Title: Evaluating the effects of caffeine and sodium bicarbonate, ingested individually or in combination, and a taste matched placebo on high-intensity cycling capacity in healthy males.

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Abstract

This study evaluated the effects of ingesting sodium bicarbonate ($NaHCO_3$) or caffeine individually, or in combination, on high-intensity cycling capacity. In a counterbalanced, crossover design, thirteen healthy non-cycling trained males (age: 21±3 years, height: 178±6 cm, body mass: 76±12 kg, W_{PEAK}: 230±34 W, $\dot{V}O_{2PEAK}$: 46±8 $ml.kg^{-1}.min^{-1}$) performed a graded incremental exercise test, two familiarisation trials and four experimental trials. Trials consisted of cycling to volitional exhaustion at 100% W_{PEAK} (T_{LIM}) 60 min after ingesting a solution containing either: (1) 0.3 $g.kg^{-1}$ body mass sodium bicarbonate (BIC), (2) 5 $mg.kg^{-1}$ body mass caffeine plus 0.1 $g.kg^{-1}$ body mass sodium chloride (CAF), (3) 0.3 $g.kg^{-1}$ body mass sodium bicarbonate plus 5 $mg.kg^{-1}$ body mass caffeine (BIC-CAF) or (4) 0.1 $g.kg^{-1}$ body mass sodium chloride (PLA). Experimental solutions were administered double-blind. Pre-exercise, at the end of exercise and 5 min post-exercise blood pH, base excess [BE] and bicarbonate ion concentration [HCO_3^-] were significantly elevated for BIC and BIC-CAF compared with CAF and PLA. T_{LIM} (median; interquartile range (IQR)) was significantly greater for CAF (399; 350-415 s; P=0.039; r=0.6) and BIC-CAF (367; 333-402 s; P=0.028; r=0.6) compared to BIC (313: 284-448 s) although not compared to PLA (358; 290-433 s; P=0.249, r=0.3 and P=0.099 and r=0.5, respectively). There were no differences between PLA and BIC (P=0.196; r=0.4) or between CAF and BIC-CAF (P=0.753; r=0.1). Relatively large inter and intra individual variation was observed when comparing treatments and therefore an individual approach to supplementation appears warranted.

Key words: Caffeine, Sodium bicarbonate, Alkalosis, Metabolic buffers, Cycling

Introduction

The individual ergogenicity of both caffeine and sodium bicarbonate ($NaHCO_3$) has been extensively evaluated *in vivo* (Higgins et al. 2013a; Meyers and Cafarelli 2005; Simmonds et al. 2010) and *in vitro* (Higgins et al. 2013b; Tallis et al. 2012; Tallis et al. 2013). Although not uniformly equivocal, recent evidence suggests that individually both caffeine (Simmonds et al. 2010) and $NaHCO_3$ (Higgins et al. 2013a) can exert ergogenic benefits on high-intensity exercise capacity (+15% at 120% $\dot{V}O_{2PEAK}$ and +17% at 100% peak power output (W_{PEAK}), respectively). With caffeine and $NaHCO_3$ eliciting performance enhancing effects by different mechanisms, a synergistic effect may occur resulting in substantial performance gains. However, to the best of our knowledge only four studies have evaluated the effects of combining caffeine and $NaHCO_3$ on exercise performance *in vivo* (Carr et al. 2011; Christensen et al. 2014; Kilding et al. 2012; Pruscino et al. 2008).

Carr et al. (2011) reported that compared with placebo (PLA) 6 $mg.kg^{-1}$ body mass caffeine improved power output in elite males during 2000-m rowing by 2.3%. This increase in PO contributed to a 3 seconds quicker completion time and in the context of the magnitude based inference statistical approach adopted by the authors was reported as very close to a substantial difference. In contrast, Carr et al. (2011) reported that the differences in PO and completion time between $NaHCO_3$ and PLA (0.6%, 0.6 s) and combined $NaHCO_3$ and caffeine and PLA (1.7%, -1.2 s) were unclear. More simply, ingestion of $NaHCO_3$ did not appear to augment 2000 m rowing performance compared to PLA. Interestingly the ingestion of caffeine with $NaHCO_3$ resulted in a greater PO (1.1%) and faster completion time (-1.8 s) compared with

$NaHCO_3$ alone suggesting that caffeine ingestion somewhat ameliorated the performance decrement reported after isolated $NaHCO_3$ ingestion, compared to PLA. However, it should be pointed out that all participants reported gastrointestinal (GI) issues including nausea, vomiting and stomach pain after $NaHCO_3$ ingestion, regardless if coingested with caffeine. When considering the *between* treatment coefficient of variation for mean performance time was 0.4%, it is plausible that these GI issues might have modulated the potential ergogenicity of $NaHCO_3$.

Pruscino et al. (2008) reported that caffeine alone negatively impacted repeated 200 m freestyle swimming performance compared with all treatments (range: -1.5 to -0.9%). Performance was significantly slower with caffeine compared to $NaHCO_3$ (-1.5±0.7%) and $NaHCO_3$ and caffeine combined (-1.2±1.0%). In contrast, performance with $NaHCO_3$ was quicker compared to all treatments (range: 0.3 to 0.7%) regardless whether ingested in isolation or with caffeine. Indeed, the largest reported performance improvement, albeit small, was for $NaHCO_3$ alone versus placebo (0.7±0.7% faster). However, it should be pointed out that the sample size for this study was relatively small (n=6) so results should be interpreted with caution. More recently Kilding et al. (2012) reported that in comparison to placebo both caffeine and $NaHCO_3$ significantly enhanced mean power output during 3-km time-trial performance in trained male cyclists (2.1% and 2.7%, respectively) although the ergogenic effects were not additive (2.4%)**.** Based on the evidence to date the combinatorial effects of $NaHCO_3$ and caffeine on physical performance are therefore equivocal.

In addition to the aforementioned GI distress it seems plausible that differences in experimental approach between studies might have contributed to the differences in results previously reported. For example, although each study adopted a dosage of

0.3 g.kg^{-1} $NaHCO_3$, the dosage of caffeine varied between studies with Pruscino et al. (2008) and Carr et al. (2011) adopting ~6 mg.kg^{-1} whereas Kilding et al. (2012) and Christensen et al. (2014) adopted 3 mg.kg^{-1}. Additionally there were differences in approaches to abstinence of caffeine intake prior to exercise, an area recognised to limit the ability to compare studies evaluating the ergogenic effects of caffeine (Tallis et al. 2015). For example, participants in Carr et al. (2011) and Pruscino et al. (2008) abstained for 48 hours prior to exercise whereas participants in Kilding et al. (2012) abstained for the duration of the study. Participants in Christensen et al. (2014) were asked to avoid caffeine drinks 36 hours prior to testing. Furthermore, differences in exercise modality and protocol (e.g. repeated 200 m freestyle swimming (Pruscino et al. 2008), 2000 m rowing (Carr et al. 2011), 3 km time trial cycling (Kilding et al. 2012) and 6 min maximal rowing (Christensen et al. 2014)) might have contributed to differences between studies.

Each of the previous studies that have examined combined caffeine and $NaHCO_3$ ingestion on exercise performance evaluated well trained participants which might also have contributed to why no clear synergistic ergogenic benefit has been observed. Indeed it is now well established that individuals who undertake high-intensity training have elevated levels of muscle carnosine compared with endurance trained and untrained individuals (Parkhouse and McKenzie 1984; Parkhouse et al. 1985). Carnosine, an intracellular buffer, is thought to play an important role in the homeostasis of muscle cells during high-intensity exercise (Derave et al. 2010) and thus might ‘offset’ any potential ergogenic contribution from $NaHCO_3$ (Aschenbach et al. 2000; Derave et al. 2010). A recent meta-analysis demonstrated that the overall mean effect of $NaHCO_3$ on exercise performance was more than 225% greater in

untrained (effect size; 95%CI: 0.59; 0.36-0.95) compared to trained (0.18; 0.13-0.33) individuals (Peart et al. 2012). The difference between untrained (0.69; -0.07-1.63) and trained (0.19; -0.58-1.07) individuals was particularly large (263%) for research using a time to volitional fatigue (T_{LIM}) protocol (i.e. exercise capacity). This is supported by Matson and Tran (1993) who suggest that using T_{LIM} is most likely to demonstrate ergogenic benefit for $NaHCO_3$ supplementation. Indeed, recent research has demonstrated that ergogenic benefit with $NaHCO_3$ ingestion is most likely observed for T_{LIM} at 100% W_{PEAK} (Higgins et al. 2013a). Similarly, Simmonds et al. (2010) demonstrated that 5 $mg.kg^{-1}$ caffeine increased T_{LIM} at 120% $\dot{V}O_{2PEAK}$ by ~15% although the ergogenic effects of caffeine are generally more pronounced in trained compared to non-trained individuals (Simmonds et al. 2010; Tallis et al. 2015).

Due to the small number of studies and myriad of differences between experimental approaches the potential synergistic benefit of $NaHCO_3$ and caffeine outside the trained population is not currently known. This is important as there is evidence that increasing numbers of recreational athletes, the vast majority males, are both using and/or want to understand the safety and presumably efficacy of performance enhancing substances (Bojsen-Møller and Christiansen 2010). Moreover, both $NaHCO_3$ and caffeine are now widely available and marketed to recreational athletes[1]. The present study therefore sought to address this gap in the literature by examining the effects of $NaHCO_3$ and caffeine, ingested individually and simultaneously, on T_{LIM} in healthy but not specifically cycling trained males. We hypothesised that ingesting $NaHCO_3$ and caffeine individually would enhance T_{LIM} versus placebo. Additionally,

[1] See http://gonutrition.com/sodium-bicarbonate and http://gonutrition.com/caffeine-200. Accessed 29th September 2015.

we hypothesised that ingesting $NaHCO_3$ and caffeine simultaneously would enhance T_{LIM} versus all experimental conditions.

Materials and methods

Participants

Thirteen healthy, non-cycling trained males (age 21 ± 3 years, height 178 ± 6 cm, body mass 76 ± 12 kg, W_{PEAK} 230 ± 34 W, $\dot{V}O_{2PEAK}$ 46 ± 8ml.kg^{-1}.min^{-1}) volunteered for this study which had received University Ethics Committee approval. All participants were recreationally active (engaging in physical activity at least twice weekly and were primarily team sport athletes or middle distance runners; International Physical Activity Questionnaire (IPAQ) score: 5679 +/- 6688 MET-min/week) although not specifically cycling trained. Participants that completed the study habitually consumed caffeine, but were not heavy caffeine users (125 ± 95 mg/day). Caffeine intake was measured using a 24 hour recall questionnaire (Maughan 1999).

Pre-experimental procedures

Participants were screened to ensure that they were not currently undertaking or had undertaken a nutritional regime involving any alkalotic buffers such as $NaHCO_3$, sodium citrate or β-alanine within the previous 3–6 months. Due to the high-intensity nature of the exercise trials participants were reminded to consume a balanced body mass maintaining diet (~50% carbohydrate, ~30% protein and ~20% fat) throughout the study. All of this nutritional information was included in the participant information sheet provided and confirmed verbally before participants gave written informed consent. Each participant also completed a general health screening questionnaire before each trial. Participants visited the laboratory on seven occasions and reported

for each trial two to three hours postprandial. Trials were conducted at the same time of day to avoid possible circadian rhythmic effects on exercise performance (Cappaert 1999).

Study design

On the first visit participants completed a graded incremental exercise test to determine $\dot{V}O_{2PEAK}$ and W_{PEAK}. After at least 48 hours rest participants undertook the first of their two familiarisation trials at a constant load equivalent to 100% W_{PEAK} (T_{LIM}; Higgins et al. 2014). On the subsequent four visits, each separated by at least 48 hours, participants completed T_{LIM} at 100% W_{PEAK} 60 mins after consuming solutions containing either: (1) 0.3 $g.kg^{-1}$ body mass sodium bicarbonate (BIC), (2) 5 $mg.kg^{-1}$ body mass caffeine plus 0.1 $g.kg^{-1}$ body mass sodium chloride (CAF), (3) 0.3 $g.kg^{-1}$ body mass sodium bicarbonate plus 5 $mg.kg^{-1}$ body mass caffeine (BIC-CAF) or (4) 0.1 $g.kg^{-1}$ body mass sodium chloride (PLA). Both 0.3 $g.kg^{-1}$ $NaHCO_3$ (Higgins et al. 2013a) and 5 $mg.kg^{-1}$ caffeine (Simmonds et al. 2010) have been demonstrated to augment high-intensity cycling capacity. Experimental solutions were administered double-blind. In addition to the relevant solute, each solution consisted of 4 $ml.kg^{-1}$ tap water and 1 $ml.kg^{-1}$ of double strength no added sugar orange squash (Sainsbury's, London, UK). A dosage of 0.1 $g.kg^{-1}$ body mass of sodium chloride (NaCl) was added to CAF and PLA drinks to taste match the $NaHCO_3$ containing solutions as closely as possible. All solutions were refrigerated overnight before consumption to enhance palatability (Higgins et al. 2013a).

Graded incremental exercise test

Before commencing the graded incremental exercise test, participants selected the seat and pedal strap positions that felt most comfortable ensuring that the leg was slightly flexed when the foot reached the bottom of each duty cycle. These settings were adopted for all subsequent trials. Participants were then seated and linked to a gas analysis system and rested quietly for five minutes. Expired gas was analysed using an online breath-by-breath system (Metamax 3B, Cortex Biophysik, Leipzig, Germany). Before each test the system was calibrated for gas concentration (5% CO_2 and 15% O_2, British Oxygen Company, Surrey, UK) using a 6 litre antistatic rebreathable bag (Harvard Apparatus Ltd, Kent, UK), volume measured using a 3 litre calibration syringe (Hans Rudolf Inc, Kansas, USA) and atmospheric pressure measured from a wall mounted mercury barometer (F. Dalton & Co Ltd, Watford, UK). Baseline data was averaged over the last sixty seconds of the rest period and for the last ten seconds of exercise. Expired gas was continually monitored and values for $\dot{V}_E$, $\dot{V}O_2$ and RER, subsequently calculated. Heart rate (HR) was measured using a telemetric HR monitor (Polar FS1, Kempele, Finland). Participants were blinded to the clock during rest to minimise any anticipatory changes in baseline physiology. Prior to commencing exercise resting blood lactate concentration ([BLa]) was obtained by means of a fingerprick capillary sample. The finger was wiped with an isopropyl alcohol swab (Medlock Medical, Oldham, UK), punctured using a 1.8 mm lancing device (Safety Lancet, Sarstedt, Germany) and the initial blood wiped away with a tissue. A 20 µl sample was collected in a sodium heparinised capillary tube (EKF Diagnostic, Magdeburg, Germany) and then added to a 2 ml Eppendorf tube which was pre-filled with 1ml of haemolysing solution (EKF Diagnostic, Magdeburg, Germany) and mixed

well. Samples were then analysed for [BLa] (Biosen C_line, EKF Diagnostic, Magdeburg, Germany).

Cycling commenced on the ergometer (Monark 824E Ergomedic, Monark, Varberg, Sweden) at a cadence of 70 rev.min^{-1} with an unloaded cradle (70 W) increasing by 35 W every three minutes until volitional exhaustion. Researchers provided verbal feedback for maintenance of the specified cadence and to complete a maximal effort. During the last five seconds of each stage HR and ratings of perceived exertion (RPE; 6-20 scale) (Borg 1982) were recorded. Heart rate and RPE were recorded at volitional exhaustion and further [BLa] samples were taken upon completion of exercise and five minutes post-exercise. W_{PEAK} was calculated as the mean power achieved during the final minute of the test (Lamberts et al. 2012). If exhaustion occurred less than one minute into a stage the appropriate duration undertaken at each power output was used to calculate a pro-rata W_{PEAK} (Higgins et al. 2013a).

Experimental Trials

After five minutes seated rest HR, perceived readiness to exercise (PRE), abdominal discomfort (AD) and gut fullness (GF; Higgins et al. 2013a) were recorded. A fingerprick capillary blood sample was collected and analysed for [BLa] as previously described. A second capillary blood sample was then collected in a 100 µl clinitube (Radiometer Medical ApS, Copenhagen, Denmark), capped at both ends and mixed well. Samples were then analysed for pH, base excess ([BE]) and bicarbonate ion concentration ([HCO_3^-]) using a blood gas analyser (ABL5 radiometer, Radiometer Medical ApS, Copenhagen, Denmark).

After baseline measurements were completed the participant consumed the experimental solution within the first 5 mins of the 60 mins pre-exercise period. Participants remained seated throughout and were allowed to consume water *ad libitum* to minimise gastrointestinal (GI) discomfort. The mean volume of water consumed was monitored and was similar between treatments: CAF (n=11): 251 ± 205 ml, BIC (n=12): 268 ± 157 ml, BIC-CAF (n=11): 381 ± 220 ml, and PLA (n=10): 236 ± 158 ml (n = number of measurements analysed per treatment). Perceived readiness to exercise, AD and GF were recorded at 30 mins and 60 mins following ingestion. Approximately 45 min following treatment ingestion participants were linked to the gas analysis system and expired gas was continually monitored and values for $\dot{V}_{E,}$ $\dot{V}O_2$ and RER, subsequently calculated. Baseline data was averaged over the last sixty seconds of the rest period and for the last ten seconds of exercise. At 60 min following treatment ingestion HR was recorded and further blood samples taken for [BLa], pH, [BE] and [HCO_3^-]. Subsequently each participant completed the T_{LIM} test at 100% W_{PEAK}. The T_{LIM} test commenced with a warm up consisting of cycling at 70 rev.min^{-1} for 4 min at 50% W_{PEAK}, 1 min at 75% W_{PEAK} and then 2 min at 70 W (unloaded ergometer). After a verbal countdown the test commenced with participants blinded to the clock throughout. The cadence of 70 rev.min^{-1} was chosen as research examining a range of power outputs (100 – 300 W) and cycling cadences (30 – 120 rev.min^{-1}) during constant load cycling found 70 rev.min^{-1} to be optimal from both metabolic and respiratory perspectives (Ansley and Cangley 2009). A stationary start was employed which has previously been used in evaluating high-intensity cycling in a laboratory setting with active but not specifically cycling trained males, similar to the present study (Wittekind et al. 2011). Ratings of perceived exertion for localised RPE

(RPE_L), representing the exercising muscles, and overall RPE (RPE_O), reflective of cardiovascular strain were recorded after 1, 2 and 3 min of exercise. Abdominal discomfort, GF and HR were recorded and blood samples taken for [BLa], pH, [BE] and [HCO_3^-] immediately post-exercise with final blood samples taken 5 minutes post-exercise. The test was ceased the second time the cadence dropped below 70 rev.min^{-1} for more than 3 or 4 seconds or if the participant was unable to re-establish the required cadence within 3 to 4 seconds (Higgins et al. 2013a). Upon completion of the test, the participant was encouraged to warm down for 5 minutes by cycling at 70 W. Familiarisation trials were similar to experimental trials but excluded the treatment and subsequent 60 min ingestion period.

Statistical analysis

Statistical analysis was completed using SPSS (IBM v21, Chicago, USA). For all data normality (Shapiro-Wilk) and homogeneity of variance/sphericity (Mauchly) were checked prior to choosing the appropriate parametric or non-parametric statistical tests. In limited instances a parametric test was chosen despite the majority of data not being normally distributed (i.e. RPE_L, RPE_O and AD). This was decided so as to minimise the potential for type I error due to multiple individual (non-parametric) comparisons and/or to ensure consistency and comparable analysis to other similar variables.

Where data was normally distributed this is presented as the mean ± SD. Where data is not normally distributed, this is presented as the median and interquartile range (IQR). The IQR range highlights where the middle 50% of the data lies and is the range

between the bottom and top quartiles. Similar to the median, the IQR is appropriate when data are not symmetrically distributed (Whitley and Ball 2002). For any violations of sphericity, degrees of freedom were corrected using Huynh-Feldt ($\varepsilon > 0.75$) or Greenhouse-Geisser ($\varepsilon < 0.75$) values for ε, where applicable (Field 2005). For 2-way repeated measures ANOVAs Bonferroni corrections for multiple comparisons were applied. Tukeys' HSD post hoc analysis was undertaken for interactions by calculating the difference required between means for significance at the minimum level of P=0.05 (Vincent and Weir 2012). The time points considered for HR and blood variables were pre-ingestion (-60 min), pre-exercise (0 min), at the end of exercise and five minutes post-exercise. Respiratory data ($\dot{V}O_2$, $\dot{V}_E$ and RER) was considered at rest and during the final 10 seconds of exercise. Values for RPE_L and RPE_O were analysed at 1 min, 2 min, and 3 min during exercise and at volitional exhaustion. Abdominal discomfort and GF were analysed pre-ingestion, 30 min post-ingestion, pre-exercise (60 min post-ingestion) and at the end of exercise. Finally, PRE was analysed pre-ingestion, 30 min post-ingestion and pre-exercise (60 min post-ingestion).

Data were analysed and quantified using a mixture of effect sizes (ES), P values (minimum requirement of $P \leq 0.05$) and, where appropriate, 95% confidence intervals. For ANOVA main effects and interactions the ES is reported as the partial η^2 value. Otherwise, for normally distributed data the ES (d) was calculated using the difference in means divided by the pooled SD of the compared trials (Nakagawa and Cuthill 2007). For non-normally distributed data the ES (r) was calculated as $Z / \sqrt{n}$ (Ivarsson et al. 2013). For non-normally distributed data confidence intervals were calculated as described by Conover (1980).

Results

Preliminary tests

$\dot{V}O_2$, $\dot{V}_E$, HR, [BLa] and RPE at the end of the graded incremental exercise test were 3.42±0.35 l.min^{-1} (46±8 ml.kg^{-1}.min^{-1}), 129.9±14.3 l.min^{-1}, 184±10 bpm^{-1}, 12.5±3.0 mmol.l^{-1} and 19±1, respectively (NB: $\dot{V}O_2$ and $\dot{V}_E$ data are n=12 due to equipment failure during data collection). This data supports the criteria for achievement of valid *peak* oxygen uptake tests (Bird and Davison 1997). Mean minute peak power output (W_{PEAK}) was 230±34 W.

Exercise Capacity

Figure 1 highlights T_{LIM} data (median; IQR) for the experimental treatments (CAF: 399 s (350-415 s), BIC: 313 s (284-448 s), BIC-CAF: 367 s (333-402 s) and PLA: 358 s (290-433 s)). With the exception of CAF vs. BIC (P=0.039, r=0.6) and BIC-CAF vs. BIC (P=0.028; r=0.6) there were no differences between treatments at the group level. However, there was relatively large inter and intra individual variation (Table 1).

*** *Figure 1 and Table 1 near here* ***

Cardio-respiratory variables

A treatment * time interaction (P<0.001; η^2=0.3) was observed for HR. At the end of exercise HR for both BIC-CAF (183±11 bpm^{-1}; P=0.01; d=0.7) and CAF (182±11 bpm^{-}

^{1}, P<0.05; d=0.6) were greater than PLA (175±11 bpm^{-1}), respectively. Five minutes post-exercise BIC-CAF (123±10 bpm^{-1}) was greater than PLA (111±9 bpm^{-1}; P<0.01; d=1.3), BIC (113±9 bpm^{-1}; P<0.01; d=1.0) and CAF (115±10 bpm^{-1}; P<0.01; d=0.8). Main effects for time (P<0.001; η^2=1.0) were observed for $\dot{V}O_2$, $\dot{V}_E$ and RER with mean values at the end of exercise of 3.59±0.70 l.min^{-1}, 134.2±26.8 l.min^{-1} and 1.11±0.10, respectively.

Blood variables

A treatment * time interaction (P<0.001; η^2=0.4) was observed for [BLa]. Post-hoc analysis revealed that at the end of exercise [BLa] for BIC-CAF (15.8±3.5 mmol.l^{-1}) was greater than PLA (12.6±3.3 mmol.l^{-1}, P<0.01; d=0.9) and CAF (13.5±3.4 mmol.l^{-1}, P<0.01; d=0.7). In addition [BLa] for BIC (14.7±3.3 mmol.l^{-1}) was greater than PLA (P<0.01; d=0.6). Five minutes post-exercise [BLa] for BIC-CAF (15.2±3.7 mmol.l^{-1}) was greater than CAF (12.8±3.2 mmol.l^{-1}, P<0.01; d=0.7) and PLA (11.4±3.4 mmol.l^{-1}, P<0.01; d=1.1). Finally, BIC (13.7±3.3 mmol.l^{-1}) was greater than PLA (P<0.01; d=0.7). Treatment * time interactions were observed for [HCO_3^-] (P<0.001; η^2=0.6), [BE] (P<0.001; η^2=0.5) and pH (P<0.001; η^2=0.6). Compared to CAF and PLA, acid-base balance significantly increased pre-exercise (60 min post-ingestion) for BIC and BIC-CAF and remained elevated at the end of exercise and 5 min post-exercise (Table 2, Figure 2). Interestingly, pH 5 min post-exercise was greater for BIC compared to CAF (P<0.01, d=2.0), BIC-CAF (P=0.01, d=0.7) and PLA (P<0.01, d=1.6; Figure 2).

*** *Table 2 and Figure 2 near here* ***

Perceptual variables

There were no differences for PRE over time or between treatments. With the exception of CAF pre-exercise (8±2) and PLA pre-ingestion (6±2) PRE at all time points was 7±2-3 units. In contrast, there were significant main effects for time for RPE_L ($P<0.001$, $\eta^2=0.9$) and RPE_O ($P<0.001$, $\eta^2=0.9$). Largely, the patterns of change for both RPE_L and RPE_O were similar for all treatments over time although, interestingly, mean RPE_L for BIC-CAF was ~1 unit lower across all time points compared with all other treatments (Table 3). Similarly, mean RPE_O for BIC-CAF was ~1 unit lower compared with all treatments after 1 min of T_{LIM} and 1 unit lower than BIC and PLA after 2 min of T_{LIM} (Table 3).

*** *Table 3 near here****

A significant time * treatment interaction was observed for abdominal discomfort (AD; $P=0.012$; $\eta^2=0.2$). Abdominal discomfort was significantly greater for both BIC-CAF and BIC pre-exercise (3±2) and at the end of exercise (3±3) compared to PLA (1±2 and 1±2, respectively; $P<0.01$). With the exception of PLA, AD was significantly greater pre-exercise when compared to pre-ingestion (1±1 for all treatments) for CAF ($P<0.05$) and both BIC and BIC-CAF ($P<0.01$). Similarly, AD was also significantly greater at the end of exercise for CAF, BIC and BIC-CAF ($P<0.01$) with a typical increase of ~2 units from pre-ingestion. There were no differences in AD over time for PLA. There were no differences for gut fullness (GF) over time or between treatments. The highest mean ratings of GF were for BIC-CAF (4±1) and BIC (4±1) 30 min post-

ingestion. All other mean ratings of GF across all time points, regardless of treatment, ranged between 2 and 3 units.

Discussion

To the best of our knowledge this is the first study to evaluate the effects of ingesting caffeine (CAF) or sodium bicarbonate (BIC) individually and in combination (BIC-CAF) on high-intensity cycling capacity at 100% W_{PEAK} in healthy non-cycling trained males. In contrast to our original hypothesis at the group level ingesting CAF and BIC individually did not enhance T_{LIM} compared with ingestion of a sodium chloride placebo (PLA). Such results are in contrast to the previously reported individual ergogenicity of CAF (Simmonds et al. 2010) and BIC (Higgins et al. 2013a). Similarly, ingestion of BIC-CAF at the group level did not enhance T_{LIM} compared with PLA or CAF although T_{LIM} was significantly greater for CAF and BIC-CAF compared to BIC. However, it should be acknowledged that there was reasonably significant inter and intra individual variation when comparing treatments (Table 1). For example, although both CAF and BIC-CAF had greater T_{LIM} than BIC at the group level, this was not the case for 4/13 (30%) of participants. Moreover, the range of individual responses for those who improved with CAF and BIC-CAF compared to BIC (29 to 170 s and 29 to 280 s, respectively) and those who didn't (-95 to 6 s and -163 to 11s, respectively) was considerable. Similarly, although not significant at the group level T_{LIM} was enhanced beyond daily variation in 8/13 (70%) participants for BIC-CAF vs. PLA. Finally, there was no fixed pattern when examining intra individual responses. For example, participant 9 reported greater T_{LIM} for CAF vs BIC but not for CAF vs. PLA or BIC-CAF. Similarly, participant 1 reported greater T_{LIM} for BIC-CAF vs. BIC but not vs. PLA. Such inter and intra individual variation might not necessarily be obvious when examining the individual responses in Table 1 as they are ordered low:high to allow reporting of

95%CI and median data. In summary, although there appear to be some relatively definitive trends, we suggest an individual approach to supplementation is warranted.

Interestingly, differences between treatments in the present study are virtually opposite to those reported by Kilding et al. (2012) who evaluated 3km time trial (TT) cycling performance in trained males. The authors reported that, CAF (-1.0%), BIC (-1.2%) and BIC-CAF (-1.2%) substantially improved TT performance compared to PLA. In contrast, differences between CAF and BIC (0.3%) CAF and BIC-CAF (0.2%) and BIC-CAF and BIC (0.0%) were insubstantial. However, both the present study and Kilding et al. (2012) appear to agree that differences between BIC-CAF and CAF are most likely trivial at best. The differences in results in the present study to those of Kilding et al. (2012) versus PLA are likely to be related to differences in training status (untrained vs. trained, respectively), caffeine dosage (5 $mg.kg^{-1}$ vs. 3 $mg.kg^{--1}$, respectively), exercise protocol (T_{LIM} vs. 3 km TT, respectively) choice of PLA (taste matched NaCl vs. corn-flour, respectively), method of treatment ingestion (powder mixed with fluid based on body mass vs. capsules with fixed fluid amount, respectively) and timing of ingestion (single bolus with fluid and treatment related to body mass vs. serial ingestion with fixed fluid amounts). In summary, the results of the present study suggest that at the group level ingestion of $5.mg.kg^{-1}$ CAF enhances T_{LIM} in healthy non-cycling trained males compared to 0.3 $g.kg^{-1}$ BIC but a PLA might be equally as effective.

As both BIC and PLA contain sodium (Na^{+}) it is also plausible that changes in electrolyte balance could have influenced performance, at least in some individuals, as Na^{+} is a principal component of extracellular strong ion difference (Badr and

Nightingale 2007; Story et al. 2004). Indeed, Siegler and Gleadall-Siddall (2010) reported that ingesting 0.045 $g.kg^{-1}$ PLA reduced Na^+ levels by 1.7 $mmol.l^{-1}$ compared to 0.3 $g.kg^{-1}$ $NaHCO_3$ when examining repeated swim performance in male and female University swimmers. In contrast, PLA resulted in 0.3 $mmol.l^{-1}$ greater potassium (K^+; also considered a strong cation) compared to $NaHCO_3$ due to differences between treatments of 0.6 $mmol.l^{-1}$ pre and post-exercise (i.e. no difference was observed pre-ingestion). In contrast to the present study Siegler and Gleadall-Siddall (2010) reported an ergogenic benefit for $NaHCO_3$ compared to PLA (-2% total swim time). The disparity in results to the present study could be related to differences in PLA dosage, participant cohort and exercise modality between studies. As electrolytes or strong ion difference were not measured in the present study further investigation as to if/how Na^+/strong ion difference influences high-intensity cycling performance appears warranted.

In the present study HR was greater for BIC-CAF and CAF compared to PLA at the end of exercise. Similarly, five minutes post-exercise HR was greater for BIC-CAF compared to all other treatments. Given the well known sympathetic nervous stimulating properties of CAF (and the moderate dose administered) it is unsurprising that HR was elevated for BIC-CAF and CAF vs. PLA pre-exercise. Moreover, Higgins et al. (2013a) reported that the same dosage of BIC in the present study elevated HR by 5 bpm^{-1} compared to PLA. As such, it also seems unsurprising that BIC-CAF reported the greatest HR 5-min post-exercise compared with other treatments. With no differences between treatments for $\dot{V}O_2$, $\dot{V}_E$ and RER it appears that, at the group level, changes in cardiovascular physiology don't explain the apparent diversity of T_{LIM} between treatments.

It has previously been suggested that a difference of more than 2 mmol.l^{-1} peak [BLa] is needed to observe a significant improvement after BIC ingestion versus PLA (Ibanez et al. 1995). In the present study there were a number of instances at the end of exercise (BIC-CAF vs. PLA, BIC vs. PLA and BIC-CAF vs. CAF) and 5 min post-exercise (BIC-CAF vs. PLA, BIC vs. PLA and BIC-CAF vs. CAF) where a differential of 2 mmol.l^{-1} [BLa] was observed. However, given that T_{LIM} was greatest after CAF and that BIC-CAF and BIC did not increase T_{LIM} versus PLA, it is suggested that augmented metabolic flux as indicated by differences in peak [BLa] is not necessarily an accurate marker for enhanced exercise performance when BIC is ingested individually or with CAF (Higgins et al. 2013a).

After ingesting BIC and BIC-CAF pre-exercise pH was significantly elevated in comparison to both pre-ingestion values and pre-exercise values for PLA and CAF. The increases of 5 and 6 units compared to pre-ingestion for BIC-CAF and BIC, respectively, and 6 and 7 units compared to pre-exercise CAF and PLA, respectively, demonstrate pre-exercise alkalosis was successfully achieved. A similar pattern was observed for [HCO_3^-] with increases of 7 and 8 mmol.l^{-1} compared with pre-ingestion for BIC-CAF and BIC, respectively, and increases of between 7 and 8 mmol.l^{-1} compared with pre-exercise CAF and PLA, respectively. However, despite augmented pre-exercise alkalosis BIC does not appear to have positively influenced T_{LIM}. Indeed median T_{LIM} for BIC was the lowest for all treatments in the present study (313 s) and substantially lower than a similar cohort measured previously in our laboratory (383 s; Higgins et al. 2013a). Interestingly, recovery of pH 5 min post-exercise was greater for BIC compared to all treatments. Further investigation to understand the differences in

T_{LIM} between studies for BIC and if/how improved recovery of pH might affect subsequent exercise performance is warranted.

In contrast, to previous research (Higgins et al. 2013a) there were no significant differences between treatments for RPE_L or RPE_O indicating at the group level differences in RPE don't appear to explain differences in T_{LIM}. However, mean RPE_L for BIC-CAF was ~1 unit lower at all time points compared with all other treatments and mean RPE_O for BIC-CAF was ~ 1 unit lower compared with all treatments after 1 minute T_{LIM} and ~1 unit lower than BIC and PLA after 2 minutes T_{LIM} (Table 3). These differences might have contributed to the increased T_{LIM} for BIC-CAF compared to BIC in some individuals (Table 1).

Mean ratings of AD were significantly greater for both BIC-CAF and BIC pre-exercise (3±2) and at the end of exercise (3±3) compared to PLA (1±2 and 1±2, respectively). Prima facie, as these values are low/mild it could be suggested that at the group level AD has had minimal effects on T_{LIM}. These data are consistent with previous work in our laboratory using the same dosage of $NaHCO_3$ (Higgins et al. 2013a). Moreover, post-hoc correlational analysis revealed no group level relationship between AD and T_{LIM} for any treatment. However, when analysing the data across all post-ingestion time points for BIC containing trials (i.e. 3 * 13 = 39 scores), 9 out of 39 (23%) and 5 out of 39 (13%) ratings of AD were rated 6 (moderate discomfort) or greater (range 6 to 8) for BIC and BIC-CAF, respectively. In contrast, there was only one rating of 6 or above for CAF and none for PLA. Furthermore, AD was lowest overall for PLA highlighting that the dosage of NaCl used was well tolerated when used in isolation (Higgins et al. 2013a). It seems plausible that AD might have negatively influenced

some individuals in some BIC or BIC-CAF trials which might help to explain, at least in part, the relatively large inter and intra variation in T_{LIM} between treatments. Moreover, although $NaHCO_3$-based solutions have been used in a variety of contemporary literature (Higgins et al. 2013a; Price and Simons 2010; Siegler and Gleadall-Siddall 2010), it is also possible that the use of solutions as opposed to capsules might have increased AD for some individuals. However, it should also be acknowledged that GI distress does not always negatively impact exercise performance (Higgins et al. 2013a; Price and Simons 2010).

In conclusion when considering nutritional supplementation prior to high-intensity exercise at the group level, 5 $mg.kg^{-1}$ caffeine, 0.3 $g.kg^{-1}$ $NaHCO_3$ or their co-ingestion do not appear to augment exercise capacity in healthy but not specifically cycling trained males when compared with a sodium chloride placebo. However, based on the inter and intra variation of results presented in this study and previously reported ergogenic effects of individually ingested caffeine and $NaHCO_3$ we believe an individual approach to supplementation is warranted.

Conflict of interest statement

The authors declare that there is no conflict of interest associated with this study.

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Table 1 – Individual and median differences in T_{LIM} (s) between treatments.

	CAF vs. BIC[a]	BIC-CAF vs. BIC[b]	PLA vs. BIC[c]	CAF vs. PLA[d]	CAF vs. BIC-CAF[e]	BIC-CAF vs. PLA[f]
	-95	-163	-116	-139	-251	-84
	-49	-15	-81	-66	-54	-47
Lower 95% CI	-9	3	-32	-34	-38	-25
	6	11	-15	12	-34	-3
	29	**29**	-13	**19**	-28	0
	29	**44**	**17**	**20**	-12	**18**
Median	**39**	**54**	**47**	**21**	-11	**35**
	84	**61**	**54**	**22**	-6	**50**
	100	**67**	**64**	**23**	**18**	**57**
Upper 95% CI	**102**	**106**	**88**	**56**	**23**	**71**
	107	**118**	**89**	**60**	**48**	**110**
	145	**199**	**95**	**116**	**68**	**112**
	170	**280**	**168**	**183**	**141**	**135**

[a] CAF > BIC; P =0.039; r=0.6, [b] BIC-CAF > BIC; P=0.028; r=0.6, [c] P=0.196, r=0.4, [d] P=0.249, r=0.3, [e] P=0.753, r=0.1, [f] P=0.099, r=0.5

NB: data in **bold** represents greater than daily variation of 16 s (Higgins et al. 2014)

Table 2 – Bicarbonate ion concentration [HCO_3^-] and base excess [BE] over time. $^{\#}$ > CAF and PLA (P<0.01), * > CAF (P<0.01), $^{\$}$ > PLA (P<0.05), $^{+}$ > CAF (P<0.05).

	Treatment	Pre-Ingestion	Pre-Exercise	End of Exercise	5 Mins Post-Exercise
[HCO_3^-] mmol.l^{-1}	CAF	25 ± 2	25 ± 2	13 ± 3	13 ± 3
	BIC	25 ± 2	33 ± 1 $^{\#}$	18 ± 4 $^{\#}$	18 ± 4 $^{\#}$
	BIC-CAF	24 ± 2	31 ± 2 $^{\#}$	16 ± 4 * $^{\$}$	16 ± 4 *
	PLA	24 ± 3	24 ± 2	14 ± 3	13 ± 4
[BE] mmol.l^{-1}	CAF	1 ± 1	1 ± 1	-14 ± 3	-14 ± 4
	BIC	1 ± 1	9 ± 1 $^{\#}$	-7 ± 4 $^{\#}$	-6 ± 5 $^{\#}$
	BIC-CAF	0 ± 2	7 ± 2 $^{\#}$	-9 ± 4 $^{+}$	-7 ± 9 $^{\#}$
	PLA	1 ± 2	0 ± 1	-13 ± 4	-13 ± 5

Table 3 – Ratings of perceived exertion localised to the leg musculature (RPE_L) and overall cardiovascular strain (RPE_O) over time (* all time points significantly different from each other; P<0.001).

	Treatment	1 min T_{LIM}	2 min T_{LIM}	3 min T_{LIM}	End of Exercise
RPE_L	CAF	12 ± 2	14 ± 2	16 ± 2	20 ± 1
	BIC	12 ± 1	14 ± 1	16 ± 1	20 ± 1
	BIC-CAF	11 ± 2	13 ± 2	15 ± 2	19 ± 1
	PLA	12 ± 2	14 ± 2	16 ± 1	20 ± 1
	Mean *	12 ± 2	14 ± 2	16 ± 1	20 ± 1

	Treatment	1 min T_{LIM}	2 min T_{LIM}	3 min T_{LIM}	End of Exercise
RPE_O	CAF	11 ± 2	12 ± 2	13 ± 2	19 ± 2
	BIC	11 ± 2	13 ± 2	14 ± 2	19 ± 2
	BIC-CAF	10 ± 2	12 ± 2	14 ± 3	19 ± 1
	PLA	11 ± 2	13 ± 2	14 ± 2	19 ± 2
	Mean *	11 ± 2	12 ± 2	14 ± 2	19 ± 1

Figure Captions

Figure 1 – Time to volitional exhaustion (T_{LIM}) at 100% W_{PEAK}. Error bars represent the full range of scores for each treatment. The intersection between the open and closed boxes represents the median whereas the overall box represents the IQR.

Figure 2 – pH over time. Error bars represent ± 1SD (some omitted for clarity). * BIC-CAF and BIC > CAF and PLA (P<0.01). # BIC > CAF and PLA (P<0.01), BIC-CAF (P=0.01).