



Effects of Plyometric Training and Beta-Alanine Supplementation on Maximal-Intensity Exercise and Endurance in Female Soccer Players

by

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Plyometric training and beta-alanine supplementation are common among soccer players, although its combined use had never been tested. Therefore, a randomized, double-blind, placebo-controlled trial was conducted to compare the effects of a plyometric training program, with or without beta-alanine supplementation, on maximal-intensity and endurance performance in female soccer players during an in-season training period. Athletes (23.7 ± 2.4 years) were assigned to either a plyometric training group receiving a placebo (PLACEBO, $n = 8$), a plyometric training group receiving beta-alanine supplementation (BA, $n = 8$), or a control group receiving placebo without following a plyometric training program (CONTROL, $n = 9$). Athletes were evaluated for single and repeated jumps and sprints, endurance, and change-of-direction speed performance before and after the intervention. Both plyometric training groups improved in explosive jumping (ES = 0.27 to 1.0), sprinting (ES = 0.31 to 0.78), repeated sprinting (ES = 0.39 to 0.91), 60 s repeated jumping (ES = 0.32 to 0.45), endurance (ES = 0.35 to 0.37), and change-of-direction speed performance (ES = 0.36 to 0.58), whereas no significant changes were observed for the CONTROL group. Nevertheless, compared to the CONTROL group, only the BA group showed greater improvements in endurance, repeated sprinting and repeated jumping performances. It was concluded that beta-alanine supplementation during plyometric training may add further adaptive changes related to endurance, repeated sprinting and jumping ability.

Key words: muscle strength, strength training, ergogenic aids, female athletes.

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Introduction

Aside from endurance activity, female soccer players must also perform numerous explosive actions (Turner et al., 2013), including jumping, kicking, accelerating, decelerating and changing of direction, with most of these preceding goal opportunities in competitive leagues (Faude et al., 2012). The ability to repeat these explosive actions throughout a 90 min game may be associated with intramuscular buffering capacity (Trexler et al., 2015). Therefore, investigating methods to enhance single and repeated explosive actions in soccer players seems to be essential, especially in female players, for whom less research is available (Datson et al., 2014). Plyometric training in female athletes may improve single and repeated explosive actions, although its interaction with other factors that may mediate adaptations to power, speed and endurance performances, such as dietary supplements (Ramírez-Campillo et al., 2016), is unclear.

A popular dietary supplement among athletes is beta-alanine, a non- proteinogenic amino acid produced endogenously in the liver and acquired mainly through the consumption of chicken, beef, pork and fish (Trexler et al., 2015). Beta-alanine supplementation may increase both fast-twitch and slow-twitch muscle carnosine (Harris et al., 2006), which improves the ability of the muscle to buffer protons (Tipton et al., 2007) and delays muscle fatigue. Therefore, beta-alanine supplementation may increase the amount of work performed during high-intensity exercise and is regarded as a potential ergogenic aid for sprints (Tipton et al., 2007) and endurance capacity (Glenn et al., 2015a), both of which are relevant to soccer performance.

Previous research in soccer has demonstrated conflicting results regarding the effects of beta-alanine supplementation on physical performance. Intermittent beep test performance (Saunders et al., 2012) improved after beta-alanine supplementation in soccer players. Conversely, beta-alanine supplementation did not affect match specific repeated-sprint performance under normal conditions (Ducker et al., 2013) or performance during a soccer-specific intermittent treadmill protocol performed under hypoxia (Saunders et al., 2014). To our knowledge, no study has

previously analyzed the effects of beta-alanine supplementation on performance adaptations after a plyometric training intervention in soccer players. Therefore, it remains unknown whether in-season beta-alanine supplementation combined with plyometric training can elicit soccer-specific performance improvements in female players when compared to plyometric training alone.

Methods

Material and methods followed methodological recommendations (Ramírez-Campillo et al., 2016) corresponding to the highest quality requirements (Stojanovic et al., IN PRESS). To the author's knowledge, this is the first study to incorporate this high-standard methodological quality to analyze the effects of beta-alanine supplementation combined with plyometric training.

Participants

After attaining written informed consent, thirty amateur female soccer players were initially recruited. Two participants were deemed unfit to participate in the study and three were excluded for lack of compliance, leaving 25 participants for analysis. The participants were not involved in regular strength or plyometric training during the three months prior to the study and had never undertaken beta-alanine supplementation prior to the study. The sample size was determined according to changes in vertical jumping performance in a group of soccer players subjected to a control ($\Delta = 0.5$ cm; SD = 1.1) or a short-term plyometric training protocol ($\Delta = 2.6$ cm; SD = 1.6) (Ramirez-Campillo et al., 2015) comparable with that applied in this study. Eight participants per group would yield a power of 95% and $\alpha = 0.01$, with a detectable ES of 0.2.

The study participants were randomly assigned to either a plyometric training group receiving placebo (PLACEBO, $n = 8$), a plyometric training group receiving beta-alanine supplementation (BA, $n = 8$) or a control group receiving placebo without following a plyometric program (CONTROL, $n = 9$). At baseline, no differences were observed in any descriptive or dependent variable between the groups (Table 1). The study was conducted in accordance with the Declaration of Helsinki and was approved by the ethics committee of the Physical Activity Sciences Department from the University of Los Lagos.

Measures and procedures

The participants had routinely performed the tests before as part of the team policy. Measurements (i.e., body height, body mass, squat jump, countermovement jump, 20 m sprint test, running anaerobic sprint test [RAST], 40 cm drop jump reactive strength index, peak jump power, change-of-direction speed [i.e., *Illinois* test], 20 m multistage shuttle run, 60 s countermovement jump) were taken one week before and after the intervention within a 4 day window, in the same order, at the same time of the day and by the same investigators, who were blinded to each participant's group assignment. Ten minutes of standard warm-up exercises were performed before testing. Three maximal trials were allowed for all performance tests with the exception of the 20 m multistage shuttle run test, peak jump power test, 60 s countermovement jump test, and RAST measurements. At least two minutes of rest were permitted between each maximal trial to reduce the effects of fatigue.

Protocols for the 60 s countermovement jumps (Bosco et al., 1983), squat jumps, countermovement jumps, drop jumps, 20 m sprints, change-of-direction speed and shuttle run tests were performed as previously described (Ramírez-Campillo et al., 2016). For the jumps, players executed maximal effort jumps on a mobile contact mat (Ergojump; Globus, Codogne, Italy) with arms akimbo. Take-off and landing were standardized to full knee and ankle extension on the same spot. The participants were instructed to maximize jump height. In addition, for the 40 cm drop jump reactive strength index, the players were instructed to minimize ground contact time after dropping down from a 40 cm box, respectively. For the 20 m sprints, the participants had a standing start with the toe of the preferred foot forward and just behind the starting line. For the change of direction speed test, the timing system and procedures were the same as for the 20 m sprint test, except that players started supine and completed a circuit with several changes of direction.

The peak jump power measurements employed the same equipment and movement patterns as the countermovement jump measurements; however, instead of adopting arms akimbo, the participants held a weighted bar positioned behind the neck (as in the back squat

exercise). A previously established testing protocol and equation were used to estimate lower body power (W) (Ramírez-Campillo et al., 2016). Unloaded peak jump power was determined with a broomstick; the loads were increased by 5 kg for each attempt, and the tests were stopped when, after four attempts, the reductions in power output were greater than 50 W compared to the previous jump load measurements.

Participants performed six 35 m maximal sprints with 10 s of rest for the RAST, as previously described (Ramírez-Campillo et al., 2016). The sprint times were measured using single-beam infrared photoelectric cells (Globus, Codogne, Italy) leveled ~0.7 m above the floor (i.e., hip level). The starting position was standardized to a split position with the toe of each preferred foot forward and behind the starting line. Mean RAST times were used for the analyses.

Training program and supplementation protocol

All groups participated in the same soccer training program, yielding similar training loads measured via the session rating of perceived exertion [~ 709 /session (arbitrary units)], as previously described (Ramírez-Campillo et al., 2016). The experiments were completed during the regular season, which was similar between groups (Table 1). Participants in the plyometric training groups performed plyometric drills immediately after the warm-up and as a substitute for some soccer drills (i.e., mainly technical) within the usual 2 h practice twice per week for six weeks. Before the training period, the participants were accustomed to all exercises completed in the plyometric program, and all training sessions were supervised (coach to player ratio of 1:4), with particular attention paid to movement patterns. The plyometric training sessions were separated by a minimum of 48 h. The description of the training program can be found in a previous study (Ramírez-Campillo et al., 2016). Briefly, plyometric training included the following: unilateral and bilateral horizontal and vertical jumps with both cyclic and acyclic arm swings, in addition to bounce drop jumps from 40 cm boxes. The participants were instructed to aim toward maximal vertical heights and horizontal distances for acyclic jumps and minimum ground contact times for cyclic jumps. The total number

of jumps performed per training session increased from 140 in the first week to 260 in the last week.

The BA group received 4.8 g/day (~84 mg per kg of body mass) of beta-alanine (GNC Pro Performance, USA) divided into six equal doses of 0.8 g (~14 mg per kg of body mass) and consumed it every 2 h each day for the six weeks of intervention (Trexler et al., 2015), for a total of 201.6 g. Participants in the PLACEBO and CONTROL groups were given the same dosages of microcrystalline cellulose. Supplements were presented in capsules, and the participants were asked to take the supplements with juice to mask the taste and texture of the supplements provided to them. Compliance to supplementation was monitored weekly via compliance forms. Five athletes in the BA group reported mild paresthesia symptoms. The supplement capsule provided no information regarding its composition, so that neither the investigators nor the participants were aware of the contents until the completion of the analyses. The supplements were distributed by a staff member blinded to the group distribution.

One week immediately before and after the intervention, each participant's energy and macronutrient intakes were determined through a 24-h food recall questionnaire conducted on three different days of the week, as previously described (Ramírez-Campillo et al., 2016).

Statistical analysis

All values are reported as means \pm standard deviations. The Shapiro-Wilk and Levene's tests yielded non-significant values for all data before and after the intervention. To determine the effects of the intervention on performance absolute mean differences between groups were compared using repeated-measures analysis of variance (ANOVA), with Tukey post hoc procedures. In addition, a one-way ANOVA compared changes between groups (i.e., the differences between the scores before and after the intervention). The α level was set at $p < 0.05$ for statistical significance. In addition, the magnitudes of the mean differences were analyzed using Cohen's d effect sizes (ES). Threshold values for qualitatively assessing the magnitudes of ES were 0.20, 0.60, 1.2, and 2.0 for small, moderate, large, and very large, respectively (Hopkins et al., 2009). The magnitudes of differences in the training effects

between groups were evaluated non-clinically (Hopkins et al., 2009): if the confidence interval overlapped with the thresholds for substantial positive and negative values, the effect was deemed unclear (i.e., trivial). The effect was otherwise clear and reported as the magnitude of the observed value with a qualitative probability, as above (i.e., small, moderate, large, and very large). The reliability of the assessments was determined using the typical error of measurement expressed as a percentage of the mean (i.e., coefficient of variation) ranging from 1.6 to 7.6%.

Results

The energy, carbohydrate, lipid and protein intake did not differ before, during and after the intervention for the CONTROL, PLACEBO or BA group (Table 1). Similarly, body mass and the body mass index were not different before, during or after the intervention for all studied groups of athletes (Table 1).

Both plyometric training groups increased the squat and countermovement jump, drop jump reactive strength index and jump power performance ($p < 0.05$; ES = 0.27 to 1.0), and both achieved a greater increase compared with the CONTROL group in these tests (Table 2). No differences were found between the BA and PLACEBO groups in training effects for jumping and power performance, except for the 60 s countermovement jump power test, where the BA group attained a greater training effect compared with the PLACEBO group (Table 2).

Both plyometric training groups increased ($p < 0.05$) their performance in the RAST, change of direction speed, 20 m sprint and 20 m multistage shuttle run tests (ES = 0.31 to 0.9); however, only the BA group had greater training effects in the RAST and 20 m multistage shuttle run tests compared to the CONTROL group (Table 3). No significant changes were observed in the CONTROL group

Discussion

Our results suggest that the replacement of several soccer drills with specific plyometric training is an effective strategy for increasing maximal-intensity and endurance performance in female soccer players. Furthermore, as a novelty, the current study demonstrated that beta-alanine

supplementation may add further adaptive responses in endurance and in repeated sprinting and jumping performances.

Considering that neither group changed its dietary intake during the experimental period, the maintenance of body mass and the body mass index in the CONTROL, PLACEBO and BA groups was not surprising. In general, these variables do not change in female soccer players during short-term in-season soccer training periods (e.g., six weeks) (Ramirez-Campillo et al., 2016b) or periods comprising soccer specific drills

plus plyometric training (Ramírez-Campillo et al., 2016). In addition, it has been already observed that there is no significant direct effect of beta-alanine supplementation on the individual's body mass (Trexler et al., 2015). However, it should be noticed that, compared with previous research dealing with beta-alanine in soccer (Trexler et al., 2015), in this study subjects' dietary habits were controlled, thus increasing the reliability of this research.

Table 1

Descriptive data of the control group (CONTROL, n = 9), plyometric training group receiving placebo (PLACEBO, n = 8) and plyometric training group receiving beta-alanine supplementation (BA, n = 8).

	CONTROL	PLACEBO	BA
Age (y)	24.0 ± 2.7	22.8 ± 2.1	24.3 ± 2.5
Body mass (kg)	58.5 ± 7.2	61.1 ± 8.3	58.1 ± 6.3
Height (m)	1.62 ± 0.04	1.64 ± 0.08	1.62 ± 0.05
Body mass index (kg.m ⁻²)	22.2 ± 1.8	22.5 ± 1.2	22.0 ± 1.3
Session rating of perceived exertion ^a	747 ± 267	690 ± 299	690 ± 179
Soccer experience (y)	9.1 ± 3.9	7.5 ± 3.1	8.0 ± 3.8
Competition games during the experimental period	4.4 ± 2.1	4.8 ± 1.8	4.0 ± 2.2
Weekly participation in other sport or training modalities (h)	1.1 ± 0.6	1.1 ± 0.5	1.0 ± 0.3
Energy intake (kcal.day ⁻¹)	2,737 ± 339	2,456 ± 348	2,426 ± 255
Carbohydrate intake (g.day ⁻¹)	424 ± 77.6	356 ± 67.4	344 ± 73.8
Lipid intake (g.day ⁻¹)	80.7 ± 12.6	74.0 ± 14.7	68.6 ± 12.2
Protein intake (g.day ⁻¹)	80.0 ± 17.3	81.8 ± 8.8	86.4 ± 12.6

^a: soccer training load was determined by multiplying the minutes of soccer training by the rating of perceived exertion after each soccer training session.

Table 2
 Training effects (with 95% confidence limits) for the jump performance variables for the control group (CONTROL, n = 9), plyometric training group receiving placebo (PLACEBO, n = 8) and plyometric training group receiving beta-alanine supplementation (BA, n = 8).

	Baseline Mean \pm SD	Post Mean \pm SD	Change (%)	Effect sizes
Peak jump power (W)				
CONTROL	2003 \pm 341	1989 \pm 272	0.0 (-5.7, 5.7)	0.01 (-0.29, 0.27)
PLACEBO	1974 \pm 259	2140 \pm 250	8.6 (5.2, 12.0) ^{b, c}	0.59 (0.41, 0.76)
BA	1944 \pm 340	2122 \pm 318	9.6 (5.6, 13.5) ^{b, c}	0.54 (0.36, 0.71)
Squat jump (cm)				
CONTROL	25.4 \pm 5.3	25.5 \pm 4.9	0.8 (-1.6, 3.1)	0.04 (-0.05, 0.12)
PLACEBO	23.4 \pm 2.4	25.0 \pm 2.9	5.9 (3.0, 8.8) ^{b, c}	0.46 (0.28, 0.64)
BA	25.4 \pm 5.2	27.0 \pm 5.5	6.1 (4.0, 8.1) ^{b, c}	0.27 (0.20, 0.34)
Countermovement jump (cm)				
CONTROL	28.9 \pm 5.8	29.4 \pm 6.3	1.6 (-1.1, 4.3)	0.07 (-0.03, 0.16)
PLACEBO	24.8 \pm 3.4	26.4 \pm 3.0	8.7 (4.4, 12.9) ^{b, c}	0.56 (0.35, 0.76)
BA	28.1 \pm 3.5	30.6 \pm 3.1	9.3 (6.1, 12.5) ^{b, c}	0.68 (0.50, 0.87)
40 cm reactive strength index (mm.ms⁻¹)				
CONTROL	1.33 \pm 0.3	1.33 \pm 0.5	-2.0 (-20.7, 16.7)	-0.12 (-0.52, 0.28)
PLACEBO	1.24 \pm 0.4	1.67 \pm 0.6	36.6 (13.4, +59.8) ^{b, c}	0.74 (0.41, 1.08)
BA	1.11 \pm 0.2	1.53 \pm 0.5	36.8 (18.3, 55.2) ^{b, c}	1.00 (0.67, 1.34)
60 s countermovement jump power (W.kg⁻¹)				
CONTROL	15.3 \pm 0.7	15.4 \pm 0.8	0.9 (-0.2, 1.9)	0.16 (0.0, 0.31)
PLACEBO	15.1 \pm 0.7	15.3 \pm 0.7	1.7 (1.0, 2.4) ^a	0.32 (0.21, 0.43)
BA	14.8 \pm 1.0	15.3 \pm 1.1	3.6 (2.3, 4.9) ^{b, c, d}	0.45 (0.32, 0.58) ^e

a, b: denote significant change pre to post training ($p < 0.05$ and $p < 0.01$, respectively). c: denotes greater change compared to CONTROL ($p < 0.05$); d: denotes greater change compared to PLACEBO ($p < 0.05$); e: denotes small meaningfully greater effect compared to PLACEBO.

Table 3

Training effects (with 95% confidence limits) for the running anaerobic sprint test (RAST), change of direction speed, 20 m sprint and endurance performance for the control group (CONTROL, n = 9), plyometric training group receiving placebo (PLACEBO, n = 8) and plyometric training group receiving beta-alanine supplementation (BA, n = 8).

	Baseline Mean \pm SD	Post Mean \pm SD	Performance change (%)	Effect sizes
RAST mean sprint time (s)				
CONTROL	7.18 \pm 0.9	7.17 \pm 0.8	-0.1 (-2.6, 2.4)	-0.01 (-0.17, 0.14)
PLACEBO	7.49 \pm 0.8	7.18 \pm 0.6	-4.0 (-6.8, -1.2) ^a	-0.39 (-0.62, -0.17)
BA	7.61 \pm 0.5	7.10 \pm 0.5	-6.6 (-9.9, -3.3) ^{b, c, d}	-0.91 (-1.27, -0.53) ^e
20 m sprint (s)				
CONTROL	3.78 \pm 0.3	3.83 \pm 0.4	1.0 (-1.5, 3.5)	0.1 (-0.11, 0.30)
PLACEBO	3.89 \pm 0.4	3.77 \pm 0.4	-3.3 (-5.4, -1.2) ^{a, c}	-0.31 (-0.46, -0.15)
BA	3.92 \pm 0.2	3.80 \pm 0.1	-3.1 (-4.4, -1.3) ^{a, c}	-0.78 (-1.05, -0.50)
Change of direction speed test time (s)				
CONTROL	18.7 \pm 0.4	18.7 \pm 0.4	-0.1 (-0.7, 0.6)	-0.02 (-0.25, 0.20)
PLACEBO	18.5 \pm 0.3	18.2 \pm 0.4	-1.3 (-1.8, -0.8) ^{a, c}	-0.58 (-0.81, -0.44)
BA	18.9 \pm 0.7	18.6 \pm 0.8	-1.6 (-2.1, -1.1) ^{a, c}	-0.36 (-0.45, -0.27)
20 m multi stage shuttle run test (min)				
CONTROL	7.9 \pm 1.8	7.9 \pm 2.0	0.3 (-5.9, 7.9)	0.01 (-0.09, 0.10)
PLACEBO	7.1 \pm 1.1	7.5 \pm 1.0	6.9 (0.0, 28.6) ^a	0.37 (0.07, 0.68)
BA	7.9 \pm 1.7	8.5 \pm 1.7	8.8 (5.1, 13.0) ^{b, c}	0.35 (0.26, 0.44)

a, b: denote significant change pre to post training ($p < 0.05$ and $p < 0.01$, respectively). c: denotes greater change compared to CONTROL ($p < 0.05$); d: denotes greater change compared to PLACEBO ($p < 0.05$); e: denotes small meaningfully greater effect compared to PLACEBO.

Both plyometric training groups showed greater ($p < 0.05$) increases in their maximal jumping and power performances compared with the CONTROL group (Table 2). These results are in accordance with a previous study that used a similar training regime (Ramírez-Campillo et al., 2016), although longer interventions may lead to even greater improvements (Stojanovic et al., IN PRESS). Regarding repeated jump performance, both PLACEBO and BA plyometric training groups showed enhanced ($p < 0.05$ and $p < 0.01$, respectively) performance after the plyometric intervention; however, only the BA group showed a significantly greater ($p < 0.05$) increase compared with the CONTROL group (Table 2). Moreover,

female soccer players from the BA group showed a greater ($p < 0.05$) increase in repeated jumping ability compared with the PLACEBO group. This result is similar to data reported previously in physically active male and female participants subjected to plyometric training (Carpentier et al., 2015), but this is the first study to present such results in female soccer players. Considering that repeated jumps might alter myoplasmic free calcium concentration and reduce fiber tension capacity in vitro (Allen et al., 1989), beta-alanine may aid in calcium handling (Hannah et al., 2015). This metabolic adaptation may result in greater jump heights (Carpentier et al., 2015), reduced contact times (Invernizzi et al., 2015) or increased

aerobic energy production (Gross et al., 2014) during repeated jumps and may explain the greater enhancement of repeated jump performance of the BA group compared to the PLACEBO group. Also, the greater improvements in repeated jumping ability observed in the BA group might be indirectly related to smaller decrements in power output (i.e., greater training intensity) during plyometric training sessions (Gross et al., 2014; Invernizzi et al., 2015) (especially during the latter part of the sessions - Derave et al., 2007), due to the metabolic adaptations associated with beta-alanine supplementation.

To our knowledge, this is the first study to report the in-season effects of plyometric training and beta-alanine supplementation on sprint and change-of-direction speed performance of female soccer players. Both plyometric training groups improved ($p < 0.05$) sprinting and change-of-direction speeds at the end of the interventions; on the other hand, no changes were observed in the CONTROL group (Table 3). These results are similar to those previously reported in female soccer players after a horizontal-based plyometric training intervention (Ramírez-Campillo et al., 2016), a key component for sprinting performance improvement during plyometric interventions. The improvements observed after plyometric training in unidirectional (i.e., sprint) (Chimera et al., 2004) or maximal-intensity, change-of-direction drills (Zebis et al., 2008) may have been mediated by rapid (i.e., ≤ 6 weeks) neuromuscular adaptations of targeted muscle groups (Markovic and Mikulic, 2010), which may occur during the competitive period (Ramírez-Campillo et al., 2016). As previously suggested (Harres and Sale, 2012), no additional effect of beta-alanine supplementation was observed for the 20 m sprint or change-of-direction speed tests. Since beta-alanine may raise muscle carnosine levels (Harris et al., 2006) and thus the muscle's buffering ability (Tipton et al., 2007), its ergogenic effects could be more pronounced in performance tasks that challenge the muscle's buffering ability (anaerobic exercise) (Trexler et al., 2015).

The RAST mean sprint times improved for both the PLACEBO ($p < 0.05$) and BA ($p < 0.01$) groups after plyometric training (Table 3). The lack of data regarding plyometric training's effects on the RAST performance of female soccer

players makes it difficult to echo previous findings. However, our results are similar to those previously reported in male soccer (Hammami et al., 2016) and handball players after performing plyometric exercises (Cherif et al., 2012). From a mechanical perspective, it is reasonable to suggest that a reduced foot-ground contact time during sprinting could contribute to this improvement (Paavolainen et al., 1999). Alternatively, because electromyographic activity usually diminishes with fatigue during repeated sprints (Brocherie et al., 2014), it may be suggested that plyometric training, through its effect on increasing electromyographic activity (Markovic and Mikulic, 2010), may have counteracted the fatigue effects throughout the repeated sprints. Although both plyometric training groups showed a significant RAST performance enhancement, to our knowledge this is the first study to demonstrate that only plyometric training plus beta-alanine supplementation induced greater ($p < 0.05$) improvements in RAST mean sprint times compared to the CONTROL group (Table 3). Moreover, a significantly greater ($p < 0.05$) RAST improvement was observed in the BA group (ES = -0.91) compared to the PLACEBO group (ES = -0.39) (Table 3). The ergogenic aid of beta-alanine in repeated sprinting ability has been previously reported in soccer players (Saunders et al., 2012), which could be explained by increased buffering ability of muscles (Girard et al., 2011). Alternatively, beta-alanine may have increased fatigue-resistance during sets of jump training (i.e., repeated jumping ability, Table 2), allowing greater training intensity during the latter part of plyometric training sessions (Derave et al., 2007; Gross et al., 2014; Invernizzi et al., 2015), thus increasing chances for greater RAST-related performance adaptations, such as neuromuscular-related explosive improvements (Girard et al., 2011; Markovic and Mikulic, 2010).

The time to exhaustion in the 20 m multistage shuttle run test was improved in both the PLACEBO ($p < 0.05$) and BA ($p < 0.01$) groups after plyometric training (Table 3), similarly to previous studies with female soccer players (Ramirez-Campillo et al., 2016b). The observed improvements in endurance due to the plyometrics might have occurred due to neuromuscular-mediated changes in the athletes' running economy (Yamamoto et al., 2008) or the

neuromechanical improvements (Markovic and Mikulic, 2010) that may positively affect the athletes' change-of-direction endurance results. Plyometric-induced improvements may also have occurred (to a lesser extent) by means of cardiovascular (i.e., $\text{VO}_{2\text{max}}$) adaptations (Grieco et al., 2012). However, this is the first study to demonstrate that only the combination of BA plus plyometric training induced a greater ($p < 0.05$) increase in 20 m multistage shuttle run test times compared to the CONTROL group (Table 3). The ergogenic effects of beta-alanine on endurance exercise performance, especially in open-ended exercise tasks (Hobson et al., 2012) until volitional fatigue (Trexler et al., 2015), such as in this study, could occur through increased oxygen utilization and work production in later stages of the test (Smith et al., 2009), an increased ventilatory threshold (Stout et al., 2007) or a reduced rating of perceived exertion (Glenn et al., 2015b).

The present study is limited by the lack of laboratory measurements of resting and post-

exercise blood pH, HCO_3^- and lactate concentration, in order to determine whether the glycolysis rate improved through increased buffering capacity. Therefore, the ergogenic effects of BA on anaerobic endurance during the RAST and in the later stages of the 20 m shuttle run cannot necessarily be attributed to underlying (and fundamental) physiological mechanisms. Certainly, this discussion remains speculative and further research is needed to examine this important question.

In conclusion, compared to soccer-specific training alone, the use of plyometric exercises, during a six-week in-season period, induced greater jumping, sprinting and endurance improvements in female soccer players. Moreover, beta-alanine supplementation increased the magnitude of the adaptive responses to endurance running as well as repeated sprinting and jumping abilities.

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References

- Allen DG, Lee JA, Westerblad H. Intracellular calcium and tension during fatigue in isolated single muscle fibres from *Xenopus laevis*. *J Physiol*, 1989; 415: 433-458
- Bosco C, Luhtanen P, Komi PV. A simple method for measurement of mechanical power in jumping. *Eur J Appl Physiol Occup Physiol*, 1983; 50: 273-282
- Brocherie F, Millet GP, Girard O. Neuro-mechanical and metabolic adjustments to the repeated anaerobic sprint test in professional football players. *Eur J Appl Physiol*, 2015; 115: 891-903
- Carpentier A, Olbrechts N, Vieillevoye S, Poortmans JR. beta-Alanine supplementation slightly enhances repeated plyometric performance after high-intensity training in humans. *Amino Acids*, 2015; 47: 1479-1483
- Cherif M, Said M, Chaatani S, Nejlaoui O, Gomri D, Abdallah A. The effect of a combined high-intensity plyometric and speed training program on the running and jumping ability of male handball players. *Asian J Sports Med*, 2012; 3: 21-28
- Chimera NJ, Swanik KA, Swanik CB, Straub SJ. Effects of plyometric training on muscle-activation strategies and performance in female athletes. *J Athl Train*, 2004; 39: 24-31
- Datson N, Hulton A, Andersson H, Lewis T, Weston M, Drust B, Gregson W. Applied physiology of female soccer: an update. *Sports Med*, 2014; 44: 1225-1240
- Derave W, Ozdemir MS, Harris RC, Pottier A, Reyngoudt H, Koppo K, Wise JA, Achten E. Beta-alanine supplementation augments muscle carnosine content and attenuates fatigue during repeated isokinetic contraction bouts in trained sprinters. *J Appl Physiol*, 2007; 103: 1736-1743
- Ducker KJ, Dawson B, Wallman KE. Effect of Beta alanine and sodium bicarbonate supplementation on

- repeated-sprint performance. *J Strength Cond Res*, 2013; 27: 3450-3460
- Faude O, Koch T, Meyer T. Straight sprinting is the most frequent action in goal situations in professional football. *J Sports Sci*, 2012; 30: 625-631
- Girard O, Mendez-Villanueva A, Bishop D. Repeated-sprint ability - part I: factors contributing to fatigue. *Sports Med*, 2011; 41: 673-694
- Glenn JM, Gray M, Stewart R, Moyen NE, Kavouras SA, DiBrezza R, Turner R, Baum J. Incremental effects of 28 days of beta-alanine supplementation on high-intensity cycling performance and blood lactate in masters female cyclists. *Amino Acids*, 2015; 47: 2593-2600
- Glenn JM, Smith K, Moyen NE, Binns A, Gray M. Effects of acute beta-alanine supplementation on anaerobic performance in trained female cyclists. *J Nutr Sci Vitaminol*, 2015; 61: 161-166
- Grieco CR, Cortes N, Greska EK, Lucci S, Onate JA. Effects of a combined resistance-plyometric training program on muscular strength, running economy, and $\dot{V}O_{2peak}$ in division I female soccer players. *J Strength Cond Res*, 2012; 26: 2570-2576
- Gross M, Bieri K, Hoppeler H, Norman B, Vogt M. Beta-alanine supplementation improves jumping power and affects severe-intensity performance in professional alpine skiers. *Int J Sport Nutr Exerc Metab*, 2014; 24: 665-673
- Hammami M, Negra Y, Aouadi R, Shephard RJ, Chelly MS. Effects of an in-season plyometric training program on repeated change of direction and sprint performance in the junior soccer player. *J Strength Cond Res*, 2016; 30: 3312-3320
- Hannah R, Stannard RL, Minshull C, Artioli GG, Harris RC, Sale C. Beta-alanine supplementation enhances human skeletal muscle relaxation speed but not force production capacity. *J Appl Physiol*, 2015; 118: 604-612
- Harris RC, Tallon MJ, Dunnett M, Boobis L, Coakley J, Kim HJ, Fallowfield JL, Hill CA, Sale C, Wise JA. The absorption of orally supplied beta-alanine and its effect on muscle carnosine synthesis in human vastus lateralis. *Amino Acids*, 2006; 30: 279-289
- Harris RC, Sale C. Beta-alanine supplementation in high-intensity exercise. *Med Sport Sci*, 2012; 59: 1-17
- Hobson RM, Saunders B, Ball G, Harris RC, Sale C. Effects of beta-alanine supplementation on exercise performance: a meta-analysis. *Amino Acids*, 2012; 43: 25-37
- Hopkins WG, Marshall SW, Batterham AM, Hanin J. Progressive statistics for studies in sports medicine and exercise science. *Med Sci Sports Exerc*, 2009; 41: 3-13
- Invernizzi PL, Limonta E, Riboli A, Bosio A, Scurati R, Esposito F. Effects of acute carnosine and beta-alanine on isometric force and jumping performance. *Int J Sports Physiol Perform*, 2016; 11: 344-349
- Markovic G, Mikulic P. Neuro-musculoskeletal and performance adaptations to lower-extremity plyometric training. *Sports Med*, 2010; 40: 859-895
- Paavolainen L, Hakkinen K, Hamalainen I, Nummela A, Rusko H. Explosive-strength training improves 5-km running time by improving running economy and muscle power. *J Appl Physiol*, 1999; 86: 1527-1533
- Ramirez-Campillo R, Burgos CH, Henriquez-Olguin C, Andrade DC, Martinez C, Alvarez C, Castro-Sepulveda M, Marques MC, Izquierdo M. Effect of unilateral, bilateral, and combined plyometric training on explosive and endurance performance of young soccer players. *J Strength Cond Res*, 2015; 29: 1317-1328
- Ramírez-Campillo R, González-Jurado JA, Martínez C, Nakamura FY, Peñailillo L, Meylan CMP, Caniuqueo A, Cañas-Jamet R, Moran J, Alonso-Martínez AM, Izquierdo M. Effects of plyometric training and creatine supplementation on maximal-intensity exercise and endurance in female soccer players. *J Sci Med Sport*, 2016; 19: 682-689

- Ramírez-Campillo R, Vergara-Pedrerros M, Henríquez-Olguín C, Martínez-Salazar C, Alvarez C, Nakamura FY, De La Fuente CI, Caniuqueo A, Alonso-Martinez AM, Izquierdo M. Effects of plyometric training on maximal-intensity exercise and endurance in male and female soccer players. *J Sports Sci*, 2016b; 34: 687-693
- Saez de Villarreal E, Requena B, Cronin JB. The effects of plyometric training on sprint performance. A meta-analysis. *J Strength Cond Res*, 2012; 26: 575-584
- Saunders B, Sale C, Harris RC, Sunderland C. Effect of sodium bicarbonate and Beta-alanine on repeated sprints during intermittent exercise performed in hypoxia. *Int J Sport Nutr Exerc Metab*, 2014; 24: 196-205
- Saunders B, Sunderland C, Harris RC, Sale C. beta-alanine supplementation improves YoYo intermittent recovery test performance. *J Int Soc Sports Nutr*, 2012; 9: 39
- Smith AE, Walter AA, Graef JL, Kendall KL, Moon JR, Lockwood CM, Fukuda DH, Beck TW, Cramer JT, Stout JR. Effects of beta-alanine supplementation and high-intensity interval training on endurance performance and body composition in men; a double-blind trial. *J Int Soc Sports Nutr*, 2009; 6: 5
- Stojanovic E, Ristic V, McMaster DT, Milanovic Z. Effect of plyometric training on vertical jump performance in female athletes: A systematic review and meta-analysis. *Sports Med*. DOI: 10.1007/s40279-016-0634-6
- Stout JR, Cramer JT, Zoeller RF, Torok D, Costa P, Hoffman JR, Harris RC, O'Kroy J. Effects of beta-alanine supplementation on the onset of neuromuscular fatigue and ventilatory threshold in women. *Amino Acids*, 2007; 32: 381-386
- Tipton KD, Jeukendrup AE, Hespel P, International Association of Athletics F. Nutrition for the sprinter. *J Sports Sci*, 2007; 25 Suppl 1: S5-15
- Trexler ET, Smith-Ryan AE, Stout JR, Hoffman JR, Wilborn CD, Sale C, Kreider RB, Jager R, Earnest CP, Bannock L, Campbell B, Kalman D, Ziegenfuss TN, Antonio J. International society of sports nutrition position stand: Beta-Alanine. *J Int Soc Sports Nutr*, 2015; 12: 30
- Turner E, Munro AG, Comfort P. Female Soccer: Part 1—A Needs Analysis. *Strength Cond J*, 2013; 35: 51-57
- Yamamoto LM, Lopez RM, Klau JF, Casa DJ, Kraemer WJ, Maresh CM. The effects of resistance training on endurance distance running performance among highly trained runners: a systematic review. *J Strength Cond Res*, 2008; 22: 2036-2044
- Zebis MK, Bencke J, Andersen LL, Dossing S, Alkjaer T, Magnusson SP, Kjaer M, Aagaard P. The effects of neuromuscular training on knee joint motor control during sidcutting in female elite soccer and handball players. *Clin J Sport Med*, 2008; 18: 329-337

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