

## **The Effect of Combined B-Alanine and NaHCO<sub>3</sub> Supplementation on Cycling Performance**

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**Running head:** β-alanine, NaHCO<sub>3</sub> and cycling performance

### **Acknowledgments**

The authors have no conflict of interest. There were no external funding sources for this work.

The results of the current study do not constitute endorsement by ACSM.

## ABSTRACT

**Purpose:** The purpose of this study was to investigate the effects of 28-days of  $\beta$ -alanine supplementation on four minute cycling time trial performance and to determine if there was an additive effect of combined  $\beta$ -alanine and sodium bicarbonate ( $\text{NaHCO}_3$ ) supplementation on high-intensity cycling performance. **Method:** Fourteen highly-trained cyclists (mean $\pm$ SD; age= $25.4 \pm 7.2$  yr; mass= $71.1 \pm 7.1$  kg;  $\text{VO}_{2\text{max}}$ = $66.6 \pm 5.7$  mL.kg<sup>-1</sup>.min<sup>-1</sup>) supplemented for 28-days with  $\beta$ -alanine (65 mg.kg<sup>-1</sup> body mass each day) or placebo. A maximal four minute bout of cycling was performed pre-supplementation (baseline) and twice post-supplementation: following ingestion of  $\text{NaHCO}_3$  (300 mg.kg<sup>-1</sup> body mass), and ingestion of a placebo using a randomized crossover design with two days between trials. Blood pH and  $\text{HCO}_3^-$  concentration were determined pre-loading (post-supplementation trials), pre-test and post-test. **Results:** In the acute  $\text{NaHCO}_3$  loading trials blood pH and  $\text{HCO}_3^-$  were elevated from pre-loading to pre-test and the magnitude of the change in  $\text{HCO}_3^-$  from pre-test to post-test was significantly greater compared to the acute placebo loading trial ( $P < 0.001$ ). Average power output in the four minute cycling performance trial was increased in placebo+ $\text{NaHCO}_3$  ( $+3.1\% \pm 1.8\%$ ) and  $\beta$ -alanine+ $\text{NaHCO}_3$  ( $+3.3\% \pm 3.0\%$ ) compared to baseline ( $P < 0.05$ ).  $\beta$ -alanine+placebo did not significantly improve average power output compared to baseline ( $+1.6\% \pm 1.7\%$ ;  $P = 0.20$ ), however, magnitude based inferences demonstrated that  $\beta$ -alanine+placebo was associated with a 37% likelihood of producing average power improvements. **Conclusions:** In trained cyclists  $\beta$ -alanine supplementation did not significantly improve four minute cycling performance; however, there may be a small meaningful improvement in performance. Acute  $\text{NaHCO}_3$  supplementation significantly improved four minute cycling performance. There appeared to be minimal additive effect of combined  $\beta$ -alanine and  $\text{NaHCO}_3$  supplementation.

**Keywords:** Highly-Trained, Cyclist, Sodium Bicarbonate, Ergogenic

## **INTRODUCTION**

**Paragraph 1.** Short duration, high-intensity bouts of exercise require a greater reliance on ATP generation from glycolysis and the phosphagen system. The ATP that is derived from these pathways is utilized to fuel muscle contraction and liberates proton release which is thought to be one of the causes of muscular acidosis during intense exercise (30). Both intra and extracellular buffer systems act to reduce the build-up of  $H^+$  and therefore aid in the regulation of intracellular pH. Carnosine has been identified as an important physiochemical buffer (1) and extracellular  $HCO_3^-$  is also thought to play an important role in the maintenance of intramuscular pH (7).

**Paragraph 2.** Carnosine ( $\beta$ -alanyl-L-Histidine) is a naturally-occurring dipeptide found in high concentrations in skeletal muscle and nervous tissue. Carnosine has been hypothesized to play a vital role in delaying the onset of muscular fatigue by acting as an intracellular buffer (14), neutralizing free radicals (8), regulating enzymes (20) and increasing calcium sensitivity of the contractile apparatus (4).

**Paragraph 3.** Previous literature has shown that  $\beta$ -alanine supplementation is capable of significantly elevating skeletal muscle carnosine content in both trained and untrained participants following four to ten weeks of  $\beta$ -alanine supplementation ( $4.0-6.4 \text{ g}\cdot\text{day}^{-1}$ ) (2, 3, 11, 15). The induced elevation in skeletal muscle carnosine content has been associated with improvements in short-term intense bouts of exercise performance in untrained participants ( $\sim 2.5$  min) (15). Several studies have suggested that carnosine may also play an important role in delaying the onset of muscular fatigue in trained athletes such as sprinters (11) and rowers (2);

however, these studies were unable to demonstrate a significant performance improvement following  $\beta$ -alanine supplementation. A recent study found a significant performance improvement in trained cyclists following eight weeks of  $\beta$ -alanine supplementation ( $2\text{-}4\text{ g}\cdot\text{day}^{-1}$ ) in average (5.0%) and peak (11.4%) power output in a maximal 30-second cycling sprint following a 110-min exhaustive endurance ride at varied intensities (35). The available literature investigating  $\beta$ -alanine supplementation, performance improvements have been demonstrated in intense bouts of short-duration exercise performance (1-7 min) which appears to be the ideal duration of exercise in which carnosine exhibits its role as an intramuscular pH buffer.

**Paragraph 4.** In addition to the intracellular buffering capacity, the extracellular buffering system, primarily the bicarbonate ion ( $\text{HCO}_3^-$ ), also plays an important role in the maintenance of intracellular pH even though the sarcolemma has been shown to be relatively impermeable to  $\text{HCO}_3^-$  (23). Several studies (21, 34) have been able to demonstrate that increased extracellular pH and augmented  $\text{HCO}_3^-$  content enhance the  $\text{H}^+/\text{La}^-$  efflux from the exercising muscle. This is largely due to the increase in activity of the  $\text{H}^+/\text{La}^-$  co-transporter which becomes more active when the intracellular/extracellular  $\text{H}^+$  gradient is increased during intense exercise (31). This is thought to allow more work to be completed from the exercising muscles during bouts of intense anaerobic exercise. In light of this, numerous studies have examined the exogenous administration of sodium bicarbonate ( $\text{NaHCO}_3$ ) in an attempt to enhance performance in highly-trained athletes (7).

**Paragraph 5.** A number of studies have shown significant improvements in exercise performance following supplementation with either  $\beta$ -alanine or  $\text{NaHCO}_3$  alone. To our knowledge only one study has examined the combined effect of  $\beta$ -alanine and  $\text{NaHCO}_3$  supplementation on exercise performance (33). While Sale et al. (33) reported a significant

improvement in cycling time to fatigue following supplementation with  $\beta$ -alanine, there was no further improvement in exercise performance with the addition of acute  $\text{NaHCO}_3$  supplementation in recreationally active males (33).

**Paragraph 6.** Chronic  $\beta$ -alanine supplementation has been shown to elevate skeletal muscle carnosine content and improve short-term exercise performance, predominantly in the untrained population (2, 3, 11, 15). It remains to be determined whether  $\beta$ -alanine supplementation can improve exercise performance in trained athletes using sport-specific performance measures such as a time trial which have a lower coefficient of variation than time to fatigue tests (9). Furthermore, it remains to be determined whether  $\beta$ -alanine and  $\text{NaHCO}_3$  supplementation can provide an additional ergogenic benefit in highly-trained athletes. Therefore, the first aim of the present study was to investigate the effects of 28-days of  $\beta$ -alanine supplementation on a maximal cycling performance trial lasting four minutes. The second aim was to determine if there was an additive effect of  $\beta$ -alanine and acute  $\text{NaHCO}_3$  supplementation on high-intensity cycling performance in highly-trained cyclists.

## **METHODS**

### **Participants**

**Paragraph 7.** Fourteen highly-trained male cyclists (mean  $\pm$  SD; age =  $25.4 \pm 7.2$  yr; mass =  $71.1 \pm 7.1$  kg;  $\text{VO}_{2\text{max}} = 66.6 \pm 5.7$  mL.kg<sup>-1</sup>.min<sup>-1</sup>) were recruited for the current study. All participants were informed verbally and in writing as to the requirements of the study and any possible side effects that could be experienced, and all gave their written informed consent. Participants had not taken any supplement in the three months prior to the study. The study was conducted in the University of Tasmania Exercise Science Laboratory and was approved by the Institutional Human Research Ethics Committee Network.

## **Experimental Design**

**Paragraph 8.** Participants attended the laboratory on five separate occasions (Fig. 1). The two initial visits to the laboratory consisted of a graded exercise test to determine the  $VO_{2max}$  and familiarisation of the maximal four minute cycling performance trial. The remaining three visits to the laboratory were for the completion of the performance trials. The first performance trial was a baseline trial which preceded a 28-day, double-blind  $\beta$ -alanine or placebo supplementation regimen. Participants were supplemented with either  $65 \text{ mg.kg}^{-1}$  body mass of  $\beta$ -alanine capsules each day (100% pure  $\beta$ -alanine, Vitaco Health, Auckland, New Zealand) or an equivalent amount of placebo capsules (dextrose monohydrate). This particular dosage of  $\beta$ -alanine is similar to that used by Hill and colleagues (15), whose participants consumed, on average, between  $50$  and  $80 \text{ mg.kg}^{-1}$  body mass of  $\beta$ -alanine and exhibited significant increases in skeletal muscle carnosine of 59%. Participants were asked to consume the capsules in four even daily doses in conjunction with meals. Participants were asked to report any side effects experienced and to bring in the supplement packaging on completion of the 28-day supplementation period to monitor compliance.

**Paragraph 9.** The two post-supplementation performance trials, required participants to ingest either  $\text{NaHCO}_3$  or a placebo in a randomised crossover design (see Fig. 1). The amount of  $\text{NaHCO}_3$  ingested by each participant prior to the performance trial was  $0.3 \text{ g.kg}^{-1}$  body mass. This particular dosage has previously been associated with exercise performance improvements (7). The same number of capsules were consumed in the acute placebo condition. Each dose of capsules was ingested with  $10 \text{ ml.kg}^{-1}$  body mass of plain water. Participants were supervised during the ingestion of  $\text{NaHCO}_3$  to ensure compliance with the supplementation protocol. Two days elapsed between post-supplementation performance trials for the purpose of a  $\text{NaHCO}_3$

washout period (28). The study was therefore comprised of four experimental conditions (n=7 in each group): placebo + placebo (PP),  $\beta$ -alanine + placebo (BAP), placebo +  $\text{NaHCO}_3$  (PSB) and  $\beta$ -alanine +  $\text{NaHCO}_3$  (BASB).

### **Preliminary testing**

**Paragraph 10.** Each participant first underwent an incremental test to exhaustion on an electronically braked cycle ergometer (Lode Excalibur Sport, Quinton, WA, USA) modified with clip-in pedals and low-profile racing handlebars to determine  $\text{VO}_{2\text{max}}$ , maximum heart rate and maximum aerobic power output. The saddle and handlebar position of the cycle ergometer were adjusted to accurately match the dimensions of each of the participants' road bicycle measurements and each participant warmed up at a self-selected pace for five minutes. The test consisted of an initial workload of 100 W, followed by a ramped protocol in which power output was increased by  $15 \text{ W}\cdot 30\text{-sec}^{-1}$  until volitional fatigue (22). Heart rate was continuously measured (s610, Polar Electro, Oy, Finland), and respiratory variables were measured and recorded every 30-sec (Parvomedics Trueone 2400, Utah, USA).

**Paragraph 11.** On a separate day, each participant underwent a familiarisation of the performance trial to minimize any learning effect for subsequent trials (17). The performance trial consisted of a single maximal bout of cycling lasting four minutes. Participants performed each trial on an air-braked, front access cycle ergometer (Repcycle Company, Canberra). The ergometer was connected to a custom made Power Evaluation System (PES Version 2.0, School of Human Life Sciences, Launceston, Australia) which measured the peak and average power (watts) and total work done (kJ). Prior to the study, the cycle ergometer was dynamically calibrated using a protocol that has been described elsewhere (25). Participants were given a standardised warm up consisting of three 3-min workloads (2, 2.5 and  $3\text{W}\cdot\text{kg}^{-1}$  body mass) on a

friction-braked cycle ergometer (Ergomedic 828E, Monark-crescent, Varberg, Sweden). Heart rate was continuously measured, and respiratory variables were measured and recorded every 30-sec.

### **Performance Trials**

**Paragraph 12.** Prior to reporting to the laboratory for the performance trials, each participant was asked to abstain from caffeine and alcohol and avoid strenuous exercise for the 24-h preceding each performance trial. Each participant was also required to record a 24-h diet diary leading up to the baseline performance trial which was then replicated in the 24-h preceding each subsequent performance trial. When the participants reported to the laboratory on the day of each performance trial, they were weighed and then had a finger-prick blood sample collected into a pre-heparinised capillary tube. Blood samples were analysed for pH and  $\text{HCO}_3^-$  using the i-STAT<sup>®</sup> blood-gas analyzer (i-STAT<sup>®</sup>, Princeton, USA). During the performance trial, participants were verbally encouraged and were instructed to attempt each performance trial with a maximal effort.

**Paragraph 13.** Prior to each post-supplementation performance trial, each participant ingested an acute dose of either  $\text{NaHCO}_3$  or placebo 90-min prior to exercise. The capsules were consumed in six equal doses over a one hour period. Each participant then rested for a further 30-min before a second blood sample was collected. The standardised warm up was then completed followed by the performance trial. Upon cessation of the exercise bout a final blood sample was taken for analysis of pH and  $\text{HCO}_3^-$ .

### **Training diary**

**Paragraph 14.** To standardize training intensity and volume across groups participants were instructed to complete two high intensity interval training (HIT) sessions per week over the 28-



day supplementation period and were asked to record the HIT sessions in a training diary. Each HIT session consisted of eight 2.5-min bouts aimed to achieve a heart rate corresponding to 90%  $VO_{2max}$  at the completion of the interval, interspersed with 3-min recovery intervals aimed to achieve a heart rate corresponding to 40%  $VO_{2max}$  which was determined from the graded exercise test.

**Paragraph 15.** Participants were instructed to continue their normal dietary and training regimen throughout the duration of the study and were also asked to keep an accurate record of training sessions (including their HIT sessions) and intensities over the 28-day supplementation period. Furthermore, participants recorded all types of training (eg, cycling, running, resistance training) and the duration of each training session in the training diaries for all sessions completed. Participants also gave an intensity rating following each training session using the CR10 Borg Scale (6) for rating of perceived exertion. To quantify the training that was completed during the supplementation period the current study used the session-RPE method (12).

### **Statistical analysis**

**Paragraph 16.** Sample size determination for the study was determined using a statistical spread sheet previously described (16). The spread sheet estimates sample size requirements for magnitude-based inferences when the typical error and the smallest worthwhile change for the primary performance measure (four minute cycling test) are entered. Data used for these values were obtained through previous unpublished laboratory results measuring the variation in four minute cycling test performance in similar athletes. These values were 4.0 W (0.98%) and 7.3 W (1.8%) for typical error and smallest worthwhile change, respectively. Sample size calculation using these values indicated the need for five participants in each group. We also allowed for an

attrition rate of ~30% based on experience from our previous studies when dealing with highly-trained athletes (ensuring a minimum of five participants in each group).

**Paragraph 17.** All data are presented as mean  $\pm$  SD for the seven participants in each group. An independent *t* test was used to determine any difference between the  $\beta$ -alanine and placebo groups at baseline. A 2-way repeated measures ANOVA was used to evaluate the performance data (average power and total work done) with group (placebo,  $\beta$ -alanine, NaHCO<sub>3</sub>) as the between-participants factor and trial (pre vs post supplementation trials) as the within-participants factor. A 3-way ANOVA (group x trial x time) was used to analyse the blood variables (GraphPad statistical software, Graphpad version 5.0, San Diego, CA). When appropriate, Bonferroni *post hoc* comparisons were used to examine differences between groups. Statistical significance was accepted at the  $P < 0.05$  level.

**Paragraph 18.** In addition to the use of statistical significance, magnitude based inferences were used to determine the practical significance of PP, BA, SB and BASB on four minute time trial performance. Using a Microsoft Excel spread sheet designed for sports science research (18) mean effects and the 90% confidence limits were estimated to establish the per cent likelihood of each experimental condition having a positive/trivial/negative effect on performance. The smallest worthwhile improvement in total work done was considered to be 0.2 of the between participant standard deviation established from baseline performance which was 2.31 kJ (2.37%) (19).

**Paragraph 19.** Training intensity/volume of the two groups during the 28-day supplementation/training period were compared using an independent samples *t* test to identify any differences between the two groups throughout this period. To determine if there was any order effect during the post-supplementation performance trials, performance achieved in the

first post-supplementation performance trial was compared with performance achieved during the second post-supplementation using a paired sample *t* test.

## RESULTS

### Performance trial

**Paragraph 20.** Prior to the 28-day supplementation period there was no difference in four minute cycling average power ( $P = 0.308$ ; placebo:  $420.6 \pm 49.1$  W and  $\beta$ -alanine:  $392.4 \pm 42.3$  W) and total work done ( $P = 0.308$ ; placebo:  $101 \pm 11.8$  kJ and  $\beta$ -alanine:  $94.17 \pm 11.8$  kJ) between the placebo and  $\beta$ -alanine group.

**Paragraph 21.** There was a significant effect of trial ( $P < 0.001$ ) with no significant group by time interaction ( $P = 0.076$ ). Compared with the baseline four minute cycling performance trial, there was no significant change in average power and total work done following PP (average power:  $+0.3\% \pm 2.2\%$ ,  $P = 0.77$  and total work done:  $+0.3\% \pm 2.2\%$ ,  $P = 0.77$ ) or BAP (average power:  $+1.6\% \pm 1.7\%$ ,  $P = 0.20$  and total work done:  $+1.45\% \pm 1.8\%$ ,  $P = 0.28$ ). For PSB, there was a significant increase in average power ( $+3.1\% \pm 1.8\%$ ,  $P = 0.04$ ) and total work done ( $+3.0\% \pm 2.2\%$ ,  $P = 0.04$ ). BASB resulted in a significant increase in average power ( $+3.3\% \pm 3.0\%$ ,  $P = 0.035$ ) and total work done ( $+3.2\% \pm 3.1\%$ ,  $P = 0.044$ ). In the  $\beta$ -alanine group, 6 out of the 7 participants showed a further increase in average power with the addition of  $\text{NaHCO}_3$  (BAP compared with BASB); however, the results did not reach statistical significance (average power:  $P = 0.22$ ; total work done:  $P = 0.13$ ). Table 1 provides the performance improvements in comparison with the PP experimental condition and the % likelihood that each condition was beneficial/trivial/negative when compared to the PP condition.

**Paragraph 22.** The percentage change from baseline performance is shown in Fig. 2. In comparison with baseline performance BAP was associated with a 37% likelihood of producing

a performance benefit and 63% likelihood of a trivial effect. Compared with baseline performance PSB and BASB were associated with a  $\geq 89\%$  likelihood of producing a performance benefit (Fig. 2).

**Paragraph 23.** Analysis of the completed training diaries showed that there was no significant difference in the total amount of training performed ( $P = 0.29$ ) between the  $\beta$ -alanine and placebo supplementation groups during the 28-day supplementation period, as identified by the session RPE method ( $\beta$ -alanine =  $19703 \pm 4067$  AU; placebo =  $21208 \pm 4895$  AU). There was no order effect for average power for the post-supplementation performance trials (Trial 1:  $415.7 \pm 50.2$  W, Trial 2:  $414.9 \pm 53.5$  W,  $P = 0.86$ ).

### **Blood pH and $\text{HCO}_3^-$**

**Paragraph 24.** Blood pH and  $\text{HCO}_3^-$  were elevated from pre-loading to pre-test following acute  $\text{NaHCO}_3$  supplementation (PSB and BASB) ( $P < 0.001$ ) (Table 2) compared to acute placebo supplementation (PP and BAP). There was a significant decrease in blood pH and  $\text{HCO}_3^-$  from pre-test to post-test in all four minute cycling performance trials ( $P < 0.001$ ) (Table 2). The magnitude of the change in  $\text{HCO}_3^-$  from pre-test to post-test in the acute  $\text{NaHCO}_3$  loading trials (BASB and PSB) was significantly greater compared to the acute placebo loading trial ( $P < 0.001$ ).

**Paragraph 25.** Of the participants, 2 cyclist who supplemented on  $\beta$ -alanine reported mild symptoms of paraesthesia. Following the acute  $\text{NaHCO}_3$  supplementation trials, 3 of the 14 participants reported mild gastrointestinal symptoms.

## **DISCUSSION**

**Paragraph 26.** The main findings from the present study were that 28-days of  $\beta$ -alanine supplementation did not significantly improve high-intensity cycling performance; however,

magnitude based inferences demonstrated that in highly-trained cyclists  $\beta$ -alanine supplementation was 37% likely to improve performance with 0% likelihood of a negative effect. Furthermore, acute  $\text{NaHCO}_3$  supplementation significantly improved average power and total work done in a four minute cycling trial. There appeared to be minimal additive effect following the combined supplementation of  $\beta$ -alanine and  $\text{NaHCO}_3$  with similar improvements in high-intensity cycling performance under the BASB condition and the PSB condition.

**Paragraph 27.**  $\beta$ -alanine supplementation alone did not significantly improve performance during the four minute cycling performance trial. The absence of a significant performance improvement in the current study is consistent with previous literature investigating the effects of  $\beta$ -alanine supplementation on maximal exercise efforts in trained populations (2, 11). Derave et al. (11) found no improvement in 400 m running time in trained sprinters following a similar four week  $\beta$ -alanine supplementation period ( $4.8 \text{ g}\cdot\text{day}^{-1}$ ), despite a significant increase in skeletal muscle carnosine in the soleus (47%) and gastrocnemius (37%). Furthermore, Baguet et al. (2) found no significant improvement in performance of elite rowers following seven weeks of  $\beta$ -alanine supplementation ( $5 \text{ g}\cdot\text{day}^{-1}$ ) despite a significant increase in muscle carnosine content in the soleus (45%) and gastrocnemius (28%). This may suggest that despite significant increases in the skeletal muscle carnosine content of highly-trained athletes, the already highly developed buffering capacity may not allow for further improvements in exercise performance to be attained in sport-specific competitive events (32). In the current study, muscle carnosine was not measured; however, previous research has demonstrated that a similar four week  $\beta$ -alanine supplementation regimen ( $4.8 \text{ g}\cdot\text{day}^{-1}$ ) can significantly increase muscle carnosine content in trained athletes (2). However, the participants in the current study ingested the capsules in four

equal daily doses, in contrast to six equal daily doses (2) which may have had an effect on the magnitude of increase in skeletal muscle carnosine.

**Paragraph 28.** Acute supplementation of  $\text{NaHCO}_3$  prior to the four minute cycling performance trial significantly increased average power by 3.1% (PSB) and 3.3% (BASB) compared to baseline performance. This finding is similar to previous studies that have reported exercise performance improvements following  $\text{NaHCO}_3$  ingestion prior to sport specific performance in highly-trained athletes (5, 26, 27). For example,  $\text{NaHCO}_3$  supplementation has been associated with ~3% improvements in 400 m (13), 800 m (36) and 1500 m (5) running times in middle and long-distance runners. McNaughton and colleagues also reported a ~3% improvement in a maximal six minute rowing ergometer test following a similar dose of  $\text{NaHCO}_3$  in highly-trained rowers (26). For trained cyclists, previous research has indicated that acute  $\text{NaHCO}_3$  supplementation can significantly improve total work done (+6.6%) in a one minute maximal cycling effort (27). The likely mechanism for the improved exercise performance in the current study may have been due to the increased buffering capacity. In the current study,  $\text{NaHCO}_3$  supplementation significantly elevated blood pH and  $\text{HCO}_3^-$  levels prior to the cycling performance trials compared to the placebo. A number of studies have confirmed that an increased extracellular pH and  $\text{HCO}_3^-$  concentration can raise the  $\text{La}^-$  and  $\text{H}^+$  efflux from active muscles (21, 34) and increase the activity of the  $\text{La}^-/\text{H}^+$  co-transporter. It has been suggested that this mechanism can cause a decrease in muscular fatigue and assist in maintaining intracellular pH allowing higher work intensities to be maintained for a longer period of time and more work to be completed (24). Therefore, in the current study, it can be speculated that the augmented extracellular reserve of  $\text{HCO}_3^-$  may have been the mechanism responsible for the performance improvement.

**Paragraph 29.** There appeared to be minimal additive effect of  $\beta$ -alanine and  $\text{NaHCO}_3$  (BASB) when compared to  $\beta$ -alanine alone (BAP). Sale et al. (33) reported a significant increase in cycling performance following  $\beta$ -alanine supplementation; however, there was no additional ergogenic benefit following the combined supplementation of  $\beta$ -alanine and  $\text{NaHCO}_3$ . The lack of a significant performance improvement following  $\beta$ -alanine supplementation in the current study may relate to the already highly developed buffering capacity of the trained athletes not allowing for further improvements in exercise capacity. Despite a 4.1% improvement in time to exhaustion compared to  $\beta$ -alanine alone, Sale et al. (33) found no additional ergogenic benefit following the combined supplementation of  $\beta$ -alanine and  $\text{NaHCO}_3$ . The non-significant improvement in time to exhaustion in the cycling capacity test may reflect the variability in cycling performance, or potential for improvement, of the untrained participants of the study by Sale et al (33) compared to the highly-trained cyclists in the current study (19). Furthermore, the current study employed a constant duration performance test which provides a more appropriate physiological simulation of actual performance and correlates well with sport-specific competitive events when compared to time to fatigue tests (9).

**Paragraph 30.** In the current study, there was no significant change in four minute time trial performance in PP compared to baseline following the four week supplementation period that included two high intensity interval sessions per week. A comprehensive review on chronic high intensity interval training interventions (29) has shown performance improvements of between 3.0-8.3% following interval training at maximal and supramaximal intensities. In the current study, the absence of a significant improvement in cycling performance is a testament to the training status of the participants who took part in the current study, as the inclusion of two high

intensity interval sessions per week was not sufficient to significantly improve maximal four minute cycling performance.

**Paragraph 31.** A limitation of the current study was that two participants reported mild symptoms of paraesthesia following  $\beta$ -alanine supplementation which may have compromised the blinding of the two participants to the supplementation condition. This adverse effect is thought to be triggered by a high acute dose of  $\beta$ -alanine (larger than 800 mg) (14) and can be avoided by increasing the frequency of daily doses which limits the amount consumed per dose or by ingesting a slow release  $\beta$ -alanine formula as opposed to a pure solution, which has been shown to have slower absorption kinetics, improved whole body retention and sensory side-effects that cannot be differentiated from a placebo (10).

**Paragraph 32.** The present study has shown that 28-days of  $\beta$ -alanine supplementation does not significantly improve four-minute cycling performance in highly-trained cyclists. Although the effects of  $\beta$ -alanine on performance were mostly trivial, the beneficial effects on performance provide some support for the use of  $\beta$ -alanine supplementation in trained cyclists. Furthermore, acute  $\text{NaHCO}_3$  supplementation significantly improved four minute cycling performance; however, there appeared to be minimal additive effect following the combined supplementation of  $\beta$ -alanine and  $\text{NaHCO}_3$  which was associated with similar improvements in high-intensity cycling performance compared to acute  $\text{NaHCO}_3$  supplementation alone.

### **Acknowledgments**

The authors have no conflict of interest. There were no external funding sources for this work. The results of the current study do not constitute endorsement by ACSM.



## REFERENCES

1. Abe H. Role of histidine-related compounds as intracellular proton buffering constituents in vertebrate muscle. *Biochemistry (Moscow)*. 2000;65(7):757-65.
2. Baguet A, Bourgois J, Vanhee L, Achten E, and Derave W. Important role of muscle carnosine in rowing performance. *J Appl Physiol*. 2010;109(4):1096-101.
3. Baguet A, Reyngoudt H, Pottier A, Everaert I, Callens S, Achten E, and Derave W. Carnosine loading and washout in human skeletal muscles. *J Appl Physiol*. 2009;106(3):837-42.
4. Begum G, Cunliffe A, and Leveritt M. Physiological role of carnosine in contracting muscle. *Int J Sport Nutr Exerc Metab*. 2005;15(5):493-514.
5. Bird S, Wiles J, and Robbins J. The effect of sodium bicarbonate ingestion on 1500-m racing time. *J Sport Sci*. 1995;13(5):399-403.
6. Borg G. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc*. 1982;14(5):377-81.
7. Burke L, and Pyne D. Bicarbonate loading to enhance training and competitive performance. *Int J Sports Physiol Perform*. 2007;2(1):93-7.
8. Chasovnikova L, Formazyuk V, Sergienko V, Boldyrev A, and Severin S. The antioxidative properties of carnosine and other drugs. *Biochem Int*. 1990;20(6):1097-103.
9. Currell K, and Jeukendrup AE. Validity, reliability and sensitivity of measures of sporting performance. *Sports Med*. 2008;38(4):297-316.
10. Décombaz J, Beaumont M, Vuichoud J, Bouisset F, and Stellingwerff T. Effect of slow-release  $\beta$ -alanine tablets on absorption kinetics and paresthesia. *Amino Acids*. 1-10.

11. Derave W, Ozdemir MS, Harris RC, Pottier A, Reyngoudt H, Koppo K, Wise JA, and Achten E. Beta-Alanine supplementation augments muscle carnosine content and attenuates fatigue during repeated isokinetic contraction bouts in trained sprinters. *J Appl Physiol*. 2007;103(5):1736-43.
12. Foster C, Florhaug JA, Franklin J, Gottschall L, Hrovatin LA, Parker S, Doleshal P, and Dodge C. A new approach to monitoring exercise training. *J Strength Cond Res*. 2001;15(1):109.
13. Goldfinch J, Mc Naughton L, and Davies P. Induced metabolic alkalosis and its effects on 400-m racing time. *J Appl Physiol Occup Physiol*. 1988;57(1):45-8.
14. Harris RC, Tallon MJ, Dunnett M, Boobis L, Coakley J, Kim HJ, Fallowfield JL, Hill CA, Sale C, and Wise JA. The absorption of orally supplied  $\beta$ -alanine and its effect on muscle carnosine synthesis in human vastus lateralis. *Amino Acids*. 2006;30(3):279-89.
15. Hill CA, Harris RC, Kim HJ, Harris BD, Sale C, Boobis LH, Kim CK, and Wise JA. Influence of  $\beta$ -alanine supplementation on skeletal muscle carnosine concentrations and high intensity cycling capacity. *Amino Acids*. 2007;32(2):225-33.
16. Hopkins W. Estimating sample size for magnitude-based inferences. *Sportscience*. 2006;10:63-70.
17. Hopkins WG. Measures of reliability in sports medicine and science. *Sports Med*. 2000;30(1):1-15.
18. Hopkins WG, Batterham AM. Making Meaningful Inferences About Magnitudes. *Sportsci*. 2005;9:6-13.
19. Hopkins WG, Schabort EJ, and Hawley JA. Reliability of Power in Physical Performance Tests. *Sports Med*. 2001;31:211-34.

20. Johnson P, and Aldstadt J. Effects of carnosine and anserine on muscle and non-muscle phosphorylases. *Comp Biochem Physiol B*. 1984;78(2):331-3.
21. Jones NL, Sutton JR, Taylor R, and Toews C. Effect of pH on cardiorespiratory and metabolic responses to exercise. *J Appl Physiol*. 1977;43(6):959-64.
22. Laursen PB, Shing CM, Peake JM, Coombes JS, and Jenkins DG. Interval training program optimization in highly trained endurance cyclists. *Med Sci Sports Exerc*. 2002;34(11):1801-7.
23. Mainwood G, and Worsley-Brown P. The effects of extracellular pH and buffer concentration on the efflux of lactate from frog sartorius muscle. *J Physiol*. 1975;250(1):1-22.
24. Marx JO, Gordon SE, Vos NH, Nindl BC, Gomez AL, Volek JS, Pedro J, Ratamess N, Newton RU, and French DN. Effect of alkalosis on plasma epinephrine responses to high intensity cycle exercise in humans. *Eur J Appl Physiol*. 2002;87(1):72-7.
25. Maxwell B, Withers R, Ilsley A, Wakim M, Woods G, and Day L. Dynamic calibration of mechanically, air and electromagnetically braked cycle ergometers. *Eur J Appl Physiol Occup Physiol*. 1998;78(4):346-52
26. McNaughton L, and Cedaro R. The effect of sodium bicarbonate on rowing ergometer performance in elite rowers. *Aust J Sci Med Sport*. 1991;23(3):66-9.
27. McNaughton L, Curtin R, Goodman G, Perry D, Turner B, and Showell C. Anaerobic work and power output during cycle ergometer exercise: effects of bicarbonate loading. *J Sport Sci*. 1991;9(2):151-60.
28. McNaughton L, and Thompson D. Acute versus chronic sodium bicarbonate ingestion and anaerobic work and power output. *J Sport Med Phys Fit*. 2001;41(4):456-62.

29. Paton CD, and Hopkins WG. Effects of high-intensity training on performance and physiology of endurance athletes. *Sportsmedicine*. 2004;8:25-40.
30. Robergs RA, Ghiasvand F, Parker D. Biochemistry of exercise-induced metabolic acidosis. *Am J Physiol Regul Integr Comp Physiol*. 2004;287:502-516.
31. Roth DA. The sarcolemmal lactate transporter: transmembrane determinants of lactate flux. *Med Sci Sports Exerc*. 1991;23(8):925.
32. Sahlin K, and Henriksson J. Buffer capacity and lactate accumulation in skeletal muscle of trained and untrained men. *Acta Physiologica Scandinavica*. 1984;122(3):331-9.
33. Sale C, Saunders B, Hudson S, Wise JA, Harris RC, and Sunderland CD. Effect of  $\beta$ -alanine plus sodium Bicarbonate on high-intensity cycling capacity. *Med Sci Sports Exerc*. 2011;43(10):1972-1978.
34. Sutton J, Jones N, and Toews C. Effect of PH on muscle glycolysis during exercise. *Clin Sci (Lond)*. 1981;61(3):331-8.
35. Van Thienen R, Van Proweyen K, Eynde BV, Puype J, Lefere T, and Hespel P. Beta-alanine improves sprint performance in endurance cycling. *Med Sci Sports Exerc*. 2009;41(4):898-903.
36. Wilkes D, Gledhill N, and Smyth R. Effect of acute induced metabolic alkalosis on 800-m racing time. *Med Sci Sports Exerc*. 1983;15(4):277-80.

## **TABLES:**

**Table 1** – The change in average power output and total work done in each loading condition from the baseline trial relative to the change in PP from baseline. The likelihood of a practically

substantial difference of the supplementation condition compared to PP were provided as % positive/% trivial/% negative.

**Table 2** – pH and  $\text{HCO}_3^-$  values pre-loading (90 mins prior to exercise in post-supplementation trials), pre-test (0 mins prior to exercise) and post-test (immediately following exercise) in the placebo and  $\beta$ -alanine groups during the baseline trial, acute placebo loading trial and acute sodium bicarbonate trial. \*Significantly different to acute placebo loading trial ( $P < 0.001$ ).

## **FIGURES:**

**Figure 1** – The study design required participants to attend the laboratory on five separate occasions. The two initial visits consisted of a graded exercise test to determine the  $\text{VO}_{2\text{max}}$  and to ensure familiarisation of the maximal four minute cycling performance trial. The remaining three visits to the laboratory were for the completion of the performance trials.

**Figure 2** – Improvement in average power output in the maximal four minute cycling performance trial in each treatment group. The likelihood of a practically substantial difference of the supplementation condition relative to the baseline performance trial for each group ( $\beta$ -alanine or placebo) were provided as % positive/% trivial/% negative above each bar. \*Significantly different to baseline ( $P < 0.05$ ).

Table 1

<b><math>\Delta_{\text{group}} - \Delta_{\text{PP}}</math>: Raw Difference <math>\pm</math> 90% Confidence</b>						
	<b>Limits</b>			<b>Magnitude based inferences (compared to PP)</b>		
	<b>BAP</b>	<b>PSB</b>	<b>BASB</b>	Likelihood of BAP being positive/trivial/negative	Likelihood of PSB being positive/trivial/negative	Likelihood of BASB being positive/trivial/negative
<b>Average power (W)</b>	5.05 $\pm$ 8.42	*13.53 $\pm$ 8.12	*14.29 $\pm$ 11.40	32/67/1	87/13/0	81/19/0
<b>Total work done (kJ)</b>	1.34 $\pm$ 2.42	*2.82 $\pm$ 1.95	*2.87 $\pm$ 2.89	27/72/1	72/28/0	69/31/0

Table 2

		Baseline trial		Acute placebo loading trial			Acute sodium bicarbonate trial		
		Pre-test	Post-test	Pre-loading	Pre-test	Post-test	Pre-loading	Pre-test	Post-test
<b>pH</b>	<b><math>\beta</math>-alanine</b>	7.42 $\pm$ 0.03	7.14 $\pm$ 0.07	7.45 $\pm$ 0.02	7.44 $\pm$ 0.02	7.13 $\pm$ 0.06	7.44 $\pm$ 0.02	7.53 $\pm$ 0.02	7.20 $\pm$ 0.06
	<b>Placebo</b>	7.45 $\pm$ 0.05	7.10 $\pm$ 0.08	7.45 $\pm$ 0.02	7.45 $\pm$ 0.02	7.13 $\pm$ 0.06	7.46 $\pm$ 0.02	7.50 $\pm$ 0.04	7.21 $\pm$ 0.06
<b>HCO<sub>3</sub><sup>-</sup> (mmol.L<sup>-1</sup>)</b>	<b><math>\beta</math>-alanine</b>	26.54 $\pm$ 1.25	11.37 $\pm$ 2.58	25.93 $\pm$ 1.02	25.80 $\pm$ 0.88	10.83 $\pm$ 1.73	26.83 $\pm$ 1.02	*33.79 $\pm$ 2.08	13.90 $\pm$ 2.25
	<b>Placebo</b>	26.21 $\pm$ 1.27	10.44 $\pm$ 2.39	25.67 $\pm$ 0.78	26.17 $\pm$ 1.80	10.94 $\pm$ 2.12	25.93 $\pm$ 1.43	*34.91 $\pm$ 2.81	14.36 $\pm$ 1.97

Figure 1

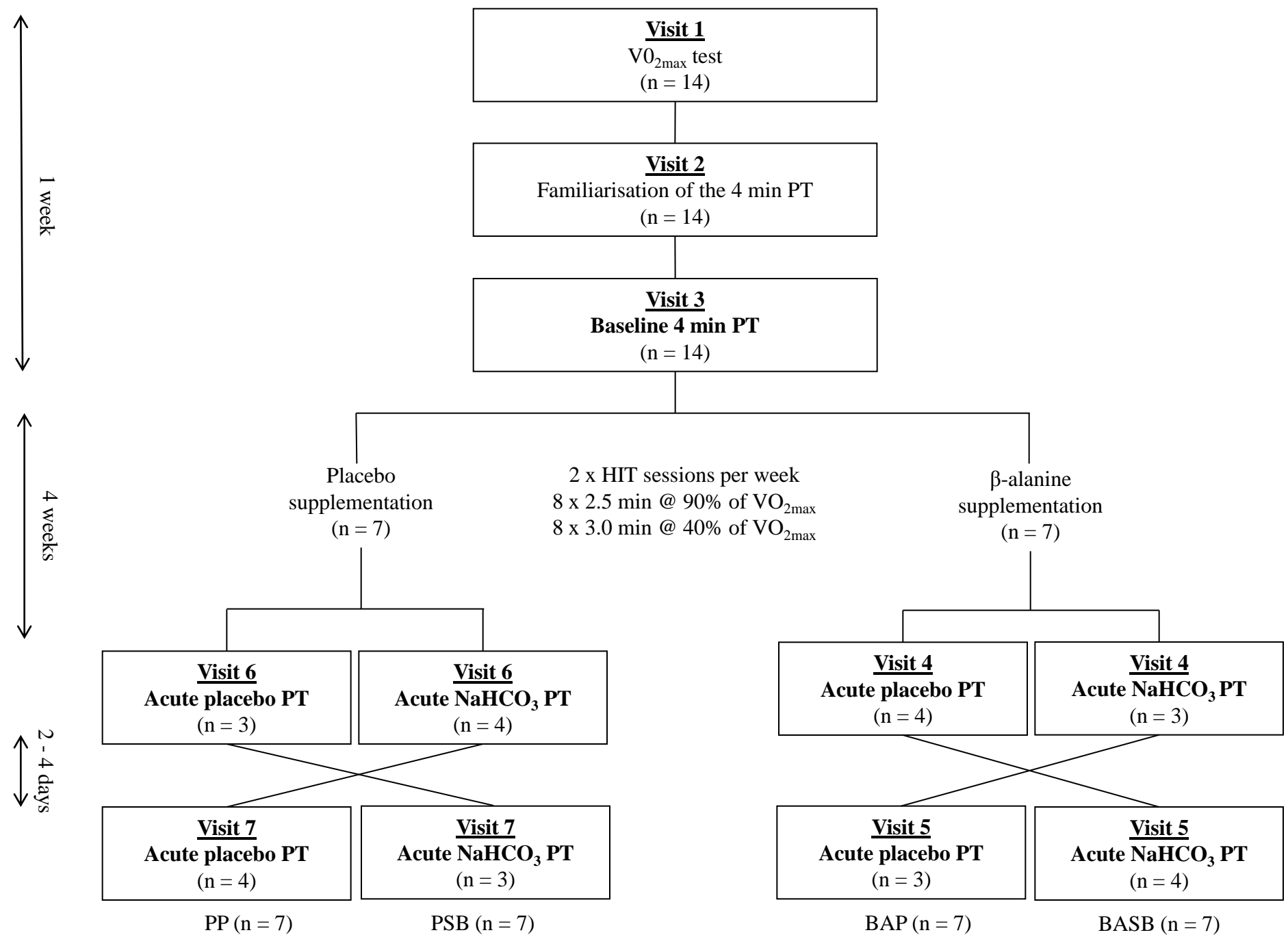




Figure 2  
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