UNIVERSITY OF
FORWARD THINKING
WESTMINSTER⊞

WestminsterResearch

http://www.westminster.ac.uk/westminsterresearch

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

Gough, L., Deb, S., Sparks, S.A. and McNaughton, L.

---

1 **Title:** The reproducibility of blood acid base responses in male collegiate athletes

2 following individualised doses of $NaHCO_3$: a randomised controlled crossover study.

3 **Authors:** [1]L. A. Gough., [1]S. K. Deb., [1]S. A. Sparks and [1]L. R. McNaughton

4 [1]Department of Sport and Physical Activity, Edge Hill University, Ormskirk, United

5 Kingdom, L39 4QP

6 **Corresponding author:** L. A. Gough, Edge Hill University, Ormskirk, Lancashire,

7 L39 4QP, Tel: 01695 657214, Email: goughl@edgehill.ac.uk

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

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).

3

1.0 Introduction

76

77 Research investigating nutritional ergogenic aid strategies that delay the occurrence

78 of metabolic acidosis during high intensity exercise have been widely investigated [4,

79 16, 41]. In particular, exogenous enhancement of the bicarbonate buffering systems

80 is thought to have an important role in offsetting the metabolite fatigue process, by

81 dampening critical rises in hydrogen cations (H^+) [17]. Ingestion of a known alkalotic

82 buffer, namely sodium bicarbonate ($NaHCO_3$), can achieve such ergogenic effects

83 through increasing blood bicarbonate [HCO_3^-] concentration within the extracellular

84 fluid of between 4-8 $mmol.L^{-1}$ [36], which typically relates to the point of peak alkalosis

85 [34]. Most common ingestion practices include doses of between 0.2 and 0.3 $g.kg^{-1}$

86 BM $NaHCO_3$, as amounts lower than this are not considered sufficient to induce a level

87 of peak alkalosis to improve performance [36]. Doses above this concentration

88 exacerbate the incidence and severity of gastrointestinal (GI) discomfort [16].

89

90 Multiple studies using group mean data have reported a high variation in time to peak

91 (TTP) alkalosis (i.e. HCO_3^-) following various doses of $NaHCO_3$ [6, 29, 33, 34]. Peak

92 HCO_3^- has previously been observed at 40 min and 60 min following 0.2 $g.kg^{-1}$ BM

93 and 0.3 $g.kg^{-1}$ BM $NaHCO_3$ respectively [33], whereas others have reported 90 [29],

94 120 [6] and 180 min [34]. Differences may be evident either as a result of sampling

95 range (20-60 min), or inter-individual variation within participants, since individual

96 absorption characteristics of blood pH and HCO_3^- have potentially been overlooked in

97 previous studies [6, 29, 33, 34]. Consequently this generic approach has led to a

98 potential reduction in the ergogenic effect on exercise performance, or caused

99 variation in performance benefits [7, 31]. More specifically, Dias et al [7] reported a

100 lack of consistency in performance response following $NaHCO_3$ during a 110% peak

101　power output cycling time to exhaustion (TTE). Fifteen recreationally active

102　participants consumed 0.3 g.kg^{-1} BM NaHCO$_3$ on four occasions, or a placebo on two

103　occasions. Only one participant produced ergogenic effects in all NaHCO$_3$ treatments,

104　with five failing to improve in any treatment. This suggests some degree of intra-

105　individual variation is evidence, which may be as a result of intra-individual blood

106　responses, although this is difficult to define as only group mean blood responses were

107　reported.

108

109　A contemporary approach involves individualising the ingestion strategy, and

110　Stannard et al. [36] reported TTP HCO$_3^-$ displayed a large inter-individual variation

111　(0.2 g.kg^{-1} BM = 40-165 min, 0.3 g.kg^{-1} BM = 75-180 min). These findings challenge

112　the aforementioned studies who reported group level analysis following NaHCO$_3$

113　supplementation at a fixed time frame [17, 31, 33]. Furthermore, variations in TTP

114　arguably provides insight to the commonly reported inter and intra-individual variations

115　following NaHCO$_3$ ingestion on performance [7, 31], as participants may not have

116　elicited peak alkalosis at the commencement of exercise [17]. Recent work by Miller

117　et al. [19] supports this claim, demonstrating during repeated sprint cycling (10 x 6 s)

118　total work done (TWD) improved by 11% with an individualised ingestion strategy, a

119　response greater than the 5% change in a similar study employing a standardised

120　ingestion strategy [3].

121

122　Further research to identify individualised NaHCO$_3$ ergogenic strategies that elicit

123　peak alkalosis are necessary. Equally for practical application in the field, a greater

124　understanding of the reproducibility of blood analytes (pH and HCO$_3^-$) following acute

125　NaHCO$_3$ is required. Daily biological variation, either short term or long term, may

126   occur in response to changes in nutritional practices and therefore effect daily acid

127   load fluxes (potential renal acid load; PRAL) [23, 27, 28] with the potential to affect the

128   reproducibility of TTP alkalosis. As a result, this may negatively affect the efficacy of

129   employing an individualised $NaHCO_3$ ingestion strategy to improve exercise

130   performance consistently. Therefore, the aim of this study was to assess the

131   reproducibility of the individual blood pH, HCO_3^- and Na^+ response following acute

132    $NaHCO_3$ ingestion in both 0.2 and 0.3 $g.kg^{-1}$ BM doses.

133

134   **2.0 Materials and Methods**

135   *2.1 Participants*

136   Participants were recruited on the basis they may gain a performance benefit from

137   enhancing their buffering capacity (McNaughton et al., 2016). As a result, sixteen team

138   and individual sports participants with backgrounds in rugby, football and running

139   volunteered for this single blind, randomised, crossover designed study. One

140   participant withdrew from the study due to GI upset (vomiting) from $NaHCO_3$ ($0.3g.kg^{-1}$

141   ^{1} BM dose; first session), therefore 15 male participants (n=5 rugby, n=7 football, n=3

142   sprinting) completed the study (height 1.81 ± 0.06 m, body mass 84 ± 8 kg, age 21 ±

143   2 years, VO_{2MAX} 52.1 ± 2.2 $ml.kg^{-1}.min^{-1}$). Participants habitually completed four

144   exercise bouts per week (4 ± 1 $p.wk^{-1}$), lasting two hours per session (2 ± 0 hr) and

145   had ten years training experience (10 ± 3 years) within their respective sports. Ethical

146   approval was obtained from Departmental Research Ethics Committee (SPA-REC-

147   2015-325) and each participant provided written informed consent and completed a

148   health screening procedure prior to data collection. The research was conducted in

149   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

174 (RER). Data was averaged over the last thirty seconds of exercise to determine the

175 VO_{2MAX}.

176

177 *2.4 Main treatment arms*

178 Administered in a block randomised method, the subsequent six treatments involved

179 two treatment arms of no treatment (CON1a, CON1b) to assess daily variation of blood

180 analytes, two treatment arms requiring ingestion of 0.2 $g.kg^{-1}$ (SBC2a, SBC2b), and

181 two with 0.3 $g.kg^{-1}$ BM $NaHCO_3$ (SBC3a, SBC3b). Solutions were mixed by a

182 laboratory technician not involved with the research by mixing 400 ml of water with 50

183 ml of flavoured sugar free squash and placed within a refrigerator to enhance

184 palatability [19]. Treatments were administered single blind and participants ingested

185 within 10 min for all treatments [36].

186

187 An arterialised finger prick capillary blood sample was obtained from the finger whilst

188 in a rested and seated state, prior to $NaHCO_3$ ingestion. Arterialisation was achieved

189 by warming the hand with a heated blanket (45°C) for 5 min prior to each individual

190 sample [12]. After $NaHCO_3$ ingestion, a further 15 blood samples were obtained over

191 a 180 min period in each treatment (Table 1). At multiple time points, a GI

192 questionnaire (VAS scale; 0 = *no instance*, 10 = *most severe*) was completed as per

193 previous research within a range of symptoms [19] (Table 3). Participants remained

194 seated throughout, with only toilet breaks permitted. No food was allowed to be

195 consumed during this period, and water was consumed *ab libitum*, with total volume

196 replicated in subsequent treatment arms. Blood samples were collected in 100 µl

197 heparin-coated clinitubes (Radiometer Medical Ltd, Denmark) and subsequently

198 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

224 Limits of agreement (LOA) with 95% percent limits and Bland-Altman plots were

225 utilised for within-subject variance and to determine if data was heteroscedastic (Bland

226 and Altman, 1986). This method is widely used [20, 35] and accounts for bias between

227 the mean differences [8]. Intraclass correlation coefficient (ICC) were displayed with *r*

228 value and significance level, as per previous recommendations [1]. Coefficient of

229 variation (CV) is reported using SD/mean*100. Correlation between HCO_3^- and pH

230 TTP was calculated using Pearson correlation, from Hopkins spreadsheet [11].

231 Statistical procedures were completed using SPSS version 22 (IBM, Chicago, USA)

232 and calculations were carried out using Microsoft Excel 2013 (Microsoft Inc., USA).

233

234 **3.0 Results**

235 *3.1 Nutritional intake*

236 Total daily calorie intake was highly reproducible for all treatments (*r* = 0.78, P <0.001;

237 Mean ± SD = 2283 ± 75), as was carbohydrate (*r* = 0.97 P <0.001; 253 ± 4 g), protein

238 (*r* = 0.98, P <0.001; 85 ± 2 g) and fat (*r* = 0.97, P <0.001; 126 ± 3 g) intake.

239

240 *3.2 Gastrointestinal upset*

241 Both the severity, and TTP GI displayed excellent reproducibility in SBC2 and SBC3

242 (severity SBC2 *r* = 0.92, P <0.001; LOA: B -0.5, -3.1, +2.2; TTP SBC2 *r* = 0.91, P

243 <0.001; LOA: B 5, -38, +47 vs. severity SBC3 *r* = 0.90, P <0.001; LOA: B -0.4, -4.7,

244 +3.8; TTP SBC3 *r* = 0.78, P = 0.005; LOA: B 7, -64, 77). In total 8/15 of the participants

245 reported symptoms of GI in both SBC2 and SBC3, and the specific symptoms are

246 depicted in Table 3. The severity of GI was decreased in SBC2 compared to SBC3

247 (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

272   Absolute change (peak change from baseline) for HCO_3^- displayed high reproducibility

273   for SBC2 compared to SBC3 (SBC2 ICC: r = 0.90, P <0.001; LOA: B 0.1, -0.9, +1.1

274   vs. SBC3 ICC: r = 0.76, P = 0.008; LOA: B 0.1, -1.9, +2.0). The absolute change in pH

275   was highly reproducible in SBC2 compared to SBC3 (SBC2 ICC: r = 0.84, P = 0.001;

276   LOA: B -0.1, -0.04, +0.03 vs. SBC3 ICC: r = 0.62, P = 0.041; LOA: B 0.01, -0.04,

277   +0.05). In contrast, the absolute change in Na^+ displayed no relationship in both SBC2

278   (ICC: r = 0.10, P = 0.562; LOA: B 0.1, -4.9, +5.1) or SBC3 (ICC: r = 0.10, P = 0.425;

279   LOA: B 1.3, -6.2, +8.7).

280

281   *3.3 Differences between treatments*

282   TTP HCO_3^- was not significantly different between SBC2 and SBC3 (all P >0.05)

283   (Table 2). Whereas, TTP pH occurred significantly later in SBC3a compared to SBC2a

284   (+17 min; P <0.026), however non-significantly later in SBC3b compared to SBC2b

285   (+8 min; P = 0.392) (Table 2). TTP Na^+ occurred significantly later in SBC3a compared

286   to SBC2a (+32 min; P = 0.027) and 25 min later for SBC3b compared to SBC2b (P =

287   0.061). A large inter-individual variation in TTP pH, HCO_3^- and Na^+ in both SBC

288   treatments was observed (Table 2).

289

290   The absolute change in blood analytes HCO_3^- and pH can be observed in Table 2.

291   Absolute change in HCO_3^- was greater in SBC3 compared to SBC2 (P <0.001; Table

292   2). Absolute pH change was significantly greater for SBC3a compared to SBC2a (+0.2;

293   P = 0.018), however not in SBC2b and SBC3b (+0.1; P = 0.242). Absolute change in

294   Na^+ was significantly greater in SBC3 compared to SBC2 (P >0.05; Figure 1). A large

295   inter-individual variation in absolute change of pH, HCO_3^- and Na^+ in both SBC2 and

296   SBC3 was observed (Table 2). Lastly, up to 60 min post $NaHCO_3$ ingestion both HCO_3^-

12

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

13

321   relevant to optimise peak alkalosis and therefore, individuals should identify their

322   respective alkalotic peak.

323

324   TTP HCO_3^- was achieved considerably earlier in the present study (<90 min),

325   compared to previous work (>95 min) who adopted the same ingestion window (10

326   min) [36]. Both studies controlled nutritional intake and employed the same 4 hr post

327   prandial strategy, however, as 10% of food is suggested to be present in the stomach

328   even after a 4 hr fast [36], small contributions from meal volume, composition and

329   texture may have produced equivocal time frames. It is more plausible however, the

330   differences in $NaHCO_3$ administration (solution vs. capsule) between studies explains

331   the discrepancies in TTP, due to the differential rapid emptying of liquids vs. the slower

332   emptying of solids [10]. In support, TTP HCO_3^- has occurred earlier in other studies

333   employing solution [5, 19, 24, 29, 31] compared to capsule $NaHCO_3$ administration [5,

334   31, 36]. In future, individuals should consider the time until competition/exercise and

335   the palatability of $NaHCO_3$ in solution; or the high amount of capsules (~20) required

336   within their respective ingestion strategies.

337

338   In some participants, the absolute HCO_3^- change lacked reproducibility (SBC3 n = 6;

339   SBC2 n = 2), with differences >1 $mmol.L^{-1}$ observed (Table 2). Participant 1 for

340   instance, elicited a 6.9 $mmol.L^{-1}$ change in HCO_3^- in SCB3a compared to a 5.6 $mmol.L^{-1}$

341   change in SBC3b. Additionally, there were two participants who failed to reproduce

342   a similar TTP HCO_3^-, with over 15 mins difference in both SBC2 and SBC3 (Table 2).

343   It is unclear why this was observed in our study considering participants replicated

344   nutritional intake. Nonetheless, some individuals may require a test-retest to evaluate

345   the reproducibility of the absolute change in HCO_3^-, which presents a logistical

14

346 limitation to the practitioner/athlete. Whether such discrepancies would translate to a

347 lack of consistency in the performance response is unknown, however, research by

348 McNaughton [16] has demonstrated that with HCO_3^- differences of around 1 mmol.L^{-1}

349 different performance responses occur. Future work should assess if discrepancies

350 in either TTP or absolute change within such individuals effects performance

351 responses.

352

353 For four of the participants, the absolute change in HCO_3^- following SBC2 was not

354 enhanced further following SBC3. For instance, participant 1 displayed a minimal

355 improvement of 0.1 mmol.L^{-1} between SBC2 and SBC3. In comparison, participant 13

356 increased nearly two fold between SBC2 (+4.8 mmol.L^{-1}) and SBC3 (+8.8 mmol.L^{-1}).

357 This suggests identification of the absolute HCO_3^- change between different doses of

358 $NaHCO_3$ is required, as some fail to display any further increase in HCO_3^- from doses

359 above 0.2 g.kg^{-1} BM $NaHCO_3$. Meaning for those individuals who display small

360 changes between $NaHCO_3$ doses, ingestion of >0.2 g.kg^{-1} BM $NaHCO_3$ may not be

361 warranted. This finding is of practical significance to individuals who suffer from GI

362 upset from a 0.3 g.kg^{-1} BM dose, considering the same acid-base response can be

363 elicited from a smaller dose. Further research may wish to evaluate if both doses

364 improve performance to a similar extent in individuals who respond this way.

365

366 This study reports HCO_3^- and pH between SBC2 and SBC3 were not significantly

367 different up to 60 min, supporting previous findings [36]. This suggests that if a limited

368 time is available prior to exercise (<60 min), it may be plausible for individuals to ingest

369 a smaller dose. This may be of significance to individuals who participate in two bouts

370 of exercise with a small amount of recovery (e.g. track and field athletes) or those who

15

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**

421   In summary, the blood analyte response following acute $NaHCO_3$ ingestion is highly

422   reproducible. The practitioner/athlete should identify both the TTP and absolute

423   change in HCO_3^- to determine both the time, and amount to ingest prior to usage in

424   training or competition. Caution should be taken however with participants who

425   displayed intra-individual variation in both TTP and absolute change in HCO_3^-, with

426   these individuals potentially not suitable for $NaHCO_3$ ingestion. Future work should

427   investigate why some participants fail to reproduce the blood analyte response from

428   $NaHCO_3$ ingestion, including investigation into the role of PRAL and longitudinal

429   nutritional practices. Lastly, based on both SBC2 and SBC3 eliciting a change in

430   HCO_3^- that may improve performance, establishing the performance response utilising

431   an individualised $NaHCO_3$ strategy is required.

References

1. Atkinson G, Nevill AM. Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. Sports Med. 1998; 26: 217-38.

2. Barnett C, Snel A, Omari T, Davidson G, Haslam R, Butler R. Reproducibility of the 13C-octanoic acid breath test for assessment of gastric emptying in healthy preterm infants. J Pediar Gastroenterol Nutr. 1999; 29: 26-30.

3. Bishop D, Edge J, Davis C, Goodman C. Induced metabolic alkalosis affects muscle metabolism and repeated-sprint ability. Med Sci Sports Exerc. 2004; 36: 807-13.

4. Cairns SP. Lactic acid and exercise performance: culprit of friend? Sports Med. 2006; 36: 279-91.

5. Carr AJ, Hopkins WG, Gore CJ. Effects of acute alkalosis and acidosis on performance: a meta-analysis. Sports Med. 2011a; 41: 801-14.

6. Carr AJ, Slater GJ, Gore CJ, Dawson B, Burke LM. Effect of sodium bicarbonate on [HCO3-], pH, and gastrointestinal symptoms. Int J Sport Nutr Exerc Metab. 2011b; 21: 189-94.

7. Froio de Araujo Dias G, da Eira Silva V, de Salles Painelli V, Sale C, Giannini Artioli G, Gualano B, Saunders B. (In)Consistencies in responses to sodium bicarbonate supplementation: a randomised, repeated measures, counterbalanced and double blind study. Plos One. 2015; 10: 1-13.

8. Giavarina D. Understanding bland altman analysis. Biochem Med (Zagreb). 2015; 25: 141-151.

9. Goel N, Calvert J. Understanding blood gases/acid-base balance. Paediatr Child Health. 2011; 22: 142-148.

10. Hellström PM, Grybäck P, Jacobsson H. The physiology of gastric emptying. Best Pract Res Clin Anaesthesiol. 2006; 20: 397-407.

11. Hopkins WG. Measures of reliability in sports medicine and science. Sports Med. 2000; 30: 1-15.

12. Johnston KR, Vickers MD, Mapleson WW. Comparison of arterialized venous with arterial blood propofol concentrations during sub-anaesthetic infusions in volunteers. Br J Anaesth. 1996; 76: 401-4.

13. MacLaren D, Morton J. Biochemistry for sport and exercise science. Champaign, IL: Human Kinetics, 2012.

14. Maughan RJ, King DS, Lea T. Dietary supplements. J Sports Sci. 2004; 22: 95-113.

15. Matson LG, Tran ZV. Effects of sodium bicarbonate ingestion on anaerobic performance: a meta-analytic review. Int J Sport Nutr. 1993; 3: 2-28.

16. McNaughton LR. Bicarbonate ingestion: effects of dosage on 60 s cycle ergometry. Journal of Sports Sciences. 1992; 10: 415–23.

17. McNaughton LR, Gough L, Deb S, Bentley D, Sparks SA. Recent developments in the use of sodium bicarbonate as an ergogenic aid. Curr Sports Med Rep. 2016; 15: 233-244, 2016.

18. McNaughton LR, Siegler J, Midgley A. Ergogenic effects of sodium bicarbonate. Curr Sports Med Rep. 2008; 7: 230-6.

19. Miller P, Robinson AL, Sparks SA, Bridge CA, Bentley DJ, McNaughton LR. The effects of novel ingestion of sodium bicarbonate on repeated sprint ability. J Strength Cond Res. 2016; 30: 561-8.

20. Myles PS, Cui J. Using the bland-altman method to measure agreement with repeated measures. Br J Anaesth. 2007; 99: 309-11.

21. Nose H, Sugimoto E, Okuno T, Morimoto T. Changes in blood volume and plasma sodium concentration after water intake in rats. Am J Physiol. 1987; 253: 15-9.

22. Paintaud G, Thibault P, Queneau PE, Magnette J, Berard M, Rumbach L, Bechtel PR, Carayon P. Intraindividual variability of paracetamol absorption absorption kinetics after a semisolid meal in healthy. European Journal of Clinical Pharmacology. 1998; 53: 355-59.

23. Poupin N, Calvez J, Lassale C, Chesneau C, Tomé D. Impact of the diet on net endogenous acid production and acid-base balance. Clin Nutr. *2012;* 31: 313-21.

24. Price MJ, Singh M. Time course of blood bicarbonate and pH three hours after sodium bicarbonate ingestion. Int J Sports Physiol Perform. 2008; 3: 240-2.

25. Radiometer Medical. 2015. *ABL 800 Reference Manual* [online]. Available from: www.radiometer.com [Accessed 2015].

26. Reilly T. Human circadian rhythms and exercise. Crit Rev Biomed Eng. 1990; 18: 165-80.

27. Remer T. 2001. Influence of nutrition on acid-base balance – metabolic aspects. Eur J Nutr. 2001; 40: 214.

28. Remer T, Manz F. Potential renal acid load and its influence on urine pH. J am Diet Assoc. 1995; 95: 791-7.

29. Renfree A. The time course of changes in plasma [H+] after sodium bicarbonate ingestion. Int J Sports Physiol Perform. 2007; 2: 323-6.

30. Rosenberg K, Durnin JV. The effect of alcohol on resting metabolic rate. Br J Nutr. 1978; 40: 293-8.

31. Saunders B, Sale C, Harris RC, Sunderland C. Sodium bicarbonate and high-intensity-cycling capacity: variability in responses. Int J Sports Physiol Perform. 2014; 9: 627-32.

32. Schwalfenberg GK. The alkaline diet: is there evidence that an alkaline pH diet benefits health? J Environ Public Health. 2012.

33. Siegler JC, Marshall PW, Bray J, Towlson C. Sodium bicarbonate supplementation and ingestion timing: does it matter? J Strength Cond Res. 2012; 26: 1953-8.

34. Siegler JC, Midgley AW, Polman RC, Lever R. Effects of various sodium bicarbonate loading protocols on the time-dependent extracellular buffering profile. J Strength Cond Res. 2010; 24: 2551-7.

35. Sparks SA, Close GL. Validity of a portable urine refractometer: the effects of sample freezing. J Sports Sci. 2013; 31: 745-9.

36. Stannard RL, Stellingwerff T, Artioli GG, Saunders B, Cooper S, Sale C. Dose-response of sodium bicarbonate ingestion highlights individuality in time course of blood analyte responses. Int J Sport Nutr Exerc Metab. [Epub ahead of print]

37. Stadlbauer V, Wallner S, Stojakovic T, Smolle KH. Comparison of 3 different multianalyte point-of-care devices during clinical routine on a medical intensive care unit. J Crit Care. 2011; 26: 433.

38. Tougas G, Eaker EY, Abell TL, Abrahamsson H, Boivin M, Chen J, Hocking MP, Quigley EM, Koch KL, Tokayer AZ, Stanghellini V, Chen Y, Huizinga JD, Ryden J, Bourgeois L, McCallum RW. Assessment of gastric emptying using a low fat meal: establishment of international control values. Am J Gastroenterol. 2000; 95: 1456-62.

39. Urwin C, Dwyer D, Carr A. Induced alkalosis and gastrointestinal symptoms after sodium citrate ingestion: a dose response investigation. Int J Sport Nutr Exerc Metab. [Epub ahead of print]

40. Vincent W, Weir J. *Statistics in Kinesiology.* 4th ed. Champaign, IL: Human Kinetics, 2012.

41. Westerblad H, Allen DG, Lännergren J. Muscle fatigue: lactic acid or inorganic phosphate the major cause? News Physiol Sci. 2002; 17: 17-21.

42. Westerterp-Platenga M, Diepvens K, Joosen AM, Bérubé-Parent S, Tremblay A. Metabolic effects of spices, teas and caffeine. Physiol Behav 2006; 89: 85-91.

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.
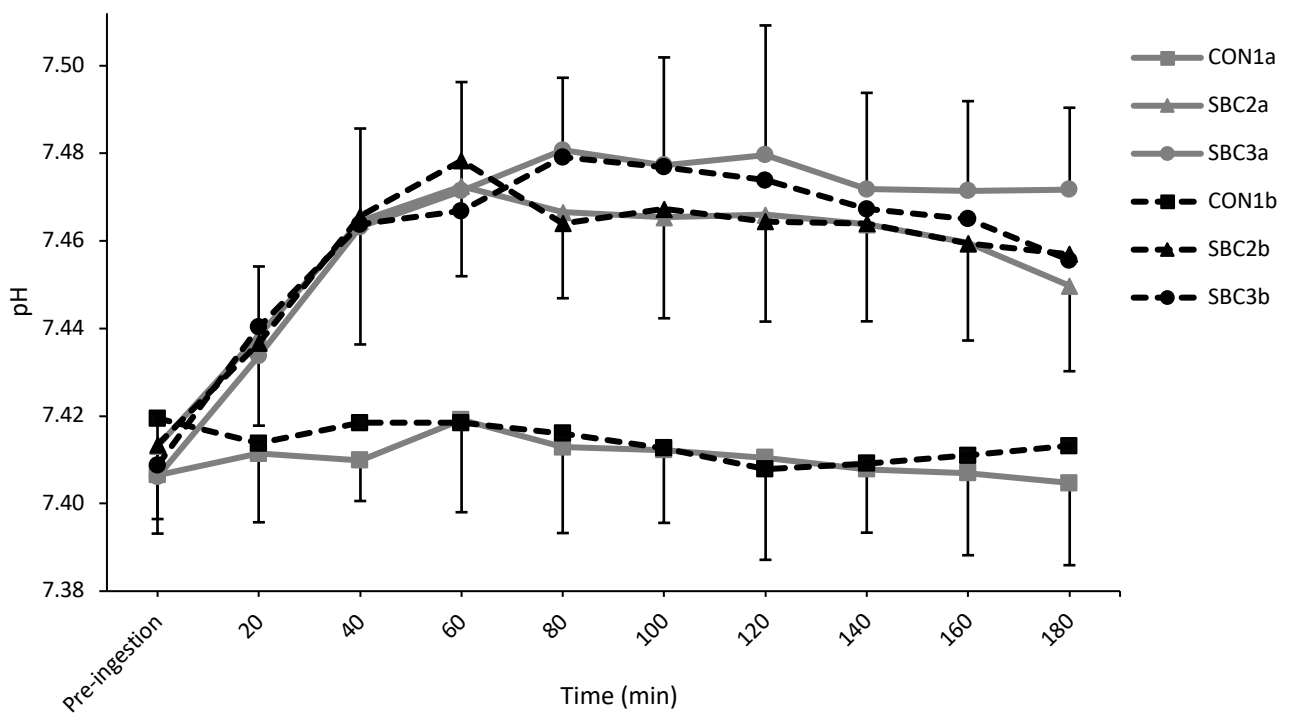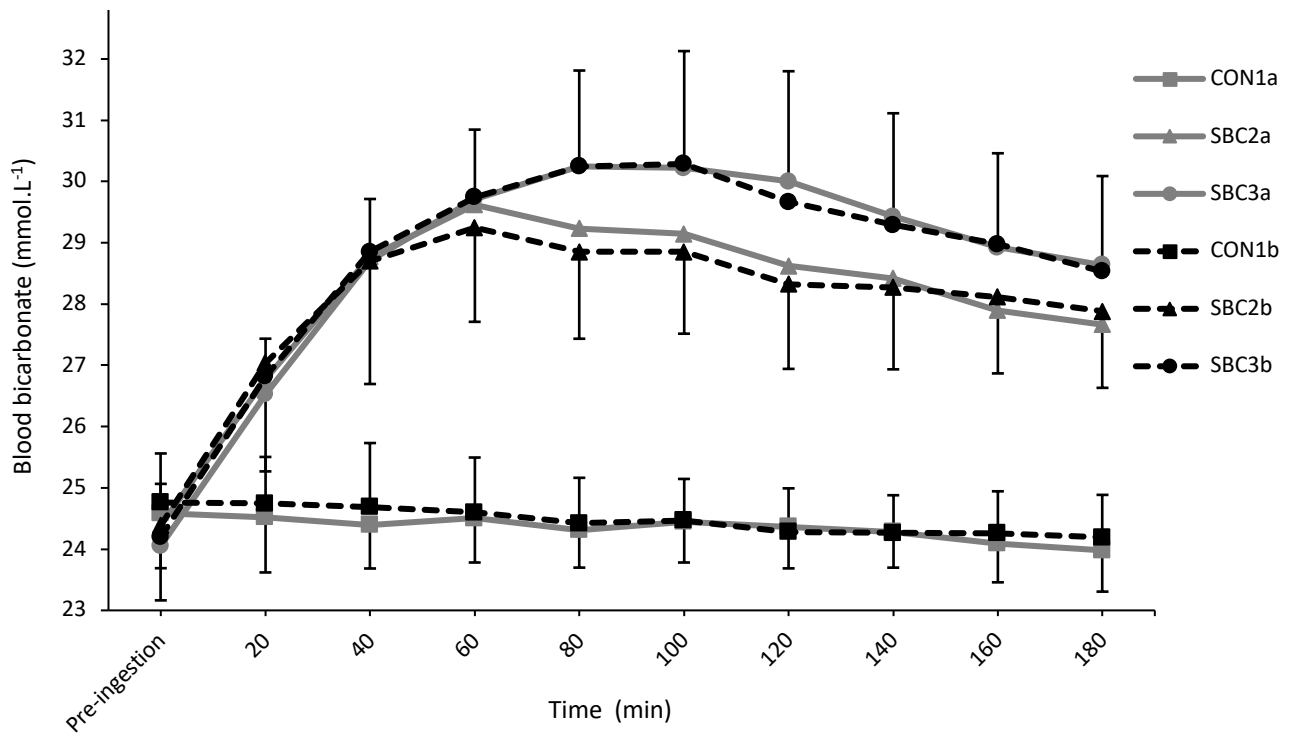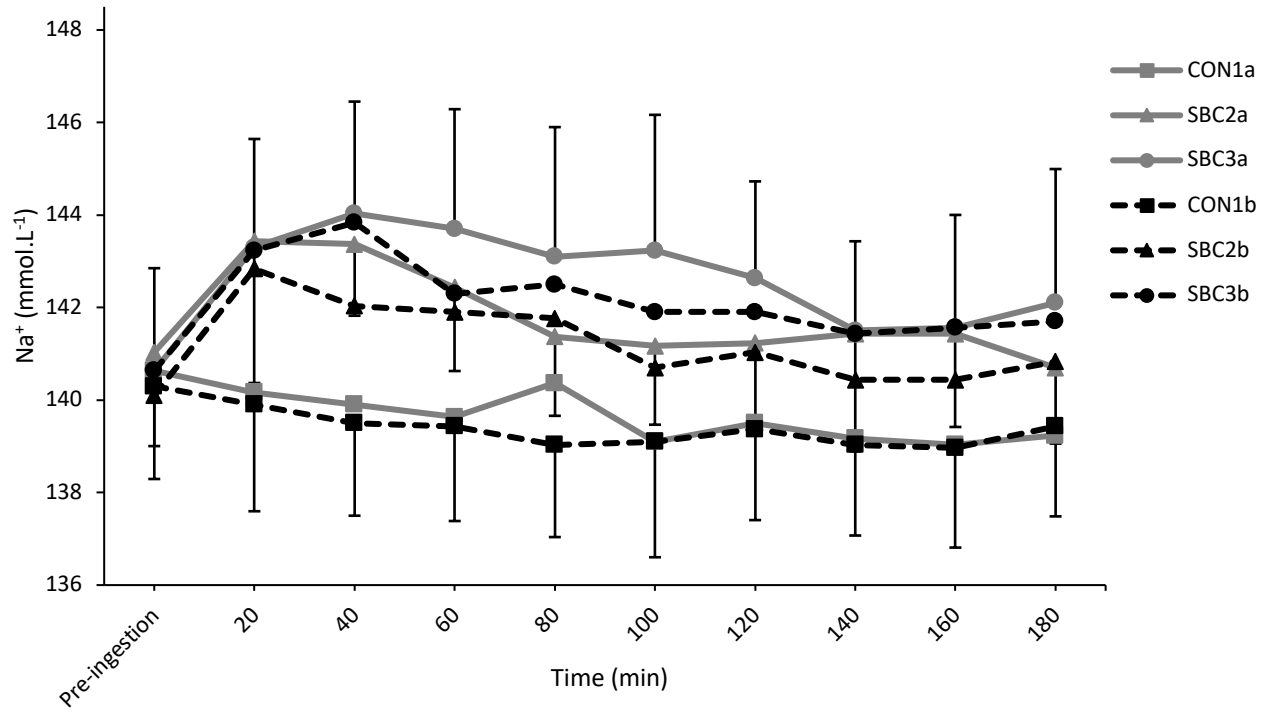
Tables

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

P.no	pH (TTP)				HCO$_3^-$ (TTP)				pH (Abs. Δ)				HCO$_3^-$ (Abs. Δ)			
	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