Edge Hill University

# Sodium Citrate: Refining Ingestion Protocols To Better Understand Its Ergogenic Potential

By

**Daniel James Tinnion**

A thesis submitted in partial fulfilment of the requirements of Edge Hill University for the degree of Doctor of Philosophy.

November 2022

**Acknowledgements**

Firstly, I would like to thank my director of studies Lars McNaughton. Thank you for giving me a shot at the start of this journey and, for having the patience to deal with me to the end. You have consistently supported me both professionally and personally, something which I will take with me into all my future endeavours.

I would also like to express my gratitude to my supervisor, Andy Sparks. Your qualities as a physiologist have had a substantial impact on my work, both as a teacher and a researcher. Whilst I am sure students I teach and will teach do not always enjoy my attempts to improve every detail, I hope that they grow to be thankful for it, as I am to you. You are a great example of what and who you can still be in this field.

Broadly I would like to thank my colleagues, GTA's and staff alike. Whilst there is too many to acknowledge independently, you have all made this a more memorable experience to say the least. I have drawn a great deal from you all both professionally and personally. You all know who you are.

Finally, I would like to give thanks to my family. To mam and dad, I have never wanted for your support or questioned your love for me, even when I have not been at my best. I never forget how fortunate I am to have had parents like you and draw strength from it every day. To my sister, whilst I believe we may always be opposites, I hope you know how much I wish I could be more like you.

This is not my work alone, many people have given me a great deal over the years, often without anything in return. It has always been about giving that back to you all.

**Abbreviations**

| | |
|---|---|
| $\Delta C_{max}$ | Change in peak blood bicarbonate concentration |
| α | Cronbach's alpha |
| $\eta_p^2$ | Partial eta squared |
| μL | microliters |
| A | Conjugate base |
| ADP | Adenosine diphosphate |
| ANOVA | Analysis of variance |
| ATP | Adenosine triphosphate |
| AU | Arbitrary units |
| AUC | Area under the curve |
| BB | Base excess |
| BM | Body mass |
| $Ca^{2+}$ | Calcium |
| CHO | Carbohydrate |
| CL | Confidence limits |
| $Cl^-$ | Chloride |
| $C_{max}$ | Peak blood bicarbonate concentration |
| $CO_2$ | Carbon dioxide |
| CON | Control |
| CrI | Credible intervals |
| CV | Coefficient of variation |
| *d* | Cohen's d |
| Delayed-release | DEL |
| Enterically-coated | ENT |
| Gelatine | GEL |
| GI | Gastrointestinal |

| | |
|---|---|
| GIS | Gastrointestinal symptoms |
| $H^+$ | Hydrogen ions |
| $H_2CO_3$ | Carbonic acid |
| HA | Weak acid |
| $HCO_3^-$ | Bicarbonate |
| HCl | Hydrochloric acid |
| HSD | Honestly significant difference |
| ICC | Intraclass correlation coefficient |
| iEMG | Imaging electromyography |
| $K^+$ | Potassium |
| kJ | Kilojoule |
| $La^-$ | Lactate |
| MAPE | Absolute percentage differences between measurements |
| MDC | Minimal detectable change |
| mEq | Milliequivalents |
| mmHg | Millimeter of mercury |
| mmol | Millimole |
| MCT | Monocarboxylate transporters |
| $Na^+$ | Sodium |
| $NAD^+$ | Nicotinamide adenine dinucleotide |
| NEAP | Net endogenous acid production |
| nM | Nanomoles |
| $O_2$ | Oxygen |
| $PCO_2$ | Partial pressure of carbon dioxide |
| PDH | Pyruvate dehydrogenase |
| $PO_2$ | Partial pressure of oxygen |
| pH | Potential of hydrogen |

| | |
|---|---|
| PFK | Phosphofructokinase |
| $pK_1$ | Apparent dissociation constant |
| PPO | Peak power output |
| ROF | Rating of fatigue |
| RPE | Rating of perceived exertion |
| $RPE_O$ | RPE overall |
| $RPE_L$ | RPE leg |
| RER | Respiratory exchange ratio |
| *S* | Solubility |
| SB | Sodium bicarbonate |
| SC | Sodium citrate |
| SD | Standard deviation |
| $T_{lag}$ | Lag time |
| $T_{max}$ | Time-to-reach peak blood bicarbonate concentration |
| TT | Time trial |
| TTP | Time-to-peak |
| VAS | Visual analogue scale |
| $VO_2$ | Oxygen uptake |
| $VO_{2max}$ | Maximal oxygen uptake |
| $VO_{2peak}$ | Peak oxygen uptake |
| W | Watts |

**Abstract**

Sodium citrate (SC) has been the subject of a relatively large quantity of research over the last 30+ years, centred around its potential as an extracellular buffering agent, which remains unclear at present. The lack of a clear performance benefit following SC use may be partly explained by inefficacious ingestion practices, whereby an ineffective dosage was provided, timing of ingestion pre-exercise was not fully considered and/or other contributing elements of the protocol were overlooked, i.e., ingestion form. To date, a protocol which simultaneously manages many of these elements has not been applied to an exercise scenario. Based on recent research using sodium bicarbonate (SB), an individualised approach to administering buffering agents has emerged as a potential method of maximising the likelihood of a performance effect. This entails supplementing SB (or SC) at a pre-determined time before exercise, allowing exercise to commence at peak pH or bicarbonate ($HCO_3^-$) levels. The utilization of this strategy is dependent on the reproducibility of these blood acid-base responses, which at present, remains undetermined following SC ingestion. Therefore, Study 1 investigated the reproducibility of blood acid-base responses following the ingestion of SC, provided in multiple quantities. This study revealed that pH and $HCO_3^-$ had limited reproducibility, irrespective of ingested dose and as such, it would be difficult to recommend an individualised approach. Despite this, SC induced potentially meaningful levels of alkalosis in the absence of significant gastrointestinal symptoms (GIS), suggesting that a positive post-ingestion response could be applied to exercise, potentially utilising a more generalised approach. Subsequent investigations were completed to elucidate the effect of different forms of encapsulation, as well as the effect of a carbohydrate (CHO) rich meal on GIS primarily, whilst also commenting on the specific effects on blood $HCO_3^-$ kinetics. Study 2 compared three different forms of encapsulation; gelatine (GEL), delayed-release (DEL) and enterically-coated (ENT) capsules. Ingestion form had no significant effect on total GIS experienced, with many participants experiencing no symptoms throughout. Peak GIS typically arose within ~100 min post-ingestion, at a similar time across encapsulation forms and notably, before mean peak blood [$HCO_3^-$] responses. Furthermore, while blood [$HCO_3^-$] was significantly higher with ENT vs. GEL, all forms induced significant changes in [$HCO_3^-$]. Collectively, the need for expensive forms of SC encapsulation was questioned. In study 3, CHO did not notably impact GIS responses or blood $HCO_3^-$ kinetics. Specifically, total GIS experienced remained similar with or without CHO and was minimal throughout. In reference to blood $HCO_3^-$ kinetics, no effect of CHO was observed on blood [$HCO_3^-$] following SC ingestion. Absolute change in $HCO_3^-$ was also comparable, with important increases well beyond reported thresholds for a positive effect. Finally, time-to-peak [$HCO_3^-$] was highly similar. An interesting finding was the emergence of peak GIS, which occurred almost exclusively in the first sample post-ingestion. While more work is required, this appeared to be the result of co-ingesting large quantities of CHO and encapsulated SC simultaneously, which has implications for future use. Taken together, CHO could be consumed alongside SC to individually support CHO metabolism, without impacting the general response to SC, although quantities consumed may provide a challenge. In study 4, findings from the preceding investigations were utilised to test the effect of ingesting 0.5 $g \cdot kg^{-1}$ body mass (BM) SC, provided in GEL capsules and alongside a CHO rich meal (1.75 $g \cdot kg^{-1}$ BM), on repeated 4 km time trial (TT) performance. Specifically, SC was ingested at a generalised time point, aiming to promote a peak [$HCO_3^-$] at commencement of the first TT or, at commencement of the second TT. Ingestion of SC in this manner was compared to a placebo and control, revealing significant effects on blood alkalosis ([$HCO_3^-$] and pH) which did not translate into performance, physiological or perceptual differences.

**Keywords:** Buffering, alkalosis, reliability, encapsulation, individualisation, time-trials, performance.

**Contents**

## List Of Tables

## List Of Figures

# CHAPTER 1 – GENERAL INTRODUCTION

At the elite levels of sport, where physical conditioning is at its peak, experience is extensive, and bespoke, periodized training and nutritional programs are the norm, 'marginal gains' often determine success or failure. For recreational athletes, marginal gains may represent substantial improvements in performance, possibly propelling them to a higher level of competition. To find such gains, athletes continuously seek to exploit every legal advantage available and for this, a role exists for the use of nutritional ergogenic aids (Peeling *et al*., 2018). As Close *et al* (2016) discuss, decades of research have investigated numerous strategies to prepare for competition (e.g., pre-exercise fuelling) (Burke *et al*., 2011), promote competition performance (e.g., carbohydrate feeding) (Stellingwerf and Cox, 2014) and recover from competition (e.g., protein feeding to promote muscle recovery) (Jager *et al*., 2017). Across all these stages of competition, particular research interest has been given to ergogenic aids that may modulate central and/or peripheral components of fatigue (Burke, 2017), namely, creatine monohydrate (Lanhers et al., 2017), beta-alanine (Saunders *et al*., 2017), SB (McNaughton *et al*., 2016) and SC (Urwin *et al.,* 2021b).

Buffering agents such as SB and SC act to enhance extracellular blood buffering by promoting blood alkalosis (marked by increased blood pH and/or [$HCO_3^-$]) (Saunders *et al.,* 2020; Lancha Junior *et al.,* 2015). And augmenting the $HCO_3^-$ buffering systems ability to protect against the excessive production of protons ($H^+$), implicated as a major cause of muscular fatigue (Edwards, 1975, 1981; Fitts, 1994). Of both the aforementioned buffering agents, SB has received significantly more research interest to date, demonstrating a clear performance effect (expressed as percentage change in mean power) of 1.7% (97% CL ± 2.0%) in exercise of 1-10 min in duration vs. an unclear performance effect of 0.0% (97% CL ± 1.3%) following SC ingestion (Carr *et al.,* 2011a). Given the disparity in research volume, the comparative efficacy of each agent following an optimised ingestion strategy is, at present, unknown (Urwin *et al*., 2021b). The two supplements generate similar levels of blood alkalosis (Heibel *et al.,* 2018; Van Montfoort *et al.,* 2004; Potteiger *et al.,* 1996) although SC appears to promote less post-ingestion GIS (Peacock *et al.,* 2021; Requena *et al.,* 2005) in some instances. Comparing SC and SB at their recommended dose, Urwin *et al.* (2022) confirmed similar peak [$HCO_3^-$] ($C_{max}$)

(34.2 (33.24-35.0) vs. 33.6 (32.8-34.5) mmol·L$^{-1}$, respectively) and found no difference in both frequency and severity of GIS ($P > 0.05$).

Maximising blood alkalosis, whilst also mitigating GIS following the ingestion of SC is largely the result of the administered dose and, until recently, the required amount necessary to promote an optimal response was not entirely clear. At present, only three dose-response studies have been conducted (McNaughton, 1990; Schabort *et al*., 2000; Urwin, Dwyer and Carr, 2016). In the earlier work of McNaughton (1990) and Schabort *et al* (2000), the highest quantities given (0.5 g•kg$^{1}$ and 0.6 g•kg$^{1}$ BM, respectively) were associated with the greatest level of induced alkalosis, with McNaughton (1990) also finding the greatest performance effect at the highest administered dose (greater total work done). This lead Urwin *et al*. (2016) to propose that higher doses (in excess of 0.6 g•kg$^{-1}$ BM) may improve performance further, something that a range of prior studies using 0.5, 0.7 up to 0.9 g•kg$^{-1}$ BM of SC had failed to clarify (Aedma, Timpmann and Oopik, 2015; Russel *et al*., 2014; Martins, Artioli and Franchini, 2010; Esau *et al*., 1996; Cox and Jenkins, 1994). Across matched doses of 0.5, 0.6 and 0.9 g•kg$^{-1}$ BM, Urwin *et al.* (2016) observed that doses in excess of 0.5 g•kg$^{1}$ BM did not provide any additional alkalosis and in turn, may elicit significantly greater gastrointestinal (GI) distress ($p = 0.004$, 0.9 g•kg$^{1}$ vs. 0.5 g•kg$^{1}$) and hinder potential performance effects. As such, current recommendations support a dose of 0.5 g•kg$^{1}$ BM to induce alkalosis and minimise GIS (Urwin *et al*., 2021b).

To elicit an ergogenic benefit from increased extracellular buffering capacity, there needs to be an increase in circulating $HCO_3^-$ following ingestion, irrespective of given dose. In theory, any increase in blood [$HCO_3^-$] would lead to an analogous increase in buffering capacity, although the minimal increase necessary to positively impact performance is unknown (Heibel *et al*., 2018). Carr *et al*. (2011a) previously proposed that a +5 mmol•L$^{-1}$ increase from baseline is required to achieve a 'likely' ergogenic benefit, whereas a +6 mmol•L$^{-1}$ increase leads to 'almost certain' ergogenic benefits. More recently, de Oliveira *et al*. (2021) linked greater effects on exercise with medium (4-6 mmol·L$^{-1}$) and large (> 6 mmol·L$^{-1}$) increases in blood [$HCO_3^-$] vs. small (≤ 4 mmol·L$^{-1}$). In a recent review, Urwin *et al*. (2021b) found that blood [$HCO_3^-$] was significantly increased following SC ingestion vs. placebo in

every case (n = 33), irrespective of ingestion protocol. Yet, despite substantial increases in blood [$HCO_3^-$], only 25% of observations reported significant improvements in performance. In this regard, Saunders *et al*. (2014) suggest that individual positive/negative responses may be only partially explained by blood [$HCO_3^-$] levels, finding no correlation between the magnitude of change in blood values ($HCO_3^-$ and pH and base excess) and high-intensity cycling capacity. Going further, not only does absolute maximal change ($\Delta C_{max}$) differ substantially between individuals, but time-to-peak (TTP) [$HCO_3^-$] ($T_{max}$) appears to vary substantially also in both SB and SC (Gough *et al*., 2017a, 2017b; Sparks *et al*., 2017; Jones *et al*., 2016; Urwin *et al*., 2016; Saunders *et al*., 2014).

Given the larger molecular weight of SC (258.06 $g.mol^{-1}$) in comparison to SB (84.007 $g.mol^{-1}$), time-to-peak alkalosis (blood [$HCO_3^-$] and pH) following SC ingestion is likely to be longer (McNaughton *et al*., 2019). When administered in solution, a peak alkalotic response following SC ingestion may arise after a minimum of 100-120 min (Potteiger *et al*., 1996) up to 180-210 min (Urwin *et al*., 2016) and varies when administered in capsule form (Urwin *et al*., 2019, Peacock *et al*., 2021). Despite this, many investigations have utilised relatively short, fixed durations (i.e., 90 min) between SC ingestion and the onset of exercise (Kumstat *et al*., 2018; Schabort *et al*., 2000; Shave *et al*, 2001). As highlighted by Urwin *et al*. (2016), this may be the negative influence of published ingestion protocols for SB, which is associated with a shorter TTP of between 30 to ~120 min (Gough *et al*., 2019; Gough *et al*., 2017a, Jones *et al*., 2016, Saunders *et al*., 2014). As a result, the unclear performance effect of SC, may be explained by a failure to reach a state of peak alkalosis at the onset of exercise and therefore, maximise the impact of increased buffering capacity across each individual (de salles Painelli and Junior, 2018).

Since Miller *et al*. (2016) first utilised an individual approach to SB ingestion, research in buffering agents has largely moved towards a more individualised approach (McNaughton *et al*., 2019), following the general trajectory of nutrition as a whole towards more personalised methods (see: Swinton *et al*., 2018; Jeukendrup, 2014). Such an individual approach requires the researcher to determine each individual's TTP ([$HCO_3^-$] and/or pH) to ensure on the next visit(s) that the exercise task is commenced at this established timepoint, when buffering capacity is theoretically at its greatest.

The usability of this approach is dictated by the reproducibility of individual TTP ([$HCO_3^-$] and/or pH) in SC, which at present remains unknown. Furthermore, whether reproducible blood responses are then able to produce equally reproducible performance benefits is also unclear. Based on existing work using SB, such an approach appears efficacious (Lassen, Lindstrøm and Lønbro, 2021; Boegman *et al*., 2020; Gough *et al*., 2017a; 2017b) but requires confirmation using SC.

Hecksteden *et al*. (2015) discuss that to appropriately quantify individual responses to an experimental treatment requires repeated administrations of the specified treatment (i.e., a buffering agent), in order to allow the proportion of reproducible and random components to be determined alongside random variation due to measurement error. Applying such a method, reproducible individual responses can be explained by differences in participants in inherited, stable characteristics and separated from any random responses attributable to changes in participant characteristics or 'status' between treatments. Importantly, this is only possible if the treatment is allowed to washout, negating any residual effects that may linger (Hopkins, 2015). Overall, successfully demonstrating reproducibility of the individual response to a treatment in this way will allow an athlete for example, to make informed choices on whether SC offers a worthwhile effect and if they can expect to gain benefits in a predictable, consistent manner across training/competition (Burke *et al*., 2017). The response to buffering agents is clearly subject to inter-individual variation, with TTP alkalotic response varying by >90 min (Urwin *et al*., 2016; 2019; Requena *et al*., 2005, Potteiger *et al*., 1996) to as much as 150 min (de Oliveria *et al*., 2020; Gough 2019; 2017a, Jones *et al*., 2016) following the ingestion of SC and SB, respectively. Therefore, the reproducibility of individual responses should not be assumed across buffering agents and is unlikely to be applicable for all individuals, however, assessing the utility of such an approach provides a logical, potentially more effective alternative to a standardised ingestion strategy.

Acute GI distress is a somewhat common side-effect following the ingestion of significant quantities of extracellular buffer, particularly when provided as an aqueous solution and may offer a partial explanation of some equivocal responses to SC (Urwin *et al*., 2021b; Cerullo *et al*., 2020; Jones *et al*., 2016, Carr *et al*., 2011a). Commonly, GIS manifests following both SB and SC ingestion as

stomach cramps, nausea, vomiting and diarrhoea amongst other symptoms (Carr *et al*., 2011b, Urwin *et al*, 2016; 2019). Although performance benefits may still be observed when GIS are reported (Carr *et al*., 2011b) a reduction in such symptoms is linked to improved performance (Costa *et al*., 2017; Wilson and Ingraham, 2015) and the appearance of these symptoms may otherwise be ergolytic for some (McNaughton *et al*., 2016; Saunders *et al*, 2014) and in this regard, often discourages individuals from supplementation entirely (Knapik *et al*., 2016; Heibel *et al*., 2018). Following ingestion of SC, the most intense GIS have been observed between 65-95 min post-supplementation (Urwin *et al*., 2016; 2019) which, when considering SC has often been administered at a standardised timepoint of 90 min pre-exercise (20 of 33 observations, see: Urwin *et al*., 2021b) suggests many individuals may have begun to exercise at the height of GIS, modifying possible ergogenic effects. Urwin *et al*. (2021b) also highlight a further 10 observations within which ingestion also occurs 65-95 min pre-exercise (75 and 85 min, n = 1 for both) or borderline to this period (60 min, n = 8). Once again, this does not entirely explain the lack of effect in some individuals (Heibel *et al*., 2018), although differing individual GIS responses to buffering agents are clear (Peacock *et al*., 2021; McNaughton, Siegler and Midgley, 2008) and should be addressed as part of an optimised ingestion strategy.

The emergence of GIS may be explained by the interaction of the ingested substance with stomach acids and as such, current research focuses on ways to impact this interaction (Peacock *et al*., 2021; Hilton *et al*., 2019; 2020a; 2020b) and to make supplementation more palatable for wider application in general (Urwin *et al*., 2019). Specifically, a range of capsules have been utilised, primarily as a means of avoiding degradation of capsules in the stomach environment and to exploit the considerable pH differences along the GI tract, causing degradation to occur predominantly in the less acidic duodenum (pH 6-7 arbitrary units (AU)) (Ibekwe *et al*., 2008a; 2008b). A key component of these capsules is often hydroxypropyl methylcellulose which resists degradation in acidic environments such as the stomach (pH -1-2 AU). Whilst de Oliveira *et al*. (2018) highlighted the possibility that reducing neutralization in the stomach may increase blood [$HCO_3^-$], reductions have instead been observed, potentially as a result of decreased time available for absorption (Hilton *et al*., 2019; 2020a). Generally, the impact various capsules may have on blood responses, such as TTP alkalotic response

appears to differ markedly, particularly vs. solution (Middlebrook *et al*., 2021; Peacock *et al*., 2021, Hilton *et al*, 2019; 2012a; Urwin *et al*., 2019).

Following SC ingestion, Urwin *et al*. (2019) observed that GEL capsules produced a greater $C_{max}$ and $\Delta C_{max}$ relative to solution for both blood pH and $HCO_3^-$. For blood pH, $C_{max}$ was ~0.018 higher (7.490 (7.482-7.498) vs. 7.472 (7.464-7.480), $P < 0.001$), with identical improvements for $\Delta C_{max}$ (0.100 (0.092-0.107) vs. 0.082 (0.074-0.089), $P < 0.001$). For blood $HCO_3^-$, $C_{max}$ was ~1.1 $mmol \cdot L^{-1}$ more (30.4 (29.8-31.1) vs. 29.3 (28.7-30.0), $P = 0.013$), reflected in significant alterations in $\Delta C_{max}$ (7.9 (7.2-8.6) vs. 6.8 (6.1-7.5) $mmol \cdot L^{-1}$, $P = 0.013$). Additionally, TTP blood pH and $[HCO_3^-]$ was also significantly later after GEL capsule ingestion than after solution (199 vs. 175 min, $P = 0.034$ and 204 vs. 164 min, $P < 0.001$). Surprisingly, although capsules were significantly more palatable than solution ($P < 0.001$), GIS did not differ between modes at any observed timepoint. This observation may be explained by the fact that both capsules and solution were co-ingested with a CHO-rich meal (1.7 $g \bullet kg^1$ BM (Thomas, Erdman and Burke, 2016), a practical method of further reducing GIS post-ingestion (Price and Cripps, 2012). Comparing multiple capsule formulations, Hilton *et al*. (2020a) administered 0.3 $g \bullet kg^1$ BM of SB in ENT, GEL and DEL capsule form. They noted fewer GIS with ENT than with GEL ($P = 0.012$) but not with DEL ($P = 0.106$) capsules. Symptom severity also decreased with DEL (4.6 ± 2.8 AU) compared to GEL capsules (7.0 ± 2.6 AU, $P = < 0.001$) and were lower with ENT (2.8 ± 1.9 AU) than both GEL ($P < 0.0005$) and DEL ($P = 0.044$) capsules. Finally, changes in peak blood pH were reduced with ENT than with GEL ($P = 0.047$) and DEL ($P = 0.047$) capsules, with no further differences between conditions. More recently, using DEL capsules only, Peacock *et al*. (2021) observed significantly higher $[HCO_3^-]$ ($P < 0.001$) and pH ($P = 0.040$) in the SB compared to SC condition. Peak blood $[HCO_3^-]$ ($C_{max}$) was significantly higher with SB compared with SC ($P < 0.001$), with the highest concentration observed at 140 and 170 min, respectively. Despite a greater, swifter change in relevant blood markers, mean GIS scores were lower ($P = 0.037$) for SC (1.5 ± 1.8 AU) than SB (2.6 ± 3.1 AU), supporting the suggestion that SC may induce less GIS (Requena *et al*., 2005). Ingestion form therefore clearly impacts GIS in terms of both frequency and severity, with encapsulation arguably representing a more palatable, safer alternative to ingestion via aqueous

solution. Whilst positive, encapsulation may also significantly influence the magnitude of any changes in blood pH and [$HCO_3^-$] and TTP alkalosis within these measures, with different formulations and different buffering agents bringing about distinct responses. To administer successfully, researchers should consider the unique responses each ingestion mode/form may have both generally and when ingesting a particular buffering agent, accounting for distinct individual responses for that buffering agent, in this case, SC.

The ingestion of SC represents a potentially valuable, but as of yet unestablished ergogenic aid and may yet provide a beneficial tool for any athlete seeking to gain a competitive advantage, specifically when mitigating fatigue is likely to be a crucial factor in determining whether the individual fails or succeeds in their given pursuit. Although valuable, the most effective use of buffering agents requires the precise development of an optimised ingestion strategy, wherein timing of an appropriate dose is managed to ensure exercise coincides with a peak alkalotic response and GIS are reduced to a minimum or eliminated. This remains to be achieved using SC and as of yet, it is not possible to conclude on its efficacy, especially when compared to SB. Furthermore, existing research supports the individual nature of the response to buffering agents and this is also evident following SC ingestion, providing a further factor for consideration in an optimised ingestion strategy - one that has yet to be addressed. This thesis will therefore address the following aims:

1) Determine the reproducibility of blood acid-base responses to individualised sodium citrate ingestion
2) Compare blood acid-base and gastrointestinal responses following individualised sodium citrate ingestion administered in different capsule formulations
3) Evaluate the effect of carbohydrate ingestion on blood acid-base and gastrointestinal responses following individualised sodium citrate ingestion
4) Evaluate the effect of sodium citrate ingestion and ingestion timing on repeated 4 km time trial performance and associated recovery.

# CHAPTER 2 – REVIEW OF LITERATURE

The following review of literature seeks to expand on the concepts discussed above, providing a greater level of understanding with which to begin the development of a more thorough investigation into the efficacy of SC. This review of the literature will begin by outlining the core concepts of acid-base balance and highlighting key mechanisms of fatigue during high-intensity exercise in an attempt to clarify simply, what buffering agents do and when/where they may do it. The potential mechanism(s) of action of SC will then be discussed, commenting individually on how both a positive and negative response to supplementation could be observed and how this may influence existing and future research. Continuing on, factors modifying the responses to extracellular buffering agents will be assessed, highlighting where researchers and athletes may look if they are to bring about the desired response. Finally, the state of the current research on SC will be critiqued, with particular attention given to research conducted using intense exercise bouts of a short duration.

## 2.1 The Concept Of Fatigue

Tracking the development of fatigue as a concept in the exercise sciences back to its inception is extremely challenging, undoubtedly stretching back centuries (Marino, Gard and Drinkwater, 2011). In his landmark work, Mosso (1904) offered two phenomena to categorise fatigue: "*The first is the diminution of the muscular force. The second is fatigue as a sensation. That is to say, we have a physical fact which can be measured and compared, and a psychic fact which eludes measurement*" (p 154). Almost 30 years later, Bainbridge (1931) noted that the limits of exercise "has often been ascribed to the capacity of the heart alone, but the facts as a whole indicate that the sum of the changes taking place throughout the body brings about the final cessation of effort" (p 176). Around this same time, a series of studies completed by Hill and Colleagues (Hill, Long and Lupton, 1924a, 1924b, 1924c; Hill and Lupton, 1923) introduced the concept of maximal oxygen consumption ($VO_{2max}$). This concept described an individual limit to oxygen uptake ($VO_2$), dictated by the cardiorespiratory systems ability to transport oxygen ($O_2$) to skeletal muscle. Furthermore, a high $VO_{2max}$ was a key contributor to endurance performance, regulated by cardiac output and elevated arteriovenous $O_2$ difference. Elite athletes have demonstrated superior $VO_{2max}$ values (Legaz-Arrese *et al*., 2007) as a functional response

to aerobic training-induced adaptations (Keul *et al*., 1996; Costill *et al*., 1976a; 1976b; MacFarlane *et al*., 1991). Basset and Howley (2000) later argued that the ability to sustain greater work rates for extended periods is associated with superior $O_2$ efficiency and exercise performance. Specifically, maintaining aerobic metabolism as the predominant energy source correlates with a delay in fatigue and preservation of improved performance. The upper limit of aerobic metabolism is termed the 'anaerobic threshold', with further increases in work rate resulting in substantial perturbations in homeostasis (Ghosh *et al*., 2004). Broadly, the function of the regulatory and support systems becomes inadequate for sustained muscular contraction, or for maintaining the bioenergetics and removal of metabolites necessary to sustain work rate (Keyser, 2010). Augmented removal of these metabolites, or greater tolerance to their accumulation is necessary to delay negative performance effects. Taken together, we return to the proposition of Mosso (1904) who categorised fatigue as being both physical and psychological or 'psychic' in nature. In this regard, the origins and purpose of fatigue remain passionately debated in the 21$^{st}$ century (Marcora and Staiano, 2010; St Clair Gibson and Noakes, 2004; Gandevia, 2001) - almost to the point of exhaustion.

### *2.1.1 Components Of Fatigue*

Fatigue may be defined as an exercise-induced reduction in muscular force generation capacity (Gandevia, 2001), coinciding with an increased perception of effort (Enoka and Stuart, 1992) and, is often addressed in terms of 'peripheral' and 'central' components (Noakes, 2007). Peripheral fatigue originates at multiple sites, including the neuromuscular junction, the sarcolemma and the contractile apparatus, with mechanisms centred around reduced excitation-contraction coupling, an accumulation of metabolites (i.e., $H^+$ and inorganic phosphate) and depletion of fuels (i.e. glycogen) (Kent-Braun, 1999). Alternatively, central fatigue originates from all structures above the neuromuscular junction (i.e., central nervous system and peripheral nerves). Mechanistically, central fatigue may be the result of reflex inhibition and disfacilitation, Renshaw cell inhibition and insufficient drive from supraspinal sites (Gandevia, 1998), possibly due to lack of subject motivation (Allen, Westerblad and Lannergren,

1995). The proportion of fatigue contributed by each component is dictated primarily by exercise intensity and thus, duration (Enoka and Stuart, 1992).

Short-duration, high-intensity exercise is limited predominantly by peripheral factors, although central factors contribute synergistically (Enoka and Stuart, 1992; Gandevia, 1992). Pertinent to the current thesis is the findings of Thomas *et al*. (2015), who found that 4 km cycling TT's were influenced to a greater degree by peripheral fatigue, as indicated by potentiated twitch force reductions of 40%, vs. 31% and 29% in 20 km and 40 km TT's, respectively. This greater contribution of peripheral fatigue was attributed to the concept of an individual critical threshold (of peripheral fatigue). Solid evidence supports that feedback from the locomotor muscles can inhibit central motor drive, with the purpose of limiting peripheral fatigue beyond an individual 'critical threshold' (Amann and Dempsey, 2008; Amman *et al*., 2009; 2011; 2013; Gagnon *et al*., 2012; Hureau *et al*., 2014). Where significant peripheral fatigue is evident via decreases in potentiated twitch force, or an increasing rate of accumulation of fatiguing metabolites (i.e., inorganic phosphate) (Duhamel *et al*., 2004; Allen, Lamb and Westerblad, 2008), there follows a reduced exercise intensity or exercise termination once threshold speeds for example, are reached (Amann and Dempsey, 2008; Amman *et al*., 2009; 2011). Within TT exercise, this may manifest in 'chosen' reductions in power output (Amann *et al*., 2011). In addition to observed reductions in potentiated twitch force, Thomas *et al* (2015) reported that the magnitude of reductions after the 4 km TT (~35%) were comparable to other studies proposing a critical threshold after similar exercise (Amann *et al*., 2006; 2009; Amann and Dempsey, 2008) and indicated that this 'threshold' was not reached after longer form time trials. In this way critical threshold marks the boundary of more sustainable vs. unsustainable exercise, characterised by distinct physiological responses (Jones *et al*., 2008; Burnley, Vanhatalo and Jones, 2012). In line with the exercise model to be used in the current thesis, peripheral factors limiting performance will be discussed solely.

### *2.1.2 Exercise Induced Metabolic Acidosis And $H^+$ Appearance*

Maintaining contractile activity during exercise is dependent on the supply of adenosine triphosphate (ATP), utilised by myosin adenosine triphosphatase (ATPase) to fuel the mechanism of cross-bridge

cycling between actin and myosin filaments that underpins force production. As intramuscular ATP stores are low, sole reliance on them would facilitate exercise for a short time only and so, other metabolic pathways are required to resynthesise ATP. These pathways involve both anaerobic, substrate-level phosphorylation, and aerobic, oxidative phosphorylation. The major determinants of the relative contribution of each pathway are the exercise duration and intensity. During exercise of a high-intensity there is a rapid, significant increase in metabolic flux (MacDougall, MacDougall and Sale, 2014) and as a result the slow rate of ATP production through aerobic pathways is insufficient to meet the increasing demand. Resultantly, anaerobic pathways are the major energy source of high-intensity exercise.

Anaerobic glycolysis resynthesises ATP utilising glycogen stores from both the liver and muscle, or free glucose (Baker, McCormick and Robergs, 2010). The use of glycogen involves nine step-wise reactions beginning with glucose-6-phosphate and 'ending' with pyruvate formation (Robergs, Ghiasvand and Parker, 2004), whilst lactate ($La^-$) production at this stage represents a fulcrum between anaerobic and aerobic metabolism (Brooks, 2020).

Increases in $La^-$ concentration ($[La^-]$), which, during high-intensity exercise, may rise up to 40 mmol/L in muscle fibres and 25 mmol/L in plasma (Sahlin *et al*., 1976; Spriet *et al*., 1987; Fitts, 1994; Lindinger, McKelvie and Heigenhauser, 1995) were traditionally used to explain the parallel decreases in pH in both the blood and muscle (Sahlin *et al*., 1976; Sahlin *et al*., 1981; Lehniger, 1982; Sahlin and Henriksson, 1984; Hagberg, 1985; Sahlin, 1986; Sahlin, Katz and Henriksson, 1987; Sahlin, Tonkonogi and Soderland, 1998) following exercise. This concept, termed lactic acidosis has been criticized widely (Gevers, 1977; 1979; Trump *et al*., 1976; Williamson *et al*., 1976; Zilva, 1978; Vaghy, 1979; Wilkie, 1979; Hochachka and Mommsen, 1933; Busa and Nuccitelli, 1984; Hagberg, 1985; Dennis, Gevers and Opie, 1991; Tofaletti, 1991) with substantial evidence indicating $La^-$ is instead crucial to exercise metabolism (Cairns, 2006; Robergs, Ghiasvand and Parker, 2004). Specifically, La- functions as a major energy source (Bergman *et al*., 1999; Chen *et al*., 2016; Hui *et al*., 2017), the major gluconeogenic precursor (Bergman *et al*., 2000; Emhoff *et al*., 2013; Meyer *et al*., 2002) and a signalling molecule (Brooks, 2009; 2002; Hashimoto *et al*., 2007).

Additionally, through the conversion of pyruvate to $La^-$ and nicotinamide adenine dinucleotide ($NAD^+$), catalysed by $La^-$ dehydrogenase (LDH), a proton is consumed, providing a slight alkalinizing effect (Robergs, Ghiasvand and Parker, 2004). Demonstrating thsis, Morris *et al*. (2011) reported a significant increase in [$HCO_3^-$] following exogenous $La^-$ supplementation (27.6 ± 1.7 vs. 29.6 ± 2.0 $mmol \cdot l^{-1}$, $P <$ 0.05). Overall, changes in acid-base balance and increases in [$La^-$] at best appear correlative rather than causative and as such, $La^-$ cannot be deemed responsible for fatigue during high-intensity exercise.

It is accepted generally that the detrimental effects of $La^-$ are more likely due to alterations in $H^+$ rather than $La^-$ (Fitts, 2003) and research routinely demonstrates that maximal force generation is correlated with declining muscle pH (Hermansen, 1981; Sahlin, 1992). Learning from the previously 'assumed causation' between $La^-$ and fatigue, evidence from numerous experimental studies summarised by Fitts. (2003) supports that an elevated muscle $H^+$ (low pH) could limit muscle function by (1) reducing the transition of the cross-bridge from the low to high force state, (2) attenuating maximal shortening velocity, (3) inhibiting myofibrillar ATPase, (4) inhibiting glycolytic rate, (5) diminishing crossbridge activation by completely inhibiting $Ca^{2+}$ binding to troponin C, and (6) lessening $Ca^{2+}$ re-uptake by inhibiting sarcoplasmic ATPase. Despite this, the role of acidosis in fatigue development is not without challenge, with Westerblad and Allen (2002) reporting no effect of increased $H^+$ in limiting $Ca^{2+}$ sensitivity, maximal tension and shortening velocity and Bangsbo (1996), arguing that muscle acidity does not reduce muscle glycogenolysis/glycolysis during intense exercise (Bangsbo, 1996). Furthermore, the precise mechanism of $H^+$ generation is also the subject of some contention. On one side, $H^+$ production is argued to be the result of ATP hydrolysis, with a 3:1 ratio between $H^+$ and $La^-$ produced (Bangsbo, Michalsik and Peterson, 1993; Juel, 1998; Robergs, 2002; Robergs *et al*., 2004). On the other, glycogenolysis is responsible for increases in $H^+$ and $La^-$ in a 1:1 ratio (glycogen + 3ADP + 3Pi → 3ATP + $2La^-$ + $2H^+$) (Juel and Halestrap, 1999; Boning *et al*, 2005; Kemp, 2005). Regardless of stance, $H^+$ is produced in significant quantities following anaerobic glycolysis particularly, and as such, changes in $H^+$ likely need to be limited/buffered based on known effects on acid-base balance discussed earlier.

As established, high intensity exercise represents a significant challenge to acid-base balance, with the $La^-$ coupled $H^+$ production representing the single largest contributor to pH disturbances during such exercise (Juel, 1998). Unsurprisingly, high intensity exercise elicits the largest increases of $H^+$ across both extracellular and intracellular compartments (Kowalchuk *et al*., 1988; Bishop, Edge and Goodman, 2004; Street *et al*., 2005). In the muscle, Kowalchuck *et al*. (1988) demonstrated that post-exercise $H^+$ levels varied by 60% compared to rest (328 $mEq \cdot L^{-1}$ vs. 132 $mEq \cdot L^{-1}$, respectively) after maximal ergometer exercise. In the interstitial space, $H^+$ was also seen to increase two-fold with knee extensor exercise to fatigue ($40 \pm 5$ vs. $80 \pm 6$ nM). Finally, in the blood, Bishop *et al*. (2004) observed significant changes in $H^+$ (~70%) following repeated sprint exercise (37.7 $mmol \cdot l^{-1}$ pre vs. 55.6 $mmol \cdot l^{-1}$ post). Collectively, elevated $H^+$ and associated reductions in pH are believed to be critical to the development of fatigue and possible exercise termination during high-intensity exercise (Cairns, 2006; Allen *et al*., 2008; Fitts, 2008; 2016).

## 2.2 Acid Base Balance

### *2.2.1 Introduction To Acid Base Balance*

Acid-base balance is predominantly the result of $[H^+]$ and is expressed in manageable quantities using the logarithm termed potential hydrogen (pH) (equation 1).

$$(1) \quad pH = -\log_{10}[H^+]$$

Acid-base balance essentially describes the precise physiological and biochemical maintenance of both plasma and muscle pH within the ranges of 7.35-7.45 (Goel and Calvert, 2012) and 7.38-7.42 (Atherton, 2003), respectively. This dynamic process must be controlled to a high degree due to the powerful effects of $H^+$ on cellular function, particularly through alterations in hydrogen bonding and protein structure, such as the structure and therefore function of glycolytic enzymes (Atherton, 2003). Biochemical sources of $H^+$ include intermediary and end-products of metabolism such as anaerobic glycolysis. Intense exercise therefore provides a substantial endogenous source of $H^+$, relative to the demands of the specific activity, requiring a range of mechanisms to preserve pH in homeostatic range.

One such example is physicochemical buffering, describing a solution containing substances that can minimise changes in pH when an acid or base is added to it (Poupin *et al*., 2012; Worthley, 1977).

### *2.2.2 Body Buffer Systems: Layman's View*

A buffer is comprised of two parts, often referred to as a *buffer pair*. In body fluid, this pair typically consists of a weak acid and the conjugate base of that acid. In short, a weak acid is an acid that demonstrates only a moderate tendency to dissociate, whereas a conjugate base describes the part that remains after the acid loses its proton ($H^+$). This relationship between a weak acid and its conjugate base can be seen below (equation 2), with 'HA' representing a weak acid and '$A^-$' its conjugate base:

$$(2) \quad HA \leftrightarrows H^+ + A^-$$

When $[H^+]$ starts to increase, some of the dissolved $A^-$ combines with free $H^+$ in what is known as the *association* reaction (equation 3) and can be expressed as:

$$(3) \quad A^- + H^+ \rightarrow HA$$

The net effect of this reaction is the removal of some of the additional $H^+$ from the solution, lessening the rise in $[H^+]$ and preventing the solution becoming too acidic. In the reverse reaction, termed the *dissociation* reaction, dissolved HA dissociates in response to declining $[H^+]$, releasing $H^+$ as shown (equation 4):

$$(4) \quad HA \rightarrow A^- + H^+$$

This reaction serves to replace removed $H^+$, limiting the fall in $[H^+]$ and preventing the solution becoming too alkaline. Taken together, $A^-$ removes $H^+$ and HA serves to control changes in $[H^+]$ in either direction. The relative rates of these reactions are governed by the *law of mass action* (Waage and Gulberg, 1864) which states that 'rate is proportional to the product of the concentrations of the reactants'. Expressed in terms of the association reaction ($H^+ + A^- \rightarrow HA$), the rate is proportional to

$[H^+]$ x $[A^-]$ therefore, if $[H^+]$ were to double but $[A^-]$ is stable, the reaction rate doubles. If both $[H^+]$ and $[A^-]$ double, the reaction rate would quadruple and so on.

To demonstrate buffering in action, consider the example of a buffered solution (HA and $A^-$), to which a strong acid such as hydrochloric acid (HCl) is added. When added, HCl rapidly dissociates ($HCl \rightarrow H^+ + Cl^-$). The resultant rise in $[H^+]$ increases the product of $[H^+]$ x $[A^-]$, so the rate of the association reaction rises. In response to greater $[H^+]$, $H^+$ and $A^-$ are converted in HA, removing some of the $H^+$ from the solution. As this reaction continues $[H^+]$ and $[A^-]$ fall and [HA] rises. The rate of association reaction now declines as the dissociation reaction increases. When these rates become equal, a new equilibrium is reached and no more $H^+$ is removed. In this state, [H+] is higher than before the addition of HCl, but lower than it would have been in the absence of buffer. Buffers then, resist changes in pH but cannot prevent them entirely.

### *2.2.3 Body Buffer Systems: Interpretation Of Acid Base Balance*

Acid-base balance has typically been interpreted using the Henderson-Hasselbalch equation and focuses the interaction between plasma carbon dioxide ($CO_2$) tension ($PCO_2$), plasma bicarbonate concentration ($[HCO_3^-]$), the negative logarithm of the apparent dissociation constant ($pK_1'$) for carbonic acid ($H_2CO_3$) in plasma, and the solubility (*S*) of $CO_2$ in plasma, to determine plasma pH. At equilibrium, this interaction can be expressed using the Henderson-Hasselbalch equation, presented below (equation 4):

$$(5) \quad pH = pK_a + \log 10 \left(\frac{[A-]}{[HA]}\right)$$

As part of this approach, the following acid-base disturbances have been defined:

- Respiratory acidosis (increased $PCO_2$)
- Respiratory alkalosis (decreased $PCO_2$)
- Metabolic acidosis (decrease extracellular base excess, actual $HCO_3^-$ concentration, or standard $[HCO_3^-]$ {concentration under standard conditions})

- Metabolic alkalosis (increased extracellular base excess, actual [$HCO_3^-$] concentration, or standard [$HCO_3^-$].

Based on these disturbances, it is evident that increasing [$HCO_3^-$] concentration would shift pH toward alkalosis. Alternatively, increasing $PCO_2$ causes the pH to decrease, shifting acid-base balance towards acidosis.

Extracellular buffering of $H^+$ that accumulates in the muscle during high-intensity efforts is predominately removed by proton-linked monocarboxylate transporters one (MCT1) and four (MCT4), through co-transport with $La^-$ in a 1:1 ratio (Juel, 1997; 1998). Based on its high expression in glycolytic type IIb muscle fibres (Halestrap and Wilson, 2012) MCT4 is the most prominent. Research also suggests that training appears to increase the abundance and activity of MCT transporters (McGinley and Bishop, 2017; 2016), unsurprisingly enhancing $La^-/H^+$ co-transport in athletes (Pilegaard, Juel and Wibrand, 1993). Furthermore, it is routinely acknowledged that increased [$HCO_3^-$] increases MCT1 and MCT4 activity, increasing $H^+$ removal (Heibel *et al*., 2018; Mainwood and Worsley-Brown, 1975).

## 2.3 The Bicarbonate Buffer System

Physicochemical buffering works to immediately attenuate changes in pH, which demonstrate an inverse relationship with [$H^+$]. In the extracellular compartment, the $HCO_3^-$ buffer system is fundamental to maintaining acid-base balance, responsible for 86% of total buffering capacity, with protein and haemoglobin accounting for the other 14% (Poupin *et al*., 2012). Within the extracellular compartment, $HCO_3^-$ is attributed with 34% of overall buffering capacity, while proteins and phosphates offer 66% of the buffering capacity combined (Atherton, 2009; Poupin *et al*., 2012).

The bicarbonate buffer system consists of two main components: (1) a weak acid, (carbonic acid ($H_2CO_3$) and (2) a bicarbonate salt, (i.e., sodium citrate (SC)) depicted in the reversible equation below (Equation 6):

$$(6)\ CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

This buffer system (and equation) describes the interplay between $CO_2$ and $HCO_3^-$. During metabolic acidosis, the increased $[H^+]$ content is buffered by $HCO_3^-$, forming more $H_2CO_3$, which in turn forms $CO_2$ and $H_2O$. Excess $CO_2$ production in this manner stimulates respiration, effectively eliminating $CO_2$ from the extracellular fluid. During metabolic alkalosis, the opposite occurs, with the overall effect of reduced $H_2CO_3$ formation, causing $CO_2$ to combine with $H_2O$ in replacement. The resultant decrease in blood $CO_2$ ultimately inhibits respiration and $CO_2$ expiration rate. The $HCO_3$- buffer is unique in that it is not reliant on acid-base status alone (Robergs, 2002; Robergs *et al*., 2004). Rather, it is dependant on $HCO_3^-$ availability, carbonic anhydrase and $CO_2$ as described above (Lehninger, Nelson, Cox and Cox, 2005). In this way, the rationale for administering buffering agents to increase circulating $[HCO_3^-]$ emerges.

### *2.3.1 Respiratory And Renal Compensation*

The $HCO_3^-$ buffer system cannot provide acid-base control in isolation beyond the initial period of exercise and therefore, additional control systems are required to expel $CO_2$. Secondary control is exerted through the respiratory system, which as highlighted above, acts to remove $CO_2$ by increasing ventilation and aiming to match the current $O_2$ demand of exercise (Stringer, Casaburi and Wasserman, 1992). Even though the lungs can maintain or minutely modify pH by changing the partial pressure of $CO_2$ ($pCO_2$), this process cannot add or remove $H^+$ (Poupin *et al*., 2012; Aguilera-Tejero *et al*., 2000). Beyond physiochemical and respiratory control, the kidneys represent the last line of $H^+$ regulation. The kidneys are capable of excreting acid or alkaline urine, thereby readjusting the extracellular fluid $[H^+]$ towards homeostatic levels. Renal compensation acts over multiple days (Poupin *et al*., 2012) and in this regard, exerts limited influence on the maintenance of acid base balance during high-intensity exercise. Considering high-intensity exercise (e.g., 4 km cycling TT) is central to the current thesis, only physicochemical and respiratory maintenance of acid-base balance will be discussed henceforth, with emphasis on the former.

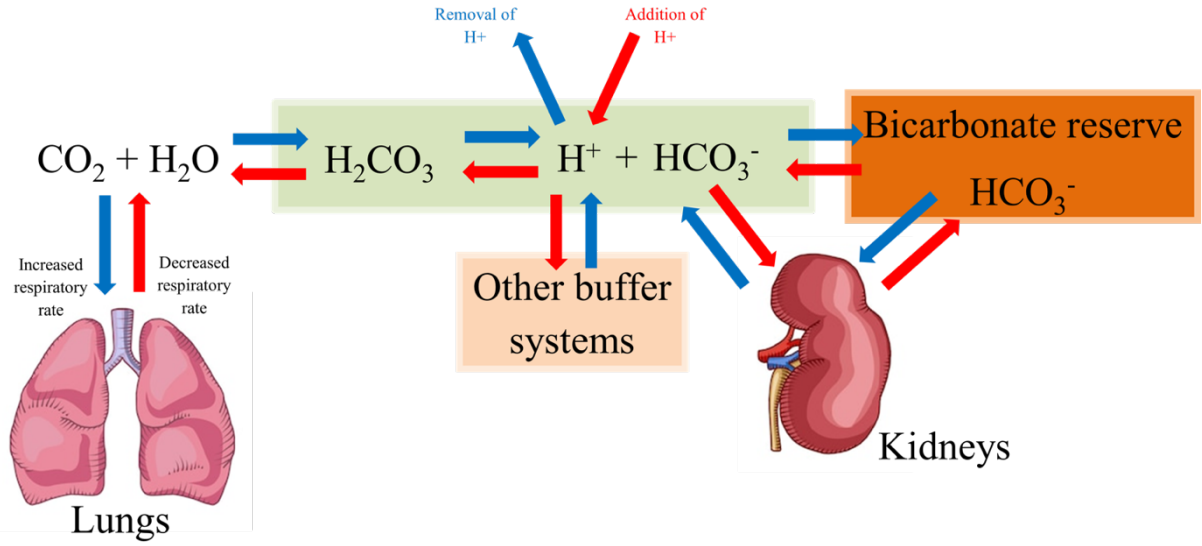


**Figure 1.1** The bicarbonate ($HCO_3^-$) buffer system and its interplay with respiratory and renal compensatory mechanism.

### 2.4 Buffering Processes And Mechanisms Of Action: Sodium Citrate

Extracellular buffering agents such as SB, calcium lactate and SC (Lancha Junior *et al*., 2015) are ingested with a shared purpose: to increase the extracellular [$HCO_3^-$], increasing H+ efflux out of the working muscle and in turn, defending acid-base balance (Heibel *et al*., 2018). Sodium citrate (SC) has not received the same research attention as SB and, for this reason, performance effects are not fully clarified (Urwin, Dwyer and Carr, 2016) and the precise mechanism by which SC may act is not fully understood. Despite an unclear effect on performance, Carr, Hopkins and Gore (2011a) demonstrated that several h post-ingestion of SC, [$HCO_3^-$] is higher than with an equimolar dose of SB. The authors explained that this greater buffering potential could be explained by citrates three negative charges that consume $H^+$ and raise [$HCO_3^-$] (Bird, Wiles and Robbins, 1995) vs. SB's one (Van Montfoort *et al*., 2004).

Immediately following ingestion, SC rapidly dissociates into $Na^+$ and $citrate^-$. Once dissociated, the citrate anion is removed from the plasma, modifying the electrical equilibrium (sum of cations and anions becomes unbalanced) (Devlin, 2011). To restore electrical neutrality there is a decrease in $H^+$

and an increase in [$HCO_3^-$], signature of the alkalotic state following buffering agent ingestion (Fabiato and Fabiato, 1978; Enoka and Stuart, 1992; Shave *et al*., 2001). Although unclear, the higher pH induced by SC ingestion may promote a greater efflux of $La^-$ through MCT1 and MCT4 and, out of the working muscle (Fabiato and Fabiato, 1978; Mainwood and Worsley-Brown, 1975; Oster *et al*., 1988; Potteiger *et al*., 1996). Despite this, it is argued that the $H^+$ consumption and $HCO_3^-$production are instead due to liver oxidation, not the intake of SC (Kamel, Halperin and Goldstein, 2010). The sarcolemma is impermeable to $HCO_3^-$ (Mainwood and Worsley-Brown, 1975; Mainwood and Cechetto, 1980; Costill *et al*., 1984; Katz *et al*., 1984) and although $citrate^-$ can penetrate this membrane by an unknown mechanism (Devlin, 1997), $HCO_3^-$ requires a specific carrier (Devlin, 1997). Within muscle fibres, $citrate^-$ is an important co-factor in multiple metabolic processes (Devlin, 1997): (1) as an intermediary for the Krebs cycle (Kowalchuk *et al*., 1989; Stewart, 1983), (2) in the transport of acetyl CoA from the mitochondria to cytosol for free fatty acid synthesis (Devlin, 1997; Linossier *et al*., 1997), (3) as a negative allosteric effector of phosphofructokinase (PFK) (Kemp and Foe, 1983; Devlin, 1997) and (4) by influencing membrane potential, it may cause a reduction in contraction threshold (Kemp and Foe, 1983; Devlin, 1997). Given SC influences $La^-$ kinetics and $La^-$ in turn blocks PFK (Faff *et al*., 1996), SC ingestion may also act through the inhibition of glycolytic mechanisms whilst $La^-$ continues to form to a greater degree post-ingestion. Alternatively, Street *et al*. (2005) found that alkalosis associated with $citrate^-$ ingestion reduced the interstitial potassium ($K^+$) accumulation during intense exercise. Given the accumulation of $K^+$ contributes to the onset of fatigue (Fitts, 1994) and reduces muscle excitability (Clausen, 2003), reducing interstitial $K^+$ accumulation during exercise is associated with improved performance (Nielsen *et al*., 2004) and may explain any potential effect of SC. Further quality studies are required to greater isolate the mechanism(s) of action following SC ingestion.

## 2.5 Sodium Citrate: Factors Modifying Individual Responses.

### *2.5.1 Increases In Circulating Bicarbonate*

To achieve the desired ergogenic effect, supplementing extracellular buffers must be followed by an increase in circulating [$HCO_3^-$], with any increases theoretically leading to a corresponding

improvement in buffering capacity (Heibel *et al*., 2018). Carr *et al*. (2011a) proposed that a >5 $mmol \cdot L^{-1}$ increase from baseline translates to a 'likely' ergogenic effect, while a + 6 $mmol \cdot L^{-1}$ increase leads to almost certain ergogenic effects. At present, the minimal change necessary to bring about performance effects is unknown, which has important implications for balancing GIS associated with extracellular buffers (Urwin *et al*., 2016). Some studies have shown mean increases as low as >2.5 $mmol \cdot L^{-1}$ to improve exercise tolerance (Morris *et al*., 2011) although ≤ 2 $mmol \cdot L^{-1}$ seems insufficient (Painelli *et al*., 2014). Interestingly, Gough *et al*. (2017b) demonstrated that although absolute differences in maximal $HCO_3^-$ increases between 0.2 and 0.3 $g \cdot kg^{-1}$ BM were ~ 1 $mmol \cdot L^{-1}$, this did not result in altered 4 km TT performance, suggesting such a difference may not be meaningful.

Arguing this, Saunders *et al*. (2014) found no correlations between magnitude of change in any blood values ($HCO_3^-$, pH and base excess (BE)) and exercise capacity, suggesting that positive/negative responses may be only partially explained by blood [$HCO_3^-$]. This supports the earlier observations of Matson and Tran (1993) who reported the difference in bicarbonate to be less than weakly associated with the resulting effect size of improvements ($r$ = 0.10, p = 0.10). Based on work surrounding SB, these discrepancies may be explained by the large individual variation and consistency in blood responses (Stannard *et al*., 2016; Dias *et al*., 2015). This is best illustrated in the work of Stannard *et al*. (2016), who found a large inter-individual variability in magnitude of the increase in [$HCO_3^-$] 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 TTP concentrations (30-150; 40-165; and 75-180 min for 0.1, 0.2 and 0.3 $g \cdot kg^{-1}$ BM). At present, reproducibility of individual blood responses (and subsequent performance) has not been investigated following SC ingestion. If any effect of SC (or lack of) on performance is to be clarified, it is important that individual TTP is investigated, providing the greatest chance for an ergogenic effect to occur which has not yet been afforded consistently.

### *2.5.2 Ingestion Timing*

Enhancing buffering capacity through optimal dosing of extracellular buffers appears relevant only if this time point coincides with the onset of exercise. To this end, numerous investigations in SB (Gough

*et al*., 2017a; 2017b, Jones *et al*., 2016, Sparks *et al*., 2017), $La^-$ (Northgraves *et al*., 2014; Painelli *et al*., 2014) and in SC, most notably through Urwin *et al*. (2016) have collectively argued that a generalised time point is unlikely to be optimal for all individuals, based on the large variance in individual TTP. Although findings have supported a minimum of 100-120 (Potteiger *et al*., 1996) to ≥180 min (Urwin *et al*., 2016; 2019) required to reach peak alkalosis, many investigations have sought to commence exercise within 90 min of SC ingestion (Kumstat *et al*., 2018; Schabort *et al*., 2000; Shave *et al*., 2001). As a result, the unclear performance effect of SC may be explained by a failure to reach a state of peak alkalosis at the onset of exercise (de salles Painelli and Lancha Junior, 2018). Of the 23 studies summarised in table 2.1, at least 12 commenced exercise before the proposed minimum period of 100-120 min (90 min or less). As suggested earlier, this may be due to the negative influence of existing SB research, which is associated with a much shorter TTP (de salles Painelli and Lancha Junior, 2018).

The value of determining individual TTP for repeat ingestion is only maintained first, if subsequent blood responses are reproducible. At present this consistency has only been demonstrated in SB. Gough *et al.* (2017a) revealed both blood pH and $HCO_3^-$ response to be reproducible in male collegiate athletes, finding $HCO_3^-$ to be the most reliable across doses of 0.2 and 0.3 $g \cdot kg^{-1}$ BM and therefore recommending its use in determining individual TTP. Second to the ability to reproduce blood responses, determining individual TTP for repeat ingestion is only of value if consistent blood responses then translate to valuable performance improvements. In this light, Miller *et al* (2016) significantly improved repeated sprint performance following 0.3 $g \cdot kg^{-1}$ BM SB vs. placebo and control, when exercise begun at each individual's predetermined ingestion peak pH response time. Developing from his earlier work, Gough *et al*. (2017b) individualised SB ingestion in eleven male, trained cyclists. Both 0.2 and 0.3 $g \cdot kg^{-1}$ BM doses reduced 4 km TT completion time (-8.3 ± 3.5 s; $p < 0.001$, $d = 0.64$ and -8.6 ± 5.4 s; $p = 0.003$, $d = 0.66$ respectively), with no difference between conditions. The authors concluded that trained cyclists may therefore benefit from individualising SB ingestion to TTP [$HCO_3^-$ ] to aid 4 km TT performance. Only one known study has directly compared exercise performance at peak circulating $HCO_3^-$ vs. a generalised time point post-ingestion. Boegman *et al*. (2020) observed a 2

s improvement in 2000 m rowing performance with individualised compared to generalised timing (367.0 ± 10.5 s vs. 369.0 ± 10.3 s). Given the lack of further evidence over a range of fatiguing exercise tasks, it remains difficult to conclude on the additive benefit of individualisation. For SC, it would appear logical to first develop a strategy to consistently facilitate performance benefits generally, before maximising these further.

### *2.5.3 Gastrointestinal Side Effects*

The occurrence of GIS following the ingestion of SC and other buffering agents is relatively common, with stomach cramps, vomiting and diarrhoea forming the most frequent complaints (Urwin *et al*., 2019; Urwin, Dwyer and Carr, 2016). Although performance benefits may still occur in the presence of GIS (Carr *et al*., 2011b), this is likely to be at least ergolytic for some (McNaughton *et al*., 2016; Saunders *et al*., 2014) or may discourage supplementation completely (Knapnik *et al*., 2016; Heibel *et al*., 2018). Earlier discussions highlighted that the most intense GIS appear to occur between 60-120 min post-supplementation (Urwin *et al*., 2016) and as such, exercise following a standardised 90-min ingestion protocol (Kumstat *et al*., 2018; Schabort *et al*., 2000; Shave *et al*, 2001) may have begun at, or around, peak GI discomfort, hindering any possible ergogenic effects (Heibel *et al*., 2018). Referring to table 2.1, it can be observed that 14 of the 21 studies included may have commenced exercise during this period, possibly clouding any results reported. Despite this, it must be considered that GIS responses do not entirely explain any effects or lack thereof (Heibel *et al*., 2018) and even where such discomfort may influence performance, this differs to an unknown degree between individuals (McNaughton, Siegler and Midgley, 2008). Nonetheless, timing of SC ingestion appears to have been sub-optimal across existing research.

Appropriate supplement timing (of the minimum effective dose) represents a crucial method in balancing the necessary increases in circulating $HCO_3^-$ whilst minimising associated side-effects. Where existing studies investigating SC have predominately standardised ingestion timing, individualising based on TTP (blood pH or [$HCO_3^-$]) would likely avoid performing exercise when peak symptoms are most likely and/or circulating $HCO_3^-$ is sub-optimal to produce a likely performance

effect. Importantly, determining individual TTP blood [$HCO_3^-$] may support the use of a lower dose, with Gough *et al*. (2017a) reporting similar maximal increases between both 0.2 $g \cdot kg^{-1}$ and 0.3 $g \cdot kg^{-1}$, with no significant differences in 4 km TT performance between doses. Given dose contributes significantly to any GIS following SC ingestion (McNaughton, 1990; Schabort *et al*., 2000; Urwin, Dwyer and Carr, 2016), optimising total quantity should be synonymous with any individualised strategy based on TTP and again, represents a further increase in the likelihood of any performance effect.

### *2.5.4 Ingestion Form*

Acute GIS associated with the ingestion of buffering agents is common, particularly when administered in an aqueous solution (Carr *et al*., 2011b). In response to large quantities of SB entering the stomach in this manner, there is an increased absorption of sodium ($Na^-$) in the small intestine and a subsequent rise in plasma $Na^-$ levels (Heigenhauser, 1990). This exchange of $Na^-$ promotes an elevated stomach $PCO_2$ and diffusion of $CO_2$ into the plasma, influencing the already acidic stomach environment (Heigenhauser, 1990) and promoting the commonly described side effects.

In an attempt to mitigate these symptoms, researchers have administered SB in capsule form, with the use of GEL capsules, co-ingested with a small CHO meal (1.5 $g \cdot kg^{-1}$ BM) currently regarded as the least likely to induce significant GIS (Hilton *et al*., 2019; Carr *et al*., 2011a). Although numerous approaches exist, the key mechanism is an attempt to exploit the significant pH changes across the GI tract, from the highly acidic stomach (pH 1-2) to the less acidic duodenum (pH 6-7) (Ibekwe *et al*., 2008a; 2008b). Formulations can ultimately be designed to respond to changes in the pH and disintegrate at the desired location, for buffering agents this involves bypassing the stomach where GIS originate (Heigenhauser, 1990).

Polymeric-coated compounds are resistant to gastric degradation and may reduce GIS induced by acid sensitive compounds, i.e. SB (Barbosa, Conway and Merchant, 2017). Hydroxypropyl methylcellulose, used in DEL capsules, can resist degradation in acidic environments (pH 1-2) and therefore provides gastro-resistant properties. Alternatively, degradation takes place in the more

alkaline duodenum and absorption can take place promptly (Hilton *et al*., 2019). As GIS are partly attributable to degradation in the stomach (Turnberg *et al*., 1970), gastro-resistant capsules may alleviate negative symptoms of buffering-agent ingestion (de Oliveira, Saunders and Artioli, 2018). De Oliveira, Saunders and Artioli (2018) expand this further, suggesting that given that less $HCO_3^-$ is lost in the stomach, smaller doses may produce similar acid-base changes to larger doses. Opposingly, reductions in gut transit time may reduce $HCO_3^-$ bioavailability when administered in this form (Barbosa, Conway and Merchant, 2017). Applying this, Hilton *et al*. (2019) found that DEL capsules containing 0.3 $g \cdot kg^{-1}$ BM SB produced a significant delay in peak alkalosis vs. an equal dose solution (~ 48 min) and reduced overall incidence and severity of GIS (mean difference = 45.1 and 47.5%, respectively, $P > 0.001$). Although these findings may simply be the result of the DEL capsules, a difference between the ingestion of buffering agents in capsule vs. solution appears to exist (Urwin *et al*., 2019). In continuation, Hilton *et al*. (2020a) sought to clarify how different encapsulation forms may alter post-ingestion GIS and acid-base balance of buffering agents (SB). Administering 0.3 $g \cdot kg^{-1}$ BM of SB in GEL, DEL and ENT capsule-form, they observed a reduced frequency of GIS was noted with ENT SB ($N = 12$) compared to GEL ($N = 14$, $P = 0.012$), but not relative to DEL capsules ($N = 12$, $P = 0.106$). Overall, ENT capsules provided the greatest reduction in symptom severity against both GEL and DEL capsules (2.8 ± 1.9 vs. 7.0 ± 2.6 ($P > 0.001$) and 4.6 ± 2.8 ($P = 0.044$) AU, respectively). Simultaneously, ENT capsules were associated with reductions in both blood pH and $[HCO_3^-]$ suggesting improved GIS outcomes may have emerged at the expense of a more substantial blood alkalotic response. In summary, encapsulation may offer greater defence against GIS following SC ingestion, with differing effects on blood alkalosis. Further work is required to determine the identical responses in SC (McNaughton *et al*., 2019).

To date, SC has been administered in a solution (dissolved in water) (Bracken, Linanne and Brooks, 2005), flavoured solution (sweetened sports drink) (Tiryaki and Atterbom, 1995; Urwin *et al*., 2016) and capsules (Vaher *et al*., 2015, Van Montfoort *et al*., 2004) with varying effects. Most recently, Urwin *et al*. (2019) found that the ingestion of 0.5 $g \cdot kg^{-1}$ BM in capsule form demonstrated a significantly greater peak and change from baseline (pH and $HCO_3^-$), which occurred later vs. solution.

This study provides the first comparison of blood and GIS responses between capsule and ingestion form, supporting the ingestion of 0.5 $g \cdot kg^{-1}$ BM in capsule form over solution, at least 180 min pre-exercise.

The work of Urwin *et al*. (2019) is novel in its indirect measurement of palatability, based on rating one's sensory experience and overall preference for a food or fluid (Yeomans, 1998). Capsules were found to be significantly more palatable than solution ($P < 0.001$) which in turn, would increase the likelihood that an athlete would adhere to a supplementation protocol, for example (Urwin *et al*., 2019). The salty taste associated with SC in solution may explain why some individuals may avoid ingestion and explain the lower palatability scores generally and vs. capsules (Bolhuis *et al*., 2011; 2012). Such outcomes are typically reported when foods or fluids provide a relatively intense stimuli for one specific taste (bitter, sweet, salty etc.) (McCrickerd and Forde, 2016) and in this regard, Urwin *et al*. (2019) speculated that the indistinct taste of the capsules may have been somewhat responsible for the improved palatability. This also supports the value in masking the salty taste of SC in a flavoured sports drink or other alternatives. vs. administering a simple solution where capsules are not available.

## 2.6 Sodium Citrate Ingestion For Exercise Performance

### *2.6.1 Overview*

Although SB represents the clear majority of extracellular buffering literature, SC has also been the subject of numerous investigations for over 30 years (Parry-Billings and MacLaren, 1986). Several studies demonstrated that SC ingestion effectively increases blood pH, inspiring exploration of its ergogenic effect (Carr, Hopkins and Gore, 2011a). McNaughton (1990) conducted the first dose-response study utilising SC and sought to highlight the subsequent impact on anaerobic exercise performance. It was concluded that a 0.5 $g \cdot kg^{-1}$ BM dose was the most effective in improving maximal cycling (1-min on a cycle ergometer) with the greatest quantity of work (44.6 ± 1.5 kJ) and peak power (1306 ± 75 W) achieved during this trial. As a result of this investigation, the use of a 0.5 $g \cdot kg^{-1}$ BM dose is commonplace in the SC literature with the more recent work of Urwin *et al* (2016) supporting this dose as one which maximises alkalosis, while doses >0.5 $g \cdot kg^{-1}$ BM may promote significantly

greater GIS. Developing this, McNaughton and Cedaro (1992) sought to then evaluate the effect of SC on exercise of varying duration (10, 30, 120 and 240 s). A 0.5 $g \cdot kg^{-1}$ BM provided no ergogenic benefit for exercise of 10 and 30 s despite significant increases in blood $HCO_3^-$ , although exercise of 120 and 240 s were significantly increased above control and placebo conditions ($P < 0.05$) post-ingestion. The work of McNaughton and Cedaro (1992) possibly provided the first example of the influence exercise task may have of the erogenicity of SC . If improvements are to be gained from increases in buffering capacity, exercise must be limited by increased $H^+$ accumulation (Heibel *et al*., 2018). In support, Bogdanis *et al*. (1998) suggested that exercise of 30 sec duration (cycling sprint) is unaffected by changes in muscle pH, where endurance-based exercise increasingly relies on aerobic metabolism without further changes in muscle acidosis. Although limited support argues that buffering agents may benefit longer duration exercise (Egger *et al*., 2014; McNaughton, Dalton and Palmer, 1999), most studies report no improvement in continuous endurance exercise following ingestion (Northgraves *et al*., 2014; Vaher *et al*., 2015; Schabort *et al*., 2000; Stephens *et al*., 2002; Freis *et al*., 2017). Of note, Higgins *et al*. (2013) observed increases in exercise capacity at 100% peak minute power, but not at 110 or 120% despite exercise falling within the range of an 'expected effect'. Clearly then, both duration and intensity should be considered in equal parts. In line with the aims of the current thesis, the following section (section 2.6.2) will critically review the effects of SC on short-duration exercise performance, taking time where possible to address previously discussed factors that may modify the individual response and have contributed to the unclear ergogenic effect of SC (Carr, Hopkins and Gore, 2011a).

### *2.6.2 Short Duration (<10 min) Performance*

Most research interested in exercise of a short-duration has been conducted within both cycling and running (Table 2.1). Early work by Cox and Jenkins (1994) investigated the physiological and ventilatory responses to repeated cycling sprints following SC ingestion. Although supplementation induced significant changes in selected ventilatory and blood variables which were comparable to similar research (Bouissou *et al*., 1988; Parry-Billings and MacLaren, 1986; Costill *et al*., 1984; Wilkes

*et al*., 1983), this was not reflected in an improved work output. Contrastingly, pre- to post-exercise blood [$HCO_3^-$] showed a 12% greater reduction following citrate$^-$ vs. the placebo, suggesting a greater extracellular $H^+$ buffering during exercise which failed to influence work output. The absence of such an improvement was attributed to the nausea reported by participants and may also be explained by the use of a fixed, 90 min ingestion strategy whereby the onset of exercise may have coincided with peak GIS (Heibel *et al*., 2018). Utilising a 90 min, fixed time point by researchers has demonstrated mixed results concerning the erogenicity of SC with performance improvements observed in some (Linossier *et al*., 1997; McNaughton, 1992; 1990), but not all applicable studies (Oopik *et al*., 2008; Van Someren, Fulcher and McCarthy, 1999; Cox and Jenkins, 1994). Discrepancies in performance improvements may have arisen from inconsistent doses utilised (0.3-0.5 g·kg$^{-1}$ BM) and well-documented inter-individual variation in blood acid-base responses (Urwin *et al*., 2016; 2019) meaning some individuals may have exercised when personal buffering capacity was sub-optimal.

Current observations propose that minimum of 100-120 (Potteiger *et al*., 1996) up to ≥180 min (Urwin *et al*., 2016; 2019) is required to promote a peak alkalotic response following SC ingestion. Interestingly, studies deploying timing of at least 120 min have also returned inconsistent results. Deploying a 180 min time-point, Parry-Billings and MacLaren (1986) significantly improved 3 x 30 s Wingate performance with only a 0.3 g·kg$^{-1}$ BM dose. Alternatively, Ibanez *et al*. (1995) found no change in 300-metre running times in elite athletes following a higher dose (0.5 g·kg$^{-1}$ BM) taken at an identical time point. Although this may be explained by a potentially lower $H^+$ accumulation over the slightly shorter running event, an argument may be made that timing is more impactful than dose, when applied to an appropriate exercise task. However, even when exercise task (5km run), dose (0.5 g·kg$^{-1}$ BM) and timing strategy (120 min prior to exercise) is matched results still remain inconsistent with no performance improvements in young male athletes (Oopik *et al*., 2008) but, significant reductions in time to completion in college runners (~30.6 s). Of note, the former was conducted using a competitive race setting vs. a lab-controlled treadmill runs in the latter which may somewhat explain differences given the tighter implementation of experimental controls and reductions in external influences.

Given such a wide mixture of ingestion strategies have been deployed, eliciting an equally mixed body the unclear ergogenic effect attributed to SC is unsurprising (Carr, Hopkins and Gore, 2011a). Based on the developing understanding of modifying factors that may positively or negatively impact any ergogenic effect of buffering agents it is too early to conclusively disregard the ability of SC to improve 4 km TT performance. Contrasting findings with previous literature where possible, future research requires a more consistent, informed approach to understanding the application of SC and as such, determining the efficacy of an individualised ingestion strategy on blood acid-base and performance reproducibility represents a logical place to start.

**Table 1.1.** An overview of key performance findings in cycling, running, swimming and 'other' exercise modes following sodium citrate ingestion**.**
*Timing given describes the time elapsed between administered dose and onset of exercise. *TT = Time Trial.

| Authors | Exercise protocol | Participants | Dose and timing | Performance change | Significant difference ($p < 0.05$) |
|---|---|---|---|---|---|
| | | | **Cycling** | | |
| Suvi *et al* (2019) | Dehydrating exercise-rest-40 km TT | 20 male endurance athletes | 0.6 $g \cdot kg^{-1}$ BM– 360+ min | No impact on subsequent 40 km TT | N |
| Feriche Fernandez-Castanys *et al* (2002) | Cycling at ~112% of peak oxygen uptake | 17 healthy male students | 0.4 $g \cdot kg^{-1}$ BM– 120 min | No difference in time to exhaustion | N |
| Schabort *et al* (2000) | 40 km TT | 8 trained male cyclists | 0.2, 0.4 and 0.6 $g \cdot kg^{-1}$ BM – 60 min | No differences across time, power output, speed or heart rate | N |
| Van Someren, Fulcher and McCarthy (1999) | Repeated 45 s cycling bouts | 12 subjects (9 male and 3 female) | 0.3 $g \cdot kg^{-1}$ BM – 90 min | Citrate did not significantly improve intermittent exercise performance | N |
| Ball and Maughan (1997) | Cycle to exhaustion at 100% of peak oxygen uptake | 6 healthy males | 0.3 $g \cdot kg^{-1}$ BM – 180 min | No performance effect | N |
| Linossier *et al* (1997) | Cycling at 120% peak oxygen uptake | 8 moderately active students (3 women and 5 men) | 0.5 $g \cdot kg^{-1}$ BM – 90 min | Increased time to exhaustion (+15%) | Y |
| Potteiger *et al* (1996) | 30 km TT | 8 trained male cyclists | 0.5 $g \cdot kg^{-1}$ BM – 90 min | Mean performance time was significantly faster (-102.7 s) | Y |
| Cox and Jenkins (1994) | 60 sec sprints (cycling) | 8 moderately active male students | 0.5 $g \cdot kg^{-1}$ BM – 90 min | No change in total work done | N |

| | | | | | |
|---|---|---|---|---|---|
| McNaughton and Cedaro (1992) | All-out cycling (10, 30, 120, 240 s) | 10 healthy men | 0.5 $g \cdot kg^{-1}$ BM – 90 min | Significant improvements in total work done over 120 and 240 s | Y |
| McNaughton (1990) | 1 min maximal cycling | 11 healthy males | 0.1, 0.2, 0.3, 0.4 and 0.5 $g \cdot kg^{-1}$ BM – 90 min | 0.5 $g \cdot kg^{-1}$ BM provided the most significant improvement in work done and peak power | Y |
| Parry-Billings and MacLaren (1986) | 3 x 30 s Wingate anaerobic tests | 6 healthy males | 0.3 $g \cdot kg^{-1}$ BM – 180 min | Citrate significantly elevated performance | Y |
| | | **Running** | | | |
| Cunha *et al* (2019) | Tennis skills and sprint ability shuttle tests | 10 nationally-ranked young male tennis | 0.5 $g \cdot kg^{-1}$ BM – 120 min | Citrate improved tennis skills and correlated test performance | Y |
| Vaher *et al* (2015) | 2 x 500 m (treadmill) | 16 endurance trained males | 0.5 $g \cdot kg^{-1}$ – BM 120 min | No performance improvement | N |
| Oopik *et al* (2008) | Competitive 1500 m | 17 female middle-distance runners | 0.4 $g \cdot kg^{-1}$ BM – 90 min | No performance effect | N |
| Oopik *et al* (2004) | Competitive 5 km run | 10 young male runners | 0.5 $g \cdot kg^{-1}$ BM – 120 min | No performance effect | N |
| Van Montfoort *et al* (2004) | Treadmill run to exhaustion test | 15 competitive male endurance runners | 0.525$g \cdot kg^{-1}$ BM - 90 min | Increased mean time to exhaustion (+0.8 s) | Y |
| Oopik *et al* (2003) | 5 km (treadmill) | 17 well trained, male college runners | 0.5 $g \cdot kg^{-1}$ BM – 120 min | Reduced mean time to completion (-30.6 s) | Y |
| Shave *et al* (2001) | 3 km run | 9 multidisciplinary athletes (2 women and 7 men) | 0.5 $g \cdot kg^{-1}$ BM – 60 min | Reduced mean time to completion (-10.7 s) | Y |
| Ibanez *et al* (1995) | 300 m sprint running | 6 elite 400m runners | 0.5 $g \cdot kg^{-1}$ BM – 180 min | No change in performance times | N |

| | | | | | |
|---|---|---|---|---|---|
| Tiryaki and Atterbom (1995) | 600 m run | 11 female track athletes | 0.3 $g \cdot kg^{-1}$ BM – 150 min | No change in performance times | N |
| | | | **Swimming** | | |
| Kumstat *et al* (2018) | 400 m freestyle test | 6 nationally ranked male swimmers | 0.3 $g \cdot kg^{-1}$ BM – 60 min | Reduced time to completion vs. placebo | N |
| Russel *et al* (2014) | 200 m TT's | 10 male swimmers | 0.5 $g \cdot kg^{-1}$ acute dose and 0.6 $g \cdot kg^{-1}$ BM chronic – 120 min | Modest time improvements (~1.03%) | Y |
| | | | **Other** | | |
| Aedma, Timpmann and Oopik (2015) | Hand crank, upper-body intermittent sprint performance | 11 trained male wrestlers | 0.9 $g \cdot kg^{-1}$ (over 17 h) – 30 min | No improvement in peak power on mean power in consecutive tests | N |

## 2.7 Reproducibility Of Blood Acid-Base Balance Responses

Individual characteristics (i.e. genetics, training status and dietary habits) may hinder or improve the physiological response to an identical nutritional intervention (Jeukendrup, 2017). Despite this, common practice seeks to determine the ergogenicity of a nutritional aid based on a single treatment, comparing the mean differences between groups/trials (Hecksteden *et al*., 2015) and ultimately overlooks inter- and intra-individual detail.

Despite a relatively large body of research supporting the use of SC in particular situations, research as a whole has been unable to demonstrate a clear ergogenic effect (Carr, Slater and Gore, 2011a). Contradictory results repeatedly emphasize that the ergogenic effect of increased [$HCO_3^-$] is variable and that this variability is in part the result of differing individual responses. This is clear in the large discrepancy in individual TTP alkalosis which may occur 100-120 (Potteiger *et al*., 1996, Requena *et al*., 1996) min post-ingestion in some individuals or up to 180+ min in others (Urwin *et al*., 2016; 2019) after equivalent SC doses. Further substantiating this, TTP alkalosis in SB has been observed to occur between 10-180 min post-ingestion (Miller *et al*., 2016). It is important to note the differing TTP values across studies may be the result of varying ingestion forms deployed, although individual variance undoubtedly remains.

A contemporary approach to this issue is to individualise the ingestion strategy, the efficacy of which remains untested with SC. Based on recent developments in SB research however, it is logical to suggest that an individual response would produce a clearer, more consistent ergogenic effect. To achieve a beneficial effect of induced alkalosis requires an appropriate time for the $H^+$ gradient to develop between the intramuscular and vascular compartments and therefore a larger effect would likely follow if peak elevations in pH (or $HCO_3^-$) are calculated prior to exercise (McNaughton and Cedaro, 1991). Stannard *et al*. (2016) reported large inter-individual variations in TTP [$HCO_3^-$] (0.2 $g \cdot kg^{-1}$ BM = 40-165 min; 0.3 $g \cdot kg^{-1}$ BM = 75-180 min), first challenging the use of group-level analysis following SB ingestion at a fixed timepoint (McNaughton *et al*., 2016; Saunders *et al*., 2014; Siegler *et al*., 2012). Miller *et al*. (2016) was the first to deploy an individualised approach to ingestion, aiming to augment repeated sprint ability. Total work completed was higher ($P < 0.05$) in the SB condition (69.8 ± 11.7

kJ) compared with both the control (59.6 ± 12.2 kJ) and placebo (63.0 ± 8.3 kJ) conditions. Furthermore, SB ingestion induced higher ($P < 0.05$) blood pH and [$HCO_3^-$] pre-exercise and during the sprints, followed by higher [$La^-$] ($P < 0.05$) after the sprints were completed. Taken together, an individualised strategy successfully increased buffering capacity which facilitated a greater total amount of work completed. Since then, a handful of further work in SB has successfully demonstrated the value of an individualised approach (Deb *et al*., 2018, Gough *et al*., 2017a; 2017b).

In athletes and coaches for example, efficiently using their finite time and resources is of utmost importance. Where nutritional aids are concerned, this requires sufficient data to guide a positive response and secondly, must have evidence that this response can be achieved consistently. Therefore, a better understanding of the reproducibility of blood analytes (pH and $HCO_3^-$) following acute SC ingestion and its interplay with daily biological variations i.e., potential renal acid load (Poupin *et al*., 2012; Remer, 2001; Remer and Manz, 1995), is highly important. Such daily biological variations may influence the erogenicity and consistency of an individualised ingestion strategy. Deb *et al*. (2018) first observed an ICC of $r = 0.60$ for the absolute change in pH from baseline across two repeated SB trials, demonstrating a fair to good level of reproducibility only and indicating some intra-individual variation. Gough *et al*. (2017a) also quantified the reproducibility of both pH and $HCO_3^-$ following 0.2 and 0.3 $g \cdot kg^{-1}$ BM doses, finding bicarbonate to display a greater reproducibility than pH for both TTP and absolute change. This provided substantial support for the ability of an individualised strategy to consistently promote peak alkalosis before exercise. Eliciting such a response is only of value if this translates to positive performance responses that are also consistent, for this Gough *et al*. (2017b) investigated the reproducibility of 4 km TT performance following individualised $HCO_3^-$ supplementation. Eleven trained cyclists completed five, 4 km TT's following the ingestion of 0.2 and 0.3 $g \cdot kg^{-1}$ BM SB twice or after no supplementation in the control trial. Based on their earlier work (Gough *et al*., 2017a) suggesting $HCO_3^-$ to be more reliable vs. pH, mean absolute change from baseline to peak [$HCO_3^-$] was good ($r = 0.68$) to excellent ($r = 0.78$) following 0.2 $g \cdot kg^{-1}$ BM and 0.3 $g \cdot kg^{-1}$ BM doses respectively. Significantly, performance responses following both doses displayed excellent

reproducibility ($r$ = 0.97 to 0.99). The use of a TTP strategy seems a valuable tool for maximising the likelihood of a consistent performance effects and identical investigations in SC are required.

### 2.8 Four-Kilometre Time Trial To Assess The Efficacy Of Sodium Citrate

Determining the efficacy of the selected exercise protocol is based on the degree to which the protocol is able to test the desired variable; SC acting mechanism. For this the 4 km TT appears appropriate, given the typical completion times of between ~4 min for world class athletes and up to 7 min for recreational athletes fall within the period whereby SC could exert an ergogenic effect (Carr, Hopkins and Gore., 2011a). Across both SB and SC research, the application of ecologically valid protocols is limited. Infrequent use of a 4 km TT, especially in SC research (0 articles), comes despite the 4 km TT representing a common distance for competing track and road cycling athletes. Nonetheless, cycling remains the most commonly researched sport in buffering agent research (Carr, Hopkins and Gore, 2011a).

Research evaluating the influence of SB on TT performance has returned mixed results concerning its erogenicity (McNaughton *et al*., 2016). A handful of studies using the 4 km TT have revealed minimal to no ergogenic effects following SB ingestion (Callaghan *et al*., 2017; Correia-Oliveira *et al*., 2017) although this may be explained in part, by the use of an ineffective ingestion strategy that may not have induced peak individual buffering capacity. Moreover, recent evidence suggests that ingesting SB based on individual TTP alkalosis is valuable in eliciting consistent ergogenic effects (Miller *et al*., 2016; Deb *et al*., 2018, Gough *et al*., 2017a, 2017b). Crucially, Gough *et al*. (2017a, 2017b) demonstrates that this strategy is associated with reproducible blood acid-base responses which are reflected in performance improvements which are also reproducible. Such evidence is yet to be obtained for SC.

Current evidence supports a greater degree of performance reproducibility in trained vs. lesser trained individuals (Gough *et al*., 2017b; Carr *et al*., 2012; Bird *et al*., 1995). This higher degree of performance reproducibility may be explained by the ability of trained athletes to better pace themselves for the exercise bout. This process is based on a greater knowledge of the distance to be completed

(Mauger, Jones and Williams, 2009), experience or practice of the exercise (cycling) (Mauger, Jones and Williams, 2010; 2009), feedback (Mauger, Jones and Williams, 2011; 2010; 2009) and differing levels of motivation to exercise to functional capacity (Mauger, Jones and Williams, 2011) in the face of the accumulating peripheral fatigue during a 4 km TT (Thomas *et al*., 2015). To this end, Ansley *et al*. (2004) found that the pacing strategies adopted by high-level cyclists during three successive 4 km TT that each elicited maximal $VO_2$ response was remarkably similar, with only distance covered provided by the researchers. This was also reflected in with similar average power outputs (TT1 447 ± 30 W; TT2 425 ± 33 W; TT3 434 ± 33 W) and imaging electromyography (iEMG) activity, which was comparable from 60 to 300 s.

### *2.9 Summary*

The numerous, key investigations that have assessed SC as ergogenic aid to augment high-intensity exercise performance have been discussed in this review. Although most research agrees that 0.5 $g \cdot kg^{-1}$ body mass of SC appears optimal for performance benefits (McNaughton, 1990; Schabort *et al*., 2000; Urwin *et al*., 2016) a number of key discrepancies in ingestion protocol and study design have rendered the ergogenic effect of SC unclear (Urwin, Slater and Gore, 2011a). Firstly, the time allowed between the ingestion of SC and the onset of exercise is varied, with many studies opting for a fixed time point, often utilising a 90 min time point as influenced by SB research (Urwin *et al*., 2016). This comes despite evidence supporting large inter-individual variation in peak responses and a minimum period of 100 min (Potteiger *et al*., 1996; Requena *et al*., 1996) up to >180 min (Urwin *et al*., 2016; 2019) necessary to induce a peak alkalotic response and simultaneously maximise possible ergogenic effects. Secondly, studies have failed to manage individual variation in GIS responses (Urwin *et al*., 2016; 2019) by selecting a fixed time point and failing to utilise appropriate methods to mitigate any symptoms, i.e., ingesting SC in capsules rather than solution (Hilton *et al*., 2019; 2020; Urwin *et al*., 2019). These key limitations come alongside a number of further modifying factors which have not received appropriate consideration or research attention, i.e., differing training status and flawed selection of an appropriate exercise task to elicit performance improvements etc.).

# Chapter 3 – GENERAL METHODS

This chapter aims to detail the procedures that are present within all studies making up the current thesis. Details will include descriptions, justification, and supporting information such as test-retest reliability data, where appropriate. Specific details for each study such as experimental design and precise methods deployed will be provided in the relevant chapters.

## 3.1 General Study Methods

### *3.1.1 Ethical Considerations*

Ethical approval for all studies within this thesis was provided by the Departmental Research Ethics Committee (DREC) for Sport and Physical Activity and, the University Research Ethics Sub-Committee (URESC) at Edge Hill University (SPA-REC-2019-252R1 and ETH2021-0301). Blood sampling was completed in compliance with the local Human Tissue Authority license regulations; accordingly, no blood samples were stored. Participation across all studies was completely voluntary and without remuneration. All participants were provided a Participant Information Sheet that detailed study commitments, experimental procedures, and any associated risks. Participants were given sufficient time to read and understand the requirements of participation. After this time, study protocols were detailed fully, and any questions were addressed prior to gaining written informed consent to participate. Participants had the right to withdraw from the study at any point, which was retained up until individual data collection was completed. No participants withdrew their consent herein. All testing took place in the physiology laboratory in the Department of Sport and Physical Activity, Edge Hill University.

Participants completed a medical screening questionnaire before taking part in any study to minimise the risk of adverse events during exercise. Accordingly, participants were required to notify the researcher if there was any change in health status throughout experimental testing. In Study 4 (Chapter 7), participants also completed a pre-exercise screening form before each exercise trial. The screening process involved measuring both resting heart rate and blood pressure. Heart rate was measured using manual palpation for one minute, whereas blood pressure was measured using an

automated sphygmomanometer. Participants were permitted to exercise only when systolic blood pressure ≤ 140 mmHg, diastolic blood pressure ≤ 90 mmHg and heart rate < 90 beats·min$^{-1}$.

### *3.1.2 Participants*

Healthy, recreationally active males between the ages of 18-30 years, who participated in sports such as boxing, cycling, and running were recruited to participate in each study. Participants were chosen based on the knowledge that performance on their sport may be limited by the accumulation of $H^+$ and peripheral fatigue and as result, they may benefit from increasing their buffering capacity through SC ingestion (de Oliveira *et al*., 2021; Grgic *et al*., 2021; Urwin *et al*., 2021a). Recreationally active participants are chosen to allow comparison with existing research in both SB (Hilton *et al*., 2019; 2020a; 2020b; Gough *et al*., 2017a) and SC (Urwin *et al*., 2021a; 2016), where recreationally athletes were used also. Training status was based on the classification criteria established by de Pauw *et al*. (2013). Participants herein are described as 'recreationally trained' based on their participation in regular exercise (> 4 h·week$^{-1}$), a peak oxygen uptake ($VO_{2peak}$) between 45.0-54.9 ml·kg·min$^{-1}$ and/or a peak power output (PPO) of 280-319 W. No participants had a history of GI disease/illness, and none were under pharmacological intervention during any study. Further exclusion criteria precluded those with hypertension, renal impairment or who were following a salt-restricted diet. Specific participant characteristics are provided in each study chapter.

## 3.2 General Experimental Procedures

### *3.2.1 Pre-Experimental Procedures*

Before each experimental trial, a range of standardised procedures were adhered to by participants, as confirmed verbally on arrival to the laboratory. All trials were commenced at same time of day (09:00-10:00) to limit the physiological effects of circadian rhythms (Ayala *et al*., 2021). Participants maintained habitual physical activity levels during each study, avoiding alcohol and caffeine consumption for 12 h (Guest *et al*., 2021; El-Sayed, Ali & Ali, 2005), and strenuous exercise for 24 h

prior to each laboratory visit (Tornero-Aguilera *et al*., 2022). Water intake was encouraged in the 24 h preceding sessions to ensure euhydration (Arnaoutis, 2022). A normal habitual diet was maintained up until the evening prior to attending the laboratory, with an overnight fast (~12 h) required thereafter. All trials took place under ambient laboratory conditions (room temperature = 18-20°C, humidity = 40-50%).

### *3.2.2 Determination Of Peak Oxygen Uptake ($VO_{2peak}$)*

Before the commencement of all experimental trials, participants performed an incremental exercise test to exhaustion to determine $VO_{2peak}$. This was performed on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands), which also allowed PPO to be calculated and used as a marker of training status alongside $VO_{2peak}$ (de Pauw *et al*., 2013). After a 5 min warm-up at 70 W, workload was increased by 1 $W \cdot s^{-2}$ (30 $W \cdot min^{-1}$) until volitional exhaustion. Throughout the protocol, participants were required to maintain a self-selected cadence (70-100 $rev \cdot min^{-1}$), with the test terminated when participants were unable to maintain >70% of this cadence for > 5 s, despite strong verbal encouragement. Breath-by-breath gases were continuously analysed using a gas analyser (Cosmed, K5, Italy) to measure oxygen uptake ($VO_2$), carbon dioxide production ($VCO_2$) and the respiratory exchange ratio (RER). Heart rate was also measured continuously using telemetry (Polar®, Kempele, Finland). Rating of perceived exhaustion (RPE) was recorded at one-minute stages on a scale of 6-20 (Borg, 1966). Data was averaged over the last 30 s of the test to determine $VO_{2peak}$, validated using a range of criteria (Midgley *et al*., 2007; 2009); heart rate $\leq 10$ $b \cdot min^{-1}$ of predicted max (220 $b \cdot min^{-1}$ – age), RER ≥ 1.15, and an RPE of 19+ at the termination of the test. Power achieved at the end of the test was also noted as PPO.

### *3.2.3 Gas Analysis*

Breath-by-breath gases were measured continuously during exercise using a portable gas analyser (Cosmed, K5, Italy). The K5 has been reported to a valid and reliable gas analyser (Guidetti *et al*., 2018; Perez-Suarez *et al*., 2018). Guidetti *et al*. (2018) found the K5 to demonstrate high agreement in

comparison to a simulation. ICC values were excellent for all variables (>0.99). Intra- and -inter K5 reliability produced excellent ICCs (>0.99), absolute percentage differences between measurements (MAPE) <2%, technical errors or ~ 1% and a minimal detectable change (MDC) ~ 3%. Prior to each use, the system was switched on and after a warm-up period (1 h), was calibrated in accordance with manufacturers guidance. As data were collected continuously, participants wore a face mask (Hans Rudolph, USA).

### *3.2.4 Heart Rate*

Heart rate was recorded continuously during exercise using a 'strap-to-watch' telemetric system (Polar®, Kempele, Finland). This required participants to wear a heart rate transmitter strap across their chest, aligned with the xiphoid process and with electrodes moistened to enhance signal conduction. Heart rate was recorded continuously during $VO_{2peak}$ testing and at a several time points during study 4 (chapter 7), described in depth therein.

### *3.2.5 Blood Sampling*

Fingertip capillary blood samples (95 μL) were taken at multiple time points throughout each study, detailed specifically in the relevant chapters. All samples were taken using a disposable lancet (AccuCheck Safe-T-Pro, Indianapolis, USA) using an aseptic technique and collected in heparin-coated capillary tubes (Radiometer Medical Ltd, Denmark). In chapters 4,5 and 6 samples were arterialised by warming the hand with a heated blanket (45°C) for 5 min prior to sample collection (Gough *et al*., 2017a; Johnston, Vickers and Mapleson, 1996). In study 4 (chapter 7) this was not completed for logistical reasons. Samples were immediately analysed using a blood gas analyser (ABL800 Basic, Radiometer Medical Ltd, Denmark) for pH, $[HCO_3^-]$, BE, $PO_2$ and $PCO_2$. The concentration of electrolytes; $[K^+]$, $[Na^+]$, $[Ca^{2+}]$ and chloride ($[Cl^-]$) were also analysed. The ABL800 BASIC radiometer demonstrates good to excellent criterion validity in comparison with alternative devices (Stadlbauer *et al*., 2011) and has shown excellent test-retest reliability (CV < 5%) in a small pilot study.

During exercise (study 4, chapter 7) a 5 μL sample was collected to obtain blood [$La^-$] using a portable analyser (Lactate Pro 2, LT-1730, Arkray, Japan), found to be accurate, reliable and valuable for applied/sports research (Crotty *et al*., 2021; Bonaventura *et al*., 2015; Pyne *et al*., 2000). In the work of Crotty *et al*. (2021) inter-Lactate Pro 2 reliability was high (CV = 3.3%); Bland Altman 95% limits of agreement ranged from ± 0.3 $mmol \cdot L^{-1}$ for blood [$La^-$] ≤ 4.0 $mmol \cdot L^{-1}$ to -1.6 to +1.4 $mmol \cdot L^{-1}$ for blood [$La^-$] > 8.0 $mmol \cdot L^{-1}$. Blood [$HCO_3^-$] were used to determine a range of post-ingestion kinetics, including lag time ($T_{lag}$), peak [$HCO_3^-$] ($C_{max}$), change in $C_{max}$ ($\Delta C_{max}$), time-to-reach $C_{max}$ ($T_{max}$) and area under the curve (AUC). Bicarbonate $T_{lag}$ was defined as the point at which blood [$HCO_3^-$] increased beyond normal daily fluctuations.

### 3.2.6 Perceptual Measures

#### *3.2.6.1 Perceptions Of Exertion And Fatigue*

During the exercise trials (study 4, chapter 7), perceived exertion and perceived fatigue were attained at regular intervals. Overall rating of perceived exertion ($RPE_O$) and leg RPE ($RPE_L$) were recorded using a 6-20 scale (Borg 1966; 1982). Both $RPE_O$ and $RPE_L$ were recorded to separate overall cardiovascular strain and localised muscular fatigue in the leg, respectively (Ritchie, 2012). This method was adopted as, in cycling particularly, localised muscular fatigue has been reported to dominate overall perception of effort and may skey independent cardiovascular responses (Demura and Nagasawa, 2003; Garcin *et al*., 1998; Borg, Ljungren and Ceci, 1985; Robertson *et al*., 1979a; 1979b). Perceive ratings of fatigue (ROF) were recorded on a 10-point Likert scale (Micklewright *et al*., 2017).

#### *3.2.6.2 Gastrointestinal Symptoms (GIS)*

Gastrointestinal symptoms (GIS) were recorded using an adapted GIS questionnaire (Carr *et al*., 2011) including nausea, flatulence, stomach cramping, belching, stomach-ache, bowel urgency, diarrhoea, vomiting and stomach bloating. Symptoms were measured on a visual analogue scale where "0 = No symptom" and "10 = severe symptom" (Miller *et al*., 2016). Visual analogue scales are deemed a valid

and reliable method to determine symptoms of discomfort (Harland, Dawkin and Marlin, 2015; Hawker *et al*., 2011) with ICC scores of between 0.90-0.99 (Bahreini *et al*., 2015; Gallagher *et al*., 2002) Symptom terminology and scoring was explained to the participants prior to each trial to ensure consistency in reporting. Frequency, severity, and time course of symptoms were established. Total symptoms experienced was also calculated, taken as the sum of each symptom reported throughout the sampling period.

# CHAPTER 4 – THE RELIABILITY OF BLOOD ACID-BASE RESPONSES FOLLOWING SODIUM CITRATE INGESTION

## 4.1 Introduction

High-intensity exercise presents a significant metabolic challenge to most athletes, with endurance intensive efforts (≥ 1 min) giving rise to the rapid production of metabolites including $H^+$ (Keyser 2010). Endogenous buffering, predominantly via the $HCO_3^-$ buffer system, removes much of the excess $H^+$ initially, however, the rate of production soon surpasses the capacity of the system (Hargreaves and Spriet 2018; Juel 2001). This reduction results in a lowering of intramuscular pH, which contributes to peripheral muscle fatigue (Kent-Braun, Fitts and Christie 2012; Sahlin, 1992), impairing high-intensity exercise performance (Thomas *et al*. 2015). In response, methods of increasing $HCO_3$- buffering capacity to resist peripheral fatigue during high-intensity exercise are widely researched (de Oliveira *et al*. 2021) with exogenous buffering agents such as SC and SB frequently ingested to augment the $HCO_3^-$ buffers and induce blood alkalosis (Heibel *et al*. 2018).

Of these agents, SB has attracted most research interest, with a recent meta-analysis concluding that SB was more effective in enhancing exercise performance compared to SC [$\beta_{SC:SB} = 0.10$ (95% CrI − 0.02 to 0.22)] (de Oliveira *et al*. 2021). Despite this, the two supplements induce similar levels of blood alkalosis (Heibel *et al*. 2018; McNaughton and Cedaro 1992; Van Montfoort *et al*. 2004), with some suggestion that SC may better reduce the occurrence of GIS in some individuals (Requena *et al*. 2005). The lesser effect of SC may be partly explained by a lack of an optimised ingestion strategy (Urwin *et al*. 2021b), with existing protocols often commencing exercise at the moment of maximal side effects and/or minimal changes in blood [$HCO_3^-$] (Painelli and Lancha Junior 2018). Furthermore, variances in individual TTP alkalosis of between 180-212 min are apparent (Urwin *et al*. 2016; 2019), suggesting that a general pre-exercise ingestion period (e.g., 60 min for all athletes) may be suboptimal.

An emerging approach to supplementation is centred around the concept of individual TTP (Gough *et al*. 2017a; 2017b; Miller *et al*. 2016), whereby the exercise task is commenced when an individual's peak blood pH or [$HCO_3^-$] occurs (Heibel *et al*. 2018). This suggests that maximally elevated circulating $HCO_3$- would improve the likelihood of performance improvements, with some evidence to support this strategy using SB (de Oliveira *et al*. 2021; Gough *et al*. 2017a; Hilton *et al*., 2020b; Miller *et al*., 2016). Promisingly, Boegman *et al*. (2020), observed an improvement 2000 m

rowing performance following an individualised TTP vs. a generalised time-point (367.0 ± 10.5 s vs. 369.0 ± 10.3 s ($p = 0.007$; d = 0.15)) suggesting this strategy not just to be effective, but more efficacious than ingestion at a generalised time point. Although more research is needed to determine the comparative efficacy of each approach on wider performance outcomes, it appears that the appropriateness of this method should at least be assessed as a potential method of optimising SC ingestion. Many metabolic reactions either produce or consume acids and bases, with net endogenous acid production (NEAP) regulated mainly by the diet. Given variations of the NEAP may influence acid-base balance, the applicability of an individualised approach based on blood alkalosis ($pH/HCO_3^-$) is dependent on the reliability of these analytes day-to-day (Poupin *et al*. 2012; Remer 2001; Remer and Manz 1995).

The reliability of the alkalotic response to SB has previously been investigated following the ingestion of 0.2 and 0.3 $g \cdot kg^{-1}$ BM doses ingested in aqueous solution (Gough *et al*. 2017). The key findings of this study supported the use of an individualised strategy, with both blood pH and $[HCO_3^-]$ demonstrating good reliability for TTP alkalosis and absolute change. Additionally, within 60 min of ingestion, blood-analyte values were similar, suggesting that where <60 min are available, smaller doses of SB may be acceptable, reducing the likelihood of GIS emerging. Despite recent interest in the use of SC to induce alkalosis (Urwin *et al*. 2016; 2019; 2021a), it is unclear if the individual responses to this exogenous buffer are reproducible. Therefore, the aim of the present study was to evaluate the reliability of individual blood pH, $HCO_3^-$ and $Na^+$ responses following the ingestion of 0.3, 0.4 or 0.5 $g \cdot kg^{-1}$ BM of SC provided in GEL capsule form.

## 4.2 Methods

### *4.2.1 Participants*

Participants were invited to take part in this study based on their engagement in any sport or activity that may benefit from an enhanced buffering capacity (Carr *et al*. 2011a; Urwin *et al*. 2021b). Fourteen recreationally active males (height 1.81 ± 0.55 m, body mass [BM] 81.4 ± 8.9 kg, age 27 ± 4 years, peak oxygen uptake [$VO_{2peak}$] 41.4 ± 13.0 $ml.kg^{-1}.min^{-1}$) volunteered for this double-blind, randomised crossover study. Ethical approval for this study was granted by the institutional research ethics committee (SPA-REC-2019-252R1), with each participant required to provide written informed consent and complete health screening before commencing data collection. Participants were also screened to confirm they had not ingested intra- or extracellular buffering agents within six months prior to the current study and were not following a sodium-restricted diet.

### *4.2.2 Pre-Experimental Procedures*

Participants reported to the laboratory on eight separate occasions, in a 4 h postprandial state and at the same time of day to minimise the effects of circadian variability (Laposky *et al*., 2008; Johnston 2014). Participants were asked to avoid alcohol and any strenuous/unaccustomed exercise during the 24 h period prior to each experimental treatment (Lieber, 2000). Caffeine was also prohibited 12 h before experimental treatments to limit any disturbance on metabolic regulation (Westerterp-Platenga *et al*., 2006) and mitigate additional risk of GIS (Boekama *et al*., 1999). Experimental trials were conducted at least 48 h apart to facilitate the washout of residual SC (Urwin *et al*., 2021a).

### *4.2.3 Determination Of Peak Oxygen Uptake ($VO_{2peak}$)*

Peak $VO_2$ ($VO_{2peak}$) was determined based on a previously utilised protocol (Deb *et al*., 2018). The RAMP test began with 5 min unloaded pedalling into a ramped increase of 0.5 $W{\cdot}s^{-1}$, equating to 30 $W{\cdot}min^{-1}$. A preferred cadence between 70-90 $revs{\cdot}min^{-1}$ was selected prior to the test, with participants asked to maintain this cadence to within 10 $revs{\cdot}min^{-1}$, until volitional exhaustion despite

strong encouragement for 10 sec. Tests were performed on an electromagnetically braked cycle ergometer (Lode Excalibur, Germany). Breath-by-breath gases were continuously analysed using a gas analyser (K5, Cosmed, Italy) to measure oxygen uptake ($VO_2$), carbon dioxide production ($CO_2$) and the respiratory exchange ratio (RER). The $VO_{2peak}$ was determined by averaging $VO_2$ over the final 30 s of exercise.

### *4.2.4 Experimental Trials*

The seven subsequent experimental trials were randomised using a Balanced Latin Square generator and involved one trial requiring the ingestion of no treatment (CON), two trials requiring the ingestion of 0.3 $g \cdot kg^{-1}$ BM SC (SC3a, SC3b), two trials requiring the ingestion of 0.4 $g \cdot kg^{-1}$ BM SC (SC4a, SC4b) and two trials requiring the ingestion of 0.5 $g \cdot kg^{-1}$ BM SC (SC5a, SC5b). Both sodium chloride and SC (Pro Athlete Supplementation, Rhymney, UK) were administered in size '0' gelatine capsules (Bulk Powders$^{TM}$, Colchester, UK), which were prepared by a laboratory technician not involved with the research. Capsules were consumed with 500 ml of water at room temperature (18°C) after an overnight fast. The mean number of capsules consumed were 33 ± 4, 44 ± 5, and 55 ± 6 for the 0.3, 0.4 and 0.5 $g \cdot kg^{-1}$ BM SC, respectively.

An initial arterialised fingertip capillary blood sample was obtained from participants whilst seated and rested prior to ingesting SC. After capsules were ingested (<10 min), blood samples were taken every 20 min for 4 h. A heated blanket (45°C) was used to warm the hand prior to each sample to assist with blood sampling (Gough *et al*., 2017a; Johnston, Vickers and Mapleson, 1996). At each interval, a GIS questionnaire was completed to assess the severity of a range of symptoms on a visual analogue scale (VAS) where 0 = no symptom and 10 = most severe (Gough *et al*., 2021). During this time, participants were asked to remain seated with only toilet breaks allowed. No food was consumed during testing although water was consumed *ad libitum* in the first trial and replicated in the remaining trials. Blood samples were collected in 100 µl heparin-coated clinitubes (Radiometer Medical Ltd, Denmark) and subsequently analysed for blood pH, $[HCO_3]^-$ and $[Na^+]$ using a blood gas analyser

(ABL800 BASIC, Radiometer Medical Ltd). This device has demonstrated good reliability for the analysis of pH, [$HCO_3$], $PCO_2$ and [$Na^+$] (Stadlbauer *et al*., 2011).

### *4.2.5 Statistical Analysis*

Assessed variables were analysed for normality (Shapiro-Wilks and Q-Q plots) and homogeneity of variance/sphericity (Mauchly) before undergoing further statistical analysis. Both one-way (treatment) and two-way (treatment/time) repeated measures analysis of variation (ANOVA) were used to deduce differences in blood parameters (pH, [$HCO_3^-$] and [$Na^-$]) with Bonferroni pairwise comparisons. Where sphericity was violated, the Greenhouse Geiser correction was applied. Effect sizes were calculated using partial eta squared ($\eta_p^2$). For nonparametric data, such as GIS scores, the Kruskal-Wallis test was used with H scores, degrees of freedom and significance reported. For GIS, 'total symptoms' (sum of each individual reported score) and 'TTP severity' (first instance at which the largest individual score occurred) were used to determine overall GIS responses. Effect sizes for Kruskal-Wallis tests were determined using eta squared ($\eta^2$). Statistical significance was set at $P < 0.05$. Heteroscedasticity was assessed using Bland-Altman plots. Where data were determined as heteroscedastic data were log transformed. Agreement between conditions was evaluated using Cronbach's alpha (α), intraclass correlation coefficients (ICCs) and coefficient of variation (CV) [(SD)/mean x 100]. Calculations were carried out using Microsoft® Excel 2019 (Microsoft Inc, Redmond, WA, USA) and statistical procedures were completed using SPSS version 27 (IBM, Chicago, IL, USA).

## 4.3 Results

### *4.3.1 Reliability Of Blood pH, $HCO_3^-$ and $Na^+$*

Resting measures of blood pH, [$HCO_3^-$] and [$Na^+$] displayed poor to moderate reliability across all experimental doses ($r$ = 0.04 to 0.63). Moderate reliability was observed at rest in pH (SC5 $r$ = 0.56, $P$ = 0.01), $Na^+$ (SC4 $r$ = 0.63, $P$ = 0.005) and $HCO_3^-$ (SC3 $r$ = 0.62, $P$ = 0.010). Reliability for pH across the 4 h sampling period ranged from poor to moderate in SC4 ($r$ = 0.01-0.72) as well as SC3 ($r$ = 0.20

to 0.71) and from poor to excellent in SC5 ($r$ = 0.01-0.96). Blood [$HCO_3^-$] demonstrated poor to good reliability in SC5 ($r$ = 0.14-0.82) and SC4 ($r$ = 0.21-0.85), whereas SC3 demonstrated poor to moderate reliability ($r$ = 0.09-0.64). Finally, [$Na^+$] displayed poor to moderate reliability in both SC4 ($r$ = 0.22-0.60) and SC3 (0.13-0.76), although SC5 produced poor to good reliability ($r$ = 0.32-0.76). Table 4.1 also provides a statistical summary including Cronbach's α analysis and coefficient of variation for pH and $HCO_3^-$.

**Table 4.1** Intraclass correlation coefficient (ICC) and Cronbach's alpha between repeated treatments (0.5 (SC5), 0.4 (SC4) and 0.3 $g \cdot kg^{-1}$ body mass sodium citrate (SC)) for blood pH and bicarbonate concentration ($[HCO_3^-]$).

| Time (min) | 68 | 85 | 102 | 119 | 136 | 153 | 170 | 187 | 204 | 221 | 238 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **pH** | | | | | | | | | | | |
| **SC5** | | | | | | | | | | | |
| **R value** | 0.91 | 0.63 | 0.49 | 0.78 | 0.65 | 0.40 | 0.01 | 0.01 | 0.49 | 0.66 | 0.42 |
| **α** | 0.95 | 0.76 | 0.64 | 0.87 | 0.77 | 0.55 | 0.02 | 0.02 | 0.68 | 0.79 | 0.62 |
| **CI** | | | | | | | | | | | |
| **Upper bound** | 0.97 | 0.87 | 0.81 | 0.93 | 0.87 | 0.76 | 0.50 | 0.52 | 0.80 | 0.88 | 0.76 |
| **Lower bound** | 0.74 | 0.14 | -0.00 | 0.42 | 0.18 | -0.18 | -0.44 | -0.49 | 0.01 | 0.23 | -0.06 |
| **CV** | 0.33 | 0.37 | 0.29 | 0.30 | 0.38 | 0.32 | 0.31 | 0.29 | 0.31 | 0.30 | 0.33 |
| **Interpretation** | Excellent | Moderate | Poor | Good | Moderate | Poor | Poor | Poor | Poor | Moderate | Poor |
| **SC4** | | | | | | | | | | | |
| **R value** | 0.01 | 0.52 | 0.35 | 0.16 | 0.72 | 0.11 | 0.45 | 0.59 | 0.34 | 0.26 | 0.62 |
| **α** | x | 0.67 | 0.53 | 0.29 | 0.90 | 0.18 | 0.61 | 0.77 | 0.51 | 0.41 | 0.76 |
| **CI** | | | | | | | | | | | |
| **Upper bound** | x | 0.85 | 0.73 | 0.60 | 0.92 | 0.60 | 0.78 | 0.84 | 0.74 | 0.71 | 0.87 |
| **Lower bound** | x | -0.11 | -0.17 | -0.32 | 0.10 | -0.49 | -0.09 | 0.14 | -0.23 | -0.34 | 0.14 |
| **CV** | 0.29 | 0.29 | 0.37 | 0.29 | 0.3 | 0.22 | 0.24 | 0.32 | 0.33 | 0.29 | 0.25 |
| **Interpretation** | Poor | Moderate | Poor | Poor | Moderate | Poor | Poor | Moderate | Poor | Poor | Moderate |
| **SC3** | | | | | | | | | | | |
| **R value** | 0.43 | x | 0.20 | x | 0.26 | 0.13 | 0.10 | x | 0.20 | 0.66 | 0.65 |
| **α** | 0.64 | X | 0.40 | x | 0.42 | 0.21 | 0.17 | 0.01 | 0.34 | 0.78 | 0.79 |
| **CI** | | | | | | | | | | | |
| **Upper bound** | 0.77 | X | 0.62 | x | 0.68 | 0.61 | 0.59 | 0.56 | 0.65 | 0.88 | 0.87 |
| **Lower bound** | -0.07 | x | -0.21 | x | -0.26 | -0.47 | -0.47 | -0.60 | -0.34 | 0.18 | 0.23 |
| **CV** | 0.23 | 0.16 | 0.25 | 0.25 | 0.27 | 0.29 | 0.40 | 0.32 | 0.38 | 0.28 | 0.19 |
| **Interpretation** | Poor | Poor | Poor | Poor | Poor | Poor | Poor | Poor | Poor | Moderate | Moderate |

***Continued below**

| Time (min) | 68 | 85 | 102 | 119 | 136 | 153 | 170 | 187 | 204 | 221 | 238 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **$HCO_3^-$** | | | | | | | | | | | |
| **SC5** | | | | | | | | | | | |
| **R value** | 0.56 | 0.59 | 0.75 | 0.71 | 0.70 | 0.73 | 0.69 | 0.79 | 0.67 | 0.75 | 0.82 |
| **α** | 0.71 | 0.01 | 0.85 | 0.83 | 0.81 | 0.85 | 0.81 | 0.88 | 0.80 | 0.86 | 0.90 |
| **CI** | | | | | | | | | | | |
| **Upper bound** | 0.84 | 0.52 | 0.91 | 0.90 | 0.89 | 0.90 | 0.89 | 0.93 | 0.88 | 0.91 | 0.94 |
| **Lower bound** | 0.06 | -0.52 | 0.39 | 0.29 | 0.27 | 0.34 | 0.27 | 0.48 | 0.24 | 0.40 | 0.54 |
| **CV** | 3.81 | 4.28 | 5.20 | 5.11 | 5.75 | 6.32 | 6.70 | 6.71 | 6.32 | 6.34 | 6.22 |
| **Interpretation** | Moderate | Moderate | Good | Moderate | Moderate | Moderate | Moderate | Good | Moderate | Good | Good |
| **SC4** | | | | | | | | | | | |
| **R value** | 0.21 | 0.30 | 0.29 | 0.68 | 0.54 | 0.85 | 0.46 | 0.75 | 0.31 | 0.66 | 0.75 |
| **α** | 0.33 | 0.48 | 0.45 | 0.81 | 0.70 | 0.92 | 0.72 | 0.87 | 0.46 | 0.78 | 0.85 |
| **CI** | | | | | | | | | | | |
| **Upper bound** | 0.68 | 0.74 | 0.69 | 0.89 | 0.82 | 0.95 | 0.78 | 0.91 | 0.73 | 0.89 | 0.92 |
| **Lower bound** | -0.42 | -0.28 | -0.24 | 0.26 | 0.05 | 0.60 | -0.03 | 0.40 | -0.30 | 0.18 | 0.35 |
| **CV** | 3.53 | 3.83 | 3.12 | 4.19 | 3.26 | 3.29 | 3.77 | 3.48 | 2.92 | 2.68 | 2.59 |
| **Interpretation** | Poor | Poor | Poor | Moderate | Moderate | Good | Poor | Good | Poor | Moderate | Good |
| **SC3** | | | | | | | | | | | |
| **R value** | 0.64 | 0.41 | 0.43 | 0.51 | 0.09 | 0.20 | 0.09 | 0.12 | 0.28 | 0.41 | 0.48 |
| **α** | 0.78 | 0.57 | 0.58 | 0.68 | 0.16 | 0.35 | 0.16 | 0.22 | 0.46 | 0.58 | 0.64 |
| **CI** | | | | | | | | | | | |
| **Upper bound** | 0.87 | 0.77 | 0.77 | 0.82 | 0.58 | 0.64 | 0.58 | 0.59 | 0.68 | 0.77 | 0.80 |
| **Lower bound** | 0.19 | 0.16 | 0.14 | 0.01 | -0.45 | 0.29 | -0.46 | -0.40 | 0.20 | 0.12 | 0.05 |
| **CV** | 3.87 | 3.36 | 3.62 | 3.62 | 3.17 | 2.90 | 2.91 | 3.56 | 3.16 | 3.07 | 2.82 |
| **Interpretation** | Moderate | Poor | Poor | Moderate | Poor | Poor | Poor | Poor | Poor | Poor | Poor |

Poor reliability of TTP [$HCO_3^-$] was observed for SC5 ($r$ = 0.10, $P$ = 0.361; α = 0.18), SC4 ($r$ = 0.17, $P$ = 0.27; α = 0.29), and SC3 ($r$ = 0.36, $P$ = 0.116; α = 0.68). Poor reliability was also demonstrated for TTP pH in SC5 ($r$ = 0.38, $P$ = 0.037; α = 0.68), SC4 ($r$ = 0.33, $P$ = 0.058; α = 0.68), and SC3 ($r$ = 0.02, $P$ = 0.472; α = > 0.50). Finally, poor reliability was also found for TTP [$Na^+$] in SC5 ($r$ = 0.10, $P$ = 0.390; α = > 0.5), SC4 ($r$ = 0.07, $P$ = 0.376; α = 0.16), and SC3 ($r$ = 0.05, $P$ = 0.404; α = 0.13).

Absolute change (peak change from baseline) for [$HCO_3^-$] displayed good reliability for SC5 (ICC: $r$ 0.72, $P$ < 0.001; α = 0.84) compared with poor reliability for both SC4 (ICC: $r$ 0.39, $P$ = 0.085; α = 0.51) and SC3 (ICC: $r$ 0.21, $P$ = 0.219; α = 0.35). Moderate reliability was observed for absolute change in pH in SC5 (ICC: $r$ = 0.63, $P$ = 0.003; α = 0.81) in comparison with poor reliability in both SC4 (ICC: $r$ = 0.38, $P$ = 0.090; α = 0.54) and SC3 (ICC: $r$ = 0.35, P = 0.109; α = 0.51). Finally, moderate reliability was found for [$Na^+$] in SC5 (ICC: $r$ = 0.58, $P$ = 0.973; α = > 0.5) vs. poor reliability in both SC4 (ICC: $r$ = 0.30, $P$ = 0.139; α = 0.512) and SC3 (ICC: $r$ = 0.16, $P$ = 0.709; α = > 0.5).

### *4.3.2 Differences Between Treatments*

There was no significant effect of condition on pH or [$HCO_3^-$] (both $P$ > 0.05). Conversely, a significant effect for condition on [$Na^+$] ($F_{2.2,8.7}$= 11.462, $P$ = 0.003, $\eta_p^2$ = 0.741) existed. Pairwise comparisons revealed small differences between SC5a and SC4a (145 vs. 142 mmol·$L^{-1}$, $P$ = 0.026). A significant effect for time was observed for [$HCO_3^-$] ($F_{2.0, 10.0}$ = 176.149, $P$ = < 0.001, $\eta_p^2$ = 0.972), pH ($F_{1.5, 4.5}$ = 54.717, $P$ = 0.001, $\eta_p^2$ = 0.948) and [$Na^+$] ($F_{2.7, 10.9}$ = 52.984, $P$ = < 0.001, $\eta_p^2$ = 0.930) although further analysis demonstrated that [$Na^+$] did not differ significantly vs. control treatment at any time point (Figure 4.1). No significant interactions between condition and time were present for pH, [$HCO_3^-$] or [$Na^+$] (all $P$ > 0.05).

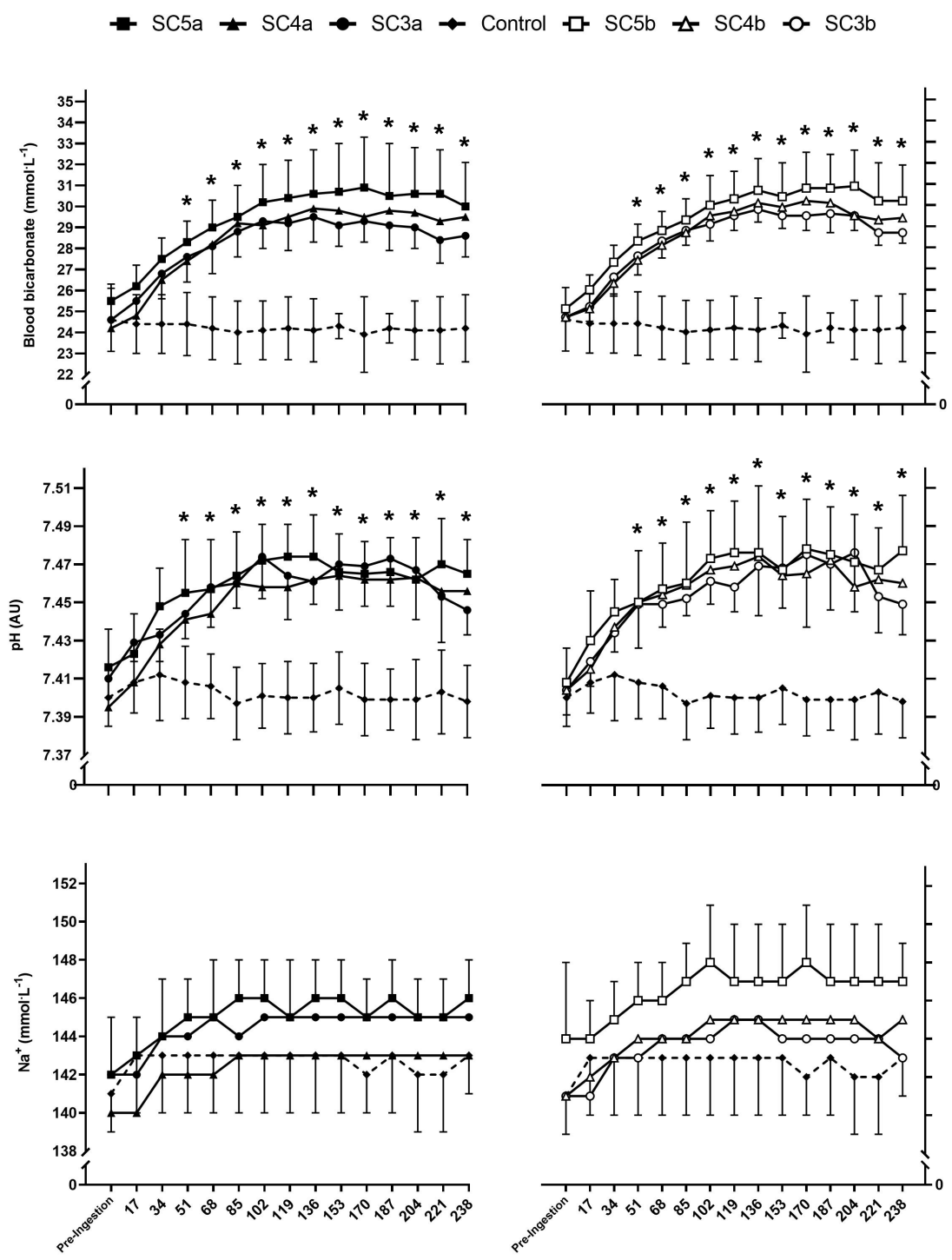


**Figure 4.1.** Mean (± SD) blood analyte responses for bicarbonate concentration ($[HCO_3^-]$) (top), pH (middle) and sodium concentration ($[Na^+]$) (bottom) following the ingestion of 0.5 (SC5), 0.4 (SC4) and 0.3 (SC3) $g{\cdot}kg^{-1}$ body mass (BM) sodium citrate (SC) vs. sodium chloride control (0.07 $g{\cdot}kg^{-1}$ BM). Some error bars are omitted for clarity. *Denotes a significant difference between SC and control conditions.

There was no significant difference between TTP pH and [$HCO_3^-$], although both TTP bicarbonate and pH varied significantly to TTP Na (both $P = < 0.001$). There was no significant difference in absolute change between conditions for bicarbonate concentration, pH and Na concentration (all $P > 0.05$). Both absolute change of pH, [$HCO_3^-$] and [$Na^+$] as well as TTP displayed larger inter-individual variation (Table 4.2).

### *4.3.3 Gastrointestinal Symptom Responses*

Total symptoms displayed good (between 0.75 and 0.9) to poor (<0.50) reliability for SC5 ($r = 0.89$, $P < 0.001$; $\alpha = 0.80$), SC4 ($r = 0.66$, $P = 0.354$; $\alpha = 0.74$) and SC3 ($r = 0.10$, $P = 0.538$; $\alpha = 0.34$). In total, 8 of 14 participants reported no GI symptoms across SC5, SC4 and SC3 (see Table 4.3 for individual symptoms). Total symptom scores were highest in SC4 (70 AU) compared with SC3 (32 AU) and SC5 (16 AU), although the difference was not significant ($H(2) = 0.366$, $P = 0.833$, $\eta^2 = 0.009$).

Time to peak (TTP) GI upset displayed moderate reliability in both SC3 and SC4 in comparison with SC5, which displayed good reliability (TTP severity: SC5 $r = 0.81$, $P = 0.135$; $\alpha = 0.47$ vs. SC4 $r = 0.58$, $P = 0.583$; $\alpha = 0.39$ vs. SC3 $r = 0.52$, $P = 0.023$; $\alpha = 0.95$). TTP was established earlier in SC3 in comparison with SC5 and SC4 (mean = 47 vs. 54 vs. 129 min, respectively), although the difference was not significant *(*$H(2) = 0.723$, $P = 0.696$, $\eta^2 = 0.018$*)*.

**Table 4.2.** Individual data displaying time-to-peak and absolute change in both blood pH and bicarbonate concentration ([$HCO_3^-$]) following the ingestion of 0.5 (SC5), 0.4 (SC4) and 0.3 (SC3) $g \cdot kg^{-1}$ body mass sodium citrate (SC).

| Participant | Time-to-peak (min) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH (-log10[H+]) | | | | | | $HCO_3^-$ ($mmol \cdot l^{-1}$) | | | | | |
| | SC5a | SC5b | SC4a | SC4b | SC3a | SC3b | SC5a | SC5b | SC4a | SC4b | SC3a | SC3b |
| **1** | 170 | 170 | 204 | 85 | 85 | 170 | 170 | 102 | 153 | 136 | 102 | 136 |
| **2** | 136 | 153 | 204 | 187 | 187 | 68 | 170 | 170 | 187 | 136 | 102 | 68 |
| **3** | 170 | 238 | 153 | 187 | 187 | 187 | 102 | 204 | 85 | 153 | 119 | 119 |
| **4** | 102 | 85 | 187 | 153 | 153 | 170 | 136 | 136 | 153 | 170 | 153 | 136 |
| **5** | 119 | 119 | 136 | 119 | 119 | 238 | 221 | 204 | 136 | 136 | 153 | 187 |
| **6** | 136 | 136 | 204 | 187 | 187 | 170 | 170 | 136 | 204 | 170 | 119 | 187 |
| **7** | 119 | 204 | 119 | 153 | 153 | 85 | 119 | 204 | 102 | 153 | 136 | 136 |
| **8** | 68 | 136 | 85 | 102 | 102 | 170 | 119 | 170 | 102 | 187 | 102 | 136 |
| **9** | 238 | 238 | 153 | 204 | 204 | 204 | 187 | 187 | 238 | 170 | 136 | 136 |
| **10** | 85 | 238 | 187 | 153 | 170 | 170 | 136 | 136 | 187 | 187 | 153 | 170 |
| **11** | 153 | 187 | 136 | 187 | 204 | 204 | 187 | 187 | 153 | 119 | 238 | 204 |
| **12** | 136 | 187 | 187 | 204 | 170 | 170 | 102 | 136 | 170 | 187 | 187 | 136 |
| **13** | 153 | 238 | 204 | 102 | 153 | 153 | 153 | 204 | 85 | 136 | 153 | 136 |
| **14** | 221 | 221 | 204 | 170 | 119 | 119 | 153 | 119 | 119 | 170 | 170 | 119 |
| **Mean** | 143 | 182 | 169 | 143 | 157 | 163 | 152 | 164 | 148 | 158 | 145 | 143 |
| **Range** | 170 | 153 | 119 | 102 | 119 | 170 | 119 | 102 | 153 | 68 | 136 | 136 |
| **SD** | 47 | 51 | 39 | 35 | 40 | 46 | 35 | 36 | 46 | 23 | 38 | 34 |
| **CV** | 32.8 | 27.7 | 22.9 | 24.5 | 25.6 | 28.0 | 23.0 | 21.8 | 31.3 | 14.3 | 26.0 | 24.1 |
| **SEM** | 12.6 | 13.5 | 10.3 | 9.4 | 10.7 | 12.2 | 9.3 | 9.5 | 12.4 | 6.0 | 10.0 | 9.2 |

| Participant | Peak absolute change from baseline | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH (-log10[H+]) | | | | | | $HCO_3^-$ ($mmol \cdot l^{-1}$) | | | | | |
| | SC5a | SC5b | SC4a | SC4b | SC3a | SC3b | SC5a | SC5b | SC4a | SC4b | SC3a | SC3b |
| **1** | 0.08 | 0.07 | 0.08 | 0.06 | 0.06 | 0.09 | 7.5 | 5.9 | 5.0 | 4.9 | 4.3 | 5.3 |
| **2** | 0.11 | 0.09 | 0.09 | 0.08 | 0.09 | 0.06 | 7.6 | 7.3 | 7.5 | 6.1 | 6.9 | 5.0 |
| **3** | 0.07 | 0.10 | 0.03 | 0.05 | 0.07 | 0.06 | 6.0 | 7.2 | 5.7 | 4.5 | 5.1 | 5.4 |
| **4** | 0.05 | 0.07 | 0.07 | 0.09 | 0.08 | 0.11 | 3.8 | 4.9 | 5.1 | 5.7 | 4.9 | 5.7 |
| **5** | 0.11 | 0.13 | 0.11 | 0.09 | 0.09 | 0.06 | 6.6 | 5.4 | 4.9 | 4.9 | 5.8 | 6.4 |
| **6** | 0.08 | 0.07 | 0.09 | 0.07 | 0.07 | 0.08 | 6.2 | 6.2 | 7.2 | 5.8 | 5.0 | 5.3 |
| **7** | 0.03 | 0.06 | 0.07 | 0.08 | 0.07 | 0.07 | 1.8 | 2.9 | 5.4 | 5.8 | 4.5 | 5.5 |
| **8** | 0.10 | 0.14 | 0.07 | 0.13 | 0.09 | 0.16 | 1.5 | 5.4 | 4.6 | 5.0 | 4.6 | 5.8 |
| **9** | 0.06 | 0.10 | 0.08 | 0.10 | 0.08 | 0.08 | 5.9 | 7.0 | 6.1 | 7.1 | 5.3 | 4.7 |
| **10** | 0.09 | 0.10 | 0.10 | 0.14 | 0.12 | 0.12 | 7.0 | 8.2 | 5.0 | 7.4 | 6.2 | 6.2 |
| **11** | 0.07 | 0.08 | 0.08 | 0.07 | 0.06 | 0.06 | 8.3 | 8.3 | 6.3 | 7.0 | 5.5 | 5.4 |
| **12** | 0.03 | 0.05 | 0.09 | 0.08 | 0.08 | 0.08 | 4.8 | 4.6 | 6.7 | 5.5 | 4.9 | 5.3 |
| **13** | 0.07 | 0.08 | 0.08 | 0.06 | 0.07 | 0.08 | 6.8 | 7.2 | 6.6 | 5.6 | 5.0 | 5.2 |
| **14** | 0.09 | 0.07 | 0.09 | 0.09 | 0.07 | 0.08 | 5.9 | 6.1 | 8.6 | 6.7 | 4.4 | 4.6 |
| **Mean** | 0.07 | 0.09 | 0.08 | 0.09 | 0.08 | 0.08 | 5.7 | 6.2 | 6.1 | 5.9 | 5.2 | 5.4 |
| **Range** | 0.08 | 0.09 | 0.08 | 0.09 | 0.06 | 0.11 | 6.8 | 5.4 | 4.0 | 2.9 | 2.6 | 1.8 |
| **SD** | 0.02 | 0.03 | 0.02 | 0.02 | 0.02 | 0.03 | 2.1 | 1.5 | 1.2 | 0.9 | 0.7 | 0.5 |
| **CV** | 33.9 | 29.8 | 23.1 | 29.0 | 20.3 | 34.2 | 36.1 | 24.1 | 19.3 | 15.4 | 14.1 | 9.2 |
| **SEM** | 0.01 | 0.01 | 0.01 | 0.01 | 0.00 | 0.01 | 0.5 | 0.4 | 0.3 | 0.2 | 0.2 | 0.1 |

*SD* = Standard Deviation, *CV* = Coefficient of Variation, *SEM* = Standard Error of Mean

**Table 4.3.** Total symptoms and most severe individual symptom of gastrointestinal upset experienced following ingestion of 0.5 (SC5), 0.4 (SC4) and 0.3 (SC3) $g \cdot kg^{-1}$ body mass (BM) sodium citrate (SC).

| Participant | SC5a | | SC5b | |
|---|---|---|---|---|
| | **Total symptoms (AU)** | **Peak symptom (AU)** | **Total symptoms (AU)** | **Peak symptom (AU)** |
| **1** | 47 | Stomach bloating | 10 | Nausea |
| **2** | 8 | Nausea/stomach ache | 0 | None |
| **3** | None | None | None | None |
| **4** | None | None | None | None |
| **5** | None | None | None | None |
| **6** | None | None | None | None |
| **7** | 8 | Nausea | 7 | Nausea |
| **8** | 23 | Vomiting | 12 | Diarrhoea |
| **9** | None | None | None | None |
| **10** | None | None | None | None |
| **11** | None | None | None | None |
| **12** | None | None | None | None |
| **13** | None | None | None | None |
| **14** | 49 | Stomach bloating | 54 | Nausea |
| **Participant** | **SC4a** | | **SC4b** | |
| | **Total symptoms (AU)** | **Peak symptom (AU)** | **Total symptoms (AU)** | **Peak symptom (AU)** |
| **1** | 14 | Nausea/stomach ache | 32 | Nausea |
| **2** | 40 | Nausea | 2 | Nausea/stomach ache |
| **3** | None | None | None | None |
| **4** | None | None | None | None |
| **5** | None | None | None | None |
| **6** | None | None | None | None |
| **7** | None | None | None | None |
| **8** | 2 | Flatulence | None | None |
| **9** | 4 | Bowel urgency | None | None |
| **10** | None | None | None | None |
| **11** | None | None | None | None |
| **12** | None | None | None | None |
| **13** | None | None | None | None |
| **14** | 16 | Bowel urgency | 238 | Bowel urgency |
| **Participant** | **SC3a** | | **SC3b** | |
| | **Total symptoms (AU)** | **Peak symptom (AU)** | **Total symptoms (AU)** | **Peak symptom (AU)** |
| **1** | 16 | Belching/stomach ache | 0 | Nausea |
| **2** | None | None | 38 | Stomach cramp/ache |
| **3** | None | None | None | None |
| **4** | None | None | None | None |
| **5** | None | None | None | None |
| **6** | None | None | None | None |
| **7** | 19 | Flatulence | None | None |
| **8** | None | None | None | None |
| **9** | None | None | None | None |
| **10** | None | None | None | None |
| **11** | None | None | None | None |
| **12** | None | None | None | None |
| **13** | None | None | None | None |
| **14** | 51 | Bowel urgency | 4 | Flatulence |

### 4.4 Discussion

This study represents the first investigation into the reliability of blood alkalosis responses to exogenous SC ingestion. The key findings were that the individual responses are highly variable irrespective of ingested dose. The reliability interpretations across all blood alkalosis variables ranged from excellent to poor, suggesting that an individualised approach to SC ingestion has limited efficacy. Poor reliability was also present at rest, demonstrating that blood acid-base regulation is dynamic and that measures of blood alkalosis are subject to significant natural fluctuations (de Oliveira *et al*., 2020), even prior to ingesting extracellular buffers under strict pre-experimental control(s). Poor reliability was observed for TTP pH, [$HCO_3^-$] and [$Na^+$] for each ingested dose. Despite similarities in overall TTP (pH and [$HCO_3^-$]), a substantial degree of inter- and intra-individual variability was observed throughout. Given this large variability in key blood measures and the low reliability of TTP blood alkalosis, it is suggested that practitioners/athletes look beyond an individualised approach to pre-exercise ingestion of SC. Data here also challenge the current recommendations for SC ingestion, which propose that 0.4-0.5 $g \cdot kg^{-1}$ BM of SC be administered ~180-200 min prior to commencing exercise (Cerullo *et al*. 2020; Urwin *et al*. 2021b). As a result, a fixed approach to pre-exercise ingestion may not be the suitable alternative. Therefore, future work needs to systematically investigate the true likely ergogenic "period" following ingestion.

Both TTP pH and [$HCO_3^-$] occurred markedly earlier overall in the present study (160 and 152 min) than previously reported (240 and 251 min, respectively) using an identical delivery method (size '0' GEL capsules) and a similar ingestion period (15 vs. 10 min) (Urwin *et al*. 2021b). Although both controlled pre-laboratory nutritional intake, upon arrival, Urwin *et al*. (2021b) provided a standardised meal (1.75 $g \cdot kg^{-1}$ BM of CHO) opting to resemble real-world practice rather than isolate the effects of supplementation through a 4 h fast. Whilst 10% of food may still be present in the stomach after this period (Stannard *et al*. 2016), meaning residual effects may have remained even when fasted, it is possible that meal volume, composition and texture may have contributed to the differing timeframes (Gough *et al*. 2017a). Furthermore, the gastric acid secretion responses to food intake, may limit the bioavailability of ingested substances (Schmidt and Dahloff, 2002) and whilst the variability in some

of the responses may be accounted for in this way, the similar peak responses (blood pH and [$HCO_3^-$]) observed in both studies do not directly support this. Alternatively, resting values reported following the ingestion of 0.5 g·kg$^{-1}$ BM were significantly higher for both blood pH (7.412 (7.365-7.449) vs. 7.256 (7.230-7.282) AU) and [$HCO_3^-$] (25.3 (24-26.8) vs. 22 (20.8-23.4) mmol·L$^{-1}$), which could explain the reduced time to attain similar levels of peak alkalosis. A final consideration is the use of different sampling frequencies (every 17 vs. 30 min), which may account for small quantities of variation at each time point, independent to variation from physiological responses to SC.

In the present study, the size of ingested dose produced no significant effect on blood pH or [$HCO_3^-$], highlighted by the similarities in peak [$HCO_3^-$] of 30 (27.2-32), 30.3 (27.8-33.9) and 31.2 (26.8-34.2) mmol·L$^{-1}$ for 0.3, 0.4 and 0.5 g·kg$^{-1}$ BM, respectively. Following the administration of 0.5 g·kg$^{-1}$ BM of SC encapsulated in GEL, similar average peak values of 30.4 (29.8–31.1) and 29.6 (28.6-30.5) mmol·L$^{-1}$ have also been reported (Urwin *et al*. 2019; 2021a), suggesting that while dose may not have offered additive improvements, meaningful changes in blood [$HCO_3^-$] (and pH) were achieved alongside a greater degree of inter-individual variation.

In order for increased extracellular buffering capacity to elicit ergogenic effects, there needs to be an increase in circulating [$HCO_3^-$] post-ingestion (Heibel *et al*. 2018). Whilst the minimal increase necessary to benefit exercise limited by peripheral fatigue remains unknown, evidence suggests that greater effects were obtained when blood [$HCO_3^-$] increases ranged from moderate (4-6 mmol·L$^{-1}$) to large (> 6 mmol·L$^{-1}$). Only two studies to date have investigated the blood $HCO_3^-$ response following the ingestion of SC in GEL capsule form (Urwin *et al*. 2019; 2021a), demonstrating $\Delta C_{max}$ in blood [$HCO_3^-$] of 7.9 (7.2-8.6) and 7.8 (6.8-8.7) mmol·L$^{-1}$, respectively. In the present study, across all doses, absolute change in blood [$HCO_3^-$] was moderate (5.7 ± 1.3 mmol·L$^{-1}$) suggesting that SC sufficiently increased [$HCO_3^-$] to levels where an ergogenic effect may be likely, although the magnitude of change was notably lower than previously reported. This may be explained by the elevated resting values presented here.

Navigating GIS, specifically by reducing their incidence and severity remains a key component of any effective exogenous buffering agent ingestion strategy. In comparison with SB, SC has been

associated with a reduced occurrence of GIS (Requena *et al*. 2005). In the present study, 8 of 14 participants indicated no GIS at any stage vs. 5 of 15 participants in the work of Gough *et al*. (2017a), although they did not quantify the total symptoms experienced by those who did report GIS. Findings herein are unsurprising given the previous use of GEL encapsulation rather than solution (Urwin *et al*. 2016) and support the use of SC to limit GIS.

In conclusion, key markers of blood alkalosis (pH and $[HCO_3^-]$ display poor reliability between repeat ingestion of multiple doses of SC, challenging the use of an individualised approach to ingestion. Despite this, all doses provided in the present study were associated with limited GIS post-ingestion and, promoted potentially meaningful, sustained changes in pH and [HCO3-], i.e. $\Delta C_{max} \geq 4$ mmol·L$^{-1}$. Such responses may support an ergogenic response to SC, although researchers should consider the high inter- and intra-individual variability in all key blood kinetics.

# CHAPTER 5 – THE BLOOD ACID-BASE AND GASTROINTESTINAL RESPONSE TO THREE DIFFERENT FORMS OF SODIUM CITRATE ENCAPSULATION

## 5.1 Introduction

Sodium citrate (SC) has been shown to improve exercise of a short duration (> 60 s and < 420 s) and very high-intensity (> 100% $VO_{2max}$) in a limited number of studies, creating ambiguity regarding its erogenicity (Cunha *et al.,* 2019; Fernandez-Castanys *et al.,* 2002; McNaughton, 1990; Oopik *et al., 2004;* Shave *et al.,* 2001; Vaher *et al.,* 2015). Both Cerullo and Urwin *et al*. (2020; 2021a) have proposed that suboptimal ingestion protocol(s), which fail to maximise blood alkalosis (pH and/or $HCO_3^-$) and manage potential gastrointestinal symptoms (GIS) could explain the lack of a clear performance effect following SC ingestion (de Oliveira *et al*., 2021). Resultantly, there remains a need to develop a protocol which would better facilitate possible performance effects (Urwin *et al*., 2019). Currently, the ingestion of 0.4-0.5 $g \cdot kg^{-1}$ BM SC ~3 h prior to exercise is recommended (Cerullo *et al*., 2020; Urwin *et al*., 2021b). Such large doses of SC are necessary as, immediately post-ingestion it rapidly dissociates into $Na^+$ and $citrate^-$ (Requena *et al*., 2005). $Citrate^-$ is then removed from the plasma, unbalancing the electrical equilibrium (Devlin, 2010). Electrical neutrality is restored by a decrease in $[H^+]$ and an increase in $[HCO_3^-]$, creating the alkalotic response observed post-ingestion (Peacock *et al*., 2021; Urwin *et al*., 2019; 2021b). At a dose of 0.4 $g \cdot kg^{-1}$ BM (28 g for a 70-kg individual) blood $HCO_3^-$ may increase by ~5 mmol/L (Cunha *et al*., 2019), equating to only 17 % of the expected increase in $HCO_3^-$ if the entire dose entered the blood (considering SC should increase $[HCO_3^-]$ by ~29.6 $mmol \cdot L^{-1}$ in 5 L of blood). Following identical calculations for SB, de Oliveira *et al*. (2018) explains that the majority of ingested $HCO_3^-$ is neutralized in gastric acids or may be removed in faeces to a smaller degree. Logically, avoiding such losses of $HCO_3^-$ would yield larger increases in $[HCO_3^-]$ and mitigate GIS.

Multiple forms of encapsulation have been trialled with the purpose of avoiding degradation in the stomach, achieved by utilizing the variable pH across the GI tract, meaning degradation of 'gastro-resistant capsules' occurs primarily in the less acidic duodenum (pH 6-7 AU) (Barbosa, Conway and Merchant, 2017). Resultantly, gastric acid neutralisation is limited, lessening GIS that would otherwise develop alongside elevated $CO_2$ tension in the stomach (Hilton *et al*., 2020a).

Hilton *et al*. (2019) observed reduced incidence/severity of GIS following the administration of DEL encapsulated SB, compared to aqueous delivery. Additionally, some individuals benefitted from augmented $HCO_3^-$ bioavailability ($\leq$2 mmol·L$^{-1}$), supporting the need for ingestion based on individual blood analyte responses. In the sole example, Peacock *et al*. (2021) recently compared the pharmacokinetic and GIS response to ingesting SB and SC in DEL capsules at an identical dose (0.3 g·kg$^{-1}$ BM). While both substances induced significant alkalosis, SB resulted in a greater change in blood [$HCO_3^-$]. Of note, SC ingestion was also associated with reduced GIS, although at a dose that has yet to be consistently evidenced as ergogenic. Given the value of reduced GIS, there is a demand for future research to understand the minimum effective dose(s) (Peacock *et al*., 2021; Urwin *et al*., 2016).

Hilton *et al*. (2020a) later demonstrated that ENT capsules have the capacity to further attenuate GIS vs. GEL and DEL capsules, providing an additional option for those who experience notable GIS, at an added financial cost. Moreover, changes in blood alkalosis ([$HCO_3^-$] and pH) were lower with ENT SB, possibly due to increased absorption across the intestinal mucosa (Turnberg *et al*., 1970) alongside declining absorption time (Hilton *et al*., 2020a). Given that the level of induced alkalosis may limit performance effects following both SB and SC (de Oliveira *et al*., 2021), ENT capsules may improve the GIS response at the expense of performance (Hilton *et al*., 2020a).

At present, no research has compared the blood acid-base and GIS response to SC ingestion between GEL and DEL capsules. Furthermore, the blood acid-base and GIS response to ENT capsule delivery of SC remains unknown. Successfully minimising GIS, while understanding the potential implications for performance (i.e., level of blood alkalosis induced) at an apparently ergogenic dose (0.4 g·kg$^{-1}$ BM) would be of high value to athletes and practitioners. Therefore, the aim of this study was to determine the blood acid-base and GIS responses to the ingestion of 0.4 g·kg$^{-1}$ BM SC, administered in GEL, DEL and ENT capsules.

## 5.2 Methods

### *5.2.1 Participants*

Fourteen recreationally active males (age 27 ± 4, BM 81.4 ± 8.9 kg, $VO_{2peak}$ 41.43 ± 13.03 $mL \cdot kg^{-1} min^{-1}$ took part in this study. Participants completed regular exercise training (≥ 3 $d \cdot week^{-1}$) for at least two years and were free of GI-related issues. Exclusion criteria also required that participants were not following a salt-restricted diet or ingesting other buffering agents simultaneous to the current study. All experimental procedures were explained fully, with questions addressed before the provision of written, informed consent. Ethical approval was given by the institutional ethics committee (SPA-REC-2019-252R1).

### *5.2.2 Experimental Overview*

In a double-blind, randomised crossover design, participants attended the laboratory on five separate occasions. Following an initial visit (detailed below), experimental trials consisted of a control (CON) and three SC trials, wherein 0.4 $g \cdot kg^{-1}$ BM SC was ingested in GEL, DEL or ENT capsules. Experimental trials were counterbalanced using a Balanced Latin Square generator and completed at least 48 h apart to facilitate washout of residual blood $HCO_3^{-1}$ (Siegler *et al*., 2012). Participants were to abstain from alcohol or caffeine beverages (El-Sayed, Ali & Ali, 2005; Guest *et al*., 2021) for 12 h and strenuous exercise 24 h (Tornero-Aguilera *et al*., 2022) before each trial.

During the initial visit, $VO_{2peak}$ was determined using a previously outlined protocol (Section 3.2.2), involving 5 min unloaded pedalling into a ramped increase of 1 W $s^{-2}$ (30 $W \cdot min^{-1}$). until volitional exhaustion was reached (Excalibur Sport, Lode, Netherlands).

Experimental trials were completed at a similar time-of-day to account for circadian variation (Ayala *et al*., 2021). Participants arrived at the laboratory following an overnight fast. Participants then ingested 0.4 $g \cdot kg^{-1}$ BM SC or CON (0.07 $g \cdot kg^{-1}$ BM sodium chloride) which was administered in size 0 opaque (white) capsules. All capsules were manually filled by a laboratory technician using a capsule filling device (Capsule Connection LLC, USA) with doses tested for accuracy regularly (Fisher,

OHAUS$^{TM}$). Capsules were consumed with 500 ml of room temperature water (18°C). After ingestion, water was permitted *ad libitum* with consumption throughout the first trial recorded and replicated as accurately as possible in subsequent trials. Excluding toilet breaks, participants remained seated throughout.

### *5.2.3 Acid-Base Balance Responses*

The response to ingestion was determined based on the time course of blood [$HCO_3^-$] and pH changes over a 4 h sampling period. Fingertip capillary blood samples (95 μL) were obtained pre-ingestion and a further 14 times, spread equally over the 238 min period. Samples were collected in heparin-coated capillary tubes (Radiometer Medical Ltd, Denmark) and analysed immediately (Radiometer ABL800 BASIC, Denmark) for blood [$HCO_3^-$] and pH. Blood $HCO_3$- measurements were then used to establish key characteristics of each form of encapsulation, lag time ($T_{lag}$), peak blood [$HCO_3^-$] ($C_{max}$), change in $C_{max}$ ($\Delta C_{max}$), time-to-reach $C_{max}$ ($T_{max}$) and area under the curve (AUC). Bicarbonate $T_{lag}$ is taken as the point at which blood [$HCO_3^-$] increased beyond normal daily fluctuation; established individually following ingestion of CON.

### *5.2.4 Gastrointestinal Symptoms*

At each interval, a GI questionnaire was completed to assess the severity of a range of GIS using a visual analogue scale ([VAS] where 0 = no symptom and 10 = most severe) (Gough *et al*., 2017). Symptoms include nausea, flatulence, stomach cramping, belching, stomach-ache, bowel urgency, diarrhoea, vomiting and stomach belching.

### *5.2.5 Statistical Analysis*

Variables were analysed for normality (Shapiro-Wilks and Q-Q plots) and homogeneity of variance/sphericity (Mauchly) before undergoing further statistical analysis. Both one-way (treatment) and two-way (treatment/time) repeated measures ANOVA were used to deduce differences in blood

and GIS parameters with Bonferroni pairwise comparisons. Where sphericity was violated, Greenhouse Geiser correction was applied. Effect sizes were calculated using partial eta squared ($\eta_p^2$). Quantifying GIS, 'total symptoms' (sum of each individual reported score) and 'TTP severity' (first instance of largest individual score) were used to determine overall GIS responses. Statistical significance was set at $P$ <0.05. Heteroscedasticity was assessed using Bland-Altman plots. Where data were determined to be heteroscedastic data were log transformed. Calculations were carried out using Microsoft® Excel 2019 (Microsoft Inc, Redmond, WA, USA), with statistical procedures completed using SPSS version 27 (IBM, Chicago, IL, USA).

## 5.3 Results

### *5.3.1 Acid-Base Responses*

Ingestion form had a significant effect on blood [$HCO_3^-$] ($F_{2,12} = 5.1$, $P = 0.025$, $\eta_p^2 = 0.459$) but not on pH ($F_{1.6,6.3} = 1.7$, $P = 0.242$, $\eta_p^2 = 0.299$) and [$Na^+$] ($F_{2,8} = 1.51$, $P = 0.278$, $\eta_p^2 = 0.274$). Blood [$HCO_3^-$] was significantly higher with ENT vs. GEL ($P$ = 0.037). In the post-ingestion period, there were significant effects on blood [$HCO_3^-$] ($F_{14,84} = 254.72$, $P$ <0.001, $\eta_p^2 = 0.977$), pH ($F_{2.9,11.6} = 64.521$, $P < 0.001$, $\eta_p^2 = 0.942$) and [$Na^+$] ($F_{14,56} = 29.026$, $P = < 0.001$, $\eta_p^2 = 0.879$). Blood [$HCO_3^-$] and pH were significantly elevated above baseline from 34 and 136 min post-ingestion and remained significantly elevated throughout the remaining sampling period. Blood [$Na^+$] was significantly elevated above baseline from 102- to-170 min post-ingestion. Significant trial x time interactions emerged for blood [$HCO_3^-$] ($F_{28,68} = 3.867$, $P < 0.001$, $\eta_p^2 = 0.942$) and [$Na^+$] ($F_{28,112} = 2.360$, $P < 0.001$, $\eta_p^2 = 0.371$). There were no significant trial x time interactions for blood pH ($F_{2.6,10.6} = 1.831$, $P = 0.204$, $\eta_p^2 = 0.314$). Time course responses for blood [$HCO_3^-$] and pH are presented in figure 5.1.

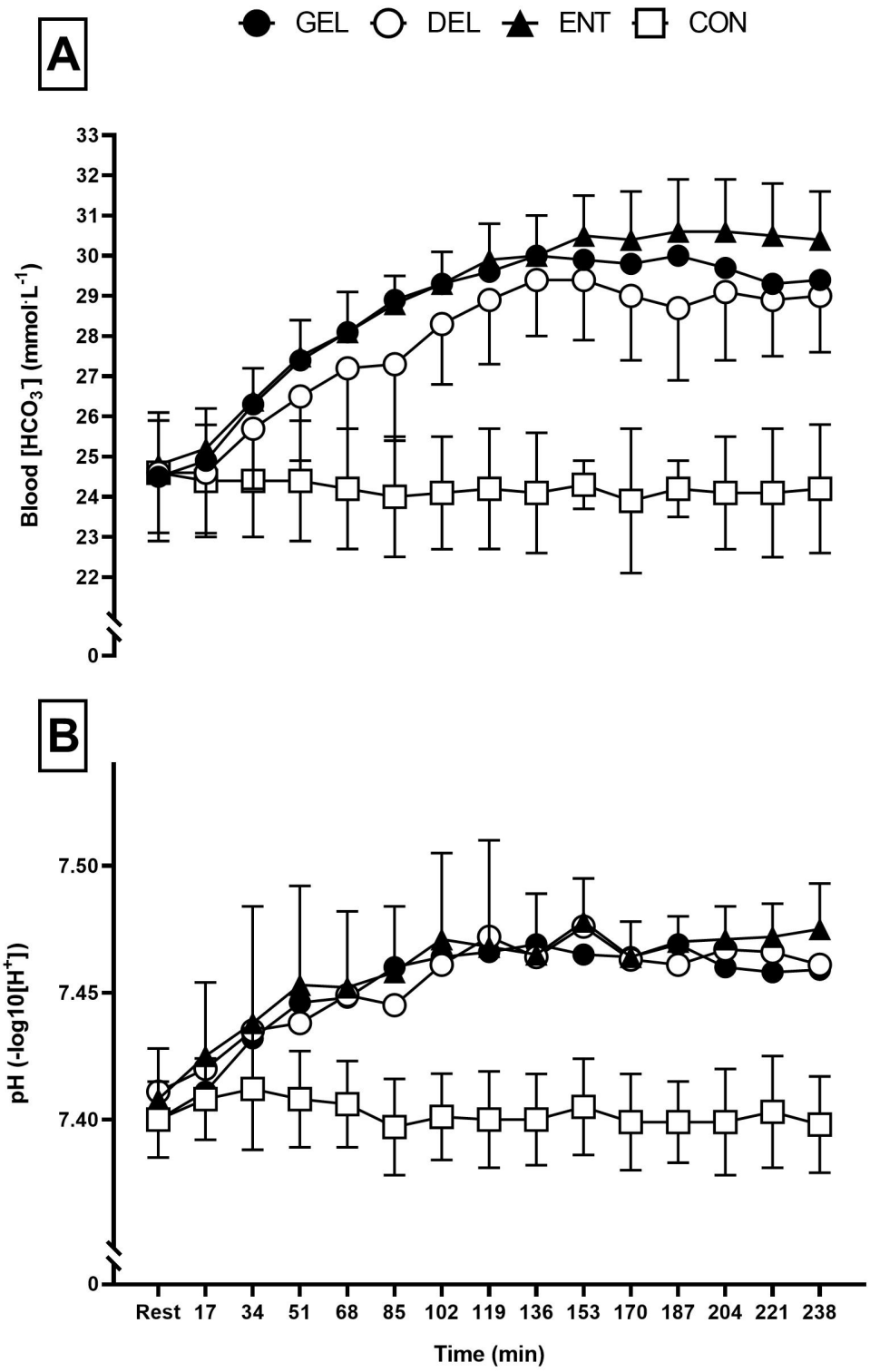


**Figure 5.1** Mean (± standard error of the mean) blood bicarbonate concentration ($[HCO_3^-]$) (A) and pH (B) pre- and post-ingestion of 0.4 $g \cdot kg^{-1}$ body mass (BM) sodium citrate (SC) in gelatine (GEL), delayed-release (DEL) and enterically-coated (ENT) capsules or, 0.07 $g \cdot kg^{-1}$ BM sodium chloride control (CON).

After SC ingestion, $\Delta C_{max,}$ was significantly higher in the ENT trial than DEL ($P$ = 0.027) however, values during the were similar between ENT and GEL ($P$ = 0.794) GEL and DEL ($P$ = 0.112) (6.2 ± 0.8 vs. 6.0 ± 0.9 mmol·L$^{-1}$ and 6.0 ± 0.9 and 5.1 ± 1.0 mmol·L$^{-1}$, respectively). $T_{max}$ occurred significantly earlier following GEL compared to ENT ($P$ = 0.023), while $T_{max}$ values were otherwise similar between trials. Blood pH peaked at similar time points ($P$ > 0.05) between GEL (153 ± 27 min), DEL (169 ± 42 min) and ENT (179 ± 33 min). Changes in peak blood pH were also similar ($P$ > 0.05) in GEL (0.084 ± 0.018), DEL (0.075 ± 0.032) and ENT (0.084 ± 0.022) trials. Blood $HCO_3^-$ kinetics for each form of encapsulation are displayed in Table 5.3.

**Table 5.1.** Within trial variation in bicarbonate ($HCO_3^-$) kinetic variables following the ingestion of 0.4 $g \cdot kg^{-1}$ body mass in gelatine (GEL), delayed-release (DEL) and enterically-coated (ENT) capsules.

| | GEL | | DEL | | ENT | |
|---|---|---|---|---|---|---|
| **Variable** | *Mean ± SD* | *CV* | *Mean ± SD* | *CV* | *Mean ± SD* | *CV* |
| $T_{lag}$ (min) | 31.0 ± 10.9 | 35.1 | 40.1 ± 21.7 | 54.2 | 34.1 ± 13.3 | 39.1 |
| $C_{max}$ ($mmol \cdot L^{-1}$) | 30.4 ± 1 | 3.3 | 29.8 ± 1.6[c] | 5.2 | 31 ± 1.1[b] | 3.6 |
| ΔCmax ($mmol \cdot L^{-1}$) | 6.0 ± 0.9 | 14.4 | 5.1 ± 1.0[c] | 20.1 | 6.2 ± 0.8[b] | 12.3 |
| $T_{max}$ ($mmol \cdot L^{-1}$) | 153 ± 27.4[c] | 17.9 | 168.8 ± 42.4 | 25.1 | 178.5 ± 32.5[a] | 18.2 |
| AUC ($mmol \cdot min \cdot L^{-1}$) | 400.5 ± 10 | 2.5 | 390.5 ± 20.6 | 5.3 | 401.1 ± 20.1 | 5 |

*Notes*: CV, coefficient of variation; $T_{lag}$, lag time; $C_{max}$, peak bicarbonate concentration; ΔCmax, change in peak bicarbonate concentration; $T_{max}$, time to peak bicarbonate concentration; AUC, area under the curve. CV was calculated as 100 x (standard deviation(SD)/mean).

### *5.3.2 Gastrointestinal Symptoms*

There was no significant effect of ingestion form on total GIS experienced ($F_{3,39} = 1.316$, $P = 0.283$, $\eta_p^2 = 0.092$). Whilst not significantly different, average total symptoms were greater following GEL (6.7 ± 13.2 AU) compared to DEL (4.8 ± 15.7 AU), ENT (1.9 ± 5.3 AU) or CON (0.3 ± 1.1 AU). Six of fourteen participants experienced no symptoms irrespective of ingestion form or sample time post-ingestion (Table 5.2).

Peak individual GIS typically occurred ≥ 100 min post-ingestion. Whilst the highest individual symptom occurred earlier following ENT (51 ± 48 min) compared to other ingestion forms (GEL = 102 ± 88 min; DEL 62 ± 20 min), ingestion form had no significant effect on the TTP GIS ($F_{2,26} = 1.857$, $P = 0.176$, $\eta_p^2 = 0.125$). Only one participant experienced GIS at the point of $T_{max}$ in the GEL trial (rated as 1/10 AU only), with no further instances of GIS at $T_{max}$. Furthermore, this participant appeared to display high GIS relative to the remaining sample, based on greater total symptom scores compared to mean values for GEL (7 vs 2 ± 7 AU).

Five of fourteen participants experienced at least one GIS in the GEL trial, whereas only three and four participants experienced GIS following DEL and ENT, respectively. One participant recorded GIS in the CON trial, which occurred around the time of ingestion. Flatulence (24.1%), stomach bloating (20.7%) and bowel urgency (19.0%) represented the most common GIS overall, with diarrhoea (5.3 ± 3.1), vomiting (4 ± 0) and bowel urgency (3.6 ± 1.2) providing the highest severity ratings overall (Table 5.3).

**Table 5.2.** Individual peak GIS reported following the ingestion of 0.4 $g \cdot kg^{-1}$ body mass (BM) sodium citrate (SC) in gelatine (GEL), delayed-release (DEL) and enterically coated (ENT) capsules or, 0.07 $g \cdot kg^{-1}$ BM sodium chloride control (CON).

| | GEL | DEL | ENT | CON |
|---|---|---|---|---|
| **Participant** | | | | |
| 1 | Nausea (3.0) | Flatulence (3.0) | Flatulence (1.0) | None (0.0) |
| 2 | Nausea (6.0) | None (0.0) | None (0.0) | None (0.0) |
| 3 | None (0.0) | None (0.0) | None (0.0) | None (0.0) |
| 4 | None (0.0) | None (0.0) | None (0.0) | None (0.0) |
| 5 | None (0.0) | None (0.0) | Flatulence (3.0) | None (0.0) |
| 6 | None (0.0) | None (0.0) | None (0.0) | None (0.0) |
| 7 | None (0.0) | None (0.0) | None (0.0) | None (0.0) |
| 8 | Flatulence (2.0) | Flatulence (3.0) | Flatulence (3.0) | None (0.0) |
| 9 | Bowel urgency (4.0) | None (0.0) | None (0.0) | None (0.0) |
| 10 | None (0.0) | None (0.0) | None (0.0) | None (0.0) |
| 11 | None (0.0) | None (0.0) | None (0.0) | Vomiting (4.0) |
| 12 | None (0.0) | None (0.0) | None (0.0) | None (0.0) |
| 13 | None (0.0) | None (0.0) | None (0.0) | None (0.0) |
| 14 | Bowel urgency (4.0) | Bowel urgency (6.0) | Bowel urgency (4.0) | None (0.0) |
| Total | 19 | 12 | 11 | 4 |
| Peak Severity | 6 | 6 | 4 | 4 |

**Table 5.3** Frequency and severity of gastrointestinal symptoms (GIS) reported following the ingestion of 0.4 $g \cdot kg^{-1}$ body mass (BM) sodium citrate (SC) in gelatine (GEL), delayed-release (DEL) and enterically coated (ENT) capsules or 0.07 $g \cdot kg^{-1}$ BM sodium chloride control (CON). Overall symptoms are presented, with the most frequent and severe GIS highlighted throughout.

| | **Overall** | | **GEL** | | **DEL** | | **ENT** | | **CON** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Symptoms** | *%* | *Mean ± SD* | *%* | *Mean ± SD* | *%* | *Mean ± SD* | *%* | *Mean ± SD* | *%* | *Mean ± SD* |
| Nausea | 8.6 | 3.4 ± 1.9 | **17.9** | **3.4 ± 1.9** | 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 ± 0.0 |
| Flatulence | **24.1** | 2.6 ± 1.2 | **17.9** | 2.0 ± 0.0 | **28.6** | **3.4 ± 1.7** | **33.3** | **2.0 ± 1.0** | 0.0 | 0.0 ± 0.0 |
| Stomach cramp | 3.4 | 1.0 ± 0.0 | 0.0 | 0.0 ± 0.0 | 9.5 | 1.0 ± 0.0 | 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 ± 0.0 |
| Bowel urgency | **19.0** | **3.6 ± 1.2** | **10.7** | **3.0 ± 1.0** | **23.8** | **4.2 ± 1.5** | **33.3** | **3.3 ± 0.6** | 0.0 | 0.0 ± 0.0 |
| Diarrhoea | 5.2 | **5.3 ± 3.1** | 7.1 | **4.0 ± 2.8** | 4.8 | **8.0 ± 0.0** | 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 ± 0.0 |
| Vomiting | 1.7 | **4.0 ± 0.0** | 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 ± 0.0 | **100** | **4.0 ± 0.0** |
| Stomach bloating | **20.7** | 2.4 ± 0.7 | **17.9** | 2.0 ± 0.0 | **19.0** | 2.8 ± 1.0 | **33.3** | **2.7 ± 0.6** | 0.0 | 0.0 ± 0.0 |
| Belching | 5.2 | 2.0 ± 0.0 | 7.1 | 2.0 ± 0.0 | 4.8 | 2.0 ± 0.0 | 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 ± 0.0 |
| Stomach-ache | 12.1 | 2.0 ± 0.6 | **21.4** | 2.3 ± 0.5 | 9.5 | 1.5 ± 0.7 | 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 ± 0.0 |
| Any | 0.8 | 2.8 ± 1.3 | 1.5 | 2.7 ± 0.5 | 1.1 | 3.3 ± 0.7 | 0.5 | 2.7 ± 0.2 | 0.1 | 0.4 ± 0.0 |

*Notes*: Overall percentage includes those who reported GIS after at least one sodium citrate form. Any percentage describes instances of reported GIS relative to total scores reported.

## 5.4 Discussion

This study represents the first investigation into the effects of capsule ingestion form on GIS and acid-base responses following individualised SC ingestion. Furthermore, the GIS and blood acid-base kinetics following the ingestion of SC in ENT capsules were measured for the first time. Whilst ENT SC resulted in a lesser average total symptom score (1.9 ± 5.3 AU) relative to DEL capsules (4.8 ± 15.7 AU) which, in turn, elicited fewer total symptoms than GEL capsules (6.7 ± 13.2 AU), these differences were not significant. Mean GIS scores for SC ingested in DEL capsules were higher than previously reported (4.8 ± 15.7 AU vs. 1.5 ± 1.8 AU) (Peacock *et al*., 2021). Irrespective of ingestion form, nearly half (6/14) of all participants experienced zero GIS, supporting the notion that SC may promote fewer GIS relative to SB (Requena *et al*., 2005). Simultaneously, the need for different forms of encapsulation for SC may be questioned. Where GIS were reported, these typically occurred within 100 min after ingestion, in relative agreement with the highest ratings around 60-90 min reported elsewhere (Urwin *et al*., 2019; Urwin *et al*., 2021a; 2021b). Given the time course of GIS, only one participant experienced symptoms at the point of peak blood [$HCO_3^-$] (1 AU). This may be critical should athletes opt to utilise an individualised approach to ingestion (Heibel *et al*., 2018). This also suggests that the minimal GIS following SC, occurred somewhat separately to the peak blood alkalotic response and can be addressed as such. Specifically, for individuals with manageable GIS, potential benefit from increased buffering capacity may be gained in the period after such symtpoms have dissipated. Change in peak [$HCO_3^-$] was significantly higher following ENT vs. DEL, although values were otherwise similar. This contrasts with previous work using SB, with Hilton *et al*. (2020a) finding that ENT capsules reduced absolute changes in [$HCO_3^-$] compared to GEL and DEL. Despite this, blood [$HCO_3^-$] was increased beyond the 4 mmol·$L^{-1}$ threshold purported to be necessary for an ergogenic response across all ingestion forms (de Oliveira *et al*., 2020). Overall, data suggests that while ingestion form did not significantly impact GIS generally, GIS symptoms remained minimal throughout SC trials, alongside potentially meaningful changes in blood [$HCO_3^-$].

GIS were reported by 28.6% of the participants across all SC trials, which is lower than previously reported (Urwin *et al*., 2019; 2021a; 2021b). This may be partly explained by the use of a

lower dose in the present study (0.4 compared 0.5 $g \cdot kg^{-1}$ BM). Flatulence, stomach bloating and bowel urgency represented the most common GIS overall, whereas the severity of diarrhoea was notably high, as noted following SB ingestion (Hilton *et al*., 2020a). Despite this, how the severity of specific GIS may impact performance remains elusive, yet may clarify why some studies have found GIS symptoms to limit performance (Cameron *et al*., 2010; Saunders *et al*., 2014; Deb *et al*., 2018) whereas others have not (Price and Simons, 2010; Miller *et al*., 2016). Future studies may aim to clarify the relationship between GIS and willingness to commence/continue exercise.

For increased extracellular buffering capacity to promote meaningful performance changes, there should be an increase in circulating $HCO_3^-$ post-ingestion (Heibel *et al*., 2018). Whilst the minimal changes necessary to induce ergogenic effects are unclear, evidence suggests greater effects occurred when blood [$HCO_3^-$] increases were moderate (4-6 $mmol \cdot L^{-1}$) to large (> 6 $mmol \cdot L^{-1}$) (de Oliveira *et al*., 2021). Whether absolute increases at TPP differ substantially from those at standardised time points, is unknown. Indeed, the mean increase shown at TTP by Gough *et al*. (2018) (+6.5 ± 1.3 $mmol \cdot L^{-1}$) resembles increases shown at 60 min (+6.1 Dias *et al*, 2015; +5.1 Jones *et al*, 2016; +5.7 Gough *et al*, 2017), 90 min (+6.5 Jones *et al*. 2016; +6.1 Gough *et al*, 2017) and 120 min (+6.5 Jones *et al*, 2016; +5.6 Gough *et al*, 2017) with a matched 0.3 $g \cdot kg^{-1}$ BM aqueous dose of SB. Pertinent to the current study, blood [$HCO_3^-$] was also similar 60-, 120- and 180-min post-ingestion in GEL capsules (Siegler *et al*. 2012). Oliveira *et al*. (2020) concludes that sustained or threshold elevations in [$HCO_3^-$] over a period time appear more relevant than maximal elevations at TTP. Whether blood [$HCO_3^-$] is consistently increased around peak, or elevated above 4 $mmol \cdot L^{-1}$ for prolonged periods post SC ingestion however, was previously unknown.

Across ingestion forms, blood [$HCO_3^-$] was significantly elevated above baseline from 34 min post-ingestion and throughout the remaining sampling period. Absolute change in blood [$HCO_3^-$] reached ≥ 4 $mmol \cdot L^{-1}$ after 85-102 min had elapsed, remaining elevated (GEL: +5.3 ± 0.8 $mmol \cdot L^{-1}$; DEL: +5.0 ± 0.8 $mmol \cdot L^{-1}$; ENT: +5.5 ± 0.8 $mmol \cdot L^{-1}$) for up to 153 min (153, 136 and 136 min, respectively) post-ingestion. Therefore, it appears that the "period of erogenicity" following SC ingestion may equate to ~153 min or more, commencing after at least 85 min. Where TTP blood

[$HCO_3^-$] has been proposed as a method of optimising the ingestion of buffering agents (Miller *et al*., 2016; Gough *et al*., 2017), it would appear unnecessary given observations here. Contesting this, Boegman *et al*. (2020) recently demonstrated significant improvements in rowing time trial performance following an individualised TTP vs. a generalised SB ingestion strategy (367.0 ± 10.5 vs. 369.0 ± 10.3 s). This suggests there may be utility in commencing performance where blood alkalosis is maximised rather than elevated, although this needs testing across a range of exercise contexts. Furthermore, the use of an individualised SC ingestion strategy remains un-investigated. Following findings herein, a broader "period of erogenicity" may be an appropriate alternative for SC, particularly where finances are limited and/or access to a blood gas analyser is not possible (de Oliveira *et al*., 2020).

In conclusion, the ingestion of 0.4 $g \cdot kg^{-1}$ BM SC appears beneficial to minimise GIS. Additionally, meaningful and sustained changes in blood [$HCO_3^-$] were observed throughout (5.1-6.2 $mmol \cdot L^{-1}$, with ingestion form exerting limited effects on blood [$HCO_3^-$]. Taken together SC may offer a large(r) period of opportunity for an ergogenic effect relative to SB, with reduced GIS. Finally, the expensive and laborious process of determining individualised time-to-peak and/or utilising costly forms of encapsulation may also be avoidable, although small benefits should be considered.

# CHAPTER 6 – THE EFFECTS OF CARBOHYDRATE CO-INGESTION ON BLOOD ACID-BASE AND GASTROINTESTINAL RESPONSES TO ACUTE SODIUM CITRATE INGESTION

## 6.1 Introduction

Carbohydrate (CHO) intake prior to high-intensity and/or endurance-intensive efforts (Chamari and Padulo, 2015) has been shown to improve subsequent performance (Galloway, Lott and Toulouse, 2014; Ormsbee, Bach and Baur, 2014; Little *et al*., 2010), likely due to increased delivery and oxidation of exogenous CHO stores and glycogen sparing (Jeukendrup, 2004). During intense activity (~85% $VO_{2max}$), energy is almost entirely generated through CHO metabolism, highlighted by a RER of ≥1.0 (van Loon *et al*., 2001; Romjin *et al*., 1993). As such, where the aim is to maximise work done in training/competition, provision of adequate CHO is pivotal (Close *et al*., 2016).

Alongside high CHO demands, high-intensity activity also promotes rapid production of $H^+$ (Keyser, 2010), leading to reductions in both blood and muscle pH, ultimately impairing performance (Thomas *et al*., 2020; Kent-Braun, Fitts and Christie 2012; Sahlin 1992). To counter these acid-base disturbances, a plethora of studies have investigated the ingestion of buffering agents such as SC (de Oliveira *et al*., 2021; Urwin *et al*., 2021a). Sodium citrate (SC) augments extracellular buffering capacity via an increase in [$HCO_3^-$] (Heibel *et al*., 2018). This alkalotic response gives rise to a greater efflux of $H^+$ generated during high-intensity activity out of the working muscle and in turn, may resist reductions in force and power production that characterise fatigue development (Keyser, 2010). Despite this, equivocal effects on exercise performance have been noted following SC supplementation (de Oliveira *et al*., 2021; Urwin *et al*., 2021a). Such outcomes may be attributed to sub-optimal ingestion protocols, where SC failed to maximise blood alkalosis or promoted excessive GIS prior to exercise (Urwin *et al*., 2021a). Presently, the ingestion of 0.4-0.5 $g \cdot kg^{-1}$ BM SC ~3 h prior to exercise is recommended (Cerullo *et* al., 2020; Urwin *et al*., 2021a). Administration in GEL capsules rather than solution is preferred (Urwin *et al*., 2019), alongside a CHO-rich meal to mitigate potential GIS (Cerullo *et al*., 2020). For the latter, 1.75 $g \cdot kg^{-1}$ BM CHO has typically been provided in SC research (Urwin et al., 2016; 2019; 2021a), based on Thomas, Burke and Erdman's (2016) recommendations for acute CHO loading. Finally, such quantities should be consumed within 45 min to improve palatability without negatively impacting blood $HCO_3^-$ kinetics relative to longer ingestion periods (Urwin *et al*., 2021b).

Independently, ingestion of both CHO and SC may be beneficial to high-intensity and/or endurance intensive efforts by augmenting CHO metabolism and counteracting acid-base disturbances, respectively. While some studies in both SC and SB have demonstrated reduced GIS following the consumption of a CHO meal (Urwin *et al*., 2019; 2016; Price and Cripps, 2012; Carr *et al*., 2011), the specific effect of CHO ingestion on $HCO_3^-$ kinetics, which may have implications for exercise performance, (Boegman *et al*., 2020; de Oliveira *et al*., 2018; Gough *et al*., 2017b) remains to be elucidated. If gaining an ergogenic benefit from SC is contingent on the absolute change in blood [$HCO_3^-$] and the time course of these changes (de Oliveira *et al*., 2021; Heibel *et al*., 2018), then clarifying the effect of CHO ingestion on these responses is crucial. Therefore, the aim of this study was to examine the effects of acute CHO intake on GIS and blood acid-base responses alongside the ingestion of an individualised dose of SC (0.5 $g \cdot kg^{-1}$ BM).

## 6.2 Methods

### *6.2.1 Participants*

Ten recreationally active males (age, 24 ± 2 years; body mass, 79.7 ± 5.7 kg; height, 1.8 ± 0.1 m; $VO_{2peak}$, 41.9 ± 10.2 $mL \cdot kg^{-1} \cdot min^{-1}$) took part in this study. Participants completed frequent exercise training (≥ 3 $d \cdot week^{-1}$ for ≥ 2 years) and were without GI issues. Participants were not included if they were following a salt-restricted diet or ingesting other buffering agents simultaneously to the current study. Participants provided written, informed consent prior to commencing. Ethical approval was granted by the institutional ethics committee (ETH2021-0301).

### *6.2.2 Experimental overview*

In a double-blind, randomised crossover design, participants attended the laboratory on five separate occasions. The initial visit included the measurement of BM (kg), height (m) and $VO_{2peak}$, replicating a previously outlined protocol (Deb *et al*., 2018). The four remaining trials consisted of two SC trials (0.5 $g \cdot kg^{-1}$ BM) and two sodium chloride trials (0.07 $g \cdot kg^{-1}$ BM) wherein each substance was ingested both with (SC-CHO and CON-CHO, respectively) or without (SC and CON, respectively) CHO (1.75 $g \cdot kg^{-1}$ BM (Thomas, Erdman and Burke, 2016; Burke, Kiens and Ivy, 2004; Hargreaves,

Hawley and Jeukendrup, 2004). Trials were counterbalanced using a Balanced Latin Square generator and completed at least 48 h apart to facilitate washout of residual blood $HCO_3^-$ (Siegler *et al*., 2012). Participants were to avoid alcohol or caffeine beverages (Guest *et al*., 2021; El-Sayed, Ali & Ali, 2005) for 12 h and strenuous exercise 24 h (Tornero-Aguilera *et al*., 2022) before each trial.

Trials were completed at a similar time-of-day to account for circadian variation (Ayala *et al*., 2021). Following an overnight fast, participants arrived at the laboratory and ingested SC or sodium chloride, administered in size 0 opaque (white) capsules, either with or without CHO (gluten-free porridge oats, Flahavans, Ireland). Based on the average BM of all participants (79.7 kg), this equated ~ 134.3 g CHO (713 kcal, 10.1 g fat and 20.2 g protein). All capsules were manually filled by a laboratory technician using a capsule filling device (Capsule Connection LLC, USA) and tested for accuracy regularly (Fisher, OHAUS$^{TM}$). Capsules were consumed with 500 ml of room temperature water (18°C) within 30 min of arrival (Urwin *et al*., 2021b). After ingestion, water was permitted *ad libitum,* with consumption during the first trial replicated closely during subsequent trials. Barring toilet breaks, participants remaining seated.

### *6.2.3 Acid-Base Balance Responses*

Ingestion response(s) were determined based on the time course of blood [$HCO_3^-$] and pH changes over a 4 h sampling period. Fingertip capillary blood samples (95 μL) were obtained pre-ingestion and for a further 14 times, spread equally over the 238 min period. Samples were collected in heparin-coated capillary tubes (Radiometer Medical Ltd, Denmark) and analysed immediately (Radiometer ABL800 BASIC, Denmark) for blood [$HCO_3^-$] and pH. Blood $HCO_3^-$ measurements were then used to establish key characteristics of each trial; lag time ($T_{lag}$), peak blood [$HCO_3^-$] ($C_{max}$), change in $C_{max}$ ($\Delta C_{max}$), time-to-reach $C_{max}$ ($T_{max}$) and area under the curve (AUC). Bicarbonate $T_{lag}$ is taken as the point at which blood [$HCO_3^-$] increased beyond normal daily fluctuation; established individually following ingestion of CON.

### *6.2.4 Gastrointestinal Symptoms*

At each interval, a GI questionnaire was completed to assess the severity of a range of GIS using a visual analogue scale ([VAS] where 0 = no symptom and 10 = most severe) (Gough *et al*., 2017a). Symptoms include nausea, flatulence, stomach cramping, belching, stomach-ache, bowel urgency, diarrhoea, vomiting and stomach belching.

### *6.2.5 Statistical Analyses*

Data were analysed for normality (Shapiro-Wilks and Q-Q plots) and homogeneity of variance/sphericity (Mauchly) before undergoing further statistical analysis. Both one-way (treatment) and two-way (treatment/time) repeated measures ANOVA were used to deduce differences in blood and GIS parameters with Bonferroni pairwise comparisons. Where sphericity was violated, Greenhouse Geiser correction was applied. Effect sizes were calculated using partial eta squared ($\eta_p^2$), characterised as trivial (<0.20), small (0.20-0.49 AU), moderate (0.50-0.79 AU) or large (≥ 0.80 AU) as previously proposed (Cohen, 1988). Quantifying GIS, 'total symptoms' (sum of each individual reported score) and 'TTP severity' (first instance of largest individual score) were used to determine overall GIS responses. Statistical significance was set at $P$ <0.05. Heteroscedasticity was assessed using Bland-Altman plots. Variables that were determined to be heteroscedastic were log transformed. Zero scores for GIS were inputted for statistical analysis as 0.01.. Calculations were carried out using Microsoft® Excel 2019 (Microsoft Inc, Redmond, WA, USA), with statistical procedures completed using SPSS version 27 (IBM, Chicago, IL, USA).

## 6.3 Results

### 6.3.1 Acid-Base Responses

Consumption of CHO had no significant effect on either blood [$HCO_3^-$] ($F_{1,2}$ = 1.080, $P$ = 0.408, $\eta_p^2$ = 0.351) or pH ($F_{1,2}$ = 3.261, $P$ = 0.213, $\eta_p^2$ = 0.620) (Figure 6.1). Where no CHO was consumed, average blood [$HCO_3^-$] was significantly higher following SC (28.7 ± 1.0) compared to CON (24.2 ± 0.02) ($F_{1,2}$ = 18.848, $P$ = 0.049, $\eta_p^2$ = 0.904). Without CHO, average blood pH was also significantly higher following SC (7.468 ± 0.015) compared to CON (7.406 ± 0.014) ($F_{1,2}$ = 20.017, $P$ = 0.047, $\eta_p^2$ = 0.909).

In the post-ingestion time-period, there were significant effects on blood [$HCO_3^-$] ($F_{14,28}$ = 23.301, $P$ < 0.001, $\eta_p^2$ = 0.921) but not pH ($F_{14,28}$ = 2.417, $P$ = 0.547, $\eta_p^2$ = 0.547). For pH alone, a significant interaction between CHO intake and no CHO trials was highlighted ($F_{1,2}$ = 28.210, $P$ = 0.034, $\eta_p^2$ = 0.934). Additional trial x time interactions were also detected following the ingestion of no CHO for both blood [$HCO_3^-$] ($F_{14,28}$ = 18.803, $P$ = < 0.001, $\eta_p^2$ = 0.904) and pH ($F_{14,28}$ = 5.994, $P$ < 0.001, $\eta_p^2$ = 0.750)

After SC ingestion, $\Delta C_{max}$ values ($HCO_3^-$) were similar during both CHO and no CHO trials (7.5 ± 0.4 mmol·$L^{-1}$ and 6.1 ± 0.6 mmol·$L^{-1}$, respectively; $P$ = 0.291) (Figure 6.2). Time to peak [$HCO_3^-$] ($T_{max}$) occurred earlier during SC (165 ± 12 min) compared to SC-CHO (197 ± 10 min), although this difference was not significant ($P$ = 0.278). Key blood $HCO_3^-$ kinetics are presented in table 6.3. Blood pH peaked earlier than blood [$HCO_3^-$], with similar $T_{max}$ noted for both SC-CHO and SC (155 ± 13 vs. 159 ± 19 min, respectively; $P$ > 0.50). Absolute change in blood pH did not differ significantly between SC-CHO and SC (0.108 ± 0.008 vs. 0.089 ± 0.013, respectively; $P$ > 0.50).

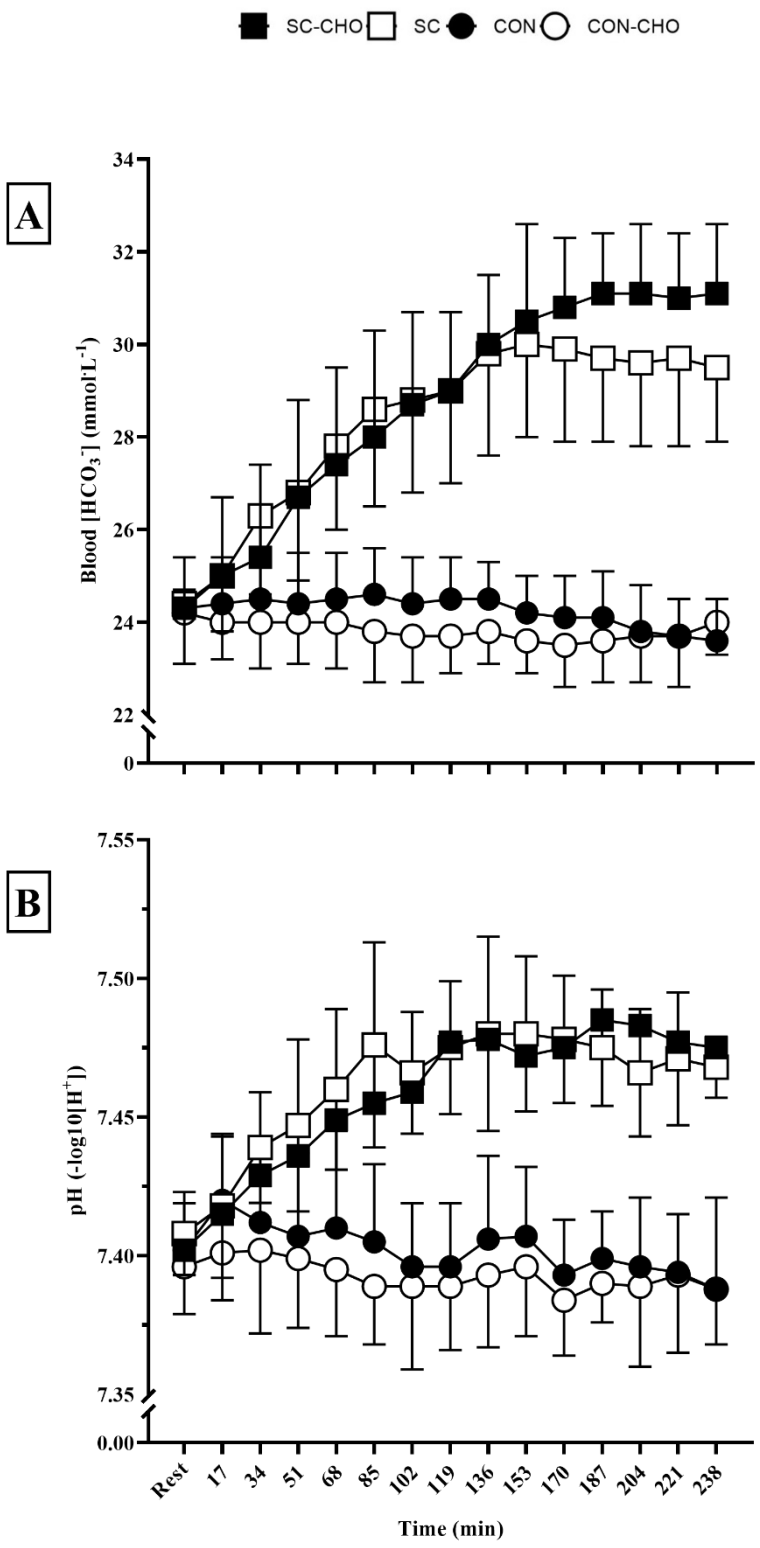


**Figure 6.1** Mean (± SD) blood [$HCO_3^-$] (A) and pH (B) pre- and post-ingestion of 0.5 $g \cdot kg^{-1}$ body mass (BM) sodium citrate (SC) or sodium chloride control (CON). CHO indicates the co-ingestion of 1.75 $g \cdot kg^{-1}$ BM of carbohydrate. Some error bars are omitted for clarity. **Denotes a significant difference between SC and CON trials ($P < 0.05$).*

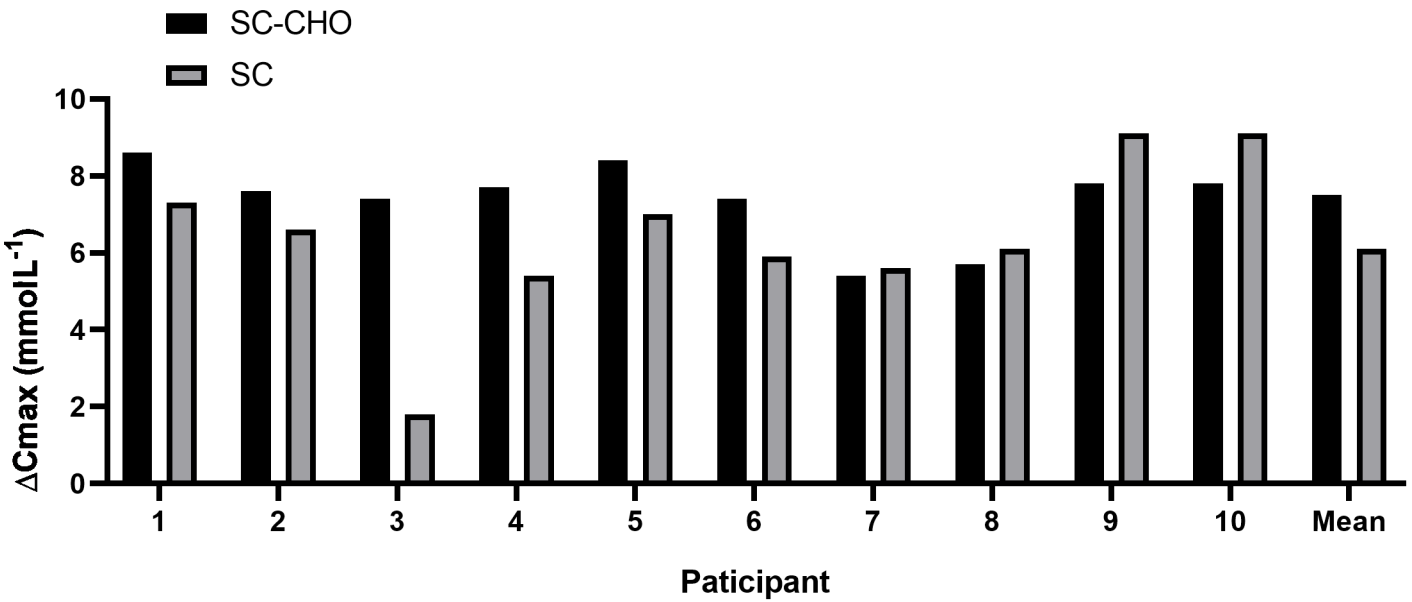


**Figure 6.2.** Absolute change (ΔCmax) in bicarbonate concentration [$HCO_3^-$] following the ingestion of 0.5 $g \cdot kg^{-1}$ body mass (BM) sodium citrate (SC) either with (SC-CHO) or without (SC) the co-ingestion of 1.75 $g \cdot kg^{-1}$ BM carbohydrate (CHO). Both individual and group level data are presented.

**Table 6.1.** Within trial variation in bicarbonate ($HCO_3^-$) kinetic variables following the ingestion of 0.5 $g \cdot kg^{-1}$ body mass (BM) sodium citrate (SC) or 0.07 $g \cdot kg^{-1}$ BM sodium chloride control (CON), both with and without the co-ingestion of 1·75 $g \cdot kg^{-1}$ BM carbohydrate (CHO).

| | SC-CHO | | SC | | CON-CHO | | CON | |
|---|---|---|---|---|---|---|---|---|
| **Variable** | Mean ± SD | CV | Mean ± SD | CV | Mean ± SD | CV | Mean ± SD | CV |
| $T_{lag}$ (min) | 24.3 ± 1.1 | 52.7 | 24.3 ± 1.3 | 55.8 | N/A | N/A | N/A | N/A |
| $C_{max}$ ($mmol \cdot L^{-1}$) | 31.8 ± 1.3 | 1.3 | 30.5 ± 1.8 | 1.8 | 25.1 ± 1.0 | 1.0 | 24.5 ± 0.9 | 0.9 |
| $\Delta C_{max}$ ($mmol \cdot L^{-1}$) | 7.5 ± 1.2 | 15.4 | 6.1 ± 1.9 | 30.3 | 0.8 ± 0.8 | 96.4 | 0.4 ± 0.5 | 132.9 |
| $T_{max}$ (min) | 197.2 ± 32.3 | 16.4 | 164.9 ± 37.6 | 22.8 | 81.6 ± 71.1 | 87.2 | 22.7 ± 28.2 | 124.3 |
| AUC ($mmol \cdot min.L^{-1}$) | 684.1 ± 37.7 | 5.5 | 675.5 ± 38.9 | 5.8 | N/A | N/A | N/A | N/A |

***Notes*:** CV, coefficient of variation; $T_{lag}$, lag time; $C_{max}$, peak bicarbonate concentration; $\Delta C_{max}$, change in peak bicarbonate concentration; $T_{max}$, time to peak bicarbonate concentration; AUC, area under the curve. CV calculated as 100 x (standard deviation(SD)/m

### *6.3.2 Gastrointestinal symptom(s) (GIS)*

There was no significant effect of CHO intake on total GIS experienced ($F_{1,9} = 0.100$, $P = 0.759$, $\eta_p^2 = 0.011$), following the ingestion of either SC or CON (Total symptom score: 2.9 ± 3.5 vs. 14.5 ± 19.9 AU, respectively). Furthermore, without CHO intake, total GIS experienced remained similar ($F_{1,9} = 0.207$, $P = 0.660$, $\eta_p^2 = 0.022$) between SC or CON trials (Total symptom score: 7.3 ± 15.2 vs. 3.5 ± 7.6 AU, respectively). Nine of ten participants experienced at least one instance of GIS in at least one trial, with the co-ingestion of CON with CHO associated with the most cases of any reported GIS (8/10 participants) compared to CON without CHO, where GIS were least frequent (3/10) (Table 6.2).

Peak individual GIS were reported almost exclusively (13/15 instances) after 17 min, irrespective of trial completed, representing the first sample post-ingestion. No relationships were highlighted in peak GIS between trials. Following the ingestion of SC alone, one participant experienced GIS at $T_{max}$ (4 AU total), with no further instances of GIS reported at $T_{max}$. However, this participant appeared to display high GIS relative to the remaining sample, based on a significantly greater total symptom score relative to group values (49 vs. 7.3 ± 15.2 AU). Nausea (15.9%), stomach cramp (9.1%) and flatulence (8.0%) emerged as the most common GIS overall, whilst diarrhoea (4.4 ± 1.6 AU), vomiting (4.0 ± 0 AU) and belching (4.0 ± 0 AU) produced the greatest average severity ratings overall (Table 6.3).

**Table 6.2.** Individual peak gastrointestinal symptom(s) (GIS) reported following the ingestion of 0.5 $g \cdot kg^{-1}$ body mass (BM) sodium citrate (SC) or 0.07 $g \cdot kg^{-1}$ BM sodium chloride (CON), both with and without the co-ingestion of 1.75 $g \cdot kg^{-1}$ BM carbohydrate (CHO). Symptom scores are displayed in parentheses and are expressed as arbitrary units (AU).

| Participant | SC-CHO | SC | CON-CHO | CON |
|---|---|---|---|---|
| 1 | None (0.0) | None (0.0) | Stomach bloating (3.0) | None (0.0) |
| 2 | Bowel urgency (3.0) | None (0.0) | Bowel urgency (3.0) | None (0.0) |
| 3 | Bowel urgency (2.0) | Nausea (3.0) | Flatulence (1.0) | None (0.0) |
| 4 | None (0.0) | Diarrhoea (7.0) | Vomiting (7.0) | None (0.0) |
| 5 | Belching (4.0) | None (0.0) | Nausea (6.0) | Vomiting (4.0) |
| 6 | None (0.0) | Stomach bloating (9.0) | Vomiting (6.0) | None (0.0) |
| 7 | None (0.0) | None (0.0) | None (0.0) | None (0.0) |
| 8 | None (0.0) | None (0.0) | Stomach cramp / ache / belching (5.0) | Stomach-ache (3.0) |
| 9 | None (0.0) | None (0.0) | None (0.0) | None (0.0) |
| 10 | None (0.0) | None (0.0) | None (0.0) | None (0.0) |

**Table 6.3.** Frequency and severity of gastrointestinal symptoms (GIS) reported following the ingestion of 0.5 $g \cdot kg^{-1}$ body mass (BM) of sodium citrate (SC) or 0.07 $g \cdot kg^{-1}$ sodium chloride (CON), both with and without 1·75 $g \cdot kg^{-1}$ BM carbohydrate (CHO). The most frequent and severe GIS are highlighted. $N = 10$.

| | **Overall** | | **SC-CHO** | | **SC** | | **CON-CHO** | | **CON** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Symptoms** | *%* | *Mean ± SD* | *%* | *Mean ± SD* | *%* | *Mean ± SD* | *%* | *Mean ± SD* | *%* | *Mean ± SD* |
| Nausea | **15.9** | 3.2 ± 1.7 | **22.2** | **4.0 ± 1.4** | **25.0** | 2.5 ± 0.8 | **13.6** | 3.3 ± 1.8 | **45.5** | **3.4 ± 2.7** |
| Flatulence | **8.0** | 2.3 ± 1.1 | 0.0 | 0.0 ± 0.0 | 4.2 | 2.0 ± 0.0 | 11.4 | 2.2 ± 1.3 | **9.1** | **3.0 ± 0.0** |
| Stomach cramp | **9.1** | 3.3 ± 1.2 | **11.1** | 3 ± 0.0 | 4.2 | **3.0 ± 0.0** | **13.6** | 3.3 ± 1.4 | 0.0 | 0.0 ± 0.0 |
| Bowel urgency | 4.5 | 2.1 ± 0.9 | **44.4** | 2.3 ± 1 | **12.5** | 2.3 ± 0.6 | **15.9** | 2.0 ± 1.2 | 0.0 | 0.0 ± 0.0 |
| Diarrhoea | 4.5 | **4.4 ± 1.6** | 0.0 | 0.0 ± 0.0 | 8.3 | **6.0 ± 1.4** | 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 ± 0.0 |
| Vomiting | 1.1 | **4.0 ± 0.0** | 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 ± 0.0 | 4.5 | **6.5 ± 0.7** | **9.1** | **4.0 ± 0.0** |
| Stomach bloating | 1.1 | 3.5 ± 2.1 | **11.1** | **5.0 ± 0.0** | **33.3** | **3.6 ± 2.3** | **27.3** | **3.8 ± 1.2** | **27.3** | 2.7 ± 1.5 |
| Belching | 1.1 | **4.0 ± 0.0** | **11.1** | **4.0 ± 0.0** | 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 ± 0.0 | 0.0 | 0.0 ± 0.0 |
| Stomach-ache | 5.7 | 3.0 ± 1.5 | 0.0 | 0.0 ± 0.0 | **12.5** | 1.7 ± 0.6 | **13.6** | **3.7 ± 1.5** | **9.1** | **3.0 ± 0.0** |
| Any | 0.5 | 3.7 ± 1.1 | 0.5 | 3.7 ± 1.1 | 1.3 | 3.0 ± 1.5 | 2.3 | 3.5 ± 1.5 | 0.6 | 3.0 ± 0.3 |

*Notes*: Overall percentage includes those who reported GIS after at least one trial. Any percentage describes instances of reported GIS relative to total scores reported.

## 6.4 Discussion

To gain a performance benefit from SC ingestion, there needs to be a rise in circulating [$HCO_3^-$], with any increases theoretically leading to a corresponding change in buffering capacity (Heibel *et al*., 2018). Although absolute change in [$HCO_3^-$] ($\Delta C_{max}$) following SC ingestion was greater with CHO (7.5 ± 0.4 mmol·$L^{-1}$) compared to without (6.1 ± 0.6 mmol·$L^{-1}$), this was not significantly different, with both trials producing potentially meaningful changes in [$HCO_3^-$] (de Oliveira *et al*., 2021). Specifically, de Oliveira *et al*. (2021) noted that the greatest ergogenic effects following the ingestion of either SC or SB were obtained when $\Delta C_{max}$ were medium (+ 4-6 mmol·$L^{-1}$) and large (> 6 mmol·$L^{-1}$) vs. small (≤ 4 mmol·$L^{-1}$). Changes in [$HCO_3^-$] presented here would therefore appear to demonstrate a 'large' change in circulating $HCO_3^-$, suitable to gain an ergogenic benefit (Heibel *et al*., 2018). Values for $\Delta C_{max}$ were similar to increases reported by Urwin *et al*. (2021b; 2019) utilising the same SC dosage (0.5 g·$kg^{-1}$ BM), provided in the same form (gelatine capsules) and ingested over the same period (30 min) (7.5 (6.5-8.4) and 7.9 (7.2-8.6) mmol·$l^{-1}$, respectively). In summation, this ingestion protocol appears to induce meaningful changes in blood [$HCO_3^-$], to levels that could support a positive ergogenic performance benefit.

Despite similarities in $\Delta C_{max}$, peak [$HCO_3^-$] in the current study occurred notably earlier than suggested by Urwin *et al*. (2021b; 2019), who observed $T_{max}$ ~204-242 (188-290) min post-ingestion, compared to ~165-197 (119-238) min following post-ingestion of SC herein. This is somewhat surprising, given Urwin and colleagues provided multiple transportable CHO in both liquid and solid forms (750 ml of sports drink, bread, jam, bananas and muesli bars), individually thought to enhance gastric emptying (Jeukendrup and Killer, 2010; Jeukendrup and Moseley, 2010), potentially leading to a faster $T_{max}$ vs. the current study, wherein only porridge oats were given. Although the precise reason for the differences in $T_{max}$ observed remain unclear, large inter-individual variation is evident across both samples (~119-290 min) which has implications for any potential individualised approach to SC ingestion.

Based on such large variability in blood acid-base responses following the ingestion of buffering agents, recent work is moving from generalised, to more individualised approaches (Gough *et al*., 2017a, 2017b; Miller, 2016). Coupled with evidence supporting a consistent intra-individual

response to the same dose provided on different days (de Oliveira *et al*., 2020; Gough *et al*., 2017a), it has been recommended that exercise be commenced at pre-determined, individual $T_{max}$, whereby buffering capacity is maximised (Heibel *et al*., 2018). Such a response is dependent on the reliability of blood acid-base balance responses across days, both as a product of daily fluctuations as the result of net endogenous acid production and, following SC ingestion, which requires investigation. In study 1 (Chapter 4), it was observed that while SC produced substantial changes in blood alkalosis (pH and $[HCO_3^-]$) alongside minimal GIS (particularly around individual $T_{max}$), blood alkalotic responses (including TTP) were highly variable, failing to support the use such a strategy.

Overall, CHO intake did not impact GIS following either SC or CON, with similar average total symptom scores, both with ($P$ = 0.759) and without CHO ($P$ = 0.660). Furthermore, only one participant reported GIS at the point of peak $[HCO_3^-]$ ($T_{max}$); a time at which exercise is likely to be commenced for individuals utilising an individualised approach to ingestion (Heibel *et al*., 2018). Intriguingly, although absolute change in $[HCO_3^-]$ (ΔCmax) following SC ingestion was greater with CHO (7.5 ± 0.4 $mmol \cdot L^{-1}$) compared to without (6.1 ± 0.6 $mmol \cdot L^{-1}$), this was not significantly different, with both trials producing potentially meaningful changes in $[HCO_3^-]$ (de Oliveira *et al*., 2021). These data suggest that whilst CHO intake does not appear to improve the GIS or blood alkalotic response to SC, CHO did not adversely affect these responses and as such, may be co-consumed to support CHO metabolism during high intensity and/or endurance intensive efforts (Close *et al*., 2016). In the present study, peak GIS emerged almost exclusively in the first measurement post-ingestion (13/15 instances), with scores from 1 to 8 AU provided (4.4 ± 2.2 AU) across all trials. GIS typically dissipated within 30 min of peak GIS emerging (31 ± 19 min), indicating GIS were largely overcome earlier than 60-90 min as previously reported for peak GIS post-ingestion (Urwin *et al*., 2019; 2016) and, before the emergence of peak $[HCO_3^-]$ after ~3 h (Urwin *et al*., 2021a; Cerullo *et al*., 2020).

Whilst encapsulation may mitigate GIS following SC ingestion compared to aqueous delivery (Urwin *et al*., 2019), ingesting the large quantity of capsules necessary to provide 0.4-0.5 $g \cdot kg^{-1}$ BM may independently present problems (Cerullo *et al*., 2020; Aedma, Timpmann and Ööpik, 2015), particularly alongside the intake of CHO (Urwin *et al*., 2019). Urwin *et al*. (2019) found that ingestion

of capsules caused “loss of appetite” more frequently than solution, attributed to the greater ingested volume/mass of capsules plus CHO. Similarly, many participants in the present study expressed difficulty consuming the large volumes of CHO and SC provided. Given that there was no notable difference in GIS experienced between SC and CON trials, with both the most (and least) severe GIS response observed in the latter, GIS may not have been attributable to SC alone. Although greater total ingested volume and/or mass have been shown to slow gastric emptying rate and potentially increase GIS (Rolls *et al*., 1998; Moore, Christian and Coleman, 1981; Hunt and Stubbs, 1975), the effect of small changes in either factor has not been directly investigated. Future research may seek to clarify any effect of small changes in ingested volume/mass. Chronic loading of CHO may also be considered as a method to achieve this (Close *et al*., 2016).

To conclude, the acute CHO intake did not negatively impact total GIS or blood $HCO_3^-$ kinetics alongside SC ingestion (i.e., $\Delta C_{max}$ and $T_{max}$) and as such, may be consumed to support CHO metabolism alongside augmented extracellular buffering capacity. Co-consuming large quantities of CHO and SC is not without risk, promoting a prompt GIS response immediately post-ingestion. While this response dissipated quickly, prior to any significant changes in $[HCO_3^-]$ ($T_{max}$), athletes may be discouraged from this approach. To this end, future research may consider methods such as chronic loading of CHO, as a means of reducing total volume/mass ingested in a single instance. Finally, given the substantial variation in $T_{max}$ within the current study independently and compared to similar ingestion protocols, it is recommended that researchers consider the utility of an individualised approach to SC ingestion. The reliability of blood acid-base responses to various doses of SC is of particular interest.

# CHAPTER 7 – THE EFFECT OF SODIUM CITRATE INGESTION TIMING ON FOUR KILOMETRE TIME TRIAL PERFORMANCE AND RECOVERY

## 7.1 Introduction

High-intensity to endurance-intensive efforts (>6 s to 1+ min) are associated with high levels of glycolytic flux (van Loon *et al*., 2001; Romjin *et al*., 1993), simultaneous to reductions in both muscle and blood pH and [$HCO_3^-$] (Fitts, 1994). Peripheral fatigue is mechanised when any of the associated regulatory/support systems are unable to promote the necessary muscular contractile force and/or sustain the bioenergetics and waste removal required (Keyser, 2010). In one example, higher $H^+$ production versus removal rate represents the limits of endogenous $HCO_3^-$ buffering capacity and as such, exogenously increasing the contribution of this system appears a logical approach to pH regulation during intense efforts (Heibel, 2018), prior to any longer-term adaptive changes (McGinley and Bishop, 2017; 2016; Pilegaard, Juel and Wibrand, 1993).

Pre-exercise ingestion of 0.5 $g \cdot kg^{-1}$ BM SC, administered in GEL capsules, may induce notable increases in blood pH (0.108 (0.092-0.126) $-\log_{10}[H^+]$) and [$HCO_3^-$] (7.7 (6.5-8.9) $mmol \cdot L^{-1}$) from baseline (Urwin *et al*., 2019; 2021a), with the latter rising to concentrations comparable with a popular alternative, SB (Urwin *et al*., 2022). Urwin *et al*. (2022) also observed a similar frequency and severity of GIS, with somesuggestions that this may even be less post-ingestion with SC (Peacock *et al*., 2021; Requena *et al*., 2005). Despite this, SB has received more research attention and appears more effective for performance compared to SC (de Oliveira *et al*., 2021). Recent reviews (Urwin *et al*., 2021; Cerullo *et al*., 2020) have discussed the role of ingestion protocols, and in the equivocal findings post-SC ingestion (see also: Carr, Hopkins and Gore, 2011b). Specifically, timing, mode and duration of ingestion have received attention, although a large proportion of the evidence originates from work conducted with SB (Hilton *et al*., 2019; 2020a; 2020b; Gough *et al*., 2017a; 2017b; Miller *et al*., 2016).

A current approach to ingestion timing involves commencing exercise at individual TTP [$HCO_3^-$] ($T_{max}$), based on highly variable inter-individual responses (Miller *et al*., 2016). Whilst the utility of maximal vs. 'meaningful' rises in [$HCO_3^-$] remains debatable (de Oliveira *et al*., 2021; 2020), this approach has demonstrated some efficacy, with Boegman *et al*. (2020) eliciting a 2 s improvement in 2000 m rowing performance with individualised timing against a generalised approach (367.0 ± 10.5 s vs. 369.0 ± 10.3 s). Furthermore, Lassen, Lindstrøm and Lønbro (2021) produced a 6 s benefit in a

3.5 km running TT following an individualised approach, although this was only compared to a placebo (775.5 ± 16.2 s vs. 781.4 ± 16.1 s) and as a result, it is difficult to isolate the influence of individualisation.

Although positive, the usability of this approach is contingent on reliable 'day-to-day' blood acid-base responses (Gough *et al*., 2017a; 2017b) and required elucidating using SC. In study 1 (chapter 4), limited reliability in key $HCO_3^-$ kinetics including both $T_{max}$ and absolute change ($\Delta C_{max}$) from baseline was identified, challenging the use of such an approach. Notwithstanding, 0.5 $g \cdot kg^{-1}$ BM promoted marked elevations in [$HCO_3^-$] (5.9 ± 1.8) throughout, with limited total GIS.

Acute GIS are a relatively common side-effect of ingesting large quantities of exogenous buffers, exacerbated when delivered as an aqueous solution (Carr *et al*., 2011b). Ergogenic effects may still occur where GIS are present (Miller *et al*., 2016; Price and Simons, 2010), although GIS may be ergolytic for some (Deb *et al*., 2018; Saunders *et al*., 2014; Cameron *et al*., 2010) and/or or discourage use entirely (Heibel *et al*., 2018). Hilton *et al*., (2020b) postulates that symptom severity may modulate the effects on performance, as opposed to total GIS. Accordingly, methods of protecting against even small changes in GIS warrant consideration. In study 2 (Chapter 5), ENT, DEL and GEL encapsulation of 0.5 $g \cdot kg^{-1}$ BM SC was compared. Ingestion form had no significant effect on total GIS, with 6/14 participants experiencing zero symptoms throughout. Significant differences in $T_{max}$ were observed between ENT (178.5 ± 32.5 min) and GEL (153 ± 27.4 min). Additionally, ΔCmax was greater in ENT (6.2 ± 0.8 $mmol \cdot L^{-1}$) vs. DEL (5.1 ± 1.0 $mmol \cdot L^{-1}$). Despite this, all forms promoted meaningful increases in [$HCO_3^-$] (5.1-6.2 $mmol \cdot L^{-1}$) which, coupled with a lack of effect on total GIS suggests that affordable GEL encapsulation may be as valuable as more expensive ENT and DEL formulations with some alterations in $HCO_3^-$ kinetics. Previous work by Urwin *et al*. (2021a) has suggested that ingestion (GEL) should take place over a period of > 60 min (15, 30 or 45 min) to avoid lessening increases in both blood and [$HCO_3^-$].

Utilising insight developed in study 1-3 (chapters 4-6), the aim of the present study was to re-apply findings to an exercise model (repeated 4 km TT) to determine the ergogenicity of acute SC ingestion. Repeated 4 km TT were completed as a means to determine the influence of SC ingestion on potential

acid-base recovery and, to allow the assessment of SC when exercise was commenced at peak [$HCO_3^-$] or, where [$HCO_3^-$] is likely to be meaningfully but not maximally elevated (+4 $mmol \cdot L^{-1}$ or more).

## 7.2 Methods

### *7.2.1 Participants*

Seven recreationally trained cyclists (de Pauw *et al*., 2013) participated in this study (mean ± SD: age, 27 ± 6.0 years; body mass, 79.4 ± 4.4 kg; height 179.0 ± 5.5 m; peak oxygen uptake ($VO_{2peak}$), 44.9 ± 9.3 $mL \cdot kg^{-1} \cdot min^{-1}$). All participants took part in regular exercise (> 4 $h \cdot week^{-1}$) and were free of GI-related disorders. Exclusion criteria precluded those with hypertension, renal impairment or following a salt-restricted diet. Participants were also not to ingest any other nutritional supplements or medications concurrent to the current study (ETH2021-0301).

### *7.2.2 Experimental Design*

In a randomised, double-blind and crossover design, participants attended the laboratory at the same time of day (Ayala *et al*., 2021) (11:00 h) on eight occasions, seperated by ≥ 48 h. Prior to the experimental conditions, participants completed a preliminary test of $VO_{2peak}$ and three separate familiarisation sessions (4 km cycling TT). In the remaining four visits, participants performed a repeated 4 km TT protocol under varied experimental conditions, administered in a counterbalanced order as determined by a Latin square generator. Experimental trials required the consumption of 0.5 $g \cdot kg^{-1}$ BM of SC on two occasions (~128 or ~153 min prior to exercise), a control (0.07 $g \cdot kg^{-1}$ BM sodium chloride and cornflour) or a placebo (cornflour only). Breakfast in the form of plain porridge oats (1.75 $g \cdot kg^{-1}$ BM) was co-consumed in each trial. Both the supplement and CHO were consumed prior to arrival at the laboratory, with verbal confirmation of adherence. Participants were instructed to abstain from alcohol (El-Sayed, Ali and Ali, 2005) or caffeine (Guest *et al*., 2021) consumption for 12 h, and strenuous exercise 24 h prior to each visit (Tornero-Aguilera *et al*., 2022). Water intake was

encouraged in the 24 h prior to experimental testing (Arnaoutis, 2022) and after an overnight fast, used to limit potential variance on gastric emptying rates (Davis *et al*., 1986) from prior food intake. On arrival to the laboratory, the protocol and procedures were reaffirmed.

### *7.2.3 Preliminary Testing*

Participants completed ramp test to volitional exhaustion on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands). Following a 5 min warm-up at a self-selected cadence (70-100 $rev \cdot min^{-1}$), workload was increased by 1 W every 2 s (30 $W \cdot min^{-1}$) until volitional cessation. Breath-by-breath gases were measured continuously throughout using a gas analyser (K5, Cosmed, Italy) alongside heart rate (Polar®, Kempele, Finland). A selection of criteria as proposed by Midgley *et al*. (2007) were used to confirm that $VO_{2peak}$ had been reached: (i) heart rate within 10 $beats \cdot min^{-1}$ of age predicted maximum (220-age); (ii) respiratory exchange ratio > 1.10 AU and (iii) RPE 18-20 AU. On three sperate days participants performed repeated 4 km cycling TT's, intervened by 35 min, to familiarise themselves with the exercise task.

In all trials but one, exercise was scheduled to commence at a generalised timepoint, based on the mean time-to-peak [$HCO_3^-$] from study 1 (chapter 4) of ~153 ± 35 min. In the remaining trial (0.5 $g \cdot kg^{-1}$ BM SC), exercise was commenced 35 min earlier, based on the inter-individual variation in mean TTP. This approach was adopted so that the effect of increased [$HCO_3^-$] could be compared, when the first vs. the second 4 km TT began around peak [$HCO_3^-$]. To ascertain the SC and sodium chloride dose, semi-nude body mass was recorded (Seca 700, Seca GmbH, Germany). Once the total quantity of capsules was calculated for the SC trials, this number was matched in both the control and placebo trial to aid blinding. Supplements were administered in size 0 opaque GEL capsules (Bulk Powders™, Colchester, UK) and were manually filled by a trained laboratory technician using a capsule filling device (Capsule Connection LL, Arizona, USA). All supplement doses were checked for accuracy pre-administration (Ohaus ®, Fisher Scientific™, Pennsylvania, USA) and were ingested with 500 ml of water within 10 min.

### *7.2.4 Experimental Trials*

Once the participants arrived at the laboratory, a resting period of 10 min was allowed before initial measures were taken. This involved determining GIS as well as resting heart rate, blood [$La^-$] and acid-base balance. Fingertip capillary blood samples (95 μL) were collected in heparin-coated glass capillary tubes (Radiometer Medical Ltd, Copenhagen, Denmark) using an aseptic technique and analysed immediately (Radiometer ABL800 Basic, Copenhagen, Denmark) for [$HCO_3^-$] and pH alongside [$Na^+$], [$K^+$] and [$Cl^-$]. A further 5 μL sample was collected from the same site to obtain [$La^-$] using a portable analyser (Lactate Pro 2, LT-1730, Arkray, Japan).

### *7.2.5 Time Trials*

Participants established a preferred handlebar and saddle position based on the Hamley method, believed to provide optimum aerobic power (Hamley and Thomas, 1967; Nordeen-Snyder, 1977; Shenum and Devries, 1976) which was recorded and utilised for all other experimental trials. After a 5 min self-selected warm-up, heart rate, GIS, [$La^-$] and blood acid-base balance was again determined. A 4 km cycling TT was then performed on an air-braked cycle ergometer (Wattbike Pro, Wattbike, Nottingham) from a static start. At a range of power outputs (100-1000 W) and sport specific cadences (70, 90, 110 and 130 rev·min$^{-1}$) the Wattbike was found to be highly reliable between bikes (0.6 and 25.5 W differences at 100 W and 1000 W, respectively) and within repeated measures on the same bike (measurement differences of 1.5 W and 1.7 W between trials at 300 W and 600 W, respectively) (Wainwright, Cooke and O'hara, 2017). Participants were instructed to complete the TT as fast as possible and were blinded from any visual feedback where possible. Participants were notified that 50% of the distance had been completed and received strong verbal encouragement during the final 1 km only (every 0.1 km). After 35 min seated rest, during which water was consumed *ad libitum*, a second 4 km TT was completed after an identical warm-up. All TTs took place under standardised laboratory conditions (temperature 18-20 °C, humidity 45 ± 5%).

### *7.2.6 Physiological And Perceptual Measures*

During each TT, physiological and perceptual measures were gathered at 1 km increments. Specifically, heart rate (Polar®, Kempele, Finland), perceived ratings of overall ($RPE_O$) and lower-limb ($RPE_L$) exertion and perceived rating of fatigue (ROF) were recorded (Micklewright *et al*., 2017; Borg, 1970). Post 4 km TT [$Bl^-$] and blood acid-base balance were measured, and GIS noted immediately, using an adapted questionnaire (Carr *et al*., 2011b). This questionnaire required participants to rate symptoms including nausea, flatulence, stomach cramping, belching, stomach-ache, bowel urgency, diarrhoea, vomiting and stomach bloating on a visual analogue scale. No symptom was indicated by a score of "0", whereas severe symptoms required a score of "10".

### *7.2.7 Statistical Analyses*

Normality was assessed using the Shapiro-Wilk test and via visual inspection of the normality plots (Grafen and Hails, 2002). Two-way (condition x time) ANOVA for repeated measures was utilised for all physiological (e.g., blood [$La^-$] and heart rate), perceptual (e.g., $RPE_O$, $RPE_L$, ROF and GIS), performance (i.e., performance times and power) and acid base-balance (e.g., blood [$HCO_3^-$], pH and electrolytes) variables. Where a significant main effect was highlighted, Bonferroni-adjusted post-hoc paired comparisons were completed (Nevill *et al*., 2002). Effect sizes are given as partial eta-squared ($\eta p^2$) for one- and two-way ANOVA, while Hedge's g and 95% confidence intervals (CI) were calculated for paired comparisons (Lakens, 2013). Effect sizes are described as small ($\eta p^2 = 0.01$; $g = 0.2$), medium ($\eta p^2 = 0.06$; $g = 0.5$) or large ($\eta p^2 = 0.14$; $g = 0.8$) (Cohen, 1988). Statistical significance was set at $P < 0.05$. Descriptive data are presented as mean ± SD throughout. Data were analysed using SPSS version 28 (IBM®, Chicago, USA).

## 7.3 Results

### *7.3.1 Exercise Performance*

Time-to-complete the 4 km TT (Figure 7.1) was similar between each trial ($F_{3,18}$ = 0.3, $P$ = 0.846, $\eta p^2$ = 0.043), with an average completion time of 382.71 ± 37.55 s in TT 1, compared to 386.95 ± 38.72 s in TT 2 ($F_{1,6}$ = 2.8, $P$ = 0.143, $\eta p^2$ = 0.321). Acute ingestion of SC had no effect on power output (Figure 7.2), with a similar average power (230 ± 59 and 219 ± 57 W in TT1 and TT2, respectively) across all trials ($F_{3,36}$ = 1.2, $P$ = 0.331, $\eta p^2$ = 0.090). No interaction was observed between condition and TT number (1 vs. 2) ($F_{3,36}$ = 0.6, $P$ = 0.609, $\eta p^2$ = 0.049), although there were significant variations in power output across the TT's ($F_{3,36}$ = 14.1, $P$ < 0.001, $\eta p^2$ = 0.540). Power output after 1 km (223 ± 87 W), 2 km (199 ± 55 W) and 3 km (205 ± 50) was similar across trials, with a notable increase in the final km (270 ± 39 W, $P$ = < 0.001).

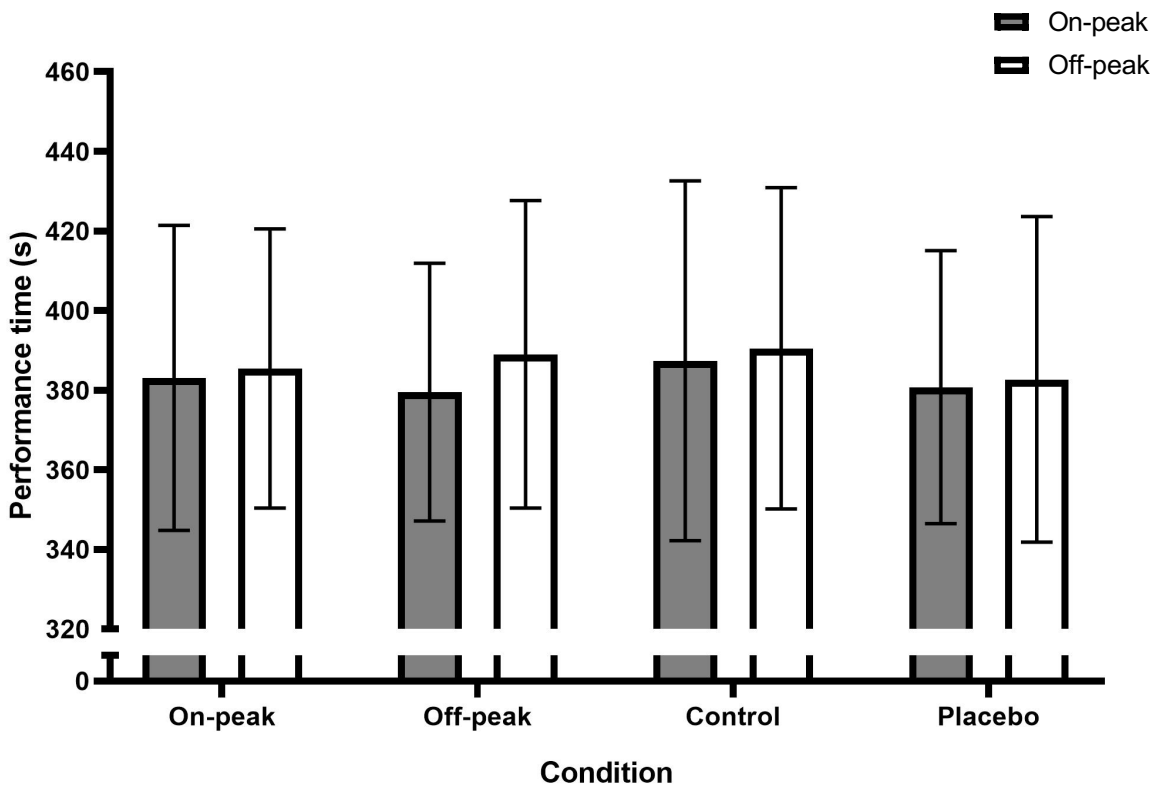


**Figure 7.1.** Mean (± SD) time-to-completion following the ingestion of 0.5 $g \cdot kg^{-1}$ body mass (BM) sodium citrate (SC), a control substance (0.07 $g \cdot kg^{-1}$ BM sodium chloride and cornflour) or a placebo (cornflour only). On-peak = TT 1 commenced at group time-to-peak (153 ± 35 min) bicarbonate concentration [$HCO_3^-$] (TT 1 153 min post-ingestion). Off-peak = TT1 commenced 35 min before group time-to-peak [$HCO_3^-$] (118 min post-ingestion).

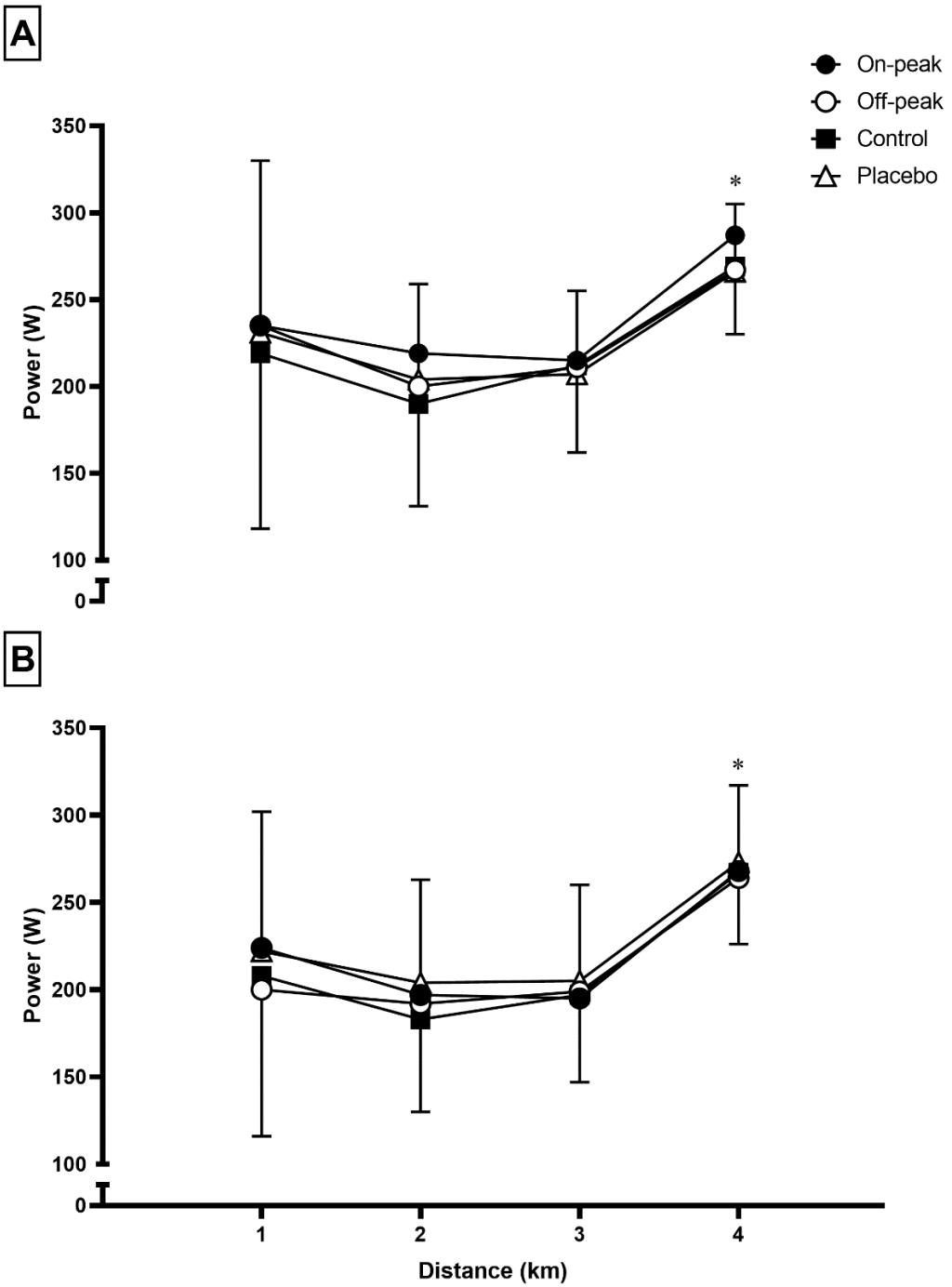


**Figure 7.2.** Mean (± SD) power output following the ingestion of 0.5 $g \cdot kg^{-1}$ body mass (BM) sodium citrate (SC), a control substance (0.07 $g \cdot kg^{-1}$ BM sodium chloride and cornflour) or a placebo (cornflour only). Time trial 1 (TT 1) is displayed above (A) and time trial 2 (TT 2) is shown below (B) On-peak = TT 1 commenced at group time-to-peak bicarbonate concentration [$HCO_3^-$] (153 min). Off-peak = TT1 commenced 35 min before group time-to-peak [$HCO_3^-$] (118 min). **Denotes a significant difference from the previous timepoint ($P < 0.05$).*

### *7.3.2 Acid-Base Balance*

A significant effect of trial on blood [$HCO_3^-$] was observed ($F_{3,36}$ = 14.2, $P$ = < 0.001, $\eta p^2$ = 0.543) (Figure 7.3). Pairwise comparisons highlighted a meaningful difference in blood [$HCO_3^-$] for off-peak SC (26.2 ± 2.0 mmol·$L^{-1}$) relative to both the control (21.9 ± 2.7 mmol·$L^{-1}$, $P$ = 0.002) and placebo trials (21.4 ± 1.9 mmol·$L^{-1}$, $P$ < 0.001). Blood [$HCO_3^-$] was similar between the on-peak (23.9 ± 2.8 mmol·$L^{-1}$), control and placebo trials, as well as for all remaining comparisons (P > 0.05). Blood [$HCO_3^-$] pre-and post-WU was similar (26.5 ± 2.3 vs. 26.1 ± 2.4 mmol·$L^{-1}$, $P$ = 0.830) but decreased substantially post-TT (17.6 ± 2.4 mmol·$L^{-1}$, $P$ = < 0.001). An interaction between time and TT number was evident ($F_{2,24}$ = 5.1, $P$ = 0.014, $\eta p^2$ = 0.300).

An effect of trial on blood pH was also evident ($F_{1,12}$ = 11.1, $P$ = 0.006, $\eta p^2$ = 0.480). Acute SC ingestion was associated with an elevated pH in the off-peak trial (7.422 ± 0.038) compared to both control (7.361 ± 0.036, $P$ = 0.004) and placebo (7.360 ± 0.030, $P$ = < 0.001), but not in the on-peak trial (7.391 ± 0.044, both $P$ > 0.05). Mirroring [$HCO_3^-$], blood pH was similar pre-and post -WU (7.430 ± 0.031 vs. 7.421 ± 0.035 -$\log_{10}[H^+]$, $P$ = 0.423) but dropped markedly post-TT (7.299 ± 0.045, $P$ < 0.001). No time x TT number interaction was apparent ($F_{2,24}$ = 2.5, $P$ = 0.106, $\eta p^2$ = 0.171).

### *7.3.3. Electrolyte Responses*

Trial notably impacted [$Na^+$] post-ingestion ($F_{1,12}$ = 16.3, $P$ = 0.002, $\eta p^2$ = 0.576) (Figure 7.3). Further analysis revealed a lower [$Na^+$] in the placebo trial (143 ± 2 mmol·$L^{-1}$) compared to SC on-peak (145 ± 2 mmol·$L^{-1}$, $P$ = 0.012), SC off-peak (146 ± 2 mmol·$L^{-1}$, $P$ = < 0.001) and control (145 ± 2 mmol·$L^{-1}$, $P$ = 0.003) trials. Meaningful changes in [$Na^+$] were demonstrated pre-WU to post-WU (both 144 ± 2 mmol·$L^{-1}$, $P$ = 0.032), which widened post-WU to post-TT (144 ± 2 vs. 147 ± 2 mmol·$L^{-1}$, $P$ = < 0.001). No time x TT number interaction was found ($F_{2,24}$ = 1.9, $P$ = 0.176, $\eta p^2$ = 0.135).

Trial had a large effect on post-ingestion [$K^+$] ($F_{3,36}$ = 3.6, $P$ = 0.023, $\eta p^2$ = 0.229). Off-peak [$K^+$] was lower compared to placebo (4.5 ± 0.5 vs. 5 ± 0.4 mmol·$L^{-1}$, $P$ = 0.016), with no other differences throughout ($P$ = > 0.50). Furthermore, [$K^+$] rose pre-WU to post-WU (4.6 ± 0.5 vs. 4.7 ± 0.5 mmol·$L^{-1}$, $P$ = 0.016) and fell post-WU to post-TT (5.0 ± 0.5 mmol·$L^{-1}$, $P$ = 0.001).

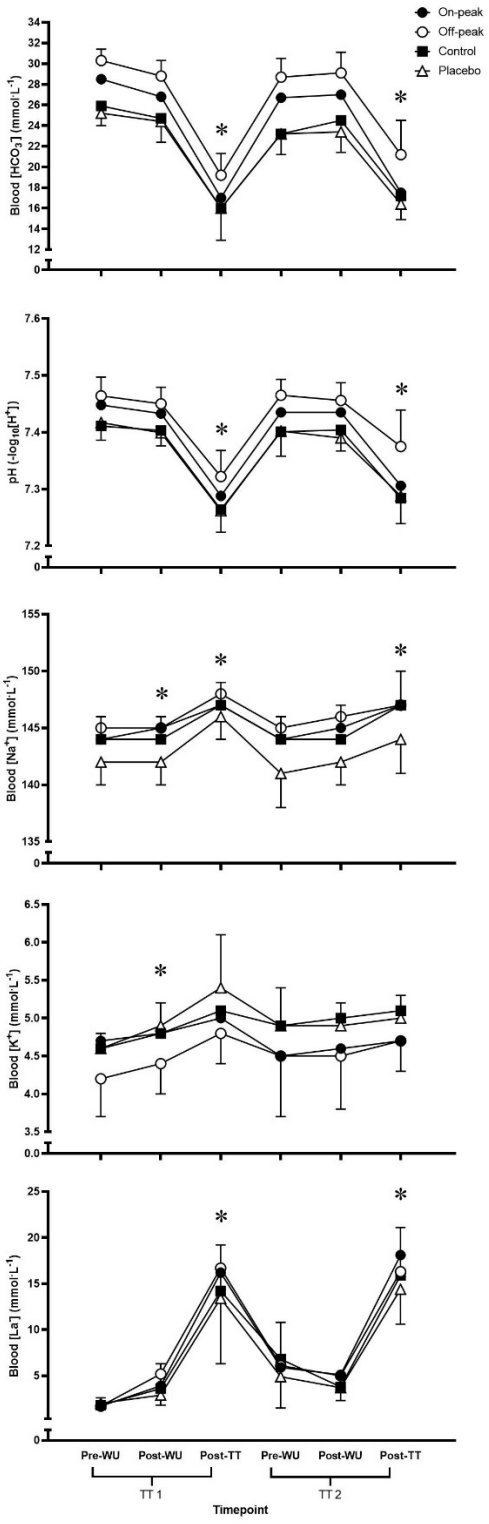


**Figure 7.3.** Mean (± SD) blood $[HCO_3^-]$ (Top), pH (second top), $[Na^+]$ (middle), $[K^+]$ (second bottom) and $[La^-]$ (bottom). Values for pre warmup (WU), post warmup (WU) and post time-trial (TT) are displayed for both TT 1 and TT 2. **Denotes a significant difference from the previous timepoint ($P < 0.05$).*

### *7.3.4 Physiological And Perceptual Responses*

Blood [$La^-$] between trials was similar ($F_{3,36}$ = 2.1, $P$ = 0.112, $\eta p^2$ = 0.152) (Figure 7.3). While blood [$La^-$] was comparable pre-to-post WU (3.9 ± 2.1 vs. 4.1 ± 2.1 mmol·$L^{-1}$, $P$ > 0.50), post TT [$La^-$] were substantially greater overall (15.6 ± 4.1 mmol·$L^{-1}$, $P$ < 0.001). Additionally, interaction between time and TT number ($F_{1,12}$ = 5.7, $P$ = 0.034, $\eta p^2$ = 0.324) was highlighted with greater blood [$La^-$] in TT2 vs. TT1 pre-WU (5.9 ± 3.4 vs 1.8 ± 0.7 mmol·$L^{-1}$), post-WU (4.4 ± 2.1 vs 4.1 ± 2.1 mmol·$L^{-1}$) and post-TT (16.2 ± 3.8 vs 15.1 ± 4.4 mmol·$L^{-1}$).

Heart rate rose progressively throughout the warm-up and continued to rise throughout the 4 km TT ($F_{1,12}$ = 735.3, $P$ = < 0.001, $\eta p^2$ = 0.984), although no differences between conditions were present ($F_{1,12}$ = 2.3, $P$ = 0.159, $\eta p^2$ = 0.158) nor was there an interaction between condition and time ($F_{1,12}$ = 0.5, $P$ = 0.487, $\eta p^2$ = 0.041). Further analysis revealed a significant time x TT number interaction ($F_{1,12}$ = 9.7, $P$ = 0.009, $\eta p^2$ = 0.448) with TT 2 demonstrating higher heart rates generally, particularly pre-WU (95 ± 15 vs. 68 ± 13 beats.$min^{-1}$). Similarly, $RPE_O$ ($F_{3,36}$ = 1.2, $P$ = 0.325, $\eta p^2$ = 0.091), $RPE_L$ ($F_{3,36}$ = 2.3, $P$ = 0.094, $\eta p^2$ = 0.161) and ROF ($F_{3,36}$ = 0.9, $P$ = 0.452, $\eta p^2$ = 0.070) did not differ between conditions, but there were noticeable increases in $RPE_O$ ($F_{3,36}$ = 73.2, $P$ = < 0.001, $\eta p^2$ = 0.859), $RPE_L$ ($F_{3,36}$ = 107.5, $P$ = < 0.001, $\eta p^2$ = 0.900) and ROF (F3,36 = 125.2, $P$ = < 0.001, $\eta p^2$ = 0.913) during the TT (Table 7.1).

### *7.3.5 Gastrointestinal Symptoms*

No GIS were reported pre-ingestion in all conditions (Table 7.2). Pre-exercise, three participants reported GIS, with fewer symptoms in the control trial (n = 1) vs. the off-peak trial (n = 3). Pre-exercise GIS scores were similar in both control (5 ± 0 AU) and off-peak (5 ± 1 AU) trials ($P$ > 0.05). Nausea was the most frequent GIS (n = 2) with both bowel urgency and diarrhoea experienced (both n = 1).

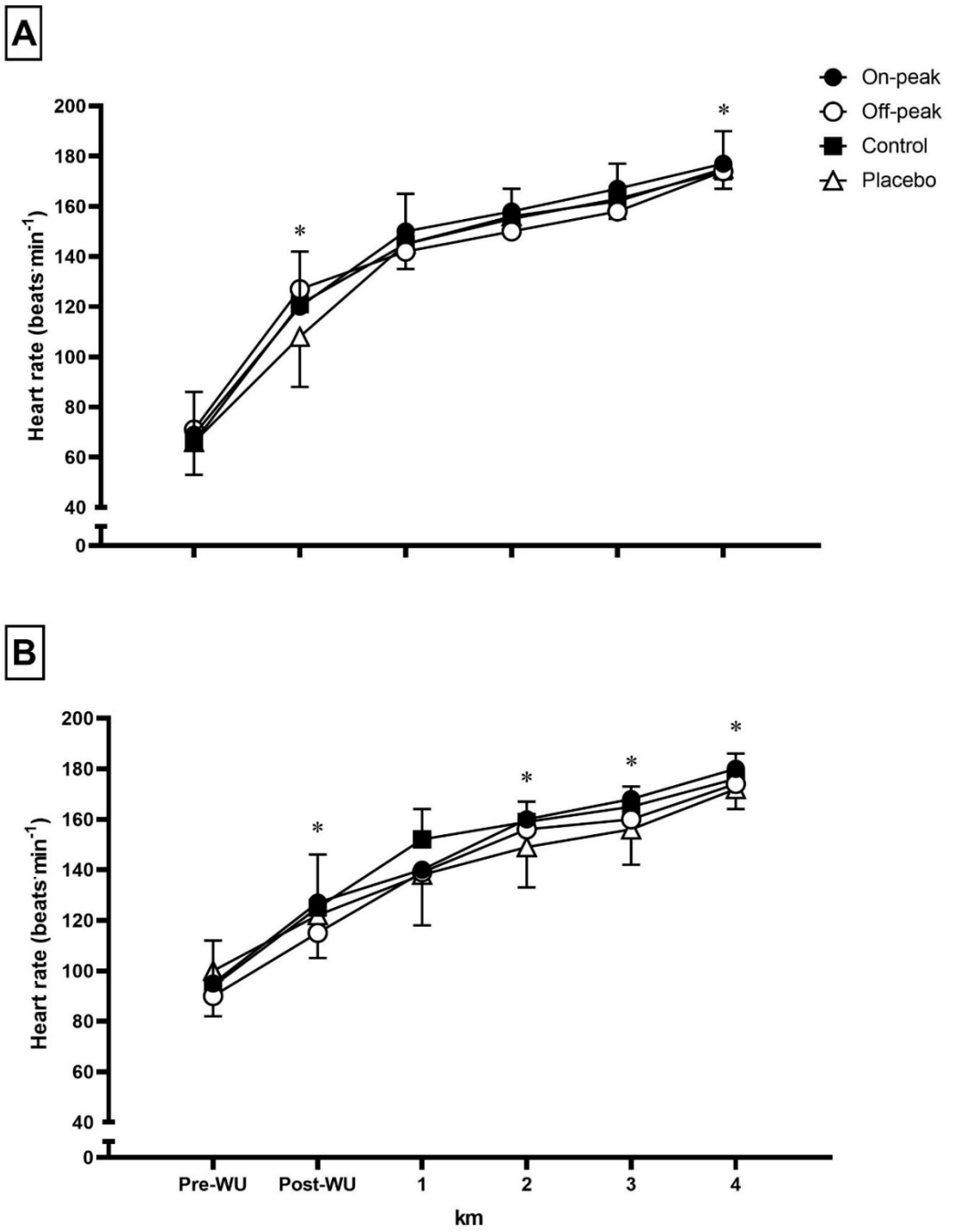


**Figure 7.4.** Mean (± SD) heart rate following the ingestion of 0.5 $g \cdot kg^{-1}$ body mass (BM) sodium citrate (SC), a control substance (0.07 $g \cdot kg^{-1}$ BM sodium chloride and cornflour) or a placebo (cornflour only). Time trial 1 (TT 1) is displayed above (A) and time trial 2 (TT 2) is shown below (B) On-peak = TT 1 commenced at group time-to-peak bicarbonate concentration [$HCO_3^-$] (153 min). Off-peak = TT1 commenced 35 min before group time-to-peak [$HCO_3^-$] (118 min). Pre- and-post warmup (WU) values are also presented. **Denotes a significant difference from the previous timepoint ($P < 0.05$).*

**Table 7.1** Mean (± SD) perceptual responses during repeated 4 km time trials (TT).

| | Condition | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **SC5 on-peak** | | **SC5 off-peak** | | **Control (Cornflour and salt)** | | **Placebo (Cornflour)** | |
| | TT 1 | TT 2 | TT 1 | TT 2 | TT 1 | TT 2 | TT1 | TT2 |
| **$REP_O$ (AU)** | | | | | | | | |
| 1 km | 13 ± 2 | 14 ± 2 | 12 ± 2 | 13 ± 2 | 13 ± 2 | 14 ± 3 | 12 ± 2 | 13 ± 2 |
| 2 km | 15 ± 2* | 15 ± 1* | 14 ± 1* | 15 ± 2* | 14 ± 2* | 15 ± 2* | 14 ± 2* | 15 ± 1* |
| 3 km | 16 ± 1* | 16 ± 1* | 16 ± 1* | 17 ± 1* | 16 ± 1* | 16 ± 2* | 16 ± 2* | 16 ± 1* |
| 4 km | 18 ± 1* | 19 ± 1* | 18 ± 1* | 19 ± 1* | 18 ± 2* | 19 ± 1* | 19 ± 2* | 19 ± 1* |
| **$RPE_L$ (AU)** | | | | | | | | |
| 1 km | 5 ± 1 | 6 ± 1 | 5 ± 1 | 6 ± 1 | 5 ± 1 | 6 ± 2 | 5 ± 2 | 6 ± 1 |
| 2 km | 6 ± 1* | 7 ± 1* | 6 ± 1* | 7 ± 1* | 6 ± 1* | 7 ± 1 | 6 ± 2* | 7 ± 1* |
| 3 km | 7 ± 2* | 7 ± 1* | 7 ± 1* | 8 ± 0* | 7 ± 1* | 8 ± 1* | 7 ± 2* | 8 ± 1* |
| 4 km | 9 ± 2* | 9 ± 1* | 8 ± 1* | 9 ± 1* | 8 ± 1* | 9 ± 1* | 9 ± 1* | 9 ± 1* |
| **ROF (AU)** | | | | | | | | |
| 1 km | 4 ± 1 | 5 ± 1 | 4 ± 1 | 5 ± 1 | 4 ± 1 | 5 ± 1 | 5 ± 1 | 5 ± 1 |
| 2 km | 6 ± 1* | 6 ± 1* | 5 ± 1 | 6 ± 1* | 5 ± 1* | 6 ± 1* | 6 ± 2* | 6 ± 1* |
| 3 km | 7 ± 1* | 7 ± 1* | 6 ± 1* | 7 ± 1* | 7 ± 1 | 7 ± 1* | 7 ± 2* | 7 ± 1* |
| 4 km | 8 ± 1* | 9 ± 1* | 8 ± 1* | 9 ± 1* | 8 ± 1* | 9 ± 1* | 8 ± 2* | 9 ± 1* |

**Notes:** SC5 = 0.5 $g \cdot kg^{-1}$ body mass (BM) sodium citrate. On-peak = TT 1 commenced at peak bicarbonate concentration ($[HCO_3^-]$). Off-peak = TT2 commenced at peak $[HCO_3^-]$. $RPE_O$ = rating of overall perceived exertion. $RPE_L$ = rating of lower-limb perceived exertion. ROF = Rating of fatigue. AU = arbitrary units.

**Denotes a significant difference from the previous timepoint ($P < 0.05$).*

**Table 7.2** Individual peak gastrointestinal symptoms (GIS) immediately before exercise. Symptoms are presented in bold for clarity and scores are displayed in parentheses.

| | **SC5 on-peak** | | **SC5 off-peak** | | **Control** | | **Placebo** | |
|---|---|---|---|---|---|---|---|---|
| **Participant** | Pre-ingestion | Pre-exercise | Pre-ingestion | Pre-exercise | Pre-ingestion | Pre-exercise | Pre-ingestion | Post-ingestion |
| 1 | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) |
| 2 | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | **Nausea (6.0)** | No symptom (0.0) | **Nausea (5.0)** | No symptom (0.0) | No symptom (0.0) |
| 3 | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | **Bowel urgency (5.0)** | No symptom (0.0) | Bowel urgency (8.0) | No symptom (0.0) | No symptom (0.0) |
| 4 | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) |
| 5 | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | **Diarrhoea (4.0)** | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) |
| 6 | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) |
| 7 | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) | No symptom (0.0) |

## 7.4 Discussion

This is the first study to investigate the effect of gelatine encapsulated SC on exercise performance and recovery. The impact of two generalised timepoints for SC ingestion on a subsequent repeated 4 km TT protocol were compared. Significant increases in [$HCO_3^-$] and pH emerged for off-peak SC ingestion but not on-peak SC, although neither had a significant effect on general TT performance (Figure 7.1). Furthermore, average performance time for TT1 increased by ~4 s only in TT2, suggesting that sufficient recovery occurred without additive benefit from SC. Lack of any meaningful differences herein are underpinned by the similar physiological and perceptual responses between trials (Figure 7.3 and 7.4). Finally, no GIS were reported pre-ingestion in all conditions throughout, with only three instances of GIS pre-exercise, largely ruling out any ergolytic effect of GIS on the performance response to SC (Table 7.2). Taken together, SC does not appear to improve 4 km TT performance or recovery, in the on-peak trial, this may be explained by markers blood alkalosis which were not significantly elevated over placebo and control conditions.

Numerous studies have investigated the effects of SC on exercise performance with equivocal outcomes (de Oliveira *et al*., 2021; Urwin *et al*., 2021b; Cerullo *et al*., 2020). Whilst some investigations have reported performance improvements (Cunha *et al*., 2019; Russel *et al*., 2014; van Montfoort *et al*., 2004), others have noted no benefit (Kumstat *et al*., 2018; Suvi *et al*., 2019; Vaher *et al*., 2015). Inconsistent ingestion protocols have been offered as an explanation for this disparity, including factors such as timing, mode and duration of ingestion. Although the value of a maximal vs. 'meaningful' change in [$HCO_3^-$] is questionable (de Oliveira *et al*., 2021; 2020), time elapsed between ingestion and the commencement of exercise influences the degree of metabolic alkalosis (blood [$HCO_3^-$] or pH). Demonstrating the effect of timing here, when TT1 was commenced one standard deviation before peak (118 min post-ingestion), a significant difference in [$HCO_3^-$] was highlighted, whilst starting TT1 on peak (153 min post-ingestion) did not significantly increase [$HCO_3^-$]. De Oliveira *et al*. (2021) observed that changes in [$HCO_3^-$] of 4-6+ mmol·$L^{-1}$ were associated with greater exercise effects than ≤4 mmol·$L^{1}$. Pre-WU [$HCO_3^-$] in the on-peak trial was ~3 mmol·$L^{-1}$ greater than both the control and placebo trials, compared to the off-peak trial ~4.5 mmol·$L^{-1}$. However, neither resulted in improvements in TT

performance, an exercise task which has previously been shown to improve with use of extracellular buffering agents (Gough *et al*., 2021; 2017b; Hilton *et al*., 2020b). Indeed, the mechanisms by which SC may impact performance generally remain poorly understood.

After ingestion, SC promptly dissociates in bodily fluids, separating into its constituent ions $Na^+$ and $citrate^-$. The citrate anion is expelled from the plasma, shifting the ratio between strong cations and strong anions, altering the electrical balance (Cerullo *et al*., 2020). To restore neutrality, $[H^+]$ is decreased and $[HCO_3^-]$ increased, marked by a rise in pH towards alkalosis (Shave *et al*., 2001; Enoka and Stuart, 1992). Greater pH results in the disposal of $La^-$ from the muscle during exercise via the monocarboxylate transporters, improving the contractile capacity of the muscle (Oster *et al*., 1988; Fabiato and Fabiato, 1978; Mainwood and Worsley-Brown, 1975). Although not significant, post-TT1 blood $[La^-]$ was higher following SC ingestion (on peak: 16.2 ± 1.9 $mmol \cdot L^{-1}$; off peak: 16.7 ± 3.4 $mmol \cdot L^{-1}$) compared to both control (14.2 ± 5.0 $mmol \cdot L^{-1}$) and placebo (13.4 ± 7.1 $mmol \cdot L^{-1}$) trials. Cerullo *et al*. (2020) adds that this may favour an increase in performance compared to a matched dose of SB, which was not evident in the current study. Nonetheless, while the sarcolemma is impermeable to $HCO_3^-$, $citrate^-$ can penetrate this membrane utilising specific transporters (Oster *et al*., 1988; Katz *et al*., 1984; Mainwood and Worsley-Brown, 1975). Collectively, these transporters permit translocation of $citrate^-$ through the inner mitochondrial membrane and absorption from the blood (Palmieri, 2014; Mycielska *et al*., 2009). Such transport facilitates the role of citrate in multiple metabolic process including as an intermediate of the Krebs cycle, the movement acetyl CoA from the mitochondria to the cytosol and, its inhibitory effect on PFK, thus interfering with glycolysis (Cerullo *et al*., 2020). Hirche *et al*. (1975) suggests this inhibitory effect may affect short-term performance (<180 s) but dissipates with long-term exercise where higher $La^-$ production occurs. Such mechanisms are unestablished in humans, but may further explain why SC benefits some, but not all exercise.

As a result of increased pH after SC ingestion, there are also reductions in interstitial $K^+$ release by the muscle during intense exercise (Street *et al*., 2005). Excessive $K^+$ production marks the emergence of fatigue (Fitts, 1994; Kjellmer, 1965) and in turn, reduces muscle excitability (Clausen, 2003). Conversely, lowering interstitial $[K^+]$ during exercise has been linked with improved

performance (Nielsen *et al*., 2004). The net effect on electrolyte balance post SC ingestion also provides a hormonal response. In one example, lower aldosterone and cortisol levels were highlighted, with increased [$Na^+$] and reduced [$K^+$] offered in explanation (Suvi *et al*., 2019; El Ghorayeb, Bourdeau and Lacroix, 2016; Bollag, 2014). No differences in either electrolyte was observed here, suggesting SC did not sufficiently promote such a response or, different, potentially counteracting mechanisms were in action.

Although ergogenic doses of SC (~0.5 $g \cdot kg^{-1}$ BM) may induce GIS, as demonstrated both in the current thesis (Chapters 4-6) and elsewhere (Urwin *et al*., 2022; 2021a; 2019; 2016), symptoms may not coincide with exercise performance. In the current thesis, GIS typically emerged ≤100 min post-ingestion, with peak [$HCO_3^-$] ($T_{max}$) ~150 min. Peak GIS have also been reported 60-90 min post-ingestion, prior to peak [$HCO_3^-$] at ~3 h as commonly reported (Urwin *et al*., 2021a; Cerullo *et al*., 2020). Such observations are also consistent with work in SB (Hilton *et al*., 2019a; 2020a; 2020b). It is therefore difficult to clarify whether GIS could have negated any beneficial effects of SC in the current study, given zero cases of GIS were reported at rest, rising to four pre-exercise. Additionally, future research should attempt to determine the perceived effects of GIS on performance to understand if any link exists between concern of GIS and actual outcome measures. Finally, perceptual measures of perceived exertion ($RPE_O$ and $RPE_L$) and fatigue (ROF) that developed during each 4 km TT (Table 7.1) were similar between SC, control and placebo trials, suggesting that differences in afferent feedback to the central nervous system were unlikely to have impacted the exercise response throughout (Siegler and Marshall, 2015; Thomas *et al*., 2015).

A key limitation of the current study is the lack of statistical power as a result of the small sample size ($N = 7$), meaning any observations made are at best suggestive and should be interpreted with caution (Cohen, 1988). An estimated four further participants or more are required to achieve appropriate statistical power. The use of recreational athletes may also be criticised given the large variability in exercise performance relative to trained athletes, i.e., when exercising on the Wattbike (CV = 6.7; 95% CI = 4.8-13.2 vs. 2.6%; 95% CI = 1.8-5.1 (Hopker *et al*., 2010). This population was

used to achieve consistency between studies, specifically those recruited to refine the SC protocol used in Study 4 (Chapter 7) and the individuals undertaking repeated 4 km cycling TT's.

### 7.5 Conclusion

This study is the first to demonstrate that 0.5 $g \cdot kg^{-1}$ BM SC does not improve 4 km TT performance when commenced either on or around average peak [$HCO_3^-$]. Despite previous suggestion that timing of ingestion may be more accurately thought of as a period (i.e., period where [$HCO_3^-$] is 4+ $mmol \cdot L^{-1}$ above resting values), manipulating the commencement of exercise by 35 min altered the blood $HCO_3^-$ (and pH) response to SC and, compared to control/placebo trials. Given the similarities in physiological and perceptual responses throughout, the lack of any ergogenic response, particularly where meaningful changes in [$HCO_3^-$] were noted, is difficult to explain. Future studies may seek to better understand the general mechanisms involving SC, before clarifying if and where it may offer a beneficial effect. A wider range of tasks that are also limited by peripheral fatigue should also be deployed to determine the efficacy of SC, guided by the information gained within the current thesis.

# CHAPTER 8 – SYNTHESIS OF FINDINGS

## 8.1 General Summary

The collective aim of the current thesis was twofold. In studies 1-3, the key aspects of any potential sodium citrate ingestion protocol were investigated in terms of both acid-base and GIS responses. Then, in study 4, findings from the preceding studies were utilised to establish the potential effect of SC on exercise performance. Before synthesising the collective findings from this thesis, a summary of the findings from each experimental chapter are offered below:

### 8.1.1 Study 1 (Chapter 4)

The aim of this study was to establish the reproducibility of blood acid-base responses to individualised doses of SC (0.5, 0.4 and 0.3 $g \cdot kg^{-1}$ BM). This represented the first investigation into the potential usability of an individualised approach to SC ingestion (timing). Whilst SC displayed limited reliability for key blood $HCO_3^-$ kinetics such as TTP and absolute change from baseline, several positive findings were highlighted. Firstly, peak [$HCO_3^-$] was comparable, irrespective of ingested dose. Secondly, meaningful and sustained changes in blood [$HCO_3^-$] (and pH) were observed post-ingestion. Thirdly, minimal GIS were experienced, with 8 of 14 participants experiencing zero GIS. Finally, any GIS experienced appeared minimal in terms of severity. While an individualised approach would not appear logical, SC may still have utility when administered using a more generalised/alternative approach.

### 8.1.2 Study 2 (Chapter 5)

The aim of this study was to determine the impact of different forms of SC encapsulation, namely, GEL, DEL and ENT capsules were compared (0.4 $g \cdot kg^{-1}$ BM) on blood acid-base and GIS responses.. Total GIS experienced was shown to be minimal and was not significantly impacted by form of encapsulation. Peak GIS typically emerged at a similar time, arising ≤100 min post-ingestion. Whilst blood [$HCO_3^-$] was significantly higher with ENT vs. GEL, meaningful and prolonged elevations in [$HCO_3^-$] were noted. Whilst minor differences were present, altering the form of encapsulation did not appear to offer substantial benefits relative to other forms.

### 8.1.3 Study 3 (Chapter 6)

The aim of this study was to elucidate the effect of CHO intake on blood acid-base and GIS responses, following individualised SC ingestion. Data suggested that while CHO intake does not appear to improve GIS and alkalotic responses to SC, they were also, not significantly worsened. Total symptoms experienced were unsubstantial throughout, emerging distinctly sperate from peak [$HCO_3^-$]. While absolute change in [$HCO_3^-$] was greater with CHO vs. without, this was not significantly different. Both CHO and NO-CHO conditions elicited meaningful elevations in [$HCO_3^-$]. Taken together, consuming CHO intake alongside SC may support metabolic outcomes, without notable alterations in the blood acid-base and GIS response.

### 8.1.4 Study 4 (Chapter 7)

The aim of this study was to apply the findings of Studies 1-3 (Chapters 4-6) to an exercise task. Using study 1, the limited reliability of blood acid-base responses underpinned the use of a generalised timepoint, supported also by the sustained, meaningful increases in [$HCO_3^-$] within the 4 h sampling period. From study 2, the lack of any beneficial changes in overall blood acid-base responses, minimal changes in [$HCO_3^-$] and limited GIS post-ingestion throughout, guided the sole use of GEL capsules. In study 3, the lack of any (positive or negative) effect of CHO co-ingestion on blood acid-base and GIS responses to SC acted as the basis for the provision of CHO in study 4 also. The potentially additive effects of CHO ingestion on metabolism and real-world nutritional applications were also considered. To further evaluate the potential for an ergogenic period, exercise commencement was manipulated around group time-to-peak [$HCO_3^-$]. Significant increases in [$HCO_3^-$] and pH emerged for off-peak SC ingestion but not on-peak SC, although neither had a significant effect on general TT performance, with limited difference in other performance, physiological and perceptual measures. Future research may investigate the general mechanisms associated with SC ingestion before concluding once again on its ergogenicity.

## 8.2 General Characteristics Of Sodium Citrate Ingestion

### *8.2.1 Ingestion Timing*

In the non-exercise chapters of this thesis (4-6), TTP blood [$HCO_3^-$] ($T_{max}$) and pH were determined following the ingestion of a potentially ergogenic doses of SC (0.4-0.5 $g \cdot kg^{-1}$ BM) (Urwin *et al*., 2021b; Cerullo *et al*., 2020). Table 8.1 displays the collective findings among recreationally trained individuals from these chapters (n = 38).

Recent reviews by both Cerullo *et al*. (2020) and Urwin *et al*. (2021) have recommended that 0.4-0.5 $g \cdot kg^{-1}$ BM SC be ingested ~180 min prior to exercise, based on peak alkalotic responses (blood [$HCO_3^-$] and pH) when ingested in GEL capsules. The current thesis demonstrates overall that peak alkalotic responses may emerge earlier, with a high frequency of individuals reaching maximal alkalosis 136-187 min post ingestion. Importantly, smaller quantities of SC (0.4 $g \cdot kg^{-1}$ BM) and the use of alternative forms of encapsulation offered had limited impact on the blood alkalotic response.

While general trends were observed for ingestion timing, individual TTP blood [$HCO_3^-$] and pH varied considerably throughout each chapter. Where 0.4 $g \cdot kg^{-1}$ BM was provided, both individual TTP blood [$HCO_3^-$] or pH ranged from 85-238 min. Where 0.5 $g \cdot kg^{-1}$ BM SC was ingested generally, individual TTP blood [$HCO_3^-$] or pH ranged from 102-238 min and 68-238 min, respectively. This differs greatly from previous findings utilising an identical quantity of SC, with Urwin *et al*. (2016; 2019) reporting peak [$HCO_3^-$] between 180 to 220 min post-ingestion. While the reasons for these differences are unclear, variations in gastric emptying rate are clear, which may be influenced by the physical state, size of the dosage form and/or the presence of food (Davis, Hardy and Fara, 1986). Furthermore, where interindividual differences exist, intraindividual differences in acid-base balance are also evident day-to-day, related to net endogenous acid production (NEAP) (Poupin *et al*., 2012; Remer 2001; Remer and Manz, 1995).

**Table 8.1.** Frequency of time-to-peak (TTP) blood bicarbonate concentration ([$HCO_3^-$]) and pH at each sample time.

| $HCO_3^-$ | Time (min) | 68 | 85 | 102 | 119 | 136 | 153 | 170 | 187 | 204 | 221 | 238 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Trial | | | | | | | | | | | |
| Study 1 (n = 14) | SC5a | 0 | 0 | 2 | 2 | 2 | 2 | **3** | 2 | 0 | 1 | 0 |
| | SC5b | 0 | 0 | 1 | 1 | **4** | 0 | 2 | 2 | **4** | 0 | 0 |
| | SC4a | 0 | 2 | 2 | 1 | 1 | **3** | 1 | 2 | 1 | 0 | 1 |
| | SC4b | 0 | 0 | 0 | 1 | **4** | 2 | **4** | 3 | 0 | 0 | 0 |
| Study 2 (n = 14) | SC4 | 0 | 2 | 2 | 1 | 1 | **3** | 1 | 2 | 1 | 0 | 1 |
| | SC4 (DEL) | 0 | 0 | 0 | 2 | **4** | 2 | 0 | 1 | 3 | 0 | 2 |
| | SC4 (ENT) | 0 | 0 | 0 | 1 | 1 | 3 | 1 | **4** | 1 | 3 | 0 |
| Study 3 (n = 10) | SC5 meal | 0 | 0 | 0 | 0 | 0 | **2** | 1 | **2** | 1 | **2** | **2** |
| | SC5 no meal | 0 | 0 | 0 | 1 | 2 | 3 | 2 | 0 | 0 | 1 | 1 |
| Pooled (n = 38) | SC5 | 0 | 0 | 3 | 4 | **8** | 7 | **8** | 6 | 5 | 4 | 3 |
| | SC4 | 0 | 4 | 4 | 6 | 11 | **13** | 7 | 12 | 6 | 3 | 4 |
| | Overall | 0 | 4 | 7 | 10 | 19 | **20** | 15 | 18 | 11 | 7 | 7 |
| pH | | | | | | | | | | | | |
| Study 1 (n = 14) | SC5a | 1 | 1 | 1 | 2 | **3** | 2 | 2 | 0 | 0 | 1 | 1 |
| | SC5b | 0 | 1 | 0 | 1 | 2 | 1 | 1 | 2 | 1 | 1 | **4** |
| | SC4a | 0 | 1 | 0 | 1 | 2 | 2 | 0 | 3 | **5** | 0 | 0 |
| | SC4b | 0 | 1 | 2 | 1 | **5** | 0 | 1 | 4 | 0 | 0 | 0 |
| Study 2 (n = 14) | SC4 | 0 | 1 | 0 | 1 | 2 | 2 | 0 | 3 | **5** | 0 | 0 |
| | SC4 (DEL) | 0 | 0 | 1 | 2 | 2 | **5** | 1 | 1 | 0 | 2 | 0 |
| | SC4 (ENT) | 0 | 0 | 1 | 2 | 2 | 2 | 0 | 1 | 1 | 1 | **4** |
| Study 3 (n = 10) | SC5 meal | 0 | 0 | 0 | **3** | 1 | 0 | 2 | 1 | 0 | 1 | 0 |
| | SC5 no meal | 0 | 1 | 0 | 2 | 2 | 1 | 0 | 0 | 0 | **3** | 0 |
| Pooled (n = 38) | SC5 | 1 | 3 | 1 | **8** | **21** | 4 | 5 | 3 | 1 | 6 | 5 |
| | SC4 | 0 | 3 | 4 | 7 | **13** | 11 | 2 | 12 | 11 | 3 | 4 |
| | Overall | 1 | 6 | 5 | 16 | **26** | 15 | 7 | 15 | 12 | 9 | 9 |

**Notes:** *SC5 = 0.5 g·kg$^{-1}$ BM sodium citrate (SC). SC4 = 0.4 g·kg$^{-1}$ BM SC. DEL = Delayed-release capsules. ENT = Enterically-coated capsules. Where capsule type is not noted, gelatine (GEL) was used. CHO = carbohydrate (1.75 g·kg$^{-1}$ BM). The most frequent results are highlighted in bold. Some timepoints are omitted for clarity.*

### *8.2.2 Absolute Change In Bicarbonate Concentration*

Augmented extracellular buffering capacity is contingent on an increase in circulating [$HCO_3^-$] (Heibel *et al*., 2018) however, the value of a maximal vs. meaningful rise has been debated (de Oliveira *et al*., 2021; 2020). Although the minimal change necessary to translate increased buffering capacity to performance improvements is unclear, evidence suggests greater effects are demonstrated when

increases are moderate (4-6 $mmol \cdot L^{-1}$) to large (> 6 $mmol \cdot L^{-1}$). Table 8.2 collates the absolute change ($\Delta C_{max}$) from baseline observed for recreationally trained individuals (n = 38) at potentially ergogenic doses of 0.4-0.5 $g \cdot kg^{-1}$ BM in the non-exercise chapters (4-6). Only two studies to date have investigated the blood $HCO_3^-$ response following the ingestion of 0.5 $g \cdot kg^{-1}$ BM SC in GEL capsule form (Urwin *et al*. 2019; 2021a), recording $\Delta C_{max}$ values of 7.9 (7.2-8.6) and 7.8 (6.8-8.7) $mmol \cdot L^{-1}$, respectively. While a large period of potential ergogenicity is demonstrated, with average $\Delta C_{max}$ shown to be 4+ $mmol \cdot L^{-1}$ above resting values from 102 min, for the remaining ~3 h, $\Delta C_{max}$ appeared less than previously reported (Urwin *et al*., 2019; 2021a). In chapter 7, it was observed that commencing exercise within this period did not translate to performance effects. For the athlete and performance nutritionist, it may be suggested that 0.5 $g \cdot kg^{-1}$ BM SC, provided in GEL capsules, can be ingested to produce substantial $\Delta C_{max}$ (with minimal GIS) however, it remains unclear if this can be translated into reproducible performance effects. Further work is required to understand why $\Delta C_{max}$ values observed here, representing an apparently 'meaningful' increase in circulating [$HCO_3^-$], did/do not bring about consistent performance benefits. Greater mechanistic insight into SC ingestion represents a logical starting point to address this question.

**Table 8.2.** Average (and range) absolute change ($\Delta C_{max}$) in bicarbonate concentration ($[HCO_3^-]$) ($mmol \cdot L^{-1}$) from baseline at each sample time.

| | Time (min) | 17 | 34 | 51 | 68 | 85 | 102 | 119 | 136 | 153 | 170 | 187 | 204 | 221 | 238 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Trial | | | | | | | | | | | | | | |
| Study 1 ($n$ = 14) | *SC5a* | 0.7 (0.0-1.5) | 1.9 (0.8-3.5) | 2.8 (1.2-3.8) | 3.5 (1.3-5.2) | 3.9 (1.1-5.9) | 4.7 (1.1-6.2) | 5.0 (1.5-6.8) | 5.1 (0.9-7.3) | 5.2 (0.6-8.2) | **5.3 (0.0-8.1)** | 5.0 (0.0-8.3) | 5.1 (0.7-6.9) | 5.1 (0.7-6.9) | 4.5 (0.0-6.8) |
| | *SC5b* | 1.0 (0.0-2.9) | 2.2 (0.4-3.3) | 3.2 (1.5-5.1) | 3.8 (1.4-5.6) | 4.2 (1.7-6.3) | 4.9 (1.3-7.0) | 5.2 (2.5-7.3) | 5.6 (1.7-8.2) | 5.4 (2.0-7.7) | **5.8 (2.1-8.1)** | 5.7 (2.8-8.3) | **5.8 (2.9-8.3)** | 5.2 (1.6-7.3) | 5.2 (1.7-7.0) |
| | *SC4a* | 0.4 (0.0-1.3) | 2.1 (0.6-3.5) | 3.1 (1.5-5.1) | 3.9 (2.7-5.3) | 4.7 (2.6-6.7) | 4.9 (3.8-7.1) | 5.3 (2.7-8.6) | **5.6 (4.0-8.6)** | 0.3 (0.0-1.4) | 5.2 (2.5-7.2) | **5.6 (3.7-7.5)** | 5.5 (4.3-7.3) | 5.1 (3.2-7.2) | 5.3 (3.8-6.7) |
| | *SC4b* | 0.4 (0.0-1.6) | 1.7 (0.1-2.8) | 2.8 (2.1-3.4) | 3.5 (2.1-4.2) | 0.6 (0.0-0.9) | 4.8 (3.1-5.8) | 5.0 (2.7-7.0) | 5.4 (4.4-6.2) | 5.2 (3.5-6.2) | **5.5 (3.9-7.1)** | 5.4 (3.6-7.4) | 4.9 (3.8-6.8) | 4.6 (3.2-7.1) | 4.8 (3.3-6.0) |
| Study 2 ($n$ = 14) | *SC4* | 0.4 (0.0-1.3) | 1.9 (0.7-2.6) | 2.9 (2.2-4.1) | 3.7 (2.7-4.4) | 4.4 (2.6-5.6) | 4.9 (3.8-6.0) | 5.2 (2.7-7.1) | **5.5 (4.5-7.1)** | 5.4 (3.5-6.7) | 5.4 (3.7-7.0) | **5.5 (3.7-7.0)** | 5.2 (4.3-6.7) | 4.8 (3.2-6.4) | 5.0 (3.8-6.2) |
| | *SC4 (DEL)* | 0.0 (0.0-0.7) | 1.0 (0.0-2.3) | 1.9 (1.0-2.7) | 2.6 (1.7-3.7) | 2.9 (1.0-4.1) | 3.7 (1.7-5.2) | 4.3 (1.4-6.2) | **4.8 (2.5-6.4)** | **4.8 (2.7-6.5)** | 4.6 (2.6-6.6) | 4.2 (2.6-6.7) | 4.5 (2.6-6.7) | 4.4 (2.4-6.5) | 4.4 (2.8-6.2) |
| | *SC4 (ENT)* | 0.4 (0.0-1.1) | 1.6 (0.3-3.0) | 2.8 (1.2-4.1) | 3.3 (1.8-4.3) | 4.0 (2.5-5.4) | 4.5 (1.9-6.2) | 5.1 (3.1-6.4) | 5.3 (3.2-7.4) | 5.8 (4.3-7.1) | 5.6 (4.4-7.7) | 5.8 (4.8-7.8) | **5.9 (4.7-7.5)** | 5.7 (4.2-7.1) | 5.4 (3.7-6.8) |
| Study 3 ($n$ = 10) | *SC5 (meal)* | 0.7 (0.0-2.9) | 1.1 (0.0-3.7) | 2.4 (0.7-5.2) | 3.1 (1.8-5.9) | 3.7 (2.3-6.4) | 4.4 (2.8-6.8) | 4.8 (2.4-6.8) | 5.7 (4.0-7.3) | 6.0 (3.4-8.1) | 6.5 (4.2-8.6) | **6.8 (3.9-8.3)** | **6.8 (5.2-8.5)** | 6.7 (4.7-8.3) | **6.8 (4.9-9.0)** |
| | *SC5 (no meal)* | 0.6 (0.0-1.5) | 1.7 (0.6-2.8) | 2.3 (0.3-4.3) | 3.3 (1.3-5.1) | 3.8 (1.3-5.3) | 4.4 (1.5-6.3) | 4.4 (1.8-6.2) | 5.4 (1.3-9.1) | **5.6 (1.3-8.5)** | 5.5 (1.4-8.3) | 5.3 (1.5-8.1) | 5.2 (0.9-8.0) | 5.2 (0.7-8.3) | 5.1 (1.0-7.7) |
| Pooled ($n$ = 38) | *SC5* | 0.8 (0.0-2.9) | 1.7 (0.0-3.7) | 2.7 (0.3-5.2) | 3.4 (1.3-6.4) | 3.9 (1.1-6.4) | 4.6 (1.1-7.0) | 4.9 (1.5-7.3) | 5.5 (0.9-9.1) | 5.6 (0.6-8.5) | **5.8 (0.0-8.1)** | 5.7 (0.0-8.3) | 5.7 (0.7-8.5) | 5.6 (0.7-8.3) | 5.4 (0.0-9.0) |
| | *SC4* | 0.3 (0.0-1.6) | 1.7 (0.0-3.5) | 2.7 (1.0-5.1) | 3.4 (1.7-5.3) | 3.3 (0.0-6.7) | 4.6 (1.7-7.1) | 5.0 (2.7-8.6) | **5.3 (2.5-8.6)** | 4.3 (0.0-7.1) | **5.3 (0.0-7.1)** | **5.3 (2.6-7.8)** | 5.2 (2.6-7.8) | 4.9 (2.4-7.2) | 5.0 (3.3-6.8) |
| | *Overall* | 0.5 (0.0-2.9) | 1.7 (0.0-3.7) | 2.7 (0.3-5.2) | 3.4 (1.3-6.4) | 3.6 (0.0-6.7) | 4.6 (1.1-7.1) | 4.9 (1.5-8.6) | 5.4 (0.9-9.1) | 4.9 (0.0-8.5) | **5.5 (0.0-8.1)** | **5.5 (0.0-8.3)** | **5.5 (0.7-8.5)** | 5.2 (0.7-8.3) | 5.2 (0.0-9.0) |

**Notes:** *SC5 = 0.5 $g \cdot kg^{-1}$ BM sodium citrate (SC). SC4 = 0.4 $g \cdot kg^{-1}$ BM SC. DEL = Delayed-release capsules. ENT = Enterically-coated capsules. Where capsule type is not noted, gelatine (GEL) was used. CHO = carbohydrate (1.75 $g \cdot kg^{-1}$ BM). Maximal values are highlighted in bold. Where negative values are noted , zero (0) is written.*

### *8.2.3 Gastrointestinal Symptoms*

A common side-effect of ingesting endogenous buffering agents is the emergence of acute GIS (de Oliveira *et al*., 2021) and, while it has been suggested that GIS may be reduced with SC ingestion (Requena *et al*., 2005), recent findings have questioned this proposition (Urwin *et al*., 2022). Although GIS experienced varies between individuals, nausea, flatulence, stomach cramp, belching, stomach ache, bowel urgency, diarrhoea, vomiting and stomach bloating are commonly reported amongst athletes (Urwin *et al*., 2016; Urwin *et al*., 2019). In chapter 4, 57% of recreationally trained individuals reported zero GIS at any time following the ingestion of 0.3, 0.4 or 0.5 $g \cdot kg^{-1}$ BM SC. In chapter 5, 43% of recreationally trained individuals reported zero GIS following the ingestion of 0.4 $g \cdot kg^{-1}$ BM SC in GEL, DEL and ENT form. In those that did experience GIS, relatively minimal total symptoms were noted, with only one instance of any GIS at the point of peak [$HCO_3^-$] ($T_{max}$). This represents a lower prevalence than has been reported in in some SB research (85-100%) (Hilton *et al*., 2019; 2020a), but greater than demonstrated in others (22-38%) (McNaughton, Siegler and Midgley, 2008; Driller *et al*., 2012). Variations between studies may relate to specific characteristics of SC v. SB or a variety of other factors, such as co-ingestion with a small CHO meal ($1 \cdot 75$ $g \cdot kg^{-1}$ BM), or ingestion with a large volume of water (14 $ml \cdot kg^{-1}$ BM) (Carr *et al*., 2011a; 2011b). In chapter 6 of the current thesis, CHO was shown to have limited impact on acute GIS, while in all experimental chapters (4-7), encapsulation appeared to limit general GIS without comparison to solution. Different methodological approaches may also underpin variance in GIS between studies, with studies opting to use different questionnaires and/or criteria to identify symptoms. In continuation, some studies have quantified the time course of GIS while others have established symptoms at discrete timepoints, rendering comparison difficult. Longer sampling periods may also increase the incidence of GIS given that side effects may emerge up to 24 h post-ingestion (Cameron *et al*., 2010). Symptoms may also be greater when endogenous buffering agents are ingested over shorter (≤ 30 min) rather than longer (30-60 min) time periods (Hilton *et al*., 2020a). Despite this, Urwin *et al*. (2021a) found minimal GIS when SC was ingested over 15-, 30-, 45- or 60-min. Future research should aim to establish a more standardised method of quantifying GIS both in time-course and exercise-type studies, permitting comparison and allowing the

identification of common characteristics. There also remains a need for investigations that establish the potential relationship between perception of GIS or 'concern' and willingness to continue, given that GIS discourages performance in some athletes but not in others. Considering that exercise independently compromises GI integrity through a variety of aetiological and pathophysiological changes also (Gaskell, Rauch and Costa, 2021a; 2021b; Costa *et al*. 2020; 2017), there is a need to better understand how these changes are impacted with the ingestion of buffering agents. The relationship between GIS scores, perception of GIS, willingness to continue and/or performance should again be considered during exercise.

**REFERENCES**

ABELOW, B., 1998. *Understanding acid-base*. United States: Lippincott Williams and Wilkins.

AEDMA, M., TIMPMANN, S. and ÖÖPIK, V., 2015. Dietary sodium citrate supplementation does not improve upper-body anaerobic performance in trained wrestlers in simulated competition-day conditions. *European journal of applied physiology*, *115*(2), pp.387-396

AGUILERA-TEJERO, E., ESTEPA, J.C., LÓPEZ, I., BAS, S., MAYER-VALOR, R. and RODRIGUEZ, M., 2000. Quantitative analysis of acid–base balance in show jumpers before and after exercise. *Research in veterinary science*, *68*(2), pp.103-108.

ALLEN, D.G., LAMB, G.D. and WESTERBLAD, H., 2008. Skeletal muscle fatigue: cellular mechanisms. *Physiological reviews*, *88*(1), pp.287-332.

ALLEN, D.G., WESTERBLAD, H. and LÄNNERGREN, J., 1995. The role of intracellular acidosis in muscle fatigue. In *Fatigue* (pp. 57-68). Springer, Boston, MA.

AMANN, M., BLAIN, G.M., PROCTOR, L.T., SEBRANEK, J.J., PEGELOW, D.F. and DEMPSEY, J.A., 2011. Implications of group III and IV muscle afferents for high-intensity endurance exercise performance in humans. *The Journal of physiology*, *589*(21), pp.5299-5309.

AMANN, M. and DEMPSEY, J.A., 2008. Locomotor muscle fatigue modifies central motor drive in healthy humans and imposes a limitation to exercise performance. *The Journal of physiology*, *586*(1), pp.161-173.

AMANN, M., ELDRIDGE, M.W., LOVERING, A.T., STICKLAND, M.K., PEGELOW, D.F. and DEMPSEY, J.A., 2006. Arterial oxygenation influences central motor output and exercise

performance via effects on peripheral locomotor muscle fatigue in humans. *The Journal of physiology*, *575*(3), pp.937-952.

AMANN, M., PROCTOR, L.T., SEBRANEK, J.J., PEGELOW, D.F. and DEMPSEY, J.A., 2009. Opioid-mediated muscle afferents inhibit central motor drive and limit peripheral muscle fatigue development in humans. *The Journal of physiology*, *587*(1), pp.271-283.

AMANN, M., VENTURELLI, M., IVES, S.J., MCDANIEL, J., LAYEC, G., ROSSMAN, M.J. and RICHARDSON, R.S., 2013. Peripheral fatigue limits endurance exercise via a sensory feedback-mediated reduction in spinal motoneuronal output. *Journal of applied physiology*, *115*(3), pp.355-364.

ANSLEY, L., LAMBERT, M.I., SCHARBORT, E., ST CLAIR GIBSON, A. and NOAKES, T., 2004. Regulation of pacing strategies during successive 4-km time trials. *Medicine & Science in Sports & Exercise*, *36*(10), pp.1819-1825.

ARNAOUTIS, G., 2022. The role of hydration in health, disease and performance. *Public Health Toxicology*, *2*(Supplement 1).

ASTORINO, T.A. and COTTRELL, T., 2012. Reliability and validity of the velotron racermate cycle ergometer to measure anaerobic power. *International journal of sports medicine*, *33*(03), pp.205-210.

ATHERTON, J.C., 2003. Acid-base balance: maintenance of plasma pH. *Anaesthesia & Intensive Care Medicine*, *4*(12), pp.419-422.

ATHERTON, J.C., 2009. Acid–base balance: maintenance of plasma pH. *Anaesthesia & Intensive Care Medicine*, *10*(11), pp.557-561.

ATKINSON, G. and NEVILL, A.M., 1998. Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. *Sports Medicine*. 26 (4), pp. 217-38.

AYALA, V., MARTÍNEZ-BEBIA, M., LATORRE, J.A., GIMENEZ-BLASI, N., JIMENEZ-CASQUET, M.J., CONDE-PIPO, J., BACH-FAIG, A. and MARISCAL-ARCAS, M., 2021. Influence of circadian rhythms on sports performance. *Chronobiology International*, *38*(11), pp.1522-1536.

BAINBRIDGE, F.A., 1931. The physiology of muscular exercise. *London.*

BAKER, J.S., MCCORMICK, M.C. and ROBERGS, R.A., 2010. Interaction among skeletal muscle metabolic energy systems during intense exercise. *Journal of nutrition and metabolism*, *2010*.

BAGUET, A., REYNGOUDT, H., POTTIER, A., EVERAERT, I., CALLENS, S., ACHTEN, E. and DERAVE, W., 2009. Carnosine loading and washout in human skeletal muscles. *Journal of applied physiology*, *106*(3), pp.837-842.

BAHREINI, M., JALILI, M. and MORADI-LAKEH, M., 2015. A comparison of three self-report pain scales in adults with acute pain. *The Journal of emergency medicine*, *48*(1), pp.10-18.

BANGSBO, J., 1996. Regulation of muscle glycogenolysis and glycolysis during intense exercise: In vivo studies using repeated intense exercise. *Biochemistry of Exercise IX*, pp.261-275.

BANGSBO, J., MICHALSIK, L. and PETERSEN, A., 1993. Accumulated O2 deficit during intense exercise and muscle characteristics of elite athletes. *International journal of sports medicine*, *14*(04), pp.207-213.

BARBOSA, J.A., CONWAY, B.R. and MERCHANT, H.A., 2017. Going natural: using polymers from nature for gastroresistant applications. *British Journal of Pharmacy*, *2*(1).

BASSETT, D.R. and HOWLEY, E.T., 2000. Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Medicine and science in sports and exercise*, *32*(1), pp.70-84.

BEN-ZAKEN, S., ELIAKIM, A., NEMET, D., RABINOVICH, M., KASSEM, E. and MECKEL, Y., 2015. Differences in MCT 1 A 1470 T polymorphism prevalence between runners and swimmers. *Scandinavian journal of medicine & science in sports*, *25*(3), pp.365-371.

BERGMAN, B.C., HORNING, M.A., CASAZZA, G.A., WOLFEL, E.E., BUTTERFIELD, G.E. and BROOKS, G.A., 2000. Endurance training increases gluconeogenesis during rest and exercise in men. *American Journal of Physiology-Endocrinology And Metabolism*, *278*(2), pp.E244-E251.

BERGMAN, B.C., WOLFEL, E.E., BUTTERFIELD, G.E., LOPASCHUK, G.D., CASAZZA, G.A., HORNING, M.A. and BROOKS, G.A., 1999. Active muscle and whole body lactate kinetics after endurance training in men. *Journal of applied physiology*, *87*(5), pp.1684-1696.

BIRD, S.R., WILES, J. and ROBBINS, J., 1995. The effect of sodium bicarbonate ingestion on 1500-m racing time. *Journal of sports sciences*, *13*(5), pp.399-403.

BLAND, J.M. and ALTMAN, D.G., 2003. Applying the right statistics: analyses of measurement studies. *Ultrasound in Obstetrics and Gynecology*. 22 (1), pp. 85–93.

BISHOP, D., EDGE, J., DAVIS, C., and GOODMAN, C., 2004. Induced metabolic alkalosis affects muscle metabolism and repeated-sprint ability. *Medicine and Science in Sports and Exercise*. 36 (5), pp. 807–13.

BOEGMAN, S., STELLINGWERFF, T., SHAW, G., CLARKE, N., GRAHAM, K., CROSS, R. and SIEGLER, J.C., 2020. The impact of individualizing sodium bicarbonate supplementation strategies on world-class rowing performance. *Frontiers in Nutrition*, *7*, p.138.

BOEKAMA, J., SAMSON, M., VAN BERGE HENEGOUWEN, G.P. and SMOUT A.J.P.M., 1999 Coffee and gastrointestinal function: facts and fiction: a review. Scandinavian Journal *Gastroenterology*. 34(230).

BOGDANIS, G.C., NEVILL, M.E., LAKOMY, H.K.A. and BOOBIS, L.H., 1998. Power output and muscle metabolism during and following recovery from 10 and 20 s of maximal sprint exercise in humans. *Acta Physiologica Scandinavica*, *163*(3), pp.261-272.

BOLLAG, W.B., 2011. Regulation of aldosterone synthesis and secretion. *Comprehensive physiology*, *4*(3), pp.1017-1055.

BOLHUIS, D.P., LAKEMOND, C.M., DE WIJK, R.A., LUNING, P.A. and DE GRAAF, C., 2011. Both longer oral sensory exposure to and higher intensity of saltiness decrease ad libitum food intake in healthy normal-weight men. *The Journal of nutrition*, *141*(12), pp.2242-2248.

BOLHUIS, D.P., LAKEMOND, C.M., DE WIJK, R.A., LUNING, P.A. and DE GRAAF, C., 2012. Effect of salt intensity in soup on ad libitum intake and on subsequent food choice. *Appetite*, *58*(1), pp.48-55.

BONAVENTURA, J.M., SHARPE, K., KNIGHT, E., FULLER, K.L., TANNER, R.K. and GORE, C.J., 2015. Reliability and accuracy of six hand-held blood lactate analysers. *Journal of sports science & medicine*, *14*(1), p.203.

BÖNING, D., STROBEL, G., BENEKE, R. and MAASSEN, N., 2005. Lactic acid still remains the real cause of exercise-induced metabolic acidosis. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *289*(3), pp. 902-903.

BORG, G., 1970. Perceived exertion as an indicator of somatic stress. *Scandinavian Journal of Rehabilitative Medicine*. 2(2), pp. 92-98.

BORG, G.A., 1982. Psychophysical bases of perceived exertion. *Medicine science sports exercise*, *14*(5), pp.377-381.

BORG, G., LJUNGGREN, G. and CECI, R., 1985. The increase of perceived exertion, aches and pain in the legs, heart rate and blood lactate during exercise on a bicycle ergometer. *European journal of applied physiology and occupational physiology*, *54*(4), pp.343-349.

BOUISSOU, P., DEFER, G., GUEZENNEC, C.Y., ESTRADE, P.Y. and SERRURIER, B., 1988. Metabolic and blood catecholamine responses to exercise during alkalosis. *Medicine and Science in Sports and Exercise*, *20*(3), pp.228-232.

BRACKEN, R.M., LINNANE, D.M. and BROOKS, S., 2005. Alkalosis and the plasma catecholamine response to high-intensity exercise in man. *Medicine & Science in Sports & Exercise*, *37*(2), pp.227-233.

BROOKS, G.A., 2009. Cell–cell and intracellular lactate shuttles. *The Journal of physiology*, *587*(23), pp.5591-5600.

BROOKS, G.A., 2020. Lactate as a fulcrum of metabolism. *Redox biology*, *35*, p.101454.

BROOKS, G.A., 2002. Lactate shuttles in nature. *Biochemical Society Transactions*, *30*(2), pp.258-264.

BRUNO, C.M. and VALENTI, M., 2012. Acid-base disorders in patients with chronic obstructive pulmonary disease: a pathophysiological review. *BioMed Research International*, *2012*.

BRYNER, R.W., HORNSBY, W.G., CHETLIN, R., ULLRICH, I.H. and YEATER, R.A., 1998. Effect of lactate consumption on exercise performance. *The Journal of sports medicine and physical fitness*, *38*(2), pp.116-123.

BURKE, L.M., 2017. Practical issues in evidence-based use of performance supplements: supplement interactions, repeated use and individual responses. *Sports Medicine*. 47 (1), pp. 79–100.

BURKE, L.M., HAWLEY, J.A., WONG, S.H. and JEUKENDRUP, A.E., 2011. Carbohydrates for training and competition. *Journal of sports sciences*, *29*(sup1), pp.S17-S27.

BURKE, L.M., KIENS, B. and IVY, J.L., 2004. Carbohydrates and fat for training and recovery. *Food, Nutrition and Sports Performance II*, pp.24-49.

BURNLEY, M., VANHATALO, A. and JONES, A.M., 2012. Distinct profiles of neuromuscular fatigue during muscle contractions below and above the critical torque in humans. *Journal of applied physiology*, *113*(2), pp.215-223.

BUSA, W.B. and NUCCITELLI, R., 1984. Metabolic regulation via intracellular pH. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *246*(4), pp. 409-438.

CAIRNS, S.P., 2006. Lactic acid and exercise performance. *Sports medicine*, *36*(4), pp.279-291.

CADY, E.B., JONES, D.A., LYNN, J. and NEWHAM, D.J., 1989. Changes in force and intracellular metabolites during fatigue of human skeletal muscle. *The Journal of Physiology*, *418*(1), pp.311-325.

CALLAHAN, M.J., PARR, E.B., HAWLEY, J.A. and BURKE, L.M., 2017. Single and combined effects of beetroot crystals and sodium bicarbonate on 4-km cycling time trial performance. *International journal of sport nutrition and exercise metabolism*, *27*(3), pp.271-278

CAMERON, S.L., MCLAY-COOKE, R.T., BROWN, R.C., GRAY, A.R. and FAIRBAIRN, K.A., 2010. Increased blood pH but not performance with sodium bicarbonate supplementation in elite rugby union players. *International journal of sport nutrition and exercise metabolism*, *20*(4), pp.307-321.

CARR, A.J., HOPKINS, W.G. and GORE, C.J., 2011a. Effects of acute alkalosis and acidosis on performance. *Sports medicine*, *41*(10), pp.801-814.

CARR, A.J., SLATER, G.J., GORE, C.J., DAWSON, B. and BURKE, L.M., 2011b. Effect of sodium bicarbonate on [$HCO_3^-$], pH, and gastrointestinal symptoms. *International journal of sport nutrition and exercise metabolism*, *21*(3), pp.189-194.

CARR, A.J., SLATER, G.J., GORE, C.J., DAWSON, B. and BURKE, L.M., 2012. Reliability and effect of sodium bicarbonate: buffering and 2000-m rowing performance. *International Journal of Sports Physiology and Performance*, *7*(2), pp.152-160.

CERULLO, G., PARIMBELLI, M., PERNA, S., PECORARO, M., LIGUORI, G., NEGRO, M. and D'ANTONA, G., 2020. Sodium citrate supplementation: An updated revision and practical

recommendations on exercise performance, hydration status, and potential risks. *Translational Sports Medicine*, *3*(6), pp.518-525.

CHAMARI, K. and PADULO, J., 2015. 'Aerobic' and 'Anaerobic' terms used in exercise physiology: a critical terminology reflection. *Sports medicine-open*, *1*(1), pp.1-4.

CHEN JR, Y., MAHIEU, N.G., HUANG, X., SINGH, M., CRAWFORD, P.A., JOHNSON, S.L., GROSS, R.W., SCHAEFER, J. and PATTI, G.J., 2016. Lactate metabolism is associated with mammalian mitochondria. *Nature chemical biology*, *12*(11), pp.937-943.

CHRISTENSEN, P.M., SHIRAI, Y., RITZ, C., and NORDSBORG, N.B., 2017. Caffeine and bicarbonate for speed. a meta-analysis of legal supplements potential for improving intense endurance exercise performance. *Frontiers in Physiology*. 8, p. 240.

CICCHETTI, D. V. and CICCHETTI, D. V., 1994. Guidelines, criteria and rules of thumbs for evaluating normed and standardized assessment instruments in psychology. *Psychological Assessment*. pp. 284--290.

CLAUSEN, T., 2003. Na+-K+ pump regulation and skeletal muscle contractility. *Physiological reviews*. 83(1), pp. 1269-1324.

CLOSE, G.L., HAMILTON, D.L., PHILP, A., BURKE, L.M. and MORTON, J.P., 2016. New Strategies in Sport Nutrition to Increase Exercise Performance. *Free Radical Biology and Medicine*, *98*, pp.144-158.

COHEN, J., 1988. *Statistical Power Analysis for the Behavioural Sciences*. L. Erlbaum Associates. 101.

CONSTABLE, P.D., 1997. A simplified strong ion model for acid-base equilibria: application to horse plasma. *Journal of Applied Physiology*, *83*(1), pp.297-311.

CORREIA-OLIVEIRA, C.R., LOPES-SILVA, J.P., BERTUZZI, R., MCCONELL, G.K., BISHOP, D.J., LIMA-SILVA, A.E. and KISS, M.A.P.D.M., 2017. Acidosis, but not alkalosis, affects anaerobic metabolism and performance in a 4-km time trial. *Medicine & Science in Sports & Exercise*, *49*(9), pp.1899-1910.

COSTA, R.J., GASKELL, S.K., MCCUBBIN, A.J. and SNIPE, R.M., 2020. Exertional-heat stress-associated gastrointestinal perturbations during Olympic sports: Management strategies for athletes preparing and competing in the 2020 Tokyo Olympic Games. *Temperature*, *7*(1), pp.58-88.

COSTA, R.J.S., SNIPE, R.M.J., KITIC, C.M. and GIBSON, P.R., 2017. Systematic review: exercise-induced gastrointestinal syndrome—implications for health and intestinal disease. *Alimentary pharmacology & therapeutics*, *46*(3), pp.246-265.

COSTILL, D.L., DANIELS, J., EVANS, W., FINK, W., KRAHENBUHL, G. and SALTIN, B., 1976a. Skeletal muscle enzymes and fiber composition in male and female track athletes. *Journal of applied physiology*, *40*(2), pp.149-154.

COSTILL, D.L., FINK, W.J. and POLLOCK, M.L., 1976b. Muscle fiber composition and enzyme activities of elite distance runners. *Medicine and science in sports*, *8*(2), pp.96-100.

COSTILL, D.L., VERSTAPPEN, F., KUIPERS, H., JANSSEN, E. and FINK, W., 1984. Acid-base balance during repeated bouts of exercise: influence of HCO3. *International journal of sports medicine*, *5*(05), pp.228-231.

COX, G. and JENKINS, D.G., 1994. The physiological and ventilatory responses to repeated 60 s sprints following sodium citrate ingestion. *Journal of sports sciences*, *12*(5), pp.469-475.

CROTTY, N.M., BOLAND, M., MAHONY, N., DONNE, B. and FLEMING, N., 2021. Reliability and validity of the Lactate Pro 2 Analyzer. *Measurement in Physical Education and Exercise Science*, *25*(3), pp.202-211.

CUNHA, V.C., AOKI, M.S., ZOURDOS, M.C., GOMES, R.V., BARBOSA, W.P., MASSA, M., MOREIRA, A. and CAPITANI, C.D., 2019. Sodium citrate supplementation enhances tennis skill performance: a crossover, placebo-controlled, double-blind study. *Journal of the International Society of Sports Nutrition*, *16*(1), p.32.

CUPEIRO, R., GONZÁLEZ-LAMUÑO, D., AMIGO, T., PEINADO, A.B., RUIZ, J.R., ORTEGA, F.B. and BENITO, P.J., 2012. Influence of the MCT1-T1470A polymorphism (rs1049434) on blood lactate accumulation during different circuit weight trainings in men and women. *Journal of science and medicine in sport*, *15*(6), pp.541-547.

CUPEIRO, R., BENITO, P.J., MAFFULLI, N., CALDERÓN, F.J. and GONZÁLEZ-LAMUÑO, D., 2010. MCT1 genetic polymorphism influence in high intensity circuit training: a pilot study. *Journal of science and medicine in sport*, *13*(5), pp.526-530.

CURRELL, K. and JEUKENDRUP, A.E., 2008. Validity, reliability and sensitivity of measures of sporting performance. *Sports medicine*, *38*(4), pp.297-316.

DAVIS, S.S., HARDY, J.G. and FARA, J.W., 1986. Transit of pharmaceutical dosage forms through the small intestine. *Gut*, *27*(8), pp.886-892.

DAVIS, S.S., STOCKWELL, A.F., TAYLOR, M.J., HARDY, J.G., WHALLEY, D.R., WILSON, C.G., BECHGAARD, H. and CHRISTENSEN, F.N., 1986. The effect of density on the gastric emptying of single-and multiple-unit dosage forms. *Pharmaceutical research*, *3*(4), pp.208-213.

DEB, S.K., GOUGH, L.A., SPARKS, S.A. and MCNAUGHTON, L.R., 2018. Sodium bicarbonate supplementation improves severe-intensity intermittent exercise under moderate acute hypoxic conditions. *European journal of applied physiology*, *118*(3), pp.607-615.

DEBOLD, E.P., FITTS, R.H., SUNDBERG, C. and NOSEK, T.M., 2016. Muscle fatigue from the perspective of a single crossbridge. *Medicine & Science in Sports & Exercise*.

DE MORAIS, H.A. and CONSTABLE, P.D., 2006. Strong ion approach to acid-base disorders. *Fluid, Electrolyte and Acid-Base Disorders in Small Animal Practice*, 3, pp.310-321.

DEMURA, S. and NAGASAWA, Y., 2003. Relations between perceptual and physiological response during incremental exercise followed by an extended bout of submaximal exercise on a cycle ergometer. *Perceptual and motor skills*, *96*(2), pp.653-663.

DENNIS, S.C., GEVERS, W. and OPIE, L.H., 1991. Protons in ischemia: where do they come from; where do they go to?. *Journal of molecular and cellular cardiology*, *23*(9), pp.1077-1086.

DEVLIN, T.M, 1997. Textbook of biochemistry with clinical correlations. John Wiley & Sons, New York, N.Y.

DEVLIN, T.M. ed., 2011. Textbook of biochemistry: with clinical correlations.

EMHOFF, C.A.W., MESSONNIER, L.A., HORNING, M.A., FATTOR, J.A., CARLSON, T.J. and BROOKS, G.A., 2013. Gluconeogenesis and hepatic glycogenolysis during exercise at the lactate threshold. *Journal of Applied Physiology*, *114*(3), pp.297-306.

FROIO DE ARAUJO DIAS, G., DA EIRA SILVA, V., DE SALLES PAINELLI, V., SALE, C., GIANNINI ARTIOLI, G., GUALANO, B. and SAUNDERS, B., 2015. (In) consistencies in responses to sodium bicarbonate supplementation: a randomised, repeated measures, counterbalanced and double-blind study. *PloS one*, *10*(11), p.e0143086.

DRILLER, M.W., 2012. *Enhancing athletic performance through high-intensity interval training and sodium bicarbonate supplementation* (Doctoral dissertation, University of Tasmania).

DRUST, B., WATERHOUSE, J., ATKINSON, G., EDWARDS, B., and REILLY, T., 2005. Circadian rhythms in sports performance--an update. *Chronobiology international*. 22 (1), pp. 21–44.

DUHAMEL, T.A., GREEN, H.J., SANDIFORD, S.D., PERCO, J.G. and OUYANG, J., 2004. Effects of progressive exercise and hypoxia on human muscle sarcoplasmic reticulum function. *Journal of Applied Physiology*, *97*(1), pp.188-196.

EDGE, J., BISHOP, D. and GOODMAN, C., 2006. The effects of training intensity on muscle buffer capacity in females. *European journal of applied physiology*, *96*(1), pp.97-105.

EDWARDS, R.H.T., 1981. Human muscle function and fatigue. *Human muscle fatigue: physiological mechanisms*, *82*, pp.1-18.

EDWARDS, R.H.T., 1975. Muscle fatigue. *Postgraduate Medical Journal*, *51*(593), pp.137-143.

EGGER, F., MEYER, T., SUCH, U. and HECKSTEDEN, A., 2014. Effects of sodium bicarbonate on high-intensity endurance performance in cyclists: a double-blind, randomized cross-over trial. *PloS one*, *9*(12), p.e114729.

EL GHORAYEB, N., BOURDEAU, I. and LACROIX, A., 2016. Role of ACTH and other hormones in the regulation of aldosterone production in primary aldosteronism. *Frontiers in Endocrinology*, *7*, p.72.

EL-SAYED, M.S., ALI, N. and ALI, Z.E.S., 2005. Interaction between alcohol and exercise. *Sports Medicine*, *35*(3), pp.257-269.

ENOKA, R.M. and STUART, D.G., 1992. Neurobiology of muscle fatigue. *Journal of applied physiology*, *72*(5), pp.1631-1648.

ESAU, T.D., HMURA, M.S., MEYER, R.E. and WORMS III, R.A., 1996. A comparative analysis of the effects of sodium bicarbonate and sodium citrate ingestion on 800-meter sprint performance. *School of Physical Therapy*. 239.

FABIATO, A. and FABIATO, F., 1978. Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. *The Journal of physiology*, *276*(1), pp.233-255.

FAFF, J., LADYGA, M., BURKHARD-JAGODZINSKA, K. and SITKOWSKI, D., 1996. Effects of alkalisation on the changes in circulatory, respiratory and biochemical indices induced by a short, intense exercise. *Biology of Sport*, *13*, pp.247-258.

FENN, W.O., 1936. Electrolytes in muscle. *Physiological Reviews*, *16*(3), pp.450-487.

FIGGE, J., ROSSING, T.H. and FENCL, V., 1991. The role of serum proteins in acid-base equilibria. *The Journal of laboratory and clinical medicine*, *117*(6), pp.453-467.

FIORENZA, M., HOSTRUP, M., GUNNARSSON, T.P., SHIRAI, Y., SCHENA, F., IAIA, F.M. and BANGSBO, J., 2019. Neuromuscular Fatigue and Metabolism during High-Intensity Intermittent Exercise. *Medicine and science in sports and exercise*, *51*(8), pp.1642-1652.

FITTS, R.H., 1994. Cellular mechanisms of muscle fatigue. *Physiological reviews*, *74*(1), pp.49-94.

FITTS, R.H., 2003. Effects of regular exercise training on skeletal muscle contractile function. *American journal of physical medicine & rehabilitation*, *82*(4), pp.320-331.

FITTS, R.H., 2008. The cross-bridge cycle and skeletal muscle fatigue. *Journal of applied physiology*, *104*(2), pp.551-558.

FITTS, R., 2016. The role of acidosis in fatigue: pro perspective. *Medicine & Science in Sports & Exercise*.

FREIS, T., HECKSTEDEN, A., SUCH, U. and MEYER, T., 2017. Effect of sodium bicarbonate on prolonged running performance: A randomized, double-blind, cross-over study. *PloS one*, *12*(8), p.e0182158.

FRITZ, C.O., MORRIS, P.E. and RICHLER, J.J., 2012. Effect size estimates: current use, calculations, and interpretation. *Journal of experimental psychology: General*, *141*(1), p.2.

GAGNON, P., BUSSIÈRES, J.S., RIBEIRO, F., GAGNON, S.L., SAEY, D., GAGNÉ, N., PROVENCHER, S. and MALTAIS, F., 2012. Influences of spinal anesthesia on exercise

tolerance in patients with chronic obstructive pulmonary disease. *American journal of respiratory and critical care medicine*, *186*(7), pp.606-615.

GALLAGHER, E.J., BIJUR, P.E., LATIMER, C., and SILVER, W. 2002. Reliability and validity of a visual analog scale for acute abdominal pain in the ED. *The American Journal of Emergency Medicine*. 20(4), pp. 287-290.

GALLOWAY, S.D.R., LOTT, M.J.E. and TOULOUSE, L.C., 2014. Pre-exercise Carbohydrate Feeding and High-Intensity Exercise Capacity: Effects of Timing of Intake and Carbohydrate Concentration. *International Journal of Sport Nutrition & Exercise Metabolism*, *24*(3), pp.258-266.

GANDEVIA, S.C., 1992. Some central and peripheral factors affecting human motoneuronal output in neuromuscular fatigue. *Sports medicine*, *13*(2), pp.93-98.

GANDEVIA, S.C., 1998. Neural control in human muscle fatigue: changes in muscle afferents, moto neurones and moto cortical drive. *Acta Physiologica Scandinavica*, *162*(3), pp.275-283.

GANDEVIA, S.C., 2001. Spinal and supraspinal factors in human muscle fatigue. *Physiological reviews*, *81*(4), pp.1725-1789.

GARCIN, M., VAUTIER, J.F., VANDEWALLE, H., WOLFF, M. and MONOD, H., 1998. Ratings of perceived exertion (RPE) during cycling exercises at constant power output. *Ergonomics*, *41*(10), pp.1500-1509.

GASKELL, S.K., RAUCH, C. and COSTA, R.J.S., 2021. Gastrointestinal assessment and management procedures for exercise-associated gastrointestinal symptoms. *Aspetar Sports Med. J*, *10*, pp.36-44.

GASKELL, S.K., RAUCH, C.E. and COSTA, R.J., 2021. Gastrointestinal assessment and therapeutic intervention for the management of exercise-associated gastrointestinal symptoms: a case series translational and professional practice approach. *Frontiers in Physiology*, *12*.

GEVERS, W., 1977. Generation of protons by metabolic processes in heart cells. *Journal of molecular and cellular cardiology*, *9*(11), pp.867-874.

GEVERS, W., 1979. Generation of protons by metabolic processes other than glycolysis in muscle cells: a critical view. *Journal of Molecular and Cellular Cardiology*, *11*(3), pp.325-330.

GIBSON, A.S.C. and NOAKES, T.D., 2004. Evidence for complex system integration and dynamic neural regulation of skeletal muscle recruitment during exercise in humans. *British journal of sports medicine*, *38*(6), pp.797-806.

GLADDEN, L.B., 2008. A "Lactatic" perspective on metabolism. *Medicine & Science in Sports & Exercise*, *40*(3), pp.477-485.

GOEL, N. and CALVERT, J., 2012. Understanding blood gases/acid–base balance. *Paediatrics and Child Health*, *22*(4), pp.142-148.

GHOSH, A.K., 2004. Anaerobic threshold: its concept and role in endurance sport. *The Malaysian journal of medical sciences: MJMS*, *11*(1), p.24.

GOUGH, L.A., DEB, S.K., SPARKS, A.S. and MCNAUGHTON, L.R., 2017a. The reproducibility of blood acid base responses in male collegiate athletes following individualised doses of sodium bicarbonate: a randomised controlled crossover study. *Sports Medicine*, *47*(10), pp.2117-2127.

GOUGH, L.A., DEB, S.K., SPARKS, A. and MCNAUGHTON, L.R., 2017b. The reproducibility of 4-km time trial (TT) performance following individualised sodium bicarbonate supplementation: a randomised controlled trial in trained cyclists. *Sports medicine-open*, *3*(1), p.34.

GOUGH, L.A., DEB, S.K., BROWN, D., SPARKS, S.A. and MCNAUGHTON, L.R., 2019. The effects of sodium bicarbonate ingestion on cycling performance and acid base balance recovery in acute normobaric hypoxia. *Journal of sports sciences*, *37*(13), pp.1464-1471.

GOUGH, L.A., WILLIAMS, J.J., NEWBURY, J.W. and GURTON, W.H., 2021. The effects of sodium bicarbonate supplementation at individual time-to-peak blood bicarbonate on 4-km cycling time trial performance in the heat. *European Journal of Sport Science*, pp.1-9.

GRAFEN, A. and HAILS, R., 2002. Modern statistics for the life sciences Oxford Univ. *Press New York, NY*.

GRGIC, J., PEDISIC, Z., SAUNDERS, B., ARTIOLI, G.G., SCHOENFELD, B.J., MCKENNA, M.J., BISHOP, D.J., KREIDER, R.B., STOUT, J.R., KALMAN, D.S. and ARENT, S.M., 2021. International Society of Sports Nutrition position stand: sodium bicarbonate and exercise performance. *Journal of the International Society of Sports Nutrition*, *18*(1), p.61.

GUEST, N.S., VANDUSSELDORP, T.A., NELSON, M.T., GRGIC, J., SCHOENFELD, B.J., JENKINS, N.D., ARENT, S.M., ANTONIO, J., STOUT, J.R., TREXLER, E.T. and SMITH-RYAN, A.E., 2021. International society of sports nutrition position stand: caffeine and exercise performance. *Journal of the International Society of Sports Nutrition*, *18*(1), p.1.

GUIDETTI, L., MEUCCI, M., BOLLETTA, F., EMERENZIANI, G.P., GALLOTTA, M.C. and BALDARI, C., 2018. Validity, reliability and minimum detectable change of COSMED K5 portable gas exchange system in breath-by-breath mode. *PloS one*, *13*(12), p.e0209925.

HAGBERG, H., 1985. Intracellular pH during ischemia in skeletal muscle: relationship to membrane potential, extracellular pH, tissue lactic acid and ATP. *Pflügers Archiv*, *404*(4), pp.342-347.

HALESTRAP, A.P. and WILSON, M.C., 2012. The monocarboxylate transporter family—role and regulation. *IUBMB life*, *64*(2), pp.109-119.

HALLGREN, K.A., 2012. Computing inter-rater reliability for observational data: an overview and tutorial. *Tutorials in Quantitative Methods for Psychology*. 8 (1), pp. 23– 34.

HAMLEY, E.J. and THOMAS, V., 1967. Physiological and postural factors in the calibration of the bicycle ergometer. *The Journal of physiology*, *191*(2), pp.55-56.

HARGREAVES, M., HAWLEY, J.A. and JEUKENDRUP, A., 2004. Pre-exercise carbohydrate and fat ingestion: effects on metabolism and performance. *Journal of Sport* Sciences, 22, pp. 31-38.

HARGREAVES, M. and SPRIET, L.L., 2018. Exercise metabolism: fuels for the fire. *Cold Spring Harbor perspectives in medicine*, *8*(8), p.a029744.

HARLAND, N.J., DAWKIN, M.J. and MARTIN, D., 2015. Relative utility of a visual analogue scale vs a six-point Likert scale in the measurement of global subject outcome in patients with low back pain receiving physiotherapy. *Physiotherapy*, *101*, pp.50-54.

HASHIMOTO, T., HUSSIEN, R., OOMMEN, S., GOHIL, K. and BROOKS, G.A., 2007. Lactate sensitive transcription factor network in L6 cells: activation of MCT1 and mitochondrial biogenesis. *The FASEB Journal*, *21*(10), pp.2602-2612.

HAWKER, G.A., MIAN, S., KENDZERSKA, T., and FRENCH, M., 2011. Measures of adult pain: Visual analog scale for pain (vas pain), numeric rating scale for pain (nrs pain), mcgill pain questionnaire (mpq), short-form mcgill pain questionnaire (sf-mpq), chronic pain grade scale (cpgs), short form-36 bodily pain scale (sf-36 bps), and measure of intermittent and constant osteoarthritis pain (icoap). *Arthritis Care & Research*. 63 (11), pp. 240-252.

HECKSTEDEN, A., KRAUSHAAR, J., SCHARHAG-ROSENBERGER, F., THEISEN, D., SENN, S. and MEYER, T., 2015. Individual response to exercise training-a statistical perspective. *Journal of applied physiology*, *118*(12), pp.1450-1459.

HEIBEL, A.B., PERIM, P.H., OLIVEIRA, L.F., MCNAUGHTON, L.R. and SAUNDERS, B., 2018. Time to optimize supplementation: modifying factors influencing the individual responses to extracellular buffering agents. *Frontiers in nutrition*, *5* (35).

HEIGENHAUSER, G.J.F., 1990. The role of the physicochemical systems in plasma in acid-base control in exercise. *International Series on Sport Sciences; Biochemistry of Exercise VII*, *21*, pp.359-374.

HERMANSEN, L., 1981, January. Effect of metabolic changes on force generation in skeletal muscle during maximal exercise. In *Ciba Found Symp*osium, *82*, pp. 75-88.

HIGGINS, M.F., JAMES, R.S. and PRICE, M.J., 2013. The effects of sodium bicarbonate (NaHCO3) ingestion on high intensity cycling capacity. *Journal of sports sciences*, *31*(9), pp.972-981.

HILL, A.V., LONG, C.N.H. and LUPTON, H., 1924a. Muscular exercise, lactic acid, and the supply and utilisation of oxygen.—Parts l-lll. *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character*, *96*(679), pp.438-475.

HILL, A.V., LONG, C.N.H. and LUPTON, H., 1924b. Muscular exercise, lactic acid, and the supply and utilisation of oxygen.—Parts IV-VI. *Proceedings of the Royal Society of London. Series B, containing papers of a biological character*, *97*(681), pp.84-138.

HILL, A.V., LONG, C.N.H. and LUPTON, H., 1924c. Muscular exercise, lactic acid and the supply and utilisation of oxygen.—Parts VII–VIII. *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character*, *97*(682), pp.155-176.

HILL, A.V. and LUPTON, H., 1923. Muscular exercise, lactic acid, and the supply and utilization of oxygen. *QJM: An International Journal of Medicine*, (62), pp.135-171.

HILTON, N.P., LEACH, N.K., SPARKS, S.A., GOUGH, L.A., CRAIG, M.M., DEB, S.K. and MCNAUGHTON, L.R., 2019. A Novel Ingestion Strategy for Sodium Bicarbonate Supplementation in a Delayed-Release Form: A Randomised Crossover Study in Trained Males. *Sports Medicine-Open*, *5*(1), p.4.

HILTON, N.P., LEACH, N.K., CRAIG, M.M., SPARKS, S.A. and MCNAUGHTON, L.R., 2020a. Enteric-Coated Sodium Bicarbonate Attenuates Gastrointestinal Side-Effects. *International journal of sport nutrition and exercise metabolism*, *1*(aop), pp.1-7.

HILTON, N.P., LEACH, N.K., HILTON, M.M., SPARKS, S.A. and MCNAUGHTON, L.R., 2020b. Enteric-coated sodium bicarbonate supplementation improves high-intensity cycling performance in trained cyclists. *European journal of applied physiology*, *120*(7), pp.1563-1573.

HIRCHE, H.J., HOMBACH, V., LANGOHR, H.D., WACKER, U. and BUSSE, J., 1975. Lactic acid permeation rate in working gastrocnemii of dogs during metabolic alkalosis and acidosis. *Pflügers Archive*, *356*(3), pp.209-222.

HOCHACHKA, P.W. and MOMMSEN, T.P., 1983. Protons and anaerobiosis. *Science*, *219*(4591), pp.1391-1397.

HOPKINS, W.G., 2000. Measures of reliability in sports medicine and science. *Sports Medicine*. 30 (1), pp. 1–15.

HOPKINS, W.G., 2015. Individual responses made easy. *Journal of Applied Physiology*, *118*, pp.1444-1446.

HOPKER, J., MYERS, S., JOBSON, S.A., BRUCE, W. and PASSFIELD, L., 2010. Validity and reliability of the Wattbike cycle ergometer. *International journal of sports medicine*, *31*(10), pp.731-736.

HUI, S., GHERGUROVICH, J.M., MORSCHER, R.J., JANG, C., TENG, X., LU, W., ESPARZA, L.A., REYA, T., ZHAN, L., YANXIANG GUO, J. and WHITE, E., 2017. Glucose feeds the TCA cycle via circulating lactate. *Nature*, *551*(7678), pp.115-118.

HUNT, J.N. and STUBBS, D.F., 1975. The volume and energy content of meals as determinants of gastric emptying. *The Journal of physiology*, *245*(1), pp.209-225.

HUREAU, T.J., OLIVIER, N., MILLET, G.Y., MESTE, O. and BLAIN, G.M., 2014. Exercise performance is regulated during repeated sprints to limit the development of peripheral fatigue beyond a critical threshold. *Experimental physiology*, *99*(7), pp.951-963.

IBANEZ, J., PULLINEN, T., GOROSTIAGA, E., POSTIGO, A. and MERO, A., 1995. Blood lactate and ammonia in short-term anaerobic work following induced alkalosis. *The Journal of sports medicine and physical fitness*, *35*(3), pp.187-193.

IBEKWE, V.C., FADDA, H.M., MCCONNELL, E.L., KHELA, M.K., EVANS, D.F. and BASIT, A.W., 2008a. Interplay between intestinal pH, transit time and feed status on the in vivo performance of pH responsive ileo-colonic release systems. *Pharmaceutical research*, *25*(8), pp.1828-1835.

IBEKWE, V.C., FADDA, H.M., MCCONNELL, E.L., KHELA, M.K., EVANS, D.F. and BASIT, A.W., 2008. Interplay between intestinal pH, transit time and feed status on the in vivo performance of pH responsive ileo-colonic release systems. *Pharmaceutical research*, *25*(8), pp.1828-1835.

IVARSSON, A., ANDERSEN, M.B., JOHNSON, U. and LINDWALL, M., 2013. To adjust or not adjust: nonparametric effect sizes, confidence intervals, and real-world meaning. *Psychology of sport and exercise*, *14*(1), pp.97-102.

JÄGER, R., KERKSICK, C.M., CAMPBELL, B.I., CRIBB, P.J., WELLS, S.D., SKWIAT, T.M., PURPURA, M., ZIEGENFUSS, T.N., FERRANDO, A.A., ARENT, S.M. and SMITH-RYAN, A.E., 2017. International society of sports nutrition position stand: protein and exercise. *Journal of the International Society of Sports Nutrition*, *14*(1), pp.1-25.

JEUKENDRUP, A., 2014. A step towards personalized sports nutrition: carbohydrate intake during exercise. *Sports Medicine*, *44*(1), pp.25-33.

JEUKENDRUP, A.E., 2004. Carbohydrate intake during exercise and performance. Nutrition, 20(7-8), pp.669-677.

JEUKENDRUP, A.E., 2017. Periodized nutrition for athletes. *Sports Medicine*, *47*(1), pp.51-63.

JEUKENDRUP, A.E. and MOSELEY, L., 2010. Multiple transportable carbohydrates enhance gastric emptying and fluid delivery. *Scandinavian journal of medicine & science in sports*, *20*(1), pp.112-121.

JOHNSTON, J.D., 2014. Physiological links between circadian rhythms, metabolism and nutrition. *Experimental physiology*, *99*(9), pp.1133-1137.

JOHNSTON, K.R., VICKERS, M.D., and MAPLESON, W.W., 1996. Comparison of arterialized venous with arterial blood propofol concentrations during sub-anaesthetic infusions in volunteers. *British Journal of Anaesthesia*. 76 (3), pp. 401–4.

JONES, S.R., CARLEY, S., and HARRISON, M., 2003. An introduction to power and sample size estimation: The importance of power and sample size estimation for study design and analysis. *Emerg Med J*. 20, pp. 453–458.

JONES, R.L., STELLINGWERFF, T., ARTIOLI, G.G., SAUNDERS, B., COOPER, S. and SALE, C., 2016. Dose-response of sodium bicarbonate ingestion highlights individuality in time course of blood analyte responses. *International journal of sport nutrition and exercise metabolism*, *26*(5), pp.445-453.

JONES, A.M., WILKERSON, D.P., DIMENNA, F., FULFORD, J. and POOLE, D.C., 2008. Muscle metabolic responses to exercise above and below the "critical power" assessed using 31P-MRS. *American journal of physiology-regulatory, integrative and comparative physiology*, *294*(2), pp. 585-593.

JUEL, C., 2001. Current aspects of lactate exchange: lactate/H+ transport in human skeletal muscle. *European journal of applied physiology*, *86*(1), pp.12-16.

JUEL, C., 1997. Lactate-proton cotransport in skeletal muscle. *Physiological reviews*, *77*(2), pp.321-358.

JUEL, C., 1998. Muscle pH regulation: role of training. *Acta Physiologica Scandinavica*, *162*(3), pp.359-366.

JUEL, C. AND HALESTRAP, A.P., 1999. Lactate transport in skeletal muscle—role and regulation of the monocarboxylate transporter. *The Journal of physiology*, *517*(3), pp.633-642.

JUNIOR, A.H.L., DE SALLES PAINELLI, V., SAUNDERS, B. and ARTIOLI, G.G., 2015. Nutritional Strategies to Modulate Intracellular and Extracellular Buffering Capacity During High-Intensity Exercise. *Sports Med*, *45*(1), pp.71-81.

KAMEL, K.S., HALPERIN, M.L. and GOLDSTEIN, M.B., 2010. *Fluid, electrolyte and acid-base physiology e-book: a problem-based approach*. Elsevier Health Sciences.

KATZ, A., COSTILL, D.L., KING, D.S., HARGREAVES, M. and FINK, W.J., 1984. Maximal exercise tolerance after induced alkalosis. *International journal of sports medicine*, *5*(02), pp.107-110.

KEMP, G., 2005. Lactate accumulation, proton buffering, and pH change in ischemically exercising muscle. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*. 289 (3).

KEMP, R.G. and FOE, L.G., 1983. Allosteric regulatory properties of muscle phosphofructokinase. *Molecular and cellular biochemistry*, *57*(2), pp.147-154.

KENT-BRAUN, J.A., 1999. Central and peripheral contributions to muscle fatigue in humans during sustained maximal effort. *European journal of applied physiology and occupational physiology*, *80*(1), pp.57-63.

KENT-BRAUN, J.A., FITTS, R.H. and CHRISTIE, A., 2012. Skeletal muscle fatigue. Comprehensive Physiology 2: 997–1044.

KEUL, J., KÖNIG, D., HUONKER, M., HALLE, M., WOHLFAHRT, B. and BERG, A., 1996. Adaptation to training and performance in elite athletes. *Research quarterly for exercise and sport*, *67*(sup3), pp.S-29.

KEYSER, R.E., 2010. Peripheral fatigue: high-energy phosphates and hydrogen ions. *Pm&r*, *2*(5), pp.347-358.

KJELLMER, I., 1965. The potassium ion as a vasodilator during muscular exercise. *Acta Physiologica Scandinavica*, *63*(4), pp.460-468.

KNAPIK, J.J., STEELMAN, R.A., HOEDEBECKE, S.S., AUSTIN, K.G., FARINA, E.K. and LIEBERMAN, H.R., 2016. Prevalence of dietary supplement use by athletes: systematic review and meta-analysis. *Sports Medicine*, *46*(1), pp.103-123.

KOO, T.K. and LI, M.Y., 2016. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *Journal of chiropractic medicine*, *15*(2), pp.155-163.

KOWALCHUK, J.M., HEIGENHAUSER, G.J., LINDINGER, M.I., SUTTON, J.R. and JONES, N.L., 1988. Factors influencing hydrogen ion concentration in muscle after intense exercise. *Journal of Applied Physiology*, *65*(5), pp.2080-2089.

KOWALCHUK, J.M., MALTAIS, S.A., YAMAJI, K. and HUGHSON, R.L., 1989. The effect of citrate loading on exercise performance, acid-base balance and metabolism. *European journal of applied physiology and occupational physiology*, *58*(8), pp.858-864.

KUMSTÁT, M.M., HLINSKÝ, T., STRUHÁR, I. and THOMAS, A., 2018. Does sodium citrate cause the same ergogenic effect as sodium bicarbonate on swimming performance? *Journal of human kinetics*, *65* (1) pp.89-98.

LAKENS, D., 2013. Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. *Frontiers in psychology*, 4(863).

LAMBERTS, R.P., SWART, J., WOOLRICH, R.W., NOAKES, T.D. and LAMBERT, M.I., 2009. Measurement error associated with performance testing in well-trained cyclists: Application to the precision of monitoring changes in training status. *International SportMed Journal*, *10*(1), pp.33-44.

LANCHA JUNIOR, A.H., DE SALLES PAINELLI, V., SAUNDERS, B. and ARTIOLI, G.G., 2015. Nutritional strategies to modulate intracellular and extracellular buffering capacity during high-intensity exercise. *Sports Medicine*, *45*(1), pp.71-81.

LANHERS, C., PEREIRA B., NAUGHTON, G., TROUSSELARD, M., LESAGE, F-X and DUTHEIL, F., 2017. Creatine Supplementation and Upper Limb Strength Performance: A Systematic Review and Meta-Analysis. *Sports Medicine,* 47 (1), pp. 163-173.

LAPOSKY, A.D., BASS, J., KOHSAKA, A. and TUREK, F.W., 2008. sleep and circadian rhythms: key components in the regulation of energy metabolism. *febs letters*, *582*(1), pp.142-151.

LASSEN, T.A., LINDSTRØM, L., LØNBRO, S. and MADSEN, K., 2021. Increased Performance in Elite Runners Following Individualized Timing of Sodium Bicarbonate Supplementation. *International journal of sport nutrition and exercise metabolism*, *31*(6), pp.453-459.

LEGAZ-ARRESE, A., MUNGUÍA-IZQUIERDO, D., NUVIALA, A.N., SERVETO-GALINDO, O., URDIALES, D.M. and MASÍA, J.R., 2007. Average VO2max as a function of running performances on different distances. *Science & sports*, *22*(1), pp.43-49.

LEHNINGER, A.L., 1982. Electron transport, oxidative phosphorylation, and regulation of ATP production. *Principles of biochemistry*.

LEHNINGER, A.L., NELSON, D.L., COX, M.M. and COX, M.M., 2005. *Lehninger principles of biochemistry*. Macmillan.

LIEBER, C.S., 2000. Alcohol: its metabolism and interaction with nutrients. *Annual review of nutrition*, *20*(1), pp.395-430.

LILJEQUIST, D., ELFVING, B. and ROALDSEN, K.S., 2019. Intraclass correlation – A discussion and demonstration of basic features. *PloS one*, *14*(7).

LINDINGER, M.I., 2003. Exercise: a paradigm for multi-system control of acid–base state. *The Journal of physiology*, *550*(Pt 2), p.334.

LINDINGER, M.I. and HEIGENHAUSER, G.J., 1991. The roles of ion fluxes in skeletal muscle fatigue. *Canadian journal of physiology and pharmacology*, *69*(2), pp.246-253.

LINDINGER, M.I., MCKELVIE, R.S. and HEIGENHAUSER, G.J., 1995. K+ and Lac-distribution in humans during and after high-intensity exercise: role in muscle fatigue attenuation? *Journal of Applied Physiology*, *78*(3), pp.765-777.

LINOSSIER, M.T., DORMOIS, D., BREGERE, P., GEYSSANT, A. and DENIS, C., 1997. Effect of sodium citrate on performance and metabolism of human skeletal muscle during supramaximal cycling exercise. *European journal of applied physiology and occupational physiology*, *76*(1), pp.48-54.

LITTLE, J.P., CHILIBECK, P.D., CIONA, D., FORBES, S., REES, H., VANDENBERG, A. and ZELLO, G.A., 2010. Effect of low-and high-glycaemic-index meals on metabolism and performance during high-intensity, intermittent exercise. *International Journal of Sport Nutrition & Exercise Metabolism*, *20*(6).

MACDOUGALL, D., MACDOUGALL, J.D. and SALE, D., 2014. *The physiology of training for high performance*. Oxford University Press.

MACFARLANE, N., NORTHRIDGE, D.B., WRIGHT, A.R., GRANT, S. and DARGIE, H.J., 1991. A comparative study of left ventricular structure and function in elite athletes. *British journal of sports medicine*, *25*(1), pp.45-48.

MACFARLANE, D.J. and WONG, P., 2012. Validity, reliability and stability of the portable Cortex Metamax 3B gas analysis system. *European journal of applied physiology*, *112*(7), pp.2539-2547.

MAINWOOD, G.W. and CECHETTO, D., 1980. The effect of bicarbonate concentration on fatigue and recovery in isolated rat diaphragm muscle. *Canadian Journal of Physiology and Pharmacology*, *58*(6), pp.624-632.

MAINWOOD, G.W. and WORSLEY-BROWN, P., 1975. The effects of extracellular pH and buffer concentration on the efflux of lactate from frog sartorius muscle. *The Journal of physiology*, *250*(1), pp.1-22.

MARCORA, S.M. and STAIANO, W., 2010. The limit to exercise tolerance in humans: mind over muscle?. *European journal of applied physiology*, *109*(4), pp.763-770.

MARINO, F.E., GARD, M. and DRINKWATER, E.J., 2011. The limits to exercise performance and the future of fatigue research. *British journal of sports medicine*, *45*(1), pp.65-67.

MARTINS, A.N., ARTIOLI, G.G. and FRANCHINI, E., 2010. Sodium citrate ingestion increases glycolytic activity but does not enhance 2000 m rowing performance. *Journal of human sport and exercise*, 5(3), pp. 411-417.

MATSON, L.G. and TRAN, Z.V., 1993. Effects of sodium bicarbonate ingestion on anaerobic performance: a meta-analytic review. *International Journal of Sport Nutrition and Exercise Metabolism*, *3*(1), pp.2-28.

MAUGER, A.R., JONES, A.M. and WILLIAMS, C.A., 2009. Influence of feedback and prior experience on pacing during a 4-km cycle time trial. *Medicine & Science in Sports & Exercise*, *41*(2), pp.451-458.

MAUGER, A.R., JONES, A.M. and WILLIAMS, C.A., 2010. Influence of exercise variation on the retention of a pacing strategy. *European journal of applied physiology*, *108*(5), pp.1015-1023.

MAUGER, A.R., JONES, A.M. and WILLIAMS, C.A., 2011. The effect of non-contingent and accurate performance feedback on pacing and time trial performance in 4-km track cycling. *British Journal of Sports Medicine*, *45*(3), pp.225-229.

MCCRICKERD, K. and FORDE, C.G., 2016. Sensory influences on food intake control: Moving beyond palatability. *Obesity Reviews*, *17*(1), pp.18-29.

MCCRUM-GARDNER, E., 2010. Sample size and power calculations made simple. *International Journal of Therapy and Rehabilitation*. 17 (1), pp. 10–14.

MCGINLEY, C. and BISHOP, D.J., 2016. Influence of training intensity on adaptations in acid/base transport proteins, muscle buffer capacity, and repeated-sprint ability in active men. *Journal of Applied Physiology*, *121*(6), pp.1290-1305.

MCGINLEY, C. and BISHOP, D.J., 2017. Rest interval duration does not influence adaptations in acid/base transport proteins following 10-week of sprint-interval training in active women. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *312*(5), pp.702-717.

MCKENNA, M.J., 1992. The roles of ionic processes in muscular fatigue during intense exercise. *Sports medicine*, *13*(2), pp.134-145.

MCNAUGHTON, L.R., 1990. Sodium citrate and anaerobic performance: implications of dosage. *European journal of applied physiology and occupational physiology*, *61*(5-6), pp.392-397.

MCNAUGHTON, L.R. and CEDARO, R., 1991. The effect of sodium bicarbonate on rowing ergometer performance in elite rowers. *Aust J Sci Med Sport*, *23*(3), pp.66-9.

MCNAUGHTON, L. and CEDARO, R., 1992. Sodium citrate ingestion and its effects on maximal anaerobic exercise of different durations. *European journal of applied physiology and occupational physiology*, *64*(1), pp.36-41.

MCNAUGHTON, L., DALTON, B. and PALMER, G., 1999. Sodium bicarbonate can be used as an ergogenic aid in high-intensity, competitive cycle ergometry of 1 h duration. *European journal of applied physiology and occupational physiology*, *80*(1), pp.64-69.

MCNAUGHTON, L.R., DEB, S., GOUGH, L., HIGGINS, M., PRICE, M., Sale, C. and SPARKS, S.A., 2019. The BASES Expert Statement on Extracellular Buffering Agents. *The Sport and Exercise Scientist, 62*, pp.6-7.

MCNAUGHTON, L.R., GOUGH, L., DEB, S., BENTLEY, D., and SPARKS, S.A., 2016. Recent developments in the use of sodium bicarbonate as an ergogenic aid. *Current Sports Medicine Reports*. 15 (4), pp. 233–44.

MCNAUGHTON, L.R., SIEGLER, J. and MIDGLEY, A., 2008. Ergogenic effects of sodium bicarbonate. *Current sports medicine reports*, *7*(4), pp.230-236.

MEYER, C., DOSTOU, J.M., WELLE, S.L. and GERICH, J.E., 2002. Role of human liver, kidney, and skeletal muscle in postprandial glucose homeostasis. *American Journal of Physiology-Endocrinology And Metabolism*, *282*(2), pp.E419-E427.

MICKLEWRIGHT, D., ST CLAIR GIBSON, A., GLADWELL, V. and AL SALMAN, A., 2017. Development and validity of the rating-of-fatigue scale. *Sports Medicine*, *47*(11), pp.2375-2393.

MIDDLEBROOK, I., PEACOCK, J., TINNION, D.J., LEACH, N.K., HILTON, N.P., SAUNDERS, B., SPARKS, S.A. and MCNAUGHTON, L.R., 2021. Capsule size alters the timing of metabolic alkalosis following sodium bicarbonate supplementation. *Frontiers in Nutrition*, *8*, p.634465.

MIDGLEY, A.W., CARROLL, S., MARCHANT, D., MCNAUGHTON, L.R. and SIEGLER, J., 2009. Evaluation of true maximal oxygen uptake based on a novel set of standardized criteria. *Applied Physiology, Nutrition, and Metabolism*, *34*(2), pp.115-123.

MIDGLEY, A.W., MCNAUGHTON, L.R., POLMAN, R. and MARCHANT, D., 2007. Criteria for Determination of Maximal Oxygen Uptake. *Sports Med*, *37*(12), pp.1019-1028.

MILLER, P., ROBINSON, A.L., SPARKS, S.A., BRIDGE, C.A., BENTLEY, D.J. and MCNAUGHTON, L.R., 2016. The effects of novel ingestion of sodium bicarbonate on repeated sprint ability. *The Journal of Strength & Conditioning Research*, *30*(2), pp.561-568.

MOORE, J.G., CHRISTIAN, P.E. and COLEMAN, R.E., 1981. Gastric emptying of varying meal weight and composition in man. *Digestive diseases and sciences*, *26*(1), pp.16-22.

MOORE, D.R., ROBINSON, M.J., FRY, J.L., TANG, J.E., GLOVER, E.I., WILKINSON, S.B., PRIOR, T., TARNOPOLSKY, M.A. and PHILLIPS, S.M., 2009. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. *The American journal of clinical nutrition*, *89*(1), pp.161-168.

MOREL, B., ROUFFET, D.M., BISHOP, D.J., ROTA, S.J. and HAUTIER, C.A., 2015. Fatigue induced by repeated maximal efforts is specific to the rugby task performed. *International Journal of Sports Science & Coaching*, *10*(1), pp.11-20.

MORRIS, D.M., SHAFER, R.S., FAIRBROTHER, K.R. and WOODALL, M.W., 2011. Effects of lactate consumption on blood bicarbonate levels and performance during high-intensity exercise. *International journal of sport nutrition and exercise metabolism*, *21*(4), pp.311-317.

MOSSO A., 1904. *Fatigue*. London: Swan Sonnenschein & Co Ltd.

MYCIELSKA, M.E., PATEL, A., RIZANER, N., MAZUREK, M.P., KEUN, H., PATEL, A., GANAPATHY, V. and DJAMGOZ, M.B., 2009. Citrate transport and metabolism in mammalian cells: prostate epithelial cells and prostate cancer. *Bioessays*, *31*(1), pp.10-20.

NEVILL, A.M., ATKINSON, G., HUGHES, M.D. and COOPER, S.M., 2002. Statistical methods for analysing discrete and categorical data recorded in performance analysis. *Journal of sports sciences*, *20*(10), pp.829-844.

NIELSEN, J.J., MOHR, M., KLARSKOV, C., KRISTENSEN, M., KRUSTRUP, P., JUEL, C. and BANGSBO, J., 2004. Effects of high-intensity intermittent training on potassium kinetics and performance in human skeletal muscle. *The Journal of physiology*, *554*(3), pp.857-870.

NIELSEN, O.B., ØRTENBLAD, N., LAMB, G.D. and STEPHENSON, D.G., 2004. Excitability of the T-tubular system in rat skeletal muscle: roles of K+ and Na+ gradients and Na+–K+ pump activity. *The Journal of physiology*, *557*(1), pp.133-146.

NOAKES, T.D., 2007. The central governor model of exercise regulation applied to the marathon. *Sports medicine*, *37*(4), pp.374-377.

NORDEEN-SNYDER, K.S., 1977. The effect of bicycle seat height variation upon oxygen consumption and lower limb kinematics. *Medicine and science in sports*, *9*(2), pp.113-117.

NORTHGRAVES, M.J., PEART, D.J., JORDAN, C.A. and VINCE, R.V., 2014. Effect of lactate supplementation and sodium bicarbonate on 40-km cycling time trial performance. *The Journal of Strength & Conditioning Research*, *28*(1), pp.273-280.

DE OLIVEIRA, L.F., SAUNDERS, B. and ARTIOLI, G.G., 2018. Is bypassing the stomach a means to optimize sodium bicarbonate supplementation? A case study with a postbariatric surgery individual. *International journal of sport nutrition and exercise metabolism*, *28*(6), pp.660-663.

DE OLIVEIRA, L.F., DOLAN, E., SWINTON, P.A., DURKALEC-MICHALSKI, K., ARTIOLI, G.G., MCNAUGHTON, L.R. and SAUNDERS, B., 2021. Extracellular buffering supplements to improve exercise capacity and performance: A comprehensive systematic review and meta-analysis. *Sports medicine*, pp.1-22.

DE OLIVEIRA, L.F., SAUNDERS, B., YAMAGUCHI, G., SWINTON, P. and Artioli, G.G., 2020. Is individualization of sodium bicarbonate ingestion based on time to peak necessary?. *Medicine and science in sports and exercise*, *52*(8).

ÖÖPIK, V., SAAREMETS, I., TIMPMANN, S., MEDIJAINEN, L. and KARELSON, K., 2004. Effects of acute ingestion of sodium citrate on metabolism and 5-km running performance: a field study. *Canadian journal of applied physiology*, *29*(6), pp.691-703.

ÖÖPIK, V., TIMPMANN, S., KADAK, K., MEDIJAINEN, L. and KARELSON, K., 2008. The effects of sodium citrate ingestion on metabolism and 1500-m racing time in trained female runners. *Journal of sports science & medicine*, *7*(1), p.125.

ORMSBEE, M.J., BACH, C.W. and BAUR, D.A., 2014. Pre-exercise nutrition: the role of macronutrients, modified starches and supplements on metabolism and endurance performance. *Nutrients*, *6*(5), pp.1782-1808.

OSTER, J.R., STEMMER, C.L., PEREZ, G.O. and VAAMONDE, C.A., 1988. Comparison of the effects of sodium bicarbonate vs. sodium citrate on renal acid excretion. *Mineral and electrolyte metabolism*, *14*(2-3), pp.97-102.

DE SALLES PAINELLI, V. and JUNIOR, A.H.L., 2018. Thirty years of investigation on the ergogenic effects of sodium citrate: is it time for a fresh start? *British Journal of Sports Medicine*, *52*(14), pp.942-943.

DE SALLES PAINELLI, V., DA SILVA, R.P., DE OLIVEIRA, O.M., DE OLIVEIRA, L.F., BENATTI, F.B., RABELO, T., GUILHERME, J.P.L.F., JUNIOR, A.H.L. and ARTIOLI, G.G., 2014. The effects of two different doses of calcium lactate on blood pH, bicarbonate, and repeated high-intensity exercise performance. *International journal of sport nutrition and exercise metabolism*, *24*(3), pp.286-295.

PALMIERI, F., 2014. Mitochondrial transporters of the SLC25 family and associated diseases: a review. *Journal of inherited metabolic disease*, *37*(4), pp.565-575.

DE PAUW, K., ROELANDS, B., CHEUNG, S.S., DE GEUS, B., RIETJENS, G. and MEEUSEN, R., 2013. Guidelines to classify subject groups in sport-science research. *International journal of sports physiology and performance*, *8*(2), pp.111-122.

PAROLIN, M.L., CHESLEY, A., MATSOS, M.P., SPRIET, L.L., JONES, N.L. and HEIGENHAUSER, G.J., 1999. Regulation of skeletal muscle glycogen phosphorylase and PDH

during maximal intermittent exercise. *American Journal of Physiology-Endocrinology and Metabolism*, *277*(5), pp. 890-900.

PARRY-BILLINGS, M. and MACLAREN, D.P.M., 1986. The effect of sodium bicarbonate and sodium citrate ingestion on anaerobic power during intermittent exercise. *European journal of applied physiology and occupational physiology*, *55*(5), pp.524-529.

PEACOCK, J., SPARKS, S.A., MIDDLEBROOK, I., HILTON, N.P., TINNION, D., LEACH, N., SAUNDERS, B. and MCNAUGHTON, L.R., 2021. Extracellular buffer choice influences acid-base responses and gastrointestinal symptoms. *Research in Sports Medicine*, pp.1-12.

PEART, D.J., SIEGLER, J.C., and VINCE, R. V, 2012. Practical recommendations for coaches and athletes: a meta-analysis of sodium bicarbonate use for athletic performance. *Journal of Strength and Conditioning Research / National Strength & Conditioning Association*. 26 (7), pp. 1975–83.

PEELING, P., BINNIE, M.J., GOODS, P.S., SIM, M. and BURKE, L.M., 2018. Evidence-based supplements for the enhancement of athletic performance. *International journal of sport nutrition and exercise metabolism*, *28*(2), pp.178-187.

PEREZ-SUAREZ, I., MARTIN-RINCON, M., GONZALEZ-HENRIQUEZ, J.J., FEZZARDI, C., PEREZ-REGALADO, S., GALVAN-ALVAREZ, V., JUAN-HABIB, J.W., MORALES-ALAMO, D. and CALBET, J.A., 2018. Accuracy and precision of the COSMED K5 portable analyser. *Frontiers in physiology*, *9*, p.1764.

PILEGAARD, H., JUEL, C. and WIBRAND, F., 1993. Lactate transport studied in sarcolemmal giant vesicles from rats: effect of training. *American Journal of Physiology-Endocrinology and Metabolism*, *264*(2), pp. 156-160.

POTTEIGER, J.A., NICKEI, G.L., WEBSTER, M.J., HAUB, M.D. and PALMER, R.J., 1996. Sodium citrate ingestion enhances 30 km cycling performance. *International journal of sports medicine*, *17*(01), pp.7-11.

POUPIN, N., CALVEZ, J., LASSALE, C., CHESNEAU, C. and TOMÉ, D., 2012. Impact of the diet on net endogenous acid production and acid–base balance. *Clinical nutrition*, *31*(3), pp.313-321.

PRICE, M.J. and CRIPPS, D., 2012. The effects of combined glucose-electrolyte and sodium bicarbonate ingestion on prolonged intermittent exercise performance. *Journal of sports sciences*, *30*(10), pp.975-983.

PRICE, M.J. and SIMONS, C., 2010. The effect of sodium bicarbonate ingestion on high-intensity intermittent running and subsequent performance. *The Journal of Strength & Conditioning Research*, *24*(7), pp.1834-1842.

PYNE, D.B., BOSTON, T., MARTIN, D.T., and LOGAN, A., 2000. Evaluation of the Lactate Pro blood lactate analyser. *European Journal of Applied Physiology*. 82 (1–2), pp. 112–116.

RADIOMETER MEDICAL. 2015. ABL 800 Reference Manual. http://www.radiometer.com. Accessed 2019.

RAMPININI, E., BOSIO, A., FERRARESI, I., PETRUOLO, A., MORELLI, A. and SASSI, A., 2011. Match-related fatigue in soccer players. *Medicine & Science in Sports & Exercise*, *43*(11), pp.2161-2170.

REILLY, T., 1990. Human circadian rhythms and exercise. *Critical Reviews in Biomedical Engineering*. 18 (3), pp. 165–180.

REMER, T., 2001. Influence of nutrition on acid-base balance–metabolic aspects. *European journal of nutrition*, *40*(5), pp.214-220.

REMER, T. and MANZ, F., 1995. Potential renal acid load of foods and its influence on urine pH. *Journal of the American Dietetic Association*, *95*(7), pp.791-797.

REQUENA, B., ZABALA, M., PADIAL, P. and FERICHE, B., 2005. Sodium bicarbonate and sodium citrate: ergogenic aids?. *The Journal of Strength & Conditioning Research*, *19*(1), pp.213-224.

RITCHIE, C., 2012. Rating of perceived exertion (RPE). *Journal of physiotherapy*, *58*(1), p.62.

ROBERGS, R.A., 2002. Blood acid-base buffering: Explanation of the effectiveness of bicarbonate and citrate ingestion. *Journal of Exercise Physiology Online*, *5*(3), pp.1-5.

ROBERGS, R.A., GHIASVAND, F. and PARKER, D., 2004. Biochemistry of exercise-induced metabolic acidosis. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *287*(3), pp. 502-516.

ROBERTSON, R.J., GILLESPIE, R.L., MCCARTHY, J. and ROSE, K.D., 1979a. Differentiated perceptions of exertion: Part I. Mode of integration of regional signals. *Perceptual and motor skills*, *49*(3), pp.683-689.

ROBERTSON, R.J., GILLESPIE, R.L., MCCARTHY, J. and ROSE, K.D., 1979. Differentiated perceptions of exertion: Part II. Relationship to local and central physiological responses. *Perceptual and motor skills*, *49*(3), pp.691-697.

ROLLS, B.J., CASTELLANOS, V.H., HALFORD, J.C., KILARA, A., PANYAM, D., PELKMAN, C.L., SMITH, G.P. and THORWART, M.L., 1998. Volume of food consumed affects satiety in men. *The American journal of clinical nutrition*, *67*(6), pp.1170-1177.

ROMIJN, J.A., COYLE, E.F., SIDOSSIS, L.S., GASTALDELLI, A., HOROWITZ, J.F., ENDERT, E. and WOLFE, R.R., 1993. Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *American Journal of Physiology-Endocrinology and Metabolism*, *265*(3), pp.380-391.

ROSENTHAL, G. and ROSENTHAL, J.A., 2011. *Statistics and data interpretation for social work*. Springer publishing company.

ROSS, A., LEVERITT, M. and RIEK, S., 2001. Neural influences on sprint running. *Sports medicine*, *31*(6), pp.409-425.

RUSSELL, C., PAPADOPOULOS, E., MEZIL, Y., WELLS, G.D., PLYLEY, M.J., GREENWAY, M. and KLENTROU, P., 2014. Acute vs. chronic supplementation of sodium citrate on 200 m performance in adolescent swimmers. *Journal of the International Society of Sports Nutrition*, *11*(1), p.26.

SAHLIN, K., 1986. Muscle fatigue and lactic acid accumulation. *Acta physiologica Scandinavica. Supplementum*, *556*, pp.83-91.

SAHLIN, K., 1992. Metabolic factors in fatigue. *Sports Medicine*, *13*(2), pp.99-107.

SAHLIN, K., EDSTROM, L., SJOHOLM, H. and HULTMAN, E., 1981. Effects of lactic acid accumulation and ATP decrease on muscle tension and relaxation. *American Journal of Physiology-Cell Physiology*, *240*(3), pp.121-126.

SAHLIN, K., HARRIS, R.C., NYLIND, B. and HULTMAN, E., 1976. Lactate content and pH in muscle samples obtained after dynamic exercise. *Pflügers Archiv*, *367*(2), pp.143-149.

SAHLIN, K. and HENRIKSSON, J., 1984. Buffer capacity and lactate accumulation in skeletal muscle of trained and untrained men. *Acta Physiologica Scandinavica*, *122*(3), pp.331-339.

SAHLIN, K., KATZ, A. and HENRIKSSON, J., 1987. Redox state and lactate accumulation in human skeletal muscle during dynamic exercise. *Biochemical Journal*, *245*(2), pp.551-556.

SAHLIN, K., TONKONOGI, M. and SÖDERLUND, K., 1998. Energy supply and muscle fatigue in humans. *Acta Physiologica Scandinavica*, *162*(3), pp.261-266.

SAUNDERS, B., ARTIOLI, G.G., DOLAN, E., JONES, R.L., MATTHEWS, J. and SALE, C., 2020. Biochemistry of Buffering Capacity and Ingestion of Buffers In Exercise and Athletic Performance. In *The Routledge Handbook on Biochemistry of Exercise* (pp. 334-352). Routledge.

SAUNDERS, B., ELLIOTT-SALE, K., ARTIOLI, G.G., SWINTON, P.A., DOLAN, E., ROSCHEL, H., SALE, C., and GUALANO, B., 2017. β-alanine supplementation to improve exercise capacity and performance: a systematic review and meta-analysis. *British Journal of Sports Medicine*. 51 (8), pp. 658–669.

SAUNDERS, B., SALE, C., HARRIS, R.C. and SUNDERLAND, C., 2014. Sodium bicarbonate and high-intensity-cycling capacity: variability in responses. *International journal of sports physiology and performance*, *9*(4), pp.627-632.

SAWCZUK, M., BANTING, L.K., CIĘSZCZYK, P., MACIEJEWSKA-KARŁOWSKA, A., ZARĘBSKA, A., LEOŃSKA-DUNIEC, A., JASTRZĘBSKI, Z., BISHOP, D.J. and EYNON, N., 2015. MCT1 A1470T: a novel polymorphism for sprint performance? *Journal of science and medicine in sport*, *18*(1), pp.114-118.

SCHABORT, E.J., WILSON, G. and NOAKES, T.D., 2000. Dose-related elevations in venous pH with citrate ingestion do not alter 40-km cycling time-trial performance. *European journal of applied physiology*, *83*(4-5), pp.320-327.

SCHLUTER, J.M. and FITTS, R.H., 1994. Shortening velocity and ATPase activity of rat skeletal muscle fibers: effects of endurance exercise training. *American Journal of Physiology-Cell Physiology*, *266*(6), pp.C1699-C1713.

SCHMIDT, L.E., and DALHOFF, K., 2002. Food-drug interactions. *Drugs*. 62:1481-1502.

SHAVE, R., WHYTE, G., SIEMANN, A. and DOGGART, L., 2001. The effects of sodium citrate ingestion on 3,000-meter time-trial performance. Journal *of strength and conditioning research*, *15*(2), pp.230-234.

SHENNUM, P.L., 1976. The effect of saddle height on oxygen consumption during bicycle ergometer work. *Medicine and science in sports*, *8*(2), pp.119-121.

SIEGLER, J.C. and MARSHALL, P., 2015. The effect of metabolic alkalosis on central and peripheral mechanisms associated with exercise-induced muscle fatigue in humans. *Experimental physiology*, *100*(5), pp.519-530.

SIEGLER, J.C., MARSHALL, P.W., BRAY, J. and TOWLSON, C., 2012. Sodium bicarbonate supplementation and ingestion timing: does it matter?. *The Journal of Strength & Conditioning Research*, *26*(7), pp.1953-1958.

SIEGLER, J.C., MIDGLEY, A.W., POLMAN, R.C. and LEVER, R., 2010. Effects of various sodium bicarbonate loading protocols on the time-dependent extracellular buffering profile. *The Journal of Strength & Conditioning Research*, *24*(9), pp.2551-2557.

SPARKS, A., WILLIAMS, E., ROBINSON, A., MILLER, P., BENTLEY, D.J., BRIDGE, C. and MCNAUGHTON, L.R., 2017. Sodium bicarbonate ingestion and individual variability in time-to-peak pH. *Research in Sports Medicine*, *25*(1), pp.58-66.

SPORER, B.C. and MCKENZIE, D.C., 2007. Reproducibility of a laboratory based 20-km time trial evaluation in competitive cyclists using the Velotron Pro ergometer. *International journal of sports medicine*, *28*(11), pp.940-944.

SPRIET, L.L., SODERLUND, K.A., BERGSTROM, M.A. and HULTMAN, E.R., 1987. Anaerobic energy release in skeletal muscle during electrical stimulation in men. *Journal of applied physiology*, *62*(2), pp.611-615.

STADLBAUER, V., WALLNER, S., STOJAKOVIC, T. and SMOLLE, K.H., 2011. Comparison of 3 different multianalyte point-of-care devices during clinical routine on a medical intensive care unit. *Journal of critical care*, *26*(4), pp.433-e1.

STANNARD, R.L., STELLINGWERFF, T., ARTIOLI, G.G., SAUNDERS, B., COOPER, S. and SALE, C., 2016. Dose-Response of Sodium Bicarbonate Ingestion Highlights Individuality in. *International Journal of Sport Nutrition*. 26(5), pp. 445-453.

STELLINGWERFF, T. and COX, G.R., 2014. Systematic review: Carbohydrate supplementation on exercise performance or capacity of varying durations. *Applied Physiology, Nutrition, and Metabolism*, *39*(9), pp.998-1011.

STEPHENS, T.J., MCKENNA, M.J., CANNY, B.J., SNOW, R.J. and MCCONELL, G.K., 2002. Effect of sodium bicarbonate on muscle metabolism during intense endurance cycling. *Medicine & Science in Sports & Exercise*, *34*(4), pp.614-621.

STEWART, P.A., 1983. Modern quantitative acid–base chemistry. *Canadian journal of physiology and pharmacology*, *61*(12), pp.1444-1461.

STREET, D., NIELSEN, J.-J., BANGSBO, J., and JUEL, C., 2005. Metabolic alkalosis reduces exercise-induced acidosis and potassium accumulation in human skeletal muscle interstitium. *The Journal of Physiology*. 566 (2), pp. 481–489.

STREINER, D.L., NORMAN, G.R. and CAIRNEY, J., 2015. *Health measurement scales: a practical guide to their development and use*. Oxford University Press, USA.

STRINGER, W., CASABURI, R., and WASSERMAN, K., 1992. Acid-base regulation during exercise and recovery in humans. *Journal of Applied Physiology,* 72 (3), pp. 954-961.

SULLIVAN, G.M. and FEINN, R., 2012. Using effect size—or why the P value is not enough. *Journal of graduate medical education*, *4*(3), pp.279-282.

SUVI, S., MOOSES, M., TIMPMANN, S., MEDIJAINEN, L., UNT, E. and ÖÖPIK, V., 2019. Influence of sodium citrate supplementation after dehydrating exercise on responses of stress hormones to subsequent endurance cycling time-trial in the heat. *Medicina*, *55*(4), p.103.

SWINTON, P.A., HEMINGWAY, B.S., SAUNDERS, B., GUALANO, B. and DOLAN, E., 2018. A statistical framework to interpret individual response to intervention: paving the way for personalised nutrition and exercise prescription. Front Nutr 2018; 5: 41.

TENAN, M.S., MCMURRAY, R.G., BLACKBURN, B.T., MCGRATH, M. and LEPPERT, K., 2011. The relationship between blood potassium, blood lactate, and electromyography signals related to fatigue in a progressive cycling exercise test. *Journal of Electromyography and Kinesiology*, *21*(1), pp.25-32.

THOMAS, D.T., ERDMAN, K.A. and BURKE, L.M., 2016. American college of sports medicine joint position statement. nutrition and athletic performance. *Medicine and science in sports and exercise*, *48*(3), pp.543-568.

THOMAS, K., GOODALL, S., STONE, M., HOWATSON, G., GIBSON, A.S.C. and ANSLEY, L., 2015. Central and peripheral fatigue in male cyclists after 4-, 20-, and 40-km time trials. *Medicine & Science in Sports & Exercise*, *47*(3), pp.537-546.

TIRYAKI, G.R. and ATTERBOM, H.A., 1995. The effects of sodium bicarbonate and sodium citrate on 600 m running time of trained females. *The Journal of Sports Medicine and Physical Fitness*, *35*(3), pp.194-198.

TOFFALETTI, J.G., 1991. Blood lactate: biochemistry, laboratory methods, and clinical interpretation. *Critical reviews in clinical laboratory sciences*, *28*(4), pp.253-268.

TORNERO-AGUILERA, J.F., JIMENEZ-MORCILLO, J., RUBIO-ZARAPUZ, A. and CLEMENTE-SUÁREZ, V.J., 2022. Central and Peripheral Fatigue in Physical Exercise Explained: A Narrative Review. *International Journal of Environmental Research and Public Health*, *19*(7), p.3909.

TRUMP, B.F., MERGNER, W.J., KAHNG, M.W. and SALANDINO, A.J., 1976. Studies on the subcellular pathophysiology of ischemia. *Circulation*, *53*(3), pp. 17-26.

TSEH, W., CAPUTO, J.L. and KEEFER, D.J., 2010. Validity and reliability of the BOD POD® S/T tracking system. *International journal of sports medicine*, *31*(10), pp.704-708.

TUCKER, L.A., LECHEMINANT, J.D. and BAILEY, B.W., 2014. Test-retest reliability of the Bod Pod: the effect of multiple assessments. *Perceptual and motor skills*, *118*(2), pp.563-570.

TURNBERG, L.A., FORDTRAN, J.S., CARTER, N.W. AND RECTOR, F.C., 1970. Mechanism of bicarbonate absorption and its relationship to sodium transport in the human jejunum. *The Journal of clinical investigation*, *49*(3), pp.548-556.

URWIN, C.S., DWYER, D.B. and CARR, A.J., 2016. Induced alkalosis and gastrointestinal symptoms after sodium citrate ingestion: a dose-response investigation. *International journal of sport nutrition and exercise metabolism*, *26*(6), pp.542-548.

URWIN, C.S., SNOW, R.J., CONDO, D., SNIPE, R.M., WADLEY, G.D., CONVIT, L. and CARR, A.J., 2022. A Comparison of Sodium Citrate and Sodium Bicarbonate Ingestion: Blood Alkalosis and Gastrointestinal Symptoms. *International Journal of Sport Nutrition and Exercise Metabolism*, 1(aop), pp.1-10.

URWIN, C.S., SNOW, R.J., ORELLANA, L., CONDO, D., WADLEY, G.D. and CARR, A.J., 2019. Sodium citrate ingestion protocol impacts induced alkalosis, gastrointestinal symptoms, and palatability. *Physiological reports*, *7*(19), p.e14216.

URWIN, C.S., SNOW, R.J., ORELLANA, L., CONDO, D., WADLEY, G.D. and CARR, A.J., 2021a. Does varying the ingestion period of sodium citrate influence blood alkalosis and gastrointestinal symptoms?. *PloS one*, *16*(5), p.e0251808.

URWIN, C.S., SNOW, R.J., CONDO, D., SNIPE, R., WADLEY, G.D. and CARR, A.J., 2021b. Factors Influencing Blood Alkalosis and Other Physiological Responses, Gastrointestinal Symptoms, and Exercise Performance Following Sodium Citrate Supplementation: A Review. *International journal of sport nutrition and exercise metabolism*, *31*(2), pp.168-186.

VÁGHY, P.L., 1979. Role of mitochondrial oxidative phosphorylation in the maintenance of intracellular pH. *Journal of molecular and cellular cardiology*, *11*(10), pp.933-940.

VAHER, I., TIMPMANN, S., AEDMA, M. and ÖÖPIK, V., 2015. Impact of acute sodium citrate ingestion on endurance running performance in a warm environment. *European journal of applied physiology*, *115*(4), pp.813-823.

VAN LOON, L.J., GREENHAFF, P.L., CONSTANTIN-TEODOSIU, D., SARIS, W.H. and WAGENMAKERS, A.J., 2001. The effects of increasing exercise intensity on muscle fuel utilisation in humans. *The Journal of physiology*, *536*(1), pp.295-304.

VAN MONTFOORT, M.C., VAN DIEREN, L., HOPKINS, W.G. and SHEARMAN, J.P., 2004. Effects of ingestion of bicarbonate, citrate, lactate, and chloride on sprint running. *Medicine and Science in Sports and Exercise.*, *36*(7), pp.1239-1243.

VAN SOMEREN, K., FULCHER, K. and MCCARTHY, J., 1999. An investigation into the effects of sodium citrate ingestion on high-intensity exercise performance. *Occupational Health and Industrial Medicine*, *2*(40), p.97.

VIU, J., JOSE-CUNILLERAS, E., ARMENGOU, L., CESARINI, C., TARANCÓN, I., RIOS, J. and MONREAL, L., 2010. Acid-base imbalances during a 120 km endurance race compared by traditional and simplified strong ion difference methods. *Equine Veterinary Journal*, *42*, pp.76-82.

VON HURST, P.R., WALSH, D.C., CONLON, C.A., INGRAM, M., KRUGER, R. and STONEHOUSE, W., 2016. Validity and reliability of bioelectrical impedance analysis to estimate body fat percentage against air displacement plethysmography and dual-energy X-ray absorptiometry. *Nutrition & Dietetics*, *73*(2), pp.197-204.

WAAGE, P. and GULBERG, C.M., 1986. Studies concerning affinity. *Journal of Chemical Education*, 63(12), pp.1044.

WAINWRIGHT, B., COOKE, C.B. and O'HARA, J.P., 2017. The validity and reliability of a sample of 10 Wattbike cycle ergometers. *Journal of sports sciences*, *35*(14), pp.1451-1458.

WEBSTER, M.J., WEBSTER, M.N., CRAWFORD, R.E. and GLADDEN, L.B., 1993. Effect of sodium bicarbonate ingestion on exhaustive resistance exercise performance. *Medicine and Science in Sports and Exercise*, *25*(8), pp.960-965.

WESTERBLAD, H. and ALLEN, D.G., 2002. Recent advances in the understanding of skeletal muscle fatigue. *Current opinion in rheumatology*, *14*(6), pp.648-652.

WESTERTERP-PLANTENGA, M., DIEPVENS, K., JOOSEN, A.M., BÉRUBÉ-PARENT, S. and TREMBLAY, A., 2006. Metabolic effects of spices, teas, and caffeine. *Physiology & Behaviour*, *89*(1), pp.85-91.

WESTON, A.R., MYBURGH, K.H., LINDSAY, F.H., DENNIS, S.C., NOAKES, T.D. and HAWLEY, J.A., 1996. Skeletal muscle buffering capacity and endurance performance after high-intensity interval training by well-trained cyclists. *European journal of applied physiology and occupational physiology*, *75*(1), pp.7-13.

WILLIAMSON, J.R., SCHAFFER, S., FORD, C. and SAFER, B., 1976. Contribution of tissue acidosis to ischemic injury in the perfused rat heart. *Circulation*, *53*(3), pp. 13-14.

WILKIE, D.R., 1979. Generation of protons by metabolic processes other than glycolysis in muscle cells: a critical view. *Journal of molecular and cellular cardiology*, *11*(3), p.325.

WILSON, P.B. and INGRAHAM, S.J., 2015. Glucose-fructose likely improves gastrointestinal comfort and endurance running performance relative to glucose-only. *Scandinavian journal of medicine & science in sports*, *25*(6), pp.613-620.

WITARD, O.C., JACKMAN, S.R., BREEN, L., SMITH, K., SELBY, A. and TIPTON, K.D., 2014. Myofibrillar muscle protein synthesis rates subsequent to a meal in response to increasing doses of whey protein at rest and after resistance exercise. *The American journal of clinical nutrition*, *99*(1), pp.86-95.

WORTHLEY, L.I.G., 1977. Hydrogen ion metabolism. *Anaesthesia and intensive care*, *5*(4), pp.347-360.

YEOMANS, M.R., 1998. Taste, palatability and the control of appetite. *Proceedings of the Nutrition Society*, *57*(4), pp.609-615.

ZILVA, J.F., 1978. The origin of the acidosis in hyperlactataemia. *Annals of Clinical Biochemistry*, *15*(1-6), pp.40-43.