

# 科技部補助專題研究計畫成果報告 期末報告

## 補充支鏈胺基酸、精胺酸與瓜胺酸對中樞疲勞與反應式技術表現的影響(第3年)

計畫類別：個別型計畫

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執行單位：國立臺灣體育運動大學運動科學中心

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中華民國 105 年 11 月 10 日

中文摘要：支鏈胺基酸、精胺酸與瓜胺酸具有多重生理功能，支鏈胺基酸可能減少色胺酸進入大腦，進而降低血清素合成；精胺酸與瓜胺酸則可能增加一氧化氮合成，並促進尿素循環，促進血氨移除，這三種胺基酸綜合補充，可能降低運動誘發之中樞疲勞。本研究群已發現補充支鏈胺基酸與精胺酸可透過抑制中樞疲勞，提升體能表現。除了體能表現之外，技術表現也是決定比賽勝負的重要關鍵，初步研究顯示中樞疲勞可能也會影響技術表現，因此本研究建立新的反應式專項技術測試，以模擬真正比賽狀況。本三年期計畫探討合併補充0.17 g/kg支鏈胺基酸、0.05 g/kg精胺酸、與0.05 g/kg瓜胺酸對網球及跆拳道模擬比賽後，中樞疲勞及反應式專項技術表現的影響，以及對連續二天耐力運動表現得影響。採用隨機交叉實驗設計，以各項目訓練有素的大學運動員為受試者，使用自覺運動強度及血清色胺酸/支鏈胺基酸濃度比率做為中樞疲勞之主觀與客觀指標。在第一年的網球部分，包括左右隨機與對角線底線抽球兩種測試，受試者需要根據對手移動的方向，再決定擊球的方向，結果顯示經過約2小時的模擬比賽，賽前補充支鏈胺基酸、精胺酸、瓜胺酸組在判斷擊球方向正確性，以及擊球準確度與一致性都顯著高於控制組。在第二年跆拳道部分，則根據訊息處理理論發展單任務與雙任務之反應式技術測試，以前動作反應時間作為評估大腦反應能力的指標，結果顯示經過3場模擬比賽後，於第三場比賽前補充支鏈胺基酸、精胺酸、瓜胺酸組可以維持單任務與雙任務之前動作反應時間，而控制組則延長了前動作反應時間。在第三年耐力運動表現方面，第一天進行5000公尺計時賽，第二天進行10000公尺計時賽，在每天賽前補充支鏈胺基酸、精胺酸、瓜胺酸組，5000及10000公尺成績皆顯著高於控制組。在上述三個實驗中都發現補充組血液血清色胺酸/支鏈胺基酸濃度比率顯著降低，氨濃度顯著降低，尿素濃度顯著增加，但自覺運動強度與控制組相同。本三年期的研究顯示，補充支鏈胺基酸、精胺酸、瓜胺酸可以透過減少大腦血清素形成，增加血氨移除，進而減緩中樞疲勞及連帶造成的判斷與反應能力下降，提升體能及技術表現，對於提升球類、技擊、耐力等型態的運動項目都有實質的幫助。

中文關鍵詞：支鏈胺基酸、精胺酸、瓜胺酸、中樞疲勞、反應式專項技術表現

英文摘要：Our group has revealed that supplementation of BCAA and arginine could improve physical performance in simulated handball and basketball games by alleviating central fatigue. In addition to physical performance, skill is also a crucial factor for athletic success. Few studies have suggested that central fatigue may also impair skill performance. This proposed study aims to establish novel reactive sport-specific skill tests to reflect real competitions in which decisions are often initiated in response to external stimuli such as the position or movement of players and/or the ball. This study investigated the effect of 0.17 g/kg BCAA, 0.05 g/kg arginine, and 0.05 g/kg citrulline on reactive sport-specific skill performance under physical and central

fatigue induced by simulated matches in tennis and taekwondo. Their effect on two consecutive days of endurance performance was also examined. This study used a randomized cross-over, placebo-control, double-blind design. The subjects will be well-trained college athletes in the respective sports. The ratings of perceived exertion and plasma tryptophan/BCAA ratio were used as the subjective and objective markers for central fatigue, respectively. The first year focused on tennis, an individual racquet sport. The test included random left-right and baseline forehand stroke. The subjects had to decide the direction to hit the ball after observing the opponent's movement. The results showed that after a 2-hr simulated match, the supplemented trial showed significantly higher rate for hitting to the correct direction, as well as higher scores of accuracy and consistency. The second year focused on taekwondo, an individual combat sport that Taiwan has the strongest international competitiveness. A single-task and double-task tests were developed. Premotor reaction time was used to reflect the time required to process the signal in the brain. The supplemented trial maintained premotor reaction time in single- and double-task tests, while the control trial showed significant decline after three simulated matches. In the third year, the subjects completed a 5000 m time trial on the first day, and a 10000 m time trial on the second day. The supplemented trial had significantly better performance on both days.

英文關鍵詞： branched-chain amino acids, arginine, citrulline, central fatigue, reactive sport-specific skill performance

# 科技部補助專題研究計畫成果報告

(期中進度報告/期末報告)

## 補充支鏈胺基酸、精胺酸與瓜胺酸對中樞疲勞與反應式技術表現的影響

計畫類別：個別型計畫 整合型計畫

計畫編號：MOST 102 — 2410 — H — 028 — 002 — MY3

執行期間：102 年 8 月 1 日至 105 年 7 月 31 日

執行機構及系所：國立臺灣體育運動大學運動科學中心

計畫主持人：張振崗

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計畫參與人員：黃玉芳、邱志暉、陳一凡、薛淳方

本計畫除繳交成果報告外，另含下列出國報告，共 \_\_\_ 份：

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出席國際學術會議心得報告

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中 華 民 國 105 年 9 月 7 日

## 摘要

支鏈胺基酸、精胺酸與瓜胺酸具有多重生理功能，支鏈胺基酸可能減少色胺酸進入大腦，進而降低血清素合成；精胺酸與瓜胺酸則可能增加一氧化氮合成，並促進尿素循環，促進血氨移除，這三種胺基酸綜合補充，可能降低運動誘發之中樞疲勞。本研究群已發現補充支鏈胺基酸與精胺酸可透過抑制中樞疲勞，提升體能表現。除了體能表現之外，技術表現也是決定比賽勝負的重要關鍵，初步研究顯示中樞疲勞可能也會影響技術表現，因此本研究建立新的反應式專項技術測試，以模擬真正比賽狀況。本三年期計畫探討合併補充 0.17 g/kg 支鏈胺基酸、0.05 g/kg 精胺酸、與 0.05 g/kg 瓜胺酸對網球及跆拳道模擬比賽後，中樞疲勞及反應式專項技術表現的影響，以及對連續二天耐力運動表現得影響。採用隨機交叉實驗設計，以各項目訓練有素的大學運動員為受試者，使用自覺運動強度及血清色胺酸/支鏈胺基酸濃度比率做為中樞疲勞之主觀與客觀指標。在第一年的網球部分，包括左右隨機與對角線底線抽球兩種測試，受試者需要根據對手移動的方向，再決定擊球的方向，結果顯示經過約 2 小時的模擬比賽，賽前補充支鏈胺基酸、精胺酸、瓜胺酸組在判斷擊球方向正確性，以及擊球準確度與一致性都顯著高於控制組。在第二年跆拳道部分，則根據訊息處理理論發展單任務與雙任務之反應式技術測試，以前動作反應時間作為評估大腦反應能力的指標，結果顯示經過 3 場模擬比賽後，於第三場比賽前補充支鏈胺基酸、精胺酸、瓜胺酸組可以維持單任務與雙任務之前動作反應時間，而控制組則延長了前動作反應時間。在第三年耐力運動表現方面，第一天進行 5000 公尺計時賽，第二天進行 10000 公尺計時賽，在每天賽前補充支鏈胺基酸、精胺酸、瓜胺酸組，5000 及 10000 公尺成績皆顯著高於控制組。在上述三個實驗中都發現補充組血液血清色胺酸/支鏈胺基酸濃度比率顯著降低，氨濃度顯著降低，尿素濃度顯著增加，但自覺運動強度與控制組相同。本三年期的研究顯示，補充支鏈胺基酸、精胺酸、瓜胺酸可以透過減少大腦血清素形成，增加血氨移除，進而減緩中樞疲勞及連帶造成的判斷與反應能力下降，提升體能及技術表現，對於提升球類、技擊、耐力等型態的運動項目都有實質的幫助。

關鍵詞: 支鏈胺基酸、精胺酸、瓜胺酸、中樞疲勞、反應式專項技術表現

## Abstract

Branched-chain amino acids (BCAA), arginine, and citrulline have multiple physiological functions. BCAA could reduce cerebral uptake of tryptophan, leading to decreased synthesis of serotonin in the brain. Arginine and citrulline could increase nitric oxide biosynthesis and reduce exercise-induced hyperammonemia by increasing urea cycle. The combination of these supplements could reduce exercise-induced central fatigue. Our group has revealed that supplementation of BCAA and arginine could improve physical performance in simulated handball and basketball games by alleviating central fatigue. In addition to physical performance, skill is also a crucial factor for athletic success. Few studies have suggested that central fatigue may also impair skill performance. This proposed study aims to establish novel reactive sport-specific skill tests to reflect real competitions in which decisions are often initiated in response to external stimuli such as the position or movement of players and/or the ball. This study investigated the effect of 0.17 g/kg BCAA, 0.05 g/kg arginine, and 0.05 g/kg citrulline on reactive sport-specific skill performance under physical and central fatigue induced by simulated matches in tennis and taekwondo. Their effect on two consecutive days of endurance performance was also examined. This study used a randomized cross-over, placebo-control, double-blind design. The subjects will be well-trained college athletes in the respective sports. The ratings of perceived exertion and plasma tryptophan/BCAA ratio were used as the subjective and objective markers for central fatigue, respectively. The first year focused on tennis, an individual racquet sport. The test included random left-right and baseline forehand stroke. The subjects had to decide the direction to hit the ball after observing the opponent's movement. The results showed that after a 2-hr simulated match, the supplemented trial showed significantly higher rate for hitting to the correct direction, as well as higher scores of accuracy and consistency. The second year focused on taekwondo, an individual combat sport that Taiwan has the strongest international competitiveness. A single-task and double-task tests were developed. Premotor reaction time was used to reflect the time required to process the signal in the brain. The supplemented trial maintained premotor reaction time in single- and double-task tests, while the control trial showed significant decline after three simulated matches. In the third year, the subjects completed a 5000 m time trial on the first day, and a 10000 m time trial on the second day. The supplemented trial had significantly better performance on both days.

Keywords: branched-chain amino acids, arginine, citrulline, central fatigue, reactive sport-specific skill performance

## 前言

支鏈胺基酸 (branched-chain amino acids, BCAA) ，包含白胺酸 (leucine) 、異白胺酸 (isoleucine) 與纈胺酸 (valine) ，以及精胺酸 (arginine, Arg) 與瓜胺酸 (citrulline, Cit) 具多重的生理功能，在不同類型的運動中可能增進運動表現。補充 BCAA 可能可以降低游離色胺酸 (free tryptophan, fTRP) 進入大腦，降低血清素 (serotonin) 合成，進而延緩中樞疲勞產生 (Blomstrand, Hassmén, Ekblom, & Newsholme, 1997) ；Arg 可能藉由合成一氧化氮 (nitric oxide, NO) 刺激血管擴張及增加血流量，增進代謝產物的移除 (Schaefer et al., 2002) 。Arg 亦為尿素循環 (urea cycle) 的中間產物，可經由精胺酸酶 (Arginase) 分解產生鳥胺酸 (ornithine) 及尿素。因此，Arg 可能提高尿素循環的速率，可促進血氨代謝 (Tsuei et al., 2005) 。Cit 則為一種強效的 NO 的前驅物，亦為尿素循環的中間產物，可促進運動後血氨的代謝 (Schwedhelm et al., 2008) 。因此，給予優秀運動員綜合補充 BCAA、Arg 與 Cit，可能可以促進機體內的生理功能、降低運動誘發之中樞疲勞並提升運動表現。

技術或判斷能力是競技運動相當重要的能力，中樞疲勞除了會影響體能，也可能造成技術或判斷能力的下降，但目前仍缺乏有關中樞疲勞對技術或判斷能力之影響的研究。另外有鑑於規律式的技術測試並無法顯著區分優秀與非優秀選手的能力，因此本計畫發展反應式技術，或雙任務技術測試的方法，以有效鑑別優秀選手技術與判斷能力的差異。本計畫以三年的時間，分別探討在各專項運動後補充 BCAA、Arg 與 Cit，對後續反應式技術能力的影響，第一年探討個人球類運動-網球，第二年探討技擊運動-跆拳道，續於第三年探討此補充品對連續二天耐力運動表現的影響。

## 文獻探討

### 一、支鏈胺基酸對運動表現與中樞神經疲勞的影響

中樞疲勞是指源自中樞神經系統內的疲勞，許多研究顯示，腦中神經傳遞物質血清素 (serotonin) 的濃度增加，可能會導致中樞疲勞反應的發生 (Blomstrand, 2006) 。亦有文獻指出血清素和運動表現具較高之關聯性，當腦內血清素濃度較低時，對運動表現是有幫助的，其可增加激發水準、增進動機及促進神經肌肉的協調反應；反之，當血清素濃度較高時，則會降低運動員的動機、讓人感覺昏睡疲勞，並使神經的協調功能降低 (Davis, Alderson, & Welsh, 2000) 。fTRP 則是調控腦中血清素合成的重要前驅物，可影響血清素合成的反應速率。色胺酸 (tryptophan, Trp) 與 BCAA 一樣為必需胺基酸，同樣經運送傳遞系統 (L-system) ，藉由血腦屏障 (blood-brain barrier, BBB) 進入腦中，其與神經突觸的接受器結合後，腦內開始分泌合成血清素，進而造成疲勞的產生 (圖四) 。血液中有 90% 的 Trp 與白蛋白 (albumin) 以結合型態存在，有 10% 以游離型態存在 (McMeeamy & Oncley, 1958) 。

在長時間的運動過程中，隨有氧代謝比例增加，促使體內分解三酸甘油脂 (triglycerides, TG)，形成脂肪酸以提供能量。當游離脂肪酸增加，會與 Trp 相互競爭白蛋白的結合位置，進而導致血液中 fTrp 的量增加。而 fTrp 則經由 L-system 通過血腦屏障進入腦內，使腦中血清素濃度增加 (Davis, Alderson, & Welsh, 2000) 。此外，經長時間的運動而導至肌肉肝醣被耗盡時，體內轉而利用蛋白質作為部份能量來源，造成血漿中 BCAA 濃度下降，使 fTrp/BCAA 比值上升，造成腦部血清素濃度增加，並導致中樞疲勞 (Blomstrand, Celsing, & Newsholme, 1988) 。

BCAA 約佔據飲食中必需胺基酸總量的三分之一，且在人體肌肉蛋白質中胺基酸總量約佔 14~18%

(Riaz et al., 2003)。BCAA 可直接代謝，做為骨骼肌的第三能量來源，僅次於醣類與脂肪 (Goldberg & Chang, 1978)。

BCAA 會與 Trp 相互競爭同一個運輸系統，若 BCAA 進入腦中與神經突觸之接受器結合，將會阻止血清素的產生 (圖四)。因此，在長時間的運動中，補充 BCAA 可增進血液中 BCAA 濃度，降低 fTrp/BCAA 比值，將可延緩腦內血清素堆積，進而降低疲勞，增進耐力運動表現 (Blomstrand et al., 1997)。Gomez-Merino 等 (2001) 於動物實驗中發現在跑步運動後 (25 公尺/分鐘，120 分鐘)，會造成海馬迴中的血清素濃度增加，而運動前給予 BCAA，則可防止血清素的合成。Mittleman, Ricci, 與 Bailey (1998) 針對受過訓練的 8 名男性與 8 名女於熱環境下進行實驗。在溫度調控於  $34.4 \pm 1.8^{\circ}\text{C}$ 、相對濕度  $39 \pm 14\%$  的環境下靜態休息 2 小時，隨後進行  $40\% \text{VO}_2\text{max}$  腳踏車運動直到衰竭。在運動期間，男性與女性受測者分別攝取 9 公克與 16 公克的 BCAA 或安慰劑。結果發現，補充 BCAA 有助於改善健康活力的表現，並在熱環境下，顯著增加中強度運動的耗竭時間，血漿中 BCAA 濃度顯著增加與 fTrp/BCAA 比值下降。Blomstrand 等 (1997) 以受過耐力訓練的男性自行車運動員進行  $70\% \text{VO}_2\text{max}$  腳踏車運動持續 60 分鐘後，隨後進行 20 分鐘最大強度運動，並於運動期間補充 BCAA 或安慰劑。結果發現，攝取 BCAA 可顯著降低運動所誘發的 fTrp/BCAA 比值、運動自覺量表 (ratings of perceived exertion, PRE) 與心理自覺量表 (rating of mental fatigue) 的分數，並改善運動後的認知表現。Davis, Welsh, De Volve, 與 Alderson (1999) 以 3 名男性和 5 名女性進行間歇性折返跑 (包括走路、 $55\% \text{VO}_2\text{max}$  慢跑、 $95\% \text{VO}_2\text{max}$  跑及衝刺) 直到衰竭，實驗交叉設計分為碳水化合物組 (CHO)、BCAA 組及安慰劑組，CHO 組於運動前 1 小時和運動前立即攝取 5 ml/kg ( $20\% \text{CHO}$ )，並於運動期間攝取 2 ml/kg ( $6\% \text{CHO}$ )，BCAA 組於運動前 1 小時攝取 5 ml/kg ( $20\% \text{CHO}$  與 7g BCAA:  $40\% \text{Val}$ 、 $35\% \text{Leu}$ 、 $25\% \text{Ile}$ ) 及運動前立即攝取 5ml/kg ( $6\% \text{CHO}$  與 7g BCAA)，結果顯示 CHO 組與 BCAA 組血漿中葡萄糖與胰島素顯著高於安慰劑組，FFA 顯著低於安慰劑組，且 CHO 組或 CHO + BCAA 組皆顯著提升運動表現。

目前 BCAA 對於增進耐力運動表現的研究仍具有許多的爭議，Van Hall 等 (1995) 以 10 名受過訓練的男性受測者，進行  $70\sim 75\%$  最大輸出功率腳踏車運動直到衰竭，並分別補充 18 克 BCAA、6 克 BCAA、3 克 Trp 與安慰劑，結果顯示補充高、低劑量 BCAA 或 Trp 對運動表現皆無影響。Cheuvron 等 (2004) 以 5 位健康的男性受測者於第一天上午進行  $50\sim 90\%$  最大輸出功率的腳踏車運動 (60 轉/分鐘，90 分鐘)，下午  $40^{\circ}\text{C}$  的熱環境下進行 2~3 小時跑步以降低體內肌肉肝醣含量，並讓身體脫水 ( $4\%$  體重)；第二天進行 60 分鐘的  $50\% \text{VO}_2\text{peak}$  腳踏車運動後，於熱環境下再進行 30 分鐘的時間測試，其中分別在運動前及運動中每隔 15 分鐘補充 BCAA 或安慰劑。結果發現，BCAA 測試 fTRP/BCAA 比值顯著降低，但在認知表現及運動表現則無顯著影響。Blomstrand, Andersson, Hassmen, Ekblom, 與 Newsholme (1995) 以 5 位受過耐力訓練的男性受測者隨機攝取  $6\%$  碳水化合物水溶液、7 克 BCAA 加上  $6\%$  碳水化合物水溶液與安慰劑，並在肝醣儲存量減少的情況下進行  $75\% \text{VO}_2\text{max}$ ，結果發現補充碳水化合物或 BCAA 並不影響運動過程淨蛋白質的降解。造成研究結果不一致的原因，可能與不同的補充時間、劑量、研究對象對於神經的反應，以及運動時間、強度與型態，和長時間運動誘發血漿氨濃度的增加有關 (Blomstrand, 2006; Meeusen, Watson, & Dvorak, 2006; Strüder et al., 1998)。

然而，近期 Stepto, Shipperd, Hyman, McInerney, 與 Pyne (2011) 以 15 位澳洲橄欖球員 (subelite) 進行實驗，採雙盲隨機交叉的方式給予含 BCAA 的胺基酸飲料或一般胺基酸飲料，經 3 小時後，受試者接受 30 分鐘的間歇性疲勞運動，進行反應運動技能測試與敏捷性測試。結果顯示於高強度間歇運動前補充 BCAA，有助於改善運動員的運動技能以及反應敏捷性。此外，補充 BCAA 後，對於 Stroop Color-Word 的認知功能測驗以及盤斯心情量表 (The Profile of Mood State, POMS) 的疲勞因子與活力

因子皆有顯著改善的效果，改善的原因可能與 fTrp/BCAA 數值下降有關。因此，由此研究可得知補充 BCAA 可能可以預防專項運動反應技術的下降。

## 二、運動與 BCAA 誘發高血氨與疲勞反應

BCAA 無法增進運動表現的原因之一可能與血漿氨濃度的增加有關 (Meeusen, Watson, & Dvorak, 2006; Strüder et al., 1998)，在激烈的運動過程中，由於單磷酸腺苷 (Adenosine monophosphate, AMP) 與次黃嘌呤核苷酸 (inosine monophosphate, IMP) 之間的脫氨作用，以及胺基酸的代謝而導致肌肉中血漿氨濃度增加 (Katz, Broberg, Sahlin, & Wahren, 1986)。此外，在耐力運動中，BCAA 的氧化會顯著增加，其先脫除胺基 (amino group) 後，利用轉胺作用 (transamination) 形成丙胺酸，於肝臟中先轉成丙酮酸，再經由醣質新生作用生成葡萄糖，經由血液輸送至肌肉中，以作為能量的來源 (圖五)。因此，經過長時間的耐力運動後，當體內肌肝醣耗盡時，則會促進 BCAA 的氧化，使血漿氨濃度增加 (Shimomura, Murakami, Nakai, Nagasaki, & Harris, 2004)。血氨可直接作用於腦中樞或通過改變腦膜對選擇性胺基酸的通透性來影響中樞功能並誘發中樞疲勞 (Mutch & Banister, 1983)。Nybo, Dalsgaard, Steensberg, Møller, 與 Secher (2005) 指出在長時間的運動中，會造成血漿氨濃度上升，並藉由神經物質的代謝而使大腦中血漿氨濃度上升並引起疲勞。

Watson, Shirreffs, 與 Maughan (2004) 利用 8 名健康男性受測者在熱環境下 (30°C) 進行 50% VO<sub>2</sub> peak 腳踏車運動至衰竭，於運動前 2 小時每 30 分鐘攝取 250 ml BCAA (12g/L)，以及在運動中每 15 分鐘攝取 150 ml，結果顯示 BCAA 組在運動前顯著增加 BCAA 濃度及降低 fTrp/BCAA 比值，但在運動期間血漿氨濃度顯著增加，運動表現、皮膚體溫、血乳酸及血糖則無顯著差異。Madsen, Maclean, Kiens, 與 Christensen (1996) 以 9 位自行車選手進行 100 公里自行車運動，並分別於運動前補充 5% 碳水化合物、5% 碳水化合物加上 18 公克 BCAA 與安慰劑。結果發現，BCAA 組於運動期間的血漿氨濃度顯著增加，對運動表現無顯著影響。MacLean, Graham, 與 Saltin (1994) 以 5 名男性進行 70% 最大做功能力的單腿伸膝動作，每回合 60 分中，共兩回合，並於中間休息的 45 分鐘內增補 BCAA (77mg/kg BW) 或安慰劑。結果發現補充 BCAA 會導致在運動過程中，肌肉會產生更高濃度的氨。MacLean, Graham, 與 Saltin (1996) 探討口服高劑量 BCAA (0.308 公克/公斤) 後，進行 64±2% 的最大伸膝運動並持續 90 分鐘。結果發現，補充 BCAA 雖可增加血漿中 BCAA 的濃度並促進運動時肌肉對 BCAA 的吸收，但肌肉中的氨濃度顯著提高，且丙胺酸與麩醯胺的含量增加，顯示在非最大運動期間，增加的血漿氨濃度主要來自於 BCAA 在肌肉中的降解。

綜合以上文獻顯示，補充高劑量 BCAA 可能會引起血漿氨濃度增加，而抵銷 BCAA 透過降低 fTrp/BCAA 的機制，延緩中樞疲勞的功用，而無法顯現增補 BCAA 的效果。目前已有多篇研究顯示，利用合併補充 BCAA 與 Arg 之方式，對於運動表現之促進更為有效 (Hsu et al., 2011; 邱名穗, 2012; 黃玫璇, 2011)。

## 三、訊息處理理論

開放性運動為隨外在刺激的不斷改變，選手必須很快地適應改變的環境並作出適當的反應。在比賽中，選手需透過身體的各感官器官，去觀察外界的環境，並做出相對應的反應。然而，在做出反應動作之前，此動作訊息則需透過中樞神經系統進行一連串的动作控制過程，方可完成整個動作。荷蘭醫師 Donders (1868~1969) 最早提出訊息理論的看法，其解釋人類在環境中，如何經由感覺、注意、辨識、轉換與記憶等內在心理活動，以吸收並運用知識的歷程。依此理論的觀點，個體處理刺激的過程類似電腦的功能，強調動作行為的產生，是經由感覺器官接收外界刺激，將資訊輸送至

中樞神經系統加以處理，經過判斷與決策後才產生行動，經歷了三項連續且不重疊的動作控制（圖三），方可完成整個動作。此外，根據動作反應的結果，又可提供下次動作前的感覺參考或動作修正的依據（林清和，2006；胡名霞，2006；Schmidt, 1991）Schmidt (1991) 認為，從刺激呈現到外顯動作的產生，包含刺激確認、反應選擇與反應編序三個階段：

### 1. 刺激確認 (stimulus identification)

此階段又為知覺 (perception) 階段，主要是透過知覺機制的運作，去組織及確認感覺器官所獲得的訊息，並將刺激訊息歸類。此階段又分為偵測刺激 (stimulus detection) 與型態分辨 (pattern recognition) 期。由於接收到的訊息相當複雜，因此知覺機制必須能找尋動作技能所需要的線索和排除無關的干擾，利用選擇性注意去選取重要有用的線索。

### 2. 反應選擇 (response selection)

此階段又為決策 (decision) 階段，在認明重要刺激之後，中樞系統依據這些訊息，將儲存在長期記憶中的知識、經驗為參照值與重要刺激比對，然後選擇適合的反應。一般認為，可能做出的反應越多，反應選擇階段所需之時間越長。

### 3. 反應編序 (response programming)

此階段又稱行動 (action) 階段，一旦確認出某刺激並選擇適當的反應方式後，先在腦幹和脊椎組成一個運動系統，即從長期記憶庫中提取並組織一個運動程式，該程式會將動作的意念轉為實際執行動作的神經指令，引導肌肉做有順序的適當收縮，進而在最佳時宜完成技能表現（林清和，2006；胡名霞，2006）。

本計畫所執行的三個研究都已經發表於國際期刊，第一年網球的研究刊登於 *Scandinavian Journal of Medicine and Science in Sports*，第二年跆拳道研究刊登於 *Journal of International Society of Sport Nutrition*，第三年中長跑的研究刊登於 *Journal of Sport Science and Medicine*，各年之研究方法、結果、討論如附件。

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

Running title: prevent cognitive decline by BCAA Arg Cit

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## Abstract

The perceptual-cognitive 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 the tennis-specific perceptual-cognitive 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 hr before the match. In the perceptual-cognitive performance test before and after the match, the subjects hit the ball to the opposite direction of the examiner's movement. The AA trial showed significantly higher rate of correct direction after the match than the PB trial (AA trial:  $93.63 \pm 1.28\%$ , PB trial:  $69.09 \pm 2.40\%$ ). The AA trials also had significantly higher post-match accuracy and consistency than the PB trial. The AA trial reported significantly lower ratings of perceive exertion during the match, concurrently with the significantly lower plasma tryptophan/BCAA ratio. The 2 trials showed similar post-match plasma  $\text{NH}_3$  concentration while the AA trial showed significantly higher  $\text{NO}_x$  concentration. This study suggested that the supplementation could prevent the decline in perceptual-cognitive performance through the alleviation of central fatigue by BCAA and prevention of excess hyperammonemia by arginine and citrulline.

**Keywords:** central fatigue, neurotransmitter, skill, exercise performance

## Introduction

The success in tennis matches requires high levels of physical, skill, and perceptual-cognitive performance that needs be maintained for over 5 hours. The perceptual-cognitive 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). The outstanding perceptual-cognitive performance has been consistently shown to be one of the most important factors that distinguish elite and non-elite tennis players (North et al. 2009). The expert tennis players used different search strategy (Williams et al. 2011), and showed higher ability to correctly anticipate the and quickly respond to the stroke intentions of their opponents than the nonexperts (Mann et al. 2007; Williams et al. 2002).

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, Lattier 2008). The significantly increased plasma free tryptophan concentrations after 4 hours of tennis match suggested that cerebral serotonin may be responsible for the

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, the loss of central drive and motivation, and impair the perceptual-cognitive 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 the 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. However, one disadvantage for BCAA supplementation is the excess hyperammonemia as the result of the increased oxidation of these amino acids during exercise (MacLean et al. 1996; Meeusen et al. 2006; Struder et al. 1998). The elevated cerebral uptake and accumulation of  $\text{NH}_3$  would nullify the potential benefit of BCAA on physical and cognitive performance (Blomstrand, Hassmen 1997; Struder, Hollmann 1998) by alterations of cerebral neurotransmission (Wilkinson et al. 2010). The addition of arginine and citrulline to BCAA supplementation may alleviate the excess  $\text{NH}_3$  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 could improve the 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 exhausting tennis training and matches. The neuromuscular fatigue in tennis has been widely documented (Hornery et al. 2007). It has been revealed that the running speed and distance covered after prolonged match were significantly decreased (Reid & Duffield 2014). Nevertheless, it is unclear whether it represents the fatigue and/or the change in match strategy to preserve the energy. The results on the impairments in skill performance after real or simulated tennis matches have been inconsistent, with accuracy in certain types of groundstrokes and/or serve may be deteriorated, while other skills may be maintained (Hornery et al. 2007; Wu et al. 2010). However, the fatigue-induced change in perceptual-cognitive performance, arguably the most important factor that distinguishes expert and nonexpert tennis players, has rarely been investigated in the sport-specific setting.

The purpose of this study was to investigate the combined supplementation of BCAA, arginine, and citrulline on tennis-specific perceptual-cognitive performance

after a 2-hr simulated match. The perceptual-cognitive performance test is designed to reflect the real match condition in which the players have to acquire and process the opponent's movement, in order to execute the racquet to hit the ball 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. All subjects have been participating in tennis training for at least 8 years and competed at the national level. The subjects have the age of  $25.56 \pm 0.67$  years; height  $1.78 \pm 0.04$  m, weight  $72.44 \pm 1.04$  kg; BMI  $23.02 \pm 0.37$  kg/m<sup>2</sup>. The exclusion criteria included major cardiovascular disease risks, musculoskeletal 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. During the 2 days prior to each trial, the subjects were provided with the same meals, purchased from local convenient stores, by the research personnel. The 3 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-cognitive performance test was conducted before and after a 2-hr simulated match.

## Supplementation

On the days of the trials, the subjects reported to the laboratory at 0700 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-cognitive performance test 60 min after finishing the breakfast. At the end of first perceptual-cognitive performance test, 2 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, capsule, General Nutrition Corporation, Pittsburgh, PA, 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) to the AA trial and 1 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 hr of ingestion (data not shown). The subjects were allowed to drink water ad libitum in the first trial, then the timing and amount of consumption were repeated in the following trial. The water consumption was  $3152.9 \pm 191.6$  mL in both trials. The temperatures was  $32.2 \pm 0.1^\circ\text{C}$  and  $32.5 \pm 0.1^\circ\text{C}$  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. (Wu, Shih 2010) with the intensity similar to real competitions. It consisted of alternating 12 receiving and 12 service games. Each game consisted of 6 points and 6 balls were hit in each point. The balls were fed at the frequency of 6 balls per 10 sec by a serving machine (Tennis Tower Competitor, Sports Tutor Inc., Burbank, CA, USA). The receiving games started with a forehand groundstroke, followed by 2 backhand groundstrokes, a forehand groundstroke, and 2 volleys. The service games started with a service, followed by 2 backhand groundstrokes, a forehand groundstroke, and 2 volleys. The participants were asked to return to the central line during the 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 after game 6, 12, 18, and 24.

### Perceptual-cognitive performance test

The perceptual-cognitive 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/hr. A 15 min rest was allowed between the two tests. A 1.5 m x 1.5 m area, marked with white athletic tape in both corners of the singles court, was used as the target for the groundstroke return.

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 the 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 m x 1.5 m target area.

The cross-court groundstroke test also consisted of 60 rounds, with 3 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 start

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 2 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 x 1.5 m target area. Only the performance in the third stroke was recorded in each round.

The direction, accuracy, consistency, and speed were recorded by a digital camera and analyzed afterwards. The accuracy score was the number of balls landed within the 1.5 m x 1.5 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, OR, USA).

#### Blood sample collection

Venous blood samples were collected before breakfast, and immediately before and after the simulated match. At each time 16 ml blood sample was collected into a

tube containing EDTA as anticoagulant. The blood samples were centrifuged at 1500 x 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, CA, USA). The absorbance at 450 nm was measured with a microplate spectrophotometer (Benchmark Plus, Bio-Rad, Hercules, CA, USA). Plasma tryptophan concentration was analyzed with a fluorescence assay (Bridge-It, Mediamics, 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 NO<sub>x</sub> concentrations were determined using Griess reagent (Green et al. 1982) and the absorbance at 450 nm was measured with a microplate spectrophotometer. Plasma concentrations of urea, glucose, lactate, NH<sub>3</sub>, 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).

## Statistical analysis

All values were expressed as mean $\pm$ SEM. The results were analyzed by 2-way (trial x time) analysis of variance with repeated measurements. If the main effect is significant, the differences were determined by post hoc Bonferroni analysis. A  $p < .05$  was considered statistically significant.

## Results

In the perceptual-cognitive performance test, the percentage of balls hit to the correct direction, i.e, opposite to the examiners movement, was significantly decreased by 27.6% after the simulated match in the PB trial, while this indicator of perceptual-cognitive 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 ( $p < .001$ ). The results were similar in both central and cross-court groundstrokes. The groundstroke consistency showed a similar trend (Fig. 3B). The post-match consistency score was unchanged after the match in the AA trial, whereas it was significantly deteriorated in the PB trial. The groundstroke accuracy was significantly decreased in both trials after the simulated match (Fig. 3C). However, the magnitude of decline was smaller in the AA trial, resulting in significantly better post-match groundstroke accuracy in the AA trial than that in the

PB trial. The unchanged perceptual-cognitive 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 other hand, the ball speed was significantly decreased after the match in the PB trial.

The supplementation of amino acids resulted in the significantly higher plasma BCAA concentrations before the simulated match (Fig. 4A). The average post-match BCAA concentration was 71.8% higher than the baseline in the AA trial. However, the difference did not reach statistical significance ( $p=.130$ ). Plasma tryptophan concentrations showed a trend of increase after the simulated match (time effect  $p=.004$ , Fig. 4B). Nevertheless, the post-hoc analysis did not reveal any significant difference between any time points. The supplementation in the AA trial resulted in significantly lower tryptophan/BCAA ratio than the PB trial before (AA trial:  $0.038\pm 0.003$ ; PB trial:  $0.073\pm 0.006$ ,  $p<.001$ ) and after (AA trial:  $0.040\pm 0.008$ ; PB trial:  $0.040\pm 0.008$ ,  $p<.001$ ) the simulated match (Fig. 4C).

The plasma biochemical parameters are presented in Table 1. The 2 trials resulted in similar plasma  $\text{NH}_3$  concentration after the simulated match. In the AA trial the post-match plasma  $\text{NH}_3$  concentration was significantly increased from the baseline ( $p=.014$ ), while it showed a trend of increase from the baseline in the PB trial ( $p=.082$ ). Plasma  $\text{NO}_x$  concentration after the match was elevated from the baseline

and was significantly higher than that in the PB trial ( $p=.048$ ). The other biochemical variables investigated were not significantly different between the 2 trials.

Throughout the simulated match, the subjects in the AA trial consistently reported significantly lower RPE than in the PB trial at the same time point (Fig. 5). Despite the less perceived 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:  $75.2\pm 1.1\%$ ,  $p=.034$ ) and set 2 (AA trial:  $76.7\pm 1.0\%$ ; PB trial:  $69.0\pm 1.2\%$ ,  $p<.001$ ) in the simulated match. The PB trial also showed a larger decline in the successful return rate between set 1 and set 2.

## Discussion

The success in tennis requires a combination of excellent motor and perceptual-cognitive performance (Williams & Ericsson 2005). This study suggested that the combined supplementation of BCAA, arginine, and citrulline could prevent the decline in perceptual-cognitive performance, as well as the accuracy and consistency of groundstrokes after a 2-hr simulated match. To our knowledge, it is the first study that investigated the beneficial effect of supplements in perceptual-cognitive performance in a tennis-specific match-like setting.

The ability to better anticipate the opponent's movements in order to select and

execute the appropriate action is a major factor that separates the experts from nonexperts in many sports (North, Williams 2009). In this study, we showed that this perceptual-cognitive performance could be deteriorated after a prolonged tennis match. Similarly, The perceptual skill, measured by anticipation of service direction of video clips, was decreased a prolonged simulated tennis match (Hornery, Farrow 2007). The decline in the perceptual-cognitive performance after the match may be the result of central fatigue as the plasma tryptophan/BCAA ratio was significantly increased in the PB trial in this study, similar to the previous report (Struder, Hollmann 1995). In the AA trial, the significantly decreased plasma tryptophan/BCAA ratio, due to the elevation in BCAA concentration, would reduce cerebral serotonin synthesis and lead to the alleviation of central fatigue (Gomez-Merino et al. 2001). In agreement, previous studies have reported that BCAA supplementation could maintain the cognitive functions (Hassmen, Blomstrand 1994) and the performance in reactive motor skills (Stepito, Shipperd 2011), while reducing the feeling of fatigue (Blomstrand, Hassmen 1997) during strenuous exercise. Furthermore, the AA and PB trials produced similar post-match plasma  $\text{NH}_3$  concentrations, indicating the absence of excess hyperammonemia from BCAA oxidation. It could be the result of increased NO synthesis, by arginine and citrulline, that enhance vasodilation in the working muscles (Clarkson, Adams 1996; Rouge,

Des Robert 2007). A previous study also showed that the same combination of amino acids could prevent the decline in the taekwondo-specific cognitive function after 3 simulated matches (Chen CY et al, submitted). The premotor reaction time, the delay between the stimulus and the electromyographic signal in the working muscle, in taekwondo roundhouse kicks was maintained after 3 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 the similar results. The results from our previous (Chen CY 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.

Previous studies have investigated potential ergogenic strategies such as carbohydrate, caffeine, and hyperhydration on neuromuscular and skill performance in tennis with limited effects (Hornery, Farrow 2007). Most studies examining the tennis skill performance using pre-determined, unopposed tasks in which the subjects knew which direction to hit before the ball is fed to them. In these 'closed' situations, the perceptual-cognitive 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, Shih 2010). On the other hand, several studies did reveal the declines in skill performance. The groundstroke accuracy was deteriorated (Hornery, Farrow 2007), while the service and forehand groundstroke consistency scores were significantly decreased after a simulated match (Wu, Shih 2010). These results indicated that, although there is evidence for neuromuscular fatigue after prolonged tennis play (Hornery, Farrow 2007), the experienced players can generally uphold the pre-determined racquet control. In a studies that using 'open' environment such as a match play, the success rate of serve return showed the trend of deteriorating toward the third hour in a match play, but the other performance variables remained unaltered (Gomes et al. 2013). It has been suggested that the study investigating fatigue in tennis would require process-based measurements such as perceptual-cognitive performance, rather than simply assessing outcome measurements such as stroke accuracy (Hornery, Farrow 2007). The present study adopted an 'open' environment in which the subjects have to anticipate and/or identify the opponent's movement before performing the groundstroke to the desired direction and location. This method is similar to the situations in real match play while controlling the variables, such as ball speed and

location, that 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-hr tennis match would not cause hypoglycemia in the subjects with adequate carbohydrate intake in the previous day (Gomes, Moreira 2014). However, the significant decline in perceptual-cognitive performance, indicated by the lower rate of ball hit to the correct direction, was still present after the simulated match in the PB trial. It suggested that the supplementation of BCAA, arginine, and citrulline could alleviate central fatigue

in addition to the effect of carbohydrate.

Using the protocol that elicit similar physiological responses to real tennis matches is one of the important factors in investigating the beneficial effects of interventions (Hornery et al. 2007). Each point in our simulated match is composed of 6 strokes within a duration of 10 s. This is similar to the 3-5 strokes per rally that lasts 6-10 s in international matches (Hornery, Farrow 2007). The post-exercise plasma lactate concentration in both trials in the current study was in line 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, the energy metabolism did not seem to be affected as the biochemical variables for carbohydrate and fat metabolism were all similar between the 2 trials. The future studies could investigate dopamine, HPA axis, cortisol, and catecholamines after BCAA supplementation.

This perceptual-cognitive performance test used in this study combined the cognitive judgement, i.e. the direction of the opponent's movement, with the motor function, i.e. 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. However, this design reflects the real match situation where the correct identification and execution are both required.

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

investigation.

## Perspectives

The perceptual-cognitive 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 while investigating the neurotransmitters that are synthesized from tryptophan and tyrosine.

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

	Trial <sup>a</sup>	Baseline	Pre-match	Post-match
NH <sub>3</sub> ( $\mu$ M)	PB	67.51 $\pm$ 11.93	71.03 $\pm$ 8.14	112.44 $\pm$ 12.37
	AA	58.63 $\pm$ 7.44	64.24 $\pm$ 6.27	114.30 $\pm$ 11.40*
NO <sub>x</sub> ( $\mu$ M)	PB	51.43 $\pm$ 11.38	46.11 $\pm$ 5.87	48.49 $\pm$ 6.73
	AA	45.71 $\pm$ 7.07	61.54 $\pm$ 14.64	80.71 $\pm$ 9.49 <sup>†*</sup>
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 <sup>b</sup> (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 ( $\mu$ M)	PB	60.89 $\pm$ 11.16	51.00 $\pm$ 5.99	274.00 $\pm$ 43.86*
	AA	77.44 $\pm$ 16.42	60.33 $\pm$ 12	304.11 $\pm$ 46.79*
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

<sup>a</sup>PB: placebo; AA: BCAA, arginine, citrulline.

<sup>b</sup>NEFA: non-esterified fatty acid.

\*Significantly different from baseline in the same trial (p<.05).

<sup>†</sup>Significantly different from the PB trial at the same time point (p<.05).

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

Figure 2. Court layout for the perceptual-cognitive 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

Figure 3. Results of perceptual-cognitive performance test before and after the simulated match in the AA and PB trials. (A) percentage of balls hit to the correct direction<sup>a</sup>; (B) accuracy score<sup>b</sup>; (C) consistency score<sup>c</sup>; (D) ball velocity<sup>d</sup>.

<sup>a</sup>Main effects: trial:  $p < .001$ ; time:  $p < .001$ ; interaction:  $p < .001$ .

<sup>b</sup>Main effects: trial:  $p = .037$ ; time:  $p < .001$ ; interaction:  $p = .002$ .

<sup>c</sup>Main effects: trial:  $p < .001$ ; time:  $p < .001$ ; interaction:  $p < .001$ .

<sup>d</sup>Main effects: trial:  $p = .129$ ; time:  $p < .001$ ; interaction:  $p < .001$ .

\*  $p < .05$ ; \*\*  $p < .01$ ; \*\*\*  $p < .001$ .

Figure 4. Plasma amino acid concentrations before and after the simulated match in the AA and PL trials. (A) branched-chain amino acids<sup>a</sup>; (B) tryptophan<sup>b</sup>; (C)

tryptophan/branched-chain amino acids ratio<sup>c</sup>.

<sup>a</sup>Main effects: trial:  $p < .001$ ; time:  $p = .004$ ; interaction:  $p = .015$ .

<sup>b</sup>Main effects: trial:  $p < .001$ ; time:  $p = .004$ ; interaction:  $p = .302$ .

<sup>c</sup>Main effects: trial:  $p < .001$ ; time:  $p = .001$ ; interaction:  $p = .001$ .

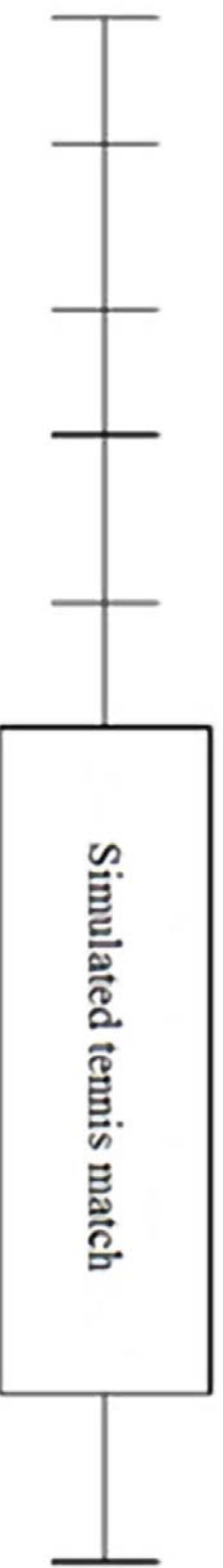
\* $p < .05$ ; \*\* $p < .01$ , significantly different from baseline in the same trial,

<sup>†</sup>Significantly different from the PB trial at the same time point,  $p < .001$ .

Figure 5. Ratings for perceived exertion during the simulated match in the AA and PB trials.

Main effects: trial:  $p = .008$ ; time:  $p < .001$ ; interaction:  $p = .371$ .

\* $p < .01$ .



Time (min)                      0                      60                      90                      150                      170                      290                      300

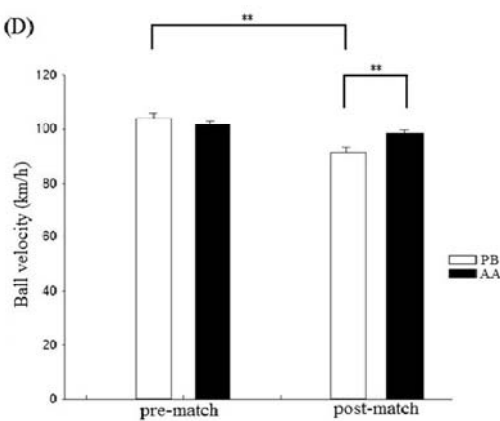
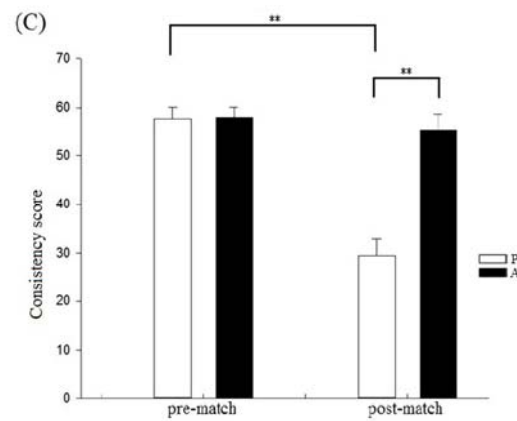
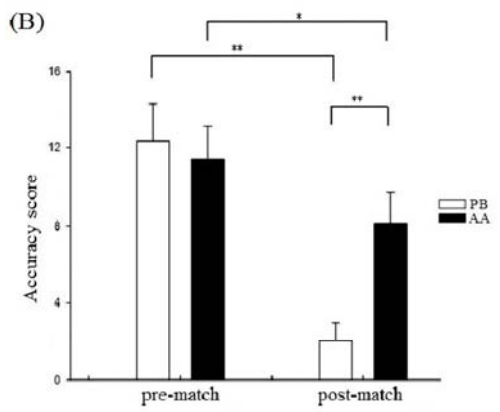
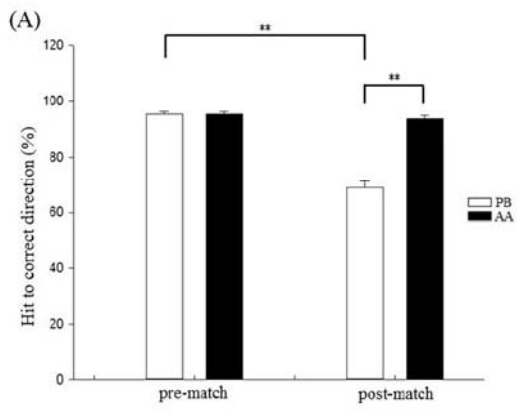
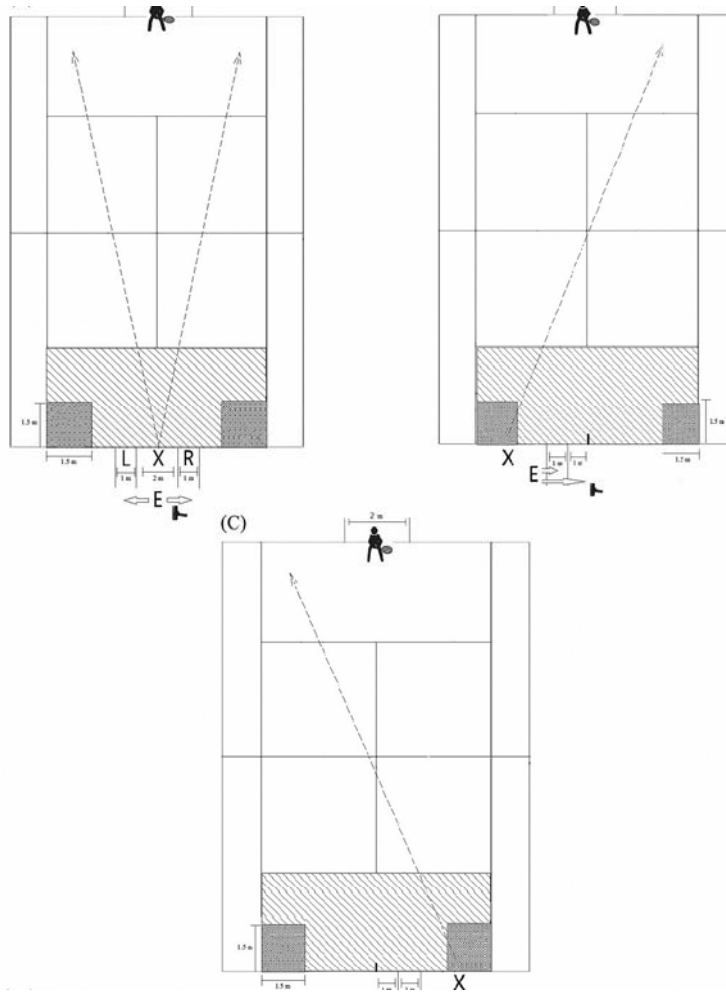
Breakfast                      ◆

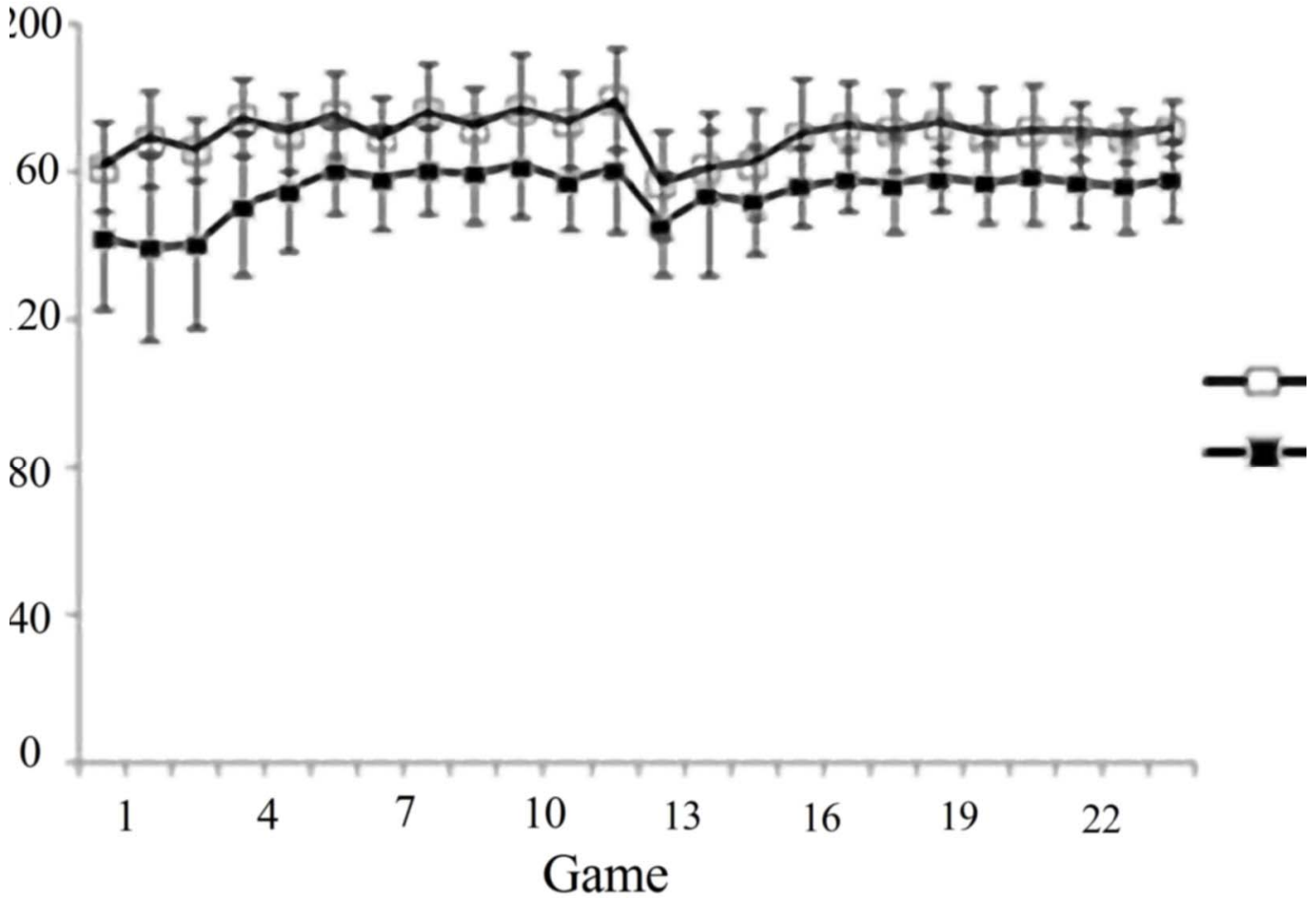
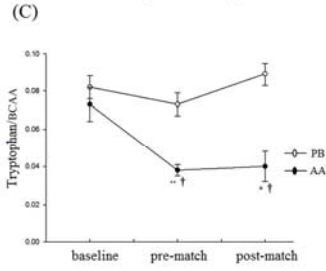
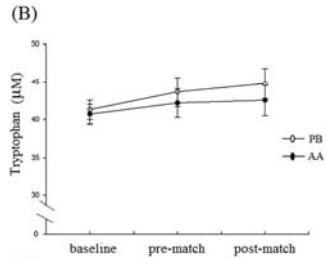
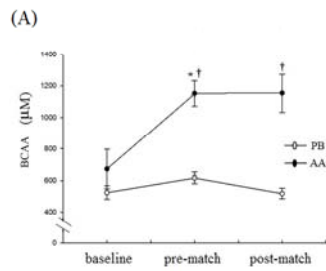
Perceptual-cognitive test                      △                      △

Supplement                      ◎

Blood collection                      ↑                      ↑                      ↑

RPE                      ★                      ★                      ★                      ★





RESEARCH ARTICLE

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# Branched-chain amino acids, arginine, citrulline alleviate central fatigue after 3 simulated matches in taekwondo athletes: a randomized controlled trial

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## Abstract

**Background:** The decline in cognitive performance has been shown after fatiguing exercise. Branched-chain amino acids (BCAA) have been suggested to alleviate exercise-induced central fatigue. Arginine and citrulline could remove the excess  $\text{NH}_3$  accumulation accompanied with BCAA supplementation by increasing nitric oxide biosynthesis and/or urea cycle. The purpose of this study is to investigate the effect of the combined supplementation of BCAA, arginine, and citrulline on central fatigue after three simulated matches in well-trained taekwondo athletes.

**Methods:** In a double-blind randomized cross-over design, 12 male taekwondo athletes performed two trials containing three simulated matches each. Each match contained three 2-min rounds of high-intensity intermittent exercise. At the end of the second match, two different supplementations were consumed. In the AA trial, the subjects ingested 0.17 g/kg BCAA, 0.05 g/kg arginine and 0.05 g/kg citrulline, while placebo was consumed in the PL trial. A validated taekwondo-specific reaction test battery was used to measure the cognitive performance after each match.

**Results:** The premotor reaction time in the three single-task tests and the reaction time in the secondary task in the dual-task test were maintained in the AA trial after three matches, while they were impaired in the PL trial, resulting in significantly better performance in the AA trial. These improvements in the AA trial coincided with significantly lower plasma free tryptophan/BCAA ratio, increased  $\text{NO}_x$  concentrations, and similar  $\text{NH}_3$  concentrations.

**Conclusions:** This study suggested that the combined supplementation could alleviate the exercise-induced central fatigue in elite athletes.

**Keywords:** Premotor reaction time, Dual task, Tryptophan, Taekwondo

**Abbreviations:** BCAA, branched-chain amino acids; CI, confidence interval; EMG, Electromyography; NEFA, non-esterified fatty acids; NO, nitric oxide; PRT, premotor reaction time

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## Background

The central nervous system plays an important role in development of exercise-induced fatigue [1]. The increased cerebral serotonin (5-hydroxytryptamine) concentration during exercise may be one of the factors responsible for central fatigue. Cerebral serotonin could result in the feeling of lethargy and tiredness, and the loss of central drive and motivation [2]. To support this hypothesis, endurance capacity was significantly decreased by the administration of serotonin agonists, while it was increased by serotonin antagonists in humans and rats [3–5].

In addition to the decreases in muscle output, it has been known that cognitive and skill performance was also impaired as exercise progresses. The studies using functional magnetic resonance imaging have shown that the brain regions involved in high-order motor tasks, such as prefrontal cortex and supplementary motor areas, were affected during fatiguing exercise [6, 7]. As the result, cognitive performance, measured by an auditory choice reaction task and reaction time to visual stimulus, were progressively impaired after exhausting exercise [8, 9]. The performance in a color-word test was also decreased after a 30-km cross-country race [10]. Furthermore, several studies reported impairments in sport-specific skill performance after strenuous exercise [11–13].

Branched-chain amino acids (BCAA), including leucine, isoleucine, and valine, have been suggested to alleviate exercise-induced central fatigue. Plasma BCAA could compete with tryptophan, the precursor for cerebral serotonin synthesis, for the L-system transporter to cross the blood brain barrier [14]. Animal studies have shown that BCAA could increase running time to exhaustion by reducing exercise-induced cerebral synthesis and release of serotonin [15, 16]. However, humans studies have failed to find ergogenic effect of BCAA supplementation [17, 18].

One possible drawback for BCAA supplementation in humans is the excess hyperammonemia due to increased BCAA metabolism during exercise [18–20]. Elevated cerebral uptake and accumulation of  $\text{NH}_3$  would offset the potential benefit of BCAA on central fatigue by alterations of cerebral energy metabolism and neurotransmission, and signaling pathways within the neuron [21]. Arginine has been suggested to reduce exercise-related accumulations of  $\text{NH}_3$  by promoting urea cycle [22] and nitric oxide (NO) biosynthesis [23]. Indeed, the combined supplementation of BCAA and arginine improved intermittent running performance in athletes in 2 consecutive days of simulated handball games by potentially alleviating central fatigue [24]. Citrulline, a NO precursor with high bio-availability [25, 26] and an intermediate in urea cycle [26], could also suppress exercise-induced hyperammonemia [27, 28].

Cognitive function is crucial for success in many sport competitions. However, most aforementioned studies

investigating the effect of exhausting exercise on cognitive functions measured the reaction time to various simple stimuli that are dissimilar in sport competitions. Several other studies used pre-determined sport tasks which are closed skills in nature and distinct from the open skills required in many sports. These limitations make it difficult to draw conclusions on the practical application to sport performance in athletes. The present study applied a validated taekwondo-specific reaction battery containing single- and dual-task tests [29] to mimic the cognitive demands in real matches. Three, instead of a single, simulated matches were applied to elicit the physical stress similar to actual competitions. In addition, premotor reaction time (PRT), the difference between the stimulus and the onset of muscle action potentials, was measured to represent the time required for the central nervous system to identify stimuli, process, and transmit signals to the muscles [30]. PRT could be viewed as an indicator for cognitive functions as it excludes the peripheral neuromuscular factors in the reaction process. Therefore, the present study investigated the effect of the combination of BCAA, arginine, and citrulline on the reaction time in a sport-specific setting after high-intensity exercise. Furthermore, the potential mechanisms, including reduced plasma tryptophan/BCAA ratio and enhanced removal of excess  $\text{NH}_3$  by increasing NO biosynthesis and/or urea cycle, were also examined.

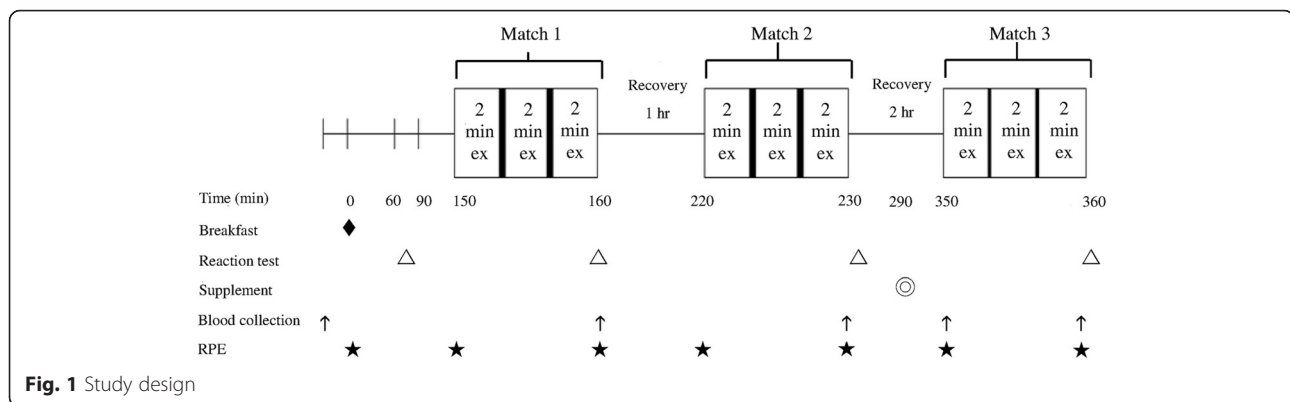
## Methods

### Participants

Twelve male taekwondo athletes were recruited from National Taiwan University of Sport, Taichung, Taiwan. All subjects have been participating in taekwondo training for at least 6 years and competed at the national or international level. The subjects have the age of  $20.0 \pm 0.8$  years, the height of  $1.77 \pm 0.04$  m, the weight of  $66.9 \pm 5.0$  kg, the body mass index of  $21.29 \pm 0.93$   $\text{kg/m}^2$ , and  $\text{VO}_2\text{max}$  of  $44.9 \pm 6.8$   $\text{ml/min/kg}$ . The exclusion criteria included cardiovascular disease risks, musculoskeletal injuries, smoking, consumption of protein supplement or under any medication in the past 3 months. The regular training schedule and diet habits were maintained during the study period. The subjects were refrained from all training 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 Research Ethics Committee of China Medical University and Hospital.

### Experimental design

This study used a double-blind, randomized cross-over design (Fig. 1). Each subject completed amino acids



(AA) and placebo (PL) trials in a random order, separated by a wash-out period of at least 7 days. The same food, purchased from local convenience stores, was provided during the 2 days prior to the trials. The meals provided approximately 1800 kcal/day with 54 % energy from carbohydrate, 30 % from fat, and 16 % 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) [24].

### Experimental procedure

Approximately 1–2 weeks prior to the first trial, cardiopulmonary function was measured. The subjects warmed up on an electrically braked cycle ergometer (Corival, Lode, Groningen, Netherland) at 50 W for 5 min, followed by incremental stages of 25 W every 3 min. The breath-by-breath gas was analyzed (Vmax 29C, Sensormedics, Yorba Linda, CA, USA). Maximal oxygen uptake ( $\dot{V}O_{2\max}$ ) was considered to be achieved when the subjects were unable to maintain the workload.

### Supplementation

On the days of the trials, the subjects reported to the laboratory at 0700 after an overnight fast. After collecting venous blood samples as the baseline, the subjects consumed the standardized breakfast. Each trial included 3 simulated matches. After the second match, 2 supplementations 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, capsule, General Nutrition Corporation, Pittsburgh, PA, USA), 0.05 g/kg arginine and 0.05 g/kg citrulline (arginine: citrulline = 1:1, tablet, General Nutrition Corporation). In the PL trial, the subjects consumed the identical amount of empty capsule and tablet containing starch (Chung-Yu Biotech Co LTD, Taichung, Taiwan) to the AA trial and 1 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). Therefore, the supplements were consumed 1 h before the third simulated match in this study. In addition, the supplements were given prior to the third match because our pilot study showed that cognitive function started to decline after 2 simulated matches (data not shown).

The subjects were allowed to drink water ad libitum in the first trial, then the timing and amount of consumption were repeated in the following trial. The water consumption was  $1133.3 \pm 548.6$  mL in both trials.

### Simulated match

The simulated match was designed to mimic the high-intensity intermittent nature of actual taekwondo competitions, modified from the previous study [31]. The exercise was performed on a cycle ergometer (894E, Monark, Varberg, Sweden). Each trial contained 3 matches with a 1-hr rest before the second match and a 2-hr rest before the third match. A match included three 2-min rounds with repeated work to rest time of 5 s and 25 s, respectively. A 1-min rest was provided between the rounds. The work to rest ratio of 1 to 5 was determined according to the analysis of international taekwondo matches [32]. During the working period, the load was set at 0.1 kp/kg body weight. The subjects were asked to pedal as fast as possible while the research personnel providing vocal encouragement. The peak and average power of each 5-s sprint was recorded. During within-round and between-round rest periods, the subjects pedaled at 60 rpm without the load.

### Reaction test battery

This taekwondo-specific reaction battery, containing 3 single- and 1 dual-task movements, has been shown to exhibit moderate to high reliability and validity in elite and sub-elite athletes. The intraclass correlation coefficients

were 0.439–0.634 in PRT in single-task movements, and 0.692 in reaction time in the secondary task in elite taekwondo athletes [29]. Each of the 4 movements was performed 5 times in a random order. A researcher told the subject which movement was to be performed prior to each task. All subjects were right-handed and performed the kicks with their right leg. Electromyography (EMG) electrodes were attached to left thenar and brachioradialis muscles.

The detailed procedure in this battery can be found elsewhere [29]. Briefly, the subjects stood in a guard position with both heels on a force platform (9260AA6, Kistler, Winterthur, Switzerland) while holding a button on the left hand. The subjects were asked to press the button on the left hand with the thumb as soon as they see a light signal from the top of the head of a dummy, then start the respective movement to attack the dummy. Three sets of single-task movements were used: (A) a roundhouse kick to the rib; (B) a roundhouse kick to the rib, a roundhouse kick to the head, then a reverse roundhouse kick to the head; and (C) a roundhouse kick to the rib, a roundhouse kick to the head, a reverse roundhouse kick to the head, a roundhouse kick to the head, a reverse roundhouse kick to the head, then a roundhouse kick to the head. The subjects can only put the right foot back to the ground after all kicks were performed in the movement. The signals from EMG, force platforms, the button, and the accelerometer (EGAXT3; Measurement Specialties, Hampton, VA, USA) in the dummy were collected through a data acquisition and analysis system (MP150, Biopac Systems, Inc., Goleta, CA, USA).

The dual-task movement D is composed of movement C, the primary task, and a secondary task. While the subjects were carrying out the primary task, research personnel turned on the light signal on the dummy again. The subjects then press the button with their left thumb as soon as they see the second light signal.

In single-task tests, PRT was determined as the time from the beginning of the light signal to the start of EMG signal of the left thenar muscles. The thenar muscles were used because they provided much clearer EMG signal compared to the leg muscles. The EMG signal from leg muscles was noisy due to the unconscious preparation for the kicks before the light, even though the subjects were asked to stand still prior to the stimulus. Motor reaction time was between the start of EMG signal of the left thenar muscles and the right leg leaving the force platform. Movement time was between the right leg leaving the force platform and the appearance of signal from the accelerometer in the dummy.

In the dual-task test, the performance of the secondary task was defined as the time between the beginning of the second light signal and pushing the button. Therefore, the

performance of the secondary task involves PRT, motor reaction time, and movement time. EMG signal was not used in measuring the secondary task because it was present throughout the first task. Therefore, it was very difficult to identify the EMG signal that triggered the movement to press the button.

#### **Blood sample collection**

Venous blood samples were collected before breakfast, immediately after each match, and immediately before the start of the third match. At each sampling time, a 16 ml blood sample was collected into a tube containing EDTA. The blood samples were centrifuged at 1500 x g (Eppendorf 5810, Hamburg, Germany) to extract plasma. The aliquoted plasma samples were stored at  $-70^{\circ}\text{C}$  until further analysis.

#### **Measurement of blood biochemical parameters**

Plasma BCAA concentration was measured enzymatically (Biovision, Milpitas, CA, USA). The absorbance at 450 nm was measured with a microplate spectrophotometer (Benchmark Plus, Bio-Rad, Hercules, CA, USA). Plasma free tryptophan concentration was analyzed with a fluorescence assay (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 NO<sub>x</sub> concentrations were determined using Griess reagent [33] and the absorbance at 450 nm was measured with a microplate spectrophotometer. Plasma concentrations of urea, glucose, lactate, NH<sub>3</sub>, glycerol, and NEFA were measured with an automatic analyzer (Hitachi 7020, Tokyo, Japan) using commercial kits (Randox, Antrim, UK). Plasma concentrations of all parameters were corrected for the changes in plasma volume using hemoglobin concentration and hematocrit in whole blood [34].

#### **Statistical analysis**

All values were expressed as mean  $\pm$  SD. The results were analyzed by 2-way (trial x time) analysis of variance with repeated measurements. If the time x trial interaction effect is significant, the difference between the 2 trials after the third simulated match was identified by one-way analysis of covariance with the pre-exercise level as the covariant. If the time effect is significant, the differences between each time points within the same trial were determined by post hoc Bonferroni analysis. A *p*-value less than .05 was considered statistically significant.

#### **Availability of data and materials**

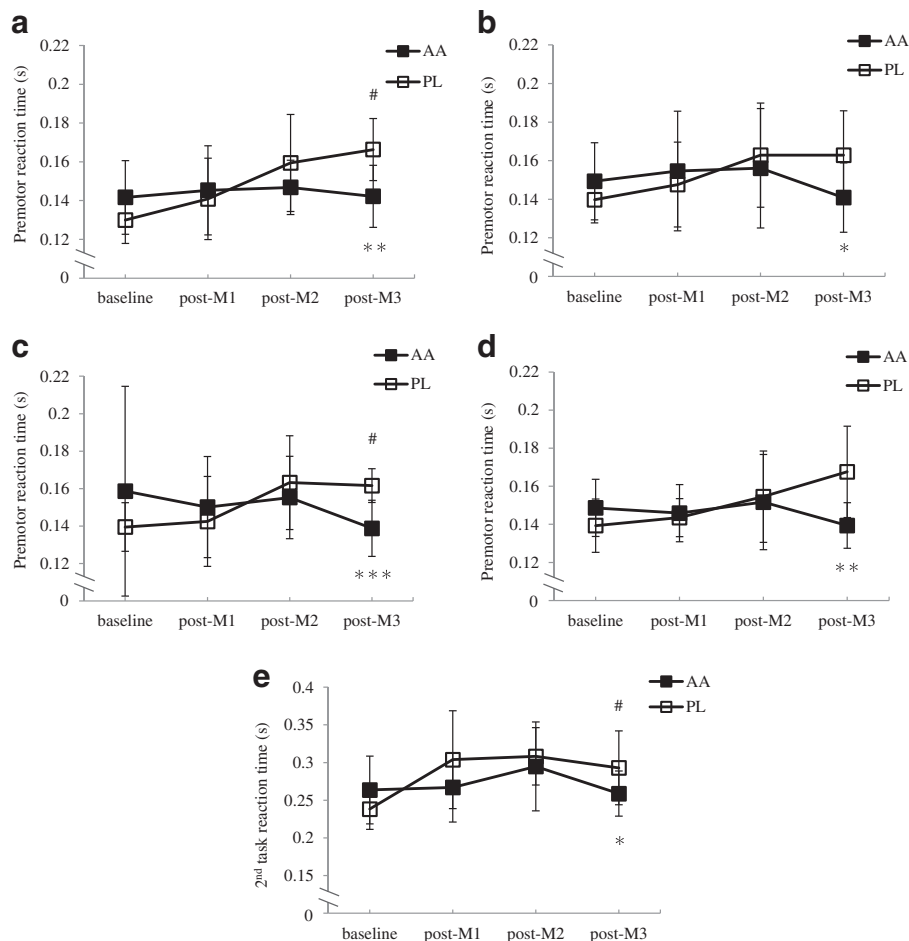
The dataset supporting the conclusions of this article is available in ResearchGate ([https://www.researchgate.net/publication/303405818\\_Branched-chain\\_amino\\_acids\\_](https://www.researchgate.net/publication/303405818_Branched-chain_amino_acids_)

arginine\_citrulline\_alleviate\_central\_fatigue\_after\_3\_simulated\_matches\_in\_taekwondo\_athletes\_a\_randomized\_controlled\_trial).

**Results**

PRT in the movement A in the AA and PL trials is shown in Fig. 2a. There were significant time and trial x time effects. PRT after the third match in the AA trial ( $0.142 \pm 0.016$  s) was significantly faster than that in the PL trial ( $0.166 \pm 0.017$  s;  $F = 13.03$ ,  $\eta^2 = 0.383$ , 95 % confidence interval (CI):  $-0.041$ ,  $-0.011$ ,  $p = .002$ ). In movement B, PRT after the third match in the AA trial ( $0.141 \pm 0.019$  s) was also significantly faster than that in the PL trial ( $0.163 \pm 0.024$  s;  $F = 6.61$ ,  $\eta^2 = 0.239$ , 95 % CI:  $-0.043$ ,  $-0.005$ ,  $p = .018$ ) (Fig. 2b). The results were alike in the most complicated movement C (Fig. 2c). In the PL trial, PRT after the third match was

significantly deteriorated compared to the baseline level, while it was maintained in the AA trial, resulting in significantly faster PRT in the AA trial (AA trial:  $0.139 \pm 0.016$  s; PL trial:  $0.162 \pm 0.010$  s;  $F = 18.15$ ,  $\eta^2 = 0.464$ , 95 % CI:  $-0.035$ ,  $-0.012$ ,  $p < .001$ ). PRT in the primary task in movement D, which is identical to movement C, exhibited the similar trend (Fig. 2d). The AA trial showed significantly faster PRT after the third match than that in the PL trial (AA trial:  $0.139 \pm 0.013$  s; PL trial:  $0.168 \pm 0.025$  s;  $F = 10.67$ ,  $\eta^2 = 0.337$ , 95 % CI:  $-0.047$ ,  $-0.010$ ,  $p = .004$ ). There was significant time and trial x time interaction effect in reaction time in the secondary task in movement D (Fig. 2e). In the PL trial it was significantly slower after the third simulated match, compared to the baseline level, while it was maintained in the AA trial, resulting in better performance in the AA trial (AA trial:  $0.259 \pm 0.031$  s; PL trial:



**Fig. 2** Premotor reaction time in movement A (a), movement B (b), movement C (c), and primary task in movement D (d), and reaction time in the secondary task in movement D (e) in the taekwondo-specific reaction test battery after each simulated matches in the AA and PL trials<sup>a</sup>. <sup>a</sup>AA: BCAA, arginine, and citrulline; PL: placebo. (a) Main effects: trial:  $p = .156$ ; time:  $p = .001$ ; interaction:  $p = .016$ ; (b) Main effects: trial:  $p = .541$ ; time:  $p = .019$ ; interaction:  $p = .025$ ; (c) Main effects: trial:  $p = .872$ ; time:  $p = .037$ ; interaction:  $p = .038$ ; (d) Main effects: trial:  $p = .113$ ; time:  $p = .152$ ; interaction:  $p = .015$ ; (e) Main effects: trial:  $p = .123$ ; time:  $p = .036$ ; interaction:  $p = .018$ ; \* $p < .05$ , \*\* $p < .01$ , \*\*\* $p < .001$ , significantly different between the 2 trials. #Significantly different from the baseline in the PL trial ( $p < .05$ )

$0.293 \pm 0.051$  s;  $F = 5.46$ ,  $\eta^2 = 0.206$ , 95 % CI:  $-0.079$ ,  $-0.005$ ,  $p = .029$ ). There was no significant effect in motor reaction time or movement time in any movement (Table 1).

The supplementation at 1 h prior to the third simulated match resulted in significantly higher plasma BCAA concentrations before and after that match in the AA trial, compared to those in the PL trial ( $F = 74.78$ ,  $\eta^2 = 0.781$ , 95 % CI:  $0.383$ ,  $0.626$ ,  $p < .001$ ;  $F = 114.22$ ,  $\eta^2 = 0.845$ , 95 % CI:  $0.466$ ,  $0.692$ ,  $p < .001$ ; respectively, Fig. 3a). Plasma free tryptophan concentrations did not show any change in either trials (Fig. 3b). The significantly elevated plasma BCAA concentration before and after the third simulated match in the AA trial resulted in the significantly lower free tryptophan/BCAA ratio than the PL trial ( $F = 62.74$ ,  $\eta^2 = 0.749$ , 95 % CI:  $-49.278$ ,  $-28.783$ ,  $p < .001$ ;  $F = 126.36$ ,  $\eta^2 = 0.857$ , 95 % CI:  $-41.763$ ,  $-28.723$ ,  $p < .001$ ; respectively, Fig. 3c).

The simulated matches significantly increased plasma  $\text{NH}_3$  concentrations by the similar magnitude in both trials (Fig. 4a). There were not significant differences between the 2 trials. The AA trial showed significantly higher  $\text{NO}_x$  concentrations after the third simulated match (AA trial:  $13.2 \pm 6.0$   $\mu\text{M}$ ; PL trial:  $8.1 \pm 4.3$   $\mu\text{M}$ ,  $F = 4.705$ ,  $\eta^2 = 0.266$ , 95 % CI:  $0.023$ ,  $11.286$ ,  $p = .049$ ; Fig. 4b). The plasma concentrations of urea, glucose, lactate, glycerol, and NEFA did not show any difference between the 2 trials (Table 2).

The average power output in each simulated match was not significantly different between the 2 trials (AA

trial:  $10.76 \pm 1.04$ ,  $11.03 \pm 0.77$ ,  $10.98 \pm 0.79$  W/kg; PL trial:  $11.00 \pm 0.46$ ,  $10.93 \pm 0.68$ ,  $10.95 \pm 0.58$  W/kg, match 1, 2, and 3, respectively). Each simulated matches significantly increased RPE scores by the similar extent, with no significant trial or trial x time interaction effect.

## Discussion

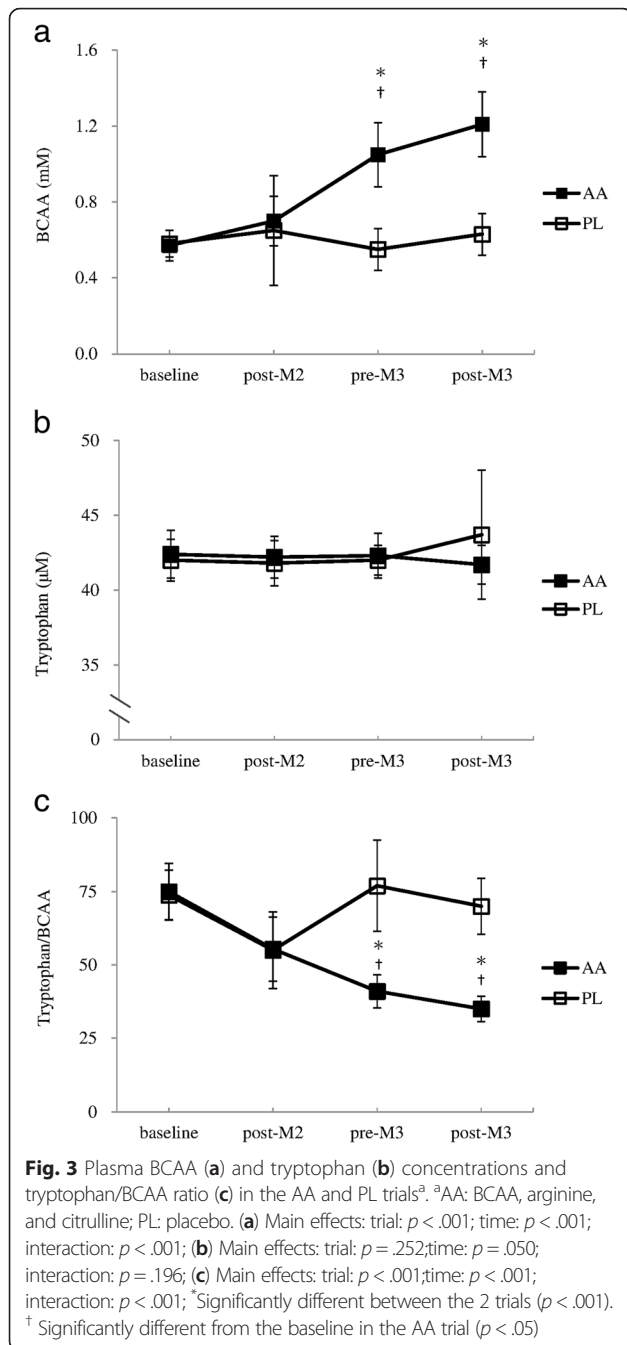
To our knowledge, this is the first study revealing that the combined supplementation of BCAA, arginine, and citrulline could prevent the exercise-induced central fatigue in a sport-specific setting in athletes. After the supplementation, the subjects in the AA trial showed significantly faster PRT in the 3 single-task movements, as well as the reaction time in the secondary task in the dual-task test, compared to those in the PL trial. These improvements coincided with a significantly lower tryptophan/BCAA ratio in the AA trial. Furthermore, the supplementation did not lead to additional  $\text{NH}_3$  accumulation, possibly mediated by an increased NO production from arginine and citrulline.

Previous studies have reported that BCAA supplementation could maintain cognitive functions [10] and the performance in reactive motor skills [35], while reducing the feeling of fatigue [17] during strenuous exercise. However, the accompanied excess  $\text{NH}_3$  accumulation could offset the effect of BCAA in most human studies [18–20]. The addition of arginine and citrulline to BCAA in this study resulted in similar plasma  $\text{NH}_3$  levels between the AA and PL trials. An increased NO production and

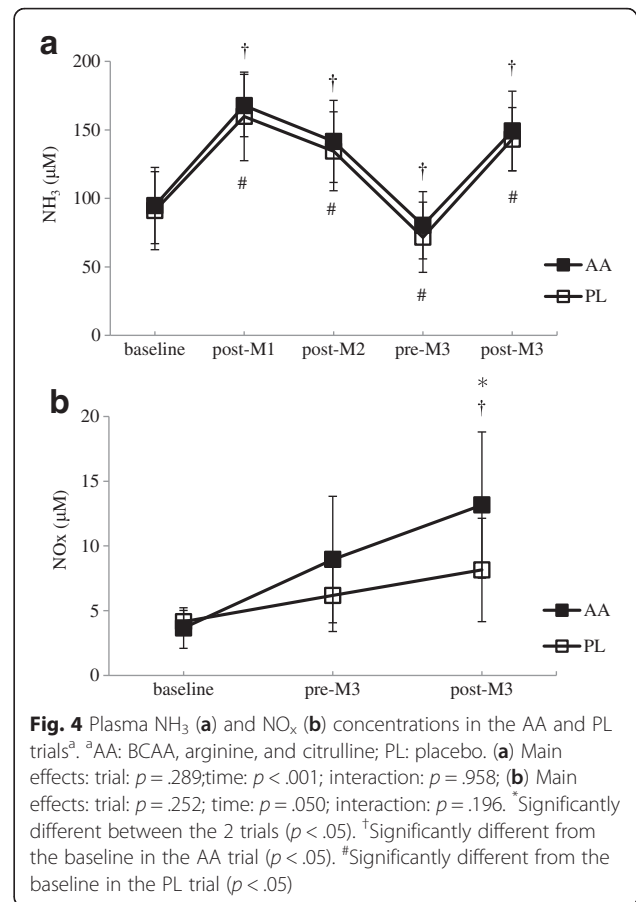
**Table 1** Motor reaction time and movement time in the taekwondo-specific reaction test battery after each simulated matches in the AA and PL trials

	Movement <sup>a</sup>	Trial <sup>b</sup>	Baseline (s)	post-M1 (s)	post-M2 (s)	post-M3 (s)
Motor reaction time	A	AA	$0.295 \pm 0.057$	$0.267 \pm 0.055$	$0.270 \pm 0.050$	$0.266 \pm 0.033$
		PL	$0.285 \pm 0.040$	$0.265 \pm 0.044$	$0.258 \pm 0.048$	$0.270 \pm 0.041$
	B	AA	$0.313 \pm 0.079$	$0.307 \pm 0.069$	$0.269 \pm 0.044$	$0.299 \pm 0.064$
		PL	$0.314 \pm 0.043$	$0.305 \pm 0.054$	$0.292 \pm 0.070$	$0.303 \pm 0.056$
	C	AA	$0.342 \pm 0.066$	$0.303 \pm 0.067$	$0.287 \pm 0.043$	$0.307 \pm 0.038$
		PL	$0.314 \pm 0.055$	$0.305 \pm 0.051$	$0.304 \pm 0.051$	$0.303 \pm 0.055$
	D	AA	$0.356 \pm 0.071$	$0.304 \pm 0.067$	$0.277 \pm 0.047$	$0.300 \pm 0.049$
		PL	$0.319 \pm 0.039$	$0.307 \pm 0.043$	$0.300 \pm 0.071$	$0.301 \pm 0.052$
Movement time	A	AA	$0.269 \pm 0.043$	$0.250 \pm 0.033$	$0.246 \pm 0.030$	$0.258 \pm 0.042$
		PL	$0.263 \pm 0.033$	$0.260 \pm 0.042$	$0.273 \pm 0.039$	$0.262 \pm 0.040$
	B	AA	$0.278 \pm 0.041$	$0.266 \pm 0.035$	$0.262 \pm 0.032$	$0.263 \pm 0.030$
		PL	$0.279 \pm 0.033$	$0.294 \pm 0.071$	$0.275 \pm 0.033$	$0.272 \pm 0.032$
	C	AA	$0.270 \pm 0.050$	$0.269 \pm 0.037$	$0.264 \pm 0.028$	$0.243 \pm 0.066$
		PL	$0.282 \pm 0.036$	$0.277 \pm 0.044$	$0.276 \pm 0.032$	$0.281 \pm 0.035$
	D	AA	$0.272 \pm 0.038$	$0.272 \pm 0.030$	$0.260 \pm 0.032$	$0.269 \pm 0.032$
		PL	$0.267 \pm 0.069$	$0.274 \pm 0.051$	$0.280 \pm 0.034$	$0.278 \pm 0.041$

<sup>a</sup>Movement A: a roundhouse kick to the rib; Movement B: a roundhouse kick to the rib, a roundhouse kick to the head, then a reverse roundhouse kick to the head; Movement C: a roundhouse kick to the rib, a roundhouse kick to the head, a reverse roundhouse kick to the head, a roundhouse kick to the head, a reverse roundhouse kick to the head, then a roundhouse kick to the head; Movement D: the primary task in a dual-task setting, same as Movement C. <sup>b</sup>AA BCAA, arginine, and citrulline, PL placebo



possibly vasodilation in the AA trial may help to remove the excess  $\text{NH}_3$  produced from the elevated BCAA metabolism. It has been reported that the supplementation of BCAA and arginine after exhaustive exercise could reduce the feeling of fatigue during recovery [36]. The combined supplementation of BCAA and arginine also significantly improved performance in high-intensity intermittent sprints on the second day of 2 consecutive days of exercise by alleviating central fatigue [31]. By incorporating citrulline, a more potent NO precursor than arginine [37], the excess hyperammonemia previously seen after BCAA



supplementation was prevented in the present study. Although the lack of a BCAA-only trial in this study precludes the direct conclusion that arginine and citrulline alleviate excess hyperammonemia, the alleviation of central fatigue by this combined supplementation regime is still prominent.

During prolonged exercise, brain regions involved in high-order motor tasks, sensory processing, and corticomotor drive are activated in order to maintain the muscular performance [6, 7, 38]. The progressively increased cerebral processing demand to maintain muscular output could lead to less brain resource that can be allocated to cognitive function. In the PL trial, significant deteriorations in PRT were apparent in all movements after the third simulated match, indicating that the accumulated physical demands resulted in central fatigue. Similarly, it has been reported that exhausting exercise could impair the reaction time in response to different types of stimuli [8, 9]. The sport-specific skill performance was also significantly decreased after exhausting exercise [11–13]. On the other hand, in the AA trial these cognitive performances were maintained and significantly better than those in the PL trial. A significantly lower plasma tryptophan/BCAA ratio, hence reduced

**Table 2** Plasma biochemical parameters and ratings of perceived exertions in the AA and PL trials

	Trial <sup>a</sup>	Baseline	post-M1	post-M2	pre-M2	post-M3
Urea (mM) <sup>1</sup>	AA	4.61 ± 1.72	8.20 ± 3.65	12.70 ± 4.50*	13.25 ± 4.10*	17.56 ± 5.30*
	PL	4.55 ± 1.90	7.36 ± 2.30	12.95 ± 5.00*	9.07 ± 4.00	16.92 ± 5.10*
Lactate (mM) <sup>2</sup>	AA	2.95 ± 0.77	13.38 ± 4.17*	12.78 ± 3.77	2.22 ± 0.53	11.93 ± 2.90
	PL	3.19 ± 0.75	13.37 ± 2.96*	12.24 ± 3.45	2.36 ± 0.55	12.57 ± 2.27
Glucose (mM) <sup>3</sup>	AA	5.26 ± 0.28	4.39 ± 0.44*	4.97 ± 0.50	5.37 ± 0.39	5.12 ± 0.50
	PL	5.25 ± 0.44	4.20 ± 0.27*	4.89 ± 0.39	5.36 ± 0.22	5.28 ± 0.39
Glycerol (μM) <sup>4</sup>	AA	27.75 ± 10.37	49.38 ± 22.00	76.48 ± 27.14*	79.81 ± 24.91*	105.78 ± 31.76*
	PL	27.42 ± 11.39	44.34 ± 13.96	78.00 ± 30.22*	54.65 ± 24.31	101.91 ± 30.93*
NEFA (mM) <sup>b,5</sup>	AA	0.33 ± 0.17	0.21 ± 0.08	0.34 ± 0.15	0.38 ± 0.12	0.47 ± 0.20
	PL	0.36 ± 0.16	0.21 ± 0.06	0.38 ± 0.10	0.58 ± 0.24	0.55 ± 0.19
RPE <sup>c,6</sup>	AA	9.3 ± 2.0	16.0 ± 2.0*	15.9 ± 2.0*	-	15.2 ± 3.0*
	PL	9.3 ± 2.0	12.8 ± 2.0*	13.6 ± 2.0*	-	14.6 ± 2.0*

<sup>a</sup>AA BCAA, arginine, and citrulline, PL placebo. <sup>b</sup>NEFA non-esterified fatty acid. <sup>c</sup>RPE ratings of perceived exertion. \*Significantly different from baseline in the same trial ( $p < .05$ )

<sup>1</sup>Main effects: trial:  $p = .329$ ; time:  $p < .001$ ; interaction:  $p = .167$

<sup>2</sup>Main effects: trial:  $p = .325$ ; time:  $p < .001$ ; interaction:  $p = .165$

<sup>3</sup>Main effects: trial:  $p = .816$ ; time:  $p < .001$ ; interaction:  $p = .520$

<sup>4</sup>Main effects: trial:  $p = .325$ ; time:  $p < .001$ ; interaction:  $p = .165$

<sup>5</sup>Main effects: trial:  $p = .117$ ; time:  $p < .001$ ; interaction:  $p = .213$

<sup>6</sup>Main effects: trial:  $p = .886$ ; time:  $p = .003$ ; interaction:  $p = .718$

cerebral serotonin synthesis [15], could be one of the mechanisms that are responsible for the alleviation of central fatigue.

This study utilized a validated dual-task protocol [29] to further identify the development of central fatigue after exercise and supplementations. The dual-task protocols carry better validity, compared to the single-task, when the primary tasks are very familiar to the participants [29]. It has been hypothesized that human brain has a fixed capacity of central processing. During dual-task situations the majority of the capacity would be distributed to the primary task. As the exercise progresses, the increased demand for the central resource for the primary task may lead to the impaired performance in the secondary task [39]. In the present study, the AA trial showed a significantly faster RT in the secondary task after the third simulated match. This result indicated that the primary task may require less cerebral processing capacity. The longer reaction time in the secondary task could also suggest a poorer ability to process multiple inputs, a common situation in many sports. The athletes in taekwondo and many other sports have to be able to read and predict opponent's next move while performing an attack or defensive task. The better performance in the secondary task implies that a greater attention capacity can be allocated to assess opponent's movements while performing the primary task. This increased ability would provide a great advantage in many sports.

The commonly measured reaction time, the sum of PRT, motor reaction time, and movement time, may be

inadequate to distinguish elite and sub-elite athletes or to identify subtle impairments in performance. This is especially true when the required movement is the main action that has been practiced numerous times by the subjects [29]. In addition, the commonly measured reaction time is a combination of central (PRT) and peripheral factors (motor reaction time, movement time), making it difficult to identify the origin of fatigue. In fact, motor reaction time, movement time, and total reaction time were similar after each match in both trials. In addition, total power output during the 3 matches was also unchanged in both trials. These results indicated that the peripheral neuromuscular system was not fatigued during the entire test period. The impairments in premotor reaction time and performance in the secondary task appeared before peripheral fatigue in this group of well-trained taekwondo athletes. By applying the protocols used in this study, we were able to identify the beneficial effect of the combined supplementation on the signal processing stage in the central nervous system, but not at the peripheral neuromuscular level. Similarly, it has been shown that well-trained wrestlers can maintain total power output in 3 simulated matches similar to the protocols used in this study [31].

The standardized diet consumed for two days prior to the trials provided less energy and carbohydrate than the subjects required. It has been suggested that an insufficient carbohydrate supply may impair cognitive function during prolonged exercise [40]. However, our subjects appeared to maintain sufficient muscle glycogen levels as they were euglycemic and plasma lactate concentration and average power output were similar across the 3

simulated matches in both trials. Thus, the effect of hypocaloric diet on the cognitive function in this study may be negligible.

## Conclusions

In conclusion, this study suggested that the combined supplementation of BCAA, arginine, and citrulline could alleviate the exercise-induced central fatigue in elite athletes. By applying validated taekwondo-specific reaction test, the performance in PRT and the secondary task provide more accurate indicator for the processing capacity in the brain. The improvements in these abilities by the supplementation could carry significant benefit in many sports. The effect of this supplementation regime on performance in more complicated skills in various sports warrants further investigation. In addition, it is noteworthy that central fatigue appears before the decline in physical performance in well-trained athletes.

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## Authors' contributions

IFC and CYC performed experiments, HJW, CYC, KMC and CKC conception and design of research, IFC and CYC analyzed data, CYC, KMC and CKC interpreted results of research, CKC drafted the manuscript, CKC, HJW, and CYC edited and revised the manuscript, CKC approved final version of manuscript. All authors read and approved the final manuscript.

## Competing interests

The authors declare that they have no competing interests.

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Research article

## The Supplementation of Branched-Chain Amino Acids, Arginine, and Citrulline Improves Endurance Exercise Performance in Two Consecutive Days

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### Abstract

The central nervous system plays a crucial role in fatigue during endurance exercise. Branched-chain amino acids (BCAA) could reduce cerebral serotonin synthesis by competing with its precursor tryptophan for crossing the blood brain barrier. Arginine and citrulline could prevent excess hyperammonemia accompanied by BCAA supplementation. This study investigated the combination of BCAA, arginine, and citrulline on endurance performance in two consecutive days. Seven male and three female endurance runners ingested 0.17 g·kg<sup>-1</sup> BCAA, 0.05 g·kg<sup>-1</sup> arginine and 0.05 g·kg<sup>-1</sup> citrulline (AA trial) or placebo (PL trial) in a randomized cross-over design. Each trial contained a 5000 m time trial on the first day, and a 10000 m time trial on the second day. The AA trial had significantly better performance in 5000 m (AA: 1065.7 ± 33.9 s; PL: 1100.5 ± 40.4 s) and 10000 m (AA: 2292.0 ± 211.3 s; PL: 2375.6 ± 244.2 s). The two trials reported similar ratings of perceived exertion. After exercise, the AA trial had significantly lower tryptophan/BCAA ratio, similar NH<sub>3</sub>, and significantly higher urea concentrations. In conclusion, the supplementation could enhance time-trial performance in two consecutive days in endurance runners, possibly through the inhibition of cerebral serotonin synthesis by BCAA and the prevention of excess hyperammonemia by increased urea genesis.

**Key words:** Central fatigue, time trial, neurotransmitter, hyperammonemia, tryptophan.

### Introduction

The elevated cerebral serotonin (5-hydroxytryptamine) level is one of the mechanisms that contribute to the central nervous system fatigue during exercise (Newsholme and Blomstrand, 2006). Serotonin, a is associated with the feeling of lethargy and tiredness that may contribute to the loss of central drive and motivation (Davis and Bailey, 1997). This hypothesis is supported by several human and animal studies. The cerebral uptake of tryptophan, the precursor for serotonin synthesis, was significantly increased in humans during 3-hr cycling (Blomstrand et al., 2005). In addition, cerebral serotonin synthesis was elevated after treadmill running in rats (Chaouloff, 1997). The running time to exhaustion was significantly decreased after the administration of a serotonergic agonist, while it was significantly improved when given a serotonergic antagonist in rats (Bailey et al., 1993).

The rate of cerebral serotonin synthesis is regulated by the transport of plasma tryptophan across the blood-brain barrier (Sharp et al., 1992). The ability of branched-chain amino acids (BCAA) to compete with tryptophan for crossing the blood brain barrier through the same transporter has provoked the hypothesis that the supplementation of these amino acids could reduce cerebral serotonin synthesis and prevent central fatigue during prolonged exercise (Blomstrand et al., 1997; Fernstrom, 2005). Indeed, the administration of BCAA prevented exercise-induced serotonin release in rat hippocampus (Gomez-Merino et al., 2001). Human studies have also shown that oral supplementation of BCAA could reduce ratings of perceived exertion and mental fatigue in maximal exercise (Blomstrand et al., 1997) and improve cognitive function after a 30-km cross-country race through reduced plasma tryptophan/BCAA ratio (Hassmen et al., 1994). However, except one study undertaken in warm conditions (Mittleman et al., 1998), most studies showed that BCAA supplementation had no effect on endurance performance (Blomstrand et al., 1995; 1997; Struder et al., 1998; van Hall et al., 1995).

One possible explanation for the lack of ergogenic effect of BCAA supplementation is the accompanied excess hyperammonemia resulted from the oxidation of these amino acids (MacLean and Graham, 1993; MacLean et al., 1994, 1996; Meeusen et al., 2006; Struder et al., 1998). It has been shown that cerebral uptake and accumulation of ammonia (NH<sub>3</sub>) was increased in humans during prolonged exercise (Nybo et al., 2005), which could induce central fatigue by alterations of cerebral energy metabolism and neurotransmission, and signaling pathways within the neuron (Wilkinson et al., 2010). Therefore, we hypothesized that incorporating arginine and citrulline with BCAA could improve endurance exercise performance by alleviating excess NH<sub>3</sub> production and reducing plasma tryptophan/BCAA ratio.

Both arginine and citrulline could reduce exercise-related accumulations of NH<sub>3</sub> by increasing the urea cycle (Curis et al., 2005; Schaefer et al., 2002) and nitric oxide (NO) biosynthesis (Clarkson et al., 1996; Curis et al., 2005). Citrulline is more potent because of its high bioavailability (Rouge et al., 2007). It has been revealed that citrulline supplementation could increase plasma urea concentration and NO production (Sureda et al., 2010), while suppressing the exercise-induced hyperammonemia

(Takeda et al., 2011) in prolonged exercise. Moreover, a combined supplementation of citrulline, arginine, and ornithine reduced plasma ammonia concentration after a single bout of exhaustive exercise in rats (Meneguello et al., 2003).

The purpose of this study was to investigate the combined supplementation of BCAA, arginine, and citrulline on endurance performance in two consecutive days in trained runners. The majority of previous studies investigating the alleviation of central fatigue in endurance exercise used a single bout of exercise. From our previous results, the effect of BCAA and arginine supplementation appears to be effective on the second consecutive day of intermittent high-intensity exercise, when the central fatigue is more apparent, in well-trained athletes (Chang et al., 2015). It is common for endurance athletes to participate in more than one event in a competition. The athletes would race in two or more consecutive days, which would lead to the accumulation of fatigue. However, to our best knowledge, the nutritional strategy to alleviate central fatigue and improve endurance performance in consecutive days has not been investigated. The use of race-like environment in performance measurement would make the results more applicable to real competitions. Each trial contained two consecutive days of exercise, with a 5000 meter (m) time trial on the first day, and a 10000 m time trial on the second day.

## Methods

### Participants

Thirteen endurance runners (10 male and 3 female) were originally recruited from the track and field team in National Taiwan University of Sport, Taichung, Taiwan. The subjects have trained and competed in events ranging from 1500 m to 10000 m. Three of them withdrew from the study because of sickness or injuries unrelated to the supplements and tests. The remaining 10 participants (7 male and 3 female) have been participating in endurance training for  $7.3 \pm 0.9$  years and competed at the national level. The 7 male participants have the age of  $20.6 \pm 1.1$  years, the height of  $1.72 \pm 0.08$  m, the weight of  $57.03 \pm 4.92$  kg, body mass index of  $19.14 \pm 1.35$  kg·m<sup>-2</sup>, and the body fat of  $12.9 \pm 2.1\%$ . The 3 female participants have the age of  $22.7 \pm 2.3$  years, the height of  $1.58 \pm 0.06$  m, the weight of  $46.80 \pm 4.57$  kg, the body mass index of  $18.67 \pm 0.58$  kg·m<sup>-2</sup>, and the body fat of  $18.6 \pm 0.8\%$ . The body composition was measured by bioelectrical impedance analysis (IOI 353, Jawon Medical, Gyeongsan-si, Korea). The exclusion criteria included major cardiovascular disease risks, musculoskeletal injuries, upper respiratory infection, smoking, and consumption of any medicine or protein/amino acids supplement in the past 3 months. The participants were advised to maintain their regular training schedule and dietary routine during the study period. The participants were instructed to refrain all training activity, any strenuous physical activity, and consumption of alcohol and caffeine-containing foods on the day prior to the trial. All participants gave their written informed consent after the experimental procedure and potential risks were explained. The study protocol

was approved by the Research Ethics Committee of China Medical University and Hospital, Taichung, Taiwan.

### Study design

This study used a single-blind, randomized cross-over design. Each subject completed amino acids (AA) and placebo (PL) trials in a random order, separated by a wash-out period of seven days. Each trial contained two consecutive days of exercise, with a 5000 m time trial on the first day, and a 10000 m time trial on the second day. During the two days prior to each trial, the participants were provided with the same three meals per day, purchased from local convenience stores. The meals provide approximately 2250 kcal·day<sup>-1</sup> with 55% energy from carbohydrate, 30% from fat, and 15% from protein, according to the manufacturer's label.

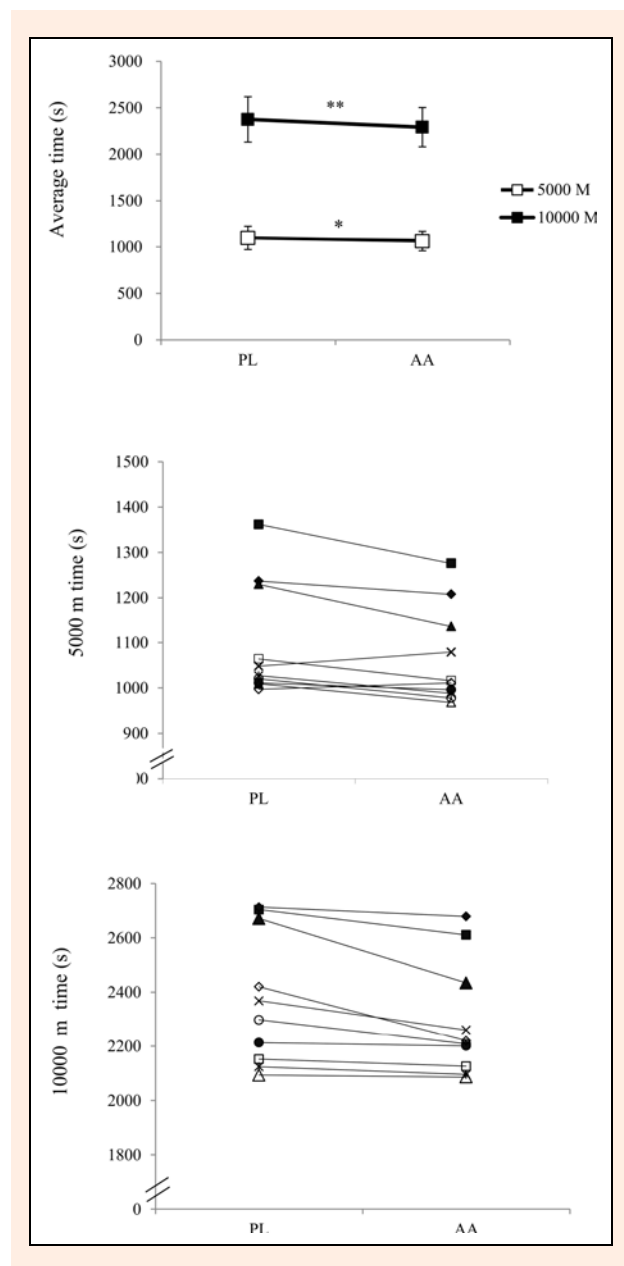
### Procedures

**Supplementation:** On the days of the trials, the participants reported to the stadium at 0630 after an overnight fast. After blood sampling, two different supplements were consumed. In the AA trial, the participants ingested 0.17 g·kg<sup>-1</sup> BCAA (leucine: isoleucine: valine = 10:7:3, containing vitamin E 6.67 IU/g BCAA, capsule, General Nutrition Corporation, Pittsburgh, PA, USA), 0.05 g·kg<sup>-1</sup> arginine and 0.05 g·kg<sup>-1</sup> citrulline (arginine: citrulline = 1:1, tablet, General Nutrition Corporation). In the PL trial, the participants consumed the identical amount of empty capsule and tablet containing starch (Chung-Yu Biotech Co LTD, Taichung, Taiwan) to the AA trial and one capsule of vitamin E (100 IU, General Nutrition Corporation). All supplements were taken with water within 10 min. The time trials started 60 min after the supplements were consumed. Our preliminary study has shown that plasma BCAA and arginine concentrations would peak after one hr of ingestion (data not shown).

**Time trial:** All subjects completed a vigorous warm-up that was identical to their pre-competition routine prior to the time trials. The 5000 m (day 1) and 10000 m (day 2) time trials were held in a certified polyurethane 400-m outdoor running track, using the international rules. All participants from both trials competed at the same time to encourage the best performance. The running time was recorded by stop watches. The subjects were aware of their performance and pace during the trials through their own watches. No food or fluid was provided during the time trial. The ratings of perceived exertion (RPE) were recorded immediately before and after each time trial using the Borg's 20-point scale (Borg, 1982). This study did not require a familiarization trial because all participants were very used to the training and competition in the early morning, and the race-like time trials from their years of experience.

**Measurement of blood biochemical parameters:** Venous blood samples were collected before the supplementation and immediately after the time trials into tubes containing EDTA. Hemoglobin and hematocrit in whole blood were measured immediately after collection by a blood cell analyzer (Sysmex Kx-21, Diamond Diagnostics, Holliston, MA, USA). After centrifugation, the plasma samples were aliquoted and stored at -70°C.

until further analysis.



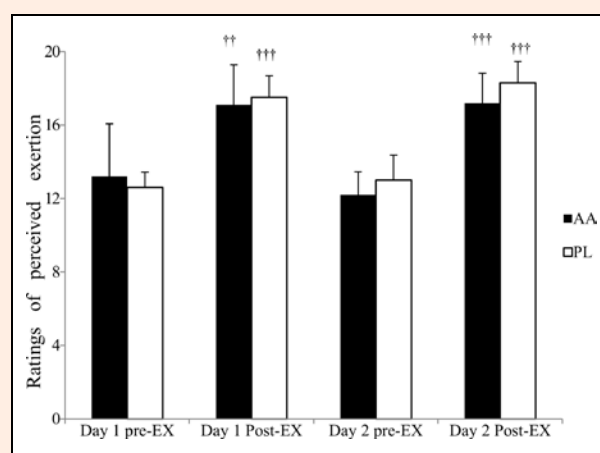
**Figure 1.** The results of 5000 m and 10000 m time trials in the AA and PL trials. (A, top) average time; (B, middle) individual 5000 m time; (C, bottom) individual 10000 m time). \*  $p < 0.05$ ; \*\*  $p < 0.01$ , significantly different between the AA and PL trials.

Plasma BCAA concentration was measured enzymatically (Biovision, Milpitas, CA, USA) with a microplate spectrophotometer (Benchmark Plus, Bio-Rad, Hercules, CA, USA). Plasma tryptophan concentration was analyzed with a fluorescence assay (Bridge-It, Medimomics, 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  $\text{NO}_x$  concentrations were determined using the Griess reagent (Green et al., 1982). Plasma concentrations of urea, glucose, lactate,  $\text{NH}_3$ , glycerol, and non-esterified fatty acids were measured with an

automatic analyzer (Hitachi 7020, Tokyo, Japan) using commercial kits (Randox, Antrim, UK). The changes in plasma volume were corrected for all blood parameters using hemoglobin concentration and hematocrit in whole blood (Costill and Fink, 1974).

### Statistical analysis

All data were expressed as mean  $\pm$  SD. The results were analyzed by two-way (trial  $\times$  time) analysis of variance with repeated measurements. If the main effect is significant, the differences were identified by Ryan-Holm-Bonferroni post hoc analysis (Atkinson, 2002). A  $p < 0.05$  was considered statistically significant.



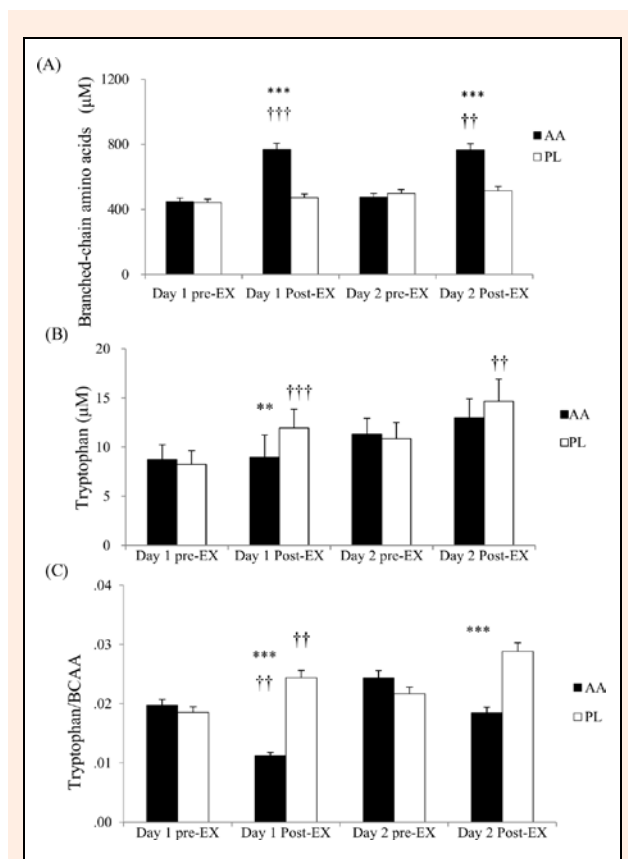
**Figure 2.** Ratings of perceived exertion before and after exercise on day 1 and day 2 in the AA and PL trials. Main effects: trial:  $p = 0.172$ ; time:  $p < 0.001$ ; interaction:  $p = 0.550$ . ††  $p < 0.01$ ; †††  $p < 0.001$ , different from the pre-EX at the same day in the same trial

### Results

The running time in 5000 m on the first day was significantly faster in the AA trial by  $2.98 \pm 3.24\%$  (AA:  $1065.7 \pm 33.9$  s; PL:  $1100.5 \pm 40.4$  s;  $p = 0.019$ ) (Figure 1A). The performance in 10000 m on the second day was also significantly better in the AA trial by  $3.38 \pm 3.10\%$  (AA:  $2292.0 \pm 211.3$  s; PL:  $2375.6 \pm 244.2$  s;  $p = 0.009$ ) (Figure 1A). The individual running time in 5000 m and 10000 m in the AA and PL trials is presented in Fig 1B and 1C, respectively. On the first day, eight participants had better performance in the AA trial (running time reduced by 1.48-7.96%), while two others were slower in the AA trial (running time increased by 1.40 and 2.96%). On the second day, all participants ran faster in the AA trial, with running time reduced by 0.33-8.87%. The percentages of performance improvement in 5000 m and 10000 m were not significantly different. Despite the improvement in running time, the post-exercise RPE were similar between the two trials (Figure 2).

The AA trial resulted in increases in post-exercise plasma BCAA concentrations by 71.1% and 60.4% on day 1 and 2, respectively, compared to the baseline (Figure 3A). Post-exercise plasma tryptophan levels were significantly increased from the baseline on both days in the PL trial, while it was increased only on day 2 in the

AA trial (Figure 3B). The larger magnitude of BCAA increase led to the significantly lower post-exercise tryptophan/BCAA ratio in the AA trial, compared to that in the PL trial (Figure 3C).



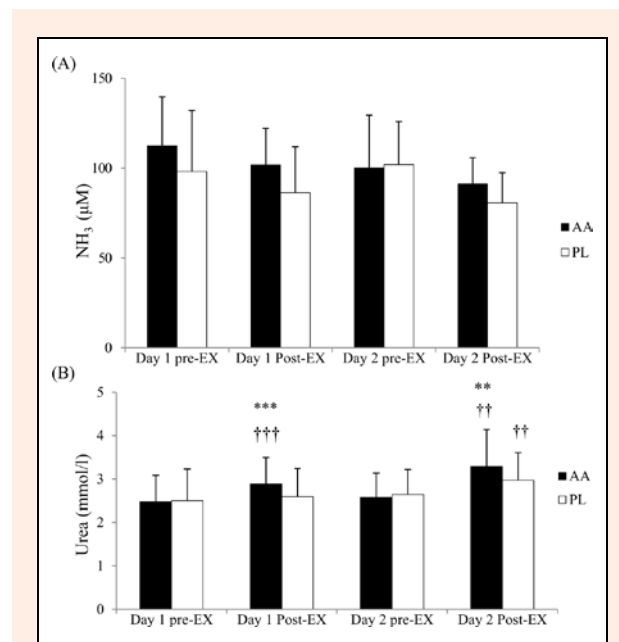
**Figure 3.** Plasma concentrations of (A) BCAA and (B) tryptophan, and (C) tryptophan/BCAA ratio in the AA and PL trials. Main effects: (A) trial:  $p < 0.001$ ; time:  $p < 0.001$ ; interaction:  $p < 0.001$ ; (B) Main effects: trial:  $p = 0.005$ ; time:  $p < 0.001$ ; interaction:  $p = 0.003$ ; (C) Main effects: trial:  $p < 0.001$ ; time:  $p = 0.040$ ; interaction:  $p < 0.001$ . \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ , AA vs PL trial at the same time point. † $p < 0.05$ ; †† $p < 0.01$ ; ††† $p < 0.001$ , different from the pre-EX at the same day in the same trial

The excess accumulation of  $\text{NH}_3$ , commonly seen after BCAA supplementations in previous studies, was absent in the AA trial as the two trials showed similar post-exercise plasma  $\text{NH}_3$  concentrations (Figure 4A). The AA trial showed significantly higher plasma urea concentration after exercise than that in the PL trial on both days (Figure 4B). Pre- and post-exercise plasma concentrations of  $\text{NO}_x$ , glucose, lactate, glycerol, and non-esterified fatty acids are presented in Table 1. These variables were not statistically different between the two trials.

## Discussion

The results of this study suggested that the combined supplementation of BCAA, arginine, and citrulline could improve endurance performance on both consecutive days of exercise. The participants in the AA trial could run faster at the same degree of perceived exertion, possibly resulting from the reduced plasma tryptophan/BCAA

ratio. In addition, the elevated urea synthesis, conceivably from arginine and citrulline supplementation, prevented the excess hyperammonemia in the AA trial.



**Figure 4.** Plasma concentrations of (A)  $\text{NH}_3$  and (B) urea in the AA and PL trials. Main effects: (A) trial:  $p = 0.182$ ; time:  $p = 0.056$ ; interaction:  $p = 0.560$ ; (B) Main effects: trial:  $p = 0.213$ ; time:  $p < 0.001$ ; interaction:  $p = 0.007$ . \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ , AA vs PL trial at the same time point. † $p < 0.05$ ; †† $p < 0.01$ ; ††† $p < 0.001$ , different from the pre-EX at the same day in the same trial

In the AA trial, the supplementation led to the significantly decreased plasma tryptophan/BCAA ratio, resulting from the elevation in BCAA concentration. The lower tryptophan/BCAA ratio would reduce cerebral uptake of tryptophan, hence decreases cerebral serotonin synthesis and alleviates central fatigue (Gomez-Merino et al., 2001). This is evidenced by the fact that the participants could run faster while feeling the same magnitude of effort. The results were similar to our previous study in which the supplementation of BCAA and arginine allowed the participants to perform better in the intermittent sprints under the same RPE (Chang et al., 2015). Although it has been suggested that BCAA could alleviate the feeling of fatigue during the exercise with fixed intensities in general populations (Blomstrand et al., 1997), this may not be the case in this study. The time-trial and race-like protocol used in this study would drive the athletes to complete the trial with their maximal effort. Thus, it is conceivable that the participants reported similar RPE in both trials.

The AA and PL trials produced similar post-exercise plasma  $\text{NH}_3$  concentrations, indicating the absence of excess hyperammonemia from BCAA oxidation. Arginine and citrulline appeared to enhance  $\text{NH}_3$  removal by increasing urea synthesis in the AA trial (Meneguello et al., 2003; Schaefer et al., 2002; Takeda et al., 2011). It is noteworthy that in the one study that showed ergogenic effect of BCAA, the supplemented trial had similar post-exercise plasma  $\text{NH}_3$  concentration to that in the control trial (Mittleman et al., 1998).

**Table 1. Plasma biochemical parameters before and after exercise in AA and PL trials. Values are means ( $\pm$ SD).**

	Trial	Day 1 pre-EX	Day 1 Post-EX	Day 2 pre-EX	Day 2 Post-EX
NO <sub>x</sub> ( $\mu$ M)	AA	100.8 (31.6)	134.4 (65.4)	89.6 (36.5)	131.1 (60.0)
	PL	93.3 (28.9)	111.4 (76.6)	100.9 (39.0)	86.3 (34.1)
Glucose (mM)	AA	5.2 (.7)	7.6 (1.8) *	6.0 (1.2)	7.9 (2.5) *
	PL	5.6 (1.4)	8.0 (1.9) *	6.3 (1.2)	7.8 (2.5) *
Lactate (mM)	AA	1.7 (.3)	7.7 (3.6) *	1.6 (.3)	6.0 (2.2) *
	PL	1.8 (.5)	7.1 (3.9) *	1.8 (.6)	5.0 (2.3) *
Glycerol ( $\mu$ M)	AA	39.3 (17.7)	127.3 (60.9) *	38.9 (26.5)	166.7 (64.2) *
	PL	39.9 (14.9)	122.8 (64.9) *	36.9 (31.0)	177.9 (96.4) *
NEFA (mM) <sup>a</sup>	AA	.66 (.33)	.54 (.30)	.72 (.50)	.93 (.61)
	PL	.74 (.26)	.63 (.39)	.64 (.45)	1.15 (.77)

<sup>a</sup> non-esterified fatty acid. \*  $p < 0.05$ , significantly different from pre-exercise on the same day in the same trial.

In our previous study that also used a two-day protocol, the ergogenic effect of BCAA and arginine was only present on the second day, presumably with accumulated central and/or peripheral fatigue (Chang et al., 2015). However, post-exercise excess hyperammonemia from BCAA oxidation was not completely prevented. By alleviating excess NH<sub>3</sub> accumulation with arginine and citrulline in the present study, the endurance performance was improved on the first and second day. It indicated that although most studies failed to show the ergogenic effect of BCAA on a single bout of endurance exercise, it was probably due to the concomitantly elevated plasma NH<sub>3</sub> concentrations that nullified the potential benefit of BCAA on alleviation of central fatigue (MacLean and Graham, 1993; MacLean et al., 1994; 1996; Meeusen et al., 2006; Struder et al., 1998). In the AA trial in this study, all but two participants had better performance in the 5000 m event on the first day, and all participants ran faster in the 10000 m event on the second day, compared to the PL trial. Although the degree of improvement varied among the participants, the general agreement among this group of trained runners indicated that the supplementation could be applicable to real endurance running events.

## Conclusion

In conclusion, the combined supplementation of BCAA, arginine, and citrulline could enhance endurance performance in two consecutive days in college runners. This supplementation could be used in multi-day competitions that are common for endurance athletes. The potential mechanisms responsible for the ergogenic effect include the alleviation of central fatigue by BCAA and the prevention of hyperammonemia through increased urea genesis by arginine and citrulline. Future studies could examine the role of other neurotransmitters such as dopamine and epinephrine. In addition, the effect of this supplementation on fed state requires further investigation.

## Acknowledgements

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### Key points

- The combined supplementation of BCAA, arginine, and citrulline could enhance performance in 5000 m and 10000 m in 2 consecutive days in competitive runners. The supplementation may be helpful in multi-day competitions.
- The supplemented BCAA may alleviate central fatigue, allowing the subjects to run faster at the same degree of perceived exertion.
- The hyperammonemia that is usually accompanied with BCAA supplementation may be prevented by arginine and citrulline through increased urea genesis.

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# Reliability and validity of a dual-task test for skill proficiency in roundhouse kicks in elite taekwondo athletes

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**Abstract:** The dual-task methodology, conducting two tasks simultaneously, may provide better validity than the traditional single-task tests in the environment that is closely related to real sport competitions. The purpose of this study is to determine the reliability and validity of a dual-task test that aims to measure the reaction time and skill proficiency in roundhouse kicks in elite and sub-elite taekwondo athletes. The dual-task results were compared to those in the single-task movements with various levels of complexity. The single-task movements A, B, and C were composed of one, three, and five roundhouse kicks, respectively. The dual-task movement D was composed of movement C and a push of a button in response to a light stimulus as the secondary task. The subjects were 12 elite and 12 sub-elite male taekwondo athletes. The test included four movements with five repeats of each movement in a randomized order. Each subject conducted the same test on two consecutive days. The intraclass correlation coefficient (ICC) showed moderate-to-high correlation in the premotor time (ICC =0.439–0.634 in elite and ICC =0.681–0.824 in sub-elite), motor time (ICC =0.861–0.956 in elite and ICC =0.721–0.931 in sub-elite), and reaction time (ICC =0.692 in elite and ICC =0.676 in sub-elite) in the secondary task in both groups. The elite athletes had significantly faster premotor time than their sub-elite counterparts in all the four movements (all  $P < 0.05$ ). The largest difference lies in the reaction time in the secondary task, in which the elite group ( $0.248 \pm 0.026$  seconds) was 33.0% faster than the sub-elite group ( $0.370 \pm 0.081$  seconds) ( $P < 0.001$ ). This study shows that the test developed in this study has reasonable reliability and validity in both single- and dual-task methods. In addition, the dual-task method may be a more appropriate way to assess the reaction time and skill proficiency in taekwondo athletes.

**Keywords:** roundhouse kick, premotor time, motor time, reaction time, elite athlete

## Introduction

Taekwondo became an official Olympic sport in 2000. The recent rule change that awards kicking to the head 3 points, compared to 1 point to the chest, has made kicking the primary attack skill in taekwondo. Roundhouse kick is usually the skill that can be conducted with the fastest speed<sup>1</sup> and has the greatest efficacy<sup>2</sup> in most taekwondo athletes.

Reaction time is a crucial factor in success in taekwondo and other combat sports for quick response to opponent's movements.<sup>3</sup> Total reaction time, the time between the stimulus and the initiation of action, can be divided into two parts according to electromyography (EMG) results. The period of time between the onset of the stimulus and the beginning of the EMG signal is called the premotor time. The second component is called the motor time, defined as the period from the increase in EMG signal

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to the actual beginning of the observed muscle movement.<sup>4</sup> Although the reaction time is routinely measured when establishing the physiological profile of athletes, the difference between the elite and sub-elite athletes may be small. This is especially true when the required movement is the main action of the respective sport that has been practiced numerous times. Indeed, it has been shown that the difference in the total reaction time in roundhouse kick between experts and novice taekwondo athletes was insignificant, with an average difference of ~0.05 seconds.<sup>3</sup> In addition, the total reaction time in female athletes of Germany national taekwondo squad was not significantly different from their regional counterparts of the same age.<sup>5</sup> It is very likely that even the sub-elite athletes have spent significant amount of time in practicing the main task in their respective sport. The movements have become automatic, making it difficult to distinguish the reaction time between the elite and sub-elite athletes.

Owing to the limitation of single-task reaction time in distinguishing the elite and sub-elite athletes, the dual-task methodology may be adopted to measure the attention needed for the primary task in athletes. It has been suggested that the central processing capacity of attention is fixed. When performing the two tasks simultaneously, the majority of the processing capacity is distributed to the primary task. If the primary task is relatively easy or proficient, more attention capacity can be allocated to the secondary task, resulting in better performance in the latter.<sup>6</sup> Therefore, the performance in the secondary task can be considered as an indirect reflection of the proficiency of the primary task.<sup>7,8</sup> In addition, dual tasking is necessary in many sport competitions. For example, taekwondo athletes have to be able to read and predict the opponents' next move while performing an attack or defensive task. The better performance in the secondary task may indicate a greater attention capacity that can be allocated to assess the opponent's movements. Despite its benefit, the application of dual-task methodology in skill evaluation of elite athletes is still scarce. The purpose of this study is to determine the reliability and validity of a dual-task test that aims to measure the reaction time and skill proficiency in roundhouse kicks in elite and sub-elite taekwondo athletes. In addition, the dual-task results were compared to the single-task movements with various levels of complexity.

## Materials and methods

### Subjects

Twelve elite and 12 sub-elite male taekwondo black-belt athletes were recruited for the study. The subjects in both

groups have been receiving training for taekwondo competitions for at least 6 years. The elite athletes have won medals in international and/or national competitions during high school or university. The sub-elite athletes have never won any medal in international or national competitions during the same period. The basic characteristics of the subjects are shown in Table 1. The elite group was significantly taller than the non-elite group. The study protocol was approved by the Research Ethics Committee, China Medical University and Hospital, Taichung, Taiwan. All the subjects gave their written informed consent after the experimental procedure, and potential risks were explained.

### Single task

A dummy was set up as the attack target. A signal light was attached on the head of the dummy (Figure 1A), and a three-axial accelerometer (EGAXT3; Measurement Specialties, Hampton, VA, USA) was installed at the back (Figure 1B). EMG electrodes were attached to the left thenar and brachioradialis muscles (Figure 2A). The subjects stood firmly on flat ground with each foot on a force platform (9260AA6; Kistler, Winterthur, Switzerland), while holding a button in the left hand (Figure 2B). The signals from accelerometer, EMG, force platforms, and the button were collected through a data acquisition and analysis system (MP150; BIOPAC Systems, Inc., Goleta, CA, USA).

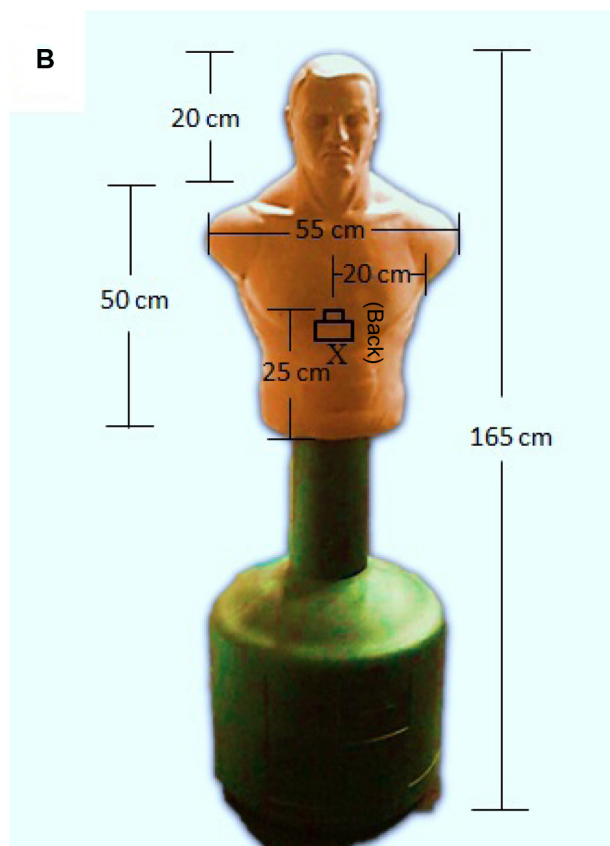
The subjects stood in a guard position with both heels on the ground and waited for the light signal. The subjects were asked to press the button on the left hand with the thumb as soon as they see the light signal, and then start the respective movement to attack the dummy. Three sets of movement were used: A) a roundhouse kick to the rib (Figure 3); B) a roundhouse kick to the rib, a roundhouse kick to the head, and then a reverse roundhouse kick to the head (Figure 4); and C) a roundhouse kick to the rib, a roundhouse kick to the head, a reverse roundhouse kick to the head, a roundhouse kick to the head, a reverse roundhouse kick to the head, and

**Table 1** Basic characteristics of subjects in the elite and sub-elite groups (mean±SD)

	Elite (n=12)	Sub-elite (n=12)
Age (years)	20.1±0.8	20.4±0.7
Height (m)	1.77±0.04	1.71±0.06*
Body weight (kg)	66.9±5.0	65.3±8.8
Body mass index (kg/m <sup>2</sup> )	21.3±0.9	22.2±2.3
VO <sub>2peak</sub> (mL/kg/min)	44.9±6.8	41.8±7.0
Experience (years)	9.9±2.2	9.9±2.3

**Note:** \*Significantly different between the elite and sub-elite groups ( $P<0.05$ ).

**Abbreviations:** SD, standard deviation; VO<sub>2peak</sub>, peak oxygen consumption rate.



**Figure 1** The position of the light (A) and accelerometer (B) in the dummy. **Abbreviation:** X, accelerometer.

**Figure 2** The position of EMG electrodes in the left hand (A) and the handheld button (B). **Abbreviation:** EMG, electromyography.



**Figure 3** Movement A: a roundhouse kick to the rib.

then a roundhouse kick to the head (Figure 5). All the subjects used the right leg as the dominant side in roundhouse kicks. Therefore, all the movements were carried out using the right leg in all the subjects. The subjects could only put the right foot back to the ground after all the kicks were performed in the movement.

## Dual task

The dual-task movement D is composed of movement C and a secondary task. When the subjects were carrying out the movement C, research personnel turned on the light signal on the top of the dummy again. The subjects had to press the button on the left hand with the thumb as soon as they see the second light signal, while conducting movement C.

Each of the four movements was performed five times in a random order. A set of 20 random numbers was generated using Microsoft Excel 2010 to represent the 20 movements in one test session. The order of the 20 movements was written in a piece of paper. A total of 20 pieces were prepared and stored in an opaque box. Before the start of each test session, the subject drew a piece of instruction from the box and gave it to a researcher. The researcher then told the subject which movement has to be performed prior to each movement.

## Determination of variables

The premotor time was determined as the time from the beginning of the light signal to the start of EMG signal of the left thenar muscles. The thenar muscles were used because they provide much clearer EMG signal compared to that of the leg muscles. The EMG signal from the leg muscles was noisy due to the unconscious preparation for the kicks before the light, even though the subjects were asked to stand still prior to each test. The movement time is between the right leg leaving the force platform and the appearance of signal in the accelerometer in the dummy.

The performance of the secondary task is defined as the time between the beginning of the second light signal and pushing the button. So the performance of the secondary task involves the premotor time and movement time. EMG signal is not used in measuring the secondary task because it is present throughout the duration of the first task. Therefore, it is very difficult to identify the EMG signal that triggers the movement to press the button.

## Statistical analysis

Intraclass correlation coefficient (ICC) was calculated using the results of ten trials on days 1 and 2 to examine test–retest reliability. ICC above 0.75 is considered high, that in the range 0.50–0.75 is considered moderate, and that below 0.50 is considered low.<sup>9</sup>

The validity was examined by analyzing the difference between the elite and sub-elite subjects by *t*-test. The average value of ten trials on both the days was used for the comparison. The difference in the premotor time and movement time among the four movements in the same group was analyzed by one-way analysis of variance with repeated measurement (rANOVA). If the main effect was significant, the Tukey's method was used to identify the difference. A *P*-value less than 0.05 was considered statistically significant.

## Results

### Test–retest reliability

The ICCs of premotor time, motor time, and reaction time in the secondary task in elite and sub-elite taekwondo athletes are shown in Table 2. In the premotor time, the results of ten trials in each movement showed moderate correlation as most ICCs were between 0.50 and 0.75. The elite group showed lower ICCs than the sub-elite group in all the four movements. In the motor time, most ICCs showed high correlation in both groups of athletes. The results of the secondary task were also moderately correlated.



**Figure 4** Movement B: (A) a roundhouse kick to the rib, (B) a roundhouse kick to the head, and (C) a reverse roundhouse kick to the head.

### Discriminant validity

The elite athletes had significantly faster premotor time than their sub-elite counterparts in all the four movements (Table 3). However, the elite and sub-elite groups had similar motor time in all the four movements.

The largest difference lies in the reaction time in the secondary task, in which the elite group was 33.0% faster than the sub-elite group. In the elite group, the premotor time was similar across the movements with different levels of complexity (Table 3). On the other hand, the

premotor time was the fastest in the simplest movement A compared to the other movements in the sub-elite group. In the motor time, both the groups were significantly faster in movement A than the other more complicated movements.

## Discussion

The results of this study showed significant test–retest reliability as the ICCs of premotor, motor, and movement times in single- and dual-task methods in both the elite and sub-elite taekwondo athletes were substantial. In addition,

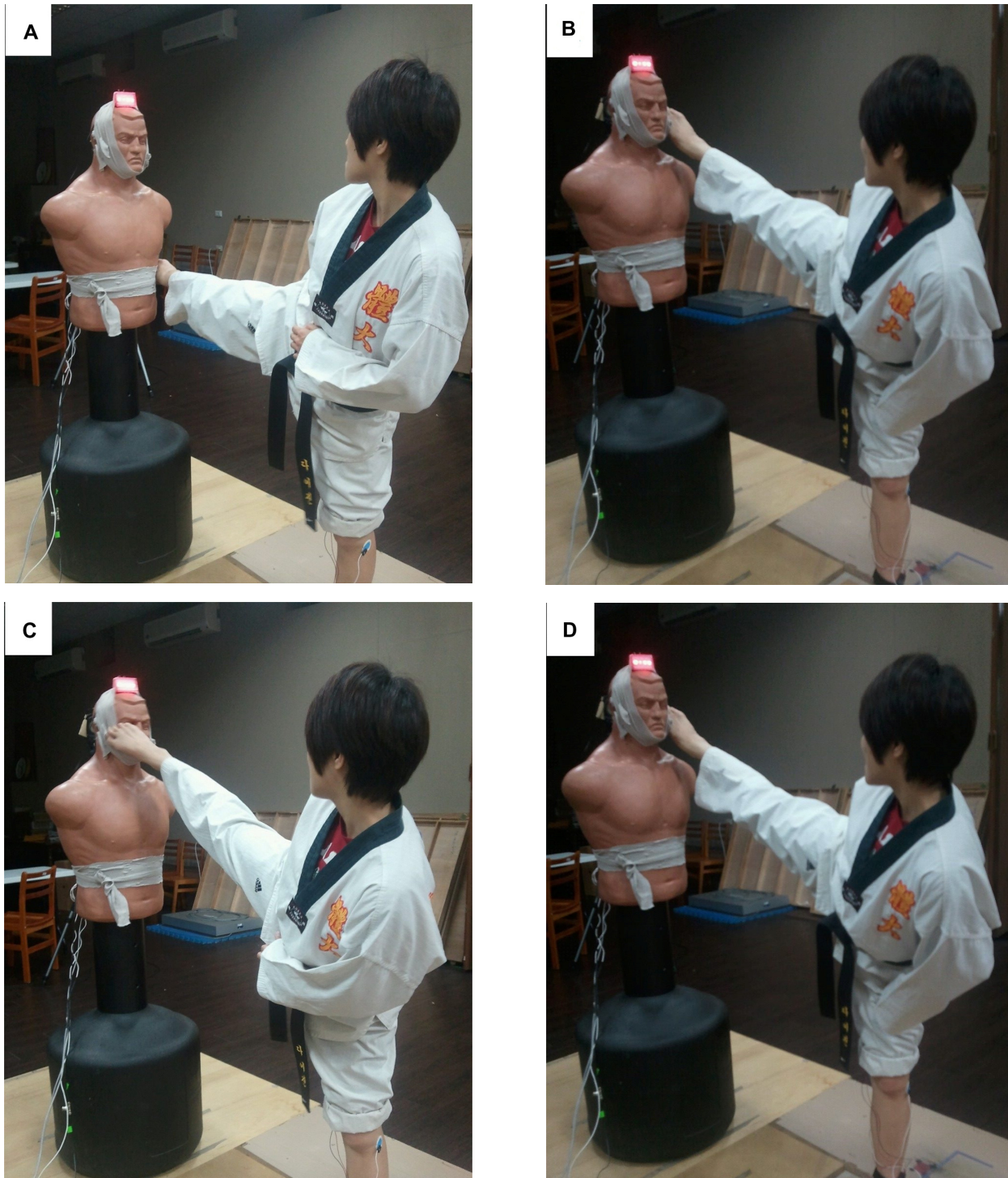
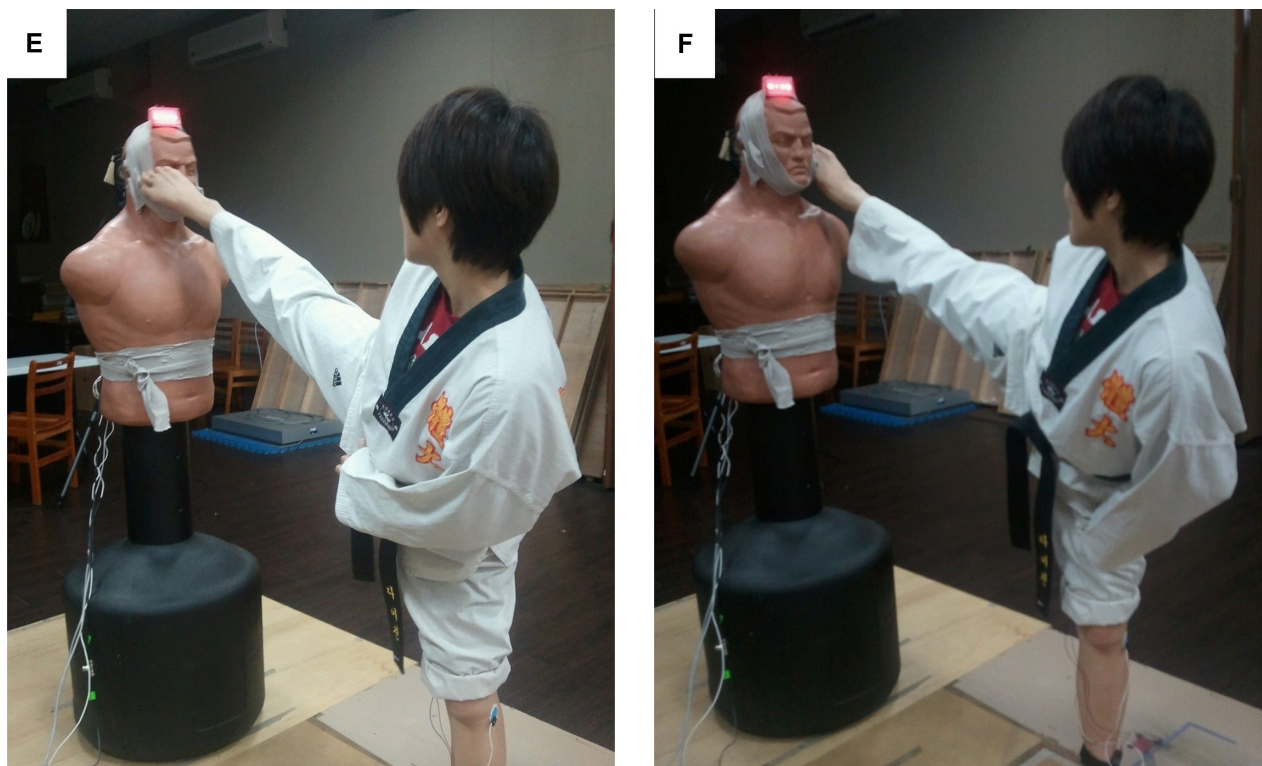


Figure 5 (Continued)



**Figure 5** Movement C: (A) a roundhouse kick to the rib, (B) a roundhouse kick to the head, (C) a reverse roundhouse kick to the head, (D) a roundhouse kick to the head, (E) a reverse roundhouse kick to the head, and (F) a roundhouse kick to the head.

both the methods could successfully distinguish elite and sub-elite athletes, with the dual-task method showing greater discriminant validity.

The premotor time in roundhouse kicks showed moderate-to-high levels of correlation in both single- and dual-task situations in both the groups of subjects. The reaction time in the secondary task also showed moderate-to-high levels of consistency. The performance in the secondary task in

movement D also showed high levels of consistency in both the groups.

The average premotor time in movement A in elite athletes was 14.0% faster than that in the sub-elite athletes. The difference increased to 16.5%–18.2% in the more complicated movements B, C, and D. On the other hand, the motor time in all the movements was similar in both the groups. The results suggested that the difference in the premotor time

**Table 2** ICCs of premotor time, motor time, and secondary task in days 1 and 2 in the elite and sub-elite athletes<sup>a</sup> (mean±SD)

	Elite			Sub-elite		
	Day 1 (s)	Day 2 (s)	ICC <sup>b</sup>	Day 1 (s)	Day 2 (s)	ICC <sup>b</sup>
Premotor time						
A	0.141±0.012	0.145±0.015	0.634	0.165±0.031	0.148±0.016	0.694
B	0.143±0.013	0.146±0.016	0.439	0.186±0.037	0.166±0.021	0.681
C	0.146±0.018	0.147±0.015	0.634	0.189±0.035	0.168±0.034	0.725
D	0.143±0.013	0.149±0.019	0.547	0.182±0.040	0.169±0.031	0.824
Motor time						
A	0.261±0.034	0.257±0.026	0.926	0.268±0.026	0.279±0.043	0.721
B	0.291±0.026	0.271±0.032	0.861	0.293±0.036	0.287±0.031	0.907
C	0.292±0.032	0.282±0.031	0.942	0.313±0.041	0.295±0.029	0.834
D	0.294±0.037	0.282±0.038	0.956	0.303±0.032	0.299±0.034	0.931
Secondary task						
D	0.243±0.029	0.252±0.030	0.692	0.389±0.136	0.351±0.063	0.676

**Notes:** <sup>a</sup>The data of each day represent the average of five trials on the same day; <sup>b</sup>calculated from the total of ten trials in 2 days.

**Abbreviations:** ICC, intraclass correlation coefficient; s, seconds; SD, standard deviation.

**Table 3** The comparison of premotor time, motor time, and secondary task performance in different movements in the elite and sub-elite taekwondo athletes<sup>a</sup> (mean±SD)

	Elite(s)	Sub-elite(s)	P-value
Premotor time			
A	0.143±0.012	0.156±0.020*	0.072
B	0.144±0.010	0.176±0.027	0.002
C	0.147±0.014	0.176±0.033	0.005
D	0.146±0.011	0.176±0.033	0.011
Motor time			
A	0.259±0.029†	0.274±0.028¶	0.210
B	0.281±0.027	0.290±0.030	0.483
C	0.287±0.009	0.304±0.031	0.187
D	0.288±0.037	0.301±0.031	0.367
Secondary task			
	0.248±0.026	0.370±0.081	<0.001

**Notes:** <sup>a</sup>The data represent the average of ten trials on both the days; \*significantly different from movement C in the sub-elite group; †significantly different from movements B, C, and D in the elite group; ‡significantly different from movements C and D in the sub-elite group.

**Abbreviations:** s, seconds; SD, standard deviation.

may contribute to the success in taekwondo competitions. In addition, the elite and sub-elite subjects have similar ability in conducting the roundhouse kicks after many years of practice. The elite subjects were significantly taller than the sub-elite subjects in the present study. To our best knowledge, there is no study investigating the effect of height on the premotor time. Even if height is a factor in the premotor time, the taller subjects would be expected to be slower due to longer transduction of the neural signal. However, the present study showed that the taller elite subjects had faster premotor time than the shorter sub-elite subjects. These results indicated that the small difference (3.3%) in height may not be an important factor in determining the premotor time.

The performance in the secondary task appeared to be the variable with the highest discriminative validity in this study. The average time in the secondary task in elite athletes was 33.0% faster than that in the sub-elite athletes. It suggested that the elite athletes were more proficient, and thus required less attention, in conducting the complicated movement. It is reasonable to assume that, with more attention capacity that can be freed from the primary task, the elite athletes may be better in analyzing and predicting opponent's response while conducting the movement. This ability would lead to more success in taekwondo competitions. Several studies on various sports have also shown that the experts performed better under dual-task situations than the novice, even though the performance may be indistinguishable in the single-task situation. The proficiency of 2-on-1 draw-and-pass drill under the dual-task situation was significantly higher in the professional rugby league players than in their

semiprofessional counterparts. The secondary task required the subjects to identify as quickly and accurately as possible the frequency of a verbal tone. The proficiency of the same 2-on-1 drill performed under the single-task situation did not discriminate the professional and semiprofessional players.<sup>10</sup> In addition, the proficiency of off-field draw-and-pass task under the dual-task situation was significantly correlated with the number of successful draw-and-pass in real competitions in professional rugby league players, while the proficiency of the same task under the single-task situation was not correlated with the success on the field.<sup>11</sup> Beilock et al<sup>12</sup> used the identification of a target word as the secondary task during the golf putting task. The results showed a positive correlation between the error in the secondary task and the inaccuracy in the primary putting task. It indicated that the less-skilled golfers allocated more attention to the putting task, resulting in the decreased performance in the secondary task.

One potential drawback for dual-task methodology is that the subjects may sacrifice the primary task performance to execute the secondary task.<sup>13</sup> The dual-task cost, the difference in primary task performance between the single- and dual-task scenarios, has been shown to increase with the increased complexity of dual-task situations.<sup>14</sup> However, the premotor time and movement time were similar between movements C and D in both the groups. Since the primary task in movement D is identical to movement C, these results indicated that our subjects maintained the performance in the primary task in the dual-task test. The dual-task cost in the present study is nonexistent.

The simple reaction time may not have high discriminative validity in taekwondo athletes. It has been suggested that elite taekwondo athletes showed significantly faster reaction time than their sub-elite counterparts in roundhouse kick. The difference was smaller in the dominant leg (elite 0.44±0.10 seconds vs sub-elite 0.51±0.15 seconds) than in the nondominant leg (elite 0.39±0.03 seconds vs sub-elite 0.49±0.02 seconds).<sup>15</sup> The total reaction time in Germany national taekwondo male athletes was also significantly faster than that in the recreational taekwondo participants, although the differences were small, ranging from 20 milliseconds to 50 milliseconds in shoulder, hip, and ankle joints. However, the total reaction time in female athletes in the national team was not significantly different from that of recreational athletes.<sup>5</sup> Furthermore, the reaction time for roundhouse kick to the chest or head was not significantly different between experts and novice taekwondo athletes.<sup>3</sup> It is noteworthy that the reaction time in the aforementioned

studies included the premotor time and motor time, making it longer than the premotor time in our results.

The elite athletes showed similar premotor time across the four movements with different levels of complexity. The sub-elite athletes also showed similar premotor time in the three more complicated movements, although the premotor time in the simplest movement A was faster than the other three movements. The lack of difference in the premotor time between movements B, C, and D is contradictory to the memory drum theory proposed by Henry and Rogers.<sup>16</sup> The memory drum theory has been challenged recently, but the alternative model that can fully explain the situations has yet been established.<sup>17</sup> This exact mechanism underlying this phenomenon requires further investigation.

In conclusion, this study establishes an easy-to-apply method to measure the attention demand and skill proficiency in taekwondo athletes. Our method is similar to that in real taekwondo competitions in which the athletes have to track and predict the opponent's movements while performing the current attack or defensive task. In most situations, the instruments and/or the time of the athletes are limited. The performance in the secondary task can be measured without force platform and EMG while providing the highest validity. Only the light signal and the handheld button are required. The athletes with better performance in the secondary task may undergo training in more complicated situations in which he/she has to read and predict the opponent's movements. On the other hand, the athletes with lower performance in the secondary task may require more practice in the main skills in the single-task situation or in less complicated dual-task environment.

## Disclosure

The authors report no conflicts of interest in this work.

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## 科技部補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現（簡要敘述成果是否有嚴重損及公共利益之發現）或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

達成目標

未達成目標（請說明，以 100 字為限）

實驗失敗

因故實驗中斷

其他原因

說明：

本計畫所執行的三個研究都已經發表於國際期刊，第一年網球的研究刊登於 Scandinavian Journal of Medicine and Science in Sports，第二年跆拳道研究刊登於 Journal of International Society of Sport Nutrition，第三年中長跑的研究刊登於 Journal of Sport Science and Medicine。本三年期計畫成功發展並驗證評估網球與跆拳道反應式技術的測試方法，並根據這些測試方法，顯示補充 BCAA、arginine、citrulline 可以減緩長時間運動後的中樞疲勞以及反應式技術的衰退，這是國際上獨有的研究成果，除了學理與生化機轉的驗證之外，此研究結果也可直接應用至技擊、球類、中長跑的多項運動，提升運動表現，對於學術研究及實務應用都有相當的貢獻。本研究方向及成果與當初撰寫計畫時的內容相當符合，都是探討補充 BCAA、arginine、citrulline 對長時間運動後中樞疲勞及反應式技術表現的影響，使用的各項生化指標也都於計畫書中提及，顯示本計畫的研究假設是合理的，本研究團隊也完成預計的各項實驗內容和文章撰寫。

2. 研究成果在學術期刊發表或申請專利等情形：

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技轉：已技轉 洽談中 無

其他：(以 100 字為限)

1. Yang CC, Wu CL, Chen IF, **Chang CK\***. (2016) Prevention of perceptual-motor decline by branched-chain amino acids, arginine, after tennis match. **Scandinavian Journal of Medicine and Science in Sports** Accepted, doi: 10.1111/sms.12717 (SCI).
2. Cheng IS, Wang YW, Chen IF, Hsu GS, Hsueh CF, **Chang CK\***. (2016) The supplementation of branched-chain amino acids, arginine and citrulline improves endurance exercise performance in two consecutive days. **Journal of Sports Science and Medicine** 15:509-515 (SCI).
3. Chen IF, Wu HJ, Chen CY, Chou KM, **Chang CK\***. (2016) Branched-chain amino acids, arginine, citrulline alleviate central fatigue after 3 simulated matches in taekwondo athletes: a randomized controlled trial. **Journal of the International Society of Sports Nutrition** 13:28 (SCI).
4. Chen CY, Dai J, Chen IF, Chou KM, **Chang CK\***. (2015) Reliability and validity of a dual-task test for skill proficiency in roundhouse kicks in elite taekwondo athletes. **Open Access Journal of Sports Medicine**. 6:181-189.

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性），如已有嚴重損及公共利益之發現，請簡述可能損及之相關程度（以 500 字為限）

本研究建立了具有合理信效度的網球反應式技術測試流程，可應用於網球選手訓練、評估能力等方向。本研究亦建立了跆拳道前動作反應時間的測試流程，可使用單任務或雙任務模式，可用於訓練、評估能力等方向。研究成果也符合原先之假設，即合併補充支鏈胺基酸、精胺酸與瓜胺酸可減緩中樞疲勞，進而提升反應式技術表現，有助於運動員在長時間的比賽中，維持技術表現；亦可提升連續二天中長跑比賽的成績，具有相當顯著的學術及實務貢獻。本研究至今未有嚴重損及公共利益之發現。

# 科技部補助計畫衍生研發成果推廣資料表

日期:2016/09/07

科技部補助計畫	計畫名稱: 補充支鏈胺基酸、精胺酸與瓜胺酸對中樞疲勞與反應式技術表現的影響
	計畫主持人: 張振崗
	計畫編號: 102-2410-H-028-002-MY3      學門領域: 運動生理學
無研發成果推廣資料	

102年度專題研究計畫成果彙整表

計畫主持人：張振崗			計畫編號：102-2410-H-028-002-MY3				
計畫名稱：補充支鏈胺基酸、精胺酸與瓜胺酸對中樞疲勞與反應式技術表現的影響							
成果項目			量化	單位	質化 (說明：各成果項目請附佐證資料或細項說明，如期刊名稱、年份、卷期、起訖頁數、證號...等)		
國內	學術性論文	期刊論文		0	篇		
		研討會論文		0			
		專書		0	本		
		專書論文		0	章		
		技術報告		0	篇		
		其他		0	篇		
	智慧財產權及成果	專利權	發明專利		申請中	0	
					已獲得	0	
			新型/設計專利			0	
		商標權			0	件	
		營業秘密			0		
		積體電路電路布局權			0		
		著作權			0		
		品種權			0		
		其他			0		
				0			
	技術移轉	件數			0	件	
		收入			0	千元	
	國外	學術性論文	期刊論文		3	篇	1. Yang CC, Chang CK*. (2016) Prevention of perceptual-motor decline by branched-chain amino acids, arginine, after tennis match. Scandinavian Journal of Medicine and Science in Sports Accepted, doi: 10.1111/sms.12717 (SCI). 2. Cheng IS, Chang CK*. (2016) The supplementation of branched-chain amino acids, arginine and citrulline improves endurance exercise performance in two consecutive days. Journal of Sports Science and Medicine 15:509-515 (SCI). 3. Chen IF, Chang CK*. (2016) Branched-chain amino acids, arginine, citrulline alleviate central fatigue after 3 simulated

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		研討會論文		1	Chang CK, Chang Chien KM, Chang JH, Wu CL, Huang MH, Liang YC, Liu TH. (2014) Branched-chain amino acids and arginine improve performance in two consecutive days of simulated handball games in male and female athletes. 19th Annual Congress of the European College of Sport Science, July 2-5, mini-oral presentation, Amsterdam, Netherland.	
		專書		0	本	
		專書論文		0	章	
		技術報告		0	篇	
		其他		0	篇	
	智慧財產權及成果	專利權	發明專利	申請中	0	件
			已獲得	0		
			新型/設計專利	0		
			商標權		0	
			營業秘密		0	
			積體電路電路布局權		0	
			著作權		0	
			品種權		0	
			其他		0	
	技術移轉	件數		0	件	
		收入		0	千元	
參與計畫人力	本國籍	大專生		0	人次	
		碩士生		0		
		博士生		3		
		博士後研究員		0		
		專任助理		1		
	非本國籍	大專生		0		
		碩士生		0		

	博士生	0	
	博士後研究員	0	
	專任助理	0	
<p style="text-align: center;">其他成果</p> <p>(無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)</p>		<p>本三年期計畫成功發展並驗證評估網球與跆拳道反應式技術的測試方法，並根據這些測試方法，顯示補充BCAA、arginine、citrulline可以減緩長時間運動後的中樞疲勞以及反應式技術的衰退，這是國際上獨有的研究成果，除了學理與生化機轉的驗證之外，此研究結果也可直接應用至技擊、球類、中長跑的多項運動，提升運動表現，對於學術研究及實務應用都有相當的貢獻。</p>	

## 科技部補助專題研究計畫成果自評表

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達成目標

未達成目標（請說明，以100字為限）

實驗失敗

因故實驗中斷

其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形（請於其他欄註明專利及技轉之證號、合約、申請及洽談等詳細資訊）

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4. 主要發現

本研究具有政策應用參考價值： 否  是，建議提供機關

（勾選「是」者，請列舉建議可提供施政參考之業務主管機關）

本研究具影響公共利益之重大發現： 否  是

說明：（以150字為限）