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High-dose beta-alanine supplementation for two weeks did not enhance intermittent endurance or sprint performance in trained futsal players

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ABSTRACT

Background: This study aimed to investigate the effects of a high-dose beta-alanine (BA) supplementation on physical performance, blood lactate concentration, and ratings of perceived exertion (RPE) in trained futsal players.

Methods: Sixteen trained futsal players participated in a randomized, parallel, double-blind experiment, completing a two-week supplementation period with either 12 g/day of BA ($n = 8$; age: 19 ± 2 years; height: 1.78 ± 0.03 m; body mass: 68.5 ± 5.4 kg) or a placebo (PLA) ($n = 8$; age: 18 ± 1 years; height: 1.74 ± 0.08 m; body mass: 65.6 ± 6.4 kg). Athletes completed the futsal intermittent endurance test (FIET) and a 30-m speed test before and after supplementation. Plasma lactate levels were measured before, immediately after, and three minutes post-FIET, while RPE was assessed during each FIET stage.

Results: There was a significant main effect of time for distance covered in the FIET (Pre-BA: 1618.13 ± 268.14 m; Post-BA: 1857.50 ± 277.81 m; Pre-PLA: 1519.13 ± 243.19 m; Post-PLA: 1621.88 ± 323.65 m; $p = 0.003$; $\eta p^2 = 0.73$), while no significant interaction effect was revealed for FIET ($p = 0.147$; $\eta p^2 = 0.27$). Furthermore, no significant interaction effects were found in the 30-m sprint test ($p = 0.149$; $\eta p^2 = 0.27$, Pre-BA: 4.33 ± 0.25 s; Post-BA: 4.22 ± 0.124 s; Pre-PLA: 4.33 ± 0.26 s; Post-PLA: 4.37 ± 0.22 s). Neither plasma lactate levels nor RPE showed significant main effects of treatment or timepoint (all $p > 0.5$). Conclusion: Two weeks of high-dose BA supplementation did not have an ergogenic impact on the distance covered during the intermittent endurance test, sprint performance, blood lactate, or RPE in trained futsal players.

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Exercise Performance; Beta-Alanine supplementation; Sprint test; Futsal intermittent endurance test

1. Introduction

Futsal is a high-intensity indoor sport demanding repeated sprint efforts and rapid recovery, making it physiologically taxing [1]. The game is on a reduced playing surface (approximately 40 × 20 m) and is characterized by repetitive high-intensity bursts, including accelerations, decelerations, and cutting movements, interspersed with brief recovery periods [2]. Matches consist of two 20-min halves, with a 10-min break [2]. Unlimited substitutions enable players to participate in high-intensity activities for 3–6 min before being replaced [3]. Consequently, the physiological demands of the sport result in sustained high heart rates and anaerobic stress [4]. Studies indicate that professional futsal players spend between 5% and 12% of their game time sprinting and running at high intensities (ie speeds exceeding 15 km/h) while also reaching 90% of their maximal heart rate (HR_{max}) and 75% of their maximal oxygen uptake (VO_{2max}) [3]. Thus, high aerobic fitness (above 60 ml. kg^{-1}. min^{-1}) and a developed anaerobic pathway are crucial for excelling in this team sport [5].

Intramuscular hydrogen ions (H^+) accumulation has been associated with fatigue development and physical performance decrement [6]. Specifically, overproduction of H^+ leads to a decline in intramuscular pH, potentially causing fatigue during high-intensity and sprinting efforts in team sports [7]. Thus, implementing nutritional strategies designed to increase intracellular buffering

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capacity may attenuate potential declines in physical performance among futsal players while optimizing on-court futsal demands. Beta-Alanine (BA) is a non-proteogenic amino acid that, after ingestion, combines with L-histidine within skeletal muscle and other organs to form carnosine [8]. Carnosine (β-alanyl-L-histidine) is a dipeptide naturally found in significant amounts in skeletal muscle [9], with a pKa of 6.83. It is an effective buffer during exercise-induced intramuscular pH changes [9]. Supplementation with BA has been shown to elevate muscle carnosine levels, potentially enhancing exercise performance and capacity by mitigating the accumulation of H^+ [10]. Saunders et al. (2017) meta-analysis showed that BA supplementation is an effective nutritional strategy for increasing high-intensity efforts between 30 s and 10 min [11].

To increase muscle carnosine levels induced by BA supplementation and exercise performance benefits, a daily dose of 4.6 to 6.4 g of BA is suggested over 4–8 weeks [12]. Interestingly, a study by Church et al. (2017) investigated the potential of increasing carnosine levels in a shorter time than the traditional supplementation period [13]. They compared a regimen of 12 g of BA daily for two weeks with 6 g daily for four weeks. The results showed that both supplementation groups experienced a significant increase in muscle carnosine levels (around 50%), with no notable difference between the two doses. This indicates that short-term, high-dose BA supplementation (12 g/day) can accelerate muscle carnosine synthesis [13]. However, whether this strategy can enhance performance or merely prevent its decline remains unclear.

Recent studies have shown that one week of BA supplementation with a dosage of approximately 20–22 g daily attenuated the decrease in a ten-minute cycling test and improved uphill cycling performance in world tour cyclists during the training camp period [14,15]. These studies revealed that the amount of BA supplementation is a key factor in enhancing performance rather than the timing of its intake. Thus, short-term, high-dose BA supplementation (12 g daily) may be an effective strategy for athletes who lack sufficient time to raise their muscle carnosine levels through traditional BA supplementation or who are engaged in a high-load weekly training regimen and seeking to prevent performance decline. Despite promising findings in cyclists, it remains unclear whether short-term, high-dose BA benefits team-sport athletes. To our knowledge, there has been no research on the effectiveness of a short-term, high-dose BA supplementation strategy among futsal players, a sport that involves high-intensity interval efforts. Therefore, the present study aimed to investigate the effects of a two-week supplementation protocol using a daily dose of 12 g of BA on physical performance, blood lactate levels, and perceived exertion in trained futsal players.

2. Methods

2.1. Participants

Twenty trained futsal players showed interest in participating in the study and were randomly assigned to the BA or PLA group using a computer-generated randomization list. Sixteen trained futsal players were included in the current study, whose descriptive characteristics are presented in Table 1. They had not used BA for the previous six months before their participation. Participants were also restricted to using buffering agents, such as sodium bicarbonate and sodium citrate, throughout the study. Goalkeepers were also excluded due to their different movement patterns. Participants gave their informed written consent to participate, and the University of Tehran Ethics Committee approved the study using the latest version of the Declaration of Helsinki (Approval ID: IR.UT.SPORT.REC.1403.134)

2.2. Experimental design

An a priori sample size calculation using G*Power (version 3.1.9.2; University of Dusseldorf; Dusseldorf, Germany) indicated that 12 players were needed to obtain statistically significant differences in FIET as the

Table 1. Y = years; *m* = meters; kg = kilograms; BMI = body mass index; PLA = placebo; BA = beta-alanine. All data are reported as mean ± SD.

Group	N	Age (y)	Height (m)	Body mass (kg)	BMI	Training experience (y)	FIET performance (m)
BA	8	19 ± 2	1.78 ± 0.03	68.5 ± 5.4	21.4 ±1.8	6 ± 2	1618 ± 268
PLA	8	18 ± 1	1.74 ± 0.08	65.6 ± 6.4	21.5±1.7	4 ± 2	1519 ±243

primary dependent variable of the study using an effect size of 0.50, β = 0.80, and two-tailed α = 0.05. A randomized, parallel, double-blind experiment was conducted in blocks with equal groups according to the distance covered in the FIET in the familiarization session. Participants performed the same experimental session twice, 2 weeks apart. The trials were accomplished before (Pre) and after 2 weeks of supplementation (Post). In addition, no competition was scheduled 14 days before and after the experimental trials. Participants' performance was assessed in each trial using a 30-m sprint test (Sprint Test) and the FIET test. In addition, the perceived exertion scale (RPE) in each stage of FIET and plasma lactate concentration during baseline, immediately after, and 3 min post-FIET were recorded. All experimental sessions were performed simultaneously (ie 4:00 pm) to avoid the possible effects of circadian rhythms on physical performance [16]. Participants were instructed to abstain from consuming any caffeine and alcohol and not to perform any vigorous physical activity 24 hours before the testing. A graphical representation of the study design is illustrated in Figure 1.

2.3. Supplementation protocol

For two weeks, participants were supplemented with either 12 g per day of BA (Bulk, Nashville, United States) or a PLA (ie glucose; Iran Dextrose, Tehran, Iran; irandextrose.com). The supplementation protocol involved four daily doses of 3 g each, taken immediately after meals (ie breakfast, lunch, afternoon snack, and dinner). To ensure blinding, both powders were flavored with orange powder. Previous studies have indicated that a daily intake of 12 g of BA, divided into four doses over two weeks, can effectively increase skeletal muscle carnosine content [13]. Participants were instructed to record each dose in a designated logbook, and compliance was monitored by comparing the total amount dispensed with the quantity remaining at the end of the study. The success of the blinding procedure was evaluated by asking participants to identify the supplement they believed they had received. Additionally, subjective symptoms of paresthesia were recorded on four occasions: before the first intake of the product (Baseline), after the first day of intake, after day 7 of intake, and after the final day of intake. The questionnaire consisted of a quantitative question about intensity feelings, a qualitative question about the type of sensory sensation, and an empty box to report any other perceived sensation of side effect [14].

2.4. Diet and training load

Each participant completed a recorded food regimen two days before each testing session. This allowed them to review their nutrition and attempt to replicate it as closely as possible for each trial. All recorded dietary information was analyzed using a nutritional tracking application (http://www.myfitnesspal.com).

Figure 1. Graphical representation of the study design. RPE: rating of perceived exertion.

Additionally, all participants followed a standardized futsal training regimen designed by the team coaches and supervised by research staff. The training load during this period was calculated by multiplying the duration of training sessions by the perceived exertion (Duration × RPE) [17].

2.5. Futsal intermittent endurance test

According to Barbero-Alvarez et al. (2005), FIET is a test designed to assess the intermittent endurance of futsal players [18]. The test involves running a shuttle over a distance of 45 m (3 × 15 m) at progressively increasing speeds, as dictated by prerecorded audio cues until the participant reaches exhaustion. During the test, participants are required to take an active rest break after every 45 min. Furthermore, after completing each set of 8 × 45 m (except for the first level of 9 × 45 m), they must take a passive rest for 30 s before continuing with the remaining part of the test. The speed increases by 0.33 km/h after the first 45 m during the initial level and then by 0.2 km/h for each subsequent 45 m. The starting speed is set at 9 km/h [18].

2.6. Speed testing

In the 30-m sprint test, athletes ran at maximal speed for 30 m in a straight line, and the time needed to cover the distance was measured. Participants performed three 30-m sprint trials, standing with their forward foot 0.5 m behind the 0-m timing gates, with at least 3-min rest between each test. Their best performance was recorded. Participants performed the 30-m sprint test 5 min after completing the warm-up. The time was recorded for 30 m to the nearest millisecond and assessed using a mobile device (iPad Pro 5th Gen 2017, Model A1701/A1709, Apple Inc.) and validated Runmatic App (v3.2.1, Runmatic LLC) [19].

2.7. Lactate measurement and rating of perceived exertion recording

Finger-prick blood lactate samples were measured before (baseline), immediately after, and 3 min post-FIET using a lactometer (Lactate Plus, Nova Biomedical, Waltham, MA, USA) [20]. Participants were asked immediately after completing each stage of the FIET test to provide the rate of perceived effort (ie 6 to 20 RPE scale of Borg) [21].

2.8. Statistical analysis

Data are presented as mean ± SD. The Shapiro–Wilk test revealed that the data were normally distributed. Independent T-tests were used to compare training load, blinding, complaints of paresthesia, and compliance percentage between groups during the supplementation period. Data of FIET, the Sprint test, and dietary intakes were analyzed using a 2 × 2 [treatment (BA vs. PL) × timepoint (Pre vs Post)] repeated measures analysis of variance (ANOVA) to detect the difference between treatment, timepoint (from Pre to Post supplementation), and the interaction between treatment × timepoint. A three-way repeated-measures ANOVA was conducted to analyze lactate concentration and perceived exertion (RPE) rating. The model measured the effect of treatment (BA vs. PL), timepoint (Pre vs. Post), and time (with three levels for lactate: Baseline, Post-FIET, 3 min post-FIET, or six levels for RPE during FIET). Bonferroni-adjusted post-hoc analyses were performed when significant differences occurred. Generalized partial eta squared (ηp^2) and Hedge's (g) values were reported to provide an estimate of standardized effect size (small $d = 0.2$, $\eta p^2 = 0.01$; moderate $d = 0.5$, $\eta p^2 = 0.06$; and large $d = 0.8$, $\eta p^2 = 0.14$) values were used as reference. All analyses were conducted using SPSS software (IBM® SPSS® Statistics v 27.0.1, Chicago, United States), and effect sizes were calculated as previously recommended [22]. Figures were developed using GraphPad Prism software (version 8.0.1, GraphPad Software, San Diego, United States). The statistical significance was set at $p < 0.05$.

3. Results

3.1. Diet and training load

Analysis of the training load revealed no significant difference (p = 0.176) in the training loads completed by each group throughout the testing period (BA: 1241 ± 59.518 arbitrary units; PLA: 1176 ± 115.65 arbitrary units). In addition, no significant interactions were reported among the groups for total energy, protein, carbohydrate, and fat intakes (Table 2)

3.2. Futsal intermittent endurance test

There was a significant main effect of timepoint for the distance covered in the FIET (Pre-BA: 1618.13 ± 268.14, Post-BA: 1857.50 ± 277.81, CV : 7.7%; Pre-PLA: 1519.13 ± 243.19, Post-PLA: 1621.88 ± 323.65, CV : 9.95%; p = 0.003; $\eta p^2 = 0.73$), while no significant main effect of treatment was reported ($p = 0.262$; $\eta p^2 = 0.17$). In addition, no significant interaction effect of treatment × timepoint was revealed ($p = 0.147$; ηp^2: 0.27) (Figure 2).

3.3. Lactate response

There was a significant main effect of time for plasma lactate, indicating lactate significantly increased from baseline to after and 3 min after FIET ($p = 0.001$; $\eta p^2 = 0.98$) (Figure 3). Plasma lactate values showed substantial increases across all conditions: (Pre-PLA baseline: 2.91 ± 0.94 mmol/L, Post-PLA baseline: 4.45 ± 1.68 mmol/L, CV: 35%; Pre-PLA after FIET: 12.86 ± 4.74 mmol/L, Post-PLA after FIET: 12.40 ± 1.67 mmol/L, CV: 19.04%; Pre-PLA 3-min after FIET: 11.43 ± 4.14 mmol/L, Post-PLA 3-min after FIET: 11.54 ± 3.05 mmol/L, CV : 13.12%, Pre-BA baseline: 4.88 ± 1.39 mmol/L, Post-BA baseline: 4.25 ± 2.14 mmol/L, CV : 31.52%, Pre-BA after FIET: 14.14 ± 5.5 mmol/L, Post-BA after FIET: 14 ± 4.33 mmol/L, CV : 13.50%, Pre-BA 3-min after FIET: 9.85 ± 3.07 mmol/L, Post-BA after 3-min FIET: 12.61 ± 3.91 mmol/L, CV : 30.81%). However, no main effect of treatment ($p = 0.507$; $\eta p^2 = 0.07$) or timepoint ($p = 0.307$; $\eta p^2 = 0.15$) was reported for plasma lactate. In addition, no significant interaction of treatment × time ($p = 0.851$; $\eta p^2 = 0.05$), treatment × timepoint ($p = 0.819$; $\eta p^2 = 0.08$), time × timepoint ($p = 0.659$; $\eta p^2 = 0.13$) and treatment × time × timepoint ($p = 0.195$; $\eta p^2 = 0.42$) was revealed for plasma lactate.

Table 2. Average daily nutrient intake before (Pre) and following (Post) supplement ingestion.

Variables	Pre-BA	Post-BA	Pre-PLA	Post-PLA	Interaction p value
Energy (Kcal)	1431 ± 376	1449 ± 370	1735 ± 381	2072 ± 980	.234
Carbohydrate (g)	170 ± 70	164 ± 53	227 ± 60	252 ± 110	.495
Carbohydrate (g/kg)	2.41 ± 1.12	2.32 ± 0.93	3.54 ± 0.99	3.91 ± 1.94	.423
Protein (g)	69.21 ± 18.24	66.54 ± 17.18	78.23 ± 17.23	86.23 ± 28.23	.375
Protein (g/kg)	0.98 ± 0.25	1.13 ± 0.61	1.25 ± 0.33	1.51 ± 0.63	.582
Lipid (g)	52.34 ± 15.11	58.43 ± 15.21	56.23 ± 19.23	80.12 ± 30.15	.251
Lipid (g/kg)	0.76 ± 0.24	0.84 ± 0.25	0.85 ± 0.26	1.61 ± 2.43	.062

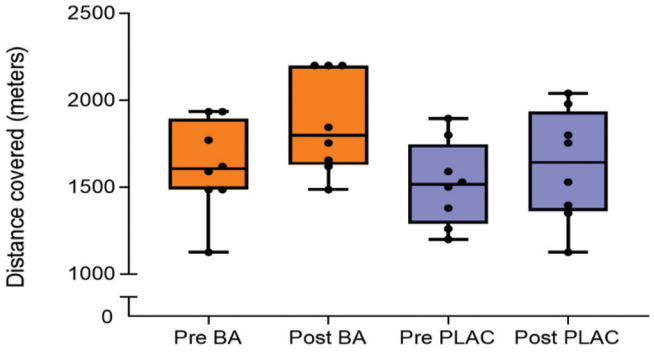

Figure 2. Change in distance covered in the FIET test of beta-alanine (ie orange color) and placebo (ie blue color) before (Pre) and after (Post) supplementation period.

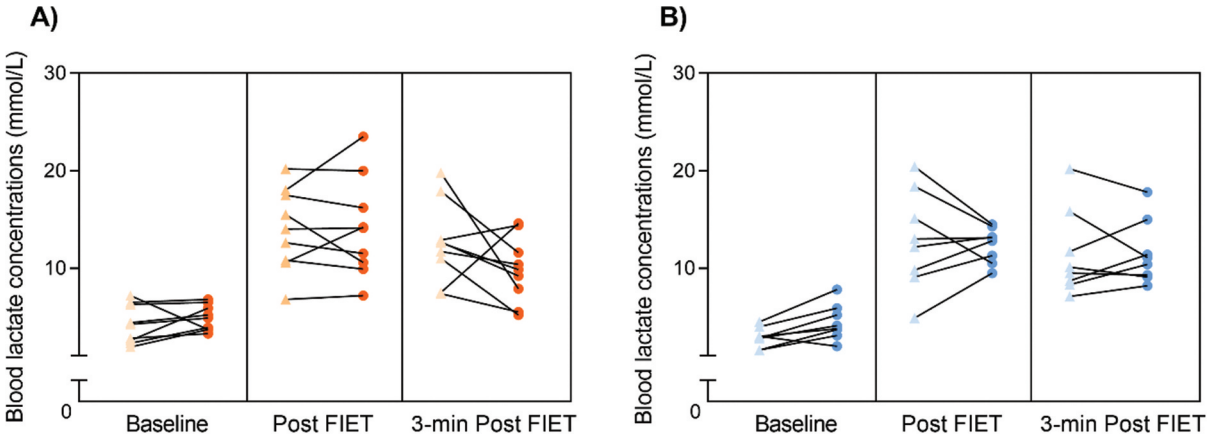

Figure 3. Changes in blood lactate at A) baseline, post FIET, and 3 min post-FIET before (triangle) and after (circle) with beta-alanine supplementation period A). * B) baseline, post FIET, and 3 min post-FIET before (triangle), after (circle) with placebo supplementation period.

3.4. Rating of perceived exertion

There was a significant main effect of time on ratings of perceived exertion (RPE), indicating that RPE increased significantly from level 1 to level 6 ($p = 0.001$; $\eta p^2 = 0.99$). However, no main effect of treatment ($p = 0.450$; $\eta p^2 = 0.08$) or timepoint ($p = 0.560$; $\eta p^2 = 0.05$) was found. In addition, no significant interactions for treatment × time ($p = 0.479$; $\eta p^2 = 0.36$), treatment × timepoint ($p = 0.373$; $\eta p^2 = 0.11$), time × timepoint ($p = 0.470$; $\eta p^2 = 0.37$), or treatment × time × timepoint ($p = 0.897$; $\eta p^2 = 0.1$) were observed (Table 3).

3.5. Speed test

There was no significant main effect of timepoint for the 30-m sprint test, suggesting no significant change from the initial to the final test (Pre-BA: 4.33 ± 0.25 s, Post-BA 4.22 ± 0.124 s, CV : 4.43%; Pre-PLA: 4.33 ± 0.26 s, Post-PLA: 4.37 ± 0.22 s, CV : 1.91%; $p = 0.442$; $\eta p^2 = 0.08$) (Figure 4). No significant main effect of treatment for

Table 3. Rate of perceived exertion (RPE) scale during the FIET test before (Pre) and after (Post) supplementation period. For both conditions (BA and PLA), along with the coefficient of variation (CV%).

Variable RPE	Pre-BA (n)	Post-BA (n)	CV%	Pre-PLA (n)	Post-PLA (n)	CV%
Level 1	9 ± 2.07 (8)	8.62 ± 2.13 (8)	12.91%	8.50 ± 2.56 (8)	8.88 ± 0.83 (8)	14.13%
Level 2	12.50 ± 2.33 (8)	11.63 ± 1.78 (8)	13.01%	12.13 ± 2.53 (8)	12.25 ± 1.03 (8)	10.86%
Level 3	15.63 ± 2.06 (8)	14.63 ± 1.76 (8)	8.21%	16.38 ± 2.44 (8)	16.13 ± 1.95 (8)	6.87%
Level 4	17.83 ± 1.83 (7)	17.17 ± 1.47 (8)	5.37%	17.8 ± 1.64 (8)	18 ± 1.87 (7)	8.71%
Level 5	19 ±1 (3)	19.14 + 0.89 (7)	4.89%	19.2 ± 0.97(5)	19 ± 0.70 (5)	3.66%
Level 6	20 (2)	19.8 + 0.44 (4)	0 %	20 (1)	20 (2)	0 %

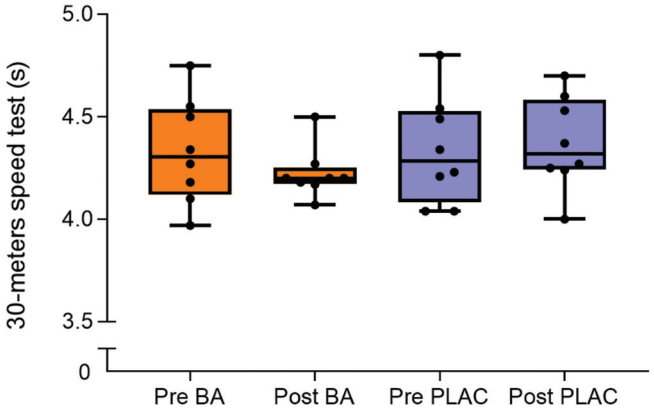

Figure 4. Change in time of the 30-m sprint test of beta-alanine and placebo before (Pre) and after (Post) supplementation period.

30-m was reported ($p = 0.654$; ηp^2: 0.03). In addition, no interaction effect timepoint × treatment was observed in the 30-m sprint test ($p = 0.149$; ηp^2: 0.27).

3.6. Blinding, compliance, and side effects

Four participants were excluded from the study: two for personal reasons (one from each group) and two due to gastrointestinal discomfort (one from the BA group and one from the PLA group). A total of sixteen participants completed the study and were included in the final analysis. Of the sixteen participants, two participants reported missing the BA powder during two meals, while the remaining participants fully complied with the protocol, with no difference between groups ($p = 0.149$). In the BA group, three participants experienced mild paresthesia only on the first day of consumption, while no participants in either group reported any paresthesia thereafter (no difference between groups; $p = 0.060$). Additionally, five out of eight participants (62.5%) correctly predicted receiving a PLA, while four accurately identified that they were receiving BA (50%). One participant from the BA group and one from the PLA group reported being unsure of what they had ingested (no difference between groups; $p = 0.642$).

4. Discussion

BA is recognized as a beneficial ergogenic aid for enhancing cardiovascular [23,24] and neuromuscular performance in team sports [25,26]. However, to our knowledge, no previous studies have examined the effects of high-dose BA consumption on intermittent endurance, associated metabolic and perceptual responses, or sprint performance in trained futsal players. Thus, this study aimed to assess the impact of a high-dose BA ingestion on futsal-physical performance testing. Based on our findings, BA supplementation at a dose of 12 g per day did not have an ergogenic impact on the total distance covered during the FIET test, sprint performance, blood lactate concentrations, or RPE; however, a main effect of time was observed for FIET performance. These results suggest that high-dose BA supplementation is unlikely to be an effective ergogenic strategy for enhancing endurance performance in futsal players when administered over a short-term period (ie less than 4 weeks).

In futsal, aerobic fitness ($VO2_{max}$) is one key variable closely associated with sport-specific performance, with values around 60 $mL{\cdot}kg^{-1}{\cdot}min^{-1}$ generally recommended for professional-level players [27]. Although no previous studies have analyzed the effect of high-dose BA in futsal players, the effectiveness of this ergogenic aid has been demonstrated in similar endurance tests, such as the Yo-Yo intermittent recovery test [23] or running-based anaerobic sprint test [28]. However, some studies reported no ergogenic effect of BA on athletes' performance [29,30]. For instance, Saunders et al. (2012) reported that 12 weeks of BA supplementation improved performance in the Yo-Yo IR2 test [23]. In contrast, Milioni et al. (2017) found that six weeks of BA supplementation did not improve repeated sprint ability [29]. One possible explanation for these discrepancies is the duration of the loading phase, suggesting that shorter supplementation periods may not be sufficient to enhance muscle carnosine levels required to improve performance. Supporting this, Smith et al. [31] observed improvements in cycling capacity after three weeks of high-intensity interval training in both BA and PLA groups; however, greater improvement of endurance and high-intensity interval performance from weeks 3 to 6 was seen in the BA group. Furthermore, a recent meta-analysis indicated that BA supplementation protocols lasting between 6 and 12 weeks are more likely to yield ergogenic benefits [32]. It is currently unclear what the minimal dose and duration required to increase muscle carnosine concentration are to improve performance [33]. Any definitive conclusions here are impossible due to the current study's lack of muscle carnosine content analysis.

In futsal, possessing an excellent neuromuscular profile (ie muscle power output and velocity values) is essential for optimal performance [33]. The special futsal characteristics, such as a reduced number of players on reduced pitch dimensions and the allowance for unlimited substitutions, demand rapid, explosive movements and quick recovery, creating an environment where the ability to accelerate, decelerate, and change direction swiftly can be the difference between winning and losing [34]. Thus, developing these explosive physical qualities is paramount to optimizing futsal performance. An increase in muscle carnosine content using BA supplementation can influence the calcium sensitivity of the contractile apparatus or modulate calcium release from the sarcoplasmic reticulum, potentially enhancing maximal explosive

voluntary contractions [11]. Our data, however, showed no benefits associated with BA ingestion in trained futsal players during a 30-m sprint test compared to PLA. This finding could be partially explained by the fact that H^+ accumulation is unlikely to be the primary cause of fatigue during short-duration (< 30 s) maximal exercise, with the efficacy of BA in this type of effort reduced [35]. Our findings align with previous studies that reported no benefits of short-term beta-alanine supplementation (ie 3 weeks) on explosive efforts, such as vertical jump performance, in male soccer players [36]. Therefore, although further research is warranted, it is unlikely that BA supplementation has an ergogenic impact on sprint performance.

BA supplementation increases muscle carnosine concentration, which enhances the muscle's ability to buffer pH during high-intensity exercise [37]. That could affect metabolic (eg lactate production) and perceptual (ie rating of perceived exertion) responses, potentially reducing or postponing lactate production during exercise [38,39]. However, outcomes can vary depending on the dose, supplementation duration, and type of exercise [40]. Regarding our data, there were no significant differences between BA or PLA ingestion in blood lactate production or RPE. Our results are consistent with previous studies conducted on soccer players, which found no significant effect of BA supplementation on reducing metabolic recovery markers (eg creatine kinase, lactate dehydrogenase) compared to PLA conditions [10]. However, regarding the RPE, our findings contradict Barahona-Fuentes et al. (2023), who reported that BA ingestion resulted in a lower post-exertion RPE in middle-distance athletes [39]. A possible explanation for this discrepancy is the difference in testing protocols (ie futsal intermittent endurance test vs. 6-minute race test) and the differing training statuses of the groups studied (trained futsal players vs. male middle-distance runners). In addition, middle-distance running relies more heavily on aerobic and anaerobic energy systems in a continuous manner, whereas futsal involves intermittent high-intensity efforts with frequent changes of direction, which may influence RPE differently. One commonly reported side effect of BA supplementation is paresthesia, a transient tingling sensation linked to the release of L-histidine and subsequent carnosine formation [40]. This can often be mitigated by dividing BA doses into smaller portions throughout the day [41]. In the current study, three participants experienced mild paresthesia only on the first day of supplementation. Additionally, one participant from the BA group and one from the placebo PLA group were excluded due to gastrointestinal discomfort, despite splitting BA doses into four daily servings.

In contrast, studies by Ávila-Gandía et al. (2021), which utilized sustained-release BA formulations during one-week high-dose loading protocols, reported no cases of gastrointestinal discomfort or paresthesia [14]. This difference may stem from the controlled, gradual release of BA in sustained-release formulations, reducing the risk of adverse effects [15]. Therefore, it seems that formulation type significantly influences tolerability, with sustained-release options offering potential benefits for minimizing side effects during both short-term high-dose BA supplementation.

4.1. Limitation

The current investigation has several limitations. First, we did not measure muscle carnosine concentration or any mechanisms related to the main finding of this study. Second, this study only analyzed physical performance (ie specifically intermittent endurance and speed simulated) and not real futsal context scenarios (ie futsal match); thus, fatigue induced by the physical performance battery could be lower than reported during an official futsal match, potentially limiting the ergogenic effects of BA. Future investigations should be conducted to determine the impact of BA in a real competitive futsal context. Third, we only studied the impact of a high dose of BA (ie 12 g per day) during two-week periods, and it is unknown if a more extended duration period, generally used in the literature (ie 4–8 weeks), could produce greater benefits in both futsal-specific testing and match play. Fourth, the findings of this study should be applied only to trained futsal players, and future studies should investigate whether the effects associated with BA are similar in elite or professional male futsal players with a higher training background. Fifth, we used a powder-based BA supplementation that may contribute to mild gastrointestinal discomfort or participants dropping out. It has been suggested that sustained release of BA could prevent GI discomfort during short-term high doses or usual supplementation [15]. Finally, while G*Power was used to estimate the sample size for adequate statistical power, the small cohort ($n = 16$) might impact the robustness of the results, particularly given the inherent variability in performance-based data measures. Given these limitations, practitioners may want to reconsider short-term, high-dose BA supplementation strategies for intermittent

sports, such as futsal, as the current protocol did not demonstrate clear performance benefits in this context. Future work should explore alternative dosing regimens and real-world competitive scenarios to optimize BA's ergogenic potential.

5. Conclusion

A two-week period of high-dose BA supplementation, at 12 g per day, did not improve the distance covered during the intermittent endurance test compared to the PLA group. Additionally, no significant differences were observed in sprint velocity (eg a 30-m sprint), metabolic responses (such as blood lactate levels), or perceptual responses (including RPE) among trained futsal players.

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