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ORIGINAL ARTICLE

Branched-chain amino acids and arginine improve physical but not skill performance in two consecutive days of exercise

Les acides aminés à chaîne ramifiée et l'arginine améliorent les performances physiques, mais n'améliorent pas les compétences en deux jours d'exercice consécutifs

Y.-T. Lin[a], M.-S. Chiu[b], C.-K. Chang[c,*]

[a] *Physical Education Office, Asia University, 500, Lioufeng Rd, Wufeng, Taichung 413, Taiwan*
[b] *Department of Exercise Health Science, National Taiwan University of Sport. 16, Sec 1, Shuan-Shih Rd, Taichung 404, Taiwan*
[c] *Department of Sport Performance, National Taiwan University of Sport. 16, Sec 1, Shuan-Shih Rd, Taichung 404, Taiwan*

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KEYWORDS
Tryptophan;
Central fatigue;
Skill performance;
High-intensity intermittent exercise

Summary
Objectives. — Tryptophan is the precursor for cerebral serotonin, which could result in exercise-induced central fatigue. Branched-chain amino acids supplementation could alleviate central fatigue by competing with tryptophan for crossing blood-brain barrier. This study investigated the effect of branched-chain amino acids and arginine on physical and skill performance in 2 consecutive days of exercise.
Equipment and methods. — Eleven male college basketball players participated in this double-blind cross-over study. Each trial lasted two days with one session of basketball drills and two basketball skill tests on each day. The subjects consumed 0.17 g/kg BCAA and 0.04 g/kg arginine (AA trial) or placebo.
Results. — Total time to complete vertical jumps, ladder suicide sprint, key combination, and full court combination was significantly faster in the second half of the basketball drill session on day 2 in the AA trial than that in the placebo trial (AA: 256.3 ± 4.8 s; placebo: 266.7 ± 6.4 s, P = 0.031). The improvement coincided with significantly lower post-exercise free tryptophan/BCAA ratio in the AA trial (AA: 0.105 ± 0.043; placebo: 0.177 ± 0.019, P < 0.001). Shooting percentage during the drills, time to complete a basketball skill test, and plasma markers for carbohydrate and fat metabolism, muscle damage, and balance between anabolism

* Corresponding author.
E-mail address: wspahn@seed.net.tw (C.-K. Chang).

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and catabolism were not significantly different between the trials. This study suggested that branched-chain amino acids and arginine could improve physical performance in the second half of the second consecutive days of exercise by alleviating central fatigue, but had no effect on skill performance.

MOTS CLÉS
Tryptophan ;
Fatigue centrale ;
Performance des compétences ;
Exercice intermittent de haute intensité

Résumé
Objectifs. — Le tryptophane est le précurseur de la sérotonine cérébrale qui pourrait entraîner une fatigue centrale induite par l'effort. La supplémentation en acides aminés à chaîne ramifiée pourrait atténuer la fatigue centrale en faisant concurrence au tryptophane pour traverser la barrière hémato-encéphalique. Cette étude a étudié l'effet des acides aminés à chaîne ramifiée et de l'arginine sur les performances physiques et les compétences lors d'exercice pendant 2 jours consécutifs.
Équipement et méthodes. — Onze joueurs masculins de basket-ball ont participé à cette étude en double insu. Chaque essai a duré deux jours avec une séance d'exercices de basket-ball et deux tests de compétence en basket-ball chaque jour. Les sujets ont consommé 0,17 g/kg de BCAA et 0,04 g/kg d'arginine (essai AA) ou un placebo.
Résultats. — Le temps total pour terminer les sauts verticaux, le sprint suicide à l'échelle, la combinaison-clé et la combinaison en plein terrain était significativement plus rapide dans la deuxième moitié de la séance de basket-ball au jour 2 dans l'essai AA que dans l'essai placebo (AA : 256,3 ± 4,8 s ; placebo : 266,7 ± 6,4 s, $p = 0{,}031$). L'amélioration a coïncidé avec un rapport tryptophane/BCAA libre significativement plus faible après l'effort dans l'essai AA (AA : 0,105 ± 0,043 ; placebo : 0,177 ± 0,019, $p < 0{,}001$). Le pourcentage de tir au cours des exercices, le temps requis pour terminer un test de compétence en basket-ball et les marqueurs plasmatiques pour le métabolisme des glucides et des graisses, les dommages musculaires et l'équilibre entre l'anabolisme et le catabolisme ne sont pas significativement différents entre les essais. Cette étude a suggéré que les acides aminés à chaîne ramifiée et l'arginine pourraient améliorer la performance physique dans la deuxième moitié des deuxièmes jours consécutifs d'exercice en allégeant la fatigue centrale, mais n'a eu aucun effet sur les performances.

1. Introduction

Exercise-induced fatigue is a complex mechanism that involves central and peripheral factors. Serotonin is hypothesized to be one of the neurotransmitters that induces central fatigue because it could lead to the feeling of tiredness and the loss of central drive and motivation [1]. An elevated cerebral serotonin concentration during exercise has been reported in rats [2]. In addition, cerebral uptake of tryptophan, the precursor for serotonin synthesis, was increased during prolonged exercise in humans [3].

Transport of plasma tryptophan across blood-brain barrier is the rate-limiting step for cerebral serotonin synthesis [4]. Branched-chain amino acids (BCAA), including leucine, isoleucine, and valine, could alleviate central fatigue by competing with tryptophan for the same carrier, L-system transporter [5]. Several animal studies have shown that BCAA could increase running time to exhaustion, which was accompanied by reduced plasma free tryptophan/BCAA ratio and cerebral serotonin synthesis [6,7]. However, most human studies failed to find ergogenic effects of BCAA on physical performance in a single bout of endurance exercise [8,9]. Recently, we have discovered that supplementation of BCAA and arginine improved physical performance on the second day of consecutive days of simulated handball games in well-trained male and female athletes [10]. This ergogenic effect appears to be associated with alleviated central fatigue as it is concurrent with lower plasma free tryptophan/BCAA ratio.

In addition to physical performance, several studies have revealed that sport-specific skill performance could also decline during exhausting exercise. For example, accuracy and consistency of tennis service and groundstroke were significantly decreased after fatigue [11]. Passing accuracy was also deteriorated after a simulated rugby game [12]. Skill performance is as important as physical capacity in winning in many sports. However, this aspect of performance has received little attention in the literature. We hypothesized that by alleviating central fatigue, the decline in skill performance could be prevented. In this study, we extend from our previous findings to investigate the effect of BCAA and arginine on physical and skill performance in 2 consecutive days in male college players. Total time to complete a combination of basketball-specific drills was used to measure physical performance, while shooting percentage during the drills and the time to complete a basketball-specific skill test were used as the indicators for skill performance.

2. Materials and methods

2.1. Subjects

Eleven male basketball players (age 20.0 ± 1.1 years, height 178.4 ± 6.2 cm, weight 71.8 ± 8.0 kg) were recruited from a

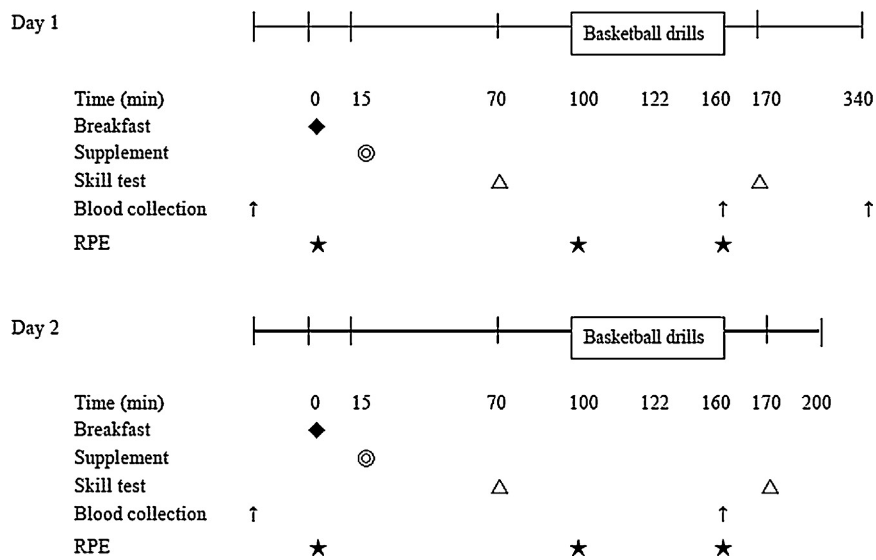

Figure 1 Study design.

division II university to participate in this study. All subjects have undergone regular basketball training for at least 3 years. The exclusion criteria included cardiovascular disease risks, musculoskeletal injuries, smoking, and consumption of any medicine or protein supplement in the past 3 months. The regular training schedule and dietary habits were maintained during the study period. The subjects were refrained from all training activity on the day prior to the trials. All subjects gave their written informed consent after the experimental procedure and potential risks were explained. The study protocol was approved by the Medical Research Ethics Committee of Asia University, Taiwan. The experiment procedure is in accordance with the Declaration of Helsinki.

2.2. Experimental design

This study used a double-blind, randomized cross-over design. Each subject completed amino acids (AA) and placebo (PL) trials in a random order, separated by a washout period of at least 7 days. Each trial lasted 2 days with 1 session of basketball drills and 2 skill tests on each day (Fig. 1). The same food, purchased from local convenience stores, was provided 1 day prior to and during the trials. The lunch and dinner each day combined to provide approximately 2556 kcal/day with 47% energy from carbohydrate, 38% from fat, and 15% from protein, according to the manufacturer's label. The breakfast on the days of trials included white bread 1.2 g/kg, jam 0.1 g/kg, butter 0.1 g/kg, and soybean milk 5 mL/kg (6.2 kcal/kg, containing carbohydrate 1.0 g/kg, protein 0.24 g/kg, and fat 0.14 g/kg) [10].

2.3. Supplementation

On the days of the trials, the subjects reported to a basketball arena at 07:00 after an overnight fast. After collecting venous blood samples as baseline, the subjects consumed the standard breakfast. After finishing the breakfast, 2 different supplements were consumed. In the AA trial, the subjects consumed 0.17 g/kg BCAA (leucine: isoleucine: valine = 2:1:1, powdered form, Optimum Nutrition, INC, Sunrise, FL, USA) and 0.04 g/kg arginine (in capsule, General Nutrition Corporation, Pittsburgh, PA, USA). In the placebo (PL) trial, the subjects ingested the same amount of starch powder and identical number of capsules containing starch. BCAA or starch power was dissolved in 250 mL of grape juice to mask the taste. All supplements were taken within 5 min. The trials started 60 min after the supplements were consumed. Our preliminary study has shown that plasma BCAA and arginine concentrations would peak after 1 h of ingestion (data not shown).

The subjects were allowed to drink water ad libtum in the first trial, and the timing and amount of consumption were repeated in the second trial. The average water consumption on day 1 and 2 was 467 ± 44 and 503 ± 49 mL, respectively.

2.4. Basketball drills

The basketball drills were modified from previous studies [13,14] to mimic college competitions. The session was consisted of four 10-min quarters, with 10 basketball drills and two 60-s rests in each quarter. There was a 2-min rest after the first and third quarter, and a 15-min rest after the second quarter. The drills included (1) vertical jump × 10: the subject repeatedly touched a mark set at 70% of his maximum vertical jump 10 times as quickly as possible (time to completion); (2) ladder suicide sprint (time to completion); (3) baseline jump shot × 20: started at half court, sprinted to baseline, received pass from an investigator standing on foul line, attempted a 4.5-m baseline jump shot, sprinted to opposite sideline at half court, repeated after 6 s (number of shots made); (4) rest for 60 s; (5) half court zigzag and foul line jump shot: started at corner, zigzag defensive slides to half court, sprinted to center court, pickup basketball, dribbled to foul line, attempted a foul line jump shot, sprinted to opposite corner, repeat (number of shots made in 2 min); (6) free throw × 5: each shot had to be completed within

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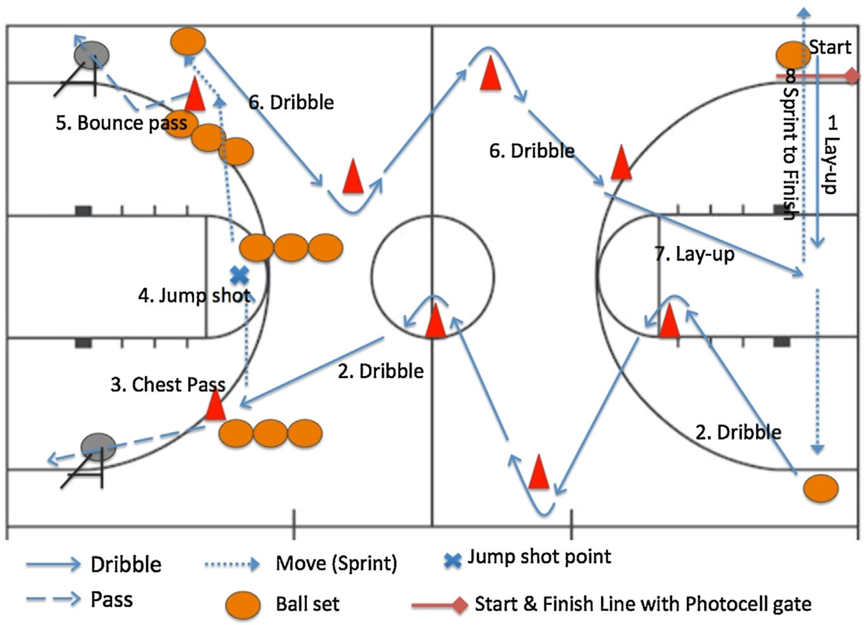

Figure 2 Layout of the basketball skill test.

5 s (number of shots made); (7) key combination × 5 (time to completion); (8) rest for 60 s; (9) full court combination (time to completion); (10) around-the-world shooting: continuous jump shots from 4.5 m from 5 positions: 0, 45, 90, 135, and 180 degree, 5 shots in each position (number of shots made in 2 min); (12) court-width sprint × 6 (time to completion).

Total movement time, the sum of time to complete vertical jumps, ladder suicide sprint, key combination, and full court combination, was used as an indicator for physical performance. Shooting percentage of all shots was also calculated. The number of total shots ranged from 223 to 236. The time to complete 10 consecutive jumps was record by a stopwatch (Casio HS-30W, Tokyo, Japan), while the time to complete other drills was measured by photocell gates (Newtest Powertimers 300-series, Oulu, Finland).

The indoor temperature was 26.4 ± 0.7 °C and humidity was 73.6 ± 1.8% at beginning of the drills with no significant difference between the two trials.

2.5. Basketball skill test

The basketball skill test measuring dribbling and passing abilities was performed before and after the drills. The skill test is based on the Skill Challenge in National Basketball Association (NBA) all-star weekend, 2011 (https://www.youtube.com/watch?v=At0cavZPPCw). The layout of the test is shown on Fig. 2. The circular targets for chest and bounce passes had a diameter of 60 cm and their center was 120 cm above the floor. The time to complete the test was record by photocell gates.

2.6. Blood sampling and biochemical parameters measurement

The time points for venous blood sample collection were shown on Fig. 1. Ten milliliters of blood sample were collected into tubes containing EDTA each time. Hemoglobin and hematocrit were measured with a hematology analyzer (Sysmex Kx-21, Diamond Diagnostics, Holliston, MA, USA) to correct for the changes in plasma volume [15]. Plasma samples were collected after centrifugation at 1500 × g (Eppendorf 5810, Hamburg, Germany) for 20 min at 4 °C. The aliquoted plasma samples were stored at −70 °C until further analysis.

Plasma BCAA concentration was measured enzymatically (Biovision, Milpitas, CA, USA) with a microplate spectrophotometer (Benchmark Plus, Bio-Rad, Hercules, CA, USA). Plasma free tryptophan concentration was analyzed with a fluorescence assay according to manufacturer's recommendation (Bridge-It, Mediomics, St. Louis, MO, USA). The fluorescence at excitation 485 nm and emission 665 nm was read by a microplate fluorescence reader (Plate Chameleon, Hidex, Turku, Finland). Plasma concentrations of testosterone and cortisol were measured with Electrochemiluminescence immunoassay (Elecsys 2010, Roche, Mannheim, Germany). Plasma concentrations of lactate, glucose, glycerol, and non-esterified fatty acids and activities of creatine kinase and lactate dehydrogenase were measured with an automatic analyzer (Hitachi 7020, Tokyo, Japan) using commercial kits (Randox, Antrim, UK).

2.7. Statistical analysis

All values were expressed as mean ± SD. The differences in total movement time and shooting percentage in the basketball drills, as well as the time in skill test between the 2 trials were analyzed by paired *t*-test. The differences in biochemical parameters were analyzed using two-way analysis of variance with repeated measurements. If the time effect was significant, post-hoc Bonferroni analysis was used to identify the difference within the same trial. If the interaction effect was significant, the difference between the 2 trials at the same time point was identified by one-way

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Table 1 Performance in the AA and PL trials.

	Trial	Day 1		Day 2	
		First half	Second half	First half	Second half
Total movement time (s)	AA	265.75 ± 16.76	261.41 ± 16.97	262.18 ± 17.93	256.25 ± 15.75[a]
	PL	264.42 ± 19.46	265.85 ± 18.04	265.12 ± 20.80	266.72 ± 21.14[a]
Shooting (%)	AA	68.5 ± 8.1	0.307 ± 0.069	0.269 ± 0.044	0.299 ± 0.064
	PL	68.5 ± 12.2	68.9 ± 9.9	67.7 ± 7.4	67.5 ± 11.8
Skill test (s)	AA	33.52 ± 5.40	34.18 ± 4.78	31.14 ± 3.55	31.93 ± 4.32
	PL	35.31 ± 6.36	33.61 ± 5.35	33.31 ± 5.33	33.55 ± 4.48

AA: branched-chain amino acids and arginine; PL: placebo.
[a] Significantly different between the 2 trials at the same time point ($P < 0.05$).

analysis of covariance with the pre-exercise level as the covariant. All analyses were performed using PASW Statistics 18.0 for Windows (SPSS, Chicago, IL). The significance level was set at $P < 0.05$.

3. Results

Total movement time in the second half of the basketball drills on day 2 in the AA trial was significantly faster than that in the PL trial (AA trial: 256.3 ± 4.8 s; PL trial: 266.7 ± 6.4 s, $P = 0.031$, Table 1). Shooting percentage in the basketball drills, as well as time to complete the basketball skill test, were not significantly different between the trials (Table 1).

The supplementation resulted in significantly higher post-exercise plasma BCAA concentrations in the AA trial than that in the PL trial on day 1 (AA trial: 895.0 ± 126.0 μmol/L; PL trial: 573.9 ± 77.2 μmol/L, $F = 32.58$, $\eta^2 = 0.685$, $P < 0.001$) and day 2 (AA trial: 897.6 ± 194.6 μmol/L; PL trial: 529.0 ± 48.8 μmol/L, $F = 23.00$, $\eta^2 = 0.605$, $P < 0.001$, Fig. 3A). The AA trial also had significantly lower post-exercise free tryptophan concentration than the PL trial (AA trial: 88.7 ± 5.9 μmol/L; PL trial: 102.9 ± 7.2 μmol/L, $F = 14.07$, $\eta^2 = 0.484$, $P = 0.002$, Fig. 3B). As the result, the AA trial had significantly lower post-exercise free tryptophan/BCAA ratio compared to the PL trial on day 1 (AA trial: 0.101 ± 0.016; PL trial: 0.183 ± 0.031, $F = 60.63$, $\eta^2 = 0.802$, $P < 0.001$) and day 2 (AA trial: 0.105 ± 0.043; PL trial: 0.177 ± 0.019, $F = 45.96$, $\eta^2 = 0.754$, $P < 0.001$, Fig. 3C).

Plasma levels of lactate, glucose, glycerol, NEFA, creatine kinase, lactate dehydrogenase, and cortisol were significantly increased, while testosterone concentration and testosterone/cortisol ratio were decreased after exercise (Table 2). However, these markers for carbohydrate and fat metabolism, muscle damage, and balance between anabolism and catabolism were not significantly different between the trials. Ratings of perceived exertion after exercise were also similar on both days between the trials (Table 2). Average heart rates during the basketball drills on day 1 and 2 were 169.8 ± 2.2 and 164.4 ± 2.2 beats/min in the AA trial, and 169.1 ± 2.2 and 163.8 ± 2.3 beats/min in the PL trial, respectively, with no difference between the trials.

4. Discussion

This study suggested that BCAA and arginine supplementation could improve physical performance in basketball-specific drills in the second half on day 2 of consecutive days of exercise. The improvement coincided with lower plasma free tryptophan/BCAA ratio, an indicator of reduced central fatigue. The subjects in the AA trial were able to maintain better drive to run and/or tolerate the feeling of fatigue better by moving faster at the same degree of perceived exertion. However, neither shooting percentage nor the time required to complete the skill test was affected by the supplements.

The present and a previous study results [10] have shown that high-intensity intermittent exercise performance on day 1 was not affected by BCAA and arginine. In agreement, it has been suggested that BCAA had no effect on performance in intermittent high-intensity shuttle running [16]. It has also been reported that a serotonin-depleting supplementation, including BCAA, phenylalanline, and tyrosine, had no effect on the distance covered in Yo-Yo intermittent endurance test in Australian Rules Football players [17]. However, physical performance on day 2 of the consecutive days was increased in this study. It is noteworthy that post-exercise free tryptophan/BCAA ratio in the AA trial was significantly lower than that in the PL trial on both days. It appears that the supplementation only became evident after the accumulation of physiological and/or psychological stress from the previous day. A further analysis of our data failed to find any significant correlation between free tryptophan/BCAA ratio at any sampling point and high-intensity intermittent exercise performance on day 1 or day 2.

To compete in consecutive days is common in international or national tournaments in basketball and many other sports. The decline in physical performance after multiple days of competition has been documented, possibly due to the accumulation of both central and peripheral fatigue. It has been shown that elite field hockey players sprinted less frequently, while standing more often, after playing 3 games within 4 days [18]. Decreases in speed, agility, and flexibility were evident after a 3-day basketball tournament [19]. Our subjects also showed a slight increase in total movement time on day 2 in the PL trial. The unique experimental design of this study revealed that BCAA and arginine improved physical performance on day 2, which could have significant practical applications.

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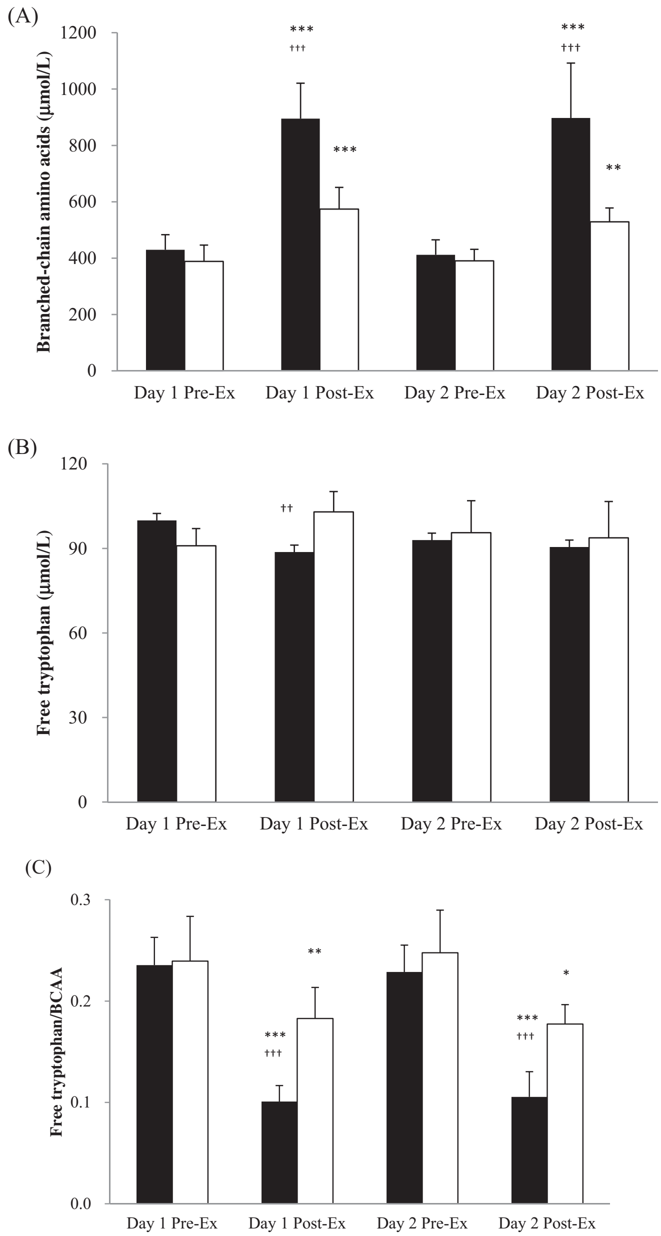

Figure 3 Plasma BCAA (A) and free tryptophan (B) concentrations and free tryptophan/BCAA ratio (C) in the AA (■) and PL (□) trials. $^{**}P < 0.01$, $^{***}P < 0.001$: significantly different from baseline in the same trial. $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$: significantly different between the 2 trials at the same time point.

A recent study has shown that a serotonin-depleting high-dose BCAA supplementation containing BCAA, phenylalanine, and tyrosine could attenuate the decline in performance in sport-specific reactive motor skills and reactive agility [17]. This effect coincided with lower plasma tryptophan/large neutral amino acids ratio, suggesting that cerebral serotonin is involved in these skill performances. The skill tests in the present study included shooting, dribbling, and passing, the most important skills in basketball. Although a case study has reported that shooting mechanics were altered after fatiguing basketball drills in a professional player [20], our subjects appeared to be able to maintain shooting accuracy throughout the drills on day 1 and 2 in both trials. This contradicted to our assumption that the fatigue induced by the basketball drills would impair the shooting ability. One possible explanation is that since all shots made in the trials were uncontested in relatively stress-free conditions, it would require less mental concentration. In addition, the subjects may have extra time to set their footings before the shots and/or may not need to jump as high as in real competitions. Another possibility is that these non-elite players are more susceptible to day-to-day variations in skills. Some of the subjects reported 'hot hands', or better feelings of shooting, at the beginning of the drills in either AA or PL trials. Similar to our results, it has been shown that Division I college basketball players could maintain shooting accuracy after a simulated game while losing 2.3% body weight by dehydration [21].

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Table 2 Plasma biochemical parameters and ratings of perceived exertion in the AA and PL trials.

	Trial	Day 1 pre-Ex	Day 1 post-Ex	Day 1 post-Ex 2 h	Day 2 pre-Ex	Day 2 post-Ex
Lactate (mM)	AA	2.20 ± 0.90	9.82 ± 2.83[a]	1.57 ± 0.37	1.79 ± 0.37	10.23 ± 2.90[a]
	PL	1.80 ± 0.32	9.28 ± 2.62[a]	1.61 ± 0.60	1.90 ± 0.64	9.34 ± 2.85[a]
Glucose (mM)	AA	4.38 ± 0.40	4.85 ± 0.52[a]	4.59 ± 0.48	4.68 ± 0.68	4.95 ± 0.77[a]
	PL	4.22 ± 0.44	4.55 ± 0.87	4.74 ± 0.58	4.62 ± 0.35	4.68 ± 0.47
Glycerol (μM)	AA	43.09 ± 10.95	101.23 ± 27.49[a]	91.54 ± 25.85[a]	48.81 ± 10.82	113.61 ± 35.11[a]
	PL	47.36 ± 25.55	112.18 ± 50.36[a]	89.62 ± 41.88[a]	54.73 ± 23.15	120.28 ± 29.71[a]
NEFA (mM)	AA	0.27 ± 0.13	0.23 ± 0.09	0.74 ± 0.31[a]	0.33 ± 0.14	0.29 ± 0.13
	PL	0.30 ± 0.18	0.30 ± 0.22	0.69 ± 0.43[a]	0.40 ± 0.17	0.37 ± 0.17
Creatine kinase (U/L)	AA	143.4 ± 104.9	191.5 ± 113.3	209.4 ± 134.9[a]	176.2 ± 114.4[a]	228.0 ± 125.2[a]
	PL	139.4 ± 139.2	208.9 ± 236.1[a]	222.4 ± 211.5[a]	176.9 ± 149.7[a]	212.5 ± 147.3[a]
LDH (U/L)	AA	187.1 ± 34.8	222.7 ± 58.4	223.5 ± 41.5[a]	216.5 ± 43.8	234.5 ± 53.9[a]
	PL	184.1 ± 43.1	198.4 ± 55.6	220.0 ± 54.3[a]	201.8 ± 59.1	220.9 ± 57.8[a]
Testosterone (nmol/L)	AA	25.80 ± 7.13	19.62 ± 7.20[a]	18.91 ± 7.37	24.09 ± 7.38	18.57 ± 9.97[a]
	PL	25.90 ± 7.07	22.44 ± 8.49[a]	21.69 ± 7.76	27.50 ± 9.96	22.80 ± 10.00[a]
Cortisol (nmol/L)	AA	615.1 ± 128.0	742.0 ± 175.0	428.2 ± 80.0[a]	689.6 ± 75.7	587.5 ± 174.3
	PL	615.2 ± 167.1	752.5 ± 232.2	477.3 ± 145.4	693.2 ± 114.2	598.7 ± 131.9
Testosterone/cortisol ratio	AA	0.044 ± 0.015	0.028 ± 0.014[a]	0.046 ± 0.020	0.035 ± 0.011[a]	0.033 ± 0.020
	PL	0.045 ± 0.018	0.032 ± 0.017[a]	0.051 ± 0.027	0.040 ± 0.017	0.041 ± 0.023
RPE	AA	10.5 ± 2.3	13.3 ± 2.7[a]	–	10.5 ± 2.6	13.6 ± 2.3[a]
	PL	9.5 ± 2.2	13.2 ± 2.3[a]	–	10.4 ± 2.2	13.3 ± 2.3[a]

AA: branched-chain amino acids and arginine; PL: placebo; NEFA: non-esterified fatty acid; LDH: lactate dehydrogenase; RPE: ratings of perceived exertion.
[a] Significantly different from baseline in the same trial ($P < 0.05$).

BCAA have been suggested to reduce markers for muscle damage such as creatine kinase and lactate dehydrogenase after intensive exercise [22,23]. BCAA could also result in higher post-exercise plasma testosterone/cortisol ratio, an indicator for the balance between anabolic and catabolic states [23,24]. However, these parameters were similar in both trials after exercise in the present study. It is possible that the basketball drills were well-tolerated by the subjects as these parameters did not reach abnormally high levels.

5. Conclusions

This study showed that the supplementation of BCAA and arginine could improve the time to complete a combination of basketball drills on the second half on day 2 of the consecutive exercise. The improvement could result from alleviated central fatigue by lowering plasma free tryptophan/BCAA ratio. Shooting, dribbling, and passing skills were not affected. Future studies could investigate skill performance under more competition-like and stressful conditions.

Disclosure of interest

The authors declare that they have no competing interest.

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