Dose-response of sodium bicarbonate ingestion highlights individuality in time course of blood analyte responses.

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ABSTRACT

To defend against hydrogen cation accumulation and muscle fatigue during exercise, sodium bicarbonate ($NaHCO_3$) ingestion is commonplace. The individualised dose-response relationship between $NaHCO_3$ ingestion and blood biochemistry is unclear. The present study investigated the bicarbonate, pH, base excess and sodium responses to $NaHCO_3$ ingestion. Sixteen healthy males (23±2 years; 78.6±15.1 kg) attended three randomised order-balanced, non-blinded sessions, ingesting a single dose of either 0.1, 0.2 or 0.3 $g \cdot kg^{-1}$BM of $NaHCO_3$ (Intralabs, UK). Fingertip capillary blood was obtained at baseline and every 10 min for 1 h, then every 15 min for a further 2 h. There was a significant main effect of both time and condition for all assessed blood analytes ($P \leq 0.001$). Blood analyte responses were significantly lower following 0.1 $g \cdot kg^{-1}$BM compared with 0.2 $g \cdot kg^{-1}$BM; bicarbonate concentrations and base excess were highest following ingestion of 0.3 $g \cdot kg^{-1}$BM ($P \leq 0.01$). Bicarbonate concentrations and pH significantly increased from baseline following all doses; the higher the dose the greater the increase. Large inter-individual variability was shown in the magnitude of the increase in bicarbonate concentrations following each dose (+2.0-5; +5.1-8.1; and +6.0-12.3 $mmol \cdot L^{-1}$ for 0.1, 0.2 and 0.3 $g \cdot kg^{-1}$BM) and in the range of time to peak concentrations (30-150; 40-165; and 75-180 min for 0.1, 0.2 and 0.3 $g \cdot kg^{-1}$BM). The variability in bicarbonate responses was not affected by normalisation to body mass. These results challenge current practices relating to $NaHCO_3$ supplementation and clearly show the need for athletes to individualise their ingestion protocol and trial varying dosages prior to competition.

Key words: Extracellular buffering, pH, fatigue

INTRODUCTION

High-intensity exercise increases hydrogen cation (H^+) production in the working muscle (Hill and Lupton, 1923). The majority of these H^+ are buffered, with only a small fraction being free in the cytosol to cause a decline in intracellular pH (Sahlin, 2014). It has been proposed that the decreased intracellular pH is a critical factor in the development of fatigue during high-intensity exercise, either via a direct effect on the muscle contractile machinery or by disruption to muscle energetics (Fitts, 1996). The ability to deal with this proton production is an important determinant of exercise performance and capacity. Two defence mechanisms against intramuscular acidosis are evident, namely, intramuscular physicochemical buffers and dynamic buffering (*i.e.*, the ability to transport H^+ out of the muscle and into the blood). Whilst the first line of defence is intramuscular physicochemical buffering, the main controller of pH during high-intensity exercise is dynamic buffering, this process allows the bicarbonate buffering system to minimise disruption to intramuscular pH (McNaughton et al. 2008; Carr et al. 2011)

Supplementation with sodium bicarbonate ($NaHCO_3$) increases the efflux of H^+ out of the muscle and the extracellular buffering capacity, thus delaying the onset of muscle fatigue, and maintaining exercise performance (Cairns, 2006; Thomas et al. 2005). It is unsurprising that $NaHCO_3$ ingestion has been a focus of researchers and athletes for over 30 years (Matson & Tran, 1993; Linderman & Gaosselink, 1994), with mixed findings in regards to the ergogenic efficacy of $NaHCO_3$ (for review see Peart et al. 2012). Some of these differences might be explained by differences dosing strategies. Several dosing strategies are employed within with the sporting field, with $NaHCO_3$ doses of 0.2-0.5 $g \cdot kg^{-1}$BM being consumed to enhance exercise performance (McNaughton et al. 1991). Nonetheless, ingestion of 0.3 $g \cdot kg^{-1}$BM is most commonplace, consumed 60-90 min prior to exercise (Renfree, 2007; Price & Singh, 2008; Siegler et al. 2010) in flavoured water or capsules (Peart et al. 2012). Consumption of 0.3 $g \cdot kg^{-1}$BM typically increases blood bicarbonate concentrations by ~5–6 $mmol \cdot L^{-1}$ from baseline (Matson & Tran, 1993; Price et al. 2003; Robergs et al. 2005; Saunders et al. 2014; Miller et al. *in press*), which

has been suggested to enhance the buffering process sufficiently to result in an ergogenic benefit (Carr et al. 2011).

Ingestion strategies can result in significant alterations in blood parameters, with peak acid-base disturbances occurring between 60 and 90 min post ingestion of 0.3 $g \cdot kg^{-1}$BM of $NaHCO_3$ (Renfree, 2007; Price & Singh, 2008; Siegler et al. 2010). Although there remains uncertainty as to how different doses affect the inter-individual variability in blood acid-base responses. Siegler et al. (2010) showed that blood bicarbonate peaked 65 min post ingestion of 0.3 $g \cdot kg^{-1}$BM, although due to blood samples assessed at 20 min intervals some important aspects of the temporal pattern in acid-base responses might have been overlooked. It has been proposed that the blood buffering responses to $NaHCO_3$ ingestion are highly individual (Peart et al. 2012; Saunders et al. 2014b) and in order to optimise ergogenic potential, individualising the timing of exercise based on acid-base responses to $NaHCO_3$ ingestion should be undertaken (Miller et al. *in press*). This highlights the need to examine how individuals respond to varying $NaHCO_3$ doses.

$NaHCO_3$ ingestion can result in gastrointestinal (GI) distress (Carr et al. 2011; Siegler et al. 2012; Peart et al. 2012), with 10% of participants not tolerating the doses needed to gain a beneficial performance effect (McNaughton et al. 2008). As dose increases, GI discomfort is more commonplace, often without additional performance improvements (McNaughton, 1991; Kahle et al. 2013). To combat GI symptoms, stacking dose strategies has been implemented (Sale et al. 2011; Saunders et al. 2014a); splitting larger doses (0.3 $g \cdot kg^{-1}$BM) into smaller separate doses across a longer timeframe (0.2 $g \cdot kg^{-1}$BM followed by 0.1 $g \cdot kg^{-1}$BM). How blood bicarbonate concentrations are altered following different dosages of $NaHCO_3$ requires further investigation.

Therefore, the present study investigated bicarbonate, pH, base excess and sodium (Na^+) responses to three different doses of $NaHCO_3$ to determine the time course of changes and the inter-individual variability in responses.

METHODS

Participants

Eighteen participants volunteered to participate in this non-blinded, order-balanced, crossover study. Two participants withdrew due to GI distress, meaning that sixteen healthy males (age, 23±2 years; height, 1.80±0.07 m; body mass, 78.6±15.1 kg) completed all aspects of the study. Participants provided written informed consent and completed a health screen questionnaire prior to taking part in the study, which was first approved by the Nottingham Trent University Ethical Advisory Committee. Participants had not ingested any nutritional supplement or suffered from any GI problems in the previous 6 months.

Protocol and measurements

Participants attended three supplementation sessions at the same time of day, in at least a 4 h post-prandial state and having replicated 24 h dietary intake. Participants were instructed to abstain from alcohol and strenuous/unaccustomed exercise for 24 h prior to each assessment, with caffeine prohibited on test days. Compliance with these requests was verbally confirmed prior to each session. Participants ingested a single dose of either 0.1, 0.2 or 0.3 $g \cdot kg^{-1}$BM of $NaHCO_3$ (Intralabs, UK) in clear gelatine capsules. Supplements were independently tested by HFL Sports Science, UK, ensuring no contamination with steroids or stimulants according to ISO 17025 accredited tests.

Fingertip capillary blood was obtained before participants ingested $NaHCO_3$ with 500 ml of water. Following ingestion, blood was obtained every 10 min for 1 h, and then every 15 min for a further 2 h, during which time participants rested in a seated position. 80 µL of whole blood was collected in a heparin-coated clinitube (Radiometer Ltd, UK), and immediately analysed for pH, bicarbonate and Na^+ concentrations with base excess being calculated (Radiometer ABL 900, UK).

Statistical Analysis

Based on an *a priori* power calculation (using Ducker et al. 2013); a minimum of 12 participants were required to achieve 95% power at $P<0.01$, with 18 participants recruited to allow for dropouts. Statistical analyses were completed using SPSS version 22 (SPSS Inc., Chicago, IL, USA) and Microsoft Excel (Microsoft Inc., USA). Data were analysed using two-way (condition X time) repeated measures ANOVA. Assessed variables were tested for normality using the Shapiro–Wilks test, and for homogeneity using the Levene test. A Greenhouse-Geisser correction was applied when Mauchly's test indicated that sphericity assumptions were violated. Blood analytes at each time-point were compared using a one-way ANOVA, with significance based on Bonferroni-corrected p-values. Net area under the curve (AUC) was calculated (as per Gannon et al. 1989), and compared using a one-way ANOVA with Bonferroni-corrected *post hoc* analysis. Linear regression analyses were performed to investigate relationships between baseline and absolute changes in bicarbonate concentrations. Statistical significance was accepted at $P\leq0.05$, with data presented as mean ± 1 standard deviation (SD).

RESULTS

The AUC was significantly greater for bicarbonate (0.1 $g \cdot kg^{-1}$BM: 314.4±96.1 $mmol \cdot L^{-1} \cdot 180min^{-1}$; 0.2 $g \cdot kg^{-1}$BM: 697.7±122.8 $mmol \cdot L^{-1} \cdot 180min^{-1}$, 0.3 $g \cdot kg^{-1}$BM: 915.7±182.2 $mmol \cdot L^{-1} \cdot 180min^{-1}$), pH (0.1 $g \cdot kg^{-1}$BM: 5.05±2.60 pH units$\cdot 180min^{-1}$; 0.2 $g \cdot kg^{-1}$BM: 9.03±2.93 pH units$\cdot 180min^{-1}$; 0.3 $g \cdot kg^{-1}$BM: 10.35±3.97 pH units$\cdot 180min^{-1}$), base excess (0.1 $g \cdot kg^{-1}$BM: 379.6±122.0 $mEq \cdot L^{-1} \cdot 180min^{-1}$; 0.2 $g \cdot kg^{-1}$BM: 824.4±156.7 $mEq \cdot L^{-1} \cdot 180min^{-1}$; 0.3 $g \cdot kg^{-1}$BM: 1078.6±210.3 $mEq \cdot L^{-1} \cdot 180min^{-1}$) and Na^+ (0.1 $g \cdot kg^{-1}$BM: -48.7±195.7 $mmol \cdot L^{-1} \cdot 180min^{-1}$; 0.2 $g \cdot kg^{-1}$BM: 111.4±223.5 $mmol \cdot L^{-1} \cdot 180min^{-1}$; 0.3 $g \cdot kg^{-1}$BM: 358.6±292.5 $mmol \cdot L^{-1} \cdot 180min^{-1}$) following 0.3 $g \cdot kg^{-1}$BM compared to 0.2 $g \cdot kg^{-1}$BM (with the exception of pH responses; $P \leq 0.05$) and 0.1 $g \cdot kg^{-1}$BM doses ($P \leq 0.05$). Overall responses to 0.2 $g \cdot kg^{-1}$BM were significantly greater than 0.1 $g \cdot kg^{-1}$BM ($P \leq 0.05$).

Baseline bicarbonate ($F_{(2,30)}=2.0$; $P=0.20$), pH ($F_{(2,30)}=0.7$; $P \leq 0.51$), base excess ($F_{(2,30)}=1.7$; $P \leq 0.20$) and Na^+ ($F_{(2,30)}=0.3$; $P \leq 0.78$) levels (Table 1) were not significantly different between doses. There was a significant main effect of time for bicarbonate ($F_{(14,210)}=72.6$; $P \leq 0.001$), pH ($F_{(14,210)}=39.8$; $P \leq 0.001$), base excess ($F_{(14,210)}=70.5$; $P \leq 0.001$) and Na^+ ($F_{(14,210)}=11.3$; $P \leq 0.001$) levels, with increases following $NaHCO_3$ ingestion under all supplemental conditions (Table 1).

There was a significant main effect of $NaHCO_3$ dose on bicarbonate ($F_{(2,30)}=53.0$; $P \leq 0.001$), pH ($F_{(2,30)}=18.4$; $P \leq 0.001$), base excess ($F_{(2,30)}=56.2$; $P \leq 0.001$) and Na^+ ($F_{(2,30)}=27.0$; $P \leq 0.001$) levels. *Post hoc* analysis showed that the responses of all blood analytes were significantly lower following 0.1 $g \cdot kg^{-1}$BM than following 0.2 $g \cdot kg^{-1}$BM (all $P \leq 0.001$ with the exception of pH [$P>0.05$]) and 0.3 $g \cdot kg^{-1}$BM doses ($P \leq 0.003$; Table 1). Bicarbonate concentrations ($P \leq 0.01$) and base excess ($P \leq 0.001$) were significantly higher following 0.3 $g \cdot kg^{-1}$BM compared to 0.2 $g \cdot kg^{-1}$BM, although there were no significant differences in pH and Na^+ concentrations between these doses. There were significant dose by time interactions for bicarbonate ($F_{(28,420)}=17.2$; $P \leq 0.001$), pH ($F_{(28,420)}=5.4$; $P \leq 0.001$), base excess

156 ($F_{(28,420)}$=18.4; P≤0.001), and Na^+ ($F_{(28,420)}$=5.0; P≤0.001) responses; time point comparisons for blood
157 analytes are displayed in Table 1.

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159 Across each time interval there was large variability in the responses of blood analytes following each
160 $NaHCO_3$ dose (Table 1). From this point the results will focus solely on blood bicarbonate
161 concentrations in the interests of brevity and given that this is the primary outcome measure of interest.
162 With respect to bicarbonate concentrations, the greatest variability in responses occurred between ~20
163 and 75 min after ingestion (Table 1). Variability was not reduced when data were normalised for body
164 mass (data not shown). Individual blood bicarbonate responses are displayed in Figure 1.

165

166 The absolute increases in bicarbonate concentrations from baseline to peak values (Figure 2) were
167 significantly greater following the ingestion of 0.3 g·kg^{-1}BM (8.2±1.4 mmol·L^{-1}) than ingestion of 0.1
168 g·kg^{-1}BM (3.6±0.8 mmol·L^{-1}; P≤0.001) or 0.2 g·kg^{-1}BM (6.1±0.9 mmol·L^{-1}; P≤0.001). The magnitude
169 of responses ranged from 2.0-5.0 mmol·L^{-1} for 0.1 g·kg^{-1}BM, 5.1-8.1 mmol·L^{-1} for 0.2 g·kg^{-1}BM and
170 6.0-12.3 mmol·L^{-1} for 0.3 g·kg^{-1}BM doses (Figure 2). One participant achieved an increase of 5 mmol·L^{-}
171 ^{1} from baseline, with none achieving an increase of 6 mmol·L^{-1} from baseline following 0.1 g·kg^{-1}BM
172 (Table 2). With the ingestion of 0.2 g·kg^{-1}BM, all 16 participants achieved an increase of 5 mmol·L^{-1}
173 from baseline and 9 participants achieved an increase of 6 mmol·L^{-1} from baseline (Table 2). All
174 participants achieved an increase of 6 mmol·L^{-1} from baseline following the ingestion of 0.3 g·kg^{-1}BM
175 (Table 2).

176

177 Individual magnitudes of responses between baseline and peak values were ranked, with only three
178 participants (1, 7 and 10) consistently in the greatest 8 responders and three participants (4, 6 and 15)
179 consistently in the least 8 responders on each dose (Table 2). The magnitudes of the responses were
180 more consistent within participants when comparing 0.2 and 0.3 g·kg^{-1}BM doses (Table 2); 6
181 participants (1, 7, 9, 10, 12, 16) were in the greatest 8 responders and 6 participants (4, 6, 8, 13, 14, 15)

were in the least 8 responders. There was a significant difference in time-to-peak blood bicarbonate concentrations ($F_{(2,30)}$=15.7, P≤0.001) between doses (Table 2). The time between ingestion and peak responses of blood bicarbonate demonstrated high inter-individual variability, with times ranging from 30-150 min (mean: 78 min; CoV: 44%) following 0.1 $g \cdot kg^{-1}$BM, 40-165 min (mean: 98; CoV: 32%) following 0.2 $g \cdot kg^{-1}$BM and 75-180 min (mean: 123 min; CoV: 29%) following 0.3 $g \cdot kg^{-1}$BM. No relationship between baseline bicarbonate concentrations and the subsequent increase in response to $NaHCO_3$ supplementation was shown for any dose (0.1 $g \cdot kg^{-1}$BM: R^2=0.01; 0.2 $g \cdot kg^{-1}$BM: R^2=0.19; 0.3 $g \cdot kg^{-1}$BM: R^2=0.01).

DISCUSSION

This is the first study to report blood analyte responses from 15 time points over 3 hrs, with a high temporal frequency of sampling, following $NaHCO_3$ ingestion at three differing doses. Despite individualising $NaHCO_3$ dosing (based on individual body mass) a high degree of inter-individual variability existed with regards to the magnitude of change in blood analyte levels and the time to peak. The magnitude of the increase in blood analytes was dose-dependent, with greater increases achieved with larger doses of $NaHCO_3$, although the range in responses was also greater at these highest dose. These data challenge the most commonly suggested supplementation protocol of 0.3 $g \cdot kg^{-1}$BM administered ~60 min prior to performance (McNaughton, 1991; Siegler et al. 2012; Duncan et al. 2014), which is unlikely to result in optimal blood biochemistry for all individuals. It is difficult to compare the time-course relationship following ingestion due to existing data being focused on either pre- to post-exercise comparisons, or due to infrequent sample collection (Renfree et al. 2007; Siegler et al. 2010; Carr et al. 2011; Miller et al. *in press*). Here we extend previous work examining the effect of $NaHCO_3$ ingestion on acid-base responses (Renfree et al. 2007; Siegler et al. 2010; Carr et al. 2011; Miller et al. *in press*), by employing a much greater temporal resolution (every 10 min) in sampling. The mean time-to-peak for bicarbonate and pH responses following ingestion of 0.3 $g \cdot kg^{-1}$BM was greater than the 60-90 min previously documented (Renfree, 2007; Price & Singh, 2008; Siegler et al. 2010); even when ingesting smaller doses (>60 min). Time-to-peak for all variables increased in a step-wise manner relative to dose; blood pH peaked at 75 (0.1 $g \cdot kg^{-1}$BM), 105 (0.2 $g \cdot kg^{-1}$BM) and 120 min (0.3 $g \cdot kg^{-1}$BM) post-ingestion. Our data suggest that the time intervals used in previous studies might lead to some misinterpretation of findings relating to optimal blood analyte responses. It remains unclear as to why high variability exists in time-to-peak when ingestion of $NaHCO_3$ was conducted within a small and structured time period (10 min). Numerous factors which could explain this variability, thus providing an avenue for future investigation.

If we use the 6 $mmol \cdot L^{-1}$ above baseline cut-off for blood bicarbonate responses, as suggested by Carr et al. (2011) to provide an ergogenic effect, it is clear that a dose of 0.3 $g \cdot kg^{-1}$BM remains the most

relevant to ensure that all individuals reach this zone (Figure 2). Following 0.3 $g \cdot kg^{-1}$BM, absolute changes in blood bicarbonate ranged between 6.0 and 12.3 $mmol \cdot L^{-1}$, with time-to-peak varying between 75 and 180 min. This demonstrates that the time taken for individuals to achieve peak concentrations or even performance relevant blood bicarbonate changes (Carr et al. 2011) is highly variable, suggesting a need to consider individual responses to $NaHCO_3$ supplementation (Figure 1). Practically, an *a priori* knowledge of an individual's blood responses following ingestion is required to optimise outcomes. What is not yet clear is whether or not individuals respond consistently to the same dose of $NaHCO_3$ or what factors influence bicarbonate release (*e.g.*, nutritional impact of gastric emptying), providing an avenue for further work.

The current investigation might also help to explain discrepancies previously shown in relation to the ergogenic effect of $NaHCO_3$ ingestion (for review see Carr et al. 2011), where numerous methodological differences relating dosing strategy were employed. In the current study, to provide consistency, participants were instructed to consume all capsules within 10 min, as per Siegler et al. (2010). The time taken to ingest $NaHCO_3$ is often unreported or is >30 min (Carr et al. 2011), which would theoretically cause more variability in individual peak responses that those reported in the current study, as such comparisons to previous blood analyte responses are confounded. Gastric emptying has shown considerable inter-individual variation (Paintaud et al. 1998; Barbosa et al. 2005), although there is some consistency in intra-individual responses (Paintaud et al. 1998; Barbosa et al. 2005). These findings suggest that it might be important to replicate dietary intake prior to ingestion in order to develop a more consistent response to $NaHCO_3$ ingestion. Participants in the current investigation replicated their 24 h dietary intake and remained fasted for 4 hrs prior to supplementation, where 90% of food would be emptied from the stomach (Tougas et al. 2000). Meal volume, composition and texture would, however, influence gastric emptying rates (Donohoe et al. 2009). An overnight fast would not be representative of athlete behaviour and so we decided to use a 4 h fast to provide a balance between experimental control and ecological validity. It should, however, be noted that the results of future studies might differ with alternative dietary intake patterns. During the current investigation non-

arterialised fingertip capillary blood samples have been used to assess blood analyte responses. The PO_2 values for the current investigation were 75.38 ± 2.14 for the 0.1 g·kg^{-1}BM condition, 74.14 ± 2.61 for 0.2 g·kg^{-1}BM and 73.18 ± 2.63 for the 0.3 g·kg^{-1}BM condition as an average across all time points. Non-warmed capillary blood samples are a useful and practical tool, reporting a strong correlation with arterial samples for pH, HCO_3 and base excess variables (Yildizdas et al. 2004). This method is also in-line with a number of previous investigations (Price & Simons, 2010; Bellinger et al. 2012; Siegler et al. 2013; Saunders et al. 2014). In a small independent study, we confirmed that blood arterialisation via warming the hand in a water bath (42^{o}C) for 10 minutes did not alter blood gas parameters. Nonetheless, it is important to suggest caution when comparing non-arterialised with arterialised samples.

Blood bicarbonate concentrations were similar over the first 30 min following ingestion of all $NaHCO_3$ doses; blood pH also followed a similar pattern for the first 60 min post-ingestion. These findings questions the use of doses above 0.1 g·kg^{-1}BM when the time between ingestion and performance is relatively short (*i.e.*, following a high-intensity warm-up or when an athlete has multiple events over a short period of time), especially when the same level of bicarbonate manipulation is achievable. In these situations it would also be advisable to consume 0.1 g·kg^{-1}BM of $NaHCO_3$, given that lower doses reduce the intensity and/or frequency of negative GI symptoms (McNaughton, 1992; Kahle et al. 2013), which would benefit athletes in the competitive setting. Some athletes require co-ingestion of $NaHCO_3$ with food and fluid in order to reduce GI symptoms, therefore lowering the dose could lead to a reduction in the amount of food/fluid ingested, vital for athletes competing numerous times within a short period.

Following large quantities of $NaHCO_3$, carbonic acid formation occurs in the stomach and Na^+ absorption and Na^+ plasma concentration both increase (Heigenhauser, 1991). As the physiochemical equilibrium shifts, water and CO_2 increase in the blood, thereby increasing CO_2 partial pressure (as described by the Henderson-Hasselbalch equation). This mechanism alters the already acidic

environment of the stomach, which can result in GI distress, including stomach bloating, nausea, and diarrhoea (McNaughton, 1992; Siegler et al. 2012). In the present study following $NaHCO_3$ ingestion we have shown increased plasma Na^+ concentrations, with the mean change being two times greater following 0.3 $g \cdot kg^{-1}$BM (4 $mmol \cdot L^{-1}$) compared to 0.1 $g \cdot kg^{-1}$BM (2 $mmol \cdot L^{-1}$). The peak change in Na^+ concentrations following 0.2 and 0.3 $g \cdot kg^{-1}$BM occurred ~105 minutes post-ingestion, which broadly corresponds to the timeframe of the greatest incidence of GI distress (~90 min following ingestion; Carr et al. 2011). The inter-individual variability in the magnitude of change in Na^+ concentrations might explain why some individuals report GI distress, whilst others do not, even at the same $NaHCO_3$ dose.

In conclusion, the present data challenges the most commonly implemented $NaHCO_3$ supplementation protocol and its efficacy to enhance buffering capacity and exercise performance for all individuals. Due to the large inter-individual responses shown, individual and mean responses should be included in future research and knowledge of the individual responses to $NaHCO_3$ supplementation is essential in the applied setting. For individuals needing to ingest $NaHCO_3$ $\leq$30 min prior to the onset of exercise, smaller doses can be ingested with no negative consequences for the additional extracellular buffering potential.

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296 preparation were undertaken by RLS, TS, SC, GGA, BS and CS. All authors approved the final version
297 of the paper.

298 **REFERENCES**

299 Afman, G., Garside, R.M., Dinan. N., Gant. N., Betts, J.A., & Williams, C. (2014). Effect of
300 carbohydrate or sodium bicarbonate ingestion on performance during a validated basketball simulation
301 test. *International Journal of Sports Nutrition Exercise Metabolism,* 24, 6, 632-644.

302 Barbosa, L., Vera, H., Moran, S., Del Prado, M., & Lopez Alarcon, M. (2005). Reproducibility and
303 reliability of the 13C-acetate breath test to measure gastric emptying of liquid meal in
304 infants. *Nutrition,* 21, 289–294.

305 Bruton, J.D., Lannergren, J., & Westerblad, H. (1998). Effects of CO_2-induced acidification on the
306 fatigue resistance of single mouse muscle fibers at 28°C. *Journal of Applied Physiology,* 85, 478–483.

307 Burke, L.M., & Pyne, D.B. (2007). Bicarbonate loading to enhance training and competitive
308 performance. *International Journal of Sports Physiology and Performance,* 2, 93-97.

309 Cairns, S.P. (2006). Lactic acid and exercise performance: culprit or friend? *Sports Medicine,* 36, 279–
310 291.

311 Carr, A.J., Hopkins, W.G., & Gore, C.J. (2011). Effects of acute alkalosis and acidosis on performance:
312 A meta-analysis. *Sports Medicine*, 41, 10, 801-814.

313 Carr, A.J., Slater, G.J., Gore, C.J., Dawson, B. & Burke, L.M. (2011). Effect of sodium bicarbonate on
314 [HCO_3^-], pH, and gastrointestinal symptoms. *International Journal of Sport Nutrition and Exercise*
315 *Metabolism*, 21, 189-194.

316 Duncan, M.J., Weldon, A., & Price, M.J. (2014). The effect of sodium bicarbonate ingestion on back
317 squat and bench press exercise to failure. *Journal of Strength and Conditioning Research,* 1358–1366.

318 Flinn, S., Herbert, K., Graham, K., & Siegler, J.C. (2014). Differential effect of metabolic alkalosis and
319 hypoxia on high-intensity cycling performance. *Journal of Strength and Conditioning Research*, 28,
320 2852–2858.

Forbes, S.C., Raymer, G.H., Kowalchuk, J.M., & Marsh, G.D. (2005). $NaHCO_3$-induced alkalosis reduces the phosphocreatine slow component during heavy intensity forearm exercise. *Journal of Applied Physiology*, 99, 1668–1675.

Gannon, M.C., Nuttall, F.Q., Westphal, S.A., Neil, B.J., & Seaquist, E.R. (1989). Effects of dose of ingested glucose on plasma metabolite and hormone responses in type II diabetic subjects. *Diabetes Care*, 12, 544–552.

Heigenhauser, G.J.F. (1991). Ergogenic enhancement of performance in exercise and sport: Bicarbonate loading. In: D. R. Lamb and M. H. Williams (Ed.), *Prospective in Exercise Science and Sports Medicine* (Vol. 4; pp. 183–212). Carmel, In: Cooper Publishing Group LLC.

Hill, A.V. & Lupton, H. (1923). Muscular exercise, lactic acid, and the supply and utilization of oxygen. *QJM*, 16, 135-171.

Joyce, S., Minahan, C., Anderson, M., & Osborne, M. (2011). Acute and chronic loading of sodium bicarbonate in highly trained swimmers. *European Journal of Applied Physiology*, 112, 1–9.

Kahle, L., Kelly, P., Eliot, K., & Weiss, E. (2013). Acute sodium bicarbonate loading has negligible effects on resting and exercise blood pressure but causes gastrointestinal distress. *Nutrition Research*, 33, 479–86.

Kozac-Collins, K., Burke, E.R., & Schoene, R. (1994). Sodium bicarbonate ingestion does not improve performance in women cyclists. *Medicine and Science in Sports and Exercise*, 26, 12, 1510-5.

Kupcis, P.D., Slater, G.J., Pruscino, C.L., & Kemp, J.G. (2012). Influence of sodium bicarbonate on performance and hydration in lightweight rowing. *International Journal of Sport Physiological Performance*, 7, 11–18.

Linderman J.K., & Gosselink K.L. (1994). The effects of sodium bicarbonate ingestion on exercise performance. *Sports Medicine*, 18, 75–80.

Matson, L.G., & Tran, Z.V. (1993). Effects of sodium bicarbonate ingestion on anaerobic performance: A meta-analytic review. *International Journal of Sport Nutrition*, 3, 1, 2–28.

346 Maughan, R.J., King, D.S., & Lea, T. (2004). Dietary supplements. *Journal of Sports Sciences*, 22, 95–
347 113.

348 McNaughton, L.R. (1992). Bicarbonate ingestion: Effects of dosage on 60s cycle ergometry. *Journal*
349 *of Sports Sciences*, 10, 415-423.

350 McNaughton, L.R., Siegler, J.C., & Midgley, A. (2008). The ergogenic effect of sodium bicarbonate.
351 *Current Sports Medicine Reports*, 7, 230–236.

352 Miller, P., Robinson, A., Sparks, A., Bridge, C.A., Bentley, D., & McNaughton, L.R. (*in press*). The
353 effects of novel ingestion of sodium bicarbonate on repeated sprint ability. *Journal of Strength and*
354 *Conditioning Research*.

355 Paintaud, G., Thibault, P., Queneau, P.E., Magnette, J., Berard, M., Rumbach, L., Bechtel, P.R., &
356 Carayon, P. (1998). Intraindividual variability of paracetamol absorption kinetics after a semisolid meal
357 in healthy subjects. *European Journal of Clinical Pharmacology*, 53, 355–359.

358 Peart, D., Siegler, J., & Vince, R. (2012). Practical recommendations for coaches and athletes: a meta-
359 analysis of sodium bicarbonate use for athletic performance. *The Journal of Strength & Conditioning*
360 *Research*, 26, 1975–1983.

361 Pilegaard, H., Domino, K., Noland, T., Juel C., Hellsten Y. & Halestrap A.P. (1999). Effect of high-
362 intensity exercise training on lactate/H transport capacity in human skeletal muscle. *American Journal*
363 *of Physiology Endocrinology and Metabolism*, 276, 2, 255–261.

364 Price, M.J., & Singh, M. (2008). Time course of blood bicarbonate and pH three hours after sodium
365 bicarbonate ingestion. *International Journal of Sports Physiology and Performance*, 3, 240–242.

366 Renfree, A. (2007). The time course for changes in plasma [H^+] after sodium bicarbonate ingestion.
367 *International Journal of Sports Physiology and Performance*, 2, 323–326.

368 Robergs, R.A. (2002). Blood acid-base buffering: explanation of the effectiveness of bicarbonate and
369 citrate ingestion. *Journal of Exercise Physiology*, 5, 3, 1-5.

Sale, C., Hill, C.A., Ponte, J., & Harris, R.C. (2012). β-alanine Supplementation Improves Isometric Endurance of the Knee Extensor Muscles. *Journal of the International Society of Sports Nutrition*, 9, 1, 26.

Sale, C., Saunders, B., Hudson, S., Wise, J.A., Harris, R.C., & Sunderland, C.D. (2011). Effect of β-alanine plus sodium bicarbonate on high-intensity cycling capacity. *Medicine and Science in Sports and Exercise*, 43 (10), 1972–1978.

Saunders, B., Sale, C., Harris, R.C., & Sunderland, C. (2014a). Effect of sodium bicarbonate and Beta-alanine on repeated sprints during intermittent exercise performed in hypoxia. *International Journal of Sport Nutrition and Exercise Metabolism*, 24, 196–205.

Saunders, B., Sale, C., Harris, R.C., & Sunderland, C. (2014b). Sodium Bicarbonate and High-Intensity-Cycling Capacity: Variability in Responses. *International Journal of Sports Physiology and Performance*, 9, 627-632.

Siegler, J.C., Marshall, P.W.M., Bray, J., & Towlson, C. (2012). Sodium bicarbonate supplementation and ingestion timing. *Journal of Strength and Conditioning Research*, 26, 1953–1958.

Siegler, J.C., Midgley, A.W., Polman, R.C., & Lever, R. (2010). Effects of various sodium bicarbonate loading protocols on the time-dependent extracellular buffering profile. *Journal of Strength and Conditioning Research*, 24, 2551–2557.

Stellingwerff, T., Boit, M.K., & Res, P.T. (2007). Nutritional strategies to optimize training and racing in middle distance athletes. *Journal of Sports Sciences*, 25, S1, S17-S28.

Thomas, C., Perrey, S., Lambert, K., Hugon, G., Mornet, D., & Mercier, J. (2005). Monocarboxylate transporters, blood lactate removal after supramaximal exercise, and fatigue indexes in humans. *Journal of Applied Physiology*, 98, 804-809.

Vanhatalo, A., McNaughton, L.R., Siegler, J., & Jones, A.M. (2010). Effect of induced alkalosis on the power-duration relationship for "All-out" Exercise. *Medicine and Science in Sports and Exercise*,, 42, 563–570.

Westerblad, H., Bruton, J.D., & Katz, A. (2010). Skeletal muscle: Energy metabolism, fiber types, fatigue and adaptability. *Experimental cell research,* 316, 3093-3099.

Westerblad, H., Bruton, J.D., & Lannergren, J. (1997). The effect of intracellular pH on contractile function of intact, single fibres of mouse muscle declines with increasing temperature. *Journal of Physiology,* 500, 193–204.

Yildizdas, D., Yapicioglu, H.L., Yilmaz, H.L. & Sertdemir, Y. (2004). Correlation of simultaneously obtained capillary, venous and arterial blood gases of pateints in a paediatric intensive care unit. *Archives of Disease in Childhoof,* 89, 176-180.

Zabala, M., Requena, B., Sanchez-Munoz, C., Gonzalez-Badillo, J.J., Garcia, I., Oopik, V., & Paasuke, M. (2008). Effects of sodium bicarbonate ingestion on performance and perceptual responses in a laboratory-simulated BMX cycling qualification series. *Journal of Strength and Conditioning Research,* 22, 1645–1653.

407 **TABLES**

408 **Table 1:** Blood bicarbonate, pH, base excess and Na^+ responses across the 3 h duration following
409 $NaHCO_3$ ingestion. Mean time point comparisons are displayed for each blood analyte; ^{X} denotes a
410 significant difference between 0.1 and 0.2 $g \cdot kg^{-1}$BM. $^{\Delta}$ denotes a significant difference between 0.2 and
411 0.3 $g \cdot kg^{-1}$BM. All comparisons are based on Bonferroni-corrected p-values of ≤ 0.003.

			Time post ingestion (min)														
			0	**10**	**20**	**30**	**40**	**50**	**60**	**75**	**90**	**105**	**120**	**135**	**150**	**165**	**180**
Bicarbonate (mmol·L^{-1})	**0.1 g·kg^{-1}BM**	Mean	25.7	25.5	26.4	27.1	27.5*	27.9*	28.0^{*X}	27.9^{*X}	28.0^{*X}	28.1^{*X}	27.9^{*X}	27.4^{*X}	27.4^{*X}	27.2^{*X}	27.2^{*X}
		SD	1.0	1.2	1.7	1.8	1.9	1.9	1.6	1.3	1.0	1.2	1.1	1.1	1.1	0.8	0.7
	0.2 g·kg^{-1}BM	Mean	25.1	25.3	25.9	27.3	28.4	28.9	29.5	30.1	30.5$^{\Delta}$	30.5$^{\Delta}$	30.1$^{\Delta}$	29.9$^{\Delta}$	29.6$^{\Delta}$	29.3$^{\Delta}$	29.1$^{\Delta}$
		SD	0.87	1.27	1.58	1.74	1.71	1.62	1.19	1.04	0.97	0.85	1.19	1.18	1.24	0.84	0.92
	0.3 g·kg^{-1}BM	Mean	25.5	25.6	26.7	27.9	29.4	30.0	30.6	31.7	32.1	32.3	32.4	32.2	32.2	31.7	31.4
		SD	1.34	1.40	1.65	1.80	1.90	1.96	2.06	1.94	1.98	1.87	2.14	1.89	2.26	1.53	1.40
pH	**0.1 g·kg^{-1}BM**	Mean	7.42	7.43	7.44	7.44	7.45	7.46	7.46*	7.46*	7.46^{*X}	7.46*	7.45^{*X}	7.45^{*X}	7.45*	7.45*	7.44
		SD	0.01	0.02	0.03	0.02	0.03	0.02	0.02	0.02	0.01	0.02	0.02	0.02	0.03	0.02	0.02
	0.2 g·kg^{-1}BM	Mean	7.42	7.42	7.43	7.45	7.46	7.47	7.47	7.47	7.48	7.48	7.48	7.48	7.48	7.47	7.46
		SD	0.02	0.02	0.03	0.03	0.03	0.04	0.03	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03
	0.3 g·kg^{-1}BM	Mean	7.42	7.42	7.44	7.45	7.47	7.48	7.48	7.49	7.50	7.50	7.50	7.50	7.49	7.49	7.49
		SD	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.02
Base Excess (mEq·L^{-1})	**0.1 g·kg^{-1}BM**	Mean	1.59	1.32	2.51	3.37*	3.84*	4.28^{*X}	4.35^{*X}	4.23^{*X}	4.45^{*X}	4.48^{*X}	4.23^{*X}	3.73^{*X}	3.63^{*X}	3.51^{*X}	3.47^{*X}
		SD	1.29	1.54	2.02	2.23	2.27	2.25	1.93	1.70	1.26	1.41	1.30	1.28	1.28	0.92	0.84
	0.2 g·kg^{-1}BM	Mean	0.96	1.12	1.71	3.43	4.71	5.28	6.09$^{\Delta}$	6.68$^{\Delta}$	7.18$^{\Delta}$	7.29$^{\Delta}$	6.90$^{\Delta}$	6.74$^{\Delta}$	6.40$^{\Delta}$	6.16$^{\Delta}$	5.87$^{\Delta}$
		SD	1.10	1.55	1.86	2.10	2.11	2.00	1.57	1.30	1.24	1.04	1.32	1.40	1.49	1.08	1.11
	0.3 g·kg^{-1}BM	Mean	1.44	1.53	2.89	4.34	6.01	6.76	7.54	8.66	9.07	9.34	9.58	9.22	9.29	8.75	8.43
		SD	1.66	1.76	2.05	2.25	2.26	2.35	2.41	2.23	2.31	2.12	2.38	2.09	2.43	1.68	1.53
NA^{+} (mmol·L^{-1})	**0.1 g·kg^{-1}BM**	Mean	143	142	142	142	143	143	142*	143^{*X}	142^{*X}	143*	142*	143^{*X}	143^{*X}	142*	142*
		SD	1	1	2	2	1	1	1	1	1	1	2	1	1	1	1
	0.2 g·kg^{-1}BM	Mean	142	141	142	142	143	143	143	143	144	144	144	144	143	143	143
		SD	2	1	2	2	2	2	2	1	1	2	1	2	1	1	1
	0.3 g·kg^{-1}BM	Mean	142	142	142	143	144	144	144	145	145	145	145	145	145	145	145
		SD	2	3	2	2	2	2	2	2	1	1	1	1	2	1	2

412 **Table 2:** Individual blood bicarbonate responses following $NaHCO_3$ ingestion across supplemental condition. Absolute and percentage change in
413 bicarbonate responses refer to the difference between baseline and peak concentrations; absolute changes of ≥5 $mmol \cdot L^{-1}$ are highlighted in bold.
414 Position based on response ranks participants on absolute change in descending order, highest response equates to 1, whilst lowest absolute change
415 equates to 16. Significant differences between supplementation conditions for absolute change and time-to-peak are denoted by * (0.1 and 0.3 $g \cdot kg^{-1}$BM) ^{X} (0.1 and 0.2 $g \cdot kg^{-1}$BM) and $^{\Delta}$ (0.2 and 0.3 $g \cdot kg^{-1}$BM; $P \leq 0.05$).
416

Participant number	0.1 g·kg^{-1}BM					0.2 g·kg^{-1}BM					0.3 g·kg^{-1}BM				
	Baseline (mmol·L^{-1})	Absolute Change (mmol·L^{-1})	Percentage change (%)	Time-to-peak (min)	Position based on response	Baseline (mmol·L^{-1})	Absolute Change (mmol·L^{-1})	Percentage change (%)	Time-to-peak (min)	Position based on response	Baseline (mmol·L^{-1})	Absolute Change (mmol·L^{-1})	Percentage change (%)	Time-to-peak (min)	P b r
1	23.7	3.9	16.5	90	5	23.2	**6.9**	29.7	90	3	23.7	**8.9**	37.6	165	
2	25.1	4.4	17.5	50	3	25.7	**6.0**	23.3	90	8	24.8	**8.9**	35.9	90	
3	25.9	2.7	10.4	120	14	25.5	**6.0**	23.5	120	9	25.5	**8.1**	31.8	105	
4	24.6	2.0	8.1	90	16	23.9	**5.5**	23.0	105	12	25.4	**7.0**	27.6	90	
5	25.3	3.8	15.0	120	6	24.7	**5.6**	22.7	105	10	25.1	**8.5**	33.9	150	
6	25.9	3.1	12.0	90	12	25.2	**5.3**	21.0	120	13	25.2	**6.9**	27.4	150	
7	25.1	3.8	15.1	50	7	24.9	**6.4**	25.7	90	6	27.1	**8.8**	32.5	120	
8	26.6	4.9	18.4	105	2	25.7	**5.1**	19.8	105	15	28.5	**7.7**	27.0	120	
9	26.6	2.6	9.8	75	15	25.3	**7.1**	28.1	120	2	26.7	**12.3**	46.1	150	
10	24.9	4.3	17.3	150	4	24.9	**6.6**	26.5	135	5	23.1	**8.6**	37.2	180	
11	25.0	3.1	12.4	90	13	24.9	**6.4**	25.7	90	7	24.2	**7.0**	28.9	180	
12	26.4	3.3	12.5	50	10	24.5	**8.1**	33.1	90	1	25.9	**8.6**	33.2	105	
13	24.9	**5.0**	20.1	30	1	26.7	**5.5**	20.6	40	11	26.7	**6.0**	22.5	90	
14	27.6	3.7	13.4	50	8	26.6	**5.2**	19.5	165	14	26.1	**8.3**	31.8	120	
15	26.8	3.2	11.9	40	11	25.1	**5.1**	20.3	60	16	24.9	**6.6**	26.5	75	
16	26.2	3.4	13.0	50	9	25.2	**6.7**	26.6	50	4	25.4	**8.4**	33.1	75	
Mean	25.7	3.6 ^{*X}	14.0	78 *		25.1	6.1 $^{\Delta}$	24.3	98 $^{\Delta}$		25.5	8.2	32.0	123	
SD	1.0	0.8	3.3	34		0.9	0.9	3.9	32		1.3	1.4	5.6	36	
Min	23.7	2.0	8.1	30		23.2	5.1	19.5	40		23.1	6.0	22.5	75	
Max	27.6	5.0	20.1	150		26.7	8.1	33.1	165		28.5	12.3	46.1	180	

417

FIGURE LEGENDS

Figure 1: Individual blood bicarbonate responses across the 3 hr following $NaHCO_3$ ingestion at 0.1(A), 0.2 (B) and 0.3 $g \cdot kg^{-1}$BM (C).

Figure 2: Mean absolute change in bicarbonate concentrations across 15 intervals (3 hr) following ingestion of 0.1 (open circles), 0.2 (solid square) and 0.3 $g \cdot kg^{-1}$BM (open triangle) of $NaHCO_3$. Zone of ergogenic effect (+6 $mmol \cdot L^{-1}$) is based on concentrations from Carr et al. (2011).

FIGURES

Figure 1

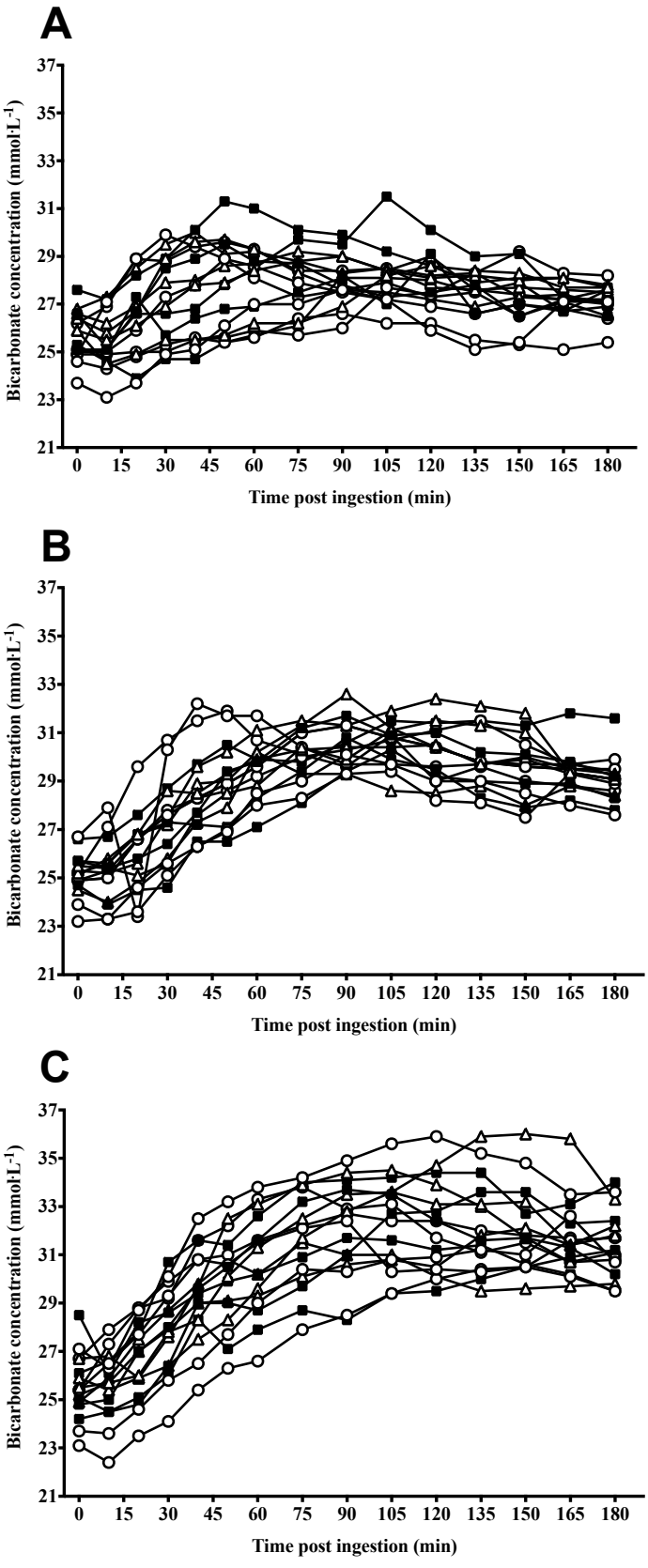
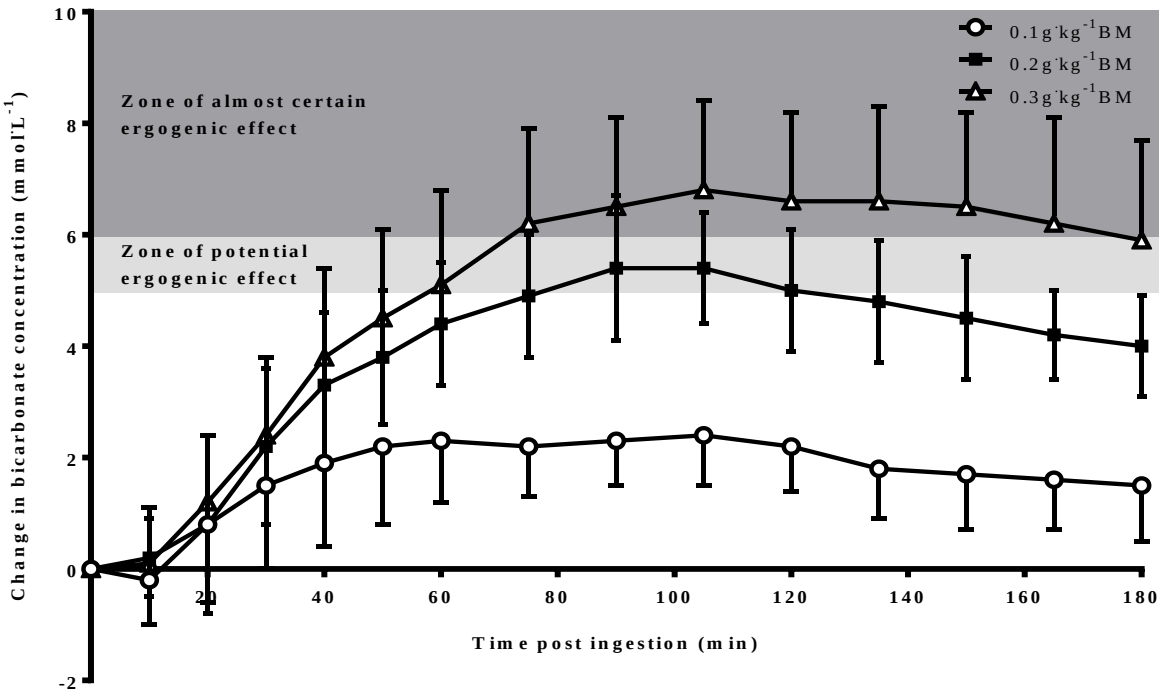


Figure 2


Zone of almost certain ergogenic effect
Zone of potential ergogenic effect
0.1g·kg-1BM
0.2g·kg-1BM
0.3g·kg-1BM
Change in bicarbonate concentration (mmol·L-1)
Time post ingestion (min)
10
8
6
4
2
0
-2
20
40
60
80
100
120
140
160
180