Extraceullular buffer choice influences acid-base responses and gastrointestinal symptoms

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1 **Extracellular buffer choice influences acid-base responses and gastrointestinal symptoms**

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ABSTRACT

Aim: To compare the pharmokinetic and gastrointestinal (GI) symptom responses between an equal dose of sodium bicarbonate and sodium citrate using delayed-release capsules. **Methods:** Thirteen active males (age 20.5 ± 2.1 y, height 1.82 ± 0.1 m and body mass 76.5 ± 9.6 kg) consumed either 0.3 g·kg^{-1} BM sodium bicarbonate, sodium citrate or a placebo, using a double-blind, randomised crossover design. Blood bicarbonate [HCO_3^-]) concentration, pH and GI symptoms were measured pre-ingestion and every 10 min for 180 min post-consumption. **Results:** [HCO_3^-] concentration ($P < 0.001$) and pH ($P = 0.040$) were significantly higher in the sodium bicarbonate condition compared with sodium citrate condition up to 3 h post-consumption. Peak blood HCO_3^- concentration was significantly higher with sodium bicarbonate compared with citrate ($P < 0.001$). Mean GI symptom scores were lower ($P = 0.037$) for sodium citrate (1.5 ± 1.8 AU) than bicarbonate (2.6 ± 3.1 AU), with considerable inter-individual variability. No GI symptoms were reported following consumption of the placebo. **Conclusion**: Both substances alter [HCO_3^-] values significantly, with sodium bicarbonate causing significantly higher pH and [HCO_3^-] values than the same dose of sodium citrate, but results in slightly more severe GI symptoms.

Keywords: Alkalosis, Sodium bicarbonate, Sodium citrate, delayed-release capsules

INTRODUCTION

The use of exogenous extracellular buffering agents has been widely investigated in the literature (McNaughton 1992; Matson and Tran, 1993; McNaughton et al., 2016) and across a range of sporting activities (Shave et al., 2001; Saunders et al., 2014; Kumstát et al., 2018). Typically, these agents have been used for their potential ergogenic effects on short duration, high intensity exercise (Grgic, 2020). The most commonly used extracellular buffers are sodium bicarbonate and sodium citrate (Carr et al., 2011), since they have the potential to increase base excess by increasing blood bicarbonate concentration and increasing blood pH. Many studies seeking to investigate the effects of sodium citrate and sodium bicarbonate on subsequent performance measures, often commence exercise at a standardised time of around 60-90 min post-ingestion (Kumstát et al., 2018; Schabort et al., 2000; Shave et al., 2001). This comes despite individual-level data supporting a minimum of 100-180+ minutes (Potteiger et al., 1996; Requena et al., 1996; Urwin et al., 2016; Urwin et al., 2019) or 30-180 minutes (Gough et al., 2019; Gough et al., 2017; Jones et al., 2016; de Oliveira et al., 2020) in order to achieve a peak alkalotic responses with sodium citrate and sodium bicarbonate respectively. Heibel at al., (2018) have previously suggested that this peak should result in blood bicarbonate concentration increases of >5 $mmol \cdot L^{-1}$ for the greatest chance of an ergogenic effect. However, the different ingestion times for sodium bicarbonate and sodium citrate are due to the longer post-ingestion time for peak pH and bicarbonate (time to peak) changes to occur in the blood. This is likely to be a result of the higher molecular weight of sodium citrate ($C_6H_5O_7Na_3$) compared to sodium bicarbonate ($NaHCO_3$) and this too may influence total blood bicarbonate changes.

What is clear, is that the timing of optimal pre-exercise ingestion has received considerable attention recently (Miller at al., 2016; Gough et al., 2017; Gough et al., 2019) and has been shown to exhibit considerable inter-individual variability (Jones et al., 2016; Sparks et al., 2017), suggesting that individual timing of ingestion is important. Indeed, Boegman et al., (2020) have recently demonstrated that individualising ingestion time provides an important competitive advantage in elite rowers compared to a standard ingestion time. Furthermore, what also appears to be important in the use of these buffering agents, is the potentially ergolytic effect for those individuals that suffer from

gastrointestinal side-effects (Deb et al., 2018), suggesting that careful individual assessments are needed prior to ingesting them before exercise.

Indeed, acute gastrointestinal (GI) distress is a relatively common side-effect of ingesting large quantities of exogenous buffers, particularly when provided as an aqueous solution. Since gastrointestinal distress appears partly attributable to interaction with acids in the stomach, contemporary research has focused on ways to reduce this discomfort (Hilton et al., 2019a; Hilton et al., 2019b), while simultaneously attempting to make supplementation more palatable for application in a practical context (Urwin et al., 2019). To achieve this, a range of capsules have been utilised as a means of avoiding degradation of capsules in the stomach and exploiting the substantial pH differences across the gastrointestinal tract, causing degradation to occur predominantly in the less acidic duodenum (pH 6-7 arbitrary units (AU)) (Ibekwe et al., 2008). Many of these capsules contain hydroxpropyl methylcullulose which resists degradation in acidic environments (pH ~1-2 AU). Whilst it was thought that reducing neutralisation in the stomach may increase blood concentrations (de Oliveira et al., 2018), reductions in blood HCO_3^- concentration have been observed, potentially due to reductions in the time available for absorption (Hilton et al., 2019a; Hilton et al., 2019b).

To date, no previous literature has assessed the time to peak of acid-base variables (bicarbonate and pH) and GI distress following administration of sodium citrate, provided within delayed release (DR) capsules. Successfully minimising gastrointestinal distress following sodium citrate/bicarbonate ingestion may improve the usability of these agents for athletes who would otherwise be discouraged by potential side-effects. Furthermore, no studies have directly compared blood acid-base responses following sodium citrate and sodium bicarbonate ingestion using an identical population and administration strategy. Whereas limited comparisons between buffering agents have been made to date, any observations made may further guide an athlete's choice to ingest one buffering agent over the other. Therefore, the aim of this study was to determine the pharmokinetic and gastrointestinal symptom responses to an orally ingested dose of sodium bicarbonate and sodium citrate, administered in delayed release capsules.

METHODS

Participants

Thirteen recreationally active males (age 20.5 ± 2.1 y, height 1.82 ± 0.1 m and body mass 76.5 ± 9.6 kg) were recruited for the current study. All participants were familiar with high intensity or intermittent exercise and took part in physical activity > 3 h wk^{-1}. Participants who had taken any nutritional supplements (e.g. beta-alanine) which may influence the response to sodium bicarbonate/citrate within the last 6 months (Baguet et al., 2009) were not eligible for the study. Written informed consent was obtained after verbal and written explanations of the benefits and potential side effects associated with the investigation. The study was approved by the departmental research ethics committee of the University (SPA-REC-2019-004).

Experimental Design

Participants attended the laboratory on three separate occasions and consumed 0.3 $g \cdot kg^{-1}$ body mas (BM) sodium bicarbonate, sodium citrate and a placebo (cornflour) in a double-blind, randomised crossover design. Experimental trials were counterbalanced in the order of administration and were completed under standard laboratory environmental conditions. All trials were separated by at least 48 h and took place at the same time of day (09:00) to minimise the physiological effects of circadian rhythms (Reilly, 1990).

Participants were asked to refrain from ingesting alcohol and undertaking any form of unaccustomed, intense exercise for at least 24 h prior to experimental testing. Within this 24 h period, participants were required to maintain their normal, diet, and keep a record of dietary intake ensure intake was replicated in the 24 h period before each subsequent trial to minimise any changes this may have on acid-base balance (Bishop and Spencer, 2004; McNaughton, 1992). On the day of testing, to further minimise potential changes in acid-base balance and standardise the possible occurrence of gastrointestinal (GI) symptoms, participants arrived at the laboratory in a fasted state (8 h).

Experimental procedures

Acid-base balance

On arrival to the laboratory, semi-nude body mass was recorded after bladder evacuation and after completing a medical screening questionnaire. Participants consumed either the experimental supplements or a placebo (cornflour) within 10 min. All supplements were administered in size 00 opaque (white) delayed release (DR) (DRCaps, Lonza, France) capsules and the same number of capsules (mean ± SD = 36 ± 4 capsules) were provided. During the testing period, all participants remained seated. During this time, fingertip capillary blood samples (95 µL) were obtained pre-consumption and then every 10 min for 180 min post-consumption. Blood bicarbonate ion [HCO^{-3}] concentration, and pH were measured using a blood gas analyser (Radiometer ABL800, Denmark) which was calibrated immediately prior to all testing sessions. This equipment has been used extensively in similar research studies and is deemed to provide valid and reliable measurements (Fagoni et al., 2018; Gough et al., 2017, Miller et al., 2016). The time to the first significant change in HCO_3^- concentration (T_{lag}), peak HCO_3^- concentration (C_{max}), absolute change in HCO_3^- concentration (ΔC_{max}), time-to-peak HCO_3^- concentration (T_{max}) and area under the concentration-time curve (AUC) were calculated.

Gastrointestinal symptoms

Symptoms of GI distress were recorded pre-consumption and every 10 min post-consumption for 180 min using a visual analogue scale (Miller et al., 2016) ranging from 0 (i.e. no symptom) to 10 (i.e. severe symptom). Participants were instructed to rate symptoms including nausea, flatulence, stomach cramping, belching, stomach ache, bowel urgency, diarrhoea, vomiting, and stomach bloating. Symptoms were described in lay terms to participants before the experimental trials commenced to ensure that symptoms were reported consistently. Aggregated GI symptom scores were calculated, as well as the highest GI symptom reported post-consumption.

Statistical analysis

Data normality was assessed using the Shapiro–Wilk test and by visual inspection of the normality plots (Grafen & Hails, 2002). Blood acid-base (HCO_3^- and pH) profiles were analysed using two-way (trial

× time) analysis of variance (ANOVA) with repeated-measures. A correction factor (Huynh-Feldt) was applied when Mauchly's test indicated that the sphericity assumption was not plausible. One-way ANOVA with repeated measures were used to compare all other variables (GI symptom scores, T_{lag}, C_{max}, ΔC_{max}, T_{max} and AUC) between trials. Where a significant main-effect was shown, Sidak-adjusted post hoc tests were used for pairwise comparisons. Effect sizes were reported as partial eta-squared (ηp^2) for one- and two-way ANOVA (Cohen, 1988), whereas Hedge's *g* (± 95% confidence intervals (CI)) were calculated for paired comparisons (Lakens, 2013). The α-level of statistical significance was set at $P < 0.05$. Data were analysed using the Statistical Package for the Social Sciences (SPSS®) version 25 software.

RESULTS

Acid–base balance

There were significant increases in blood HCO_3^- concentration ($F = 142$, $P < 0.001$, $\eta p^2 = 0.90$) in the bicarbonate and citrate conditions compared with pre-consumption, with no change in placebo (Figure 1A). Blood HCO_3^- concentration was highest at 140 min ($P < 0.001$) in the bicarbonate (mean difference = 5.3 mmol·L^{-1}; 95% CI 3.9–6.8 mmol·L^{-1}) and 170 min ($P < 0.001$) in the citrate (mean difference = 3.5 mmol·L^{-1}, 95% CI 1.9–5.0 mmol·L^{-1}) conditions compared with pre-consumption. Blood [HCO_3^-] concentration was significantly higher in the bicarbonate condition ($F = 142$, $P < 0.001$, $\eta p^2 = 0.92$) compared with the citrate condition ($P < 0.001$), with a significant condition × time interaction ($F = 31.7$, $P < 0.001$, $\eta p^2 = 0.73$; Figure 1a); blood bicarbonate was significantly higher for bicarbonate compared to citrate from 30 min to 170 min post-ingestion.

[INSERT FIGURE 1 NEAR HERE]

Blood pH increased in the bicarbonate and citrate conditions ($F = 21.1$, $P < 0.001$, $\eta p^2 = 0.64$) compared with pre-consumption (Figure 1b) , with no change in placebo, The highest values occurred at 110 min ($P < 0.001$) in the bicarbonate and 170 min ($P = 0.007$) in the citrate conditions. The ingestion of sodium

bicarbonate or citrate had a significant effect on blood pH (F = 51.2, $P < 0.001$, $\eta p^2 = 0.81$) compared with placebo. Blood pH was significantly higher ($P = 0.040$) with sodium bicarbonate than with citrate (mean difference [MD] = 0.014 AU; 95% CI 0.001–0.027 AU), with a significant condition × time interaction (F = 7.0, $P < 0.001$, $\eta p^2 = 0.37$; Figure 1B); blood pH was significantly higher for bicarbonate compared to citrate at several timepoints post-ingestion.

[INSERT TABLE 1 NEAR HERE]

Bicarbonate kinetics

Consuming either sodium bicarbonate or sodium citrate had a significant and large effect on all bicarbonate kinetic variables (Table 1). Bicarbonate T_{lag} was significantly longer with citrate (MD = 15 ± 21 min; 95% CI 2–28 min) compared with the bicarbonate, as was T_{max} (22 ± 26 min; 95% CI 6–38 min). Similarly, C_{max} (1.9 ± 1.0 mmol·L^{-1}; 95% CI 1.2–2.5 mmol·L^{-1}), ΔC_{max} (2.1 ± 1.2 mmol·L^{-1}; 95% CI 1.4–2.8 mmol·L^{-1}) and AUC (246 ± 141 mmol·min^{-1}·L^{-1}; 95% CI 161–331 mmol·min^{-1}·L^{-1}) were significantly higher for bicarbonate compared with the citrate condition.

[INSERT TABLE 2 NEAR HERE]

Gastrointestinal symptoms

No GI symptoms were reported following consumption of the placebo. More participants reported GI symptoms in the bicarbonate ($n = 8$) compared with the citrate ($n = 5$) condition (Table 2). Furthermore, GI symptom scores were significantly higher (Figure 2, $P = 0.037$) with sodium bicarbonate (2.6 ± 3.1 AU) than with sodium citrate (1.5 ± 1.8 AU).

[INSERT FIGURE 2 NEAR HERE]

Discussion

This is the first study to investigate and compare the blood-acid base responses and gastrointestinal symptoms to the same dose of sodium bicarbonate and sodium citrate ingested in delayed-release capsules. The outcomes of this work indicate that both sodium bicarbonate and sodium citrate significantly increase blood HCO_3^- using the DR capsule delivery method; however, this response is greater following sodium bicarbonate ingestion when both buffers were ingested using a 0.3 $g \cdot kg^{-1}$ BM dose. This study also showed that HCO_3^- kinetics are prolonged with sodium citrate, potentially due to its larger molecular weight. This larger molecular weight is likely to slow the digestion and absorption of sodium citrate and lead to the delayed blood HCO_3^- response which is of a lower magnitude. The majority of the blood bicarbonate kinetic responses (C_{max}, ΔC_{max}, T_{max} and AUC) were also greater with sodium bicarbonate, except T_{lag}, which was greater for sodium citrate. These data suggest that sodium bicarbonate may be the more favourable buffering supplement to ingest, considering measures of acid-base balance, when ingested using a buffering agent at 0.3 $g \cdot kg^{-1}$ BM, since it induces larger increases in blood bicarbonate (>5 $mmol \cdot L^{-1}$), more likely to result in ergogenic effects (Heibel et al., 2018). This notion is supported by meta-analytical data which showed a significant effect of sodium bicarbonate on exercise outcomes (+1.7% [90% CL ±2.0%]) with a typical dose of 0.3 $g \cdot kg^{-1}$, but no overall effect of sodium citrate (0.0% [–1.3%] albeit with a typical dose of 0.3 $g \cdot kg^{-1}$ (Carr et al., 2011).

In order to achieve higher similar blood HCO_3^- responses, a larger dose of sodium citrate of 0.5 $g \cdot kg^{-1}$ has been suggested to be suitable and potentially tolerated by the GI system (Urwin et al., 2019). These desirable >5 $mmol \cdot L^{-1}$ increases in blood HCO_3^- may explain the efficacy of coinciding exercise with T_{max}, and subsequently therefore with ΔC_{max} (Miller et al., 2016; Gough et al., 2017; Boegman et al., (2020). Sodium bicarbonate is generally considered a more effective ergogenic supplement than sodium citrate (Carr et al., 2011). However, discrepancies may occur due to discrepancies in timing of ingestion relative to exercise (de Salles Painelli & Lancha Junior, 2018). Studies commonly request participants ingest sodium bicarbonate and sodium citrate 90 min prior to exercise. While this appears to be suitable for sodium bicarbonate since peak HCO_3^- concentration occurs around this time (de Oliveira et al.,

2020), it likely takes up to 3 h to reach peak values following sodium citrate ingestion (Urwin et al., 2017; Urwin et al., 2019). Our data support these assertions, since peak HCO_3^- concentration occurred much earlier for sodium bicarbonate, while HCO_3^- only peaked at 170 min with sodium citrate. The pharmacokinetics here suggest that HCO_3^- concentration was decreasing after reaching its peak with sodium bicarbonate, although it remained elevated above concentrations with sodium citrate from 30 min until 170 min post-ingestion. This suggests that although sodium bicarbonate might be considered the more effective supplement when taken 30-170 min prior to exercise, if taken 3 h pre-exercise, there may not be differences between sodium citrate and sodium bicarbonate. In fact, had we continued data collection beyond 180 min, bicarbonate concentration may remain elevated for an extended period with sodium citrate (Urwin et al., 2019).

It is important however, to acknowledge that many previous performance comparison studies investigating both sodium citrate and sodium bicarbonate have not used an individualised ingestion strategy, nor have they suitably quantified the GI symptoms. Establishing which is the most effective ingestion strategy is likely to be highly individualised. Individuals experiencing severe GI symptoms are unlikely to use exogenous buffers prior to competition, and yet most studies have failed to account for this in their evaluation of the ergogenicity of their supplement, which may reduce the overall estimate of the effectiveness of these buffering agents (Deb et al., 2018). Indeed, this work is the first study to compare GI symptoms for both sodium bicarbonate and sodium citrate in the same participants using DR capsules. More GI discomfort was apparent with sodium bicarbonate compared to sodium citrate, although absolute levels of discomfort were relatively low, compared to more traditional ingestion methods (Hilton et al., 2020). Whilst lower levels of discomfort with both supplements were reported than is typically reported with gelatine capsules (Hilton et al., 2020) there were some participants that still experienced quite severe GI symptoms. This variability in GI response is consistent with previous work that has observed considerable inter-individual variability (Gough et al., 2017; Deb et al., 2018) even when DR capsules are employed (Hilton et al., 2019a). Clearly, those individuals susceptible to GI symptoms that may be so pronounced as to be ergolytic, would be unlikely to use such a pre-exercise ingestion strategy.

For those that can use the DR capsule effectively, it likely avoids degradation of the capsule in the stomach, reducing interaction with stomach acids, in turn minimising the uncomfortable bloating in particular. Hilton et al., (2020) have previously shown reduced GI symptoms using DR compared to gelatine capsules following ingestion of 0.3 $g \cdot kg^{-1}$ of sodium bicarbonate in over 90% of participants. Delayed-release capsules have also previously been shown to lead to lower GI symptoms with sodium bicarbonate ingestion when compared to aqueous solution (Hilton et al., 2019a; Hilton et al., 2019b). Greater discomfort with sodium bicarbonate might also be associated with the different molecular masses of the respective compounds; the lighter sodium bicarbonate compound (84.007 $g \cdot mol^{-1}$) might suffer quicker breakdown in the gut compared to the heavier sodium citrate (258.07 $g \cdot mol^{-1}$), causing this discomfort. These differences must be taken with caution, however, since the literature suggests that a 0.5 $g \cdot kg^{-1}$ dose of sodium citrate might be considered more effective to increase bicarbonate concentration and improve performance (McNaughton, 1992). Future studies should investigate a variety of doses in order to determine if the use of DR capsules to deliver sodium citrate alters the pharmokinetic responses.

A limitation of this study is that we only analysed the pharmacokinetics of blood HCO_3^- and pH changes following sodium bicarbonate and sodium citrate supplementation in a fasted state. Whilst we see this as an important first study in determining the likely effects of these exogenous buffers consumed in DR capsules, it is likely that ingestion with a high carbohydrate meal would further reduce the severity of GI symptoms (Carr et al., 2011). Furthermore, the ingestion of a pre-exercise meal is also likely to more accurately reflect the pre-competition/training routine of athletes. We also wanted to determine the pharmacokinetics following administration of identical 0.3 $g \cdot kg^{-1}$ doses of sodium bicarbonate and sodium citrate but appreciate that a 0.5 $g \cdot kg^{-1}$ dose of sodium citrate might be considered optimal for HCO_3^- and performance changes (McNaughton, 1992). Therefore, future work should investigate the effects of these buffers delivered in DR capsules, at a variety of doses, following the ingestion of a pre-exercise meal strategy more likely to be used by athletes.

Conclusion

This study investigated the pharmokinetic and GI symptom effects of ingesting sodium bicarbonate and sodium citrate contained in delayed-release capsules at the same 0.3 $g \cdot kg^{-1}$ BM dose. Ingestion of sodium bicarbonate using this ingestion strategy resulted in a greater change in blood HCO_3^- compared to sodium citrate, but both exogenous buffering substances significantly alter HCO_3^- values. Ingestion of sodium citrate resulted in a smaller number of GI issues albeit at a dose that is smaller than previously reported to be tolerable and ergogenic. Future work should now focus on implementing dose responses studies, following feeding, prior to exercise.

Competing Interest Statement

The authors declare that they have no competing interests.

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Figure Legends

Figure 1. Mean (±SD) **A** blood [HCO_3^-] and **B** pH following the consumption of 0.3 g·kg^{-1} body mass sodium bicarbonate, sodium citrate or a placebo (cornflower). *Denotes significant difference between sodium bicarbonate and sodium citrate ($P < 0.05$).

Figure 2. Mean (±SD) gastrointestinal symptom scores following the consumption of 0.3 g·kg^{-1} body mass sodium bicarbonate and sodium citrate. *Denotes significant difference between conditions ($P < 0.05$).

Table Legends

Table 1. Mean (SD) bicarbonate kinetic variables following the consumption of 0.3 g·kg^{-1} body mass sodium bicarbonate and sodium citrate.

Table 2. Overall and maximal gastrointestinal (GI) symptoms experienced for each participant following the consumption of 0.3 g·kg^{-1} body mass sodium bicarbonate and sodium citrate. Symptom severity scores are displayed in ( ). Time or time range of maximal symptom are displayed in [ ].

Table 1

Variable	Sodium Bicarbonate	Sodium Citrate	*P*-value	Effect size [95%CI]
T_{lag} (min)	30 ± 8	45 ± 19	0.024*	1.0 [0.2, 1.9]
C_{max} (mmol·L^{-1})	30.9 ± 1.2	29.0 ± 0.7	< 0.001**	1.9 [1.0, 2.8]
ΔC_{max} (mmol·L^{-1})	5.9 ± 0.7	3.8 ± 0.9	< 0.001**	2.5 [1.5, 3.6]
T_{max} (min)	123 ± 17	145 ± 28	0.010*	0.9 [0.1, 1.7]
AUC (mmol·min^{-1}·L^{-1})	5165 ± 168	4919 ± 109	< 0.001**	1.7 [0.8, 2.6]

Notes: T_{lag}, lag time; C_{max}, peak bicarbonate concentration; ΔC_{max}, change in peak bicarbonate concentration; T_{max}, time to peak bicarbonate concentration; AUC, area under the curve. Effect size is reported using the Hedge's *g* correction.

Table 2

	Sodium Bicarbonate		Sodium Citrate	
Participant (#)	**GI Symptoms Experienced**	**Maximal GI Symptom**	**GI Symptoms Experienced**	**Maximal GI Symptom**
1		No symptom (0)		No symptom (0)
2	N/SC/SA/BU	Nausea (3) [100]	N	Nausea (3) [30]
3	B	Belching (2) [30]	F	Flatulence (3) [90]
4	BU/D	Bowel urgency (7) [90]	BU	Bowl urgency (5) [70-90]
5		No symptom (0)		No symptom (0)
6		No symptom (0)		No symptom (0)
7		No symptom (0)	N/F	Nausea/Flatulence (2) [60]
8		No symptom (0)		No symptom (0)
9	D/F	Diarrhoea (6) [70]	F	Flatulence (2) [30]
10		No symptom (0)		No symptom (0)
11	N/N/SC/B/SA/BU	Diarrhoea (9) [60-80]		No symptom (0)
12		No symptom (0)		No symptom (0)
13		No symptom (0)	F/N/SC/SB/SA	Flatulence (4) [90]

Belching = B; Bowel Urgency = BU; Diarrhoea = D; Flatulence = F; Nausea = N; Stomachache = SA; Stomach Bloating = SB; Stomach Cramping = SC; Vomiting = V.

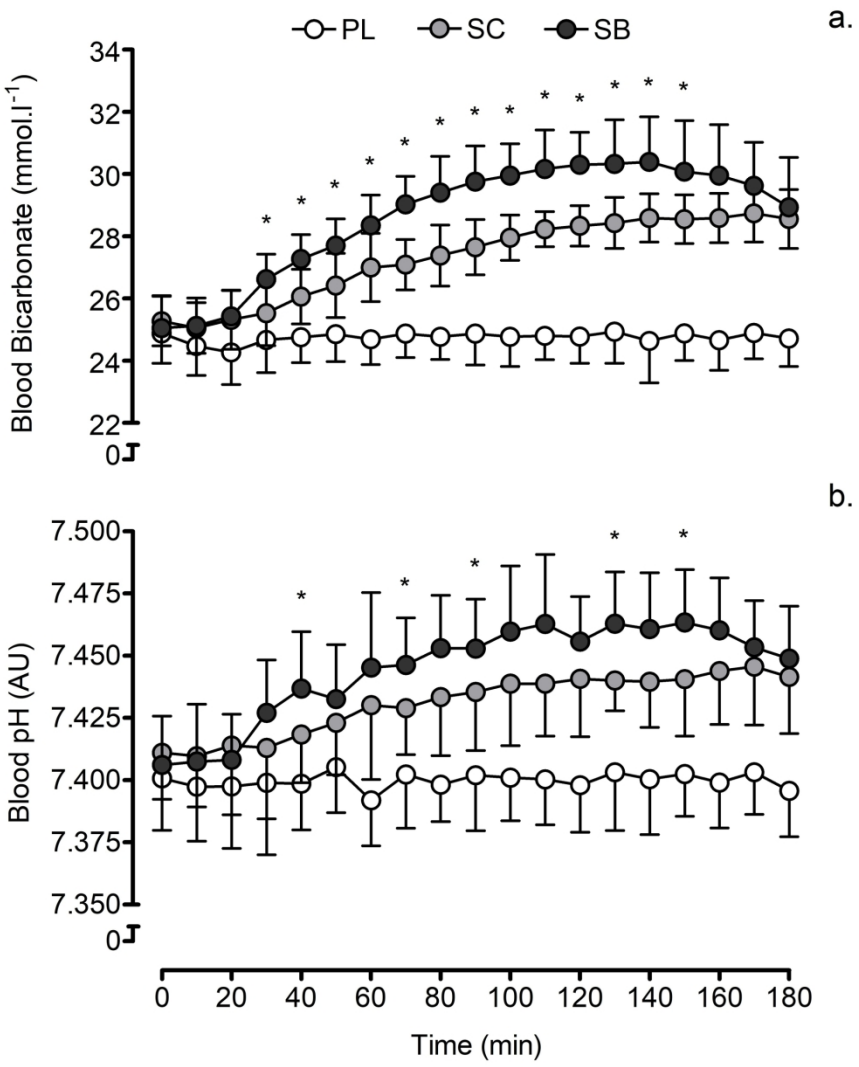

Figure 1. Mean (±SD) a. blood [HCO3–] and b. pH following the consumption of 0.3 g□kg–1 body mass sodium bicarbonate, sodium citrate or a placebo (cornflower). *Denotes significant difference between sodium bicarbonate and sodium citrate ($P < 0.05$).

187x239mm (300 x 300 DPI)

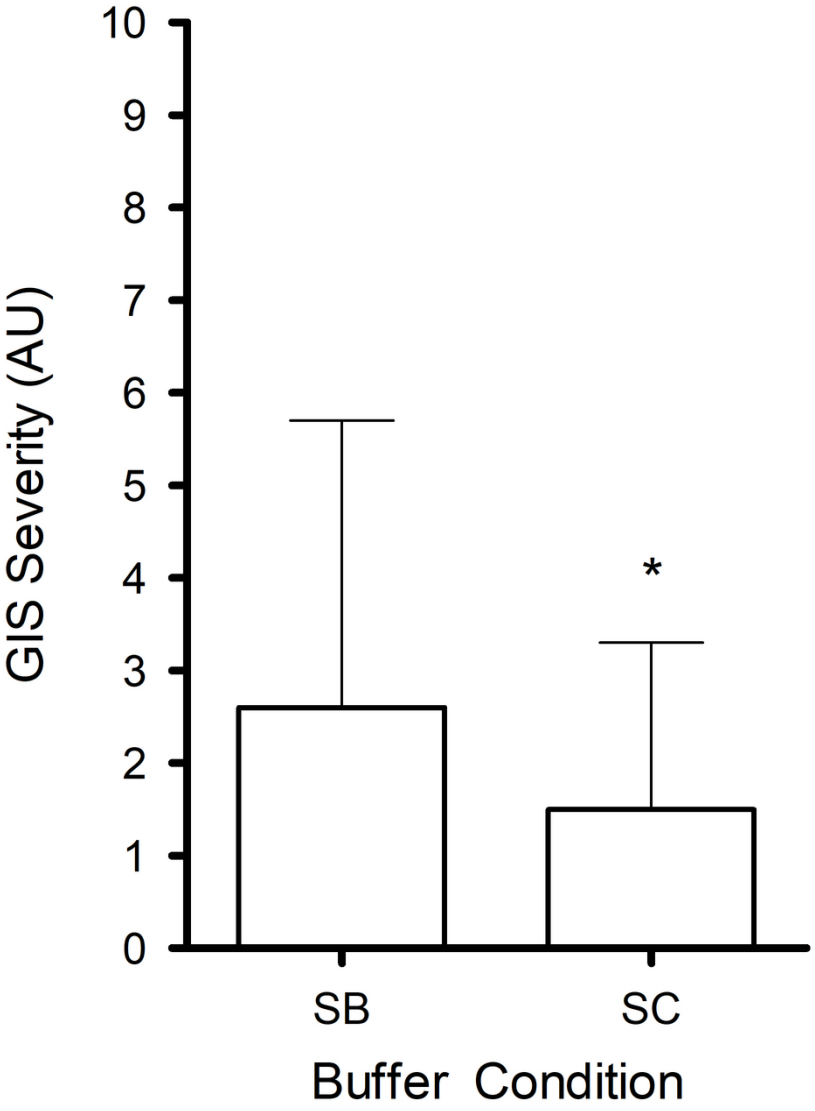

Figure 2. Mean (±SD) gastrointestinal symptom scores following the consumption of 0.3 g□kg −1 body mass sodium bicarbonate and sodium citrate. *Denotes significant difference between conditions (P < 0.05).

94x116mm (300 x 300 DPI)

Response to Reviewer's Comments

We would like to thank the reviewers for their time and efforts invested in reviewing and our manuscript. We have made considerable efforts to consider and revise the manuscript and we now feel that it is much improved. We also provide individual responses to each comment and question raised by the reviewers, which are bullet pointed under each of the comments. All changes to the manuscript have also been highlighted in red font.

Reviewer(s)' Comments to Author:

Reviewer: 1

Comments to the Author
This article concerns the effects of different pro alkalotic loading substances on firstly acid base balance status/ kinetics specifically pH and blood alkalosis and secondly the effects on GI discomfort. The study has the outcome of showing that when moderately trained young participants consume a bicarbonate like supplement either as sodium citrate or sodium bicarbonate the sodium citrate results in lower gastrointestinal discomfort but a lower absolute change in pH and buffering capacity. The study is effective in furthering the research concerning optimisation of bicarbonate like loading strategies to promote clients into the exercise performance. However, it is a question for this reviewer that perhaps the inclusion of an exercise task (line 177) would make the dataset more robust and ultimately sodium bicarbonate loading is used to promote changes in exercise performance. Therefore, the differences shown are needed in context to high intensity exercise performance.

- Thank you for this comment. Yes, we can appreciate that in many studies the approach is to prescribe an extracellular buffering agent and then look at the performance effects. The focus of these studies is exercise performance, not typically pharmokinetics and GI symptoms. However, our primary focus here is to look at the differences in the pharmokinetics of administering both sodium bicarbonate and sodium citrate using delayed-release capsules. The key focus therefore is on establishing if these buffers cause the same or different responses, so that we can use this information in subsequent work. We have already started work on a study using this method that also includes an exercise task. The focus of that study is the application of this study's method/findings on an exercise task that we have previously demonstrated can be improved using sodium bicarbonate in DR capsules. We hope to submit that study for publication later this year. Given both reviewers comments on the context and the introduction we hope that our extensive revision to this section frames the study better and addresses these issues.

Generally speaking, the first part of the introduction requires some context with regards to acid base balance changes with anaerobic exercise and how ergogenic aids such as sodium citrate and bicarbonate will influence exercise performance and metabolism. In addition, there requires some background evidence highlighting the discrepancies and variation of time of ingestion prior to intensity exercise and subsequent changes in alkalinity with those different timing strategies.

- We really appreciate these suggestions, and on reflection, agree that the introduction was a little to abrupt and lacked some context initially. We have therefore included more background information, additional references for evidence of application, context, and trends in current research work on this topic. We feel strongly that the introduction is considerably better following this review and revision. We hope that the

reviewer agrees that this section is much improved and addresses the reviewer's comments and suggestions.

The methodology and statistical analysis is well described.

- We very much appreciate this positive feedback.

Specific comments

Introduction

The first line of the introduction is confusing modify what is meant by 'time to a peak'?

- We agree that this could have been clearer. In light of the other whole scale changes to the introduction, we have also ensured that this comment is address. We have now amended reference to time to peak to ensure that the variables and that this refers to post-ingestion time to the peak changes in pH and HCO3.

The objective statement at the end of the introduction requires modification. This statement doesn't appear to be an informative or accurate statement of study intent merely to 'assess' conceivably there is a comparison between different methods of ingestion of sodium bicarbonate.

- We agree with the reviewer, that the aim could have been clearer. We have now amended the aim to read:

  "Therefore, the aim of this study was to determine the pharmokinetic and gastrointestinal symptom responses to an orally ingested dose of sodium bicarbonate and sodium citrate, administered in delayed release capsules".

Methodology
Line 65 typo 'trial'

- This has now been amended.

Discussion
Lone 157 change to 'symptoms'

- This has now been amended.

Reviewer: 2

Comments to the Author
The current study looked to assess the pharmacokinetics of equal doses of sodium bicarbonate and sodium citrate. While simplistic in design and study measures, the study appears to have been robustly undertaken with sufficient statistical analysis. For that, I wish to congratulate the authors. While it is generally well written, I believe some of the interpretation in the discussion could be re-examined.

- We would like to thank the reviewer for their kind words and comments. It is always good to get a different perspective on a manuscript during the review process and we

recognise that this positively effects the final manuscript. We have therefore taken care to address each od the comments and suggestions.

Introduction

Line 20 - Given the range of time for pre-exercise ingestion, I think “Many” would be more appropriate than “Most”.

- The introduction has been extensively re-written in order to address the comments of reviewer 1. We do however agree that “many” is a more appropriate choice of terms, so we have changed this ass suggested in the revision.
-

Line 25 - need to close the parentheses

- This has now been amended.

Methods
Line 66-67 - for completeness, I believe the range of report times to the laboratory should be reported. Not just that they arrived at the same time for each trial.

- This has now been added to the Experimental Design section of the method.

You have not reported as to what participants did during the three hour measurement period. Did they remain in the laboratory? Did they remain seated, etc?

- This has now been amended to read:

“During the testing period, all participants remained seated. During this time, fingertip capillary blood samples (95 µL) were obtained pre-consumption and then every 10 min for 180 min post-consumption.”

Results

Appears to be an error with the submission as there are two Table 1s (identical though) and two Table 2s (I imagine the correct one is the second one).
Table 2 - columns need slight reformatting. Not all of the rows line up for P4.
Table 2. I don’t understand how P4 can have a range of aggregate GI scores (70-90) for sodium citrate when you’ve only reported one symptom for them.

- Many thanks for pointing this error out. We are a little bemused as to how this has happened to be honest. We have now ensured that only one version (the correct version) of each table has been uploaded for submission. We have reformatted the existing tables to ensure each line is formatted correctly. The date in the square parentheses represents the time range during which the maximal symptom was experienced. We have now amended the table title to make this clearer.

Discussion

Line 168-174 - could this not be interpreted slightly differently? The difference in performance outcomes between bicarbonate and citrate in previous studies may be due to the use of standardised pre-exercise ingestion times. You have already highlighted in the introduction that the times used are often shorter than what may be optimal. I would conclude this paragraph, not by suggesting that bicarbonate may be the better ergogenic aid, but by

suggesting that studies should therefore look to optimise and prescribe timings of each supplement before then studying their comparative efficacy as ergogenic aids.

- The reviewer raises an interesting point here. From a GI perspective, symptoms appear to be slightly less severe with citrate (with some variability), but the likely change in blood bicarbonate is not as high as it is with bicarbonate. Based on previous observations across multiple studies (summarised by Heibel et al., 2018), those studies observed changes in blood bicarbonate of >5 $mmol.l^{-1}$ are most likely to see an ergogenic effect. That said, it is also possible that some of the previous studies have not fully investigated the deleterious effects of severe GI symptoms. Indeed, many studies that do not show ergogenic effects, do not also suitably quantify the GI symptoms at all and only report mean responses. This makes it very difficult to evaluate the likely interaction of ergogenicity and GI symptoms. Reporting individual responses is important since individuals that experience severe GI symptoms (despite strategies to reduce them) are highly unlikely to actually use these ergogenic aids. This is highlighted nicely in the study by Deb et al., (2018) which shows that the only participant to not benefit from sodium bicarbonate ingestion was also the participant with the most severe GI symptoms. Clearly more work needs to be done to investigate these issues further, but we have amended this section of the discussion to reflect these issues.

Line 179-193 - as above. The narrative in this paragraph should weave with that above more effectively. To better highlight your point, it may be pertinent to highlight data from a particular that has used standardised ingestion timings, as well as the pre-exercise bicarbonate concentrations.

- We agree with the reviewer. The original discussion was rather awkwardly phrased. We have made considerable efforts to revise and restructure the discussion in light of the constructive comments following review. We hope that the resubmitted manuscript addresses these concerns appropriately.

Line 196-198 - I would argue that symptom severity was not “low” but instead had large inter-participant variation. The participant that reported a peak score of 9 for diarrhoea probably felt that their symptoms were quite severe, not “low”. I would discuss this variability, and why it might be observed, not that symptoms were mostly low.

- We agree with the reviewer that this point needed a little more context. The original statements in the manuscript are slightly misleading, given the severe GI symptoms observed in a minority of participants. However, the use of DR capsules clearly reduces the severity of GI symptoms (shown by all three studies by Hilton et al.,) compared to more traditional methods of ingestion. That said, without that acknowledgement, a score of 9 is clearly not low. We have therefore revised this section to improve the context and to highlight the inter-individual differences.

Line 195-208 - Given previous data showing reductions in GI symptoms with carbohydrate feeding (e.g. Carr et al., 2011), this should be highlighted here in relation to the fact that your protocol required participants to be fasted. Equally, how feeding would potentially affect any pharmacokinetics should be explored, or at least mentioned.

- We agree with the reviewer that this is an important consideration, that we overlooked in the first version of the manuscript. We have now acknowledged this issue in the discussion and listed the fasted pre-ingestion protocol as an important first step, but

also a potential limitation. We have therefore recommended that future work look at meal ingestion which more accurately reflects pre-exercise nutrient ingestion strategies alongside buffer ingestion.

Line 210 - I disagree slightly here. I do not believe that a study can be limited by something it could have done next. How could you have assessed the effect on exercise performance without rerunning each trial and exercising at the pre-assessed timings? I would use this paragraph to highlight that this is something future studies should do. As well as look at the effect of different doses, and the effect of feeding.

- We agree again with the reviewer. As per our response to the previous comment, the discussion has been revised to reflect actual limitations and distinguish those from future research directions. We hope that this now suitably addresses the reviewer's comments and suggestion.