Title: The effects of sodium bicarbonate supplementation at individual time-to-peak blood bicarbonate on 4-km cycling time trial performance in the heat

Authors: Dr Lewis A Gough^{1*}., Mr Jake J Williams¹., Mr Josh W Newbury¹., and Mr William H Gurton²

Affiliations: ¹Human Performance and Health Research Group, Centre for Life and Sport Sciences Research Centre, Birmingham City University, Birmingham, United Kingdom ²Department of Sport, Exercise and Rehabilitation Sciences, Canterbury Christ Church University, Canterbury, United Kingdom.

*Corresponding author: Dr Lewis Gough, lewis.gough@bcu.ac.uk

ABSTRACT

The purpose of this study was to explore the effect of individualised sodium bicarbonate (NaHCO₃) supplementation according to a pre-established individual time-to-peak (TTP) blood bicarbonate (HCO₃) on 4-km cycling time trial (TT) performance in the heat. Eleven recreationally trained male cyclists (age: 28 ± 6 years, height: 180 ± 6 cm, body mass: 80.5 ± 8.4 kg) volunteered for this study in a randomised, crossover, triple-blind, placebo-controlled design. An initial visit was conducted to determine TTP HCO₃⁻ following 0.2 g.kg⁻¹ body mass (BM) NaHCO₃ ingestion. Subsequently, on three separate occasions, participants completed a 4-km cycling TT in the heat (30 degrees centigrade; °C) (relative humidity ~40%) following ingestion of either NaHCO₃ (0.2 g.kg⁻¹ body mass), a sodium chloride placebo (0.2 g.kg⁻¹ BM; PLA) or no supplementation (control; CON) at the predetermined individual TTP HCO₃⁻. Absolute peak [HCO₃⁻] prior to the 4-km cycling TT's was elevated for NaHCO₃ compared to PLA (+2.8 mmol.l⁻¹; p = 0.002; g = 2.2) and CON (+2.5 mmol.l⁻¹; p < 0.001; g = 2.1). Completion time following NaHCO₃ was 5.6 \pm 3.2 s faster than PLA (1.6%; CI: 2.8, 8.3; p = 0.001; g = 0.2) and 4.7 ± 2.8 s faster than CON (1.3%; CI: 2.3, 7.1; p = 0.001; q = 0.2). These results demonstrate that NaHCO₃ ingestion at a pre-established individual TTP HCO₃⁻ improves 4-km cycling TT performance in the heat, likely through enhancing buffering capacity.

Keywords: alkalosis, supplements, track cycling, buffering, environmental physiology

HIGHLIGHTS

- This is the first time NaHCO₃ ingestion has been shown to improve 4-km cycling TT performance in conditions of high ambient heat.
- A smaller dose of NaHCO₃ (0.2 g.kg⁻¹ BM) is ergogenic in the heat, which is smaller than the dose typically ingested for sports performance (0.3 g.kg⁻¹ BM). This is important, as gastrointestinal discomfort is typically lower as the dose reduces.
- This study suggests that the individualised time-to-peak HCO₃⁻ ingestion strategy with lower doses of NaHCO₃ is an ergogenic strategy in conditions of high ambient heat.

1 INTRODUCTION

2 Athletes who compete indoors or internationally are frequently exposed to warm-hot ambient environments, as such events are commonly held in these climates (e.g., Summer 3 4 Olympic Games). Equally, many athletes worldwide are required to perform in warm-hot environments during the warmest season, or at the hottest part of the day. This is apparent 5 6 during indoor track cycling events where temperatures are approximately 28-34°C 7 (depending on the location and ventilation quality); whilst in most cases air conditioning is 8 not available. Indeed, at the most recent Olympic games (Tokyo, 2020) daily temperatures exceeding 30°C were common (Costa et al., 2019), which creates a greater physiological 9 challenge for the athlete compared to competing in a thermoneutral environment. 10 Specifically, exercise in warm-hot environments causes alterations to muscle energy 11 12 metabolism, such that in the scenario of high-intensity exercise over 1 min, there is a significant shift in contribution of adenosine triphosphate (ATP) supply from aerobic to 13 anaerobic energetic systems, such as anaerobic glycolysis (Fink et al., 1975; Febbraio et al., 14 1994; Febbraio et al., 2001). Combine this with greater level of extracellular lactate during 15 exercise in the heat versus thermoneutral conditions for the same given exercise, this 16 suggests a greater level of acidosis is present (Robers, 2004). Unsurprisingly, Mitchell et al., 17 (2014) reported a significant reduction in exercise capacity (time to exhaustion) in hot 18 19 environments (37°C) compared to cool environments (10°C) at both 80% and 100% VO_{2max}. It 20 is intuitive to suggest therefore that nutritional interventions that could support ATP production via anaerobic energy pathways and reduce acidic stress may mitigate the 21 deleterious physiological changes caused by exercising in the heat. 22

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24 Sodium bicarbonate (NaHCO₃) supplementation is a well-known alkalotic buffer, which can increase circulating blood bicarbonate (HCO₃⁻) within the extracellular compartments 25 (typically around 5-6 mmol.l⁻¹). Typical dosage is between 0.2-0.4 g.kg⁻¹ body mass (BM), 26 although a 0.3 g.kg⁻¹ BM dose is most widely accepted as being optimal for performance 27 28 enhancement (McNaughton et al., 2016). Whilst some authors suggest acidosis is not a contributor to fatigue (see Westerblad, 2016), ingestion of NaHCO₃ is suggested to increase 29 30 extracellular buffering capacity and therefore improve intramuscular hydrogen cation (H⁺) handling. Specifically, upregulation of the lactate-H⁺ co-transporter to efflux H⁺ from the 31

32 active musculature into circulation may occur, which subsequently maintains the acid base balance of the intracellular (muscle) compartments (Bishop et al., 2004; Mainwood and 33 Worsley-Brown, 1975). Moreover, as H⁺ provides competition at the troponin-binding site, 34 35 the greater efflux of H⁺ from the muscle cytosol caused by NaHCO₃ supplementation could facilitate improved cross-bridge cycling (Fabiato and Fabiato, 1978; Knuth et al., 2006). This, 36 37 in theory, should allow for sustained muscle contractions during high-intensity exercise. Based on these mechanisms, the supplementation of NaHCO₃ could support the increased 38 demand for anaerobic energy production that is evident under thermal stress, and in turn, 39 40 improve exercise performance. Despite this, only Mündel (2018) has investigated NaHCO₃ 41 ingestion (0.5 g.kg⁻¹ BM) in conditions of ambient heat stress (30° C) and reported that total 42 work done (kJ) was improved in the second bout of two 30 s Wingate tests compared to a placebo (effect size = 0.35; p < 0.05). Based on the encouraging findings, it is plausible that the 43 44 use of NaHCO₃ could be ergogenic for other types of performance including, but not limited 45 to, track cycling events.

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To date, several studies have reported ergogenic effects of NaHCO₃ during a 4-km cycling time 47 trial (TT) when ingestion was aligned with an individualised time-to-peak (TTP) HCO₃⁻ (Gough 48 et al., 2018a; 2018b; 2019; Hilton et al., 2020; Gurton et al., 2021a). Some of these studies 49 50 have also examined the use of 0.2 g.kg⁻¹ BM NaHCO₃, which elicited almost identical ergogenic benefits compared to the traditionally administered 0.3 g.kg⁻¹ BM NaHCO₃ dose (Gough et al., 51 2018a; 2018b; 2019). Indeed, Gough et al. (2018a) reported no difference in time to complete 52 a 4-km cycling TT between these two doses (Hege's g effect size (g) = 0.02, p = 0.870), whilst 53 both were significantly faster than the placebo ($g 0.2 \text{ g.kg}^{-1} \text{ BM} = 0.64$, $g 0.3 \text{ g.kg}^{-1} \text{ BM} = 0.66$; 54 both p <0.05). Considering that smaller doses of NaHCO₃ lead to lower gastrointestinal (GI) 55 56 discomfort and sodium load (Gough et al., 2017; Gurton et al., 2020), and that track cyclists are likely to repeatedly supplement NaHCO₃ over a few events within 48-72 hours, the smaller 57 58 dose may be a more attractive option. The aim of this study therefore, was to investigate 0.2 g.kg⁻¹ BM NaHCO₃ ingestion supplemented at each individuals peak HCO₃⁻ on 4-km cycling TT 59 performance in conditions of high ambient heat. The hypothesis of this study was that 60 NaHCO₃ ingestion would improve 4-km TT cycling performance in the heat compared to the 61 62 placebo.

64 METHODS

65 *Participants*

Eleven male recreationally trained cyclists (de Pauw et al., 2013) (180 ± 6 cm; 80.5 ± 8.4 kg; 66 67 28 ± 6 years) volunteered for this study. Whilst a priori power calculation was not conducted, 68 our sample size was identical to previous research highlighting n = 11 would be required to satisfy statistical power for a 4-km cycling TT, although this study was conducted in 69 70 thermoneutral conditions (Gough et al. 2019). All protocols were submitted to, and approved by, an institutional review board for testing of human subjects (Gough/3647/Mod/2019/Sep 71 72 /HELSFAEC), and all participants provided written informed consent prior to any experimental 73 procedure.

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75 Experimental overview

Participants visited the laboratory on five separate occasions in a block randomised, 76 77 crossover, triple-blind, placebo-controlled study. Each participant was required to record 78 nutritional intake 24 hours prior to laboratory visits and encouraged to maintain identical 79 nutritional ingestion throughout the study. During the initial laboratory visit, a 24-hour dietary 80 recall was conducted, and participants were asked to replicate their intake before subsequent visits to the laboratory. Participants arrived 3 hours post-prandial to each trial, which were 81 conducted at a similar time of day (± 1 hour) to manage the effects of circadian rhythms 82 (Reilly, 1990). Trials were separated by a minimum of three days and maximum of five days 83 to reduce the influence of training adaptations (Drust et al., 2005). Participants were not 84 85 ingesting any supplements that could interfere with the results during the study, and none 86 had ingested beta alanine previously.

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88 **Pre-experimental procedures**

The initial visit involved ingestion of 0.2 g.kg⁻¹ BM NaHCO₃ (Bicarbonate of Soda, Dr Oatker, United Kingdom) to determine TTP HCO₃^{-.} Following ingestion of all vegetarian capsules (size 00, Bulk Powders, UK) within a 5 min period, a stopwatch was started, and participants

92 remained seated for 120 min. Finger prick blood samples were collected at baseline in 70µl heparin-coated capillary tubes, following 30 min and subsequently every 15 min using a blood 93 gas analyser (ABL9, Radiometer, Denmark) to analyse blood HCO_3^- . TTP HCO_3^- was 94 determined by the highest HCO₃⁻ value (absolute-peak) and this established the ingestion 95 96 timing for each participant during experimental trials. Using a visual analogue scale (Miller et 97 al., 2016), GI discomfort was recorded at baseline and every 15 min until participants reached their respective TTP HCO₃⁻. The scale was a 20 cm horizontal line that was anchored with 'no 98 symptom' to the left and 'severe discomfort' to the right. 99

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101 Experimental procedures

102 The following four separate visits involved the completion of a 4-km cycling TT, with one of 103 those visits including a familiarisation trial on a Wattbike cycle ergometer (Nottingham, UK). 104 All trials were conducted in an environmental chamber (Altitude Centre, UK) maintained at 105 30°C (~40% relative humidity). These conditions were selected, as these were the most 106 commonly reported temperatures upon consultation with track cyclists known to the lead author during pilot work. Participants selected their preferred positions on the Wattbike (i.e., 107 108 saddle and handlebar) and remained seated (10 min) to accustom to the conditions. Following 109 an individualised warm-up that participants selected based on the knowledge they were preparing for a 4-km TT, participants then passively rested for 5 min and then performed the 110 4-km cycling TT as quickly as possible. The individualised warm-up was replicated for each 111 112 experimental trial. This protocol was selected because of its ecological validity and reproducibility (Stone et al., 2011), but also as it would cause sufficient disturbances in acid 113 base balance (Gough et al., 2018a), thus providing an appropriate protocol to examine the 114 115 operating mechanisms of NaHCO₃.

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During each trial, the Wattbike monitor was blinded from the participant, however, verbal encouragement and distance covered was provided. Time to complete (s), mean power (W), and speed (km.h⁻¹) were recorded for the total 4-km distance. Heart rate (HR) (Polar Electro, Finland) and whole-body rating of perceived exertion (RPE) were recorded at rest and every 1 km (scale, 6-20; Borg, 1982). Tympanic temperature was recorded using an infrared tympanic thermometer (Braun ThermoScan[®] 7 IRT 6520, Braun GmbH, Kornberg, Germany) outside and 10 min in the chamber along with post warm-up and post exercise. Blood 124 measures for acid-base balance (HCO₃⁻, and pH) were recorded at baseline, absolute-peak and post-exercise using a blood gas analyser (ABL9, Radiometer Medical Ltd., Denmark). 125 Blood lactate (BLa⁻) was also measured (0.3µl sample) using a portable lactate analyser 126 (Lactate Pro 2, Arkray, Japan) at the same time points. Participants repeated this experimental 127 trial three occasions apart from ingesting either: 0.2 g.kg⁻¹ BM NaHCO₃, 0.2 g.kg⁻¹ BM sodium 128 chloride (placebo; PLA) in vegetarian capsules (size 00, Bulk Powders, UK) or no 129 supplementation (control; CON). Capsules were identical in appearance and the volume of 130 water was recorded and replicated for any subsequent trial (mean = 452 ± 34 ml). A laboratory 131 132 technician that was not involved in the research had manually prepared and administered the 133 supplementation of NaHCO₃ in a block-randomised order to ensure the study remained 134 blinded. Participants remained seated following NaHCO₃ or PLA ingestion until their respective absolute-peak, with only water permitted *ab-libitum*. Once this was achieved, 135 136 participants began their individualised warm-up. Lastly, GI discomfort was recorded every 15 137 min, as per the previously defined method. At the end of each experimental trial, a supplement belief questionnaire was used to assess the blinding efficacy, as per previous 138 research (Gough et al., 2018a; 2018b; Gurton et al., 2021b). 139

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141 Statistical analysis

All data were assessed for normality using both Shapiro-Wilks tests and graphical methods 142 including skewness and kurtosis. Homogeneity of variance/sphericity were analysed using 143 144 Mauchly tests and any violations were corrected via Greenhouse-Geisser adjustments. The reproducibility of peak change in HCO₃⁻ (TTP visit vs. NaHCO₃ treatment) and 4-km cycling TT 145 performance (PLA vs CON treatments) was determined using Intraclass Correlation 146 Coefficients (ICC's) and categorised as poor ($r = \le 0.40$), fair (r = 0.40 - 0.59), good (r = 0.60 - 0.59) 147 148 0.74) or excellent ($r = \ge 0.74$). One-way repeated measures ANOVA were conducted on 4-km cycling TT performance (completion time, mean power, mean speed). To determine individual 149 150 changes in time to complete the smallest worthwhile change (SWC) statistic was used (0.2 * between-subject standard deviation; SD) (Paton and Hopkins, 2006). Two-way repeated 151 measures ANOVA were used to analyse blood metabolite responses (HCO₃⁻, pH, H⁺ and BLa⁻), 152 HR, tympanic temperature and RPE. Significant two-way interactions (treatment x time) were 153 154 explored further by conducting post hoc testing with bonferroni correction factors. The 155 assumption of normal distribution was violated for GI discomfort, therefore Wilcoxon 156 matched-paired signed-rank tests were conducted as the non-parametric alternative, with median and z values reported. For ANOVA interactions and main effects, the effect size is 157 reported as partial eta squared (η_p^2) . Between treatment effect sizes (g) were calculated by 158 159 dividing mean difference by the pooled SD (Nakagawa and Cuthill, 2007) and applying Hedge's g bias correction to account for the small sample size (Lakens, 2013). The interpretations of 160 these effect sizes were categorised as trivial (≤ 0.2), small (0.2 – 0.49), moderate (0.5 – 0.79) 161 or large (\geq 0.8) (Cohen, 1988). Effect sizes for non-normally distributed data (r) were 162 calculated from z/vn, with 0.10, 0.24 and 0.37 considered as small, medium and large, 163 164 respectively (Ivarsson et al., 2013). Data are presented as Mean ± SD (unless stated otherwise) 165 and 95% confidence intervals (CI) reported for differences in performance, with variances that 166 do not cross the zero-boundary treated as significant (Gardner and Altman, 1986). Statistical 167 significance was set at p < 0.05 and all statistical data were analysed using SPSS software 168 version 26 (IBM, Chicago, IL, USA).

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170 **RESULTS**

171 **4-km cycling time trial performance**

No trial order effect was reported from the performance data (completion time) (p = 0.972, 172 173 $\eta p^2 = 0.003$). Completion time following NaHCO₃ was 5.6 ± 3.2 s faster than PLA (1.6%; CI: 2.8, 8.3; p = 0.001; q = 0.2) and 4.7 ± 2.8 s faster than CON (1.3%; CI: 2.3, 7.1; p = 0.001; q = 0.2). 174 175 In total, six out of eleven participants reported 'true' improvements following NaHCO3 ingestion (i.e., change in performance vs. either CON or PLA above the SWC). Excellent 176 177 reproducibility was observed for completion time between PLA and CON treatments (ICC = 0.998; p < 0.001). Mean and inter-individual variation for 4-km completion time is displayed 178 in Figure 1 and Table 1. Only two participants were able to identify the NaHCO₃ treatment 179 180 correctly, with all other participants stating they 'don't know'.

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182 [INSERT Figure 1 NEAR HERE]

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184Mean power across the 4-km distance following NaHCO3 increased by 10.0 ± 7.1 W185compared to PLA (+3.6%; CI: 3.9, 16.1; p = 0.003; g = 0.2) and by 6.7 ± 8.7 W compared to CON186(+2.4%; CI: -0.8, 14.3; p = 0.085; g = 0.1). Mean speed following NaHCO3 was 0.6 ± 0.4 km.h⁻¹

higher than PLA (+1.5%; CI: 0.3, 1.0; p = 0.001; g = 0.2) and 0.5 ± 0.5 km.h⁻¹ higher than CON (+1.1%; CI: 0.1, 0.9; p = 0.025; g = 0.2).

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190 Blood metabolite response

During the preliminary TTP visit, absolute change in $[HCO_3^-]$ was 2.6 ± 0.9 mmol.l⁻¹ (range, 1.5 - 4.4 mmol.l⁻¹) and occurred between 30 and 105 min (62.7 ± 19.0 min; CV = 30%). Absolute change in blood pH was 0.04 ± 0.02 AU (range, 0.02 – 0.07 AU) and occurred between 30 and 105 min (69.5 ± 21.5 min; CV = 31%). These changes in $[HCO_3^-]$ from baseline to peak value displayed excellent reproducibility when compared to absolute change following NaHCO₃ during cycling trials (+2.6 mmol.l⁻¹; ICC = 0.976; *p* < 0.001).

197 Significant treatment x time interactions were observed during the cycling trials for $[HCO_3^{-1}]$ (p < 0.001; $\eta_p^2 = 0.628$), blood pH (p = 0.018; $\eta_p^2 = 0.359$), $[H^+]$ (p = 0.022; $\eta_p^2 = 0.350$) 198 and [BLa⁻] (p < 0.001; $\eta_p^2 = 0.708$). Absolute peak [HCO₃⁻] prior to the 4-km cycling TT's was 199 elevated for NaHCO₃ compared to PLA (+2.8 mmol.l⁻¹; p = 0.002; g = 2.2) and CON (+2.5 200 201 mmol.¹; p < 0.001; g = 2.1). Peak blood pH was also higher for NaHCO₃ compared to PLA (+0.048 AU; p = 0.007; g = 1.9) and CON (+0.061 AU; p = 0.055; g = 1.1). Prior to the 4-km 202 203 cycling TT's, [H⁺] was lower following NaHCO₃ compared to PLA (-4.5 nmol.l⁻¹; p = 0.008; g =1.8) and CON (-6.3 nmol.l⁻¹; p = 0.099; g = 1.0). Inter-individual differences for the change in 204 205 [HCO3-] from baseline to peak are displayed in **Table 1**. Absolute decline in [HCO₃⁻] during the 206 4-km cycling TT's for NaHCO₃ was 4.4 ± 3.4 mmol. l^{-1} greater than PLA (p = 0.005; g = 1.8) and 4.1 ± 2.1 mmol.¹ greater than CON (p < 0.001; g = 2.2). The total H⁺ efflux during the 4-km 207 cycling TT's for NaHCO₃ was 7.3 \pm 6.7 nmol.l⁻¹ higher than PLA (p = 0.014; g = 0.8) and 12.8 \pm 208 14.6 nmol.l⁻¹ higher than CON (p = 0.047; g = 1.0). Post-exercise [HCO₃⁻] was lower following 209 NaHCO₃ compared to PLA (-1.5 mmol. l^{-1} ; p = 0.046; q = 0.7) and CON (-1.6 mmol. l^{-1} ; p = 0.01; 210 g = 0.9). Peak [BLa⁻] after exercise was elevated following NaHCO₃ compared to PLA (+2.76) 211 mmol.l⁻¹; p = 0.001; g = 1.2) and CON (+2.53 mmol.l⁻¹; p = 0.002; g = 1.1). There were no 212 differences at any time point between PLA and CON treatments (all p > 0.05). Mean ± SD for 213 214 blood metabolite response is displayed in Figure 2 (A-D).

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216 [INSERT **Table 1** NEAR HERE]

- 217 [INSERT Figure 2 (A-D) NEAR HERE]
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219 Heart rate, tympanic temperature and perceived exertion

Significant treatment x time interactions were observed for HR (p = 0.036; $\eta_p^2 = 0.196$), but not tympanic temperature (p = 0.892; $\eta_p^2 = 0.036$) or RPE (p = 0.066; $\eta_p^2 = 0.199$). At the 3-km segment, HR was elevated following NaHCO₃ compared to PLA (+5 b.min⁻¹; p = 0.008; g = 0.6) and CON (+4 b.min⁻¹; p = 0.053; g = 0.5). Maximum HR at the end of the 4-km cycling TT was elevated following NaHCO₃ compared to PLA (+5 b.min⁻¹; p = 0.027; g = 0.7) and CON (+5 b.min⁻¹; p = 0.063; g = 0.5).

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227 Gastrointestinal discomfort

Aggregate GI discomfort was exacerbated for NaHCO₃ compared to PLA (median, 16 vs. 2 cm) and displayed a large effect size (z = -2.807; p = 0.005; r = 0.8). Severity score for peak GI symptom was higher for NaHCO₃ compared to PLA (median, 5 vs. 1 cm) and displayed a large effect size (z = -2.670; p = 0.008; r = 0.8; **Table 2**).

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233 [INSERT Table 2 NEAR HERE]

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235 DISCUSSION

This study aimed to investigate the effects of NaHCO3 ingestion on 4-km cycling TT 236 237 performance in conditions of high ambient heat. In agreement with the hypothesis, this study reports, for the first time, that NaHCO₃ ingestion improves 4-km cycling TT performance in 238 239 the heat. The current study findings are therefore more applicable to real-world track cycling, which are typically performed in higher temperatures than the thermoneutral conditions 240 241 used in previous laboratory research. These findings also suggest that exercise of a similar intensity and duration might also benefit from the use of NaHCO₃ as an ergogenic strategy 242 243 (e.g. track and field). Moreover, the current study is the first to demonstrate that an acute, lower dose of NaHCO₃ (0.2 g.kg⁻¹ BM) is a viable strategy to improve performance in high 244 ambient heat conditions; therefore, corroborating previous findings in thermoneutral 245 conditions (Gough et al., 2018a; 2018b). Based on GI discomfort typically being reduced from 246 lower doses of NaHCO₃ (i.e. 0.2 g.kg⁻¹) this might be a more attractive option in a practical 247 setting compared to larger doses (i.e. 0.3 g.kg⁻¹) (Gough et al., 2017; McNaughton, 1992). 248 249 Further research is warranted investigating the use of NaHCO₃ ingestion during other exercise 250 modalities that are often conducted in the heat (e.g., Olympic sports).

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The current study supports previous research investigating NaHCO₃ ingestion in high ambient 252 heat, whereby improvements in performance were observed during the second bout of 253 repeated Wingate tests (2 x 30 s interspersed with 5 min active recovery) following chronic 254 ingestion of 0.5 g.kg⁻¹ BM NaHCO₃ ingestion split into three equal doses across an eight hour 255 256 period (Mündel, 2018). Whilst multiple differences in experimental approach between the 257 authors and the current study exist (e.g., ingestion dose and timing, exercise mode and duration), the present study adds novel findings such that NaHCO₃ is ergogenic for longer 258 259 duration exercise in the heat, and arguably within a more ecologically valid protocol (4-km 260 cycling TT vs Wingate). The ergogenic effect in the current study was also more profound 261 compared to the findings of (Mündel, 2018), which may be due to the sub-maximal intensity 262 of the 4-km cycling TT compared to that of the supramaximal intensity Wingate protocol of 263 Mündel (2018). This is due to the potential for supramaximal intensity exercise to cause a 264 rapid decline in pH that completely saturates the monocarboxylate transporter with H⁺ and 265 therefore leaving no capacity for NaHCO₃ to cause ergogenic effects (Messonnier et al., 2007). Moreover, the current study offers novelty as a smaller, acute dose of 0.2 g.kg⁻¹ BM NaHCO₃ 266 267 was ergogenic despite mild GI discomfort. This corroborates with previous research reporting similar ergogenic effects of smaller NaHCO₃ doses during an identical exercise protocol in 268 thermoneutral and hypoxic conditions (Gough et al., 2017, 2018b, 2019). It is likely that the 269 270 use of the individualised time-to-peak HCO₃⁻ strategy ensured the NaHCO₃ dose was 271 ergogenic, as previous research using a standardised time point of ingestion (i.e., 60 min prior to exercise for all participants) has shown the 0.2 g.kg⁻¹ BM dose was not effective 272 (McNaughton, 1992). Future studies should compare various ingestion strategies and doses 273 274 to continue optimising the effectiveness of NaHCO₃ in environments of high ambient heat, 275 whilst also striving to balance the side effects and performance benefits.

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The ergogenic effect of NaHCO₃ ingestion on performance in high ambient heat corroborates with findings in thermoneutral environments (Gough et al., 2018a), which is surprising given the additional anaerobic demand in the heat that would potentially suggest this supplement would be more ergogenic compared to thermoneutral conditions. Other research supplementing NaHCO₃ in hypoxia also display similar findings (Deb et al., 2017; Gough et al., 2018b, 2019), all of which are also more demanding of anaerobic energy production. It is also 283 suggested that pre-exercise increases in plasma volume could be ergogenic in conditions of high ambient heat (Sims et al., 2007a, 2007b), to which this mechanism should be evident 284 following alkalizing agents (Vaher et al., 2015). Indeed, Vaher et al. (2015) reported following 285 286 ingestion of sodium citrate (500 mg.kg⁻¹) 120 min prior to exercise, plasma volume increased 287 by around 4%. It might have been the short duration of the current studies exercise protocol 288 that nullified any impact of plasma volume, however, as the current study and other published studies to date (Mündel, 2018) employing NaHCO₃ supplementation in the heat 289 have ranged from between 30 s to 7 min, whereas this mechanism is more likely relevant to 290 291 longer duration exercise. Future research could therefore investigate the effectiveness of 292 NaHCO₃ ingestion in conditions of heat versus temperate conditions, whilst also using longer 293 duration exercise protocols. This would help identify how far-reaching the ergogenic 294 mechanisms of NaHCO₃ ingestion could be in conditions of high ambient heat.

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296 The current study findings support previous work on cycling time trials performed in 297 thermoneutral conditions that report ergogenic effects following NaHCO₃ ingestion (Gough et al., 2018a, 2018b; Gurton et al., 2021a; Hilton et al., 2020). The rise in extracellular acid 298 299 base balance likely explains the improved performance following NaHCO₃ ingestion, as such increases are suggested to increase H⁺ efflux due to the upregulation of the lactate/H⁺ 300 301 cotransporter, and subsequently more H⁺ is buffered (Marx et al., 2002). These biochemical changes are suggested to offer protective effects to cross-bridge binding and Ca²⁺ handling 302 (Fitts, 2008; 2016). Support for this can be found from both the significantly increased 303 absolute decline in HCO₃⁻ during the 4-km cycling TT (i.e., greater amount of extracellular 304 buffering) and the elevated post-exercise lactate for the NaHCO₃ versus PLA treatment, which 305 306 are both indicative of a higher exercise intensity following NaHCO₃ ingestion. Equally, the H⁺ 307 analysis in the current study corroborates this notion, as this metabolite was lower preexercise in the NaHCO₃ condition vs. PLA/CON, but was the same at the post-exercise time 308 309 point, thus inferring a greater H⁺ efflux from muscle during the 4-km cycling TT. These findings of the current study must be interpreted with caution as the measurements were only 310 extracellular and therefore, we cannot account for changes at the muscular level. 311 Nonetheless, NaHCO₃ ingestion likely improves buffering capacity and will lead to improved 312 313 exercise performance in most participants.

315 The increase in HCO₃⁻ from baseline to absolute peak following NaHCO₃ ingestion were low $(2.6 \pm 0.9 \text{ mmol.}^{-1})$ in comparison to the typical average increase in previous research 316 (Renfree, 2007; Siegler et al., 2010; Jones et al., 2016; Gough et al., 2017). Indeed, Gough et 317 al. (2017) reported the mean increase of HCO_3^- following 0.2 g.kg⁻¹ BM NaHCO₃ was 5.7 ± 0.9 318 and 5.6 \pm 1.1 mmol.l⁻¹ in repeated trials, with 13 out of 15 participants achieving > 5 mmol.l⁻¹ 319 ¹. In fact, in the current study not a single participant reached the 5 mmol.l⁻¹ increase from 320 321 baseline to peak HCO₃⁻ that is a purported threshold to ensure the ergogenic effect from NaHCO₃ ingestion (Carr et al., 2011). Despite this, a significant improvement in performance 322 323 was reported in this study, and in particular, three participants displayed increases in HCO₃⁻ 324 of $\leq 2 \text{ mmol.}^{1}$, however, improved their performance above the SWC of the test protocol. Similarly, one participant reported a 4.5 mmol.l⁻¹ increase in HCO₃⁻, however did not improve 325 their performance above the SWC. These findings subsequently question the requirement to 326 327 ingest such large doses of NaHCO₃ (Gough et al., 2019), as reaching a threshold increase in 328 HCO_{3⁻} does not seem as important as previous research suggests (Jones et al., 2016; Oliveira 329 et al., 2020). The reason why such low increases in HCO₃⁻ were observed might be due to the known inter-individual variation in HCO₃⁻ changes following NaHCO₃ supplementation, and 330 that the current study employed a lower dose of NaHCO₃ (Jones et al., 2016; Gough et al. 331 332 2017). Future research should attempt to explore reasons why such a large inter-individual variation in bicarbonate uptake occurs (i.e., pre-exercise procedures, training status, 333 genetics), and how this uptake relates to the resulting performance effect. 334

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336 CONCLUSION

The ingestion of 0.2 g.kg⁻¹ BM NaHCO₃ is a suitable ergogenic aid for improving 4-km TT cycling performance in ambient heat. Athletes and coaches can therefore employ this strategy with a high degree of confidence that an ergogenic effect will be observed. Lower doses of NaHCO₃ may also be attractive due to the lower sodium load and the potential to reduce GI discomfort, compared to the most commonly ingested 0.3 g.kg⁻¹ BM NaHCO₃ dose. Future research should explore the use of NaHCO₃ in high ambient heat conditions in a range of other competitive events and/or training bouts.

344

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347

348 FUNDING DETAILS

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- 351 DISCLOSURE STATEMENT
- No financial interest or benefit has arisen from the direct applications of this research.

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534 **FIGURE LEGENDS**

535

Figure 1 Mean ± SD and inter-individual variation (dashed lines) for 4-km cycling TT
completion times in the heat. ** denotes NaHCO₃ significantly faster (*p* < 0.05) compared to
placebo (PLA) and control (CON).
Figure 2 (A-D) Mean ± SD blood metabolite response (A: Blood bicarbonate, HCO₃⁻; B: blood

541 pH; C: Hydrogen cations, H⁺; D: Blood lactate, BLa⁻) from baseline to post-exercise. Some error

bars have been removed for clarity. Symbols denote significant difference (p < 0.05): *

543 NaHCO₃ vs. placebo (PLA); ** NaHCO₃ vs. placebo (PLA) and control (CON).

544 **TABLES**

Particinant	4-km TT performance (s)		Change in [HCO ₃ -] (mmol.l ⁻¹)	
i articipant	NaHCO₃ vs. PLA	NaHCO₃ vs. CON	NaHCO₃ vs. PLA	NaHCO₃ vs. CON
1	-6.7	-2.8	5.7	3.6
2	-11.0	-6.7	0.5	1.1
3	-4.2	-3.3	0.5	0.2
4	-7.3	-6.6	2.7	2.2
5	-10.7	-9.7	1.7	1.7
6	-5.8	-7.8	1.5	0.5
7	-4.0	-0.6	2.1	1.0
8	-3.5	-3.4	3.9	4.1
9	-0.5	-1.6	2.5	2.3
10	-3.4	-4.5	4.8	4.3
11	-4.2	-4.7	2.8	2.4

Table 1 Inter-individual differences for changes in 4-km cycling TT performance and absolute increase in $[HCO_3^-]$ from baseline to absolute peak.

Abbreviations: HCO_3^- , blood bicarbonate; *TT*, time trial; $NaHCO_3$, sodium bicarbonate; *PLA*, placebo; *CON*, control. Changes in 4-km TT performance highlighted as bold text represent improvements that achieved the smallest worthwhile change (-4.7 s).

Participant	NaHCO ₃	PLA	CON
1	Belching (1)	Bloating (1)	Nil <i>(0)</i>
2	BUR <i>(3)</i>	AD (2)	Nil <i>(0)</i>
3	Belching (8)	Nil <i>(0)</i>	Nil <i>(0)</i>
4	Belching (7)	Nil <i>(0)</i>	Nil <i>(0)</i>
5	Vomiting (5)	Nausea (2)	Nil <i>(0)</i>
6	Nil <i>(0)</i>	Nil <i>(0)</i>	Nil <i>(0)</i>
7	Belching (10)	Vomiting <i>(9)</i>	Nil <i>(0)</i>
8	Belching (13)	AD (7)	Nil <i>(0)</i>
9	Diarrhoea (18)	AD (2)	AD (2)
10	Belching (4)	Nil <i>(0)</i>	Nil <i>(0)</i>
11	AD <i>(4)</i>	Nil <i>(0)</i>	Nil <i>(0)</i>

Table 2 Peak gastrointestinal (GI) discomfort severity score experienced byeach participant during each experimental trial

Abbreviations: *NaHCO*₃, sodium bicarbonate; *PLA*, placebo; *CON*, control; *BUR*, bowel urgency rating; *AD*, abdominal discomfort. Symptom severity score (on a scale of 0 to 20) is displayed in parenthesis.

547

548 FIGURES









555 Figure 2 (A-D)