Original Article

Effect of a five-week beta-alanine supplementation on the performance, cardio-respiratory system, and blood lactate level in well-trained rowing athletes: A double-blind randomized pre-post pilot study

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Abstract:

Intense exercise by top-level athletes significantly lowers the pH of muscle and blood, which leads to fatigue. Beta-alanine (BA) supplementation can increase carnosine levels in skeletal muscle, which can delay a decrease in pH in the muscles. Previous studies have shown that multiple doses of BA supplementation were effective for people of different physical fitness and age. The purpose of this double-blind, randomized, controlled study is to investigate the effect of a five-week BA supplementation on well-trained, experienced rowing athletes at approximately the median dose of previous studies, which determined that 50 mg day⁻¹ kg⁻¹ of body weight was an effective daily dose. Two groups were formed in the spirometric study. One group received BA supplementation, while the control group did not. Five weeks after the first test (T1), the second test (T2) occurred, and the blood lactate levels were measured before and after the tests (Pre[La]_b; Post[La]_b). The maximum mean values of different physiological load parameters showed no significant difference. In the BA group, the mean lactate level was significantly lower after the T2 measurement (Post[La]_b) compared to those of T1 (P = 0.01) and the control group, i.e., T1 (P = 0.008), T2 (P = 0.028). The mean time and performance of the second measurement of the BA group increased [T1 = 582,7 ± 88,2 (s); T2 = 636,4 ± 106,6 (s)] but the result is not significant. In summary, the five-week dose of 50 mg day⁻¹ kg⁻¹ of body weight, which is 3.845 day⁻¹ for the group average, lowers the blood lactate levels after the tests (Post[La]_b) but does not increase the athlete's performance. For well-trained athletes, during a five-week supplementation, it is not advisable to lower the value below 4–6 g day⁻¹ with the dietary supplement to achieve an ergogenic effect.

Keywords: dietary supplement, carnosine, physiological characteristics, spirometric examination, oxygen consumption

Introduction

The use of legal performance enhancers and dietary supplements is often a contentious issue. The primary goal of any performance-enhancing method is to improve the performance of athletes. Accelerating regeneration is perhaps one of the most important issues. The use of agents that provide rapid regeneration after high-intensity training is important. Training control requires adequate measurements to analyze training load and rest.

It is known that during intense exercise, lactic acid is formed in the body, especially in sports that require frequent high intensity. This causes the pH of the muscle and blood to significantly decrease, which contributes to muscular fatigue. Lactic acid enters the bloodstream. Carnosine is capable of binding hydrogen ions and exhibits proton binding properties (Blancquaert et al., 2017); thus, it is able to delay a decrease in pH in the muscles owing to intense exercise (Matthews et al., 2019). The body can produce carnosine from beta-alanine and L-histidine. Carnosine has many physiological effects. Histidine is a non-essential amino acid that occurs in high concentrations during carnosine synthesis in skeletal muscles; however, beta-alanine is present at much lower concentrations in skeletal muscles, and it appears to be a limiting factor in carnosine synthesis (Blancquaert et al., 2017). The catalyst for the process is the enzyme carnosine synthase. This enzyme is responsible for breaking down carnosine. This enzyme can be detected in blood serum and in many other human tissues but mainly in skeletal muscles. Owing to the intracellular proton binding effect of carnosine acidosis, fatigue occurs earlier in the absence of carnosine (Derave et al., 2010). Owing to their structure, nitrogen atoms

on the imidazole ring at physiological pH can immediately bind protons (Trexler et al., 2015). When beta-alanine and sodium bicarbonate are used together, an even more significant buffering effect can be achieved.

The primary way for beta-alanine to enter the body is through meat consumption but not at the same concentration as with a targeted dietary supplement. The concentration of carnosine in skeletal muscle fibers can be increased by beta-alanine supplementation (Varanoske et al., 2019; Carvalho et al., 2018), while the intake of carnosine as a dietary supplement is not effective in increasing carnosine levels in the muscle tissue. Although red meat, which is rich in carnosine, can be used to optimize human growth, development, and health (Wu, 2020). Increasing carnosine levels can improve performance during high-intensity exercise (Saunders et al., 2017). With a daily intake of 4–6 g of beta-alanine for 2–4 weeks, athletic performance can be increased (Peeling et al., 2018; Ruth et al., 2013). Choosing a dietary supplement intake strategy is important. With a daily intake of 4–6 g of beta-alanine for 2 weeks, a 20–30% increase in carnosine levels can be achieved; with a dietary supplement of 4–6 g per day for 4 weeks, a 40–60% increase in skeletal muscle carnosine levels can be achieved (Stellingwerff et al., 2012; Blancquaert et al., 2017). However, the carnosine-increasing effect of BA in the muscle has been shown for a daily dose of 1.6 g (Stellingwerff et al., 2012). Studies have investigated BA supplementation in an effort to increase exercise performance via multiple dosing strategies.

The ergogenic effect of BA has been observed at doses of 0.8–2.4 g/day for 4 weeks (McCormack et al., 2013; J. R. Stout et al., 2008; Furst et al., 2018) and at doses of 4–6 g/day for 4–6 weeks (Saunders et al., 2017; de Andrade Kratz et al., 2017). Long-term running time results and athletic performance can be improved after several weeks of daily 5 g supplementation (Santana et al., 2018). According to several previous studies, 40–60 mg kg⁻¹ of body weight daily dietary supplement for several weeks results decrease in serum lactate values after exercise (Smith et al., 2019; Glenn et al., 2015). In contrast, a previous study has reported an increase in lactate levels after exercise owing to similar beta-alanine consumption; however, the performance enhancer effect of beta-alanine has been also observed (Beckner et al., 2020). It is determined that the correct dosage of BA is not entirely clear. Previous studies used different dosages for groups of different ages, genders, and fitness levels. The aim of this study is to investigate the effect of BA on the performance in well-trained athletes with good stamina after five weeks of supplementation at a daily dose of 50 mg kg⁻¹ of body weight, which is approximately the mean dosage that was determined to be effective in previous research. We were interested to know whether the 50 mg kg⁻¹ of body weight daily dietary supplement for five weeks affected the physiological characteristics of athletes and their pre- and post-exercise lactate levels. Physiological indicators that determine the performance of top-level athletes can be measured; performance diagnostic tests are widely used. Possible changes in load physiological characteristics can be monitored by several consecutive examinations. The measuring of lactate and cardiovascular system characteristics is an important component of training and preparation for elite rowers in their pursuit of high performance. Physiological characteristics, such as minute ventilation (VE) and absolute and relative oxygen consumption (VO₂, RVO₂), are quality determinants of cardiovascular system performance. Excellent endurance in rowing is necessary for successful racing, which is why we chose rowing athletes; in addition, the effectiveness of beta-alanine has not been previously studied in such experienced rowing athletes. The aim of this study was to provide accurate advice to well-trained athletes on consuming beta-alanine to improve their performance. In the current top-level sports, tenths and sometimes hundredths of seconds are decisive; therefore, in addition to a proper training plan and diet, ergogenic supplements (i.e., only those that are not prohibited by the WADA) are also needed.

Materials and methods

Experimental design - The research was performed at the Institute of Physiotherapy and Sports Science of the Faculty of Health Sciences of the University of Pécs; the ethical authorization number of this research is ETT TUKEB 25229-5 / 2018 / EKU. All participants were provided detailed information and made a written statement agreeing to participate in the research. The double-blind, randomized, controlled research evaluated the effect of a five-week beta-alanine dietary supplement through exercise physiological parameters of performance. Two groups of participants were formed using a random number-generator page – <https://www.randomizer.org>. One group (n = 12) received BA supplementation, while the control group (n = 11) did not. Five weeks after the first test (T1), the second test (T2) was performed, and the results of both groups and measurements were compared. Blood lactate levels were determined before and 3 min after both tests.

Participants - In this longitudinal study, a total of n = 23 male rowing athletes (19.4 ± 2.2 years old) were included, who received the same training. The participants included age group champions, adult Hungarian champions, European Championship and World Championship participants, and one university world champion. The group had a mean height of 1.82 ± 0.07 m and an average body weight of 76.9 ± 10.2 kg; their body composition is characterized by an average of 13.6 ± 3.3% body fat percentage and 43 ± 2.5% muscle percentage. All procedures were in line with the 1964 Declaration of Helsinki and its subsequent amendments to ethical standards. All participants were informed orally and in writing about the research, its risks, and benefits; then, after explaining the study, all participants gave their written consent to participate in the study. The condition to participate in the study was the same as the condition in the competitions, i.e., the existence of a

valid sports doctor's certificate. Furthermore, the participants could not consume a dietary supplement 8 weeks prior to the research.

Supplementation protocol - After the first measurement, one part of the group ($n = 12$) consumed 50 mg kg^{-1} of body weight of beta-alanine daily for five weeks, while the control group ($n = 11$) did not. For the first time, the daily BA dose for athletes and the total dose of BA they ingested during the study was determined on a personalized basis; then, the powdered food supplement was given to the athletes in sachets by personal measurement; they also received a shaker and a measuring spoon. Their consumption was also supervised by their coach; they had to consume the supplement in two instalments before morning and afternoon workouts.

Anthropometric measurements and lactate level determination - Body composition was analyzed with an "Inbody 720" (Biospace Co. Inc., Seoul, South Korea) bioelectrical impedance analyzer (BIA). In both studies (T1, T2), serum lactate concentration (Accutrend® GC VD-003 GCTL) was measured from a fingertip blood sample before the test and 3 min after the test.

Spiroergometric examination and protocol - Cardio-respiratory system characteristics were measured using a "Woodway System WUS" treadmill (Waukesha, WI, USA) until complete fatigue. A "Cardiosys Ergos-stress MDE" (Walldorf, DE) instrument was used to measure the resting pulse (HR_o) (beats min^{-1}) and maximum heart rate (HR) (beats min^{-1}); a Vyaire Medical JLAB "Vyntus" (Mettava, IL, USA) instrument was used to measure the aerobic capacity ($\text{VO}_{2\text{max}}$), ventilation VE (BTPS l min^{-1}), and its components. The protocol used in the spiroergometric study was as follows: warm-up 2 min, belt speed 5 $\text{km} \times \text{h}^{-1}$ with a slope of 0%, then for 2 min at 11 km h^{-1} with a slope of 3%. Next, the slope was increased by 3% every 2 min with the belt speed of 11 km h^{-1} until the end of the study.

Statistical analyses - Statistical analysis was performed using the *Statistica for Windows* software package (version 12.1, StatSoft Inc., Tulsa, OK 74104, USA, 2006). Differences per load and organ system were analyzed by the repeated ANOVA, post hoc, and Tuckey HSD methods; the data were presented as the mean $\pm$ standard deviation; significance was assumed at the $P < 0.05$ level.

Results

The basic characteristics of all participants and both groups ($n = 23$) are shown in Table 1. The data of the groups are almost the same; there is no significant difference between them ($P > 0.05$). There was no deviation from baseline in the second study five weeks later ($P > 0.05$).

Table 1. Participant characteristics

Variable	All participants ($n = 23$)	BA ($n = 12$)	C ($n = 11$)
Age (years)	$19,4 \pm 2,2$	$19,5 \pm 1,86$	$19,2 \pm 2,7$
Body height (m)	$1,82 \pm 0,07$	$1,8 \pm 0,07$	$1,84 \pm 0,06$
Body mass (kg)	$76,9 \pm 10,2$	$74,7 \pm 8,5$	$79,4 \pm 11,8$
Body fat (%)	$13,63 \pm 3,3$	$12,9 \pm 1,9$	$14,4 \pm 4,4$
Body muscle (%)	$43 \pm 2,5$	$43,5 \pm 2,2$	$42,4 \pm 2,8$

Table 1. Baseline participant characteristics. The data are presented as the mean $\pm$ standard deviation. BA is the daily beta-alanine strategy; C is the control group. There are no significant between-group differences.

Different load physiological characteristics were compared per measurement and per intensity zone. The different characteristics of the cardio-respiratory system were compared by intensity zone; as a result of increasing load, the individual load physiological indicators showed a linear upward trend during the study. These data allowed us to determine the maximal working and oxygen uptake capacity of the study group during the two studies. The average of the peak values of each load physiological parameter per measurement and per group is shown in Table 2.

Table 2. Maximum mean $\pm$ standard deviation of performance and cardiovascular system characteristics

Variable	BA		Control	
	T1	T2	T1	T2
Test				
Performance (s)	$582,7 \pm 88,2$	$636,4 \pm 106,6$	598 ± 45	610 ± 68
HR (b min^{-1})	$198,3 \pm 2,7$	$199,4 \pm 1,9$	$197,2 \pm 2,44$	$196,2 \pm 3,9$
VO_2 (L min^{-1})	$4,31 \pm 0,73$	$4,67 \pm 0,5$	$4,61 \pm 0,58$	$4,65 \pm 0,68$
RVO_2 ($\text{mL kg}^{-1} \text{ min}^{-1}$)	$57,77 \pm 7,7$	$62,88 \pm 6,6$	$58,33 \pm 4,7$	$58,66 \pm 3,9$
VE (L min^{-1})	$155,2 \pm 12,7$	$157,7 \pm 12,3$	$150,09 \pm 8,9$	$148,9 \pm 8,8$
RER	$1,02 \pm 0,05$	$1,07 \pm 0,04$	$1,08 \pm 0,07$	$1,07 \pm 0,02$

Table 2. Data for BA and control groups during the two measurements. Abbreviations: HR heart rate, VO_2 absolute oxygen uptake, RVO_2 relative oxygen uptake, VE minute ventilation, and RER respiratory exchange ratio. The data are presented as the mean $\pm$ standard deviation. No significant between-group differences reported.

No significant difference was observed between the maximum mean values of the groups for any of the load physiological characteristics. The mean values of BA and control groups did not significantly change; the difference between the two groups is not significant ($P > 0,05$). Regarding the time spent on the treadmill, we did not find a significant difference between the average results of the measurements. Averages of the measurements: $T1 = 598 \pm 45$ s, $T2 = 610 \pm 68$ s for the control group, $T1 = 582,7 \pm 88,2$ s, $T2 = 636,4 \pm 106,6$ s for the beta-alanine group. In the measurements of the control group, the difference between the mean values of the time results is almost negligible and not significant ($P > 0,05$). The mean time result of the second measurement (T2) of the BA group was larger than that of the first measurement (T1) and that of both measurements of the control group; however, there was no significant difference ($P > 0,05$) (Fig. 1).

Fig. 1 Mean time result of BA and control groups during the two measurements (T1; T2): AP (sec). There is no significant difference between the measurements (T1; T2) and the average results of the groups.

The lactate values taken from the blood serum during the T1, T2 measurements are shown in Table 3.

Table 3. Lactate values obtained from blood serum of the subjects before and 3 min after the test. Averages $\pm$ standard deviation

Variable	BA		Control	
Test	T1	T2	T1	T2
Pre[La-] _b (mmol L ⁻¹)	1,67 $\pm$ 0,43	1,75 $\pm$ 0,34	1,76 $\pm$ 0,4	1,87 $\pm$ 0,47
Post[La-] _b (mmol L ⁻¹)	13,09 $\pm$ 1,19	11,26 $\pm$ 1,09*	13,19 $\pm$ 1,42	12,9 $\pm$ 1,47

Table 3. Abbreviations: Pre[La-]_b lactate value measured before the test, Post[La-]_b lactate value measured 3 min after the test. * = the T2 measurement of the BA group showed a significant difference: between BA T1 ($p = 0,01$), C T1 ($p = 0,008$), and between C T2 ($p = 0,028$).

Neither the BA nor the control group showed a significant difference between the average results of the measurements for lactate values before the test (Pre[La-]_b); ($P > 0,05$). The average of the measurements for lactate values 3 min after the test; for the control group: Post[La-]_b = 13,19 $\pm$ 1,42 (mmol L⁻¹) (T1); Post[La-]_b = 12,9 $\pm$ 1,47 (mmol L⁻¹) (T2). For the BA consumer group: Post[La-]_b = 13,09 $\pm$ 1,19 (mmol L⁻¹) (T1); Post[La-]_b = 11,26 $\pm$ 1,09 (mmol L⁻¹) (T2). The mean of the second measurement (T2) of the BA group showed a significant decrease compared to the first measurement (T1) ($P = 0,01$). The mean of the second measurement (T2) of the BA group showed a significant difference compared to the first measurement (T1), ($P = 0,008$) and second (T2) measurements of the control group ($P = 0,028$). The results of the control group did not significantly change ($P > 0,05$).

Discussion

Both tests (T1, T2) meet the criteria of the *vita maxima test*, according to which the maximum heart rate per person approached the value of $[220 (b \min^{-1}) - \text{age}]$ during the increasing load; the duration of the test exceeded 6 min in the case of each participant; the RER value was greater than 1, and lactate values measured after the load also indicated adequate blood acidity. Thus, the obtained results are the ones that best show the current fitness status of the subject. In untrained individuals, minute ventilation in the maximum intensity zone is $VE \leq 100$ (L min⁻¹), the oxygen uptake is $VO_2 \leq 3$ (L min⁻¹), and the relative oxygen uptake is $RVO_2 \leq 40$ (mL kg⁻¹ min⁻¹) (Pavlik, 2011). By evaluating the obtained data for the group, it is determined that the participants

were in good fitness during both measurements; thus, we measured the effect of BA in groups with good endurance at the daily dose of 50 mg kg^{-1} of body weight. We found that the treadmill test was more appropriate for the group tested against the bicycle ergometer. On the one hand, more effort is required and the study group does not have a sport-specific adaptation either, which is an important consideration when selecting a test (Nagy et al., 2020). Regarding the treadmill time, the average time result for the BA group improved by $53,7 \text{ s}$ ($T1 = 582,7 \pm 88,2 \text{ s}$; $T2 = 636,4 \pm 106,6 \text{ s}$); the difference is large; however, this is not a significant result. However, the measurement protocol shows interesting results when at 600 s the treadmill angle increased from 9% to 12% . At this load level, the control group was still able to perform for an average of 10 s for $T2$. Meanwhile, the beta-alanine group spent an average of $36,4 \text{ s}$ in $T2$ at the 12% incline of treadmill. None of the groups reached this level for $T1$.

Thus, based on the results of lactate (Post[La-]_b) measured after the test of the subjects, the beta-alanine dietary supplement of 50 mg/kg/day results in a decrease in the lactate values obtained from the blood serum after the load. These results agree with previous observations (Ruth et al., 2013; Smith et al., 2019; Glenn et al., 2015; Jagim et al., 2013). The lactate results obtained 3 min after the exercise (Post[La-]_b) must be evaluated in parallel with the time spent on the treadmill and with the maximum heart rate. After all, if the athletes had not run on the treadmill to full fatigue or with sufficient intensity, a lower lactate value would not have occurred owing to the beta-alanine supplementation because they would not have reached the submaximal and maximum load zones. As a result of increasing load, the cardiovascular system responds with a linearly increasing performance (Pryimakov, 2020). In the case of a gradually increasing *vita maxima* load, at the high-intensity load steps, there is no longer enough oxygen available; thus, the body is forced to work by accumulating oxygen debt. This results in increasing levels of performance for a longer or shorter period of time, which then is followed by a sudden decrease (Pavlik, 2011). Measurement of $T2$ in the BA group had the lowest lactate value after exercise (Post[La-]_b), $(-1,83 \text{ mmol L}^{-1})$ at $T1$. While this is larger compared to the measurements of the control group $[-1,93 \text{ mmol L}^{-1}]$ at $T1$, $(-1,64 \text{ mmol L}^{-1})$ at $T2$ and was coupled with a longer time spent on the treadmill. In contrast, a previous study has reported increases in lactate levels after exercise owing to beta-alanine consumption; however, the ergogenic effect of beta-alanine was also observed (Beckner et al., 2020). It can be seen that the lactate level after exercise (Post[La-]_b) decreased despite the fact that participants achieved higher mean time results on the treadmill although a higher load results in a higher lactate value after exercise (Da Silva et al., 2018).

Beta-alanine supplementation had no effect on the aerobic capacity ($\text{VO}_{2\text{max}}$) or relative aerobic capacity ($\text{RVO}_{2\text{max}}$) of the subjects, these results agree with previous observations (Suszter et al., 2017). The respiratory minute ventilation (VE) did not differ during the studies; thus, aerobic capacity could not change because respiratory volume is the largest limiting factor of aerobic capacity. However, our study did not cover either the determination of red blood cell counts in athletes or the examination of vascularity of their muscles and their supply to mitochondria, which are also limiting factors.

The average body weight of the studied group was $76,9 \text{ kg}$; when it is multiplied by the dose of 50 mg kg^{-1} of body weight, the beta-alanine consumption of $3,845 \text{ g day}^{-1}$ for the group is obtained. This value lags behind the $4\text{--}6 \text{ g day}^{-1}$ dose in studies that monitored past performance gains (Saunders et al., 2017; de Andrade Kratz et al., 2017); however, the value is higher than the dose value of $2,4 \text{ g day}^{-1}$, which also causes an ergogenic effect (Beasley et al., 2018). In addition, the beta-alanine supplementation of $2,4 \text{ g/day}$ for 4 weeks increases training performance and endurance in middle-aged people (Furst et al., 2018). In elderly adults, the ergogenic effect of beta-alanine was also observed at doses of $0,8\text{--}2,4 \text{ g/day}$ (McCormack et al., 2013; J. R. Stout et al., 2008). We determined that for young, well-trained athletes, these daily amounts were not sufficient to achieve an ergogenic effect. Thus, in our opinion, the amount of correct dosage depends on the age, gender, and fitness level of the person and on the sport. When planning the correct beta-alanine dietary intervention, we think that it is necessary to pre-define the target group. We believe that dosages under $4\text{--}6 \text{ g day}^{-1}$ are sufficient for older or untrained people. For well-trained athletes, during a five-week supplementation, it is not advisable to consume less than $4\text{--}6 \text{ g day}^{-1}$ of the dietary supplement to achieve an ergogenic effect. In addition, we believe that it is worthwhile to perform a test where the inclination of the treadmill changes less or not at all and athletes can perform the test more evenly.

Conclusions

Decreased lactate value after exercise (Post[La-]_b) means a reduced metabolic response to exercise due to beta-alanine consumption. In this study, the five-week beta-alanine supplementation of 50 mg kg^{-1} of body weight, which on average was $3,845 \text{ g/day}^{-1}$ for the group, was not found to be ergogenic; it only reduced the lactate level after exercise. In contrast, previous studies have observed a performance-enhancing effect from the daily consumption of a much lower dose of $0,8\text{--}2,4 \text{ g}$ (Beasley et al., 2018; Furst et al., 2018; McCormack et al., 2013; J. R. Stout et al., 2008). Our study showed that beta-alanine dosing, which had a performance-enhancing effect on untrained and older individuals, was effective only in reducing the lactate value in trained individuals. In our opinion, this discrepancy is due to the basic characteristics of the participants such as age, gender, and level of fitness. This study indicates that all of these characteristics should be considered when designing a beta-

alanine dietary intervention to achieve an ergogenic effect. For well-trained athletes, a 4–6 week 4–6 g day⁻¹ of beta-alanine is recommended. Prior to our future studies, it is necessary to analyze the components of the blood (red blood cells and hemoglobin) to further increase the duration of intervention and to analyze the biochemical changes in the blood owing to exercise.

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