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Acute Supplementation with Beta-Alanine Improves Performance in Aerobic-Anaerobic Transition Zones in Endurance Athletes

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ABSTRACT

Objective: To determine the acute effect of low and high-dose BA trials on maximal aerobic speed (MAS) in endurance athletes. We hypothesized that high doses of BA have a greater effect than low doses, both compared to baseline.

Material and Methods: Twelve male endurance athletes volunteered for the study (age = 21.8 ± 2.37 years, weight = 69.8 ± 4.36 kg, height = 174 ± 5.45 cm, maximal oxygen uptake = 59.6 ± 3.77 mL O₂ · kg⁻¹ · min⁻¹). The experimental design applied was randomized cross-over, double-blind. Treatment included three 6-minute run tests (6-MRT), the first as a baseline, then randomized 6-MRT with low (30 mg · kg⁻¹) and high (45 mg · kg⁻¹) dose BA trials. The 6-MRTs were separated by 72 hours. The main variable of the study was the distance (m) performed in the 6-MRT. Differences between tests were established through ANOVA and Tukey’s multiple comparison tests (p < 0.05).

Results: The analysis showed significant differences between baseline and both doses (p < 0.001). No significant differences were observed between low and high-dose BA trials (p > 0.05).

Conclusion: Both 30 and 45 mg · kg⁻¹ of BA increased physical performance at maximal aerobic speed in endurance athletes. The acute intake formats described in the present investigation may be helpful for endurance athletes training and competing in aerobic-anaerobic transition zones.

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Amino acids; ergogenic effect; physical performance; physical endurance; exercise test



Introduction

Beta-alanine (BA) is a non-proteogenic amino acid (aa) produced endogenously in the liver (1). Humans acquire this aa through consuming foods such as poultry, beef, and fish (2). Furthermore, scientific literature has consistently shown an increase in carnosine (CA) content in human skeletal muscle due to BA supplementation (3, 4). Indeed, CA is a dipeptide (L-histidine and BA) present in muscle tissue, which regulates intramyocellular pH during physical exercise (5, 6). Despite this evidence, only a few studies have reported increased physical performance associated with BA supplementation and the consequent increase in intramyocellular CA (4). In this sense, there is scientific background on the effect of supplementation with BA in strength training programs (7, 8), endurance exercises (9, 10) and aerobic-anaerobic transition zone tests (10, 11).

Precisely, the latter intensity corresponds to a range between the aerobic threshold and the anaerobic threshold (12, 13). However, some studies have described that the aerobic-anaerobic transition zone may include maximal

aerobic speed (MAS) and maximal oxygen uptake (VO₂ max) (14). In this sense, VO₂ max is evidenced from a plateau in its kinetics (15), while MAS corresponds to the minimum intensity to reach VO₂ max (16). Furthermore, Faude et al. (17) suggest individualized knowledge of this specific intensity zone because it evaluates endurance performance and allows precise prescription of endurance training intensities.

Concerning BA supplementation on performance in aerobic-anaerobic transition zones, in a meta-analysis developed by Ojeda et al. (14), it was concluded that this aa generates minor effects on performance in this physiological zone. In this sense, the use of BA in aerobic-anaerobic transition zones can be divided into acute (11) and chronic supplementation (10, 18). The latter supplementation format has been widely documented (1, 10). Indeed, for the increase of physical performance in tests between 1-4 minutes (min) of duration, a position stand was reported with recommendations between 4-6 g · day of BA for 2-4 weeks (1). In parallel and under chronic supplementation, Santana et al. (10) investigated the effect of BA supplementation on 10 km

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running performance, concluding that this supplementation format generates positive effects in aerobic tests. Likewise, Outlaw et al. (18) evaluated the cumulative effect between endurance training and BA supplementation on treadmill performance, concluding that both variables generate non-significant increases in aerobic tests.

Despite the existing evidence that relates the use of BA to physical performance (8–10), information on the acute effect of BA supplementation in aerobic-anaerobic transition zones is scarce (14). Indeed, to the best of our knowledge, only the research presented by Ojeda et al. (11) has studied the acute effect of BA supplementation on performance in aerobic-anaerobic transition zones. These researchers supplemented with 30 mg per kg body mass ($\text{mg}\cdot\text{kg}^{-1}$) of BA, ingested 60 min before a time-limited test at MAS, evidencing a significant increase in performance at this intensity ($p < 0.05$). Apparently, the changes in physical performance reported by Ojeda et al. (11) were due to interindividual pharmacokinetic responses generated after acute intake of BA (19). In this sense, Stautemas et al. (19) reported on the plasma pharmacokinetic responses generated after acute BA supplementation (a: fixed-dose = 1,400 mg BA and b: dose in relation to body mass [$10\text{ mg}\cdot\text{kg}^{-1}$ BA]). They concluded that both supplementation protocols elevated plasma BA concentrations after 50–100 min from ingestion. The same authors mentioned that the plasma pharmacokinetics profile variation following an acute dose of BA reflects a variable response on CA loading (19). However, these same variations (BA kinetics and consequent CA synthesis) require a search for correct and personalized acute dosing (20).

Based on the findings, it is evident that acute supplementation with BA on performance in aerobic-anaerobic transition zones is scarce (11, 14). However, the results presented by Ojeda et al. (11) and the pharmacokinetic responses generated after acute ingestion of BA presented by Stautemas et al. (19), suggest that acute supplementation with BA, 60 min before a MAS test, could be beneficial for physical performance. Despite the background presented, the effect of an acute dosage more significant than $30\text{ mg}\cdot\text{kg}^{-1}$ with this aa in aerobic-anaerobic transition zones is uncertain. Therefore, further investigations covering these variables are required (1, 14, 21). Consequently, the primary purpose of this study was to determine the acute effect of low and high-dose BA trials on MAS in endurance athletes. We hypothesized that high doses of BA have a greater effect than low doses.

Methods and materials

Approach to research

This study included 12 male endurance athletes who volunteered (convenience sample). The inclusion criterion was years of training for endurance events (participants had a minimum of two years running middle-distance and long-distance events). The exclusion criterion was the inability to perform the 6-minute run test (6-MRT). The experimental design applied was randomized cross-over and

double-blind to investigate the effect of acute supplementation with BA on performance in a MAS test. The treatments were low-dose ($30\text{ mg}\cdot\text{kg}^{-1}$) and high-dose ($45\text{ mg}\cdot\text{kg}^{-1}$) BA, contrasted with a baseline. Both the baseline and the applied treatments were 72 hours apart.

Participants

Twelve male endurance athletes volunteered to participate in this study. All participants were informed of the aim of the research and the possible risks of the experiment. All endurance athletes signed the informed consent before the application of the protocol. The study protocol and the informed consent were approved by the Scientific-Ethical Committee of the Universidad de Las Américas, Chile (CEC_PI_2020005) and developed under the ethical standards for exercise and sport sciences (22). This study was registered at ClinicalTrials.gov Identifier: NCT05096793.

Instruments

For the characterization of the sample, it was evaluated: height (evaluation performed with a stadiometer from the feet to the vertex [Frankford plane]), weight, and body fat percentage. These last variables were evaluated using a Tanita Inner Scan BC-554[®] digital scale. Participants were barefoot and wearing shorts and a light T-shirt. Finally, the body mass index (BMI) was calculated by dividing body mass (kg) by height squared (m^2).

The 12-minute run test (12-MRT) was used to evaluate VO_2 max and thus complete the characterization of the participants. This test was carried out on a 400-meter athletic track. The distance achieved in meters was converted into kilometers, and then the VO_2 max was obtained through the following equation (23):

$$\text{VO}_2\text{max} \left(\text{mLO}_2 \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \right) = \\ (22.351 \times \text{distance in kilometers} - 11.288)$$

Standardized warm-up

For the 12-MRT and the three runs of the 6-MRT, the standardized warm-up consisted of 10 min of jogging at $10\text{ km}\cdot\text{h}$. This was followed by five min of lower extremity ballistic movements. Finally, runners performed three 80-meter (m) accelerations.

6-Minute race test

The purpose of the 6-MRT is to determine, indirectly, the MAS in endurance runners (24). This test consists of running the longest possible distance in six min. The test was performed on an official 400 m athletic track simultaneously every measurement day (09:00–11:00 a.m.) and under similar climatic conditions (temperature = $16\text{--}18^\circ\text{C}$ and relative

humidity = 70–80%). Also, to simulate the reality of the competition, the 6-MRT was performed in subgroups of three participants. These subgroups were maintained throughout the intervention, modifying only the dose of BA ingested.

For the 6-MRT, the main performance parameter is each participant's distance (m) in the 360 seconds (s) that the test lasts (25). In this research, the distance control was individualized, while for precise distance control, the track was marked every 10 m. At the end of the six-min run, for both the low and high-dose BA trial (30 and 45 mg·kg⁻¹ BA intervention), the distance covered in m, the capillary lactate ([La]) concentrations produced (mmol·L⁻¹), and the end and recovery heart rate (HR) were recorded at min 1, 3, 5, 7 and 9 (11).

Lactate and HR evaluation

A lactometer (h/p/cosmos®) was used to measure capillary [La]. This lactometer generates an enzymatic-amperometric detection of lactate with an accuracy of ± 3% (minimum standard deviation of 0.2 mmol·L⁻¹), sample volume 0.2 µL, and with a measurement range of 0.5–25.0 mmol·L⁻¹. A heart rate monitor (Polar H10®) was used to measure HR, while the data was stored using the Polar Beat® application.

Nutritional timing

The nutritional timing consisted of a carbohydrate load before the execution of the 12-MRT and 6-MRT. For this, all participants were available two hours before the 12-MRT and 6-MRT under fasting conditions. The nutritional timing consisted of 2 g of rapidly absorbed carbohydrate per kg body weight (2 g·kg⁻¹), 60 min before the tests. This nutritional timing was intended to avoid paresthesia and ensure an optimal carbohydrate load for the participants' physical performance (11, 14, 26).

Experimental protocol and BA supplementation

All the evaluations of the experiment were carried out in one week. Before applying the experiment, all participants were asked not to ingest caffeine, energy supplements, or any substance that would increase metabolism during the entire intervention. Before the intervention, participants signed the informed consent, performed the anthropometric assessments and the 12-MRT (VO₂ max). On day 1, all participants completed the 6-MRT corresponding to baseline. Then, on days 2 and 3, each participant performed the 6-MRT with 30 mg·kg⁻¹ and 45 mg·kg⁻¹ of BA (low and high-dose trials, respectively). BA was purchased in powder format from a factory specializing in sports supplements. BA was colorless when diluted in water and had a characteristic taste. This format of BA (powder) allowed personalized dosing for each participant. The research team performed the personalized dosing before the application of the treatment. Between the evaluation days, there was 72 hours difference. The 30 mg·kg⁻¹ or 45 mg·kg⁻¹ BA administration was done with a double-blind method. Thus, on

day 2, 50% of the sample performed the 6-MRT supplemented with 30 mg·kg⁻¹ BA, while the other 50% performed the 6-MRT with 45 mg·kg⁻¹ BA. On day 3, those participants who completed the 6-MRT with 30 mg·kg⁻¹ BA now performed with 45 mg·kg⁻¹ BA and vice versa (Figure 1A and B).

Sixty min before the 6-MRT, supplementation with BA was performed. This supplementation had two dosages: low-dose participants ingested 30 mg·kg⁻¹ of BA, and high-dose participants ingested 45 mg·kg⁻¹ of BA. In both cases, BA was dissolved in 500 mL of distilled water. Thirty min after this BA intake, standardized warming was performed (Figure 1B).

Statistical analysis

The normal distribution of the results was determined with the Shapiro-Wilk test. Descriptive statistics were used to express individual values, means, and standard deviations (SD) of the variables studied. Differences between physical performance evidenced by baseline 6-MRT (m) and supplementation with 30 mg·kg⁻¹ BA and 45 mg·kg⁻¹ BA was performed using an ANOVA. For this analysis, effect size (ES) was performed using the Partial Eta Squared test. Post hoc analysis was performed using Tukey's multiple comparison test ($p < 0.05$).

Comparison of resting, term, and post-exertion [La] and HR were performed using a Student's t-test for related samples (27). The ES for all analyses was calculated through Cohen's d test. The latter analysis considers an insignificant ($d < 0.2$), small ($d = 0.2$ to 0.6), moderate ($d = 0.6$ to 1.2), large ($d = 1.2$ to 2.0), or very large ($d > 2.0$) effect. The SPSS version 19® statistical software, the Excel 2013® software, and a customized spreadsheet were used for tabulation and data analysis (28). The significance level for all statistical analyses was $p < 0.05$.

Results

The participants had the following characteristics: age: 21.8 ± 2.37 years, weight: 69.8 ± 4.36 kg, height: 174 ± 5.45 cm, BMI: 22.8 ± 1.63 kg·m², body fat percentage: $9.3 \pm 2.62\%$, VO₂ max: 59.6 ± 3.77 mL O₂·kg⁻¹·min⁻¹.

After the ANOVA test was applied, it was evident that both groups supplemented with BA significantly increased the distance in the 6-MRT compared to baseline ($p < 0.001$, ES = 0.68). Post hoc analysis showed significant differences between baseline and both doses of BA ($p < 0.01$). However, this same analysis did not show significant differences between both doses ($p > 0.05$). Progressions and changes are reported in Figure 2 and Table 1.

11 of the 12 cases studied showed increases in 6-MRT after 30 and 45 mg·kg⁻¹ BA supplementation in the individual analysis. Only one participant showed decreases in physical performance with both dosages of BA. The individual changes are reported in Table 2.

On the other hand, resting [La] showed no differences between low and high-dose BA trials ($p = 0.428$; ES = 0.43). However, resting HR evidenced significant differences between low and high-dose BA trials at rest ($p = 0.01$; ES = 0.90). At the end of 6-MRT, term and post-exertion (min

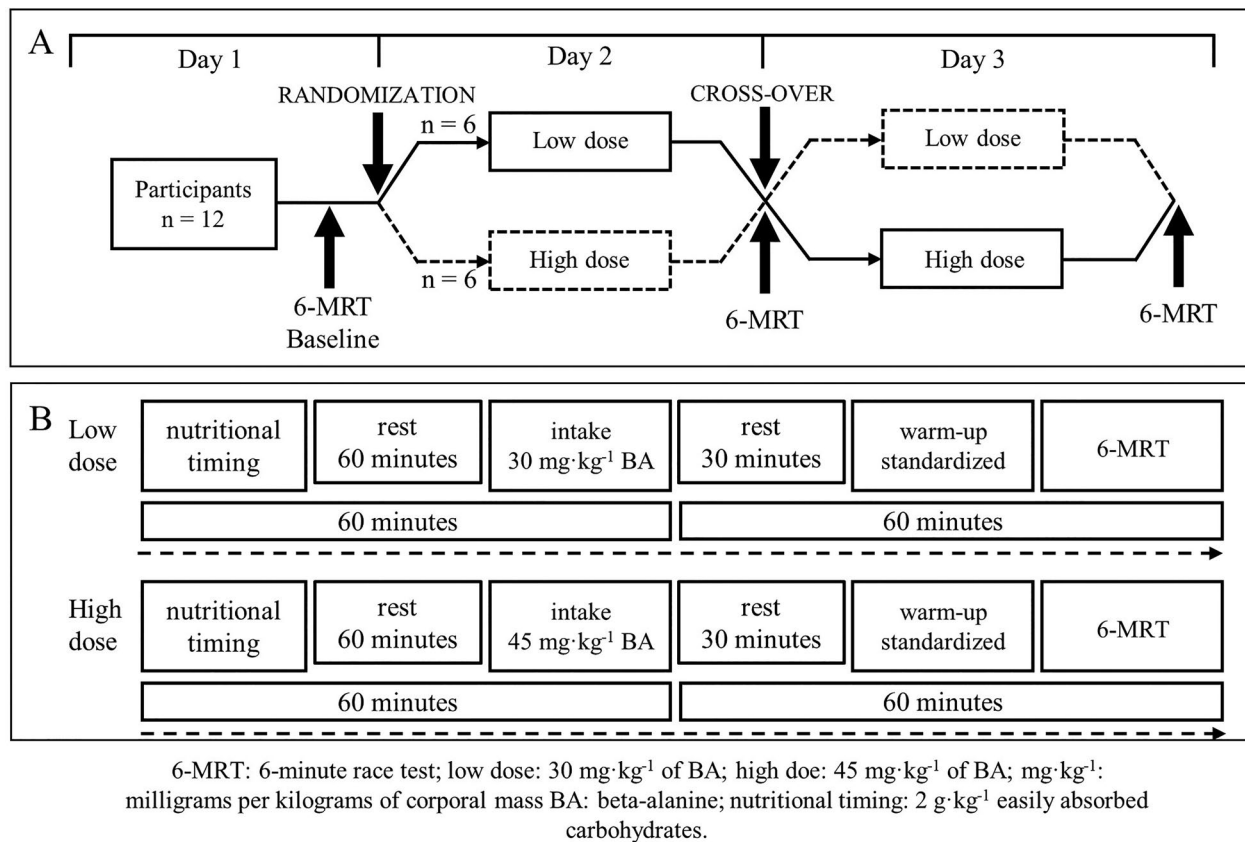


Figure 1. Research design.

Table 1. Performance in 6-MRT with Low and High-Dose BA Trials.

	BL (m) mean ± SD	Low-dose (m) mean ± SD	High-dose (m) mean ± SD	<i>p</i>	ES
ANOVA	1,828.3 ± 75.7	1,856.9 ± 66.6	1,861.7 ± 65.9	0.001	0.68
<i>post hoc: Tukey's multiple comparisons test</i>					
		Mean diff.	95% CI of diff.	<i>p</i>	
BL vs. low dose		-28.58	-46.41 to -10.76	0.003	
BL vs. high dose		-33.42	-55.31 to -11.52	0.004	
Low dose vs. high dose		-4.833	-25.22 to 15.55	0.801	

6MRT: 6-minute race test; m: meters; BL: baseline; Low-dose: 30 mg·kg⁻¹ of BA; High-dose: 45 mg·kg⁻¹ of BA; SD: standard deviation; ES: effect size; diff.: differences; CI: confidence interval; *p*: statistical significance.

Table 2. Individual Performance Mean Variation Post Supplementation with Beta-Alanine.

Participants	BL		Low dose		High dose		Δ				
	6-MRT (m)	6-MRT (m)	[La] max (mmol·L ⁻¹)	HR (bpm)	6-MRT (m)	[La] max (mmol·L ⁻¹)	HR (bpm)	Δ 6-MRT LD - BL (m)	Δ 6-MRT HD - BL (m)	Δ [La] max LW - HD (mmol·L ⁻¹)	Δ HR max LW - HD (bpm)
1	1,970	1,992	15.9	182	1,974	16.3	185	22	4	-0.4	-3.0
2	1,950	1,938	17.0	187	1,940	15.3	200	-12	-10	1.7	-13.0
3	1,900	1,922	19.2	191	1,952	15.6	193	22	52	3.6	-2.0
4	1,840	1,858	15.9	195	1,890	14.5	194	18	50	1.4	1.0
5	1,800	1,878	16.5	177	1,830	12.6	200	78	30	3.9	-23.0
6	1,800	1,822	16.0	196	1,849	17.5	194	22	49	-1.5	2.0
7	1,800	1,855	11.6	195	1,865	13.7	194	55	65	-2.1	1.0
8	1,830	1,850	14.9	196	1,840	15.8	182	20	10	-0.9	14.0
9	1,800	1,818	17.6	202	1,846	20.6	201	18	46	-3.0	1.0
10	1,770	1,790	14.9	195	1,785	14.6	192	20	15	0.3	3.0
11	1,760	1,790	14.2	202	1,768	15.0	193	30	8	-0.8	9.0
12	1,720	1,770	18.6	194	1,802	15.8	196	50	82	2.8	-2.0

6MRT: 6-minute race test; m: meters; BL: baseline; LD: Low dose (30 mg·kg⁻¹ of beta-alanine); HD: High dose (45 mg·kg⁻¹ of beta-alanine); Δ: variation delta; [La]: lactate concentrations; mmol·L⁻¹: millimoles per liter; HR: heart rate; bpm: beat per minutes.

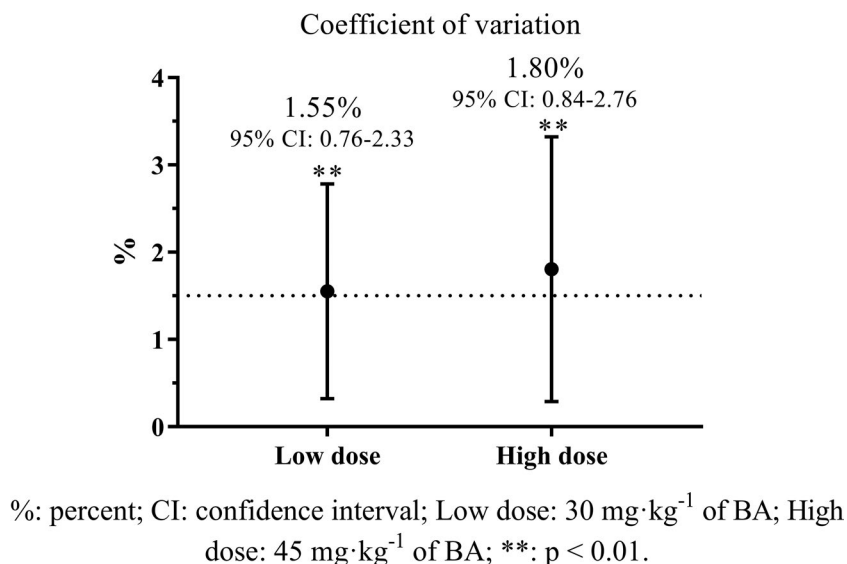
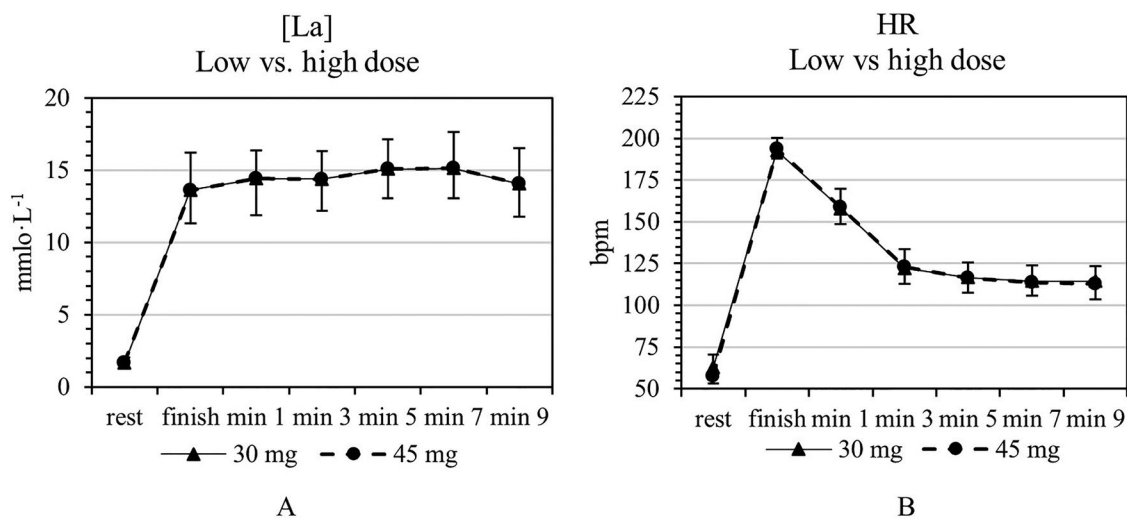


Figure 2. Coefficient of variation between baseline and low and high dose of BA.



[La]: lactate concentrations; Low dose: 30 mg·kg⁻¹ of beta-alanine; High dose: 45 mg·kg⁻¹ of beta-alanine; mmol·L⁻¹: millimoles per liter; HR: heart rate; bpm: beat per minutes.

Figure 3. Lactate concentrations and heart rate post effort.

1, 3, 5, 7, and 9), HR and [La] showed no significant differences between the two conditions (30 mg·kg⁻¹ of BA and 45 mg·kg⁻¹ of BA). The progressions and changes are reported in [Figure 3A and B](#).

Discussion

Concerning the main objective of this study, it was possible to verify that both 30 mg·kg⁻¹ (low-dose) and 45 mg·kg⁻¹ (high-dose) generated increases in the 6-MRT distance when compared to baseline ($p < 0.004$). The first point of analysis is the distance performed in the 6-MRT by the sample. In this regard, the participants performed 1828.3 ± 75.7 m on the baseline. This performance is considered "excellent" (26). Likewise, from the perspective of VO_2 max (59.6 ± 3.77 mL O₂·kg⁻¹·min⁻¹), the participants

showed "excellent" oxygen consumption (23). Based on this background, which demonstrates the participants' fitness level at the beginning of the experiment, it can be assured that the reported changes in the 6-MRT distance for low and high-dose trials were generated by BA intake (4, 11). Indeed, in the study developed by Ojeda et al. (11) the effect of 30 mg·kg⁻¹ of BA on performance in a time limit test at MAS was tested. At the end of the study, the researchers reported significant differences in the time taken in the time limit test between the group supplemented with BA and the placebo ($p = 0.047$; ES = 0.48) (11). In this regard, there is evidence on plasma pharmacokinetic responses after acute supplementation with BA (19). This background supports both the research of Ojeda et al. (11) and the findings reported in the present study. In this context and based on the information reported by Stautemas

et al. (19), both the low and high-dose BA trial protocol used in this study elevated plasma BA concentrations after 50–100 min from ingestion. In addition, the same authors reported a variable response on CA load after acute supplementation with BA (19). These results indicated the effectiveness of acutely supplementing with this aa before testing in aerobic-anaerobic transition zones, associating the increase in physical performance with a possible increase in CA load after acute intake of BA (19). However, variations in BA kinetics and consequent CA synthesis require a personalized analysis (20). Likewise, the same research group concluded the need to explore further the acute effect of BA in this specific intensity zone (14).

Considering the scarce existing background on the acute effect of BA (only one study with 30 mg·kg⁻¹ of BA (11)), in the present investigation, the ES was evaluated with a dose of 45 mg·kg⁻¹ on the distance realized in the 6-MRT, reproducing precisely the protocol described by Ojeda et al. (11). At the end of the study, both administered doses (30 and 45 mg·kg⁻¹ of BA) evidenced significant increases in the distance of the 6-MRT when compared to the baseline ($p < 0.004$). However, the high-dose group (45 mg·kg⁻¹ of BA) evidenced a higher distance in the 6-MRT than the low-dose group (low-dose: 1856.9 ± 66.6 m vs. high-dose: 1861.7 ± 65.9 m). Although there was no significant difference between the two protocols ($p > 0.05$), this difference resulted in a 4.8 m, equivalent to 0.25%. This last figure becomes relevant when analyzing the minimal differences in a footrace's final phase (14). Likewise, these differences, absolute and percentual, observed between low and high-dose BA trials (4.8 m and 0.25%) could be justified by increasing intramyocellular CA after BA intake (6, 19). Indeed, the pKa of 6.83 possessed by CA generates an effective physicochemical buffer to regulate pH during exercise (29). Expressly, the nitrogen atoms in the imidazole ring of the CA structure can readily accept a proton (H⁺) at physiological pH and thus actively participate in the buffer system during exercise (30). Despite the evidence supporting our results (19), the only way to verify the increase in intramyocellular CA after an acute dose of BA is through a muscle biopsy (31). However, this is an invasive and expensive technique, with conditions practically impossible to perform. In parallel, recent studies have shown an increase in the reliability of plasma CA evaluations (32). However, the results of these plasma analyses do not support any variation in intramyocellular CA concentrations either. This last point requires further exploration.

Despite the slight differences found between low and high-dose BA trials, we suggest a 45 mg·kg⁻¹ of BA dose to increase performance in aerobic-anaerobic transition zones. This, since a 0.25% can generate a significant difference in sports performance in endurance athletes (e.g., a performance of 4 min 20 s in 1,500 m run could improve 0.45 s with the intake of 45 mg·kg⁻¹ of BA 60 min before the race). However, individual analysis is needed to determine whether BA supplementation can improve training and competition performance (4).

Among the findings obtained in the present study, there were no significant differences in term and post-exertion [La] between low and high-dose BA trails ($p > 0.05$). However, some participants exceeded 20 mmol·L⁻¹ lactate

post-exertion (Table 1), whereas the mean values were 16.0 ± 2.04 and 15.6 ± 2.01 for EG1 and EG2, respectively. Indeed, evidence has shown an increase in post-exertion [La] following acute supplementation with BA (11). This increase in post-exertion [La] may be associated with the buffering effect generated by this aa and the consequent synthesis of intramyocellular CA (31, 33). Thus, CA would prevent enzymatic inhibition of glycolytic processes by counteracting the intramyocellular accumulation of H⁺ (34). Consequently, it may increase [La] during high-intensity exercise. There were no significant differences in term and post-exertion HR between low and high-dose BA trials ($p > 0.05$). Only resting HR showed substantial differences between low and high-dose BA trials ($p = 0.01$). Despite this, some participants exceeded 200 beats per min (bpm) at the end of 6-MRT (these values were higher than the theoretical maximal HRs of some participants: 206 – 0.7 × age (35)), while the mean values were 192.7 ± 7.44 and 193.7 ± 5.68 for low and high-dose BA trials, respectively. Concerning these values, Ojeda et al. (11) reported higher term HR in participants supplemented with 30 mg·kg⁻¹ of BA compared to the placebo group ($p > 0.05$). In this sense, because CA is present in other excitable tissues such as the brain and heart, it likely has additional physiological functions (33), including promoting an increase in HR. However, the latter point requires further exploration. Likewise, an important limitation was the absence of HR and [La] control in the 6-MRT on day 1, corresponding to the baseline. For this reason, the analysis of these variables was only between both experimental treatments (low-dose vs. high-dose).

Finally, our interest in investigating the effects of acute BA supplementation protocols in aerobic-anaerobic transition zones is based on establishing a safe dose of BA to improve performance during training and competition. Likewise, this supplementation format avoids exposure to weeks of prolonged BA use, thus decreasing the risk of exposure to paresthesia symptoms generated at each intake (36).

Conclusion

In conclusion, our findings indicate that both low and high-dose BA trials (30 mg·kg⁻¹ and 45 mg·kg⁻¹ of BA), 60 min before a maximal test at MAS, increase physical performance in endurance athletes. Also, although there were no significant differences between treatments, supplementation with 45 mg·kg⁻¹ of BA showed a more substantial effect than 30 mg·kg⁻¹ of BA. Therefore, the dosages and intake formats described in the present investigation may be helpful for endurance athletes training and competing in aerobic-anaerobic transition zones. These antecedents support our study hypothesis.

Practical applications

Endurance coaches and athletes seeking an ergogenic aid to improve physical performance in aerobic-anaerobic transition zones should consider acute and chronic supplementation with BA. However, based on our findings, we suggest a dose

of 30-45 mg·kg⁻¹ of BA. However, coaches and athletes should consult qualified professionals on the most appropriate dosage formats for each reality. Likewise, it is advisable to observe the individual responses of each athlete to determine the actual effect of BA supplementation (Table 2) (4). In this sense, the objective of any sports supplement is to optimize performance. Athletes require a correct and perhaps personalized dosage for inducing a homogeneous pharmacokinetic and ergogenic response (20). Finally, to avoid the presence of paresthesia after BA supplementation, we suggest ingesting 2 g·kg⁻¹ of rapidly absorbed carbohydrates 60-90 min before ingesting BA (11, 14, 26).

Author contributions

Álvaro Huerta Ojeda, Guillermo Barahona-Fuentes, and Sergio Galdames Maliqueo: conception, methodology, investigation, data curation, writing—original draft preparation, writing—review and editing. Marcela Guzmán Solís: methodology and investigation. María Mercedes Yeomans Cabrera: visualization and writing—review and editing. Carlos Jorquera-Aguilera: supervision and project administration. All authors have read and agreed to the published version of the manuscript.

Disclosure statement

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