Beta-alanine Supplementation Neither Reduce the Oxidative Stress nor Improve Physical Performance of Volleyball Athletes

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Abstract

This study analyzed whether the Beta-Alanine (BA) supplementation improves performance and oxidative stress indices during the tapering period. We assessed eleven volleyball athletes over 8-week. The performance was evaluated through the countermovement jump (CMJ). We evaluated Internal Training Load (ITL), questionnaires (Wisconsin Upper Respiratory Symptom Survey 21 – WURSS 21) and oxidative stress (Thiobarbituric acid reactive substances - TBARS, uric acid and nitrite). Athletes supplemented with BA or dextrose (PL) at doses of 6.4 g/day. Beta-alanine (BA) supplementation does not modulate the performance (CMJ). Along the treatment period, the internal load of the PL group remained higher than the BA group ($P=0.011$). The uric acid and nitrite were neither modulated by training nor treatment. However, the TBARS was lower at post moment than pre, without difference between groups. Thus, BA supplementation neither increased the performance nor decreased oxidative stress in volleyball athletes after a period of training intensification.

Keywords

Buffering, Carnosine, Exercise, Nutrition, Performance.

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1. INTRODUCTION

The intensification of the training load (ITL), followed by the tapering period, has been used to improve performance [1, 2]. In the period of ITL, there is a decrease in performance and immune system, increase in cell injury markers and oxidative stress [3, 4]. In the tapering period, there is a decrease in training volume, and at the end of the tapering is expected an increase in performance due to the restoration of energetic substrates, recovery of the immune system, muscle cells and improvement of cellular redox status [5].

Some ergogenic resources also improve the athletes' performance. BA supplementation enhances performance mainly by maintaining the balance of intracellular pH [6]. BA, together with L-histidine, are precursors of the synthesis of carnosine in skeletal muscle, which acts as an intracellular buffer, slowing the increase of the hydrogen ions concentration in the muscle and the decrease of the physical performance [6, 7]. In addition to the buffering action of carnage, there is evidence of an antioxidant effect by reducing oxidative stress and consequently preventing damage to proteins and lipid membranes [8, 9], acute responses that occurs during ITL period. Furthermore, we do not know whether BA could be a better response when a muscle was submitted to a greater stress in a long period of time, like carbohydrate supercompensation.

In modalities with characteristics like volleyball, BA supplementation is able to improve physical performance [10-12]. The maintenance of the stable pH and the reduction of the oxidative stress promoted by the BA supplementation enhances the quality of the training measured through the lower ITL and oxidative stress [13]. In this sense, the purpose of the present study was to evaluate whether BA supplementation reduces oxidative stress and improves the physical performance of volleyball athletes in a tapering period after the ITL. We hypothesized that performance might improve during a tapering period and have a synergic effect in a group submitted to BA supplementation.

METHODS

Subjects

Eleven adult athletes (group BA=6, Group PL=5), male, healthy, professional volleyball players, participated in this study. Anamnesis was used to identify the profile and training aspects of the athletes, as well as the use of supplements and health history. We evaluated the body mass (kg) and body composition through the Tanita® brand Tetrapolar bioimpedance [14]. Height (cm) was measured using a Sanny® portable stadiometer. The sample characterization is shown in table 1. Athletes who used dietary protein supplementation, creatine supplementation for 3 months and BA for 6 months before the study would be excluded, as well

as vegetarians or vegans. No one was excluded from the study. The study was approved by a local Research Ethics Committee registered under number 1426/2015 and was conducted following the standards set by the Declaration of Helsinki. Participation in the study was voluntary and occurred only after the signing of the Informed Consent Term. The athletes received all the information related to the study. The risks and discomforts that would be minimal were mentioned, as well as the possible benefits.

Table 1. Characteristics of subjects

	BA Group	PL Group
Age (years)	19±0.9	20±0.6
Body mass (kg)	76.7±6.1	79±3.6
Height (cm)	190±10.0	180±10.0
% Body fat	11.7±2.2	12.1±1.9

All data are presented as mean±standard deviation (SD).

Design

The study design is a double-blind, randomized, and placebo-controlled. All tests were performed at the same time of the day to avoid changes in the circadian rhythm.

Methodology

The design included the analysis of countermovement jump (CMJ), internal load (7 days of test interval), questionnaires (WURSS 21) and blood collection with a 21-day range (pre- and post-tapering period analysis). We described a schematic representation of the experimental design in figure 1.

Food consumption

For the food consumption analysis, we applied a single 24-hour reminder of a typical training day at the beginning of the tapering period. Athletes were instructed to maintain their eating habits throughout the study. We used the Dietpro 5i® software (A.S. Systems, version 5.8) to determine the energy consumption of macro and micronutrients (vitamins C and E). We evaluated the micronutrient intake according to the Dietary Reference Intakes guidelines [15].

Supplementation of β -alanine and placebo

The supplementation was given to the team's physical trainer, who oversaw distributing to the athletes. The capsules were placed in a small packet referring to each day, marked with a number that corresponded to the athletes; this numbering was randomized by a third individual who did not participate in the analyses and writing of the work. Supplementation was achieved by ingestion of 6.4 grams per day of BA (99.9% pure β -alanine; CarnoSyn®, NAI, USA) or placebo (maltodextrin, NeoNutri®) [16]. Both supplements were given in slow absorption capsules of hydroxypropyl methylcellulose, size 00, opaque white [17] (DRCaps®, Mexico), to prevent the occurrence of paresthesia, once a day, including playing days, during a period of 21 days (tapering period), consumed 30 minutes before the technical training. Through individual reports, 8 athletes (6 from the BA group and 2 from the PL group) reported small occurrences of paresthesia. Before and during the supplementation period, all athletes were instructed not to discuss any side effects to the other athletes on the team.

Analysis of Counter-Movement Jump

The athletes were submitted to a CMJ test to determine the elevation of the center of gravity about the ground [5]. The jump height was determined employing a contact mat (Hidrofite®), with the performance measured and analyzed by the software jump test 2.0®. The realigned motor gesture for such performance was the counter-movement jump (CMJ), thus, used as a standard technique for monitoring. Its technique of execution is a concentric action preceded by a preparation movement that constitutes the eccentric phase, until approximately 90° of knee flexion. All subjects were instructed to perform as much effort as possible and instructed to keep their hands on their waist all the time. The test protocol was 8 jumps with a 1-minute interval between each jump [18]. If the jump technique were not performed correctly during any attempt, that attempt would not be counted, and athletes would be invited to perform another jump again after 1 minute. The average of the 8 jumps was the value used for performance comparisons between weeks. Before each CMJ evaluation for monitoring, there was an activity on the cycle ergometer as a warm-up time of 5 minutes.

Internal Training Load

In order to control the internal training load (ITL), the session's Rating of Perceived Exertion (sRPE) was used, in which the training load was calculated through the product between the intensity, identified through the 10-point sRPE scale, adapted by Foster et al. [19], and the training volume, expressed by the total time of the training session in minutes. The sRPE was reported 30 minutes after the end of each training session, in which the athletes reported on the scale the scores for the question: "How was your training?". The values were obtained in arbitrary units (AU) to calculate the internal training load.

Questionnaire

The upper respiratory tract infection symptom questionnaire (WURSS 21), with twenty-one survey questions, was used to collect information on health-related dimensions that are negatively affected by common colds. All items are based on a Likert scale of severity from 0 to 7. The questionnaire was always applied at the beginning of the training weeks, with the individuals at rest, before the training.

Blood collection

Blood collection was performed at rest by venous puncture of the antecubital vein (week 0 and 3). Blood was transferred to blood tubes (BD Vacutainer® with EDTA K2) with 10 mL by the athlete. The samples were kept on ice until the time of centrifugation. The tubes were centrifuged at 3000rpm/8min at 4°C for separation of the plasma. After, the samples were frozen at -80°C for further analysis (dosing of thiobarbituric acid reactive species (TBARS), uric acid and plasma nitrite).

Determination of lipid peroxidation

For the analysis of TBARS, the method proposed by Janero [20]. For each assay, 11 µl of Hydroxy-butyl-toluene (HBT) diluted in 2% ethanol and 100 µl of the sample supernatant (centrifuged at 12.000 g per 1 minute) were added. Subsequently, 100 µl of thiobarbituric acid (1% in 50 mM NaOH) and 100 µl of 25% HCL were added, and 100 µl aliquots of 10-1mM 1,1,3,3-tetramethoxypropane were added to the standard. The tubes containing the mixture were incubated in a boiling bath (100°C) for 10 minutes and cooled in an ice bath. 290 µl of butanol was then added to each tube and vortexed until the full transfer of the rosy colour from the lower layer to the upper layer. After that centrifugation was performed for 5 minutes at 12.000 rpm. The butanol phase was withdrawn and the absorbance assessed at 532 nm in a spectrophotometer (SpectraMax Plus, Molecular Devices®). The TBARS concentration was then determined using the molar extinction coefficient of the MDA ($\epsilon = 1.56 \times 10^5 \text{ M}^{-1} \cdot \text{mL}$), using the formula: TBARS concentration = (absorbance/0.156) x dilution = nm/mg protein.

Dosing of uric acid in plasma

Plasma uric acid dosing was done using the commercial Bioclin® kit according to the protocol proposed by Fossati et al. [21]. Absorbance reading was performed using a spectrophotometer (Molecular Devices - Spectra Max 190®).

Evaluation of plasma nitrite content

The analysis of plasma nitrite content was performed using the method proposed by Ding et al. [22]. Absorbance reading was performed using a spectrophotometer (Molecular Devices - Spectra Max 190®).

Statistical analysis

Data are presented as mean and standard deviation. For CMJ and IL, the two-way repeated-measures analyses of variance (ANOVA) were used to detect within and between-group differences. If the assumption of sphericity was not achieved Greenhouse-Geisser corrections were applied. The analyses were completed by Bonferroni's test for multiple comparisons. Oxidative stress data have a non-normal distribution and, therefore, to verify possible differences between groups, we use the nonparametric Friedman test. For the analysis of food consumption, the Student T-test was used. The level of significance accepted was $P \leq 0.05$.

To check the qualitative outcomes and probability to find the same results again in the future (i.e., the magnitude of the evidence), we applied the Bayes factor hypothesis testing analyses. The posterior odds have been corrected for multiple testing by fixing to 0.5 the prior probability that the null hypothesis holds across all comparisons [23]. Individual comparisons are based on the default t-test with a Cauchy (0, $r = 1/\sqrt{2}$) prior. The "U" in the Bayes factor denotes that it is uncorrected [23-25]. The outcomes were classified as anecdotal ($BF_{10}=1$ to 3), moderate (3 to 10), strong (10 to 30), very strong (30 to 100) and extreme (>100) favoring the alternative hypothesis; or anecdotal ($BF_{10}=1$ to 0.33), moderate (0.33 to 0.1), strong (0.1 to 0.03), very strong (0.03 to 0.01) and extreme (<0.01) favoring the null hypothesis (Lee and Wagenmakers' classification) [25-27]. To calculate the probability to find the same results again, we divided the actual BF_{10} value by $BF_{10}+1$. We made all analysis through the JAMOV®.

RESULTS

Beta-alanine supplementation does not modulate the performance (CMJ). On the first week post intensification period (W5) there was a performance decrement in comparison with WO, returning to initial level at W6. The training strategy utilized here has the likelihood of 99% to be harmful to performance on the following week after intensification period (as expected), and 98% of probability to return to Wo level of performance at W6 and W8. As the performance was not higher than Wo in any period during the tapering (unexpected data),

and BA does not modulate the performance, we do not recommend both to improve vertical jump performance.

Figure 2. Squat jump analysis for groups for 8 weeks and mean Squat jump for groups.

The performance decrease at W5 is explained by the greater training stress showed by the higher ITL than previous periods. Also, we can see a higher internal load in a placebo group than the BA group before the supplementation period. The possibility of the internal load at W5 to be higher than W1, W2, W3 and W4 are 87%, 94%, 99% and 98%, respectively. Interesting that the ITL at W3 has 89% of probability to be lower than W1.

Figure 3. Internal load analysis for groups during the intensification period and mean internal load for groups.

The ITL at baseline was similar between groups allowing us to assess the supplementation and training efficiency. Along the treatment period, the ITL of the placebo group remained higher than the BA group ($P=0.011$). There was an increment of the internal load at W1 and decrease at W2 and W3 for both groups. The internal load decreased at W2 and W3 were in comparison with baseline and W1. The W1 and W2 had higher internal load than W3, regardless of the group. There was no difference between groups at W3. The chance of occurrence of these results is 99%.

Figure 4. Internal load analysis for groups during the tapering period and mean internal load for groups.

We observed a difference between baseline and W2 ($P=0.045$) for WURSS-21. However, this difference was not confirmed by Bayesian analyses, with the symptoms been similar along the training period regardless of the treatment. Also, the vigor and humor were not modulated along the training period regardless of the group analyzed.

The fatigue feeling was lower at W2 than baseline and W1 period. The likelihood of feeling low fatigue at W2 in comparison with baseline is 94%, regardless of treatment. However, the chance of feeling less fatigue than W1 was not confirmed by Bayesian analyses.

The uric acid and nitrite were neither modulated by training nor treatment (table 2). However, the TBARS was lower at post moment than pre, without difference between groups (figure 5). The likelihood of TBARS to be modulated by the training process is 93%.

Figure 5. TBARS analysis between groups during the tapering period.

Table 3 shows the results of energy intake, macro and micronutrients of athletes. There was a significant difference between the groups for the consumption of Vitamin E ($P = .013$), indicating a higher consumption of this antioxidant in the PL group.

Table 3. Analysis of oxidative stress for the groups.

BA GROUP					
Analysis	Week 0±SD	Week 1±SD	Week 2±SD	Week 3±SD	Δ 0-3%
TBARS (nmol/mL)	1.2±0.2	-	-	0.8±0.1	-50.0
Uric acid (mg/dL)	5.8±1	-	-	5.6±1.2	-3.5
Nitrite (uM/mL)	10.1±2.7	-	-	7.6±0.1	-32.8
PL GROUP					
Analysis	Week 0±SD	Week 1±SD	Week 2±SD	Week 3±SD	Δ 0-3%
TBARS (nmol/mL)	1.1±0.2	-	-	0.8±0.1	-37.5
Uric acid (mg/dL)	5.2±0.7	-	-	5.4±0.6	+3.7
Nitrite (uM/mL)	8.5±1.5	-	-	10.3±1.1**	+17.4

Data presented as mean±SD and delta (Δ) between week 0 and 3. TBARS: Thiobarbituric acid reactive substances. #Statistical difference for the BA group from week 0 to 1 (P=.007) and from week 0 to 3 (P=.001). *Statistical difference between groups (P=.001). The #Statistical difference in the PL group from week 0 to 1 (P=.008), week 0 to 3 (P=.014), week 1 to 2 (P<.001) and week 2 to 3 (P<.001). **Statistical difference between groups at a post-supplementation time (P<.001).

DISCUSSION

We found that BA supplementation neither increases the physical performance nor reduces the oxidative stress of volleyball athletes. Therefore, a 3-week nutritional supplementation with a single dosage of 6.4 g/day does not appear to be a good strategy for improving physical performance and reducing oxidative stress in volleyball players. The tapering process was not effective as well, contrary to the expected.

The effects of BA supplementation on the exercising muscle buffering are well described [6, 28, 29]. However, little is known about the impact of this supplement on recovery in a training process after a period of ITL. Our study is the first to analyses the effects of BA supplementation on volleyball athletes and consumption during a tapering period after the ITL. We hypothesized that the muscle function harmed by the intensification period could recovery faster with BA supplementation. On the first week post intensification period (W5), there was a performance decrement in comparison with Wo. The training strategy utilized here has the likelihood of 99% to be harmful to performance on the following week after the intensification period. The performance decrement observed is expected [2]. Therefore, the aim of the intensification period was reached. The performance returned to the initial level at

W6. (Table 2). We observed 98% of chance to return to Wo level of performance at W6 and W8. However, a super-compensation is expected during the tapering period (i.e. performance improvement). So, regardless of BA supplementation or PL, the training strategy was not good for performance improvement. Although all subjects on BA group have reported paresthesia it was not enough to improve performance through a placebo effect (positive expectancy). The tapering process followed the current guidelines [30]; however it does not promote the super-compensation as expected. The athletes' only reaches the performance level of Wo. The reason could be the nutrition aspects since the athletes consumed less carbohydrate than recommended [31].

Regarding food consumption, no difference was found in the amounts of carbohydrates and proteins between groups. Furthermore, both groups consumed a lower amount of carbohydrate than recommended for athletes (3.6×2.6 g/kg body weight), which may have also influenced performance for both groups. Regarding the statistical difference in vitamin E intake, this probably did not influence the athletes' redox status, since the BA group had its adequate consumption according to the population's needs (Recommended Dietary Allowances).

The response of the immune system and markers of cell injury to the demands of volleyball matches and training have been little explored. After a game of volleyball, there is an increase in inflammatory biomarkers and muscle damage [32]. The inflammatory biomarkers remained elevated for 37 hours after the match, and biomarkers of muscle damage for 2 days [32]. Therefore, there is a need for strategies that might improve the athletes recovering. Similar responses were observed for the oxidative stress biomarkers of our study, where athletes initially found themselves with greater stress. We expected that the greater oxidative stress caused by intensification period could speed up the BA action on positive modulation of oxidative stress (super-compensation). Unfortunately, neither BA supplementation nor the tapering process was able to benefit athletes in modulating oxidative stress positively [33]. Although the oxidative stress biomarkers point to the effect of training intensification, the WURSS-21 questionnaire showed no difference regarding intensification or BA supplementation.

The redox state of athletes can be influenced by the training load [34]. In our study, the athletes had a high training load which could explain the endogenous antioxidant capacity decrement during an intensification period, this also explains the increase in TBARS concentrations in both groups, indicating a possible training-induced oxidative stress. This response was expected and desired. Because of the training volume decrement during a tapering period, we expected an improvement of the antioxidant defense greater than Wo, and that BA supple-

mentation could have a synergistic action with tapering through a super-compensation response. However, neither the tapering period nor the BA supplementation was able to improve antioxidant defence, impacting on athletes' fatigue and recovery. Therefore, a load intensification period is not needed to improve the BA action on oxidative stress. As our assessment does not analyse the muscle buffering capacity, this question keeps open.

Here we used the amount of 6.4 g/day of BA according to established protocols [16]. This amount of BA supplemented daily increases the muscle carnosine concentration between 40% and 60% in 4 weeks [16]. The amount of BA in our study (134.4g) was higher than in other studies (112g) [16]. We used a higher load to optimize the results since BA supplementation is time and/or dose-dependent [6]. Possibly the dosage did not reflect a considerable loss via urine, since the only study that reported this loss brought an excretion of only 3%, and this value would cause a loss of the only 3.7g of the total 134.4g we offer [35]. It reinforces our data that BA supplementation neither improves performance nor antioxidant defence.

PRACTICAL APPLICATIONS

Beta-alanine supplementation does not help the athletes' in the recovery process. With this training load and for volleyball athletes, the importance of good nutrition focused on proper carbohydrate intake, with vitamins that help athletes recover, seems to be the best strategy rather than BA supplementation. Even, the training process composed of training intensification followed by the tapering period, as proposed by the current literature, does not improve performance (CMJ) in volleyball players. Therefore, other training strategies, like optimum training load, seems to be a better strategy to improve performance, reducing the risk of injury and illness.

CONCLUSION

We conclude that BA supplementation neither improve physical performance nor oxidative stress of the volleyball athletes. The daily consumption of a single dose of BA during the tapering period did not contribute to modulate oxidative stress, the perception of general stress, as well as internal training load and performance. Therefore, BA is not a good supplementation strategy for volleyball athletes during a tapering period.

AUTHORSHIPS, DECLARATIONS OF FUNDING SOURCES AND CONFLICTS OF INTEREST

The study was designed by JCS, RLSG and PHSMA; data were collected and analysed by JCS and RLSG; data interpretation and manuscript preparation were undertaken by JCS, RLSG, GJE, AMZ, RHL, and PHSMA. All authors approved the final version of the paper. The authors have no conflict of interest to declare.

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