EFFECTS OF ACUTE B-ALANINE SUPPLEMENTATION ON COUNTERMOVEMENT JUMP PERFORMANCE AFTER A 4X400 M RUNNING FATIGUE PROTOCOL: A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL

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Abstract:

This study aimed to examine the effect of acute beta-alanine (β-alanine) supplementation on jump performance after a strenuous fatigue protocol. Twelve healthy young men (age 21.4±0.5 years, body height 180.2±5.8 cm, body mass 76.6±9.2 kg) volunteered to participate in this randomized, double-blind, placebo-controlled trial. The experimental group ingested 3.2 g of β-alanine (separated into two 1.6 g dosages) mixed with 23 g of glucose, whereas the placebo group ingested two dosages containing 23 g of glucose. Following the supplementation intake, participants completed a jump protocol involving countermovement jump (CMJ) and four consecutive countermovement jumps (CMJ-4). Subsequently, a 4x400 m running fatigue protocol was carried out to produce fatigue. After the fatigue protocol, the same jumping tests were repeated, CMJ and CMJ-4, to evaluate the loss in jump height. The Mann-Whitney U test was used to analyze differences between the groups, whereas Wilcoxon signed-rank test was conducted to analyze differences within the groups with statistical significance set at $p<.05$. After β-alanine supplementation, no significant decrease in jump height was found in the experimental group in none of the tests after the fatigue protocol. Conversely, a significant decrease was noticed in the placebo group in CMJ but not in the CMJ-4 test. In conclusion, an acute β-alanine supplementation could attenuate jump height loss after the fatigue protocol. Therefore, athletes and coaches should consider acute β-alanine supplementation to attenuate sports performance decrease after high-intensity exercises in which muscle acidosis is highly increased.

Key words: *beta-alanine, countermovement jump, acute supplementation, sports performance*

Introduction

Beta-alanine (β-alanine) is a naturally occurring non-proteogenic beta-amino acid endogenously produced in the liver (Trexler, et al., 2015). The main reason for β-alanine ingestion is to increase muscle carnosine. It is essential to note that carnosine supplementation is not an efficient method for increasing muscle carnosine levels because it is metabolized before reaching skeletal muscle (Gardner, Illingworth, Kelleher, & Wood, 1991). On the other hand, β-alanine has been shown as the rate-limiting precursor to endogenous carnosine production, where this compound, combined with L-histidine, forms carnosine (Blancquaert, et al., 2017). Beta-alanine is obtained through diet by consuming foods such as poultry and meat (Trexler, et al., 2015), while the most common supplementation method is to ingest β-alanine in doses ranging from 1.6 to 6.4 g/day−1 (Saunders, et al., 2017; Saunders, Sale, Harris, & Sunderland, 2012; Stellingwerff, et al., 2012). In addition, β-alanine ingestion has been shown to increase muscle carnosine, regardless of diet or baseline carnosine levels (Stellingwerff, et al., 2012; Trexler, et al., 2015). Therefore, supplementation with β-alanine could be the most effective method to increase muscle carnosine levels.

The increase of muscle carnosine levels could play a key role in exercise. Intracellular acid-based regulation is considered the primary physiological role of carnosine. Improvement of intracellular buffer capacity causes fatigue delay and, therefore, prolongs exercise (Hobson, Saunders, Ball, Harris, & Sale, 2012). Intramuscular carnosine

reduces muscle acidity, decreasing the large production of hydrogen ions (H+) (Cady, Jones, Lynn, & Newham, 1989). Increased concentration of H+ instigates a diminution of actin and myosin cross-bridge formation, which further causes a decrement in muscle contraction (Fabiato & Fabiato, 1978). This leads to a string of metabolic processes such as a decrease in force production and a fatigue increase (Dutka & Lamb, 2004). Therefore, muscle carnosine increase could influence power performance after a fatigue protocol. In addition, this supplement could also be susceptible to a placebo effect. Previously, other nutritional ergogenic aids have shown a placebo positive influence on sports performance (Hurst, et al., 2020).

Previous studies had shown improvements in performance tests after β-alanine ingestion when fatigue protocol was carried out, probably due to the aforementioned physiological explanations. Concretely, chronic (eight weeks) oral β-alanine ingestion has been shown to significantly improve power performance after a strenuous endurance exercise, as shown in Van Thienen et al. study on endurance-trained cyclists (Van Thienen, et al., 2009). In addition, in another study (Carpentier, Olbrechts, Vieillevoye, & Poortmans, 2015), after two months of β-alanine intake, there was a slight increase in power performance, measured as countermovement jumps (CMJ). However, athletes usually ingest β-alanine as a "pre-exercise" supplement (Gonzalez, Walsh, Ratamess, Kang, & Hoffman, 2011), and there is a lack of information concerning acute β-alanine intake in sports performance (Huerta Ojeda, Tapia Cerda, Poblete Salvatierra, Barahona-Fuentes, & Jorquera Aguilera, 2020). To the best of the authors' knowledge, there are only three studies carried out with only acute β-alanine ingestion on exercise performance with diverse results, where one obtained positive results (Huerta-Ojeda, et al., 2019), and the remaining two showed no effects (Bellinger & Minahan, 2016; Glenn, Smith, Moyen, Binns, & Gray, 2015) on exercise performance. Moreover, none of the previously mentioned studies using the acute β-alanine ingestion were carried out after a strenuous exercise protocol. To the best of the authors' knowledge, there is no research investigating the effects of the acute β-alanine ingestion on performance after a strenuous exercise. Therefore, the main objective of this study was to evaluate the effects of the acute β-alanine ingestion on performance after a strenuous fatigue protocol.

Methods

Participants

Twelve healthy, physically active students from the Faculty of Sport and Physical Education, University of Novi Sad (21.4±0.5 years, 180.2±5.8 cm, 76.6±9.2 kg) volunteered for the study. None of the participants in the study consumed any dietary supplements during the four weeks prior to the study kick-off, and none of them reported any musculoskeletal injury or disease. Volunteers signed a consent form to participate in the study after receiving a complete insight into the study protocol. Each participant obtained written guidelines, including detailed information about the test protocol, time frame, and responsibilities regarding the study.

Study design

This study was based on a randomized, double-blind, placebo-controlled, parallel design of evaluating the effects of acute β-alanine ingestion. The randomization was carried out by alternating group assignment of participants into either the placebo group (n=6) or the β-alanine group (n=6). The study was approved by the Ethical Committee of the Faculty of Sport and Physical Education in Novi Sad (46-06-01-2020-1) and conducted according to the principles of the Helsinki Declaration. This experiment was divided into three different round-ups: the pre-study protocol, baseline, and experimental protocol (Fig. 1).

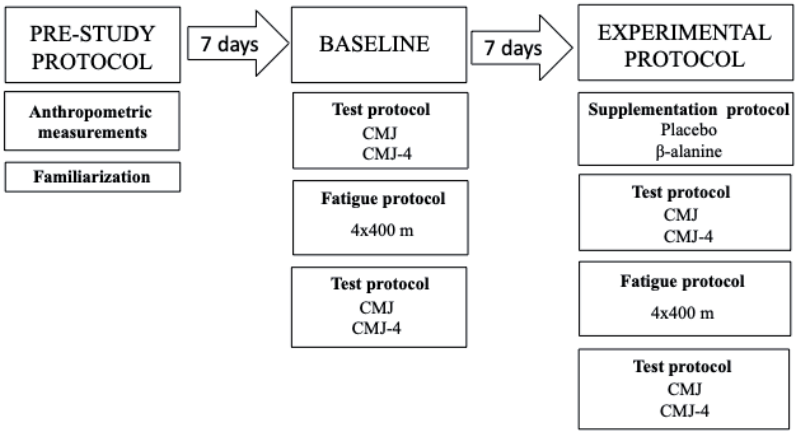

Figure 1. Study design. CMJ, countermovement jump; CMJ-4, four consecutive countermovement jumps.

The pre-study protocol was divided into two different phases. The purpose of the first phase was to obtain anthropometric measurements for all of the included participants in order to assess the homogeneity between the groups. Body mass (BM), body mass index (BMI), and body fat percentage (BF%) estimations were obtained using bioelectric impedance (model InBody 230), whereas the body height (BH) was calculated through a Seca SE213 stadiometer. The second phase of the pre-study protocol consisted of the familiarization of participants with the performance test of the study to minimize any potential learning influence on the subsequent tests.

At baseline, participants performed a standardized warm-up before any test was carried out. The warm-up consisted of five minutes of submaximal running followed by a previously established dynamic warm-up: high knee and foot walk, carioca, butt kicks, high skip, spiderman, lateral slide with floor touch, low skip + long jumps, and dynamic calf stretch (Stevanovic, et al., 2019). Following the warm-up, participants completed the countermovement jump (CMJ) and four consecutive countermovement jumps (CMJ-4). Jump heights were measured on a force plate (Just Jump System; Probotics, Huntsville, AL, US) and were executed with voluntary knee flexion and keeping the hands placed on the hips during all tests. Both tests were performed three times, with a 1-minute rest between the attempts, and only the highest result was used for the analysis. Subsequently, a fatigue protocol was performed to produce muscle tiredness in the participants. This protocol consisted of 4x400 meters running trials, interspersed with three minutes of recovery between the trials. After the fatigue protocol, the same jumping tests were repeated, CMJ and CMJ-4.

For the experimental protocol, each group ingested either a placebo or β-alanine supplementation four hours (the first dosage) and 45 minutes (the second dosage) before the warm-up. The β-alanine group ingested a total dosage of 3.2 mg/kg, as previously other authors used the same dosage (Gross, et al., 2014), and it was ingested at two different moments so as to reduce possible side effects (e.g., paresthesia). The β-alanine group ingested two dosages of 1.6 mg/kg of β-alanine mixed with 23 g of glucose, whereas the control group ingested two placebo dosages containing only 23 g of glucose. Both groups received the same amount of drink (500 ml) of similar color and smell. Beta-alanine and glucose powder were obtained from THE Nutrition®. After supplementation ingestion, volunteers carried out a warm-up and performed the same procedures as at baseline, i.e., the jump test, fatigue protocol, and repetition of jump tests.

Statistical analysis

All quantitative data were recorded into an Excel table and analyzed using Statistical Package for Social Sciences version 26.0 (SPSS Inc., Chicago, IL, USA). Data are presented as mean (M) ± standard deviation (SD). Given that the sample size of each group is lower than 10, non-parametric statistics were applied. Mann-Whitney U test was used for the differences between the groups and the Wilcoxon signed-rank test to analyze the differences within the groups between the pre- to post-test measurements. Statistical significance was set at $p<.05$.

Results

All the participants completed the intervention, and no side effects associated with supplementation were reported during the study. Participants completed all individual running trials in the range of 0:59–1:34 min, accumulating between 4.5–5.4 min of total high-intensity activity (four running trials in total). Since participants ingested the second dosage of supplement to the end of the performance measurements, the duration was less than two hours. Physical characteristics of the β-alanine group and control group were similar at baseline, with no significant differences in age, height, BM, BMI, or BF. Therefore, both groups were considered to be homogeneous. Table 1 displays the details concerning both groups' physical characteristics and body composition.

Table 1. Descriptive characteristics of β-alanine and control groups

Variable	β-alanine group (n=6)	Placebo group (n=6)	p value
Age (years)	20.9±2.9	22.0±2.9	.808
Body height (cm)	180.2±5.8	180.2±4.3	.748
Body mass (kg)	76.8±9.2	76.3±5.6	.873
BMI (kg/m^2)	23.5±1.7	23.5±1.1	.749
BF (%)	17.4±2.9	17.5±4.4	.873

Note. Data are expressed as mean ± standard deviation. BMI, body mass index; BF, body fat; kg, kilograms; cm, centimeters; m, meters; %, percentage; n, number of subjects; SD, standard deviation; p, statistical significance; bolded p values indicate statistical significance ($p<.05$).

Table 2. Jumping test performance data in baseline and after the experimental protocol

	Baseline					
	β-alanine group (n=6)			Placebo group (n=6)		
	Before	After	p value	Before	After	p value
CMJ (cm)	50.89±2.77	44.87±2.83	**.028**	58.58±6.90	52.17±7.20	**.028**
CMJ-4 (cm)	47.20±3.03	42.42±4.01	**.028**	51.92±5.71	46.52±7.06	**.046**
	Experimental protocol					
	β-alanine group (n=6)			Placebo group (n=6)		
	Before	After	p value	Before	After	p value
CMJ (cm)	49.15±4.20	46.86±3.20	.249	57.20±7.30	53.97±6.90	**.043**
CMJ-4 (cm)	44.73±2.75	42.42±2.30	.345	50.44±6.90	46.37±6.10	.075

Note. Data are expressed as mean ± standard deviation. CMJ, countermovement jump; CMJ-4, four consecutive countermovement jumps; bolded p values indicate statistical significance (p<.05).

CMJ and CMJ-4 were measured before and after the fatigue protocol in baseline and experimental protocol. In baseline, both the β-alanine and placebo groups significantly decreased their CMJ and CMJ-4 values after the fatigue protocol. In the experimental protocol, after the β-alanine supplementation, no significant decrease in height was found in none of the tests. Conversely, a significant decrease was noticed in the placebo group in CMJ but not in the CMJ-4 test. Jumping test values are depicted in Table 2.

Discussion and conclusions

This study was carried out to determine the effects of acute β-alanine supplementation on explosive strength tests after a strenuous exercise. None of the participants reported any side effects after ingestion of this ergogenic aid. The results revealed that the acute β-alanine supplementation provided positive effects on CMJ and CMJ-4 performance, attenuating the loss of jump height after the fatigue protocol. Moreover, the study results could have also been influenced by a possible placebo effect, as shown placebo's positive impact on the CMJ-4 performance.

The acute dosage of β-alanine was 3.2 g (separated into two 1.6 g dosages), and none of the participants suffered from paresthesia on the skin (Harris, et al., 2006). It has been previously reported that higher dosages than 0.8 g has been associated with this side effect (Harris, et al., 2006). In order to reduce the side effects of β-alanine, the ingestion of the supplement was divided into two different dosages taken four hours (the first dosage) and 45 minutes (the second dosage) before the warm-up. Previously, it was reported that the peak increase of β-alanine in plasma was 30-45 minutes upon this supplement intake, and it is estimated that β-alanine levels keep elevated even 3-4 hours after the ingestion (Harris, et al., 2006). Therefore, this supplementation protocol was carried out with a mission to maximize acute β-alanine/carnosine concentrations to increase the effects on exercise performance while reducing possible side effects.

Only three previous studies evaluated the effects of acute β-alanine on exercise performance (Bellinger & Minahan, 2016; Glenn, et al., 2015; Huerta-Ojeda, et al., 2019). Two studies assessed endurance performance with mixed results (Bellinger & Minahan, 2016; Huerta-Ojeda, et al., 2019). Although results in these two studies may be important, they are not comparable to our study's results due to different metabolic outcomes being measured. On the other hand, the remaining study assessing acute effects was conducted by Glenn et al. (2015), who measured anaerobic performance. Participants in that study ingested an acute dosage of 1.6 g β-alanine and did not improve exercise performance in three consecutive Wingate tests. In comparison to the results found in the present study, those authors possibly did not find significant results due to an insufficient acute dosage. The differences between the findings of both works could be explained by the fact that an acute dosage of 3.2 g might be more effective than a 1.6 g dosage. Therefore, a higher acute dosage of β-alanine could exert better results regarding sports performance. In addition, in Glenn et al.'s study (2015) participants were a trained population, while in this intervention, the population was healthy young males. Therefore, the difference between the studies' results could be explained by the following: it is more difficult to achieve a significant improvement in a trained population than in a healthy untrained population (Spurway & MacLaren, 2006). To the best of the authors' knowledge, no study was carried out after fatigue protocol with β-alanine alone. However, one study measured the effects of the mixed supplementation of β-alanine plus carnosine (2 g of both) on jump performance after a fatigue protocol (45 seconds of CMJ) (Invernizzi, et al., 2016). These

authors found similar results to ours attenuating jump performance loss after strenuous exercise, although the studies' fatigue protocols differed.

The fatigue protocol could elevate muscle acidosis and hence influence subsequent exercise performance. In this study, fatigue protocol consisted of 4x400 meter running trails, interspersed with 3-minute ecoveries between the trails. Participants completed all individual running trials in the range of ~0:59–1:34 min, accumulating between 4.5–5.4 min of total high-intensity activity (four running trials in total). This fatigue protocol was selected for the main purpose of eliciting a high amount of intra-muscle acidosis. The ATP yield from glycolysis is highest in exhaustive short-term exercise (i.e., 400 meters sprint), which increases muscle acidosis (measured by blood lactate) (Hirvonen, Nummela, Rusko, Rehunen, & Härkönen, 1992). In addition, this level of acidosis attains an individual maximum (Hirvonen, et al., 1992) and stays elevated for 3-to-5-min post-exercise (Divito, McLaughlin, & Jacobs, 2021).

Probably the physiological pathway to attenuate jump performance decrement after a fatigue protocol could be explained by the indisputable role of carnosine to act as a pH buffer under an acid environment (Swietach, et al., 2013). Carnosine is capable of binding muscle H+, reducing myocyte cytoplasm pH (Swietach, et al., 2013). Dutka and Lamb (2004) demonstrated that increased carnosine concentration inside cytoplasm could reduce cytoplasm H+, augmenting Ca2+ sensitivity in both slow-twitch and fast-twitch muscle fibers. Therefore, there could be an increase in skeletal muscle force production (Swietach, Leem, Spitzer, & Vaughan-Jones, 2014). Furthermore, the results of this work could also have been influenced by a possible β-alanine induced placebo effect. A previous meta-analysis suggested that nutritional ergogenic aids' produced placebo effect could exert a small to moderate effect on sports performance (Hurst, et al., 2020).

This study is not exempt from potential limitations. Therefore, the findings of the present experimental trial should be interpreted with caution. Firstly, the sample size (n=12) involved in the study could be considered small. Secondly, there was a lack of biochemical markers to control the β-alanine effect on exercise-induced acidity (e.g., blood lactate). Finally, the absence of muscle carnosine levels measurement could also be considered a limitation of this study. For that reason, future research might investigate the acute effects of β-alanine supplementation on CMJ performance after a fatigue protocol by increasing the sample size and measuring the blood acidity and muscle carnosine levels.

In conclusion, an acute dose (3.2 g) of β-alanine supplementation, divided in two equal dosages (four hours and 45 minutes prior to the exercise) could attenuate jump height loss after a 4x400 running bouts. Therefore, athletes, coaches, sport scientists and nutritionist should consider acute β-alanine supplementation to attenuate sports performance decrease after high intensity exercises in which muscle acidosis is highly increased. Nevertheless, future studies evaluating acute β-alanine supplementation on sport-specific performance after strenuous exercise are needed to support these results.

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