Beta-Alanine Supplementation Improves Isometric, but not Isotonic or Isokinetic Strength Endurance in Recreationally Strength-Trained Young Men.

*Diogo Bassinello[1], *Vitor de Salles Painelli[1], Eimear Dolan,[1] Manoel Lixandrão[1], Monique Cajueiro[1], Mariana de Capitani[1], Bryan Saunders[1,2], Craig Sale[3], Guilherme G Artioli[1], Bruno Gualano[1], Hamilton Roschel[1].

* These authors contributed equally to the manuscript

1. Applied Physiology & Nutrition Research Group; Faculdade de Medicina FMUSP, Escola de Educação Física e Esporte, Universidade de São Paulo, São Paulo, SP, BR
2. Institute of Orthopaedics and Traumatology, Faculty of Medicine FMUSP, University of São Paulo, Brazil.
3. Sport, Health and Performance Enhancement Research Centre; Musculoskeletal Physiology Research Group; School of Science and Technology; Nottingham Trent University; United Kingdom.

Corresponding Author:

Dr. Hamilton Roschel, Ph.D.

Escola de Educação Física e Esporte – Universidade de São Paulo

Av. Melo de Moraes, 65, Butantã – São Paulo, SP – Brazil, Postal code: 05508-030.

Phone: +55 11 3091-8783; Fax: +55 11 3091-3136

E-mail: hars@usp.br

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Abstract:

Background: β-alanine (BA) supplementation may be ergogenic during high intensity exercise, primarily due to the buffering of hydrogen cations, although the effects of beta-alanine supplementation on strength endurance are equivocal. **Aim:** To determine the effects of 4 weeks of beta-alanine supplementation on skeletal muscle endurance using a battery of performance tests. **Methods:** This study employed a parallel group, repeated measures, randomised, double-blinded and placebo-controlled design. Twenty recreationally strength-trained healthy males completed tests of isotonic strength endurance (repeated bench and leg press), along with tests of isometric and isokinetic endurance conducted using an isokinetic dynamometer. Tests were performed before and after a 4 week intervention, comprising an intake of 6.4g·day^{-1} of BA (n = 9) or placebo (maltodextrin, n = 11). **Results:** Time-to-exhaustion during the isometric endurance test improved by ~17% in the BA group ($p < 0.01$), while PL remained unchanged. No significant within-group differences ($p > 0.1$) were shown for any of the performance variables in the isokinetic test (peak torque, fatigue index, total work) nor for the total number of repetitions performed in the isotonic endurance tests (leg or bench press). **Conclusions:** Four weeks of BA supplementation (6.4 g·day^{-1}) improved isometric, but not isokinetic or isotonic endurance performance.

Key-words: carnosine, resistance, muscle function, strength, pH, acidosis.

Introduction

Carnosine (β-alanyl-L-histidine) is a histidine containing dipeptide, abundantly expressed in human skeletal muscle, that is involved in several physiological processes that contribute to exercise capacity and performance (Sale et al. 2013). Supplementation with beta-alanine has been consistently reported to increase intramuscular carnosine content (Harris et al. 2006; Saunders et al. 2017a) which should theoretically enhance intracellular buffering capacity (Artioli, Gualano, Smith, Stout, & Lancha, 2010). The buffering capacity of carnosine occurs due to the pKa of its imidazole ring (6.83), which renders it an ideal intracellular physicochemical buffer to regulate the pH of the intramuscular environment, which may reduce from ~7.1 to 6.6 during exhaustive exercise (Sahlin et al. 1976). Exercise induced acidosis has been shown to play causal roles in peripheral fatigue (Debold et al. 2016) and therefore intracellular buffers, such as carnosine, are essential to counteract changes in pH and resist fatigue. Accordingly, the intramuscular increases in carnosine content, brought about by BA supplementation, can improve exercise performance in a wide range of high-intensity exercise activities, with exercise capacity based assessments lasting between 30 seconds and 10 minutes being most amenable to supplementation (Saunders et al. 2017b). Despite ever-increasing knowledge regarding the applicability of BA to a variety of exercise modalities (Saunders et al. 2017b), little is currently known about the effects of this dietary intervention on resistance training (RT).

Resistance exercises, particularly when using high-loads and/or high-volume protocols, are characterised not only by a high energetic demand but also by restricted blood flow during time under tension (Tamaki, Uchiyama, Tamura, & Nakano, 1994), which increases reliance on anaerobic energy metabolism, and leads to subsequent elevations in intramuscular H^+ and lactate concentrations (Tesch et al. 1986). In this respect, BA supplementation may have the potential to increase muscle tolerance to high-load and high-volume resistance training bouts, due to the enhanced intracellular buffering capacity which it should theoretically provide. Repeated isotonic exercises are a commonly used strength exercise. Since training volume, and the number of repetitions performed, are essential determinants of gains to muscle strength and hypertrophy (Robbins et al. 2012; Sooneste et al. 2013), it seems reasonable to speculate that BA may be an effective ergogenic aid for resistance athletes, if it

can increase capacity to perform repeated isotonic movements, through protecting against the development of fatigue-inducing levels of acidosis. Relatively few investigations have, however, been conducted on this topic (Derave et al. 2007; Hoffman et al. 2006, Hoffman et al. 2008a; Hoffman et al. 2008b; Jones et al. 2017; Kendrick et al. 2008; Sale et al. 2012), and the results reported are equivocal. These studies display large heterogeneity in relation to factors such as participant training status, and the intensity of the resistance protocol under investigation, which may have contributed to this ambiguity in findings. Other factors including the co-supplementation of BA with creatine (Hoffman et al. 2006), examination of the combined effects of BA supplementation with resistance training (Kendrick et al. 2008), and inadequate wash-out periods during a cross-over design (Hoffman et al. 2008b), further complicate interpretation of the available literature. Additionally, contrasting results have been reported on the potential of BA supplementation to improve a sustained isometric contraction, with one study reporting a positive influence (Sale et al., 2012), while two others have reported no effect (Derave et al., 2007; Jones et al., 2017).

Given the strong theoretical potential of BA supplementation to improve strength endurance, along with the equivocality of the existing evidence base, there is a clear need for further research in this area. More specifically, this research should be designed in order to address the aforementioned limitations and discrepant results described above. The aim of this study, therefore, was to employ a double-blind, randomised and placebo-controlled parallel group trial to evaluate the effects of 4 weeks of BA supplementation on a battery of RT exercises involving lower- and upper-body isotonic, isokinetic and isometric muscular endurance tests. We hypothesised that each of the three forms of strength endurance protocols employed (isotonic, isometric and isokinetic) would be positively impacted by the BA supplementation intervention under investigation.

Methods

Participants

Young, healthy and omnivorous men with previous experience of resistance training were recruited to the study. All individuals were required to have been involved in an upper- and lower-body

resistance training program for a minimum of six months prior to their involvement in the study and were requested to maintain an identical training structure for the duration of the study. To ensure a minimal level of training, individuals needed to be capable of lifting a minimum of 1x and 3x their own bodyweight for the bench-press and 45° leg-press exercises. Exclusion criteria included use of β-alanine or creatine supplementation in the previous 6 and 3 months. In addition, current or prior use of steroids, current hypertension, type 1 or type 2 diabetes, or any cardiovascular, neuromuscular or osteoarticular issues that could prevent the performance of exercise tests also warranted exclusion. All participants were fully informed of the requirements of the study and provided written informed consent prior to the start of the study. Ethical approval was granted by the University of São Paulo's ethical committee of the School of Physical Education and Sport (#1.339.704 and 1.211.693).

Experimental design

This study comprised a parallel-group, double-blind, randomised and placebo-controlled design. Participants undertook a battery of strength endurance tests, which were conducted before (PRE) and after (POST) supplementation with beta-alanine (BA) or placebo (PL). The protocol comprised isotonic strength endurance tests of the upper and lower body (bench and leg press), along with lower body isokinetic and isometric endurance tests on an isokinetic dynamometer. Participants undertook a total of 6 experimental test sessions, *i.e.*, 3 pre and 3 post intervention. These test sessions took place in a standardized order PRE and POST the supplementation intervention, with each session separated by a minimum of 48 hours in order to allow for recovery between experimental test sessions. The order and content of the sessions were: 1) isotonic endurance (bench press); 2) isotonic endurance (leg press) and 3) isometric and isokinetic lower limb endurance. Time of day was standardised for each participant to control for the influence of circadian variation on performance (Reilly and Brooks. 1986). For safety purposes, two investigators, who were blinded to the treatment allocation, supervised each session.

Participants were matched for strength based on their 1-RM bench and leg press scores in blocks of four, and subsequently randomly allocated to receive either BA or PL (maltodextrin, Natural

Alternatives Inc., USA). The supplementation protocol required individuals to ingest two 800-mg tablets four times per day totalling 6.4 $g \cdot day^{-1}$ for a period of 4 weeks. Supplements were provided in a sustained-release formulation (Natural Alternatives Inc., USA) and doses were separated by 3 to 4 h to avoid any associated symptoms of paraesthesia. (Décombaz et al. 2012). All capsules were identical in colour and taste and were indistinguishable from each other. Enough supplement for 4 weeks was provided in an unlabelled and sealed pot separated by an independent researcher not involved in data collection. Adherence to supplementation was determined by counting the amount of supplement remaining at the post-supplementation trial, and verbally confirmed with all participants; a high degree of adherence was reported for both groups (Table 1). Similar supplementation protocols to the one employed within the current study have been reported to increase muscle carnosine concentrations by approximately 60% (Harris et al. 2006; Hill et al. 2007). Importantly, our group recently showed that the greatest average increase in muscle carnosine occurs within the first 4 weeks of supplementation (Saunders et al. 2017a). The flow of participants throughout the study is illustrated in Figure 1. Fifty-four participants initially expressed interest in the study and 36 of these were subsequently screened for eligibility. Following application of the inclusion/exclusion criteria, 23 were randomised to the study (BA n = 12, PL n = 11). Three participants from the BA group subsequently withdrew from the intervention and did not complete POST testing, two of whom experienced non-protocol related injuries, and one who did not provide a reason for withdrawal. Hence, 20 participants completed all sessions of the study (BA n = 9, PL n = 11). Participant characteristics are presented in Table 1.

Pilot Study:

Prior to the main trial, a pilot study was conducted to assess whether the isotonic test protocol was capable of inducing acidosis. Maximal strength was assessed in 5 healthy and recreationally strength-trained participants using the protocol described below. Subsequently, participants undertook the same isotonic strength endurance test protocol as was used in the main trials. Details regarding one repetition maximal (1-RM) strength testing, along with the procedures used to ascertain isotonic strength endurance are provided below. Participants undertook two pilot test sessions, with upper body (bench press) and lower body (leg press) isotonic strength assessed on separate days. Venous blood

samples were obtained from the antecubital vein at rest, post sets 2, 4, 6 and 8, and at 5 minutes post-exercise. Blood lactate, bicarbonate, and pH, as surrogates of muscle acidosis, were assessed using these samples. Blood PCO_2 and pH were immediately measured by injecting whole blood samples into an automated blood gas analyzer (Rapid Point 350, Siemens, Germany). Blood bicarbonate concentration was subsequently calculated according to the Henderson-Hasselbalch equation. Plasma lactate was determined spectrophotometrically using an enzymatic-colorimetric method (Katal, Interleck, São Paulo, Brazil) in a microplate-based assay (SpectraMax M2e, Molecular Devices LLC, California, USA). Evidence of a significant reduction in pH was shown from resting (7.34 ± 0.03 and 7.33 ± 0.01 for the leg and bench press) to post-exercise (7.24 ± 0.04 for both leg and bench press) (main effect of "set": $0.01 < P < 0.05$ for all between set comparisons). Lactate increased throughout both isotonic endurance tests ($0.01 < P < 0.05$ for both the bench and the leg press). Results from these pilot tests are reported in Supplementary Tables 1 and 2.

Maximal strength tests for the bench and leg press

Prior to the main experimental trials, one repetition maximal strength (1-RM) for both bench and leg press were assessed and the results used to determine the loads required to individualise subsequent experimental testing sessions. Maximum dynamic strength was determined as the maximum weight that could be lifted in a single repetition (*i.e.*, 1-RM test). This was evaluated for the upper and lower limbs using the bench press (Smith Machine, Hammer Strength, California, USA) and 45º leg press (Leg Press 45º, Movement, São Paulo, Brazil). Individuals self-selected the positioning of their hands on the bar of the Smith Machine for the bench press. Individual positions were recorded and reproduced throughout the study. Similarly, positioning of the feet and the flexion angle of the knees at 90º were determined and recorded for the 45° leg press, with the knee joint angle determined using a goniometer. All tests followed the recommendations of the American Society of Exercise Physiologists (Brown and Weir. 2001).

Prior to testing, participants warmed-up by jogging on a treadmill for 5 minutes at 9 $km \cdot h^{-1}$, followed by a task-specific warm-up consisting of eight repetitions at 50% of estimated 1-RM, 2 min rest, and three repetitions at 70% of estimated 1-RM. Following 2 minutes of rest, the participants had

up to 5 attempts interspersed with 3-min resting periods to achieve their individual 1-RM loads. Both 1-RM tests were performed on the same day with the bench press performed prior to the leg press for all individuals with a minimum rest interval of 30 minutes. Strong verbal encouragement was given during all attempts. Prior to the main trials, familiarization sessions to the 1-RM test were performed until the variation of each participants measurement was < 5%, which took between 2 and 5 sessions. The coefficient of variation (CV) between the last familiarization session and PRE for the 1-RM was 1.8 and 2.3% for the bench and leg press respectively.

Isotonic endurance tests of the upper and lower body:

Isotonic endurance tests for the bench and leg presses were performed on the same equipment and using the same positioning as those used for the 1-RM tests. Following a 5min warm-up on a treadmill at $9km \cdot h^{-1}$, participants performed a specific warm-up consisting of eight repetitions at 50% of the load used in the test. After 2 mins of rest they then performed three repetitions at 70% of the test load, followed by a further 2 mins of rest. All tests began with full extension of the elbows (bench press) or knees (leg press). Participants then performed eight sets of repetitions at 70% 1-RM, with each set performed until failure. A 2-min rest interval was allowed between sets. Strong verbal encouragement was provided during each set. The number of repetitions performed during each set was recorded. Prior to the main experimental trials, familiarisation sessions to the isotonic endurance tests were performed until the variation of each participants measurement was < 5%, which took between 3 and 5 sessions for both the bench and the leg presses. The CV for the total number of repetitions between PRE and the last familiarisation session was 2.3 and 3.1% for the bench and leg presses respectively.

Isokinetic and isometric endurance tests

All isokinetic and isometric fatigue tests were performed on an isokinetic dynamometer (Biodex System 3, Biomedical Systems, Newark, CA, USA) using the dominant leg and according to previously described methods (Derave et al., 2007; Sale et al., 2012). Individuals were seated upright and strapped securely to the chair across the shoulders and waist, as well as the thigh of the non-dominant leg. The ankle of the dominant leg was strapped to the equipment; the femoral epicondyle of

the knee was aligned with the centre of rotation of the dynamometer and the leg was maintained at 90º in relation to the horizontal. Participants warmed up on a treadmill for 5 minutes at 9 $km \cdot h^{-1}$. A specific warm-up was then performed and consisted of 5 sets of isometric contractions lasting 15 s at increasing absolute intensities of 40, 60, 80 and 100 Nm, with 30 s between sets. Thereafter, three maximal contractions of 5 s were performed interspersed by 90 s of rest, to determine maximal voluntary isometric contraction (MVIC). Participants then performed an isometric contraction at 45% of MVIC until exhaustion, defined as an inability to maintain 95% of the intensity required for more than 1 second. Time-to-exhaustion (TTE) in seconds was recorded, and quantified as the point at which the participants force output fell below 95% of the target force for more than 1 second. Participants were required to maintain force output as close as possible to the target force, which was indicated by a line superimposed upon the computer screen. In addition to this visual representation, participants were also given verbal feedback when their force output was "too high", "too low" or "on the line".

Thirty minutes following the isometric contraction test, individuals performed maximal voluntary isokinetic knee extensions consisting of 5 x 30 maximal repetitions at a constant angular velocity of $180° \cdot s^{-1}$. The contraction was initiated with the knee flexed to 90°, continued to the point of full knee extension, before passively returning to the same starting position at $90° \cdot s^{-1}$. Each bout of contractions was separated by a 1-min rest period. Participants received visual feedback of their produced peak torque and strong verbal encouragement throughout the test. Peak torque achieved during each contraction was measured and subsequently used to calculate the average peak torque during each set, total work per set (J) and fatigue index (the torque produced in the final 10 repetitions compared to the initial 10 repetitions of each set).

Diet and training:

Twenty-four hours prior to all laboratory visits, participants were required to refrain from alcohol, caffeine and strenuous exercise, while food intake was recorded using a 24-h food diary. Participants were asked to report for testing between 2 and 4 hours following their last meal. Additionally, food intake was assessed PRE and POST by three 24-h food diaries undertaken on separate days (two weekdays and one weekend day). Energy and macronutrient intake were

subsequently analysed by a nutritionist using specific software (Virtual Nutri, São Paulo, Brazil). To avoid the potentially confounding influence of changes to training volume or intensity, thus isolating the effect of increased muscle carnosine content on the exercise measures, participants were requested to record their training schedule in the month prior to the study and replicate the exact same regimen throughout the study period. Adherence to this was verbally confirmed with each individual on a weekly basis.

Statistical Analysis

Data were analysed using intention-to-treat principles. All participants who were randomised to the intervention, including those who subsequently withdrew from the study were included in this analysis. Mixed-models were used to determine the effect of supplementation on the total number of repetitions on the bench press and leg press, time-to-exhaustion (TTE) in the isometric test, and food consumption. 'Group' (PL or BA) and 'Time' (PRE and POST) were fixed factors, and 'Participants' a random factor. To assess the effect of supplementation on total work done, peak torque and fatigue index during the isokinetic dynamometer test, 'Set (Set 1, Set 2, Set 3, Set 4 and Set 5; or Rest, Post-set 4, Post-set 8 and 5 min post-set 8) was included as an additional fixed factor, in addition to time and group. A Tukey *post-hoc* adjustment was used in the case of a significant F-value, to identify the location of differences. Additionally, a secondary per protocol analysis was conducted through comparing delta scores between the groups using unpaired *t*-tests. The effect size (ES) of pre-post change was calculated using Cohen's d. Effect sizes were quantified using the following criteria: < 0.2: negligible effect; 0.2 – 0.39: small effect; 0.40 – 0.75: moderate effect; >0.75: large effect. The Fischer Exact Test was used to compare the proportion of participants who correctly guessed their treatment allocation between groups. Data analyses were conducted using SAS 9.3 software. Results were interpreted according to the statistical probabilities of rejecting the null hypothesis (H0) and in the following categories: $P > 0.1$: no evidence against H0; $0.05 < P < 0.1$: weak evidence against H0; $0.01 < P < 0.05$: evidence against H0; $0.001 < P < 0.01$: strong evidence against H0; $< P < 0.001$: very strong evidence against H0 (Amrhein et al. 2017).

RESULTS

Isotonic strength endurance (upper and lower body)

BA supplementation did not influence the number of repetitions performed in either of the isotonic strength endurance tests (bench or leg press). No evidence of a significant effect of 'group' nor 'group x time' were obtained ($p > 0.1$ for all comparisons). Delta score assessment showed no evidence of between-group differences for either of these isotonic endurance tests ($p > 0.1$), and the ES of pre-post change in the BA group was 'negligible' for both exercises (0.14 and 0.09 for bench and leg press respectively).

Isometric endurance test

Strong evidence of increased TTE in the isometric endurance test was shown for the BA group (+9.0 ± 3.0 s; +17.2 ± 5.4%, $p < 0.01$), but not for PLA (+0.4 ± 7.1 s; +2.1 ± 12.9%, $p > 0.1$) Delta score assessment showed a significant between-group difference for this variable ($p < 0.01$), and the effect size for the BA group was 'moderate' (0.53, see Figure 3).

Isokinetic endurance:

BA supplementation did not influence total work done, peak torque or the fatigue index calculated from performance in the isokinetic endurance test ($p > 0.1$ for all outcomes, see Figures 4 - 6). Very strong evidence of an effect of 'set' for total work, peak torque and fatigue index was shown, indicating an overall decrease in total work and peak torque over the 5 sets, as well as an increase in fatigue index ($p < 0.001$ for all comparisons), although delta score analysis confirmed that there were no differences between the groups for any of these variables ($p > 0.1$ for all comparisons). The ES for total work done in the BA group was 'neglible' (-0.11, See Figure 4).

Food consumption

Absolute and relative total energy, carbohydrate, protein and fat intake are presented in Table 2 and remained unchanged throughout the study ($p > 0.1$ for all 'group x time' interactions).

Double-blind efficacy

Five out of 9 participants correctly guessed that they were ingesting BA, while 3 of 11 participants correctly guessed that they were taking placebo. No evidence of differences between the groups for the identification of the ingested supplement was obtained (Fischer Exact Test: $p > 0.1$).

Discussion

The main findings of this study were that BA supplementation improved lower limb isometric endurance, but did not impact isokinetic or isotonic endurance. These results show that BA supplementation can convey an ergogenic effect in some, but not all strength endurance based resistance tests. An increased intracellular buffering capacity as a result of BA supplementation is the most likely mechanism underpinning its ergogenic influence (Sale et al., 2013). It seems plausible, therefore, that the extent of acidosis induced by the different strength endurance protocols investigated in the current study likely influenced the amenability of these protocols to BA supplementation.

The improved isometric endurance in the current study indicates that performance in this test is amenable to BA supplementation, a finding which agrees with previous results reported by Sale et al. (2012), but disagrees with those of Derave et al. (2007) and Jones et al. (2017). The discrepancy between our results, and those of Derave et al. (2007) is likely due to differences in hold times identified. The predicted time at which muscle fatigue occurs whilst maintaining a constant isometric contraction at 45% MVIC is approximately 78s (Ahlborg et al. 1972), which is similar to the times reported by Sale et al. 2012 (~75 secs) and somewhat higher than those reported in the current study (~55 secs). An MVIC of this intensity is likely to result in a complete occlusion of blood flow (de Ruiter, Goudsmith, Van Tricht, & de Haan, 2007), thus requiring the muscle to function as a closed unit, with little or no capacity to deliver oxygen, nor to remove the metabolic by-products of anaerobic metabolism, namely H^+. This hypoxic environment will therefore be susceptible to a rapid accumulation of hydrogen cations, rendering intracellular physicochemical buffers such as carnosine essential to the slowing of fatigue inducing levels of acidosis. In contrast, participants in the investigation by Derave et al. (2007) maintained a substantially longer isometric hold time (173 ± 55 and 201 ± 48 seconds for PL and BA

groups at baseline), than those reported in the current study, and in that by Sale et al. (2012). It seems plausible to suggest, that the actual MVIC intensity reported in that study was, in fact, substantially lower than 45%, meaning that at least some level of re-oxygenation may have occurred during the hold. In this situation, acidosis is likely to have a lower contribution to fatigue, thus explaining the lack of effect of BA supplementation on this outcome. This hypothesis is not, however, supported by the results of Jones et al. (2017) who had a similar participant group, used the same protocol, and reported similar hold times (~74 secs) to the current study, and to Sale et al. (2012), but no effect of BA supplementation. No obvious explanation was available for this discrepancy in results, and the authors advised that further research be undertaken. Our findings confirm those reported by Sale et al. (2012), and show that BA supplementation does indeed have the capacity to enhance the ability to maintain an isometric hold conducted at 45% of maximal intensity.

In contrast to our findings of improved isometric endurance, BA supplementation did not impact isokinetic endurance, as evidenced by a lack of change to dynamic knee extension torque, fatigue index or total work. These findings were unexpected, since the employed protocol is the same as the one previously used by Derave et al. (2007), who reported that BA supplementation improved peak torque in each of the five bouts compared to the pre-supplementation values, and that muscle fatigue was significantly attenuated in the later stages of exercise (bouts 4 and 5) when compared to placebo. In contrast, no changes were observed in peak torque as a result of BA supplementation in our study. Recently, an in-depth meta-analysis reported that capacity based assessments (namely those that are conducted until failure) are more amenable to supplementation than performance based assessments (namely those based on a set task, Saunders et al. 2017a). The isokinetic endurance test employed within the current study was a performance-based assessment, whereby participants completed 5 sets of 30 maximal contractions. It is plausible that task constraints, such as a failure to maintain appropriate technique, rather than metabolic factors (namely acidosis) may have been the main performance limiting factors for the recreationally strength trained athletes who took part in this study, thus explaining the lack of effect of BA on this assessment. In contrast, the highly trained participants in the previous study by Derave et al. (2007) may have been capable of maintaining a more consistent

technique, and so other factors (*e.g.*, increased acidosis) would have a greater influence on fatigue development, and thus highly trained participants may be more amenable to the effects of BA supplementation on this particular test.

The assessment of the influence of BA supplementation on isotonic endurance performance in the current study was particularly important, given that training volume is a crucial factor in the optimisation of resistance training gains including strength and hypertrophy (Robbins et al. 2012; Sooneste et al. 2013). Nutritional interventions that support the completion of larger volumes of similar loads are therefore particularly relevant for strength athletes. The number of repetitions performed in the isotonic endurance tests in the current study were not however influenced by BA supplementation. This finding contrasts with previous investigations that have reported increased resistance training volume in response to BA supplementation. (Hoffman et al. 2006, Hoffman et al. 2008a; Hoffman et al. 2008b). It is important to note however, that each of these investigations had confounding influences that make it difficult to isolate the contribution of BA supplementation *per se* to the increased training volume reported. Hoffman et al. (2006) reported that the combination of BA and creatine increased RT volume to a greater extent than creatine alone, although the isolated effect of BA only was not assessed in that study. In 2008, the same group reported that footballers supplementing with BA completed a greater number of repetitions of repeated bench press than a group receiving a placebo. The initial measurement was, however, taken after 3 weeks of supplementation, and pre-intervention values were not reported meaning that this finding cannot be isolated to the supplement (Hoffman et al. 2008a). Finally, a significantly higher number of repetitions performed during repeated squat performance at 70% 1-RM was reported in a group supplementing with BA when compared to those receiving placebo (Hoffman et al. 2008b). This study, however, employed a 4-week wash-out period, while more recent research indicates that this is not a sufficient time-period to allow carnosine content to return to baseline (Baguet et al., 2009; Stellingwerff, Decombaz, Harris, & Boesch, 2012). This means that those participants who completed the placebo condition in the second arm of the trial may have been experiencing losses of carnosine, potentially augmenting the differences in performance between the BA and PLA arms of the trial and introducing an artefact into the data analysis. More specifically, the

BA group had a higher post-supplementation training volume than the PLA group, and this was interpreted as a positive effect of supplementation. However, it is plausible that this difference may have been caused by an artificially reduced performance on this test by the PLA group, due to continued carnosine losses throughout the PLA condition in those athletes that were randomized to the active supplementation arm of the intervention first. In light of these limitations, along with recognition of the importance of training volume to optimize strength and hypertrophic gains to resistance training (Sooneste et al. 2013) we deemed it important to design our study to ensure that these aforementioned confounding influences were controlled for. This was achieved through employing a parallel-group, and placebo controlled design; through controlling for changes in training type and volume throughout the intervention, and by conducting repeated familiarisations of the isotonic endurance test until each participants performance varied by less than 5%. This rigorous experimental design allowed us to isolate the effect of BA to the exercise protocol under investigation. Therefore, we are confident in our results that BA supplementation is ineffective at improving strength endurance during isotonic endurance tests of the leg and bench presses. This was somewhat unexpected, given that pilot testing showed that our protocol did result in an accumulation of lactate, with concomitant decrease in blood pH (see Supplementary tables S1 and 2), and therefore represented an environment that should theoretically be susceptible to BA induced increases to intramuscular carnosine content and buffering capacity. The extent of lactate accumulation reported in the pilot study, was however of a lower magnitude than those previously reported for exercise protocols known to be amenable to BA supplementation (high-intensity cycling performance; Sale et al. 2011). It seems plausible to suggest, therefore, that the extent of acidosis induced by our isotonic endurance protocol, was not of a sufficient magnitude to be the main performance limiting factor for this test, supporting the lack of any effect of BA supplementation. Similarly, sodium bicarbonate, which functions to enhance dynamic buffering capacity through reducing blood bicarbonate levels (McNaughton et al. 2016) has also been reported to be ineffective at enhancing strength endurance using similar protocols (Portington et al. 1998; Webster et al. 1993).

This study is not without limitations. The tests used herein do not necessarily reflect a real-world RT session, where multiple exercises are typically performed, with a broad range of duration, intensity, repetitions, resting times, and potential combination with other type of exercises (*e.g.,* endurance or high-intensity interval training), all of which may potentially lead to increased acidosis, and therefore benefit from increased carnosine content. The effectiveness of BA supplementation in sport-specific settings, in particular using highly-trained resistance athletes, needs to be investigated. Muscle carnosine was not measured and, although substantial carnosine increases have been consistently reported with similar BA protocols, it would be important to confirm whether the ability of BA to increase carnosine content is matched by increases in performance at an individual level. Further studies should determine whether differential patterns of response to BA-supplementation (Saunders et al. 2017a) relate to discrepant RT performances. Studies directly assessing muscle acidosis in response to RT are necessary to show the relevance of muscle carnosine to this type of exercise.

In conclusion, BA supplementation improved lower limb isometric endurance, but not isokinetic or isotonic endurance. These data provide support for the use of BA supplementation, and subsequently increased intramuscular carnosine content, in some, but not all forms of RT. The applied implications of these findings should be investigated using real-life sporting and everyday activities. Further research is required to fully elucidate the specific attributes of resistance exercise that are most susceptible to the performance enhancing influence of BA supplementation, enabling more targeted interventions.

Compliance with Ethical Standards:

Conflict of Interest: The supplements for this study were provided by Natural Alternatives International (NAI) Inc, San Marcos, California. The authors have no other conflict of interest to declare.

Ethical Approval: All procedures performed in the current study were in accordance with the ethical standards of the institution and with the 1964 Helskinki declaration, and its later amendments. Ethical approval was granted by the University of São Paulo's ethical committee of the School of Physical Education and Sport (#1.339.704 and 1.211.693).

Informed Consent: All participants were fully informed of the requirements of the study and provided written informed consent prior to the start of the study.

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Table 1. Participants' characteristics

	Groups		*P*
	Beta-alanine (n = 9)	**Placebo (n = 11)**	
Age (y)	25 ± 5	24 ± 3	0.87
Height (m)	1.74 ± 0.08	1.72 ± 0.06	0.50
Body weight (kg)	78.8 ± 15.5	78.4 ± 10.5	0.95
Training experience (months)	33.55 ± 39.93	32.45 ± 27.16	0.94
Bench press maximum strength (1-RM kg^{-1}·bw)	1.14 ± 0.11	1.20 ± 0.15	0.37
Leg press maximum strength (1-RM kg^{-1}·bw)	3.95 ± 0.58	3.85 ± 0.54	0.68
Adherence to supplementation (%)	95.25 ± 9.04	91.93 ± 9.77	0.44

Data are expressed as mean ± standard deviation. No significant difference between groups was observed (all $P > 0.05$).

Table 2. Participants' food consumption throughout the study

	BETA-ALANINE		PLACEBO		
	PRE	**POST**	**PRE**	**POST**	***P****
ENERGY (kcal)	2815 ± 774	2515 ± 822	2645 ± 711	2155 ± 461	0.75
PROT (g)	144.2 ± 35.9	130.8 ± 46.2	160.7 ± 92.4	113.0 ± 22.9	0.90
PROT/kg	1.8 ± 0.2	1.6 ± 0.4	2.1 ± 1.3	1.5 ± 0.1	0.46
CHO (g)	299.4 ± 93.2	286.2 ± 118.7	276.6 ± 110.1	230.5 ± 78.3	0.43
CHO/kg	3.8 ± 0.9	3.5 ± 1.2	3.5 ± 1.5	3.0 ± 1.0	0.46
FAT (g)	115.6 ± 32.4	93.1 ± 20.3	99.5 ± 41.9	73.6 ± 33.1	0.69
FAT/kg	1.4 ± 0.2	1.1 ± 0.2	1.3 ± 0.6	1.0 ± 0.4	0.58

Data are expressed as mean ± standard deviation. PROT: protein; CHO: carbohydrate; PRE: Pre-supplementation, Post: Post-supplementation.

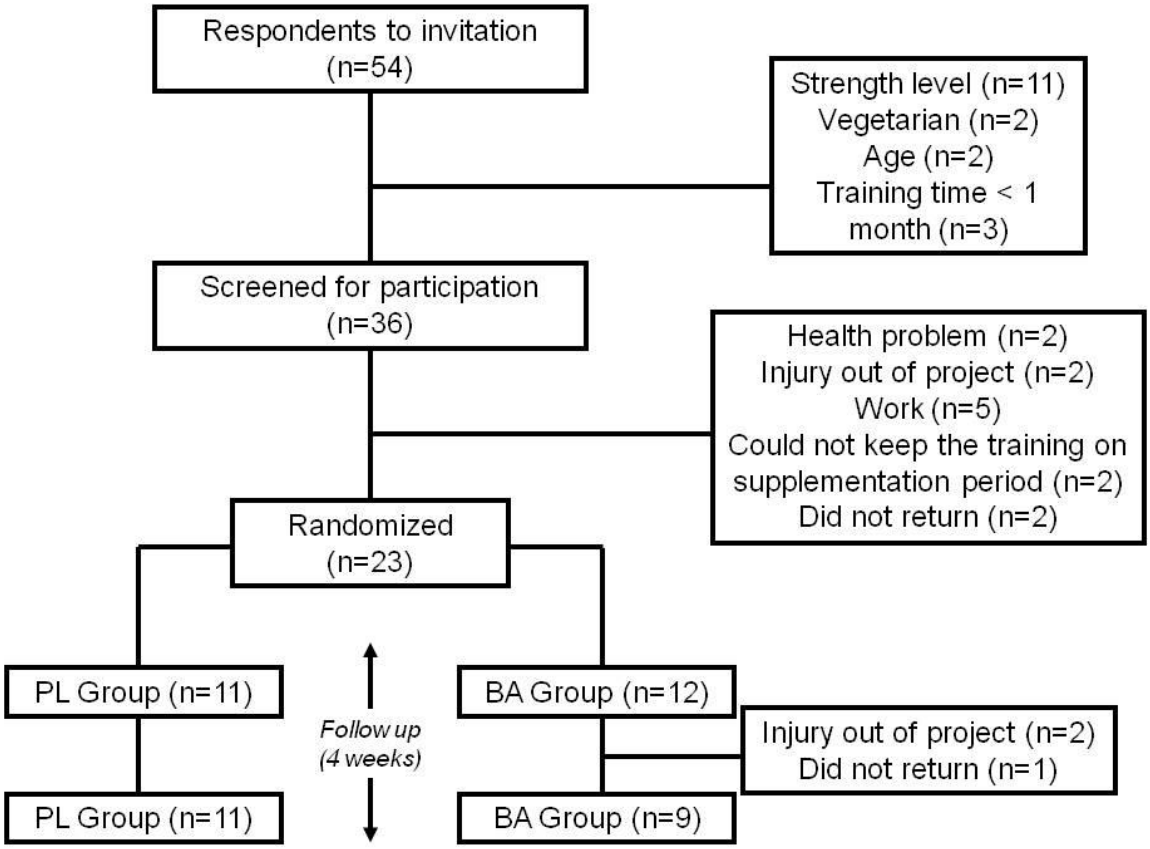

Figure 1. Fluxogram of participants

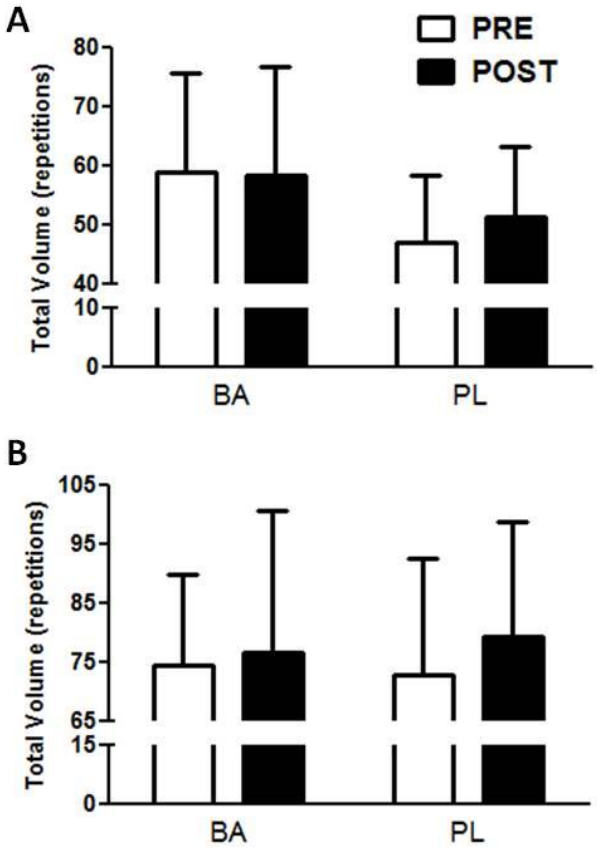

Figure 2. Total number of repetitions performed during the strength endurance test in the bench press (Panel A) and leg press (Panel B) exercises, pre-supplementation (PRE) and post-supplementation (POST) for the beta-alanine (BA) and placebo (PL) groups.

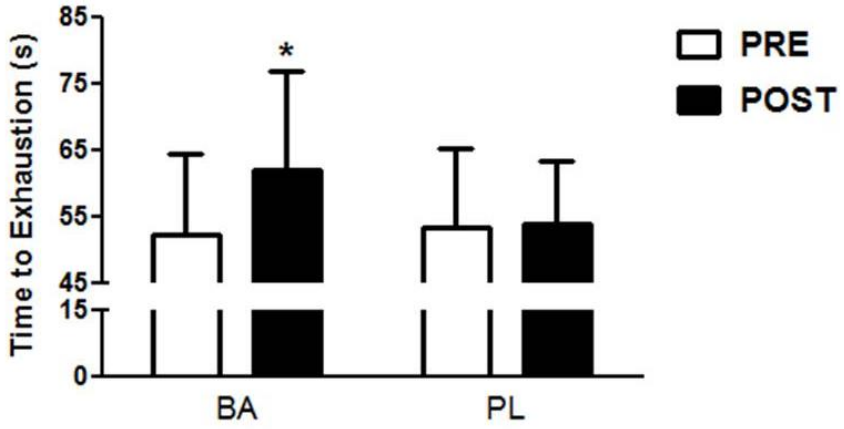

Figure 3. Time-to-exhaustion during the submaximal isometric contraction of the dominant lower limb. *P = 0.01 refers to a within-group effect. PRE: Pre-supplementation, Post: Post-supplementation

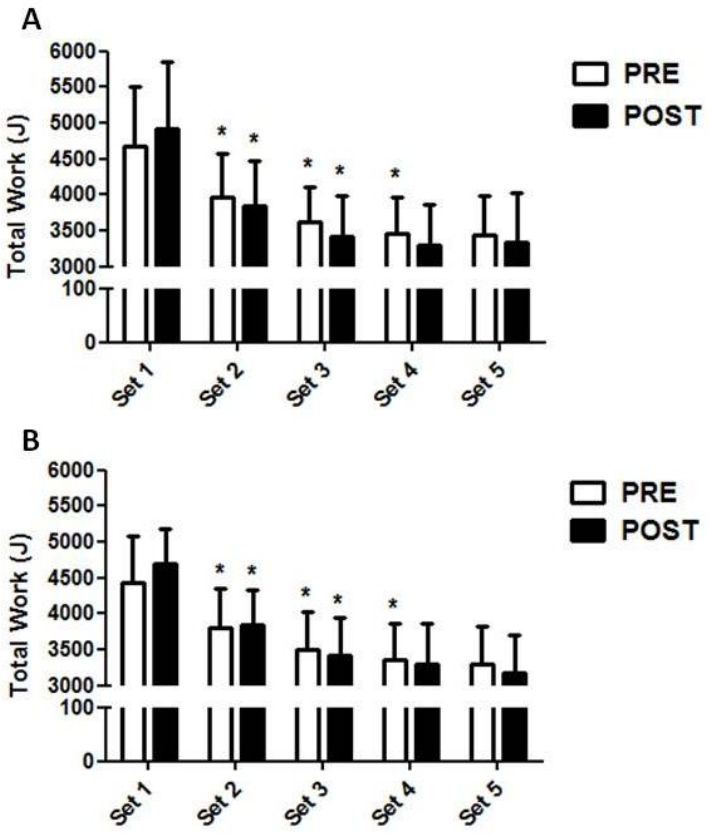

Figure 4: Total work during the strength endurance test in the isokinetic dynamometer, PRE (white bars) and POST (black bars) beta-alanine (Panel A) or placebo (Panel B) supplementation. Legend: The symbol * refers to a significant within-group difference ($0.01 < P < 0.05$) compared to the previous set (BA, n = 9; PL, n = 11).

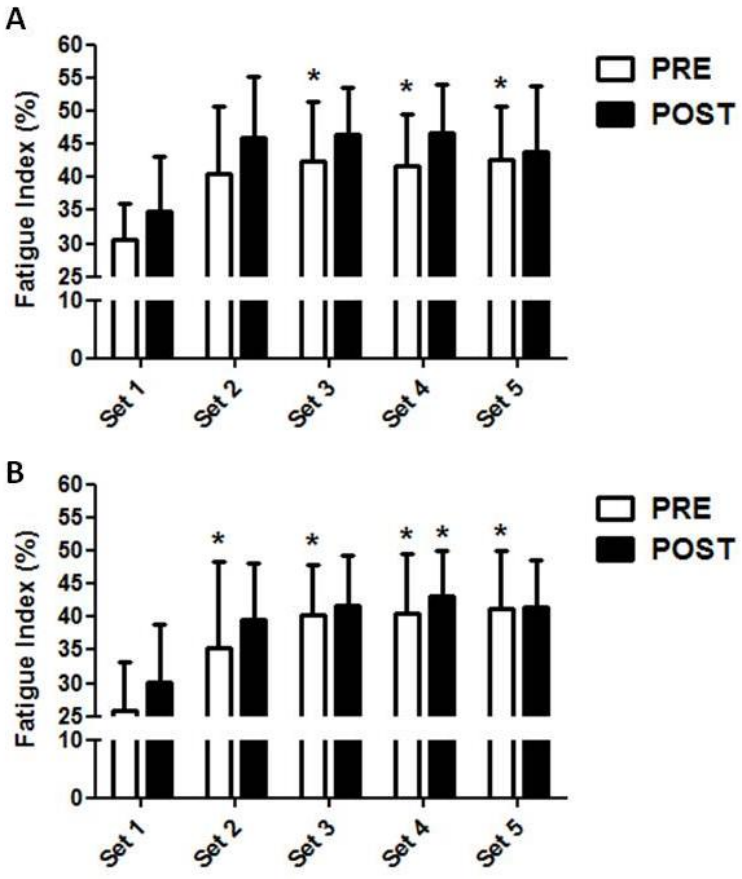

Figure 5: Fatigue index during the strength endurance test in the isokinetic dynamometer, PRE (white bars) and POST (black bars) beta-alanine (Panel A) or placebo (Panel B) supplementation. Legend: The symbol * refers to a significant within-group difference compared to set 1 (BA, n = 9; PL, n = 11).

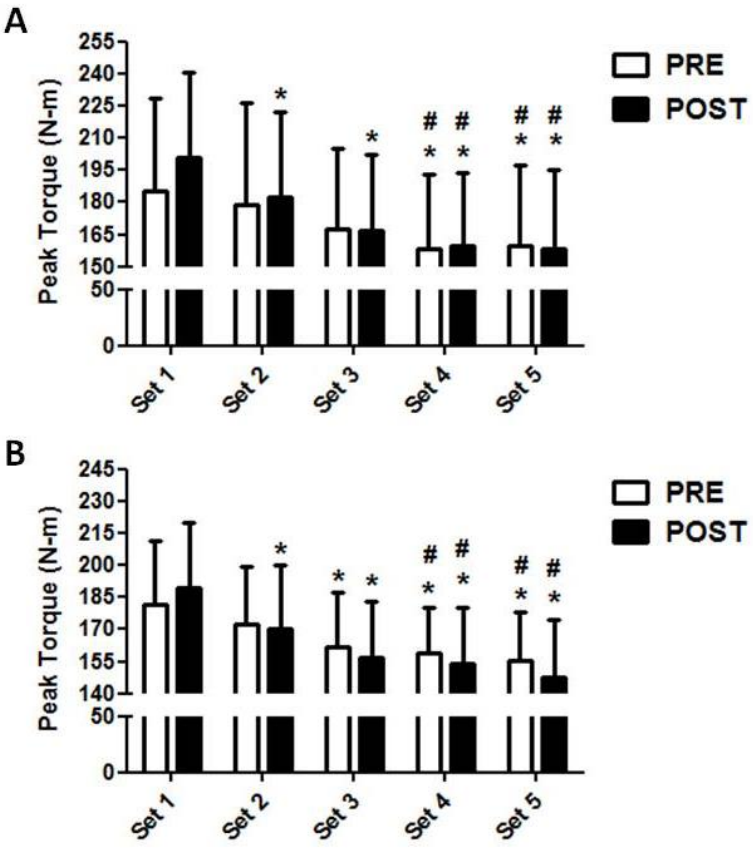

Figure 6: Peak torque during the strength endurance test in the isokinetic dynamometer, PRE (white bars) and POST (black bars) beta-alanine (Panel A) or placebo (Panel B) supplementation. Legend: The symbol * refers to a significant within-group difference compared to set 1; The symbol # refers to a significant within-group difference compared to set 2 (BA, n = 9; PL, n = 11).