

Prevention of perceptual-motor decline by branched-chain amino acids, arginine, citrulline after tennis match

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Accepted for publication 23 May 2016

Perceptual-motor performance in prolonged tennis matches may be affected by central fatigue. The purpose of this study was to investigate the supplementation of branched-chain amino acids (BCAA), arginine, and citrulline on tennis-specific perceptual-motor performance after a simulated match. Nine male tennis players consumed 0.17 g/kg BCAA, 0.05 g/kg arginine, and 0.05 g/kg citrulline (AA trial), or placebo (PB trial) 1 h before the match. In the perceptual-motor performance test before and after the match, the subjects hit balls to the opposite direction of the examiner's movement. The AA trial showed significantly higher rate of correct direction than the PB trial after the match (AA trial:

93.63 ± 1.28%, PB trial: 69.09 ± 2.40%). The AA trial also demonstrated significantly higher post-match accuracy and consistency than the PB trial. The AA trial showed significantly lower heart rate and ratings of perceive exertion during the match, concurrently with a significantly lower plasma total tryptophan/BCAA ratio. Similar post-match plasma NH₃ concentrations were found in both trials while the AA trial was significantly higher in NO_x concentration. This study suggested that the supplementation could prevent the decline in perceptual-motor performance through alleviation of central fatigue by BCAA and prevention of excess hyperammonemia by arginine and citrulline.

Success in tennis matches requires high levels of physical, skill, and perceptual-motor performance that may need to maintain for over 5 h. The perceptual-motor performance in tennis refers to the ability to acquire the information of opponent's body and racquet movements, integrate it with existing knowledge and motor capabilities, then select and execute appropriate actions (Marteniuk, 1976). Outstanding perceptual-motor performance has been consistently shown to be one of the most important factors that distinguish expert and non-expert tennis players (North et al., 2009). Expert tennis players used different search strategies (Williams et al., 2011), and showed higher ability to correctly anticipate and quickly respond to stroke intentions of their opponents than the non-experts (Williams et al., 2002; Mann et al., 2007).

Central nervous system has been suggested to play a crucial role in the development of fatigue in tennis as the central activation deficit occurs progressively during a prolonged tennis match (Girard et al., 2008). The number of voluntarily recruited motor units and the maximal discharge rate from active motor units were progressively decreased at the latter stages of an exhausting tennis match (Girard et al.,

2008). The significantly increased plasma-free tryptophan concentrations after 4 h of tennis match suggested that cerebral serotonin may be responsible for central fatigue (Struder et al., 1995). Plasma-free tryptophan can cross the blood-brain barrier and act as the precursor for cerebral serotonin which could augment the feeling of lethargy and tiredness, loss of central drive and motivation, and impair the perceptual-motor ability (Fernstrom, 2013).

It has been hypothesized that BCAA could prevent exercise-induced central fatigue by competing for the same transporter across blood-brain barrier with tryptophan (Blomstrand, 2006). Previous studies have reported that BCAA supplementation could maintain cognitive functions (Hassmen et al., 1994) and performance in reactive motor skills (Stepito et al., 2011), while reducing the feeling of fatigue (Blomstrand et al., 1997) during strenuous exercise. However, one disadvantage for BCAA supplementation is the excess hyperammonemia as the result of the increased metabolism of these amino acids during exercise (MacLean et al., 1996). The elevated cerebral uptake and accumulation of NH₃ would nullify the potential benefit of BCAA on physical and cognitive performance (Blomstrand et al., 1997;

Struder et al., 1998) by alterations of cerebral neurotransmission (Wilkinson et al., 2010). Combining arginine and citrulline with BCAA supplementation may alleviate the excess NH_3 by enhancing nitric oxide (NO) biosynthesis (Clarkson et al., 1996; Rouge et al., 2007) and/or urea cycle (Schaefer et al., 2002; Sureda et al., 2010). Indeed, the combined supplementation of BCAA and arginine improved intermittent sprint performance on the second day of consecutive days of simulated handball games by alleviating central fatigue (Chang et al., 2015). With the addition of citrulline, a potentially better NO precursor than arginine (Schwedhelm et al., 2008), the prevention of hyperammonemia may be more eminent.

Numerous studies have investigated the changes in different aspects of performance following exhaustive tennis training and matches. The neuromuscular fatigue in tennis has been documented (Hornery et al., 2007a, b, c). It has been revealed that running speed and distance covered after prolonged match were significantly decreased (Reid & Duffield, 2014). Nevertheless, it is unclear whether it represents fatigue and/or the change in match strategy to preserve energy. The results on the impairments in skill performance after real or simulated tennis matches have been inconsistent. It has been shown that accuracy in certain types of groundstrokes and/or serve may be deteriorated, whereas other skills may be maintained (Hornery et al., 2007a, b, c; Wu et al., 2010). Decision making is another important aspect of sport competitions. It has been shown that decision making accuracy was actually improved after fatigue in junior elite water-polo players (Royal et al., 2006). The arousal induced by exercise intensity up to 80% maximal aerobic power also increased the speed of decision making in soccer players (Fontana et al., 2009). However, the subjects in these studies only responded verbally to video clips and did not actually perform the task after they made the decision. The fatigue-induced change in perceptual-motor performance, arguably the most important factor that distinguishes expert and non-expert tennis players, has rarely been investigated in sport-specific settings.

The purpose of this study was to investigate the combined supplementation of BCAA, arginine, and citrulline on tennis-specific perceptual-motor performance after a 2-h simulated match. The perceptual-motor performance test is designed to reflect real match conditions in which players have to acquire and process the opponent's movement, and hit balls to the opposite direction. In addition, the simulated match elicited similar physiological responses to real competitions. The perceptual-cognitive performance test, and a match-like experimental protocol were key methodological features in this study, designed to extend the existing literature.

Materials and methods

Participants

Nine right-handed male tennis players were recruited for this study. The experience in tennis training ranged from 8 to 18 years. All subjects have competed at the national level. The subjects have the age of 25.56 ± 0.67 years; height 1.78 ± 0.04 m, weight 72.44 ± 1.04 kg; BMI 23.02 ± 0.37 kg/m^2 . The exclusion criteria included major cardiovascular disease risks, musculo-skeletal injuries, smoking, and consumption of any medicine or protein/amino acids supplement in the past 3 months. The subjects were advised to maintain their regular training schedule and dietary habits during the study period. The subjects were refrained from all training and heavy physical activity on the day prior to the trial. All subjects gave their written informed consent after the experimental procedure and potential risks were explained. The study protocol was approved by the International Review Board of China Medical University Hospital, Taichung, Taiwan.

Study design

This study used a single-blind, randomized cross-over, placebo-controlled design (Fig. 1). Each subject completed amino acids (AA) and placebo (PB) trials in a random order, separated by a wash-out period of at least 7 days. Two days prior to each trial, the subjects were provided with the same meals, purchased from local convenience stores. The three meals per day combined to provide approximately 1828 kcal with 51% energy from carbohydrate, 31% from fat, and 18% 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) (Chang et al., 2015).

The experimental procedure is outlined in Fig. 1. A tennis-specific perceptual-motor performance test was conducted before and after a 2-h simulated match.

Supplementation

On the days of the trials, the subjects reported to the laboratory at 07:00 after an overnight fast. After collecting a venous blood sample as the baseline, the subjects consumed the standardized breakfast. Then the subjects underwent the perceptual-motor performance test 60 min after finishing the breakfast. At the end of first perceptual-motor performance test, two different supplements were consumed. In the AA trial, the subjects ingested 0.17 g/kg BCAA (leucine: isoleucine: valine = 10:7:3, containing vitamin E 6.67 IU/g BCAA,

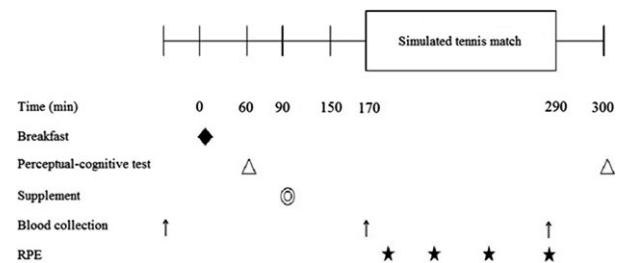


Fig. 1. Study design. ★ ratings of perceived exertion after game 6, 12, 18, and 24.

capsule; General Nutrition Corporation, Pittsburgh, Pennsylvania, USA), 0.05 g/kg arginine and 0.05 g/kg citrulline (arginine: citrulline = 1:1, tablet; General Nutrition Corporation). In the PB trial, the subjects consumed the identical amount of empty capsule and tablet containing starch (Chung-Yu Biotech Co LTD, Taichung, Taiwan) and one capsule of vitamin E (100 IU; General Nutrition Corporation). All supplements were taken with water within 10 min. 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 libitum* in the first trial, and then the timing and amount of consumption were repeated in the following trial. The water consumption was 3152.9 ± 191.6 mL in both trials. The environmental temperature was 32.2 ± 0.1 °C and 32.5 ± 0.1 °C in the AA and PL trial, respectively. The relative humidity was $49.5 \pm 0.4\%$ and $49.7 \pm 0.3\%$ in the AA and PL trial, respectively. There was no significant difference between the trials.

Simulated match

The simulated match was modified from Wu et al. (2010) with the intensity similar to real competitions. It consisted of alternating 12 receiving and 12 service games. Each game consisted of six points and six balls were hit in each point. The balls were fed at the frequency of six balls per 10 s by a serving machine (Tennis Tower Competitor; Sports Tutor Inc., Burbank, California, USA). The receiving games started with a forehand groundstroke, followed by two backhand groundstrokes, a forehand groundstroke, and two volleys. The service games started with a service, followed by two backhand groundstrokes, a forehand groundstroke, and two volleys. The participants were asked to return to the central line during groundstrokes, and to approach to the net during volleys. A 20 s break was allowed between each point, and a 90 s break was allowed after each receiving game. A 180 s break was given between game 12 and 13. Heart rate was monitored throughout the study period using a short-ranged telemeter (EXEL SPORT, Cardiosport, West Sussex, UK). The ratings of perceived exertion (RPE) were recorded using the Borg scale (Borg, 1982) after game 6, 12, 18, and 24.

Perceptual-motor performance test

The perceptual-motor performance test included a central and a cross-court groundstroke tests, modified from The Loughborough Tennis Skill Test (Davey et al., 2002). The tennis balls were served by a machine (Tennis Tower Competitor) at a frequency of 15 balls per minute at the speed of 100 km/h. A 15 min rest was allowed between the two tests. A $1.5 \text{ m} \times 1.5 \text{ m}$ area, marked with white athletic tape in both corners of the singles court, was the target for groundstroke returns.

The central groundstroke test consisted of 60 returns (Fig. 2a). The subjects stood in front of the center mark at the baseline while the balls were served to his left or right. The examiner, an experienced tennis coach, would randomly move to his left or right along the baseline after the balls were served and prior to the balls reach the participant's side of the court, while holding a racquet at hand in a preparation stance. The subjects were asked to hit either forehand or backhand groundstroke to the direction opposite to the examiner's movement, aiming at the $1.5 \text{ m} \times 1.5 \text{ m}$ target area.

The cross-court groundstroke test also consisted of 60 rounds, with three balls in each round. The first 30 rounds were the balls served to the participants' left side (Fig. 3b),

followed by 30 rounds to their right side (Fig. 3c). The subjects always started from the center mark at the baseline. After each groundstroke, the subjects were asked to return to the 2-m region in the center of the baseline. The subjects hit the first two cross-court groundstrokes while the examiner standing next to the serving machine. After the third ball was served, the examiner either moved slightly to a 1-m region marked with white athletic tape, or moved to the center mark. The subjects were asked to hit the ball away from the examiner. Namely, if the examiner moved slightly, the participants would hit the ball down the line. If the examiner moved to the center mark, the subjects would still hit the ball cross-court. The subjects were asked to aim for the $1.5 \times 1.5 \text{ m}$ target area. Only the performance in the third stroke was recorded in each round.

When the examiner considered that the machine-served ball was off the target and/or with inappropriate speed, he would shout "not count." The participant then ignored the ball. Such shots were not included in the required number of hits. The direction, accuracy, consistency, and speed were recorded by a digital camera and analyzed afterward. During the analysis of video footage, each ball served by the machine was inspected to ensure that its trajectory and speed were consistent. The accuracy score was the number of balls landed within the $1.5 \text{ m} \times 1.5 \text{ m}$ target area. The consistency score was the number of balls landed within the backcourt (excluding the target). The ball speed was measured by a radar gun (Jugs Inc., Tualatin, Oregon, USA).

Blood sample collection

Venous blood samples were collected before breakfast, and before and after the simulated match. A 16 mL blood sample was collected into a tube containing EDTA as anticoagulant at each sampling time. The blood samples were centrifuged at $1500 \times g$ (Eppendorf 5810, Hamburg, Germany) to extract plasma. The aliquoted plasma samples were stored at -70 °C until further analysis.

Measurement of blood biochemical parameters

Plasma BCAA concentration was measured enzymatically (Biovision, Milpitas, California, USA). The absorbance at 450 nm was measured using a microplate spectrophotometer (Benchmark Plus, Bio-Rad, Hercules, California, USA). Plasma total tryptophan concentration was analyzed by a fluorescence assay (Bridge-It, Mediomics, St. Louis, Missouri, USA). The fluorescence at excitation 485 nm and emission 665 nm was read by a microplate fluorescence reader (Plate Chameleon, Hidex, Turku, Finland). Plasma NO_x concentrations were determined using Griess reagent (Green et al., 1982) and the absorbance at 450 nm was measured by a microplate spectrophotometer. Plasma concentrations of urea, glucose, lactate, NH_3 , glycerol, and NEFA were measured with an automatic analyzer (Hitachi 7020, Tokyo, Japan) using commercial kits (Randox, Antrim, UK). The plasma concentrations of all parameters were corrected for the changes in plasma volume using hemoglobin concentration and hematocrit in whole blood (Costill & Fink, 1974). The changes in post-exercise plasma volume ranged from -7.3% to 7.7% .

Statistical analysis

All values were expressed as mean \pm SEM. The results were analyzed by 2-way (trial \times time) analysis of variance with

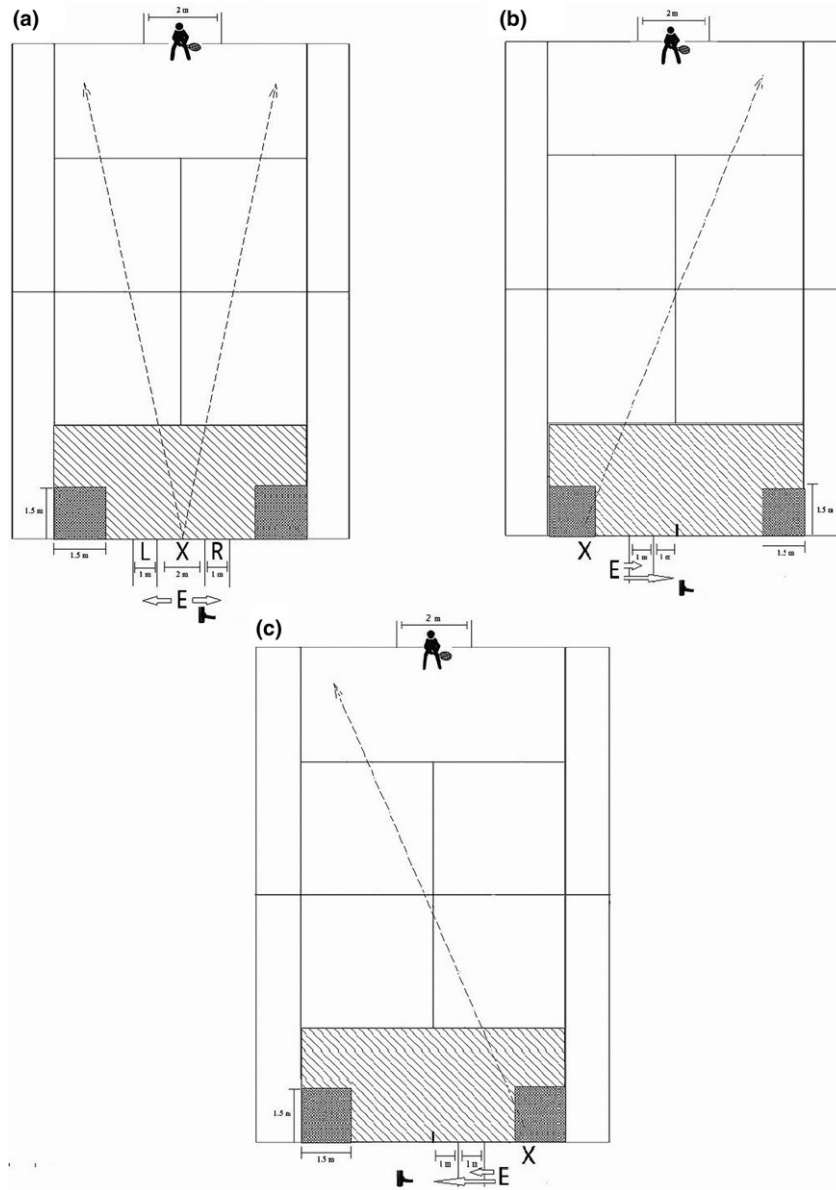


Fig. 2. Court layout for the perceptual-motor performance test. (a) Central groundstroke test; (b) cross-court groundstroke test to the right; (c) cross-court groundstroke test to the left. ■: Accuracy score area; ▨: Consistency score area; X: Tennis ball serving machine; E: Examiner; - - -: Ball fed direction; ■: Radar gun.

repeated measurements. If the time effect is significant, the differences between each time points within the same trial were determined by post hoc Bonferroni analysis. If the time \times trial interaction effect is significant, the difference between the two trials was identified by one-way analysis of covariance with the pre-match (perceptual-motor performance test) or baseline (blood biochemical parameters) level as the covariant. A $P < 0.05$ was considered statistically significant.

Results

In the perceptual-motor performance test, the percentage of balls hit to the correct direction, that is,

opposite to the examiners movement was significantly decreased by 27.6% after the simulated match in the PB trial. On the other hand, this indicator of perceptual-motor performance was maintained in the AA trial (Fig. 3a). It led to a significantly higher rate of correct direction after the simulated match in the AA trial than that in the PB trial ($P < 0.001$, effect size = 1.19). The results were similar in both central and cross-court groundstrokes. The groundstroke accuracy was significantly decreased in both trials after the simulated match (Fig. 3b). However, the magnitude of decline was smaller in the AA trial, resulting in significantly better post-match

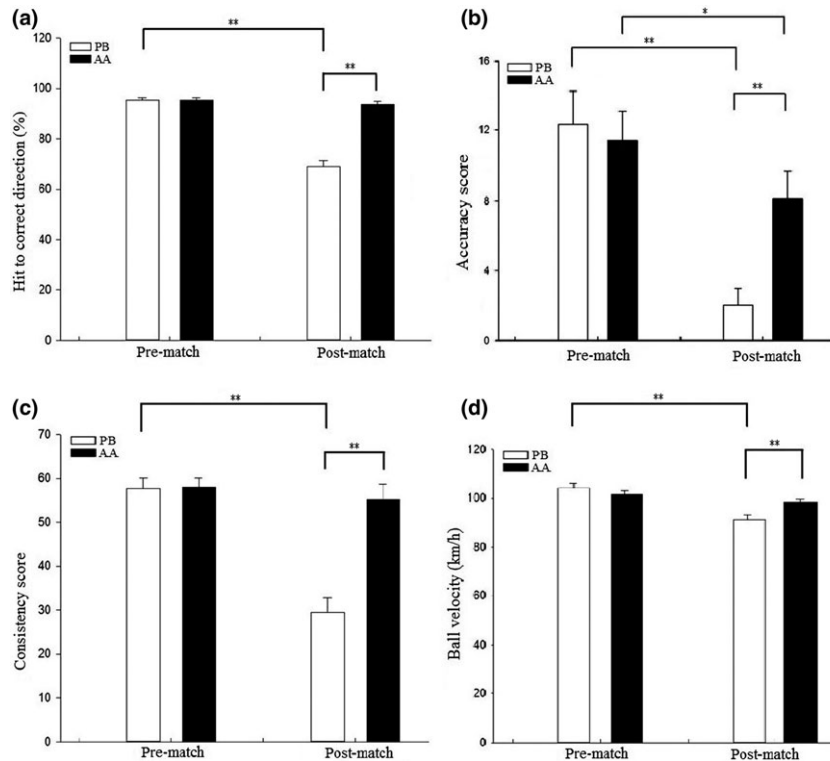


Fig. 3. Results of perceptual-motor performance test before and after the simulated match in the AA and PB trials. (a) percentage of balls hit to the correct direction^a; (b) accuracy score^b; (c) consistency score^c; (d) ball velocity^d. Notes: ^aMain effects: trial: $P < 0.001$; time: $P < 0.001$; interaction: $P < 0.001$. ^bMain effects: trial: $P = 0.037$; time: $P < 0.001$; interaction: $P = 0.002$. ^cMain effects: trial: $P < 0.001$; time: $P < 0.001$; interaction: $P < 0.001$. ^dMain effects: trial: $P = 0.129$; time: $P < 0.001$; interaction: $P < 0.001$. * $P < 0.05$; ** $P < 0.001$. PB: placebo; AA: BCAA, arginine, citrulline.

groundstroke accuracy in the AA trial than that in the PB trial ($P < 0.001$, effect size = 1.01). The post-match consistency score was unchanged after the match in the AA trial, whereas it was significantly deteriorated in the PB trial (Fig. 3c). The unchanged perceptual-motor and skill performance in the AA trial was not the result of speed-accuracy trade-off. The ball speed in the groundstrokes remained the same after the simulated match in the AA trial (Fig. 3d). On the contrary, the ball speed was significantly decreased after the match in the PB trial.

The supplementation of amino acids resulted in significantly higher plasma BCAA concentration before the simulated match (Fig. 4a). Average post-match BCAA concentration was 71.8% higher than the baseline in the AA trial ($P < 0.001$). Plasma total tryptophan concentration in the PB trial was significantly higher in the pre-match and post-match than the baseline ($P = 0.027$ and 0.009 , respectively), while it remained unchanged in the AA trial (Fig. 4b). The supplementation in the AA trial resulted in a significantly lower total tryptophan/BCAA ratio than the PB trial before (AA trial: 0.038 ± 0.003 ; PB trial: 0.073 ± 0.006 , $P < 0.001$, effect size = 1.32) and after (AA trial: 0.040 ± 0.008 ;

PB trial: 0.089 ± 0.006 , $P < 0.001$, effect size = 0.71) the simulated match (Fig. 4c).

The plasma biochemical parameters are presented in Table 1. The two trials resulted in similar plasma NH_3 concentration after the simulated match. In the AA trial post-match plasma NH_3 concentration was significantly increased from the baseline ($P = 0.014$), while it showed a trend of increase from the baseline in the PB trial ($P = 0.082$). Plasma NO_x concentration after the match was elevated from the baseline and was significantly higher than that in the PB trial ($P = 0.048$). The other biochemical variables investigated were not significantly different between the two trials.

Throughout the simulated match, the subjects in the AA trial showed significantly lower heart rate at the end of each game (Fig. 5). As the result, they also reported significantly lower RPE than in the PB trial at the same time point (Table 2). Despite less effort, the AA trial showed significantly higher successful return rate than the PB trials in both set 1 (AA trial: $79.6 \pm 1.1\%$; PB trial: $75.2 \pm 1.1\%$, $P = 0.034$) and set 2 (AA trial: $76.7 \pm 1.0\%$; PB trial: $69.0 \pm 1.2\%$, $P < 0.001$) in the simulated match. The PB trial also showed a larger decline in the successful return rate between set 1 and set 2.

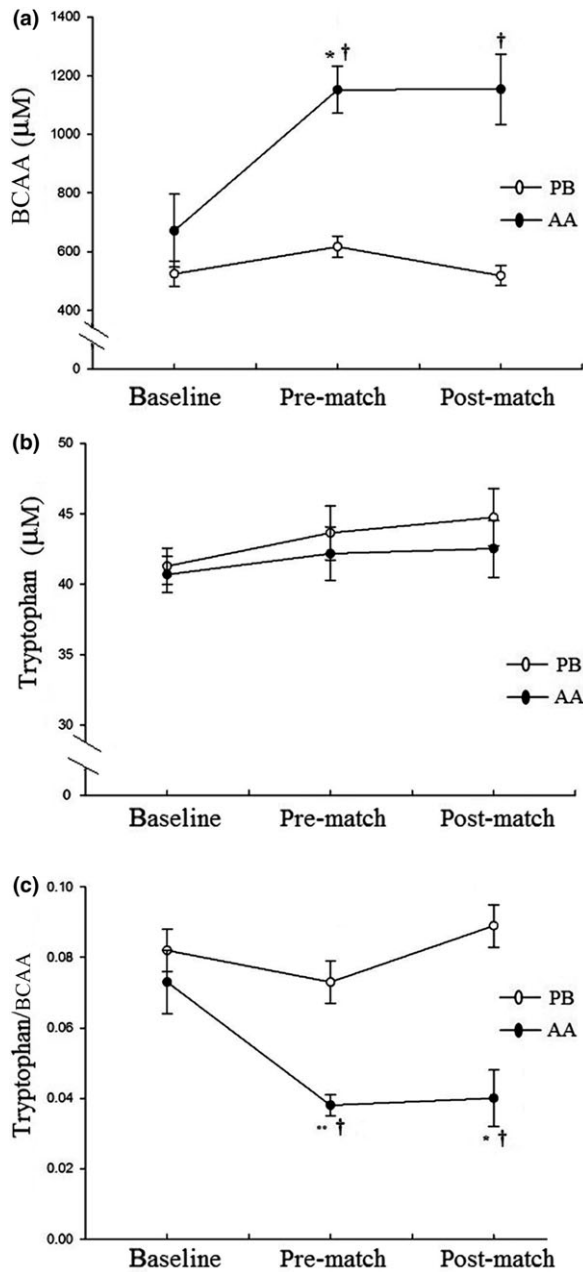


Fig. 4. Plasma amino acid concentrations before and after the simulated match in the AA and PL trials. (a) branched-chain amino acids^a; (b) tryptophan^b; (c) tryptophan/branched-chain amino acids ratio^c. Notes: ^aMain effects: trial: $P < 0.001$; time: $P = 0.004$; interaction: $P = 0.015$. ^bMain effects: trial: $P < 0.001$; time: $P = 0.004$; interaction: $P = 0.302$. ^cMain effects: trial: $P < 0.001$; time: $P = 0.001$; interaction: $P = 0.001$. * $P < 0.05$; ** $P < 0.01$, significantly different from baseline in the same trial. †Significantly different from the PB trial at the same time point, $P < 0.001$. PB: placebo; AA: BCAA, arginine, citrulline.

Discussion

Success in tennis requires a combination of excellent motor and perceptual-motor performance (Williams & Ericsson, 2005). This study suggested that the

Table 1. Plasma concentrations of biochemical parameters before and after the simulated tennis match in the PB and AA trials, mean \pm SEM

	Trial	Baseline	Pre-match	Post-match
NH ₃ (μM)	PB	67.5 \pm 11.9	71.0 \pm 8.1	112.4 \pm 12.4
	AA	58.6 \pm 7.4	64.2 \pm 6.3	114.3 \pm 11.4*
NO _x (μM)	PB	51.4 \pm 11.4	46.1 \pm 5.9	48.3 \pm 6.7
	AA	45.7 \pm 7.1	61.5 \pm 14.6	80.7 \pm 9.5†*
Urea (mM)	PB	2.41 \pm 0.14	2.73 \pm 0.17*	3.36 \pm 0.15*
	AA	2.14 \pm 0.14	2.55 \pm 0.12*	3.21 \pm 0.14*
NEFA (mM)	PB	0.62 \pm 0.12	0.39 \pm 0.14	1.56 \pm 0.23*
	AA	0.82 \pm 0.19	0.27 \pm 0.06*	1.67 \pm 0.16*
Glycerol (μM)	PB	60.9 \pm 11.2	51.0 \pm 6.0	274.0 \pm 43.9*
	AA	77.4 \pm 16.4	60.3 \pm 12.0	304.1 \pm 46.8*
Lactate (mM)	PB	2.59 \pm 0.23	3.02 \pm 0.18	3.37 \pm 0.52
	AA	2.64 \pm 0.39	2.60 \pm 0.26	3.06 \pm 0.53
Glucose (mM)	PB	5.56 \pm 0.22	5.27 \pm 0.44	5.00 \pm 0.20
	AA	5.25 \pm 0.13	4.70 \pm 0.24	5.12 \pm 0.12

PB: placebo; AA: BCAA, arginine, citrulline; NEFA: non-esterified fatty acid.

*Significantly different from baseline in the same trial ($P < 0.05$).

†Significantly different from the PB trial at the same time point ($P < 0.05$).

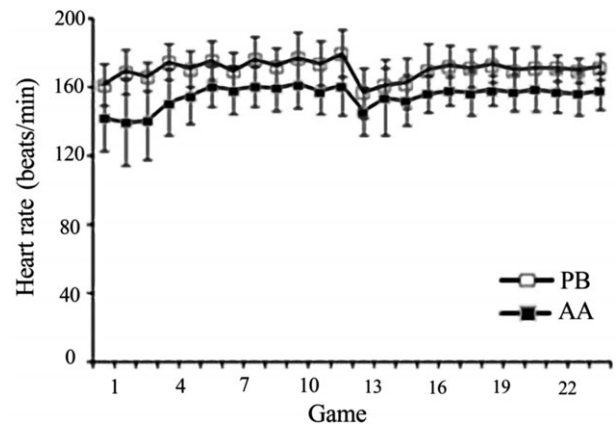


Fig. 5. Heart rate at the end of each game during the simulated match in the AA and PB trials. Main effects: trial: $P = 0.004$; time: $P < 0.001$; interaction: $P = 0.247$. PB: placebo; AA: BCAA, arginine, citrulline.

Table 2. Ratings for perceived exertion during the simulated match

Trial	Game 6	Game 12	Game 18	Game 24
PB	14.6 \pm 0.9	15.9 \pm 1.0	17.3 \pm 0.6	18.4 \pm 0.3
AA	11.0 \pm 0.3	13.8 \pm 0.8	14.9 \pm 0.8	16.2 \pm 0.7

PB: placebo; AA: BCAA, arginine, citrulline.

Main effects: trial: $P = 0.008$; time: $P < 0.001$; interaction: $P = 0.371$.

combined supplementation of BCAA, arginine, and citrulline could prevent the decline in perceptual-motor performance, as well as the accuracy and consistency of groundstrokes after a 2-h simulated match. To our knowledge, it is the first study that

investigated the beneficial effect of supplements in perceptual-motor performance in a tennis-specific match-like setting.

The ability to better anticipate opponent's movements in order to select and execute appropriate actions is a major factor that separates experts from non-experts in many sports (North et al., 2009). In this study, we showed that this perceptual-motor performance could be deteriorated after a prolonged tennis match. Similarly, the perceptual skill, measured by anticipation of service direction of video clips, was decreased during a prolonged simulated tennis match (Hornery et al., 2007a, b, c). The decline in the perceptual-motor performance after the match may be the result of central fatigue as plasma tryptophan/BCAA ratio was significantly increased in the PB trial in this study, similar to the previous report (Struder et al., 1995). In the AA trial, the significantly decreased plasma tryptophan/BCAA ratio would reduce cerebral serotonin synthesis and subsequently alleviate central fatigue (Gomez-Merino et al., 2001). In agreement, previous studies have reported that BCAA supplementation could maintain cognitive functions (Hassmen et al., 1994) and the performance in reactive motor skills (Stepito et al., 2011), while reducing the feeling of fatigue (Blomstrand et al., 1997) during strenuous exercise. Furthermore, the AA and PB trials produced similar post-match plasma NH_3 concentrations, indicating the absence of excess hyperammonemia from BCAA metabolism. It could be the result of increased NO synthesis, by arginine and citrulline, that enhanced vasodilation in the working muscles (Clarkson et al., 1996; Rouge et al., 2007). A previous study also showed that the same combination of amino acids prevented the decline in taekwondo-specific cognitive functions after three simulated matches (Chen IF et al., submitted). Pre-motor reaction time, the delay between the stimulus and the electromyographic signal in the working muscle, in taekwondo roundhouse kicks was maintained after three simulated matches in the supplemented trial, while it was significantly deteriorated in the placebo trial. The reaction time in the secondary task in a dual-task test also showed similar results. The results from our previous (Chen IF et al., submitted) and the present study clearly indicated that the combined amino acids supplementation could prevent the fatigue-induced decline in cognitive performance in sport-specific settings.

A close positive relationship between plasma NEFA and free tryptophan concentrations has been reported due to their competition to albumin binding (Curzon et al., 1973). Although only total tryptophan concentration was measured in this study, the

similar post-match plasma NEFA concentrations between the two trials suggested that free tryptophan levels were also likely to be comparable. Thus, it is reasonable to assume that the significantly elevated plasma BCAA concentration in the AA trial would lead to a lower free tryptophan/BCAA ratio and reduced cerebral serotonin synthesis.

Previous studies have investigated potential ergogenic strategies such as carbohydrate, caffeine, and hyperhydration on neuromuscular and skill performance in tennis with limited effects (Hornery et al., 2007a, b, c). Most studies examining tennis skill performance using pre-determined, unopposed tasks in which the subjects knew which direction to hit before the ball was fed to them. In these "closed" situations, the perceptual-motor ability is generally not required and the participants could maintain certain degree of skill performance. For example, there was no decline in service and groundstroke skill performance after a 2-h match (McRae & Galloway, 2012). At the moderate-intensity fatigue in which the heart rate was similar to that reported in real match play, the groundstroke accuracy was maintained in both expert and non-expert players (Lyons et al., 2013). Furthermore, the accuracy scores of service and forehand groundstroke were unchanged after a simulated match (Wu et al., 2010). On the other hand, several studies did reveal the declines in skill performance. The groundstroke accuracy was deteriorated (Hornery et al., 2007a, b, c), while the service and forehand groundstroke consistency scores were significantly decreased after a simulated match (Wu et al., 2010). These results indicated that, although there is evidence for neuromuscular fatigue after prolonged tennis play (Hornery et al., 2007a, b, c), experienced players can generally maintain the racquet control. In studies that used "open" environment such as a match play, the success rate of serve return showed a trend of deteriorating toward the third hour in a match play, but other performance variables remained unaltered (Gomes et al., 2013). It has been suggested that to investigate fatigue in tennis would require process-based measurements such as perceptual-motor performance, rather than simply assessing outcome measurements such as stroke accuracy and consistency (Hornery et al., 2007a, b, c). The present study adopted an "open" environment in which the subjects have to anticipate and/or identify the opponent's movement before hitting balls to the desired direction and location. This method is similar to the situations in real match play while controlling other variables, such as ball speed and location, which are necessary for the experimental design.

According to the speed-accuracy trade-off theory, stroke accuracy is largely maintained, whereas stroke

velocity is more likely to deteriorate under physiological stress (Fitts, 1954). However, this is not the case in the current study. The groundstroke consistency and accuracy scores were both significantly higher with faster ball speed after the match in the AA trial than the PB trial. These results indicated that the subjects in the AA trial were able to hit the balls more accurately with higher speed, providing an advantage in real matches.

It has been revealed that carbohydrate supplementation could modestly improve the affective state and perception of internal stress (Gomes et al., 2014), while maintaining stroke quality (Vergauwen et al., 1998) in prolonged tennis play. Our subjects in both trials had adequate glycogen status after 2-day dietary control and a standard breakfast containing 1.0 g/kg carbohydrate. In addition, they performed both trials at the euglycemic state. In agreement, it has been shown that a 3-h tennis match would not cause hypoglycemia in the subjects with adequate carbohydrate intake in the previous day (Gomes et al., 2014). However, the significant decline in perceptual-motor performance, indicated by the lower rate of balls hit to the correct direction, was still present after the simulated match in the PB trial.

Using the protocol that elicits similar physiological responses to real tennis matches is one of the important factors in investigating beneficial effects of interventions (Hornery et al., 2007a, b, c). Each point in our simulated match is composed of six strokes within 10 s. This is similar to the pace of 3–5 strokes per rally that lasts 6–10 s in international matches (Hornery et al., 2007a, b, c). The post-exercise plasma lactate concentration in both trials in the current study was consistent with those observed in real match plays (Reid & Duffield, 2014).

Unexpectedly, all subjects showed lower heart rate during the simulated match in the AA trial, compared to the PB trial. This was not the result of lack of effort because the subjects showed higher successful return rate in the AA trial. It has been suggested that the high plasma BCAA concentration would inhibit cerebral uptake of tyrosine, in addition to tryptophan, as these large neutral amino acids share the same transporter across the blood–brain barrier (Fernstrom, 2013). Tyrosine is the precursor for cerebral synthesis of dopamine, which could stimulate the hypothalamic–pituitary–adrenal (HPA) axis and increase the release of cortisol from adrenal cortex. The elevated cortisol would in turn stimulate the release of epinephrine from adrenal medulla by activating phenylethanolamine N-methyltransferase, the key enzyme in epinephrine synthesis (Wong et al., 2008). Therefore, the possible inhibition of cerebral dopamine synthesis by BCAA supplementation

could result in lower epinephrine release, hence, lower heart rate. This is probably another reason why the subjects in the AA trial reported lower RPE. However, energy metabolism did not seem to be affected as the biochemical variables for carbohydrate and fat metabolism were all similar between the two trials. Future studies could investigate dopamine, HPA axis, cortisol, and catecholamines after BCAA supplementation using animal models.

This perceptual-motor performance test used in this study combined cognitive judgment, that is, the direction of the opponent's movement, with motor function, that is, hit the ball to the desired direction and location. Therefore, it has to be taken into consideration that both perceptual-cognitive and neuromuscular functions contributed to the performance in this test. Although we have tried our best to reflect realistic situations in the perceptual-motor performance test, it is still possible that lack of an opponent could impact the application of this test to match performance (Pinder et al., 2011).

In conclusion, this study suggested that the combined supplementation of BCAA, arginine, and citrulline could prevent the decline in perceptual-motor performance after a prolonged simulated match in experienced tennis players. By applying the tennis-specific test that reflects real match conditions, the results of this study could provide significant practical applications. The supplementation regime may be extended to other sports in which the perceptual-motor performance is vital. The effect of this supplementation in real tennis matches warrants further investigation.

Perspectives

The perceptual-motor performance, an integral part of tennis match, is deteriorated after a prolonged simulated match. The results of this study could have impact on the prevention of such deterioration by alleviating central fatigue without excess hyperammonemia. Future studies should further explore the application of this supplementation regime in real matches. Furthermore, the cerebral synthesis of neurotransmitters from tryptophan and tyrosine could be investigated using animal models.

Key words: Central fatigue, neurotransmitter, skill, exercise performance.

Acknowledgements

This study is supported by the Ministry of Science and Technology, Taiwan (102-2410-H-028-002-MY3). The authors thank Ms Yu-Fang Huang for her technical assistance.

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