Flatulence	None	None	None
3	Flatulence	None	Bowel Urgency	Bowel Urgency
4	Stomach Cramp	Belching	Belching	Stomach Ache
5	None	None	None	None
6	None	None	None	None
7	Stomach Bloating	Stomach Cramp	Bowel Urgency	Stomach Ache
8	Stomach ache	Nausea	Stomach cramp	Diarrhoea
9	Bowel urgency	Bowel urgency	None	Stomach bloating
10	Stomach Bloating	Stomach Bloating	Stomach Ache	Stomach Ache
11	Diarrhoea	Diarrhoea	Diarrhoea	Diarrhoea
12	None	None	Bowel Urgency	None
13	Nausea	Nausea	Nausea	Nausea
14	None	None	None	None
15	None	None	None	None