Volume 38

2013

An NRC Research Press Journal

Une revue de NRC Research Press

www.nrcresearchpress.com

Applied Physiology, Nutrition, and Metabolism

Physiologie appliquée, nutrition et métabolisme

The Canadian Society for Exercise Physiology and the Canadian Nutrition Society have chosen *Applied Physiology, Nutrition, and Metabolism* as their principal medium for the publication of research papers

CSEP | SCPE

Canadian Nutrition Society
Société canadienne de nutrition

La Société canadienne de physiologie de l'exercice et la Société canadienne de nutrition ont choisi *Physiologie appliqueé, nutrition et métabolisme* comme leur principal organe de publication d'articles de recherche

NRC Research Press

NRC Research Press

ARTICLE

The ergogenic effect of beta-alanine combined with sodium bicarbonate on high-intensity swimming performance

Vitor de Salles Painelli, Hamilton Roschel, Flávia de Jesus, Craig Sale, Roger Charles Harris, Marina Yázigi Solis, Fabiana Braga Benatti, Bruno Gualano, Antonio Herbert Lancha, Jr., and Guilherme Giannini Artioli

Abstract: We investigated the effect of beta-alanine (BA) alone (study A) and in combination with sodium bicarbonate (SB) (study B) on 100- and 200-m swimming performance. In study A, 16 swimmers were assigned to receive either BA (3.2 g·day^{-1} for 1 week and 6.4 g·day^{-1} for 4 weeks) or placebo (PL; dextrose). At baseline and after 5 weeks of supplementation, 100- and 200-m races were completed. In study B, 14 were assigned to receive either BA (3.2 g·day^{-1} for 1 week and 6.4 g·day^{-1} for 3 weeks) or PL. Time trials were performed once before and twice after supplementation (with PL and SB), in a crossover fashion, providing 4 conditions: PL-PL, PL-SB, BA-PL, and BA-SB. In study A, BA supplementation improved 100- and 200-m time-trial performance by 2.1% ($p = 0.029$) and 2.0% ($p = 0.0008$), respectively. In study B, 200-m time-trial performance improved in all conditions, compared with presupplementation, except the PL-PL condition (PL-SB, +2.3%; BA-PL, +1.5%; BA-SB, +2.13% ($p < 0.05$)). BA-SB was not different from BA-PL ($p = 0.21$), but the probability of a positive effect was 78.5%. In the 100-m time-trial, only a within-group effect for SB was observed in the PL-SB ($p = 0.022$) and BA-SB ($p = 0.051$) conditions. However, 6 of 7 athletes swam faster after BA supplementation. The probability of BA having a positive effect was 65.2%; when SB was added to BA, the probability was 71.8%. BA and SB supplementation improved 100- and 200-m swimming performance. The coingestion of BA and SB induced a further nonsignificant improvement in performance.

Key words: performance, buffer, acidosis, ergogenic aid, athlete, supplementation.

Résumé : Dans cette étude, on analyse les effets du bêta-alanine (« BA ») seul (étude A) et en combinaison avec du bicarbonate de sodium (« SB ») (étude B) sur la performance à la nage sur une distance de 100 m et de 200 m. Dans l'étude A, on répartit 16 nageurs dans deux groupes : BA recevant 3,2 g·jour^{-1} durant 1 semaine; 6,4 g·jour^{-1} durant 4 semaines ou placebo (« PL »; dextrose). Au début et après 5 semaines de supplémentation, les sujets participent aux épreuves de 100 m et de 200 m. Dans l'étude B, 14 sujets reçoivent soit BA (3,2 g·jour^{-1} durant 1 semaine; 6,4 g·jour^{-1} durant 3 semaines) ou le PL (dextrose). Les épreuves contre-la-montre sont réalisées une fois avant la supplémentation et deux fois après (incluant PL et SB), et ce, selon un plan croisé procurant 4 conditions : PL-PL, PL-SB, BA-PL, BA-SB. Dans l'étude A, la supplémentation en BA améliore de 2,1 % ($p = 0,029$) et 2,0% ($p = 0,0008$) respectivement la performance aux épreuves de 100 m et de 200 m contre-la-montre. Dans l'étude B, à l'épreuve de 200 m contre-la-montre, on observe une amélioration de la performance dans toutes les conditions, sauf en PL-PL comparativement à l'épreuve avant la supplémentation : PL-SB : +2,3 %; BA-PL : +1,5 %, BA-SB : +2,13 %; $p < 0,05$. On n'observe pas de différence entre BA-SB et BA-PL ($p = 0,21$), mais la probabilité d'un effet positif est de 78,5 %. Dans l'épreuve de 100 m contre-la-montre, on observe seulement un effet intragroupe de SB après PL-SB ($p = 0,022$) et BA-SB ($p = 0,051$). Toutefois, 6 athlètes sur 7 nagent plus vite après la supplémentation en BA; la probabilité d'un effet positif de BA est de 65,2 % et celle de SB est de 71,8 % lorsqu'ajoutée à BA. La supplémentation en BA et en SB améliore la performance à la nage sur des distances de 100 m et de 200 m. L'apport combiné de BA et de SB améliore encore la performance, mais de façon non significative. [Traduit par la rédaction]

Mots-clés : performance, tampon, acidose, facteur ergogène, athlète, supplémentation.

Introduction

Muscle acidosis is a major cause of fatigue during high-intensity exercise. Possible mechanisms for this include competition between hydrogen ions (H^+) and calcium ions (Ca^{2+}) at the troponin-binding site, limiting the ability of the muscle contractile machinery to operate effectively (Donaldson et al. 1978; Fabiato and Fabiato 1978); impairment of energy production through the inhibition of key glycolytic enzymes (Sutton et al. 1981); inhibition of recovery of phosphorylcreatine (Harris et al. 1976); and disruption of its role as a temporal buffer of ADP accumulation (Sahlin and Harris 2011). In this context, nutritional strategies capable of increasing muscle-buffering capacity and attenuating the increase in H^+ concentration during high-intensity exercise might benefit performance.

To date, 2 nutritional aids have been shown to reduce the rate of intramuscular H^+ accumulation: sodium bicarbonate (SB) and beta-alanine (BA). Whereas supplementation with SB increases the extracellular buffering capacity and the dynamic buffering (i.e., H^+ efflux from muscle cells to blood) by increasing the blood bicarbonate concentration (for a review, see Requena et al. 2005), supplementation with BA increases intracellular buffering capacity by augmenting intramuscular carnosine (β-alanyl-L-histidine) synthesis (Harris et al. 2006). In addition to the increased muscle-buffering capacity induced by elevated muscle carnosine, a recent study with human muscle fibres showed that high carnosine lev-

Received 19 July 2012. Accepted 1 November 2012.

V. de Salles Painelli, H. Roschel, F. de Jesus, M.Y. Solis, F.B. Benatti, B. Gualano, A.H. Lancha, Jr., and G.G. Artioli. University of Sao Paulo, School of Physical Education and Sports – Department of Biodynamics, Laboratory of Applied Nutrition and Metabolism, Av. Mello de Moraes, 65 - Butanta, 05508-030, Sao Paulo, SP, Brazil.
C. Sale. Biomedical, Life and Health Sciences Research Centre, Nottingham Trent University, Nottingham NG11 8NS, UK.
R.C. Harris. Junipa Ltd., Newmarket, Suffolk, UK.
Corresponding author: Vitor de Salles Painelli (e-mail: vitor.painelli@usp.br).

Table 1. Participant characteristics in studies A and B.

	Study A		Study B	
Variable	BA (n = 9)	PL (n = 7)	BA (n = 7)	PL (n = 7)
Age (y)	18.62±1.99	20.16±5.91	20.71±3.35	18.71±2.75
Height (m)	1.80±0.05	1.77±0.06	1.83±0.05	1.79±0.06
Body mass (kg)	69.82±10.90	68.43±9.67	71.92±15.15	67.82±8.00
200-m TT performance (s)	139.13±16.24	144.28±18.19	131.62±8.84	137.38±9.79
100-m TT performance (s)	62.15±7.26	64.79±8.51	60.00±4.31	63.26±5.55

Note: No differences between groups in the 2 studies. BA, beta-alanine; PL, placebo; TT, time trial.

els can improve the Ca^{2+} sensitivity of the contractile apparatus and enhance Ca^{2+} release from sarcoplasmic reticulum when Ca^{2+} release is inhibited, such as during fatigue (Dutka et al. 2012). This mechanism might help to explain part of the beneficial effects of BA supplementation, with or without SB ingestion, that have been reported in the literature (Sale et al. 2011).

There are various studies showing the beneficial effects of both acute and chronic SB ingestion on high-intensity exercise capacity (McNaughton et al. 2008) and on performance in various sports, such as judo (Artioli et al. 2007), swimming (Lindh et al. 2008), and boxing (Siegler and Hirscher 2010). Several qualitative reviews are available on the effects of BA on exercise performance and capacity (Artioli et al. 2010; Derave et al. 2010; Sale et al. 2010). Hobson and colleagues (2012) recently provided a quantitative review of available studies using meta-analysis, and reported a positive effect (p = 0.002) of BA on the outcome of 57 exercise tests from 15 published papers. Much of the positive effect was explained by significant improvements (p = 0.013) in exercise capacity, although BA did not significantly improve exercise performance (p = 0.204). However, it should be noted that few studies have addressed the effect of BA on sports-specific tasks, and the findings of those that have are conflicting (Derave et al. 2007; Van Thienen et al. 2009; Baguet et al. 2010; Bellinger et al. 2012; Saunders et al. 2012).

High blood lactate (9–15 mmol·L^{-1}) has been shown after 100- and 200-m freestyle swimming races, suggesting that acidosis is a key feature of short-distance swimming events (Bonifazi et al. 1993). Therefore, it seems reasonable to expect that athletes engaged in these events will benefit from an enhanced ability to buffer reductions in intracellular pH. A combination of strategies capable of increasing intracellular (with BA supplementation) and extracellular and dynamic buffering capacity (with SB supplementation) could further enhance the ability of the muscle to buffer H^+. Also, the recently demonstrated effect of carnosine on calcium sensitivity (Dutka et al. 2012) could provide an additional theoretic mechanism for the additive ergogenic effect of BA and SB.

Some studies have examined the ergogenic potential of SB on short-distance swimming performance (i.e., 100 and 200 m or 100 and 200 yards) and interval swimming performance (Gao et al. 1988; Pierce et al. 1992; Lindh et al. 2008; Pruscino et al. 2008; Siegler and Hirscher 2010; Joyce et al. 2012). However, results are quite conflicting; some studies reported no effect of SB on time-trial performance (Pierce et al. 1992; Pruscino et al. 2008; Joyce et al. 2012), one reported a positive effect on swimming trials (Lindh et al. 2008), and others reported improved interval swimming performance (Gao et al. 1988; Siegler and Hirscher 2010). However, no study has yet investigated the effects of BA supplementation on swimming performance. Likewise, the potential for coingestion of BA and SB to further improve swimming performance, compared with either BA or SB alone, has not been studied. Existing studies on the combined effects of BA and SB on exercise capacity (Sale et al. 2011) and performance (Bellinger et al. 2012) are equivocal, and further information is required from well-controlled exercise performance studies. Therefore, we performed 2 independent studies aimed at examining the effect of BA supplementation alone (study A) and in combination with SB supplementation (study B) on high-intensity swimming performance (i.e., 100- and 200-m races).

Materials and methods

Study A: Effects of BA on swimming performance

Participants

Eighteen junior-standard swimmers (12 male, 6 female) volunteered to participate in the study. Participant characteristics are presented in Table 1. All swimmers were well-trained athletes who were actively participating in state-level official competitions at the time of data collection. All athletes were involved in a regular training program that focused on 100- and 200-m distance training. The periodization phase was the same during the course of the study, and training regimens consisted of 6 × 1.5 h pool-based and 2 × 1 h land-based training sessions per week. Participants had not consumed creatine in the 3 months prior to the study or BA in the 6 months prior to the study. All participants were fully informed of any risks and discomforts associated with the study, and all gave their written informed consent. The study was approved by the institution's Ethical Advisory Committee.

Experimental design and dietary supplementation

A randomized, double-blind, placebo-controlled, parallel-group study was conducted. The randomization was stratified by gender and by individual best time in the 200-m race.

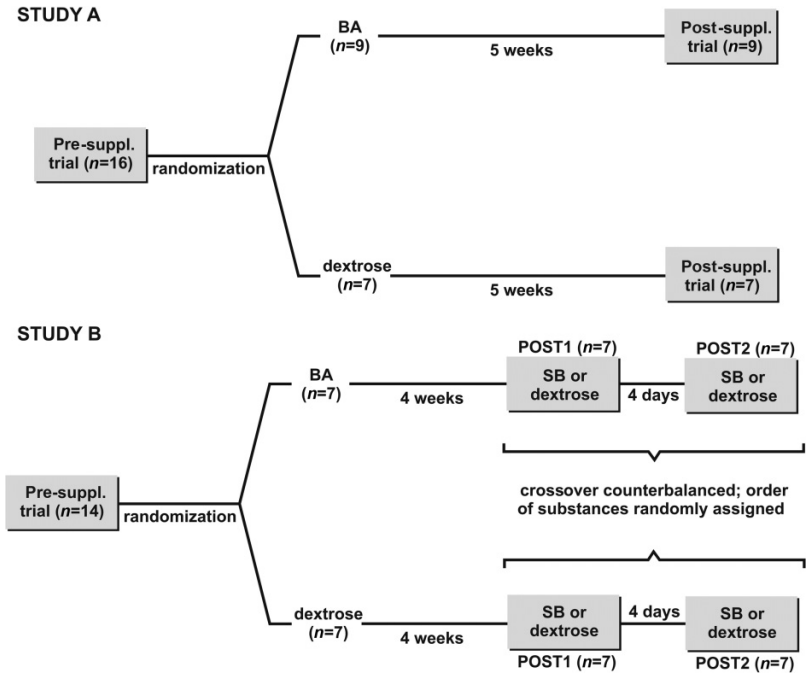
The athletes were assigned to receive either BA (n = 9) or placebo (PL; n = 9). However, 2 volunteers from the PL group dropped out before completing the study. Athletes in the BA group received 3.2 g·day^{-1} of BA (CarnoSyn, Compound Solutions Inc., Vista, Calif., USA) during the first week (800 mg gelatin capsules, taken 4 times per day) and 6.4 g·day^{-1} during the subsequent 4 weeks (2 × 800 mg gelatin capsules, taken 4 times per day). Athletes in the PL group received the exact same amount of placebo (i.e., dextrose), which was given as identical capsules (Ethika Inc., Sao Paulo, Brazil). Carboxymethyl cellulose was added to both BA and dextrose capsules to slow the absorption of BA and minimize paraesthesia.

Figure 1 illustrates the experimental design. Participants underwent the same experimental procedures on 2 different occasions, 5 weeks apart. The trials were completed at baseline (PRE) and after 5 weeks of supplementation (POST). In each trial, the athlete's performance was assessed with 2 simulated swimming time trials (i.e., 100- and 200-m freestyle races).

Simulated swimming time trials

All simulated swimming time trials took place in the same swimming pool (2 m deep × 25 m long × 20 m wide) at the same time of day (1500–1700 h). The athletes were familiar with competing over the set distances and performed each race in ability-matched pairs, as assigned by their coach. This procedure was adopted to introduce a competitive element to the protocol, which helps to make the simulation closer to a real competitive situation (Lindh et al. 2008). The order of racing was kept the same in the PRE and POST trials. Each pair completed a standardized warm-up prior to each trial. Ten minutes after the warm-up, the

Fig. 1. Experimental design of studies A (top panel) and B (bottom panel). BA, beta-alanine; SB, sodium bicarbonate; suppl., supplementation; POST1, performance assessment immediately after the 4 weeks of supplementation; POST2, performance assessment immediately after the 4 weeks and 4 days of supplementation.



pairs performed the 200-m freestyle time-trial performance. After the 200-m trial, the athletes recovered for 30 min before the same pairs performed the 100-m freestyle time-trial performance. All races started with the athletes diving off diving blocks and were timed using an electronic timing system (Omega Swiss Timing, Bern, Switzerland).

Food intake assessment

To control for intervening variables, food intake was assessed during the supplementation period by means of three 24-h dietary recalls undertaken on separate days (2 weekdays and 1 weekend day), with the aid of a visual photo album of real-sized foods and portions. The 24-h dietary recall consisted of listing the foods and beverages consumed during the 24 h before the assessment. Nutritional supplements were also recorded. Energy and macronutrient intake were analysed with Virtual Nutri software (Sao Paulo, Brazil).

Study B: Effects of the coingestion of BS and SB on swimming performance

Participants

Fourteen junior-standard swimmers (7 male, 7 female), not engaged in study A, volunteered for participation in this study (athletes' main characteristics are presented in Table 1). The periodization phase was the same during the course of the study, and training regimens consisted of 8 × 2.5-h pool-based and 3 × 1.5-h land-based training sessions a week. Exclusion criteria, randomization and blinding procedures, and dietary assessment and performance tests were identical to those described in study A. All participants were fully informed of any risks and discomforts associated with the study, and all gave their written informed consent. The study was approved by the institution's Ethical Advisory Committee.

Experimental design

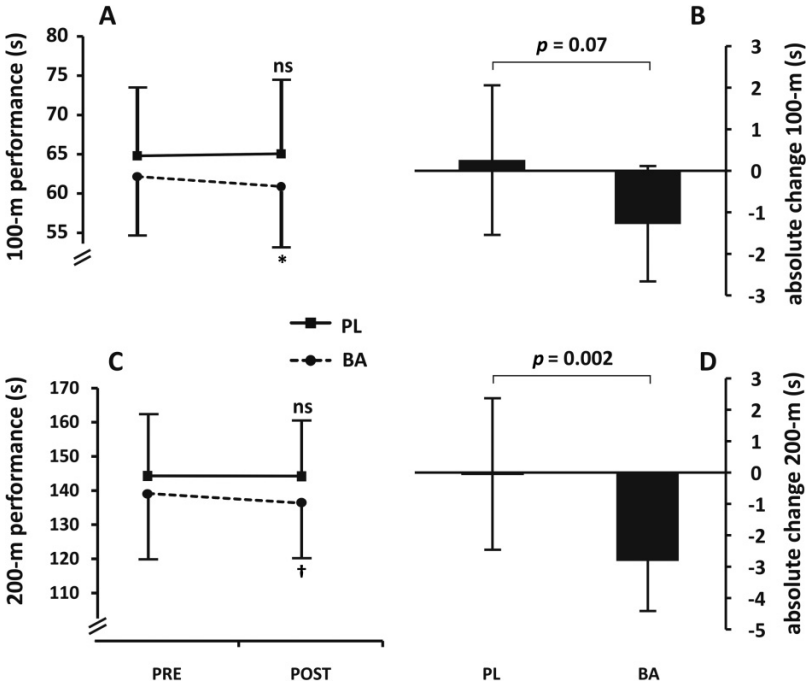
The experimental design (Fig. 1) was adapted from Sale et al. (2011). The performance tests were identical to those applied in study A. The first trial (PRE) was completed prior to the supplementation period. The athletes were then randomly assigned to receive either BA (n = 7) or PL (n = 7), and were supplemented for 4 weeks and 4 days. The BA group received 3.2 g·day^{-1} of BA (CarnoSyn, Compound Solutions Inc.) during the first week (800 mg gelatin capsules, taken 4 times per day) and 6.4 g·day^{-1} during the subsequent 3 weeks and 4 days (2 × 800 mg gelatins capsules, taken 4 times per day). Athletes in the PL group received the exact same amount of placebo (i.e., dextrose), which was given as identical capsules (Ethika Inc.). Carboxymethyl cellulose was added to both BA and dextrose capsules to slow the absorption of BA and minimize paraesthesia.

After 4 weeks of BA or PL supplementation, the athletes acutely ingested either SB (0.3 g·kg^{-1} body weight) or placebo (same dose of dextrose) in a crossover fashion (POST1 and POST2 assessments (POST1 and POST2, the first and second assessment of swimming performance, respectively, after the supplementation begun)). There was a 4-day interval between trials, during which the participants continued the BA or PL supplementation. SB and placebo were acutely consumed over a 10-min period, with 500 mL of plain water, commencing 90 min prior to the start of the trials. This design allowed for 4 experimental conditions: PL-PL, PL-SB, BA-PL, and BA-SB.

Statistical analysis

In both studies, unpaired t tests were used to compare performance, food intake, and participant characteristics between groups at the PRE trial. Swimming performance was tested with repeated-measures mixed models, followed by single-degree-of-freedom contrast analysis. In study A, the absolute changes (i.e., POST-PRE) in performance were compared in the experimental groups using an unpaired t test. In study B, absolute changes were compared in the experimental conditions with a 1-way analysis of variance, followed by a Tukey's post hoc test. Additionally, a magnitude-based inference was conducted, following the recommendations of Batterham and Hopkins (2006), to detect small effects of practical importance in an applied setting. This establishes the likelihood (in percentage terms) of each experimental manipulation having a positive, trivial, or negative effect. All

Fig. 2. Time-trial performance (A, C) and absolute changes in time-trial performance (B, D) for the beta-alanine (BA) and placebo (PL; dextrose) conditions in both 100-m and 200-m races (study A). ns, not significant. *, $p = 0.029$ for within-group effect; †, $p = 0.0008$ for within-group effect.



other analyses were conducted using SAS software, version 9.2. The significance level was set at $p < 0.05$.

Results

Study A

No between-group differences were observed prior to supplementation (PRE) for the 100- and 200-m freestyle races (Table 1).

In the 100-m trial, mixed-model analysis revealed a trend toward a significant effect of BA supplementation on the time-trial performance (interaction effect, $F = 3.75$; $p = 0.07$) (Fig. 2A). Also, contrast analysis revealed a significant within-group effect after BA supplementation ($p = 0.029$) (Fig. 2A). Absolute changes in performance in the BA group, compared with the PL group, approached a statistically significant difference in the 100-m race ($p = 0.07$) (Fig. 2B). Individual data show that all 9 athletes swam faster after BA supplementation (average change, –2.1%), whereas only 4 of 7 in the PL group swam faster in the 100-m race (average change, +0.3%) (Table 2).

In the 200-m trial, a significant interaction effect was observed in the mixed-model analysis ($F = 7.59$; $p = 0.015$) (Fig. 2C). Also, contrast analysis revealed a significant within-subject effect in the BA group ($p = 0.0008$) (Fig. 2C). Absolute changes in performance observed in the BA group were statistically different from those in the PL group in the 200-m race ($p = 0.002$) (Fig. 2D). Individual data showed that 8 of 9 athletes swam faster in the BA group (average change, –2.0%), whereas 4 of 7 swam faster in the PL group (average change, –0.1%) (Table 2).

Magnitude-based inference analysis revealed that the positive performance changes after BA supplementation in the 100- and 200-m trials were approximately 96% and 99%, respectively (Table 3).

Food intake

The intake of energy (BA, 3499 ± 1327 kcal; PL, 3335 ± 1035 kcal; $p = 0.80$), carbohydrate (BA, 52.8% ± 5.9%; PL, 50.4% ± 8.2%; $p = 0.55$), lipid (BA, 30.8% ± 7.7%; PL, 29.8% ± 5.2%; $p = 0.77$), and protein (BA, 16.7% ± 2.3%; PL, 19.9% ± 6.2%; $p = 0.23$) did not significantly differ within or between groups.

Table 2. Individual time-trial performance (in seconds) in the 100- and 200-m swimming races (study A).

Athlete no.	Placebo		Athlete no.	Beta-alanine	
	PRE	POST		PRE	POST
100-m time trial					
1	59.97	59.28	8	53.58	52.62
2	55.85	55.22	9	70.12	69.50
3	81.21	83.82	10	76.03	75.89
4	63.60	62.16	11	61.87	61.09
5	61.87	64.77	12	64.16	59.47
6	60.46	60.84	13	57.43	56.16
7	70.59	69.30	14	56.96	56.45
—	—	—	15	56.28	55.56
—	—	—	16	63.19	61.20
Mean ± SD	64.79±8.52	65.06±9.37		62.18±7.23	60.88±7.42
200-m time trial					
1	132.19	129.60	8	118.22	115.05
2	122.35	123.60	9	162.08	158.57
3	175.75	177.32	10	165.94	162.87
4	142.97	139.78	11	139.86	135.59
5	154.38	158.06	12	145.00	145.00
6	130.10	130.06	13	130.88	128.00
7	152.23	151.21	14	129.12	126.21
—	—	—	15	124.25	123.65
—	—	—	16	136.88	132.00
Mean ± SD	144.3±18.2	144.2±19.1		139.1±16.2	136.3±16.1

Note: PRE, baseline; POST, after 5 weeks of supplementation.

Study B

At PRE, there were no significant differences between the BA and PL groups in either 100- or 200-m freestyle time-trial performance (Table 1).

100-m swimming performance

In the 100-m time trial, the mixed-model analysis showed no interaction effect ($F = 1.36$; $p = 0.28$). Contrast analysis revealed significant within-group effects when SB was ingested (i.e., in the PL-SB and BA-SB conditions; $p = 0.022$ and $p = 0.051$, respectively),

Table 3. Magnitude-based inference analysis of results from studies A and B.

Comparators	Probability of being positive, %	Probability of being trivial, %	Probability of being negative, %
Study A (100 m)			
BA vs. PRE	96.2	3.3	0.5
PL vs. PRE	18.0	34.4	47.6
Study A (200 m)			
BA vs. PRE	99.2	0.7	0.1
PL vs. PRE	32.1	40.1	27.8
Study B (100 m)			
BA vs. PRE	65.2	25.9	8.9
SB vs. PRE	97.0	2.6	0.4
BA-SB vs. PRE	93.6	5.5	0.9
PL vs. PRE	12.6	30.0	57.4
BA-SB vs. BA	71.8	21.7	6.5
Study B (200 m)			
BA vs. PRE	92.3	6.4	1.3
SB vs. PRE	100	0.0	0.0
BA-SB vs. PRE	100	0.0	0.0
PL vs. PRE	2.6	11.0	86.4
BA-SB vs. BA	78.5	16.8	4.7

Note: All probabilities refer to the POST trial (after supplementation) vs. the PRE trial (baseline), except BA-SB vs. BA, which refers to the addition of sodium bicarbonate (SB), compared with beta-alanine (BA) alone.

but no effect was observed in the BA-PL condition ($p = 0.49$) (Fig. 3A). The combination of BA plus SB did not elicit further improvements in the 100-m performance, compared with BA alone ($p = 0.29$) (Fig. 3A). However, the likelihood of BA having a positive effect on performance was ~65%, and the likelihood of SB exerting additive effects to BA was ~72% (Table 3).

Absolute change analysis revealed no differences in the 4 conditions ($F = 2.01$; $p = 0.14$) (Fig. 3B). Two of the 7 athletes swam faster after placebo ingestion (i.e., PL-PL condition; average change, +1.1%; Δ mean time, +0.67 ± 2.77 s) than before supplementation. In contrast, 100-m performance improved in 6 of the 7 athletes after acute SB supplementation (i.e., PL-SB condition; average change, –2.6%; Δ mean time, –1.70 ± 2.07 s) and after chronic BA supplementation (i.e., BA-PL condition; average change, –1.4%; Δ mean time, –0.84 ± 1.47 s). All 7 athletes were faster after chronic BA combined with acute SB supplementation (average change, –2.8%; Δ mean time, –1.59 ± 1.71 s) than before supplementation. All 7 athletes showed further improvement in the 100-m performance after acute SB supplementation combined with chronic BA supplementation (BA-SB), compared with placebo (BA-PL) (average change, –1.4%; Δ mean time, –0.76 ± 0.43 s). Table 4 presents data for the individual athletes.

200-m swimming performance

There was a significant improvement in the 200-m time-trial performance after chronic supplementation with BA, after acute ingestion of SB, and after the combination of these 2 (interaction effect, $F = 5.17$; $p = 0.024$) (Fig. 3C). According to the contrast analysis, BA supplementation (i.e., BA-PL condition) tended to improve 200-m performance ($p = 0.06$), compared with the PRE performance (Fig. 3C), whereas both PL-SB and BA-SB supplementation significantly improved 200-m performance ($p = 0.0001$ and $p < 0.0001$, respectively) (Fig. 3C). The combination of BA plus SB did not elicit further significant improvements in the 200-m performance, compared with BA alone ($p = 0.21$) (Fig. 3C). However, the likelihood of BA having a positive effect on the 200-m performance was ~92%, and the likelihood of SB having additive effects to BA was ~78% (Table 3).

Absolute change analysis revealed that the BA-PL, PL-SB, and BA-SB conditions yielded greater performance improvements than PL-PL ($F = 8.5$; $p = 0.001$), although no differences were observed between conditions (Fig. 3D). Three of the 7 athletes swam faster after placebo ingestion (i.e., PL-PL condition; average change, +0.9%; Δ mean time, +1.31 ± 2.77 s) than before supplementation. All 7 athletes showed improved 200-m performance after acute SB supplementation (i.e., PL-SB condition); average change, –2.3%; Δ mean time, –3.29 ± 3.29 s) and after chronic BA supplementation (i.e., BA-PL condition; average change, –1.5%; Δ mean time, –1.97 ± 1.01 s). All 7 athletes were also faster after chronic BA combined with acute SB supplementation (average change, –2.13%; Δ mean time, Δ2.78 ± 0.82 s) than before supplementation. Additionally, all 7 athletes showed further improvements in the 200-m performance when acute SB supplementation followed chronic BA supplementation (BA-SB), compared with placebo (BA-PL) (average change, –0.63%; Δ mean time, –1.18 ± 0.62 s). Table 4 presents data for the individual athletes.

Food intake analysis

The intake of energy (BA, 3418 ± 822 kcal; PL, 3219 ± 794 kcal; $p = 0.66$), carbohydrate (BA, 57.1% ± 6.8%; PL, 53.2% ± 5.6%; $p = 0.29$), lipid (BA, 24.2% ± 4.5%; PL, 26.3% ± 6.4%; $p = 0.51$), and protein (BA, 18.5% ± 6.8%; PL, 20.4% ± 3.8%; $p = 0.56$) did not significantly differ within or between groups.

Side effects and blinding efficacy

In study A, 4 of 9 subjects reported mild paraesthesia with BA supplementation; in study B, 4 of 7 subjects reported the same symptoms. None of the subjects in study B reported severe gastrointestinal symptoms related to SB ingestion, but all of them experienced eructation with SB supplementation. In study A, 7 of 16 subjects successfully predicted the substance ingested (Fischer's exact test, $p = 0.61$). In study B, 6 of 14 subjects successfully predicted BA or PL during the 4 weeks (Fischer's exact test, $p = 0.59$), whereas 8 of 14 subjects successfully predicted SB or PL after acute ingestion (Fischer's exact test, $p = 0.59$).

Discussion

To our knowledge, this is the first study to examine the ergogenic effects of BA supplementation on swimming performance. Furthermore, this is one of the few studies to examine the effects of BA, with or without SB supplementation, in well-trained competitive athletes.

Our main finding is that 4 and 5 weeks of BA supplementation effectively improved the 200-m freestyle swimming performance of trained athletes, and tended to improve the 100-m performance. Although the effect of BA on the 100-m performance did not reach statistical significance, 15 of 16 athletes (all 9 in study A and 6 of 7 in study B) performed better after BA supplementation than before, which is likely to be relevant in real competitive settings (probability of positive effect was 96.2% in study A and 65.2% in study B). Combining data from the 2 studies (while noting the slightly shorter supplementation period in study B), we estimate that the 100-m swim time improved with 4–5 weeks of BA supplementation by –1.08 ± 1.39 s, compared with a presupplementation time of 61.22 ± 6.06 s, in contrast to a mean change in the combined placebo group of +0.46 ± 2.26 s. Similarly, the combined 200-m swim data allowed us to estimate an improvement of –2.28 ± 1.47 s after 4–5 weeks of BA supplementation, in contrast to a mean change in the combined placebo group of +0.63 ± 2.60 s.

It is interesting to note that BA supplementation was somewhat more effective in study A than in study B, especially for the 100-m time trials. The most likely explanation for this relates to the statistical power of the 2 studies; although study A assessed 3 more athletes than study B, the number of levels in the statistical analysis in study B was considerably higher, which certainly hindered our ability to detect the slight, though meaningful, improvements in performance elicited with BA. To overcome this

Fig. 3. Time-trial performance (A, C) and absolute changes in time-trial performance (B, D) for the beta-alanine (BA) and placebo (PL) conditions in both 100- and 200-m races before and after the acute ingestion of sodium bicarbonate (SB) and placebo (study B). ns, not significant. *, Significantly different from baseline ($p < 0.05$ for within-group effect); p values that tended to be significantly different from baseline are displayed in the figure.

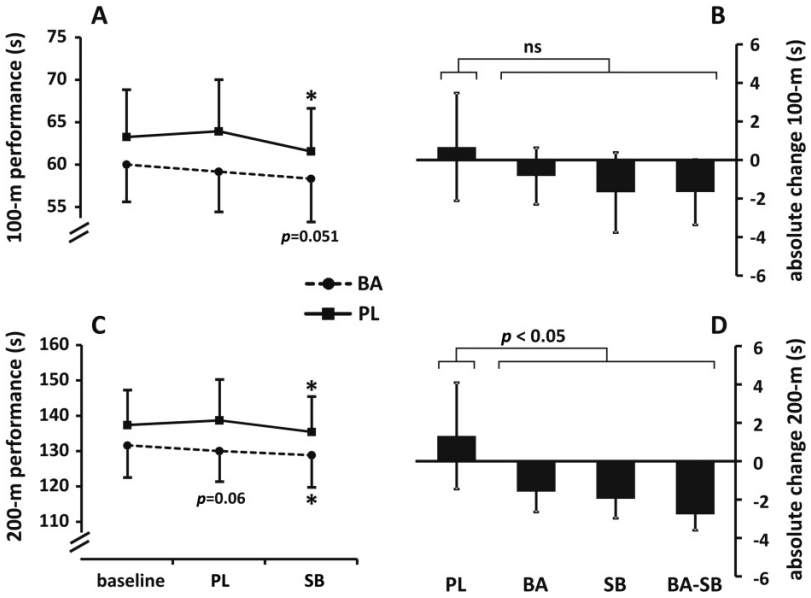


Table 4. Individual time-trial performance (in seconds) in the 100- and 200-m swimming races (study B).

	Placebo condition				BA condition		
Athlete no.	Baseline	PL-PL	PL-SB	Athlete no.	Baseline	BA-PL	BA-SB
100-m time trial							
17	65.45	70.91	65.29	24	55.73	55.76	54.06
18	65.85	61.99	60.59	25	55.82	55.78	54.79
19	57.94	59.81	55.63	26	56.91	56.47	56.37
20	58.33	58.97	58.84	27	64.38	64.16	63.43
21	56.47	56.47	56.17	28	63.62	63.28	62.72
22	69.28	68.78	68.41	29	65.51	64.78	64.34
23	69.45	70.59	66.01	30	58.07	53.94	52.63
Mean±SD	63.25±5.55	63.93±6.02	61.56±5.05	60.01±4.31	59.17±4.67	58.33±4.97	
200-m time trial							
17	150.09	152.88	148.32	24	123.79	121.41	120.31
18	138.18	137.44	136.23	25	120.45	119.71	118.37
19	126.81	126.12	123.41	26	134.86	133.19	131.72
20	127.78	127.35	127.18	27	139.02	138.15	137.29
21	128.73	129.54	126.77	28	138.87	135.26	135.01
22	147.94	148.44	146.90	29	141.26	140.19	139.22
23	142.19	149.14	139.10	30	123.12	122.22	119.95
Mean±SD	137.4±9.8	138.7±11.4	135.4±10.0	131.6±8.8	130.0±8.6	128.8±9.0	

Note: BA, beta-alanine; PL, placebo; SB, sodium bicarbonate.

issue, we used a magnitude-based inference analysis, which may be a more appropriate approach in this type of study (Batterham and Hopkins 2006). In contrast to the 100-m time trials, the ergogenic effect of BA on the 200-m performance was more consistent in the 2 studies. This can be explained by the duration of the 200-m trial, which falls into the range that gets more benefit from BA supplementation (i.e., exercise lasting 60 to 240 s) (Hobson et al. 2012). Alternatively, it could be argued that the higher training volume and the better competitive indexes of athletes in study B hindered the effect of BA supplementation. This possibility cannot be ruled out because there is indirect evidence in the literature indicating that the putative increase in muscle-buffering capacity induced with high-volume, high-intensity long-term training regimens diminishes the importance of the BA-induced increase in muscle carnosine (Bellinger et al. 2012).

Study B indicates that SB was also effective in improving both the 100- and 200-m swimming performance, and to a similar extent as that achieved with BA. Moreover, acute SB decreased the swim time in athletes already supplemented with BA by a further –0.76 ± 0.43 s (71.2% likelihood of a positive effect) and –1.18 ± 0.62 s (78.5% likelihood of a positive effect) over the 100-m and 200-m trials, respectively. The combined effect of BA + SB supplementation were –1.59 (±1.71) s and –2.78 (±0.82) s, respectively. Our results are in accordance with those of Sale et al. 2011, which indicated that the coingestion of BA plus SB, compared with either one alone, led to a 4.1% increase in time to exhaustion, which related to a 69% probability that this is a meaningful difference in exercise capacity. Taken together, these 2 studies suggest a slight but probably important additive effect of BA and SB. Further studies are needed to examine whether or not different dosing regimens

of BA (e.g., for periods longer than 4 or 5 weeks, which could result in higher muscle carnosine accretion (Hill et al. 2007)) and SB (e.g., chronic supplementation) display more evident additive ergogenic effects.

More and more studies have shown that BA supplementation can improve high-intensity exercise capacity (Hobson et al. 2012), but fewer studies have examined the effect on exercise performance. Exercise performance studies are required to provide direct evidence of the efficacy of BA supplementation as an ergogenic aid in real sports settings. The few existing studies involving the application of BA to relevant sports activities have revealed a potential ergogenic effect in the final sprint of a cycling race (Van Thienen et al. 2009) and in rowing (Baguet et al. 2010), but not in a soccer-specific test (Saunders et al. 2012) or 400-m running trials (Derave et al. 2007). Indeed, further investigations are warranted to determine which sports could theoretically benefit from the ergogenic effect of BA (e.g., 400-m swimming races, 800-m running races, team sports, and combat sports).

It is important to emphasize that most investigations involving BA supplementation have enrolled physically active individuals, rather than well-trained athletes (Hill et al. 2007; Stout et al. 2007; Zoeller et al. 2007; Smith et al. 2009). Despite the methodologic constraints inherent in studies involving athletes (e.g., reduced sample size, competition schedule constraints, inability to perform invasive assessments), they are important to substantiate the efficacy of nutritional aids in sports. In this sense, our investigation corroborates the ergogenic effects of BA. In fact, by examining the intraindividual variances in the most recent Brazilian national-level 100- and 200-m freestyle competitions (i.e., the same competitive level as the athletes enrolled in our study), we observed that the improvements seen with both BA and SB are much greater than the normal variance of the real competition (normal variance, 0.4% for 100-m and 0.01% for 200-m trials, compared with improvements ranging from 1.4% to 2.8% after supplementation). This strengthens the concept that both strategies can provide meaningful advantages in actual competitions. Furthermore, considering the 100- and 200-m racing times of men and women competing at the semifinals and finals in the 2012 Olympic Games in London, the absolute improvements promoted by both BA (–1.28 s over 100 m and –1.60 s over 200 m) and SB (–1.69 s over 100 m and 1.97 s over 200 m) would have elevated the sixth-place athlete to first place in most events. Hence, the improvements observed in our investigation could represent a useful competitive advantage in the "real-world" scenario. However, it is important to highlight the fact that our athletes had no Olympic indexes at the time of data collection, although all of them were well trained. Thus, caution should be exercised when extrapolating these findings to top-level elite athletes.

An important limitation of this study is the lack of muscle and blood analyses to confirm the ability of BA to increase muscle carnosine and of SB to increase blood bicarbonate. However, because only well-trained competitive athletes volunteered for the investigation, data collection had to include only minimally invasive procedures. Despite the lack of these measurements, every human study using 1.6 to 6.4 g·day^{-1} of BA for 4 weeks or longer has found increases of >40% in muscle carnosine (Stellingwerff et al. 2012). Likewise, many studies have consistently demonstrated that acute ingestion of 300 mg·kg^{-1} of SB results in a significant increase in blood bicarbonate concentration and pH (Requena et al. 2005). Therefore, the supplementation protocols used for both BA and SB in our study are very likely to have promoted increased muscle carnosine and blood bicarbonate, respectively, which minimizes the weight of these lacking data.

Finally, it is important to note that more than 50% of the athletes ingesting BA reported mild paraesthesia, which is a symptom commonly described when 1600 mg of BA in powder is acutely ingested (Harris et al. 2006; Decombaz et al. 2011). The same symptoms are not observed when 1600 mg of BA is provided in controlled-release tablets (Stellingwerff et al. 2012), suggesting that the strategy adopted in our study to slow the absorption of BA (i.e., the addition of carboxymethyl cellulose to the gelatin capsules) was only partially successful in preventing paraesthesia. Despite this, our data show that the athletes were not able to correctly guess the substance that they were taking, which suggests that the blinding remained intact.

In conclusion, BA and SB supplementation improved the 200-m freestyle swimming performance in highly trained athletes, and tended to improve the 100-m performance. The coingestion of BA and SB further increased the 200-m time-trial performance by –1.18 s and the 100-m time-trial performance by –0.76 s, compared with BA alone, which makes the effect of supplementation, respectively, ~80% and ~70% likely to be meaningful. Future studies involving trained athletes are warranted to investigate the effect of BA, with and without SB, in the various sports in which performance is limited by acidosis.

Acknowledgements

The contribution of Vanessa Fujimoto Galves, Natália de Oliveira, and César Augusto do Carmo is gratefully acknowledged. Vitor de Salles Painelli is supported by CNPq (grant no. 130601/2011-0), and Guilherme G. Artioli is supported by FAPESP (grant no. 2011/17059-2). The authors are grateful to FAPESP (grant nos. 2010/51428-2 and 2010/11221-0), and to all athletes and coaches who participated in the study. Roger C. Harris is an independent consultant to Natural Alternatives International. The other authors declare that they have no conflict of interest.

References

Artioli, G.G., Gualano, B., Coelho, D.F., Benatti, F.B., Gailey, A.W., and Lancha, A.H., Jr. 2007. Does sodium-bicarbonate ingestion improve simulated judo performance? Int. J. Sport Nutr. Exerc. Metab. **17**(2): 206–217. PMID:17507744.

Artioli, G.G., Gualano, B., Smith, A., Stout, J., Lancha, A.H., Jr . 2010. Role of beta-alanine supplementation on muscle carnosine and exercise performance. Med. Sci. Sports Exerc. **42**(6): 1162–1173. PMID:20479615.

Baguet, A., Bourgois, J., Vanhee, L., Achten, E., and Derave, W. 2010. Important role of muscle carnosine in rowing performance. J. Appl. Physiol. **109**(4): 1096–1101. doi:10.1152/japplphysiol.00141.2010. PMID:20671038.

Batterham, A.M., and Hopkins, W.G. 2006. Making meaningful inferences about magnitudes. Int. J. Sports. Physiol. Perform. **1**(1): 50–57. PMID:19114737.

Bellinger, P.M., Howe, S.T., Shing, C.M., and Fell, J.W. 2012. Effect of Combined beta-Alanine and Sodium Bicarbonate Supplementation on Cycling Performance. Med. Sci. Sports Exerc. **44**(8): 1545–1551. doi:10.1249/MSS.0b013e31824cc08d. PMID:22330016.

Bonifazi, M., Martelli, G., Marugo, L., Sardella, F., and Carli, G. 1993. Blood lactate accumulation in top level swimmers following competition. J. Sports Med. Phys. Fitness, **33**(1): 13–18. PMID:8350602.

Decombaz, J., Beaumont, M., Vuichoud, J., Bouisset, F., and Stellingwerff, T. 2011. Effect of slow-release beta-alanine tablets on absorption kinetics and paresthesia. Amino Acids, **43**(1): 67–76. doi:10.1007/s00726-011-1169-7. PMID:22139410.

Derave, W., Ozdemir, M.S., Harris, R.C., Pottier, A., Reyngoudt, H., Koppo, K., et al. 2007. beta-Alanine supplementation augments muscle carnosine content and attenuates fatigue during repeated isokinetic contraction bouts in trained sprinters. J. Appl. Physiol. **103**(5): 1736–1743. doi:10.1152/japplphysiol.00397.2007. PMID:17690198.

Derave, W., Everaert, I., Beeckman, S., and Baguet, A. 2010. Muscle carnosine metabolism and beta-alanine supplementation in relation to exercise and training. Sports Med. **40**(3): 247–263. doi:10.2165/11530310-000000000-00000. PMID:20199122.

Donaldson, S.K., Hermansen, L., and Bolles, L. 1978. Differential, direct effects of H^+ on Ca^{2+}-activated force of skinned fibers from the soleus, cardiac and adductor magnus muscles of rabbits. Pflugers Arch. **376**(1): 55–65. doi:10.1007/BF00585248. PMID:30066.

Dutka, T.L., Lamboley, C.R., McKenna, M.J., Murphy, R.M., and Lamb, G.D. 2012. Effects of carnosine on contractile apparatus Ca2+ sensitivity and sarcoplasmic reticulum Ca2+ release in human skeletal muscle fibers. J. Appl. Physiol. **112**(5): 728–736. doi:10.1152/japplphysiol.01331.2011. PMID:22174397.

Fabiato, A., and Fabiato, F. 1978. Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiace and skeletal muscles. J. Physiol. **276**: 233–255. PMID:25957.

Gao, J.P., Costill, D.L., Horswill, C.A., and Park, S.H. 1988. Sodium bicarbonate ingestion improves performance in interval swimming. Eur. J. Appl. Physiol. Occup. Physiol. **58**(1–2): 171–174. doi:10.1007/BF00636622. PMID:2849539.

Harris, R.C., Edwards, R.H., Hultman, E., Nordesjo, L.O., Nylind, B., and Sahlin, K. 1976. The time course of phosphorylcreatine resynthesis during recovery of the quadriceps muscle in man. Pflugers Arch. **367**(2): 137–142. doi:10.1007/BF00585149. PMID:1034909.

Harris, R.C., Tallon, M.J., Dunnett, M., Boobis, L., Coakley, J., Kim, H.J., et al. 2006. The absorption of orally supplied beta-alanine and its effect on muscle carnosine synthesis in human vastus lateralis. Amino Acids, **30**(3): 279–289. doi:10.1007/s00726-006-0299-9. PMID:16554972.

Hill, C.A., Harris, R.C., Kim, H.J., Harris, B.D., Sale, C., Boobis, L.H., et al. 2007. Influence of beta-alanine supplementation on skeletal muscle carnosine concentrations and high intensity cycling capacity. Amino Acids, **32**(2): 225–233. doi:10.1007/s00726-006-0364-4. PMID:16868650.

Hobson, R.M., Saunders, B., Ball, G., Harris, R.C., and Sale, C. 2012. Effects of beta-alanine supplementation on exercise performance: a meta-analysis. Amino Acids, **43**(1): 25–37. doi:10.1007/s00726-011-1200-z. PMID:22270875.

Joyce, S., Minahan, C., Anderson, M., and Osborne, M. 2012. Acute and chronic loading of sodium bicarbonate in highly trained swimmers. Eur. J. Appl. Physiol. **112**(2): 461–469. doi:10.1007/s00421-011-1995-z. PMID:21584683.

Lindh, A.M., Peyrebrune, M.C., Ingham, S.A., Bailey, D.M., and Folland, J.P. 2008. Sodium bicarbonate improves swimming performance. Int. J. Sports Med. **29**(6): 519–523. doi:10.1055/s-2007-989228. PMID:18004687.

McNaughton, L.R., Siegler, J., and Midgley, A. 2008. Ergogenic effects of sodium bicarbonate. Curr. Sports Med. Rep. **7**(4): 230–236. doi:10.1249/JSR.0b013e31817ef530. PMID:18607226.

Pierce, E.F., Eastman, N.W., Hammer, W.H., and Lynn, T.D. 1992. Effect of induced alkalosis on swimming time trials. J. Sports Sci. **10**(3): 255–259. doi:10.1080/02640419208729924. PMID:1318391.

Pruscino, C.L., Ross, M.L., Gregory, J.R., Savage, B., and Flanagan, T.R. 2008. Effects of sodium bicarbonate, caffeine, and their combination on repeated 200-m freestyle performance. Int. J. Sport Nutr. Exerc. Metab. **18**(2): 116–130. PMID:18458356.

Requena, B., Zabala, M., Padial, P., and Feriche, B. 2005. Sodium bicarbonate and sodium citrate: ergogenic aids? J. Strength Cond. Res. **19**(1): 213–224. doi:10.1519/13733.1. PMID:15705037.

Sahlin, K., and Harris, R.C. 2011. The creatine kinase reaction: a simple reaction with functional complexity. Amino Acids, **40**(5): 1363–1367. doi:10.1007/s00726-011-0856-8. PMID:21394603.

Sale, C., Saunders, B., and Harris, R.C. 2010. Effect of beta-alanine supplementation on muscle carnosine concentrations and exercise performance. Amino Acids, **39**(2): 321–333. doi:10.1007/s00726-009-0443-4. PMID:20091069.

Sale, C., Saunders, B., Hudson, S., Wise, J.A., Harris, R.C., and Sunderland, C.D. 2011. Effect of beta-alanine plus sodium bicarbonate on high-intensity cycling capacity. Med. Sci. Sports Exerc. **43**(10): 1972–1978. doi:10.1249/MSS.0b013e3182188501. PMID:21407127.

Saunders, B., Sale, C., Harris, R.C., and Sunderland, C. 2012. Effect of beta-alanine supplementation on repeated sprint performance during the Loughborough Intermittent Shuttle Test. Amino Acids, **43**(1): 39–47. doi:10.1007/s00726-012-1268-0. PMID:22434182.

Siegler, J.C., and Hirscher, K. 2010. Sodium bicarbonate ingestion and boxing performance. J. Strength Cond. Res. **24**(1): 103–108. doi:10.1519/JSC.0b013e3181a392b2. PMID:19625976.

Smith, A.E., Walter, A.A., Graef, J.L., Kendall, K.L., Moon, J.R., Lockwood, C.M., et al. 2009. Effects of beta-alanine supplementation and high-intensity interval training on endurance performance and body composition in men; a double-blind trial. J. Int. Soc. Sports Nutr. **6**(5). doi:10.1186/1550-2783-6-5. PMID:19210788.

Stellingwerff, T., Decombaz, J., Harris, R.C., and Boesch, C. 2012. Optimizing human in vivo dosing and delivery of beta-alanine supplements for muscle carnosine synthesis. Amino Acids, **43**(1): 57–65. doi:10.1007/s00726-012-1245-7. PMID:22358258.

Stout, J.R., Cramer, J.T., Zoeller, R.F., Torok, D., Costa, P., Hoffman, J.R., et al. 2007. Effects of beta-alanine supplementation on the onset of neuromuscular fatigue and ventilatory threshold in women. Amino Acids, **32**(3): 381–386. doi:10.1007/s00726-006-0474-z. PMID:17136505.

Sutton, J.R., Jones, N.L., and Toews, C.J. 1981. Effect of PH on muscle glycolysis during exercise. Clin. Sci. (Lond.), **61**(3): 331–338. PMID:7261554.

Van Thienen, R., Van Proeyen, K., Vanden Eynde, B., Puype, J., Lefere, T., and Hespel, P. 2009. Beta-alanine improves sprint performance in endurance cycling. Med. Sci. Sports Exerc. **41**(4): 898–903. doi:10.1249/MSS.0b013e31818db708. PMID:19276843.

Zoeller, R.F., Stout, J.R., O'Kroy, J.A., Torok, D.J., and Mielke, M. 2007. Effects of 28 days of beta-alanine and creatine monohydrate supplementation on aerobic power, ventilatory and lactate thresholds, and time to exhaustion. Amino Acids, **33**(3): 505–510. doi:10.1007/s00726-006-0399-6. PMID:16953366.