Doubling of muscle carnosine concentration does not improve laboratory 1-h cycling time trial performance

Weiliang Chung[1,2], Audrey Baguet[1], Tine Bex[1], David J. Bishop[2], Wim Derave[1]

1. Department of Movement and Sports Sciences, Ghent University, Watersportlaan 2, 9000 Ghent, Belgium.
2. Institute of Sport, Exercise and Active Living (ISEAL), Victoria University, Ballarat Road, Footscray Park, VIC 3011, Australia.

Name and address for correspondence:

Weiliang Chung

Institute of Sport, Exercise and Active Living (ISEAL)

Victoria University

Ballarat Road, Footscray Park

VIC 3011, Australia

Phone: +61 3 9919 4793

+61 402 365 111

Email: weiliang.chung@hotmail.com

Abstract

Muscle carnosine loading through chronic oral beta-alanine supplementation has been shown to be effective for short-duration, high-intensity exercise. This randomised, placebo controlled study explored whether the ergogenic effect of beta-alanine supplementation is also present for longer duration exercise. Subjects (27 well-trained cyclists/triathletes) were supplemented with either beta-alanine or placebo (6.4 g/day) for six weeks. Time to completion and physiological variables for a 1-h cycling time-trial were compared between pre- and post-supplementation. Muscle carnosine concentration was also assessed via proton magnetic resonance spectroscopy before and after supplementation. Following beta-alanine supplementation, muscle carnosine concentration was increased by 143 ± 151% (mean ± SD; $p < 0.001$) in the gastrocnemius and 161 ± 56% ($p < 0.001$) in the soleus. Post-supplementation time trial performance was significantly slower in the placebo group (60.6 ± 4.4 to 63.0 ± 5.4 min; $p < 0.01$) and trended towards a slower performance following beta-alanine supplementation (59.8 ± 2.8 to 61.7 ± 3.0 min; $p = 0.069$). We found an increase in lactate/proton concentration ratio following beta-alanine supplementation during the time-trial (209.0 ± 44.0 (beta-alanine) vs. 161.9 ± 54.4 (placebo); $p < 0.05$), indicating that a similar lactate concentration was accompanied by a lower degree of systemic acidosis, even though this acidosis was quite moderate (pH ranging from 7.30-7.40). In conclusion, chronic beta-alanine supplementation in well-trained cyclists had a very pronounced effect on muscle carnosine concentration and a moderate attenuating effect on the acidosis associated with lactate accumulation, yet without affecting 1-h time-trial performance under laboratory conditions.

Keywords: beta-alanine, supplement, exercise, buffering, magnetic resonance spectroscopy

Introduction

Synthesised from beta-alanine and histidine, carnosine (β-alanyl-L-histidine) is present in high concentrations within human skeletal muscle (Boldyrev, Aldini, & Derave, in press). This dipeptide has an important role in maintaining homeostasis in the muscle due to its pH buffering and calcium sensitizing properties (Derave, Everaert, Beeckman, & Baguet, 2010; Dutka, Lamboley, McKenna, Murphy, & Lamb, 2012). Potential additional roles of carnosine in myocytes, such as antioxidant and metal-chelating capacity, are still to be demonstrated.

As muscle carnosine synthesis is rate-limited by beta-alanine (Harris et al., 2006), supplementing with beta-alanine (4-6 g/day) has been reported to increase muscle carnosine concentrations by 40-60% after 4 weeks and 80% by 10 weeks. These increases were accompanied by an increase in total work done in a high-intensity, cycling capacity test ($CCT_{110\%}$) by 13% and 18% respectively in physically active students (Hill et al., 2007).

Trained sprinters have higher muscle carnosine concentration compared to untrained individuals as they have a higher proportion of fast-twitch muscle fibers (Baguet, Everaert, De Naeyer, et al., 2011; Parkhouse, McKenzie, Hochachka, & Ovalle, 1985). Beta-alanine supplementation for 4 weeks (4.8 g/day) showed increased carnosine concentration by 37% and 47% in the gastrocnemius and soleus muscles in trained sprinters, along with improved performance in repeated maximal bouts of isokinetic knee extensions (Derave et al., 2007). Other studies have also shown ergogenic benefits of carnosine loading on a 30-s maximal sprint at the end of a simulated cycling race (Van Thienen et al., 2009) and rowing performance (Baguet, Bourgois, Vanhee, Achten, & Derave, 2010; Ducker, Dawson, & Wallman, 2012). However, there is a need for more field-based research to investigate if these positive findings from laboratory tests can be transferred into real-world environments. Trained sprinters from the previously-mentioned study did not receive an ergogenic effect from beta-alanine supplementation in a simulated 400-m race despite attenuating fatigue in isokinetic knee extensions (Derave et al., 2007). Highly-trained Australian swimmers supplemented with beta-alanine for 10

weeks were also unable to see clear benefits in training and competition performance (Chung et al., 2012). Conversely, Brazilian swimmers posted positive benefits in swimming time trial performance after 5 weeks of beta-alanine supplementation (Painelli et al., 2013).

Most exercise protocols in the current research have been limited to short duration, high-intensity exercise as emphasis is put on the role of muscle carnosine as a pH buffer (Harris, Marlin, Dunnett, Snow, & Hultman, 1990; Sewell, Harris, Marlin, & Dunnett, 1992). All 15 studies included in a recent meta-analysis utilized exercise protocols lasting between 1 and 7 minutes (Hobson, Saunders, Ball, Harris, & Sale, 2012). Sprint exercises that last less than 1 minute probably derive no benefit from beta-alanine supplementation. In incremental exercise testing, beta-alanine supplementation did not increase VO_{2max} in both sexes (Stout et al., 2007; Zoeller, Stout, O'Kroy J, Torok, & Mielke, 2007) but was able to increase the ventilatory threshold in men (Stout et al., 2007) which could be indicative of the ergogenic potential of beta-alanine in exercise activities beyond 15 minutes.

In addition, some research studies have been able to establish a direct role for carnosine on muscle contractile behaviour. Australian researchers first investigated the effect of carnosine on the excitation-contraction coupling process in mammalian skeletal muscle fibres (Dutka & Lamb, 2004) following positive results from an earlier study on frogs and cod (Lamont & Miller, 1992). They concluded that carnosine augments force production solely by increasing Ca^{2+} sensitivity in the muscle contractile apparatus of rats. Further exploration into human skeletal muscle also yielded positive findings for a similar role of carnosine in increasing Ca^{2+} sensitivity of the contractile apparatus in both muscle fibre types as well as potentially aiding Ca^{2+} release in the slow-twitch, type I fibres (Dutka et al., 2012). Although the above-mentioned studies were performed in skinned single fibres, similar conclusions were drawn from studies on contracting intact skeletal muscles (Everaert, Stegen, Vanheel, Taes, & Derave, 2013).

The carnosine content of human slow-twitch muscle fibers is only half of fast-twitch fibers (Harris et al., 2006) and endurance athletes, with a predominantly slow-twitch musculature, have lower

muscle carnosine concentration than untrained individuals (Baguet, Everaert, De Naeyer, et al., 2011; Parkhouse et al., 1985). The above-mentioned study of Dutka et al. (2012) suggests that carnosine also contributes to contractile behaviour in slow-twitch fibers. Therefore, we aim to investigate whether beta-alanine supplementation can increase muscle carnosine stores in endurance-trained athletes and whether carnosine loading can improve their endurance performance. We hypothesized that muscle carnosine loading would improve performance in a 1-h cycling time-trial, a reliable and practically relevant measure of endurance performance (Jeukendrup, Saris, Brouns, & Kester, 1996).

Materials and Methods

A total of 28 well-trained male cyclists/triathletes volunteered to participate in this double-blinded study and were recruited in three cohorts (Figure 1). These subjects undertook cycling training for an average of ~8 h/wk and participated regularly in amateur or semi-professional competitions. Within each cohort, subjects were matched for VO_{2peak}, W_{max} and baseline muscle carnosine concentration and placed into two equal groups. An independent individual, not involved with data collection, subsequently allocated subgroups of each cohort randomly into either beta-alanine (CarnoSyn™, sustained-release beta-alanine, Natural Alternatives International, San Marcos, USA) or placebo (maltodextrin, Natural Alternatives International, San Marcos, USA) supplementation. The supplement batch tested negative for contamination from prohibited substances by an independent drug surveillance laboratory (HFL Sport Science, Cambridgeshire, UK).

The supplementation protocol lasted 6 weeks and involved ingesting 6.4 g/day (two 800 mg tablets, four times daily at least two hours apart) of beta-alanine or placebo with meals or snacks. All supplements were contained in sealed opaque containers and were distributed to the subjects at the end of the baseline testing session. All subjects returned the supplement containers at the post-supplementation testing session. Muscle carnosine concentration, cycling time-trial performance and exercise biochemistry were analysed before and after 6 weeks of supplementation.

Subjects were asked to keep a training diary and to maintain similar training loads throughout the supplementation period to avoid any confounding factors from training differently. A short questionnaire about supplementation grouping, side effects and any difference in training load was completed after the post-supplementation tests. One subject of the placebo group dropped out midway during the study, citing reasons of lacking in time to maintain training status and compliance with supplementation. This research was approved by the local ethics committee (Ghent University Hospital, Belgium).

Preliminary incremental cycling test

On the first visit, subjects were screened to be medically fit before performing an incremental cycling protocol (Kuipers, Keizer, Brouns, & Saris, 1987) to exhaustion to determine peak oxygen consumption (VO_{2peak}) and maximal workload (W_{max}). After a 5-minute warm up at 100 W, workload was increased by 50 W every 2.5 minutes until a heart rate of 160 beats per minute was reached. The workload was then increased by 25 W every 2.5 minutes until volatile exhaustion or when cycling cadence was less than 60 r.p.m.

Maximal workload was determined using the following equation: $\boldsymbol{W_{max} = W_{out} + (t/150) * 25}$, where W_{out} is the last completed workload and t is the number of seconds sustained in the last workload (Jeukendrup et al., 1996). All exercise tests were performed on an electrically-braked cycling ergometer (Lode, Groningen, Netherlands). Oxygen consumption was measured continuously via a computerised breath-by-breath system (Jaeger Oxycon Pro, Hoechberg, Germany).

Cycling time-trial

Although all the subjects were accustomed to cycling at moderate intensities for extended periods, a familiarisation time-trial was conducted at least 3 days before baseline data collection. Endurance cycling performance was determined before and after supplementation by a cycling time-trial with an individualised amount of work ($\boldsymbol{Work = 0.75 * W_{max} * 3600}$). The time-trial was performed with

the ergometer set up in linear mode according to the following formula: $\boldsymbol{W = L * (r.p.m)^2}$, where the linear factor (*L*) is calculated from the subject's preferred cadence (r.p.m) at 75% W_{max} (Jeukendrup et al., 1996). In summary, they would be able to complete the time-trial in exactly 1-hour if they cycled constantly at their preferred cadence. However, all subjects were asked to complete the time-trial in the fastest time possible with no encouragement or feedback, except for the amount of work completed displayed on the computer screen. Each subject was asked to refrain from exercising 24 h before cycling time trials and performed the time-trials at the same time of day in pre- and post-supplementation conditions. They were also asked to avoid caffeinated products and to ingest the same pre-exercise meals 2 hours prior to testing. Each cycling time-trial was performed under laboratory conditions with ad-libitum water intake.

At rest, 25%, 50%, 75% and 100% of the cycling time trial, heart rate (Polar RS400, Kempele, Finland), and RPE (Borg, 1982) were recorded and blood parameters of pH, lactate (amperometric electrode using the enzyme, lactate oxidase) were determined from a capillary blood sample from the fingertip with an automated cartridge-based gas analyser (ABL 90, Radiometer, Copenhagen, Denmark). Blood bicarbonate was calculated from blood pH and pCO_2 values (Henderson-Hasselbach equation) while proton concentration was calculated with the formula: proton concentration = 10^(-pH), where pH is blood pH derived from the capillary blood sample.

The coefficient of variation (CV) between the familiarisation and baseline time trials in this study was 3.2%, compared to a CV of 3.4% from the original validity study (Jeukendrup et al., 1996).

Muscle carnosine determination

Muscle carnosine concentration was determined non-invasively via proton magnetic resonance spectroscopy (^{1}H-MRS) in the gastrocnemius and soleus muscles as described by Derave and colleagues (2007). Each subject was laid supine and the right lower leg was fixed in a holder with the ankle at 20° of plantar flexion. All MRS measurements were performed on a 3-T whole body MRI

scanner (Siemens Trio, Erlangen, Germany) equipped with a spherical knee coil. Single-voxel point-resolved spectroscopy was used with the following parameters: repetition time (TR) = 2000 msl; echo time (TE) = 30 ms; number of excitations = 128; 1024 data points; spectral bandwidth = 1200 Hz and a total acquisition time of 4.24 min. The average voxel size of the gastrocnemius and soleus was 40 mm x 12 mm x 30 mm. Following shimming procedures, the line width of the water signal was on average 25.7 and 24.8 Hz for gastrocnemius and soleus, respectively. A 500 ml spherical container filled with an aqueous solution of 20 mM carnosine (Sigma-Aldrich) was used as an external reference for absolute quantification. The following equation was used to determine the concentration of C2-H (at 8 ppm) carnosine in vivo:

$$[C_m] = [C_r] \cdot (S_m \cdot V_r \cdot C_{T1r} \cdot C_{T2r} \cdot T_m) / (S_r \cdot V_m \cdot C_{T1m} \cdot C_{T2m} \cdot T_r)$$

$[C_m]$: carnosine concentration in vivo; $[C_r]$: carnosine concentration of the external reference phantom (20 mM); S_m and S_r: estimated signal peak areas of the muscle and reference phantom; V_m and V_r: voxel volumes of the muscle and reference phantom; C_{T1r}, C_{T2r}, C_{T1m} and C_{T2m}: correction factors for the T1 and T2 relaxation times in the muscle and in the reference phantom; T_m and T_r: temperatures in the muscle and in the reference phantom.

The CV for repeated measurements within the same day (Ozdemir et al., 2007) were 7.6% (gastrocnemius) and 4.3% (soleus), while the biological variability within a 6 week period (Baguet et al., 2009) were 14.2% (gastrocnemius) and 9.8% (soleus).

Statistical analyses

A repeated measures ANOVA (2 conditions x 2 time points) was used to evaluate muscle carnosine, cycling time-trial performance with “group” (beta-alanine vs. placebo) as between-subjects factor and “time” (pre- vs. post-supplementation) as within-subjects factor. Repeated measures ANOVA were used to also compare power output (2 conditions x 8 time points), training load (2 conditions x 6 time points); heart rate, RPE and blood parameters of pH, bicarbonate and lactate (2 conditions x 5

time points) between pre- and post-supplementation. An independent t-test was used to evaluate the lactate vs. proton concentration ratio between beta-alanine and placebo groups. All statistical analysis was performed using a statistical package (SPSS 19.0, Chicago, IL, USA). Values are presented as means ± SD with significance assumed at $p < 0.05$.

Results

Supplementation and training

All supplement containers were returned empty and compliance was verbally confirmed by all subjects. The sustained-release formula of the beta-alanine supplementation was not tested but there were no reports of paraesthesia from any subjects. From the questionnaire, 10 out of 14 subjects in the beta-alanine group and 12 out of 13 subjects in the placebo group thought that they were supplemented with placebo.

There were no significant differences between groups in training load ($p > 0.05$), quantified by distance or duration spent in moderate-high intensity (Table 1). Qualitatively, from the questionnaire, 7 subjects (4 beta-alanine and 3 placebo) trained more during the study, 9 subjects (5 beta-alanine and 4 placebo) had similar training load and 11 subjects (5 beta-alanine and 6 placebo) trained less during the study.

Muscle carnosine concentration

In the beta-alanine group, carnosine concentration was increased by 143 ± 147% ($p < 0.001$) and 161 ± 60% ($p < 0.001$) in the gastrocnemius and soleus respectively. There were no significant differences in the absolute increase in carnosine concentration between the gastrocnemius and soleus ($p = 0.347$). Carnosine concentration also increased slightly in the placebo group by 25 ± 40% ($p = 0.09$) in the gastrocnemius and 18 ± 24% ($p = 0.05$) in the soleus (Figure 2).

Post supplementation performance

Subjects in the beta-alanine group tended to be slower by 1.9 min ($p = 0.069$) while the placebo group were slower by 2.4 min ($p < 0.01$) in the post-supplementation time-trial. These slower performances were matched by differences in power output during the pre- and post-supplementation time-trial (Figure 4). There was no beneficial effect of treatment on performance parameters, as indicated by the lack of interaction effects ($p = 0.621$). There was no relationship between mean change in muscle carnosine concentration and cycling time-trial performance in both beta-alanine ($p = 0.615$; $r = 0.147$) and placebo ($p = 0.09$; $r = 0.487$) groups. There were also no significant differences in peak heart rate, average heart rate and RPE between the pre- and post-supplementation time-trials in both groups (Table 2).

Exercise biochemistry

No significant differences were present at any time point between beta-alanine and placebo groups during the post-supplementation time-trial for blood pH, bicarbonate and lactate concentrations (Table 2). However, higher values were recorded in both groups for pH and blood bicarbonate at the end of the post-supplementation time-trial when compared to pre-supplementation. When the ratio of lactate (mmol/L) over proton (µmol/L) concentrations was calculated, this ratio was significantly higher at the end of the time-trial in the beta-alanine compared to placebo group post-supplementation, whereas both groups did not differ pre-supplementation (see figure 5).

Discussion

Despite the large increases in muscle carnosine concentration, there was no ergogenic benefit with 6 weeks of beta-alanine supplementation (total dosage of 280 g) on 1-h time-trial performance. In fact, post-supplementation cycling performance in both the beta-alanine and placebo groups was slightly decreased compared to the pre-supplementation trial. We also did not find any relationship between the magnitude of increase in muscle carnosine concentration and the change in time-trial performance. To our knowledge, this study is the first to document the lack of effect of beta-

supplementation on an actual endurance exercise performance test as previous studies utilised incremental exercise tests (Stout et al., 2007; Zoeller et al., 2007) and high-intensity efforts at the end of a prolonged cycling protocol (Van Thienen et al., 2009).

A key finding in this study is the largest ever-reported relative increase in muscle carnosine concentration following beta-alanine supplementation. So far, the highest reported relative increases in muscle carnosine following chronic beta-alanine supplementation were 80-85% following 10-12 weeks (Del Favero et al., 2012; Hill et al., 2007). We now document an increase of ~150% after only 6 weeks of supplementation. Stellingwerff and colleagues (2012) combined all published data into one analysis and concluded that the total amount of consumed beta-alanine is the primary determinant of the degree of increase in muscle carnosine. The relationship between consumed beta-alanine and increment in muscle carnosine from this analysis would predict an increase of 60% in response to the 280 g of beta-alanine ingested in the current study, which is far below the actual measured increase of 140-160%.

One possible explanation could be insulin-related as subjects were co-ingesting beta-alanine with their meals. A recent study from Stegen et al. (2013) showed that carnosine loading (3.2 g/day for 6-7 weeks) was more pronounced when beta-alanine was consumed together with a meal (64% increase in carnosine) than between meals (41% increase in carnosine). Another possible reason for the large increases could be that endurance-trained athletes have lower baseline muscle carnosine concentrations (Baguet, Everaert, Hespel, et al., 2011).as a given absolute increase in carnosine concentration will evidently equate to a larger relative increase with a low baseline value. Furthermore, recent unpublished research (Bex et al., 2013) from our laboratory comparing beta-alanine supplementation between controls and athletes showed that carnosine loading was more pronounced in the trained vs. untrained muscles of athletes.

Although our results at first sight did not reveal any meaningful differences in exercise-induced blood lactate concentration and pH, we established an interesting corresponding relationship

between blood pH and lactate. The lactate/proton concentration ratio was increased following beta-alanine supplementation (Figure 5), indicating that a similar lactate concentration was accompanied by an attenuated degree of systemic acidosis. This was only significant at the end of the time-trial where the highest blood lactate concentrations would occur due to a high-intensity finish. Our finding here further supports the evidence for carnosine as an important pH buffer, with functional relevance for acid-base balance during exercise in humans (Baguet, Koppo, Pottier, & Derave, 2010).

Even though we showed an effect of carnosine loading on lactic acidosis, no effect was observed on exercise performance, which indicates that pH buffering is not a limiting or performance determining factor in a 1-h cycling time-trial. Similarly, induced alkalosis via sodium bicarbonate supplementation also showed no ergogenic effect on intense endurance cycling performance (Stephens, McKenna, Canny, Snow, & McConell, 2002). At the same time, our results may suggest that alternative roles of carnosine on muscle homeostasis and function, such as the calcium release and sensitivity, although not measured in the present study, may not markedly affect endurance exercise performance. Despite the lack of a direct beneficial effect on aerobically fuelled muscle work, the aerobic athlete may still benefit from beta-alanine supplementation as a way to augment the sprint capacity during or at the end of aerobic events (Van Thienen et al., 2009).

In the current study, both groups displayed reduced exercise performance following supplementation. In the post-test, they started at the same power output, but failed to maintain a high power in the midsection until the end of the trial. This is likely attributable to a lower training status, as heart rate and RPE throughout the time-trial was unchanged, indicating similar degree of perceived exertion and motivation to attain maximal results in the post- vs. pre-supplementation trials.

In conclusion, chronic beta-alanine supplementation in well-trained cyclists had a very pronounced effect on muscle carnosine concentration and a moderate attenuating effect on the change in pH associated with lactate accumulation. However, these factors did not affect 1-h cycling time-trial

performance within laboratory settings. This is in agreement with pH buffering as the primary mechanism explaining the ergogenic effects of carnosine loading. Therefore, the role of beta-alanine supplementation as an ergogenic aid is probably limited to short duration (1-15 min), high-intensity exercise.

Acknowledgements

We would like to express our gratitude to all the subjects for volunteering their time and effort to participate in this study. We are greatly appreciative of the help from Anneke Volkaert for their assistance with data collection, the MR research group at Ghent University Hospital and Lander Vanhee for his medical support. We also thank Natural Alternatives International for their invaluable support in providing the beta-alanine and placebo supplements for this study.

This study was financially supported by grants from the Research Foundation – Flanders (FWO G035213N and G024311).

Table 1. Weekly log of training load (distance and duration) spent in moderate-high intensity during the study

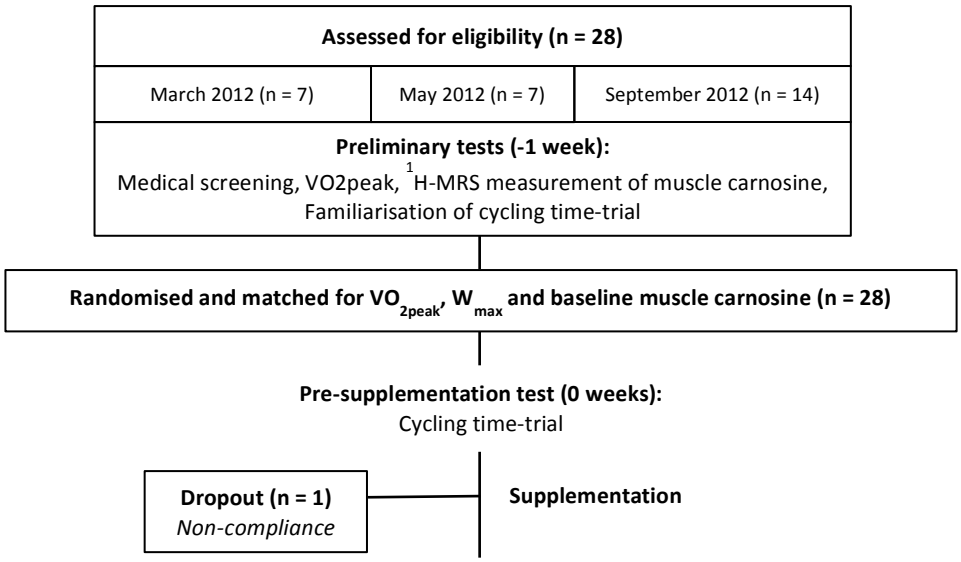
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Distance (km)	**Beta-alanine (n = 13)**	299 ± 184	239 ± 134	206 ± 139	186 ± 141	194 ± 119	198 ± 174
	Placebo (n = 12)	208 ± 128	134 ± 115	161 ± 122	141 ± 128	167 ± 110	182 ± 141
Duration (min)	**Beta-alanine (n = 13)**	637 ± 324	524 ± 278	438 ± 260	391 ± 220	387 ± 224	465 ± 366
	Placebo (n = 12)	512 ± 288	384 ± 273	481 ± 257	446 ± 230	425 ± 260	534 ± 262

Values expressed as mean ± SD.

Table 2. Blood chemistry and physiological variables during the cycling time-trial

			Time-trial completion				
			0%	**25%**	**50%**	**75%**	**100%**
pH	**Beta-alanine (n = 14)**	**Pre**	7.404 ± 0.016	7.343 ± 0.045	7.358 ± 0.048	7.374 ± 0.043	7.311 ± 0.058
		Post	7.405 ± 0.023	7.339 ± 0.053	7.370 ± 0.034	7.389 ± 0.027	7.339 ± 0.054*
	Placebo (n = 11)	**Pre**	7.396 ± 0.015	7.333 ± 0.045	7.357 ± 0.055	7.381 ± 0.041	7.322 ± 0.053
		Post	7.402 ± 0.013	7.342 ± 0.050	7.372 ± 0.049	7.392 ± 0.037	7.359 ± 0.053*
HCO_3 ($mmol.L^{-1}$)	**Beta-alanine (n = 14)**	**Pre**	24.8 ± 1.4	19.9 ± 2.7	20.2 ± 3.0	20.8 ± 2.7	17.9 ± 2.2
		Post	25.4 ± 1.0*	19.6 ± 3.5	20.6 ± 2.6	21.8 ± 2.1	19.1 ± 2.5*
	Placebo (n = 11)	**Pre**	25.5 ± 1.3	19.3 ± 3.0	19.9 ± 3.5	21.4 ± 3.3	18.8 ± 3.2
		Post	25.3 ± 1.0	19.5 ± 3.0	21.1 ± 3.8	22.5 ± 3.4	20.8 ± 3.1*
Lactate ($mmol.L^{-1}$)	**Beta-alanine (n = 14)**	**Pre**	1.5 ± 0.4	7.6 ± 3.1	7.1 ± 3.4	6.2 ± 3.2	10.0 ± 2.7
		Post	1.4 ± 0.2	8.8 ± 4.2	7.1 ± 3.3	5.7 ± 2.8	9.7 ± 2.6
	Placebo (n = 11)	**Pre**	1.5 ± 0.3	7.9 ± 3.1	6.8 ± 3.1	5.5 ± 2.8	8.5 ± 3.1
		Post	1.4 ± 0.3	8.2 ± 3.5	6.7 ± 3.8	5.1 ± 3.1	7.3 ± 3.2
Heart rate (b.p.m)	**Beta-alanine (n = 14)**	**Pre**	68 ± 12	168 ± 9	170 ± 10	170 ± 10	181 ± 6
		Post	69 ± 11	168 ± 12	169 ± 12	168 ± 12	181 ± 8
	Placebo (n = 13)	**Pre**	72 ± 8	170 ± 12	171 ± 12	170 ± 14	181 ± 11
		Post	71 ± 13	168 ± 8	171 ± 10	171 ± 11	180 ± 9
RPE	**Beta-alanine (n = 14)**	**Pre**	6.4 ± 0.6	14.5 ± 1.8	15.9 ± 1.7	16.7 ± 1.6	19.0 ± 2.1
		Post	6.1 ± 0.4	14.5 ± 2.0	16.0 ± 1.9	17.0 ± 1.5	19.0 ± 0.8
	Placebo (n = 13)	**Pre**	6.2 ± 0.6	14.6 ± 1.7	16.0 ± 1.8	16.8 ± 1.3	18.8 ± 1.2
		Post	6.0 ± 0.0	15.0 ± 1.6	16.3 ± 1.7	17.0 ± 1.6	18.8 ± 1.4

Values expressed as mean ± SD. * indicates difference from pre-supplementation time-trial ($p < 0.05$).



Beta-alanine (n = 14)	
Age (y)	30.3 ± 7.4
Mass (kg)	72.8 ± 7.1
VO_{2peak} ($ml.min^{-1}.kg^{-1}$)	60.4 ± 9.6
W_{max} (W)	360.0 ± 36.0
Work to be completed	973.0 ± 98.0

Placebo (n = 13)	
Age (y)	31.6 ± 8.3
Mass (kg)	69.8 ± 5.3
VO_{2peak} ($ml.min^{-1}.kg^{-1}$)	61.0 ± 5.3
W_{max} (W)	351.0 ± 44.0
Work to be completed	948.0 ± 119.0

Figure 1. Experimental design and subject characteristics.

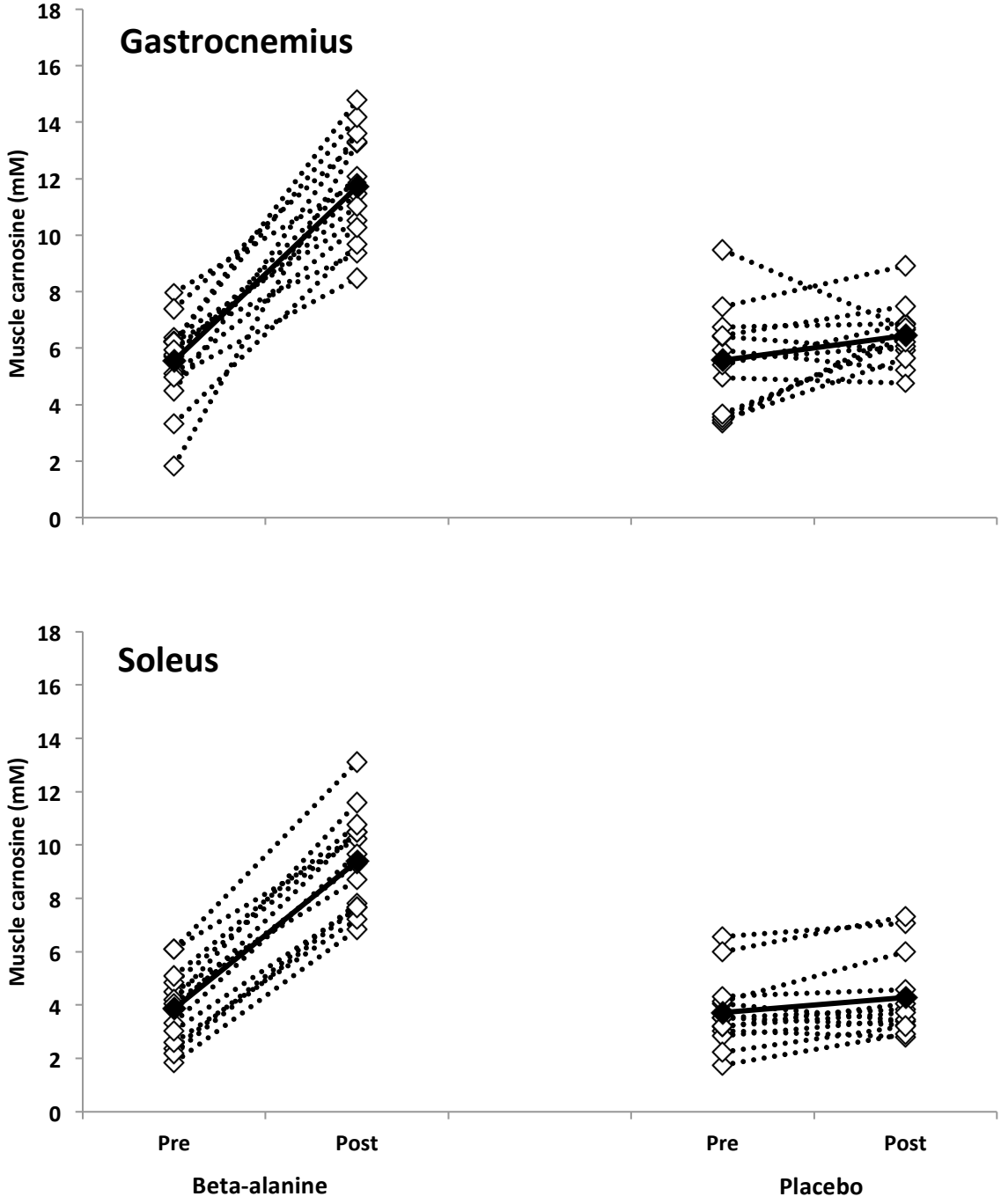


Figure 2. Pre- and post-supplementation carnosine concentration in gastrocnemius and soleus muscles. Open markers denote individual responses and closed markers denote group mean.

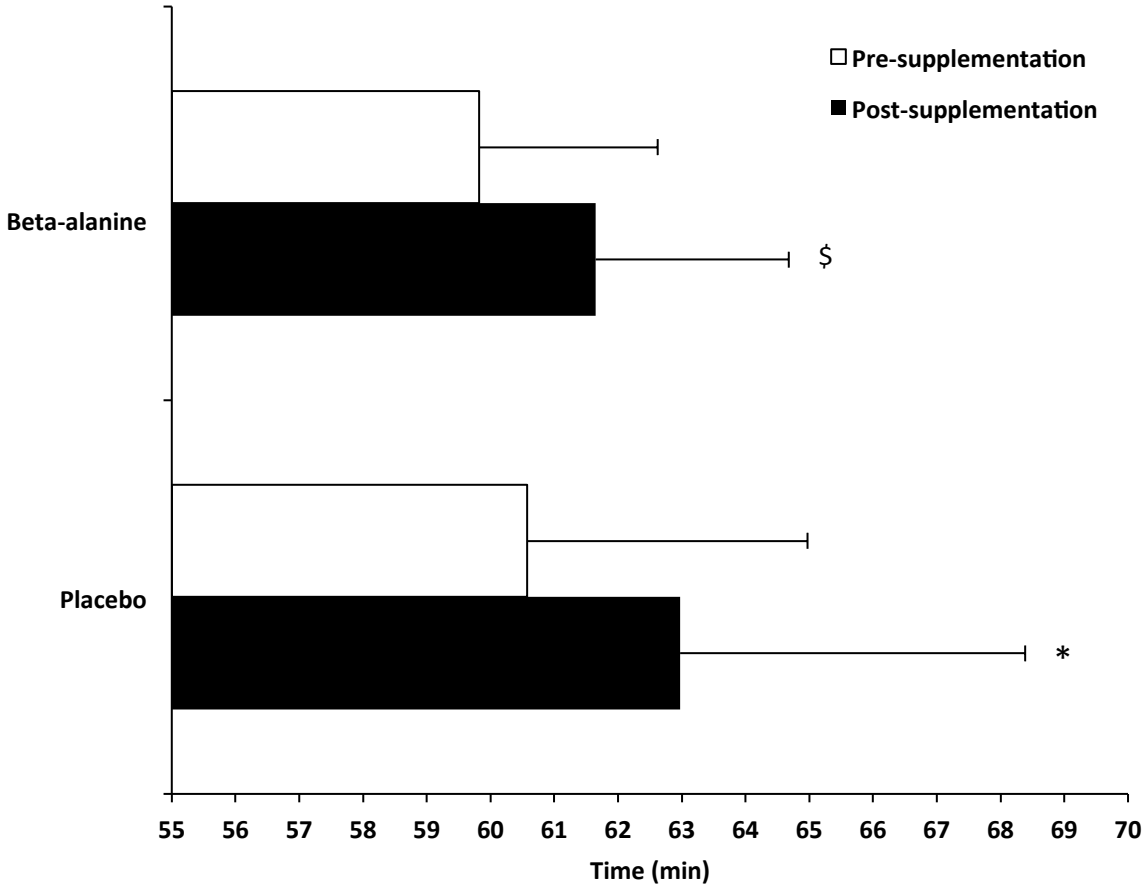


Figure 3. Time to complete an individualised cycling time-trial pre- and post-supplementation with either beta-alanine or placebo. * indicates significant difference from pre-supplementation time-trial in the placebo group ($p < 0.05$). $ indicates approaching significant difference from pre-supplementation time-trial in the beta-alanine group ($p = 0.069$).

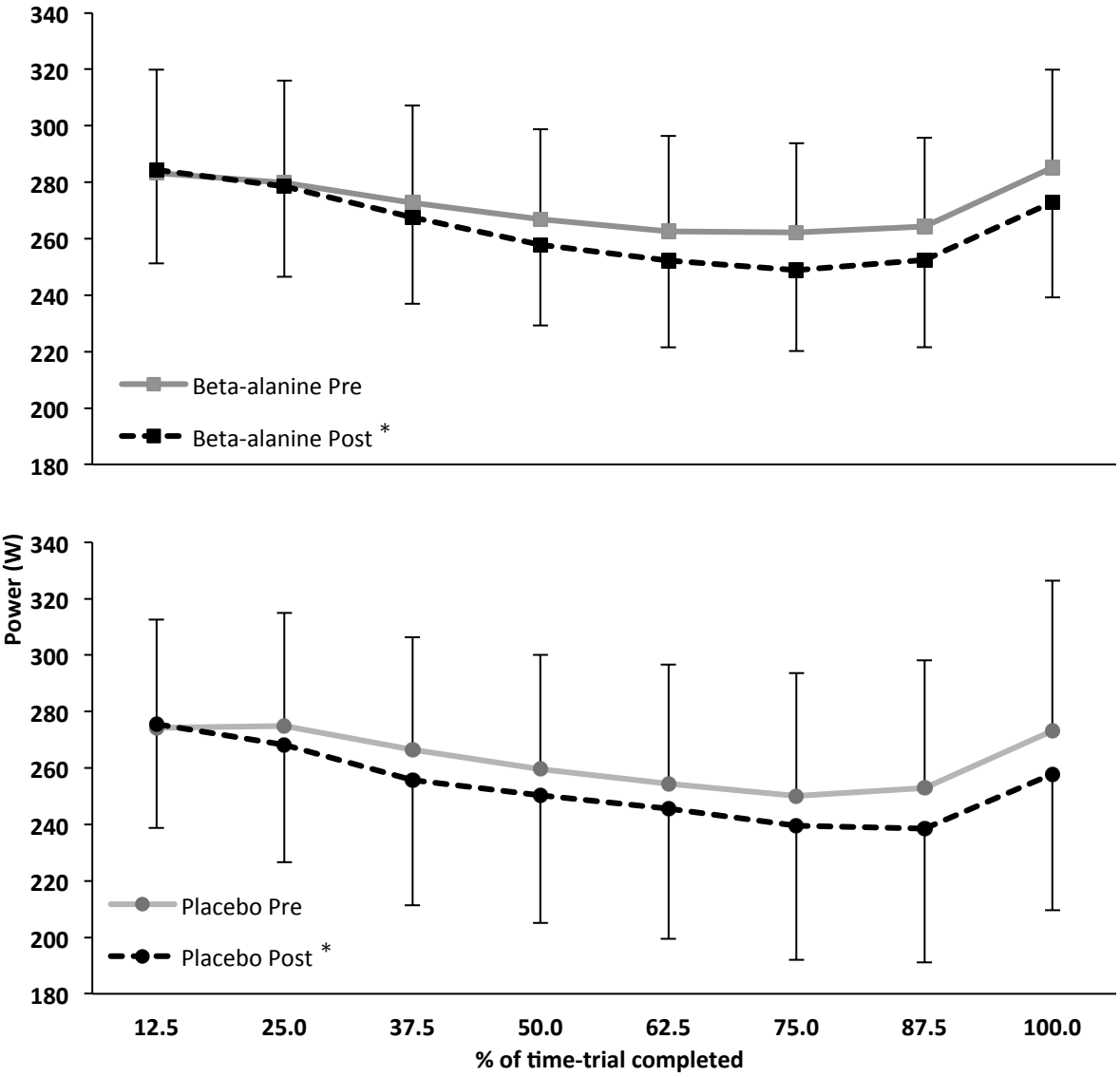


Figure 4. Power output during cycling time-trial performance before and after 6 weeks of beta-alanine or placebo supplementation. * indicates difference between pre- and post-supplementation ($p < 0.05$).

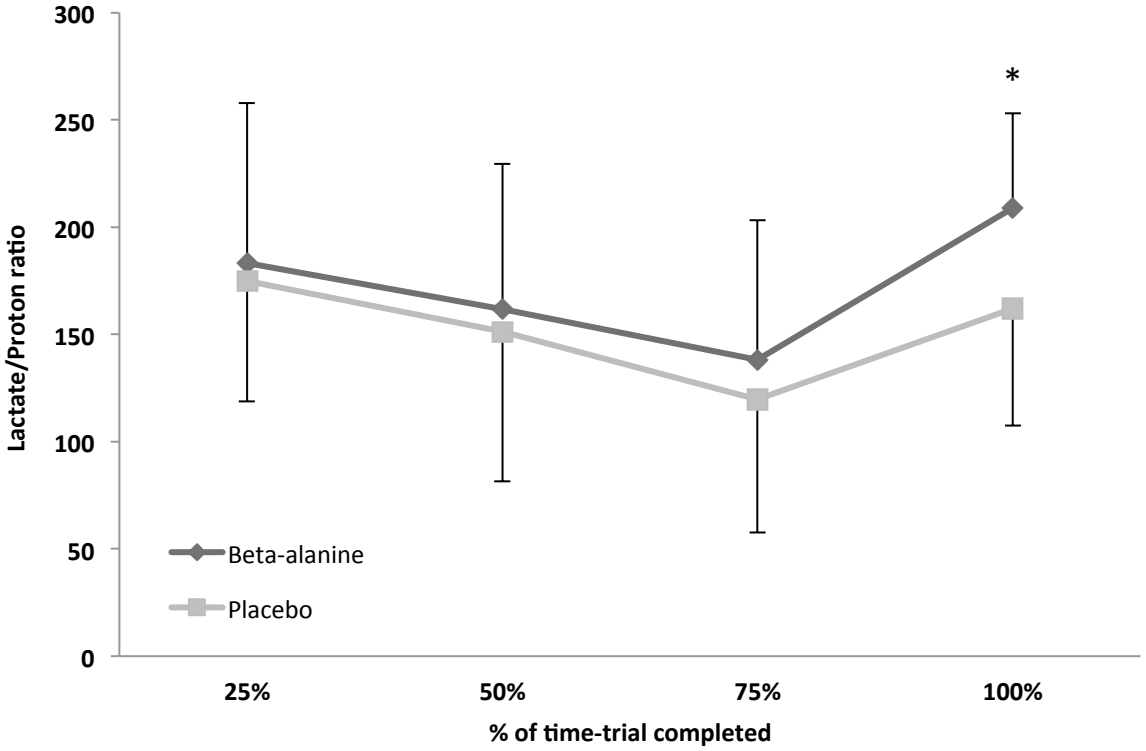


Figure 5. Post-supplementation lactate (mmol/L) to proton (µmol/L) concentration ratio during the time-trial. * indicates significant differences between beta-alanine and placebo groups ($p < 0.05$).

Baguet, A., Bourgois, J., Vanhee, L., Achten, E., & Derave, W. (2010). Important role of muscle carnosine in rowing performance. *Journal of Applied Physiology, 109*(4), 1096-1101. doi: 10.1152/japplphysiol.00141.2010

Baguet, A., Everaert, I., De Naeyer, H., Reyngoudt, H., Stegen, S., Beeckman, S., . . . Derave, W. (2011). Effects of sprint training combined with vegetarian or mixed diet on muscle carnosine content and buffering capacity. *European Journal of Applied Physiology, 111*(10), 2571-2580. doi: 10.1007/s00421-011-1877-4

Baguet, A., Everaert, I., Hespel, P., Petrovic, M., Achten, E., & Derave, W. (2011). A new method for non-invasive estimation of human muscle fiber type composition. *PloS one, 6*(7), e21956.

Baguet, A., Koppo, K., Pottier, A., & Derave, W. (2010). Beta-alanine supplementation reduces acidosis but not oxygen uptake response during high-intensity cycling exercise. *European Journal of Applied Physiology, 108*(3), 495-503. doi: 10.1007/s00421-009-1225-0

Baguet, A., Reyngoudt, H., Pottier, A., Everaert, I., Callens, S., Achten, E., & Derave, W. (2009). Carnosine loading and washout in human skeletal muscles. *Journal of Applied Physiology, 106*(3), 837-842. doi: 10.1152/japplphysiol.91357.2008

Bex, T., Chung, W., Baguet, A., Stegen, S., Stautemas, J., Achten, E., & Derave, W. (2013). *Muscle carnosine loading by beta-alanine supplementation is more pronounced in trained vs. untrained muscles.* Paper presented at the Congress of the European College of Sport Science.

Boldyrev, A. A., Aldini, G., & Derave, W. (in press). Physiology and pathophysiology of carnosine. *Physiological Reviews*.

Borg, G. A. (1982). Psychophysical bases of perceived exertion. *Medicine and Science in Sports and Exercise, 14*(5), 377.

Chung, W., Shaw, G., Anderson, M. E., Pyne, D. B., Saunders, P. U., Bishop, D. J., & Burke, L. M. (2012). Effect of 10 week beta-alanine supplementation on competition and training performance in elite swimmers. *Nutrients, 4*(10), 1441-1453. doi: 10.3390/nu4101441

Del Favero, S., Roschel, H., Solis, M. Y., Hayashi, A. P., Artioli, G. G., Otaduy, M. C., . . . Gualano, B. (2012). Beta-alanine (Carnosyn™) supplementation in elderly subjects (60–80 years): effects on muscle carnosine content and physical capacity. *Amino Acids, 43*(1), 49-56. doi: 10.1007/s00726-011-1190-x

Derave, W., Everaert, I., Beeckman, S., & Baguet, A. (2010). Muscle Carnosine Metabolism and β-alanine Supplementation in Relation to Exercise and Training. *Sports Medicine, 40*(3), 247-263. doi: 10.2165/11530310-000000000-00000

Derave, W., Özdemir, M. S., Harris, R. C., Pottier, A., Reyngoudt, H., Koppo, K., . . . Achten, E. (2007). β-alanine supplementation augments muscle carnosine content and attenuates fatigue during repeated isokinetic contraction bouts in trained sprinters. *Journal of Applied Physiology, 103*(5), 1736-1743. doi: 10.1152/japplphysiol.00397.2007

Ducker, K., Dawson, B., & Wallman, K. E. (2012). Effect of beta-alanine supplementation on 2000 m rowing ergometer performance. *International Journal of Sport Nutrition and Exercise Metabolism, In press*.

Dutka, T. L., & Lamb, G. D. (2004). Effect of carnosine on excitation-contraction coupling in mechanically-skinned rat skeletal muscle. *Journal of Muscle Research and Cell Motility, 25*(3), 203-213.

Dutka, T. L., Lamboley, C. R., McKenna, M. J., Murphy, R. M., & Lamb, G. D. (2012). Effects of carnosine on contractile apparatus Ca(2)(+) sensitivity and sarcoplasmic reticulum Ca(2)(+) release in human skeletal muscle fibers. *Journal of Applied Physiology, 112*(5), 728-736. doi: japplphysiol.01331.2011

Everaert, I., Stegen, S., Vanheel, B., Taes, Y., & Derave, W. (2013). Effect of beta-alanine and carnosine supplementation on muscle contractility in mice. *Medicine and Science in Sports and Exercise, 45*(1), 43-51. doi: 10.1249/MSS.0b013e31826cdb68

Harris, R. C., Marlin, D. J., Dunnett, M., Snow, D. H., & Hultman, E. (1990). Muscle buffering capacity and dipeptide content in the thoroughbred horse, greyhound dog and man. *Comparative Biochemistry and Physiology A, 97*(2), 249-251.

Harris, R. C., Tallon, M. J., Dunnett, M., Boobis, L., Coakley, J., Kim, H. J., . . . Wise, J. A. (2006). The absorption of orally supplied β-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

Hill, C. A., Harris, R. C., Kim, H. J., Harris, B. D., Sale, C., Boobis, L. H., . . . Wise, J. A. (2007). Influence of β-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

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

Jeukendrup, A., Saris, W. H., Brouns, F., & Kester, A. D. (1996). A new validated endurance performance test. *Medicine and Science in Sports and Exercise, 28*(2), 266-270.

Kuipers, H., Keizer, H. A., Brouns, F., & Saris, W. H. M. (1987). Carbohydrate feeding and glycogen synthesis during exercise in man. *Pflugers Archiv (European Journal of Physiology), 410*(6), 652-656. doi: 10.1007/bf00581327

Lamont, C., & Miller, D. J. (1992). Calcium sensitizing action of carnosine and other endogenous imidazoles in chemically skinned striated muscle. *The Journal of Physiology, 454*(1), 421-434.

Ozdemir, M. S., Reyngoudt, H., De Deene, Y., Sazak, H. S., Fieremans, E., Delputte, S., . . . Achten, E. (2007). Absolute quantification of carnosine in human calf muscle by proton magnetic resonance spectroscopy. *Physics in Medicine and Biology, 52*(23), 6781-6794. doi: 10.1088/0031-9155/52/23/001

Painelli, V., Roschel, H., de Jesus, F., Sale, C., Harris, R. C., Solis, M. Y., . . . Artioli, G. G. (2013). The ergogenic effect of beta-alanine combined with sodium bicarbonate on high-intensity swimming performance. *Applied Physiology, Nutrition, and Metabolism, 38*(5), 525-532. doi: 10.1139/apnm-2012-0286

Parkhouse, W. S., McKenzie, D. C., Hochachka, P. W., & Ovalle, W. K. (1985). Buffering capacity of deproteinized human vastus lateralis muscle. *Journal of Applied Physiology, 58*(1), 14-17.

Sewell, D. A., Harris, R. C., Marlin, D. J., & Dunnett, M. (1992). Estimation of the carnosine content of different fibre types in the middle gluteal muscle of the thoroughbred horse. *The Journal of Physiology, 455*(1), 447-453.

Stegen, S., Blancquaert, L., Everaert, I., Bex, T., Taes, Y., Calders, P., . . . Derave, W. (2013). Meal and beta-alanine coingestion enhances muscle carnosine loading. *Medicine and Science in Sports and Exercise, 45*(8), 1478-1485. doi: 10.1249/MSS.0b013e31828ab073

Stellingwerff, T., Anwander, H., Egger, A., Buehler, T., Kreis, R., Decombaz, J., & Boesch, C. (2012). Effect of two β-alanine dosing protocols on muscle carnosine synthesis and washout. *Amino Acids, 42*(6), 2461-2472. doi: 10.1007/s00726-011-1054-4

Stephens, T. J., McKenna, M. J., Canny, B. J., Snow, R. J., & McConell, G. K. (2002). Effect of sodium bicarbonate on muscle metabolism during intense endurance cycling. *Medicine and Science in Sports and Exercise, 34*(4), 614-621.

Stout, J. R., Cramer, J. T., Zoeller, R. F., Torok, D., Costa, P., Hoffman, J. R., . . . O'Kroy, J. (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

Van Thienen, R., Van Proeyen, K., Eynde, B.V., Pyuype, J., Lefere, T., & Hespel, P. (2009). β-alanine improves sprint performance in endurance cycling. *Medicine and Science in Sports and Exercise, 41*(4), 898-903.

Zoeller, R. F., Stout, J. R., O'Kroy J, A., Torok, D. J., & 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