Sodium bicarbonate ingestion and individual variability in time to peak pH

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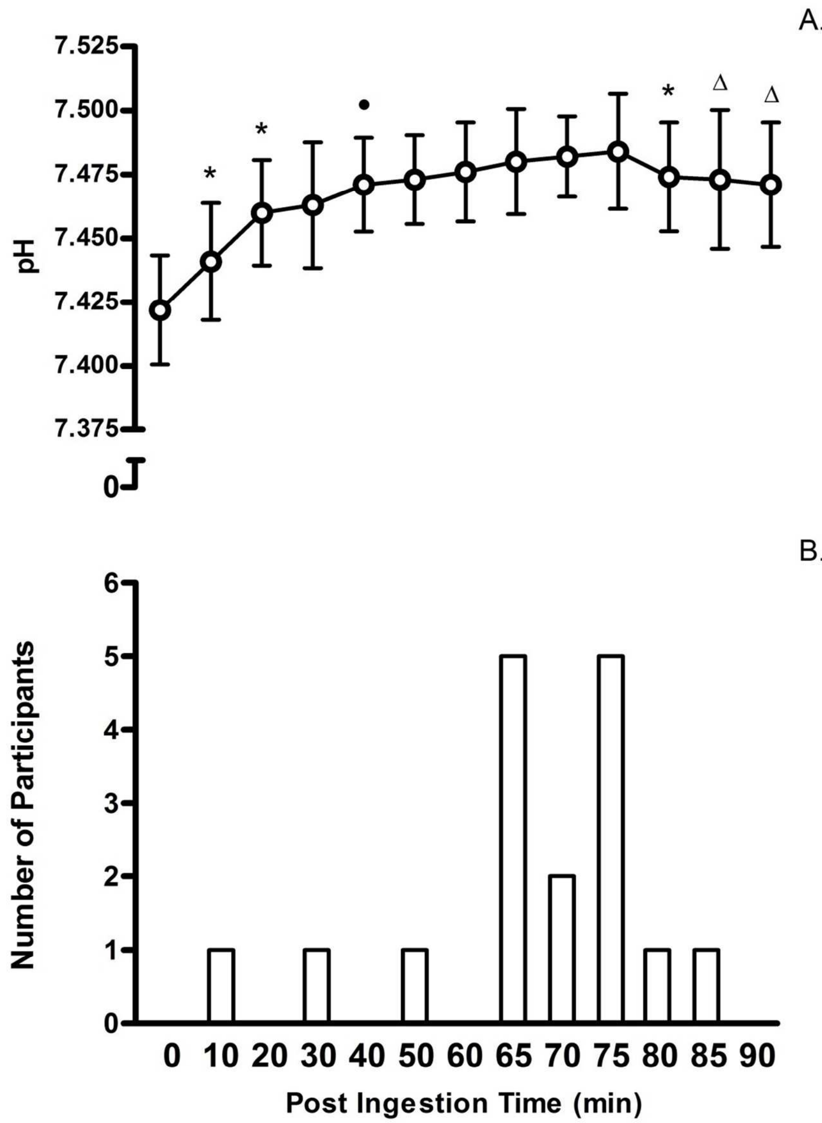

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Abstract

This study determined variability in time to peak pH after consumption of 300mg.kg-1 of sodium bicarbonate. Seventeen participants (mean ± SD: age 21.38 ± 1.5y; mass 75.8 ± 5.8kg; height 176.8 ± 7.6cm) reported to the laboratory where a resting capillary sample was taken. Then, 300 mg.kg-1 of NaHCO3 in 450ml of flavoured water was ingested. Participants rested for 90 min and repeated blood samples were procured at 10 min intervals for 60 mins and then every 5 min until 90 min. Blood pH concentrations were measured. Results suggested that time to peak pH (64.41±18.78 min) was variable with a range of 10-85 min and a coefficient of variation of 29.16%. A bi-modal distribution occurred, at 65 and 75 min. In conclusion athletes, when using NaHCO3 as an ergogenic aid, should determine, their time to peak pH to best utilise the added buffering capacity this substance allows.

Key Words: Performance, individual response, buffering, acidity

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Introduction

Sodium bicarbonate ingestion is **used as a** method of improving buffering against hydrogen ions induced by high intensity short duration exercise. There have been a number of review articles which have reaffirmed its effectiveness as an ergogenic aid when consumed prior to exercise performance lasting up to **10 min** in duration (Mc Naughton, Siegler, and Midgley, 2008; Peart et al 2012; Burke 2013). A relatively recent meta-analysis of the effects of sodium bicarbonate ingestion on high intensity exercise performance suggested that the most effective pre-exercise doses should be between 0.3-0.5g/kg/BM, which is likely to improve mean power by 1.7±2.0% (Carr et al 2011) in appropriate exercise, **such as in high intensity exercise, including repeated sprint activity (RSA), usually lasting less than a total of 10 minutes (Mc Naughton, Siegler, and Midgley, 2008).**

Although some evidence suggests that, at physiological temperatures, direct inhibition of force production by acidification is not as great as previously thought **(Westerblad et al. 1997; Tobias et al., 2013; Wallimann et al., 2011)**, interventions that minimize intracellular H^+ accumulation may improve RSA. H^+ accumulation depends on both the production and removal of H^+. The intra- and extracellular buffer systems act to reduce the build-up of free H^+ during high-intensity exercise and may therefore be important in maintaining repeated-sprint performance. Indeed, Bishop et al., (2003) have reported a significant relationship between RSA and both change in blood pH and *in vivo* muscle buffer capacity (Edge et al. 2002). The intracellular accumulation of H^+ also depends on the extracellular H^+ concentration. H^+ efflux out of the muscle cell has been reported to be inhibited by extracellular acidosis (Hirche et al. 1975) and enhanced by a greater extracellular buffer concentration (Mainwood and Worseley-Brown 1975). It is therefore frequently hypothesized that increases in the extracellular buffer concentration, via the ingestion of an alkaline solution such as sodium bicarbonate ($NaHCO_3$), may improve

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H^+ efflux out of the muscle cell and improve repeated-sprint performance (Miller et al. 2015).

It has been proposed that exercise increases hydrogen cation (H+) production (Hill and Lupton, 1923) thus decreasing intracellular pH (Sahlin 2014) which is a crucial factor in the development of fatigue during and after high-intensity exercise, either by directly affecting muscle contractile properties or by disrupting to muscle energetics (Fitts, 2008, Spriet, Matsos, Peters, Heigenhauser, Jones 1985). Early work in the field of acid base balance by Sutton et al. (1981) studied five males after oral administration of a control, an acidotic or alkalotic substance. Participants exercised on a cycle ergometer for 20 min at 33% VO_{2max}, followed by 20 min at 66% and at 95% VO_{2max} until exhaustion. Endurance at 95% VO_{2max}. was shortest with acidosis, longest with alkalosis and intermediate in the control trial. They concluded that any situation which leads to low bicarbonate levels, for example a preceding bout of exercise, may limit the muscle glycolytic capacity.

It is now however generally accepted that the increased H^+ production causes competition on the ionisable binding sites of the actin / myosin complex, as well as sarcoplasmic reticulum dysfunction with regard to Ca^{2+} release and uptake (Allen, Lamb, Westerblad; 2008; Fitts, 2008; Stephenson, 1998), **which can then lead to fatigue. Hence, attenuating the increase in muscle (and subsequently blood) acidosis should help delay the onset of fatigue during repeated bouts of high intensity exercise.**

Although not conclusive, it appears that increasing the blood buffering potential via $NaHCO_3$ ingestion either creates an electro-chemical gradient between the intra- and extracellular milieu, thus allowing for greater facilitation of proton removal from inside the cell; or sustains Ca^{2+} release and re-sequestering in the sarcoplasmic reticulum by increasing the strong ion difference (Kemp, 2006). Sustaining these mechanisms may

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prolong skeletal muscle function and perhaps maintain exercise performance, but the degree of efficacy in enhancing physical performance remains equivocal (McNaughton Siegler and Midgley, 2008).

As a result of the mechanisms by which this supplement may act to delay fatigue, many laboratory investigations have used a variety of relevant exercise models including running (Bird and Robins, 1995; Goldfinch et al, 1988, Tiryaki and Atterbon, 1995;), cycling (McNaughton et al. 1991; McNaughton 1992a, Miller et al. 2015), boxing and swimming (Siegler and Gleadall-Siddall, 2010; Lindh et al., 2008; Gao et al., 1988) in order to assess its effectiveness. Indeed, such is the wealth of published studies on sodium bicarbonate, that recently, researchers have started to focus on its co-ingestion with other active ingredients such as caffeine (Kilding et al. 2012; Pruscino et al., 2008) and β-Alanine (Saunders et al. 2014b; **Tobias et al., 2013**) in order to assess the potential additive effects in order to provide further performance enhancements via the activation of different ergogenic mechanisms simultaneously.

Given the positive effects reported in the use of $NaHCO_3$ there is also a body of evidence that has found no ergogenic benefit using this supplement (Katz et al., 1984; Horswill et al., 1988; Linderman et al., 1991; Driller et al., 2013; Higgins et al., 2013; Saunders et al., 2014), which probably contributes to its small effect (1.7 ± 2.0%). The absence of an ergogenic effect in these studies is not yet fully understood and has multiple possible causes: administration of different doses (Horswill et al., 1988); employment of exercise models that are not limited by intramuscular acidosis (Linderman and Fahey, 1991; Linderman and Gosselink, 1994); individual variation in the blood responses to supplementation (Price & Simons, 2010; Saunders et al, 2014.); and gastrointestinal upset (McNaughton Siegler and Midgley, 2008

Interestingly, there is some variety in the timing of pre-exercise administration in the literature which typically ranges from 60-90 min (Egger et al. 2014; Higgins et al. 2013; Christiensen et al 2014; Martiott et al 2015). In some cases a multiple acute dose has been used starting at 90 mins and continuing until 50 min pre-exercise (Krustrup et al. 2015) or more chronic supplementation across several days (Mueller et al. 2013). This range of pre-exercise ingestion times are likely to influence the effectiveness of the supplement and therefore the magnitude of the potential performance benefits which are reported. Presently there is no standardised pre-exercise ingestion time which has been determined as most effective, and there are also some suggestions that training status, diet and activity may affect buffering capacity. We hypothesize that these factors lead to considerable inter-individual variation in the time at which optimal buffering may occur following the ingestion of supplements designed to alter the pH of the blood. Therefore the aim of this experiment was to determine the variability in individual responses to a single bolus of sodium bicarbonate.

Methods

Participants

Seventeen male active team and individual sports participants (mean ± SD: age 21.38 ± 1.5y; mass 75.8 ± 5.8kg; height 176.8 ± 7.6cm) volunteered to take part in the study. All participants were familiar with high-intensity exercise and on took part in a minimum of two hours of intermittent team or individual sporting activity per week. All participants were informed of both the benefits and the potential side effects associated with the study (both verbally and in writing), before they provided written informed consent and then underwent screening. The study was approved by the institutional Departmental Ethics Committee. **Following health screening, all individuals were deemed free of any illness likely to affect performance, for example, asthma and cardiovascular**

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disease and no individuals were taking any performance enhancing supplements and had never done so.

Procedures

The participants attended the laboratory once in order to obtain basic anthropometric measurements and to determine each individuals resting blood pH responsiveness to $NaHCO_3$ ingestion. Following the screening and anthropometric data collection participants ingested 300 mg·kg^{-1} (BM) of $NaHCO_3$ taken in 400 ml of water with 50 ml of flavoured cordial (Robinsons Fruit Squash, UK). This method has previously been used by Price et al., (2003) as it has been shown to improve drink palatability (Lavender and Bird, 1989). Participants were asked to refrain from maximal exercise, to maintain a typical diet and avoid consuming alcohol and beverages other than water for the 24 hour period prior to their laboratory trial in order to minimise disturbances to normal acid-base status (McNaughton et al 2011, Bishop et al 2004, Lavender and Bird, 1989). **At this visit participants were also asked to replicate their pre-trial diet at each subsequent visit.**

At the visit, participants reported to the laboratory where a 300 µl resting capillary blood sample was taken aseptically from the fingertip. **Their pre-trial diet was then reported and checked prior to the commencement of each trial.** The participants then consumed 300 mg·kg^{-1} of $NaHCO_3$ in 400ml of water with 50ml of flavoured cordial within a 5 min period. This dose has previously been found to improve individual anaerobic performance (Goldfinch et al., 1988; McNaughton, 1992; McNaughton and Cedaro 1992; McNaughton et al., 1991) as well as repeated sprint performance (Bishop et al 2004; Gaitanos et al., 1990; Lavender and Bird, 1989) in men and women (McNaughton et al 1997). Participants then rested quietly for a 90 min period following the completion of ingestion. During this time additional capillary blood samples were procured at 10 min intervals for the first 60 min and then at 5 min intervals until 90 min. Blood pH concentrations were measured using a blood gas analyser (Radiometer

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ABL800, Denmark). The instrument was calibrated prior to and after each test sessions as well as after every 10 samples. In our laboratory the instrument has a CV of 1.2%.

Statistical Analysis

All data were assessed for normality using standard graphical methods prior to analysis (Grafen and Hails 2002). Blood pH responses over the post ingestion period were assessed using repeated measures ANOVA. Post hoc pair-wise comparisons were made using a Bonferroni adjustment and statistical significance was assumed as $p < 0.05$. Calculations of effect sizes were done using partial eta squared (ηp^2) for ANOVA. The conventional interpretations of Cohen (1988) were used to evaluate effect sizes where < 0.20 = trivial, 0.20-0.49 = small, 0.50-0.79 = moderate, and large ≥ 0.80 = large. All data are presented as mean ± SD and were analysed using SPSS v22 for Windows (SPSS Inc., Chicago, IL, USA).

Results

The ingestion of the sodium bicarbonate bolus had a significant effect on pH (**F = 16**, $p < 0.001$, $\eta p^2 = 0.50$). Indeed the post ingestion pH values were all significantly **greater** than the pre-ingestion sample ($p < 0.05$). Most notably there was a significant increase in pH at the 10 ($p = 0.007$) and 20 min ($p < 0.001$) sample points compared to the pre ingestion values (Figure 1A). There was a further increase in pH after 40 min compared to the 20 min value ($p = 0.01$) after which pH did not significantly change until a decrease occurred between 75-80 min ($p = 0.03$). There were further significant decreases in pH between 75-85 min ($p = 0.006$) and 75-90 min ($p = 0.018$). Mean time to peak pH was 64.41±18.78 min with a coefficient of variation of 29.16%. Furthermore between subject effects analysis revealed that there was significant variation in the pH responses ($F = 5830237$, $p < 0.001$, $\eta p^2 = 1.00$). The times to peak pH to determine the optimum loading period strategy, are shown in Table 1 with the range of times

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spread between 10-85 min (Figure 1B). Time to peak pH frequency was bi-modally distributed between 65 and 75 min. Peak pH achieved was not correlated to weight, with a low correlation (r=0.07, p=0.79) and neither was weight correlated to change in minimum-maximum pH achieved, r=0.124, p=0.64).

Discussion

The results of this study suggest that after ingestion of a bolus of $300 mg \cdot kg^{-1}$ body mass of sodium bicarbonate, the time to reach peak pH is variable, with a range of 10-85 min. This suggests that when used as an ergogenic aid to improve sprint performance, in studies that have either used 60 or 90 min after ingestion (**see Mc Naughton, Siegler, and Midgley, 2008**), the time lag is probably too short (60 min) or too long. This is supported by the fact that the mean time to peak pH, across all subjects is 65.0±18.4 min, conforming that an exercise time of 60-90 min post ingestion is either too short, or too long respectively. Hence, this would then suggest that these subjects are not making the most of the possible ergogenic, buffering capacity allowed by the ingestion of $NaHCO_3$.

As the correlation between body mass and peak pH was low (r=0.07), it is not possible to predict pH on the basis of a subject's body mass. This could have been a simpler method to estimate approximate peak pH, but it is in itself, not surprising given that the sodium bicarbonate ingestion is based on a body mass relationship.

This study supports the previous work of Price and Singh (2008) who examined increases in blood pH and bicarbonate concentration after ingestion of an NaHCO3 solution. They found, similar to this work, that peak blood pH and bicarbonate concentration occurred between 60 and 90 min. Values then decreased over the remainder of the ingestion period though, like the results of this study still remained elevated above pre ingestion levels.

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In conclusion, researchers, athletes and coaches should endeavour to undertake testing to ensure that if sodium bicarbonate is being used as an ergogenic aid, that their time to peak pH is known so that performance can be maximised at the time when peak pH is achieved.

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Table 1. Individual responses to ingestion of 300mg·kg-1 sodium bicarbonate

	Time (mins)												
Participant	**0**	**10**	**20**	**30**	**40**	**50**	**60**	**65**	**70**	**75**	**80**	**85**	**90**
1	7.409	7.433	7.441	7.465	7.485	7.49	7.495	7.495	7.489	7.496	7.499	**7.528**	7.520
2	7.410	7.431	7.453	7.476	7.485	7.473	7.467	7.483	7.506	**7.517**	7.512	7.508	7.498
3	7.390	7.429	7.452	7.459	7.475	7.475	7.497	7.485	7.491	**7.518**	7.482	7.513	7.489
4	7.429	7.472	7.462	7.443	7.472	7.447	7.495	**7.517**	7.509	7.501	7.499	7.478	7.476
5	7.431	7.45	7.457	7.477	7.468	7.47	7.472	**7.484**	7.484	7.468	7.465	7.463	7.448
6	7.388	7.416	7.448	7.429	7.466	7.468	7.457	7.460	7.465	7.477	**7.484**	7.450	7.450
7	7.414	**7.482**	7.480	7.474	7.473	7.468	7.461	7.459	7.454	7.451	7.447	7.445	7.442
8	7.411	7.454	7.457	7.460	7.481	7.477	7.494	**7.518**	7.494	7.513	7.495	7.503	7.498
9	7.439	7.459	7.471	**7.486**	7.468	7.456	7.476	7.465	7.472	7.471	7.457	7.465	7.469
10	7.454	7.474	7.494	7.488	7.508	7.48	7.500	**7.501**	7.496	7.483	7.486	7.473	7.472
11	7.42	7.434	7.454	7.428	7.439	7.469	7.453	7.451	**7.471**	7.449	7.455	7.44	7.471
12	7.468	7.452	7.516	7.521	7.507	**7.516**	7.510	7.459	7.462	7.466	7.443	7.445	7.44
13	7.417	7.423	7.441	7.445	7.45	7.44	7.457	**7.465**	7.462	7.463	7.451	7.446	7.441
14	7.447	7.407	7.448	7.482	7.457	7.486	7.48	7.477	**7.486**	7.465	7.485	7.461	7.49
15	7.403	7.406	7.456	7.438	7.46	7.464	7.444	7.462	7.481	**7.487**	7.448	7.452	7.441
16	7.431	7.444	7.461	7.447	7.46	7.488	7.477	7.497	7.487	**7.509**	7.474	7.49	7.487
17	7.419	7.426	7.431	7.446	7.454	7.467	7.464	7.483	7.477	**7.49**	7.48	7.481	7.477
Mean	**7.422**	**7.441**	**7.460**	**7.463**	**7.471**	**7.473**	**7.476**	**7.480**	**7.482**	**7.484**	**7.474**	**7.473**	**7.471**
SD	**0.021**	**0.023**	**0.021**	**0.025**	**0.018**	**0.017**	**0.019**	**0.021**	**0.016**	**0.023**	**0.021**	**0.027**	**0.024**

Note: Peak pH is illustrated in bold font.

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Figure 1. Mean (±SD) changes in pH following sodium bicarbonate ingestion (A) and individual participant time to peak pH frequency (B). (*) Denotes a significant increase in pH from the previous time point ($p < 0.01$). (●) Denotes a significant increase in pH from the 20 min sample $p \leq 0.01$. (∆) Denotes a significant decrease in pH from the 75 min sample ($p < 0.05$).

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Sodium bicarbonate ingestion and individual variability in time to peak pH

Manuscript ID GSPM-2015-0169

Author's responses

We would like to thank the reviewers for the time and effort put into the review of our manuscript. We have responded to each of the comments where appropriate (or have given a response as to why we have not changed the manuscript). We have then made changes ot the manuscript and have **bolded** these within the manuscript.

Reviewer 1

Reviewer's report:

This study is an example of how relevant problems/questions in the literature can be solved with simple designs, bringing an overview and approach of an intriguing nutritional strategy in the sports nutrition and exercise physiology fields. Namely, blood pH responses to acute sodium bicarbonate supplementation.

The manuscript is well written, with an interesting introduction and a refined review of the literature on the biochemistry of acidosis-induced fatigue and sodium bicarbonate ergogenic effects. The methods proposed to achieve the objectives are simple but seem appropriate to this reviewer. The discussion could be extended, providing more future perspectives. However, this reviewer has some doubts and notes which require appropriate answers before the manuscript can be accepted, and therefore, a major review is required.

THANKYOU FOR YOUR POSITIVE COMMENTS, WE HOPE THAT WE HAVE ADDRESSED YOUR CONCERNS INT E REVISE DMANUSCRIPT AND WE LOOK FORWARD TO SEEIGN IT IN PRINT IN THE NEAR FUTURE.

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Page 4, 1st paragraph - At this paragraph, the authors provided a very brief and appropriate overview of the mechanisms by which muscle acidosis could induce muscle fatigue. This reviewer would also appreciate in this paragraph a brief inclusion and description of how H+ accumulation could interfere with the energy production process, inhibiting key glycolytic enzymes (see *Sutton, J.R., Jones, N.L., and Toews, C.J. 1981. Effect of PH on muscle glycolysis during exercise. Clin. Sci., v. 61, n. 3, p. 331–338*), as well as the recovery of phosphorylcreatine (see *Harris, R.C., Edwards, R.H., Hultman, E., Nordesjo, L.O., Nylind, B., and Sahlin, K. 1976. The time course of phosphorylcreatine resynthesis during recovery of the quadriceps muscle in man. Pflugers Arch., v. 367, n. 2, p. 137–142*).

We have added a short paragraph to the Introduction and have cited the Sutton paper (above) as well as some other. We have not used the Harris et al. (1976) paper as we feel the other papers are a better indication of the work!

Page 4, 2nd paragraph - Another good paragraph including a brief review of the available literature on the ergogenic effects of sodium bicarbonate across different sports modalities. Regarding swimming performance, Gao et al. (1988) included an interval protocol, which is not an usual swimming competition event. I suggest the authors to remove it and reference *Lindh, A.M., Peyrebrune, M.C., Ingham, S.A., Bailey, D.M., and Folland, J.P. 2008. Sodium bicarbonate improves swimming performance. Int. J. Sports Med., v. 29, n. 6, p. 519–523*. As well, Danaher et al. (2014) employed a cross-over design that does not help to visualize the real effect of BA + SB supplementation. I suggest the authors to remove it and reference *Tobias, G., Benatti, F.B., de Salles Painelli, V., Roschel, H., Gualano, B., Sale, C., Harris, R.C., Lancha, A.H.Jr, Artioli, G.G. (2013). Additive effects of beta-alanine and sodium bicarbonate on upper-body intermittent performance. Amino Acids, v. 45, p. 309–317.*

We have left the Gao et al (1988) paper in the manuscript as we are aware of a number of swimmers/athletes, who use sodium bicarbonate in training to accomplish more high intensity work during interval training. We have added the Lindh et al (2008) study as suggested. We have also deleted the Danaher et al. (2014) paper and replaced it with the Tobias et al. (2013) paper.

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Page 5, 1st paragraph - Indeed, sodium bicarbonate has an ergogenic effect. There is plenty of evidence supporting this fact, as the authors already cited in the previous paragraph. However, the authors must agree that there is also a large body of evidence that does not support the positive effects of this supplement (Katz et al., 1984; Horswill et al., 1988; Linderman et al., 1992; Driller et al., 2013; Higgins et al., 2013; Saunders et al., 2014), which probably contributes to its small effect (1.7 ± 2.0%). The absence of an ergogenic effect in these studies is not yet fully understood and has multiple possible causes: administration of different doses (Horswill et al., 1988); employment of exercise models that are not limited by intramuscular acidosis (Linderman et al., 1992); individual variation in the blood responses to supplementation (Price & Simons, 2010; Saunders et al, 2014.); and gastrointestinal discomfort affecting some individuals (McNaughton, 1992). In the present investigation, the authors are based on the hypothesis of the individual variation in blood responses as a putative factor of the misconceptions in the literature. An interesting and recent study published by De Araujo Dias et al. (Plos One - 2015) somehow may support this idea. Specifically, the authors investigated the consistency of blood and performance responses during repeated trials with sodium bicarbonate. And interestingly, despite of an inconsistency in the performance improvement, the mechanism that supports the ergogenic effects of sodium bicarbonate was consistently present in all trials. Perhaps the increased blood pH and bicarbonate levels were increased during the trials, but their peaks in blood were individual and occurred at different moments (according to what the the present investigation seems to suggest)...? Instead of reporting so specifically Christensen et al. (2014), this paragraph would substantially benefit from the discussion here employed by this reviewer, and therefore, I suggest the authors to include it.

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We have included some of the above, as you will see, in the relevant space in the Introduction. We have also removed a paragraph with respect to the work of Christensen et al. (2014), on rowing (with bicarbonate and caffeine) as we are concerned, I think you would agree, that the Introduction is becoming rather long and the other reviewer wished it to be shortened!

Page 6, 1st paragraph - What were the inclusion and exclusion criteria employed by the authors? This should be included. In addition, the paragraph has a final sentence '*Following screening...*' without an end. The authors are required to amend this.
This has been amended, though the inclusion criteria was they simply had ot be involved, as wwe have stated as a team sports player of an individual athlete who were used to activities with repetituive sprint activity./

Page 6, 2nd paragraph - What is the origin and purity of the sodium bicarbonate used in this study? The authors are invited to include information on this issue. Also, I agree with the requests made to the participants (abstinence from alcohol, exercise, ...), but how compliance with this was confirmed by the authors? The standardisation of the diet prior to the supplement ingestion would have been important to prevent variations resulting from the different diets. Since this was not done, did the authors at least promoted a standardised time to consume the last meal before attending to the lab? And the moment of the day that the supplement was ingested, was it standardised?
The sodium bicarbonate is food grade and readily available over the counter (OTC) from any supermarket. It is always used in our work and is important as this will be the method whereby athletes will acquire the substance for their personal use.
We have added some information pertaining to diet which we asked the participants to replicate prior to each trial and we verbally check prior to the trial!

Page 7, 1st paragraph - Most of the studies investigating sodium bicarbonate effects on performance usually examine its effect between 90 and 120 minutes after the supplement ingestion. What was the reason for choosing 90 minutes as a final measure instead of a longer period? Similarly to blood pH, why the authors did not provide the blood bicarbonate data? This would definitely enrich the manuscript. The authors are invited to do it. In addition, do the authors have data on the reliability of the equipment employed in blood analysis?
We are not using the blood bicarbonate data as this is the topic of another paper!
Pilot data on several subjects confirmed that NO pilot subject tested had a peak over 90 minutes. Thus, this time was chosen.
The analyser used in this test is calibrated regularly and has high reliability and a CV of 1.2% in our lab which we have added.

Page 8, 1st paragraph - What was the statistical analysis employed to evaluate the '*between subject effects analysis*'?

Page 8, 2nd paragraph - At this point, the blood bicarbonate data was cited by the authors. Was it analyzed or not? If so, the authors must include it in the manuscript.
We have deleted mention of blood bicarbonate analysis.

Page 8, 3rd paragraph - Here, the authors presented a discussion which seems, at the very least, unfinished. The absence of references for it are proof of that. Therefore, the authors are invited to write a proper discussion, highlighting the original results of this investigation and how they help to overcome the prior limitations in the literature, as well as perspectives for future studies

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Reviewer 2

Comments to the Author
General
Introduction is greatly overlong. I do not think this is the format of the journal. Much of the content does not provided evidence for the rationale, but a more general overview of the area. The title should reflect the content more specifically by stating ‘inter-individual variability’. The intra-individual variability is also of interest, but for future research.
DONE with respect to title The other reviewer wished us to add significantly ot the Introduction which we have included.

Abstract
It may be worth stating the study considers the ’between participant’ variability, otherwise it may be taken as within participant variability.
DONE
Line 2: Could refer to participants as athletes as in line 9? Must be addressed in methods though if group characteristics are actually reflective of trained status.
WE HAVE CHANGED THE TERMINOLOGY TO PARTICIPANTS AS IS THE CASE RATHER THAN ATHLETES

Introduction
Line 6: ‘is used [as] a…’.
DONE
Line 22/23: What is appropriate exercise for Sodium bicarbonate ($NaHCO_3$) to be effective?
DONE
Line 26/27: Old reference (1985) for fatigue being caused by H+ ions. Fatigue is multidimensional and not just / predominantly H+ ions. Please use a more up to date reference. The field of fatigue has moved on since then.
THIS IS A CLASSIC REFERENCE AND WE BELIEVE STILL RELEVANT. HOWEVER WE HAVE ADDED TWO ADITIONAL REFERENCES TO SUPPORT OUR COMMENT.
Paragraph 2 onwards does little to develop the research question which should focus on the duration to peak blood pH values / inter-individual variation in blood pH kinetics following ingestion.
REVIEW 1 LIKES THIS PARAGRAPH SO WE HAVE LEFT IT IN!
Paragraph 3; Line 28/29. Here the authors are quite correct, the evidence is equivocal. Prior to this statement it seems that the authors are of the view that ingestion of $NaHCO_3$ is predominantly positive re performance.
INDEED, WE DO BELIEVE THAT FOR MOST ATHLETES SODIUM BICABONATE IS EFFECTIVE UNDER THE RIGHT CIRCUMSTANCES.
Paragraph 4: Considers exercise models / co-ingestion, the current study is pre-exercise so not relevant .
WE DISAGREE AS THIS PARAGRAPH DISCUSSES (BRIEFLY) THE DIFFERENT MODELS WHICH HAVE BEEN USED TO ATTENUATE FATIGUE, WE HAVE SIMPLY USED THE CO-INGESTION ROUTINE TO SPECIFY THE DIFFERENT MODELS AND THEORIES PROPOSED.
Paragraph 5: Again this appears off topic.
The authors need to critique previous studies for whether there was a performance enhancement and what the ingestion times actually were. So, did previous studies miss the potential peak in blood pH – is this necessary for improved performance, how do blood values reflect inter-cellular values?
The authors will benefit from including two studies (Price and Singh, 2008, Renfrew 2008, both in the International Journal of Sports Physiology and Performance). These two studies have effectively already presented the optimal ingestion times / time course of blood pH following ingestion of $NaHCO_3$. The novelty of the current study is the greater number / more frequently taken blood samples for greater precision re peak blood pH, and the

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consideration of the individual differences. These points are of importance to those researchers in the area of NaHCO3 efficacy and should be developed in the rational for this study.

SEE BELOW FOR PRICE AND SINGH (2008)

Method

Paragraph 1: Last sentence is incomplete.

DONE

What anthropometry was undertaken and why? This has not been considered as a factor affecting absorption of NaHCO3 / dosage etc. in the rationale. Anthropometry is also not noted in the results and only superficially in the discussion.

WE SIMPLY USE THIS TO GIVE ADDITIONAL DATA TO THE PARTICIPANTS

Why use the previously standard fixed volume of fluid. Most authors in the field may have begun with the approach but subsequently adopted fluid per kg of body mass. The possible range of concentrations can be calculated per body mass and noted in the discussion.

WE DO NOT FEEL THIS IS RELEVANT AND IN REAL TERMS MAKES NO DIFFERENCE TO THE RESEARCH AND INDEED IN OUR COMBINED 40 YEARS OF EXPERIENCE WORKING WITH SODIUM BICABONATE, MAKES NO DIFFEENCE WHATSOEVER TO THE OUTCOME!

Were the HCO3 concentrations and base excess values considered? Please add if data was collected (as intimated at the end of the results) or state why if not.

THAT DATA IS SIMPLY SUPERFLUOUS TO THE WORK HERE.

There were a large number of capillary blood sample, was there any participant discomfort? There are many more samples than expected for, for example, lactate threshold testing etc.

NO, NO SUBJECT COMPLAINEDABOUT THE TESTINGPROCESS AND ALL SIGNED INFORMED CONSENT AND NO SUBJECTS DROPPED OUT OF THE STUDY

Results

Use the descriptor ‘greater’ rather than ‘higher’.

DONE

Pg 8, line 24/25: is the decimal for the f value really needed?

DONE

How many participants experience gastrointestinal distress? This is important for such studies providing ingestion recommendations and is usually reported for NaHCO3 studies.

WHIST WE AGREE WITH YOU RE: GI UPSET, THI SIS OFF TOPIC FOR THIS PAPER AND WE ARE CURRENTLY WRITING A PAER WITH RESPECT TO ONLY GI UPSET AFTER SODIUM BICABONATE INGESTION WHICH WILL FULY DEAL WITH THE ISSUE

How could the ratings of gut fullness and/or abdominal discomfort as used in other studies be helpful here? It may help validate them as in indirect measure or help determine responders or non –responders?

Discussion

The discussion is currently very brief and should be far more critical (see the points already noted above) The results should really be compared to the Price and Singh and Renfrew studies (also showing peak between 60-90 minutes).

WE BELIEVE A LONG DISCUSSION IS NOT NECESSARY SINCE THE WORK SUPPORTS PREVIOUS WORK HOWEVER, WE AHEV ADDED THE PRICE AND SINGH PAPER THOUGHW E WERE UNABLE TO FIND RENFREW IN 2008!

The authors have not closely proof read the manuscript as they have left their reference reminders (‘REFS’) in the text.

DONE

I think that the 65-75 minute window is quite short when the intra-individual variation has not been assessed. Do the authors have any views ono intra-individual variability?

OUR WORK WAS BASED ON A SMALL PILOT STUDY, BUT IN ANSWER TO YOUR QUESTION, NOT AT THIS STAGE THOUGH WE ARE CURRENTLY UNDERTAKING A STUDY WITH ATHLETES OVER A FIVE DAY PERIOD (5 TIME IN 10 DAYS) AND MEASURED FOR 90 MIN WITH THE SAME PRE-TEST DIETARY INTAKE.

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There is a lone sentence regarding correlation with body mass – this should be in the results – but needs some greater explanation and discussion.

CHANGED

How practical is the authors' recommendation that researchers, athletes and coaches should undertake testing – only to former would likely have access to the appropriate facilities (likely what was meant). In my experience, I am not convinced that the intra-individual variation is that good or consistent, hence why NaHCO3 ingestion studies vary so much and are generally equivocal.

THERE ARE A NUMBER OF REASOBALY CHEAP BLOOD GAS ANALYSERS (RADIOMETER) AS WELL AS BLOOD pH METERS (HORIBA) NOW AVAILABLE WHICH WOULD HELP!

If your participants were athletes, would NaHCO3 be beneficial with the known improved cellular buffering capacity in this group?

THESE ARE ATHLETES ALBEIT RECREATIONAL, IMPROVED MUSCLE BUFFERIGNIS ONLY LIKELY TO OCCUR WITH ELITE, INDIVIDUALS WHO UNDERTAKE LARGE VOLUME HIGH INTENSITY TRAINING

How close are blood pH values and intracellular values?

DIFFICULT TO SAY IN REAL TERMS AND AT TH EMOMENT THERE IS NO EVIDENCE THAT SODIUM BICABONATE BUFFERS INTRACELLULARLY

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