行政院國家科學委員會專題研究計畫 成果報告

補充精胺酸與支鏈胺基酸對運動表現的影響(II)-分子機轉（第 3 年）

研究成果報告(完整版)

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計 畫 主 持 人 ： 張振崗
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公 開 資 訊 ： 本計畫涉及專利或其他智慧財產權，2 年後可公開查詢

中 華 民 國 101 年 11 月 01 日

中文摘要： 此三年期研究探討補充精胺酸與支鏈胺基酸對運動表現的影響，以及可能的機轉。精胺酸可能可以提升內皮細胞依賴性之血管擴張，促進葡萄糖吸收，以及透過產生 NO，進而刺激粒線體合成。支鏈胺基酸可能可以減少運動造成的肌肉損傷，促進葡萄糖吸收，降低中樞疲勞，兩者合併使用，可能可以產生加成性的效果，進而提升運動表現。本研究第一與第二年均以訓練有素的運動員為研究對象，以獲得最具實用價值之成果。

第一年以 15 名男子手球選手為對象，採用交叉實驗設計，探討補充精胺酸與支鏈胺基酸對連續二天模擬手球比賽表現的影響，於運動前補充 0.17 g/kg BCAA 與 0.04 g/kg arginine (AA trial)，或安慰劑 (placebo trial)。結果顯示，AA trial 在第二天的下半場，20 公尺衝刺跑的速度顯著增加，而 placebo trial 則無顯著改變，顯示補充精胺酸與支鏈胺基酸可能可以在連續二天的間歇性球類運動時，提升第二天的運動表現。

第二年以 10 名男子角力選手為對象，採用交叉實驗設計，探討補充精胺酸與支鏈胺基酸對一天中連續三場模擬角力比賽表現的影響，於運動前補充 0.17 g/kg BCAA 與 0.04 g/kg arginine (AA trial)，或安慰劑 (placebo trial)。結果顯示，兩個 trial 之間，三場模擬比賽的總做功，以及總做功減少率，均無顯著差異，而運動後與恢復期間血漿葡萄糖、胰島素濃度，以及醣類與脂肪代謝指標等，亦無顯著差異，顯示顯示補充精胺酸與支鏈胺基酸對間歇性高強度之技擊性運動表現並無顯著影響。

第三年以成熟分化之肌小管細胞 C2C12 為模式，探討精胺酸的產物 NO 對粒線體合成的影響，並探討合併低氧處理，是否可產生加成性的效果。結果顯示，NO 確實可以透過活化粒線體合成的調控途徑，包括 PGC-1a、NRF-1、mtTFA，進而刺激粒線體合成，但與低氧處理並無加成性的效果。

整體而言，本計畫成功建立了連續兩天球類運動、以及連續場次技擊性運動的運動表現分析流程，這在過去的文獻上均未出現，但卻是與實際運動情況相當接近，可做為往後研究的方法。而本研究也顯示顯示補充精胺酸與支鏈胺基酸可能可以提升連續二天的間歇性球類運動之表現，對運動員具有實際應用之效果。第三年細胞實驗的結果也顯示，透過補充精胺酸，增加 NO 合成，可能可以刺激粒線體合成，進而提升耐力運動表現，此現象需要進一步於運動員身上探討。由於新穎的研究設計，本研究也相當具有學術價值，成果已陸續發表中。

中文關鍵詞： 精胺酸、支鏈胺基酸、運動表現、肌肉損傷、醣類代謝、一氧化氮、粒線體合成

英文摘要： This 3-year study investigated the effect of arginine and BCAA on exercise performance and the potential mechanisms. The combination of arginine and BCAA may have additive effects on exercise performance. The first and second year of this study use well-trained athletes as subjects. The first year investigated male handball players with a cross-over design. The results indicated that AA trial was significantly faster in 20-m sprints in the second half of the second day. The speed remained unchanged in placebo trial. It indicated that arginine and BCAA could enhance exercise performance on the second day of consecutive days of handball competitions. The second year investigated wrestlers with cross-over design. Each trial contained 3 consecutive matches on the same day. The results showed that total power, power decrements, plasma concentrations of glucose and insulin, oxidation rates of carbohydrate and fat were all similar between the trials. Therefore, arginine and BCAA may not have effect on performance in combat sports. The third year investigated the effect of nitric oxide and hypoxia on mitochondria biogenesis in C2C12 myotubes. The results showed that NO could increase mitochondria biogenesis by stimulating PGC-1a、NRF-1、mtTFA. However, the combination of hypoxia did not produce additive effect. In conclusion, this project established the protocol of 2 consecutive days of ball games, and consecutive matches on the same day in combat sports. These are all novel protocols that can be used in future studies. This study also showed that argninine and BCAA could increase exercise performance in consecutive days of ball games. It can be practically applied to athletes. Year 3 also showed that arginine could stimulate mitochondria biogenesis by increasing nitric oxide production. This result need to be verified in athletes. Part of the results has been published, others are submitting.

英文關鍵詞： arginine, branched-chain amino acids, exercise

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行政院國家科學委員會補助專題研究計畫期末成果報告

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計畫類別：■ 個別型計畫　　□ 整合型計畫

計畫編號：98-2320-B-028-001-MY3

執行期間：　98 年 8　　月　1　日至　101　年　7　月　31 日

計畫主持人：張振崗

共同主持人：巫錦霖、程一雄

計畫參與人員：　陳一凡、曾玫蕙、黃玉芳

成果報告類型(依經費核定清單規定繳交)：□精簡報告　■完整報告

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□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

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■涉及專利或其他智慧財產權，□一年■二年後可公開查詢

執行單位：國立臺灣體育運動大學

中　華　民　國　101　年　10 月　30　日

目錄

摘要

此三年期研究探討補充精胺酸與支鏈胺基酸對運動表現的影響，以及可能的機轉。精胺酸可能可以提升內皮細胞依賴性之血管擴張，促進葡萄糖吸收，以及透過產生 NO，進而刺激粒線體合成。支鏈胺基酸可能可以減少運動造成的肌肉損傷，促進葡萄糖吸收，降低中樞疲勞，兩者合併使用，可能可以產生加成性的效果，進而提升運動表現。本研究第一與第二年均以訓練有素的運動員為研究對象，以獲得最具實用價值之成果。

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Keywords: arginine, branched-chain amino acids, exercise performance, muscle damage, carbohydrate metabolism, nitric oxide, mitochondria biogenesis

Abstract

This 3-year study investigated the effect of arginine and BCAA on exercise performance and the potential mechanisms. The combination of arginine and BCAA may have additive effects on exercise performance. The first and second year of this study use well-trained athletes as subjects. The first year investigated male handball players with a cross-over design. The results indicated that AA trial was significantly faster in 20-m sprints in the second half of the second day. The speed remained unchanged in placebo trial. It indicated that arginine and BCAA could enhance exercise performance on the second day of consecutive days of handball competitions. The second year investigated wrestlers with cross-over design. Each trial contained 3 consecutive matches on the same day. The results showed that total power, power decrements, plasma concentrations of glucose and insulin, oxidation rates of carbohydrate and fat were all similar between the trials. Therefore, arginine and BCAA may not have effect on performance in combat sports. The third year investigated the effect of nitric oxide and hypoxia on mitochondria biogenesis in C2C12 myotubes. The results showed that NO could increase mitochondria biogenesis by stimulating PGC-1a、NRF-1、mtTFA. However, the combination of hypoxia did not produce additive effect. In conclusion, this project established the protocol of 2 consecutive days of ball games, and consecutive matches on the same day in combat sports. These are all novel protocols that can be used in future studies. This study also showed that argninine and BCAA could increase exercise performance in consecutive days of ball games. It can be practically applied to athletes. Year 3 also showed that arginine could stimulate mitochondria biogenesis by increasing nitric oxide production. This result need to be verified in athletes. Part of the results has been published, others are submitting.

前言

This is the final report of the 3-year study. Arginine and branched-chain amino acids (BCAA) leucine, isoleucine, and valine have a wide range of physiological functions that may improve performance in various types of exercise. Arginine may stimulate endothelium-dependent vasodilation, glucose transport, and mitochondria biogenesis via nitric oxide (NO) and AMP-activated protein kinase (AMPK) pathways and increase ammonia removal via urea cycle. BCAA may increase glucose transport and increase mitochondria biogenesis via mammalian target of rapamycin (mTOR) pathway, and decrease cerebral serotonin synthesis by competing with tryptophan for cerebral uptake. Peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α) has been shown to be a crucial regulator in mitochondria biogenesis and may be regulated by NO and AMPK. Exercise performance is the result of integration of various physiological systems. Therefore, a combination of supplementation strategy may be required to improve several physiological systems in order to enhance performance in well-trained athletes. Arginine and BCAA, with their multiple physiological functions, have been suggested individually to play important roles in post-exercise recovery, adaptation to endurance training, and endurance exercise performance. However, very few studies have investigated the ergogenic effects of arginine and BCAA combination in well-trained athletes and the underlying mechanisms.

In Year 1, we hypothesized that arginine and BCAA could enhance the performance in handball during the 2 consecutive days of simulated games. The potential mechanism of muscle damage and biochemical moderations were investigated.

In Year 2, we hypothesize that supplementation of arginine and BCAA would increase post-exercise glycogen recovery and endurance exercise performance through their additive and synergistic effects on energy metabolism systems.

In Year 3, we hypothesized that arginine and BCAA could show additive and synergistic effects on energy metabolism systems, specifically in the enhancement of mitochondrial biogenesis.

研究目的

Year 1:

1. To investigate the effect of arginine and BCAA supplementations on performance recovery after simulated handball games on consecutive days.
2. To investigate the effect of arginine and BCAA supplementations on the mechanisms responsible for the recovery after a simulated handball game in athletes.

Year 2:

1. To examine whether BCAA and arginine could restore the performance on 3 consecutive matches in well-trained wrestlers.
2. To investigate whether BCAA and arginine could provide additive effect on glucose disposal during the recovery and the performance in the subsequent matches in well-trained wrestlers.

Year 3:

1. To investigate the effects of nitric oxide, one of the products of arginine, on markers for mitochondria biogenesis and oxidative.
2. To investigate the mechanisms of NO-induced mitochondrial biogenesis, including PGC-1α, mtTFA, and NRF-1.

文獻探討

Post-exercise glycogen recovery

Muscle glycogen is the most important energy source during high-intensity intermittent exercise such as Taekwondo. Taekwondo tournaments usually finish in one day, containing multiple contests with 1-2 hours of rest in between. The insufficient recovery of muscle glycogen after previous contests may impair the performance in the subsequent contest. Therefore, it is essential to establish the optimal post-exercise muscle glycogen recovery protocol following high-intensity intermittent exercise.

It has been shown that consumption of at least 0.5 g carbohydrate/kg/hr is required to reach the maximal post-exercise glycogen recovery [1], while higher doses up to 1.5 g/kg/hr have been used in other studies [2, 3]. The consumption of arginine and leucine along with glucose could result in higher insulin response compared to glucose alone in healthy subjects at rest [4] and after exercise [5]. The supplementation of leucine in combination with carbohydrate resulted in higher post-exercise insulin concentration and greater muscle glycogen recovery compared to carbohydrate alone in highly trained athletes [6]. Other studies also suggested that supplementation of carbohydrate and protein hydrolysates could increase post-exercise muscle glycogen resynthesis compared to isocaloric carbohydrate [7, 8]. Several in vitro studies have also confirmed the stimulatory effect of arginine and leucine on insulin secretion in isolated beta-cells [9-11]. In addition, it has been suggested that NO may increase the expression and translocation of GLUT-4 in rat skeletal and heart muscles [12, 13]. The vasodilation effect of arginine may increase blood flow and substrate delivery to muscle and further increase glycogen recovery.

Post-exercise recovery for subsequent exercise performance

Only limited studies have investigated the effect of dietary supplementation during post-exercise recovery period on the subsequent exercise performance. Fallowfield et al. suggested that during the 4 hr recovery period after running for 90 min at 70% $\dot{V}O_{2max}$, carbohydrate supplementation could increase the time to exhaustion in the subsequent exercise [14]. The consumption of carbohydrate-protein drink after glycogen-depleting exercise also increased the time to exhaustion by 55% in the subsequently cycling exercise at 85% $\dot{V}O_{2max}$, compared to carbohydrate alone [15]. Betts et al. revealed that carbohydrate-protein supplementation during the 4-hr post-exercise recovery period significantly increased time to exhaustion in the subsequent running exercise at 70% $\dot{V}O_{2max}$ compared to carbohydrate alone [16]. However, the carbohydrate-protein supplementation did not show any additional effect compared to isocaloric carbohydrate [16]. On the other hand, several studies have failed to observe the beneficial effect of carbohydrate-protein supplementation during post-exercise recovery on the performance of subsequently exercise compared to carbohydrate alone [17, 18].

BCAA and glycogen recovery

BCAA and arginine may also facilitate the insulin-dependent phase by inducing insulin secretion [9, 11]. The consumption of leucine and arginine along with glucose could result in higher insulinemic response compared to glucose alone in healthy subjects at rest [4]. In addition, The supplementation of leucine in combination with carbohydrate resulted in higher post-exercise insulin concentration and greater muscle glycogen recovery compared to the same amount of carbohydrate in athletes [5, 6]. Arginine supplementation after endurance exercise could also increase glucose and insulin concentrations during the recovery period in trained athletes [19]. Another study revealed that arginine increased insulin-mediated whole-body glucose disposal in healthy subjects [20], which might help to increase post-exercise glycogen resynthesis.

Wrestling is a sport characterized by high-intensity bouts interspersed with brief periods of mild- to moderate-intensity work or rest [21]. It requires whole-body strength and explosiveness, as well as isometric component for technical performance [22]. Olympic and international wrestling events require athletes to compete in multiple matches in one day. The rest between matches are usually 1-3 hrs. it has been shown that a free-style wrestling match decreased the glycogen level in the vastus lateralis muscle by 21.5% [23]. Several studies have reported post-match blood lactate concentration at 10.5-20 mM [23-26], indicating that carbohydrate is the major energy source in wrestling. It is hypothesized that the low muscle glycogen level resulted from previous matches would impair the performance in the subsequent match.

NO, mTOR, PGC-1α, and mitochondria functions

It has been suggested that NO may also be involved in PGC-1α pathway. A 10-week supplementation of arginine enhanced NO production and expression levels of AMPK and PGC-1α, resulting in elevated lipolysis and the oxidation of glucose and fatty acids in Zucker diabetic obese rats [27]. Long-term exposure of cells to low concentrations of NO induced mitochondrial biogenesis through increased expression of PGC-1α in several cell types [28]. The process is mediated by cGMP as the activation of NO-cGMP pathway increased mitochondria content and oxidative phosphorylation in various cell lines [29]. Furthermore, inhibition of NOS by ingestion of L-NAME decreased expression levels of cytochrome c oxidase I and IV some in soleus in rats. [30]. The hypoxia-induced PGC-1α expression and mitochondria biogenesis was absent in nNOS-/- mice [31].

It has also been revealed that mitochondria function may be related by mTOR. The inhibition of mTOR by rapamycin decreased mitochondrial membrane potential, oxygen consumption, and ATP synthesis ability in Jurkat T cells [32] and expression levels of PGC-1α and mitochondrial oxidative enzymes in C2C12 myotubes [33]. The knockdown of mTOR partner raptor by small interfering RNA decreased aerobic ATP production in cell models [32].

The mechanism through which mTOR regulating mitochondria function appeared to involve yin-yang 1 and PGC-1α transcriptional pathway [33], independent from the traditional $P70^{S6K}$ and 4E-BP1 pathway [32].

Year 1

研究方法

Subjects

The subjects were 15 male handball players of National Taiwan College of Physical Education. The average age is 21.1 years. The height is 179.6±6.4 cm, the weight is 78.3±11.3 kg. The VO2max is 52.3±4.4 ml/kg/min. All subjects gave their informed consent before the study.

Experimental protocol

This a randomized cross-over design. Each subject completed 2 trials: BCAA (0.17 g/kg BCAA and 0.04 g/kg arginine) and placebo (starch). Each trial contains 2 days. The protocol is shown in Figure 1. The procedure of each trial is shown in Figure 2.

Biochemical analyses

Plasma concentrations of glucose, lactate dehydrogenase, and creatine kinase were measured by automatic analyzers.

Statistical analysis

The average time of 5 20-m sprint was calculated. The percent change was calculated as (Day2 – Day1) * 100/Day 1. The time of sprint in the 2 trials was compared by paired t-test. The changes in plasma markers were analyzed by 2-way ANOVA with repeated measurement. The post-hoc analysis was performed with Ryan-Holm-Bonferroni method.

結果

The average time of 5 sprints in the 2 trials are shown in Figure 3. There was no significant difference between the 2 trials. However, when the results were expressed as the percent change, The 21-25 sprints in the BCAA trial was significantly faster than that in the trial (Figure 4, -0.011±0.3% vs 0.005±0.02%，p=0.023).

The plasma concentrations of creatine kinase and lactate dehydrogenase were similar between the 2 trials at all time points analyzed (Figure 5 and 6). Although they were elevated after exercise, suggesting minor muscle damage. The plasma glucose concentrations in the 2 trials were shown in Figure 7. There was no difference between the 2 trials at any time point analyzed.

討論

The results of this study suggested that the supplementation of BCAA and Arg may improve the performance in the second day of simulated handball games. The markers for muscle damage were similar between the 2 trials. Therefore, it is unlikely that BCAA and Arg could reduce muscle damage at these settings. One of the potential mechanisms responsible for the enhancement in performance may be the reduction in central fatigue as suggested by several studies [34, 35].

This study is of significant practical value as the handball games are usually held on consecutive days in tournaments. The faster recovery from the competition on the previous day will ensure the players have better speed on the following days.

Year 2

研究方法

Subjects

Nine well-trained male wrestlers were recruited from National Taiwan College of Physical Education, Taichung, Taiwan. Their age was 19.2±0.4 (mean±SEM) years, the height was 1.69±0.02 m, the body weight was 72.18±2.71 kg, and the body fat was 15.46±1.64%.

Study design

This study used a randomized cross-over design. The procedure of exercise tests and blood and gas samplings is shown in Figure 8. Each subject completed 3 trials in a random order according to their order of admission to this study. Each trial was separated by at least 2 weeks. The same food was provided in the lunch and dinner on the day before, and the breakfast on the day of each trial. The lunch and dinner were meal boxes purchased from a local restaurant. The 2 meals combined to provide approximately 1434 kcal, with 49.7% energy from carbohydrate, 30.1% from fat, and 20.2% from protein. The breakfast contained white bread 1.2 g/kg, jam 0.1 g/kg, butter 0.l 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).

Experimental procedure

The subjects reported to the laboratory in the early morning after an overnight fast. A cannula was put in the antecubital vein by licensed personnel. After a blood sample was taken to serve as the baseline, the subjects consumed the standardized breakfast. The exercise test started 1 hr after the breakfast was consumed. Each trial contained 3 matches. At the end of the second match, 3 different supplementations were consumed: 1.2 g/kg glucose (CHO trial), 1 g/kg glucose + 0.1 g/kg Arg + 0.1 g/kg BCAA (leucine: isoleucine: valine = 2:1:1, CHO+AA trial), or water (placebo trial). All supplementations were dissolved in 600 ml lemon flavored water to make the tastes similar. The subjects were allowed to drink water ad libtum in the first trial, while the timing and amount of consumption were recorded. The timing and amount of water consumption were repeated in the following trials.

Exercise tests

The high-intensity intermittent exercise test was designed to mimic the actual wrestling competition. The tests were performed on a Monark cycle ergometer (894E, Monark, Varberg, Sweden). Each trial contained 3 matches with a 1-hr rest between match 1 and 2, and a 2-hr rest between match 2 and 3. Each match contained 3 exercise periods interspersed with 1-min rests. The subjects alternated 10-s all-out sprints and 20-s rests in each exercise period. The load was 0.1 kp/kg body weight. The subjects were asked to pedal as fast as possible with vocal encouragement by research personnel. In the rest periods the load was removed and the subjects were asked to pedal at 60 rpm. The peak and average power of each sprint was recorded.

Expired air analysis

The O_2 consumption and CO_2 production during a 2-min test period was measured using a breath-by breath gas analyzer (Vmax 29C, Sensormedics, Yorba Linda, CA, USA). The average values during the 2-min period were used to estimate the carbohydrate and fat oxidation rates according to the published equations [36].

Biochemical and hormone measurements

The research personnel who conducted the analysis were blind to the group of the samples. Hemoglobin concentration and hematocrit in whole blood was measured by a hematology analyzer (KX-21N, Sysmex Corporation, Kobe, Japan) to correct for change in plasma volume [37]. Plasma NO_x concentration was measured with modified Griess reaction using a commercial kit (Sigma, St. Louis, MO, USA). The absorbance at 540 nm was measured with a microplate spectrophotometer (Benchmark Plus, Bio-Rad, Hercules, CA, USA). Plasma concentrations of insulin were measured by electrochemiluminescence (Elecsys 2010, Roche Diagnostics, Basel, Switzerland) with the kit provided by the manufacturer. Plasma glucose, glycerol and non-esterfied fatty acid (NEFA) were measured with an automatic analyzer (Hitachi 7020, Tokyo, Japan) using commercial kits (Randox, Antrim, UK).

Statistical analysis

All values were expressed as means±SEMs. The area under the curve (AUC) was calculated for plasma concentrations of glucose and insulin, as well as total carbohydrate and fat oxidation, during the 2-hr recovery period after the second match. The changes in exercise performance, plasma concentrations of metabolites, and substrate oxidation rates were analyzed by a two-way analysis of variance with repeated measures. The analysis was performed with SPSS for Windows 15.0 (SPSS, Chicago, IL, USA). A P value less than .05 was considered statistically significant.

結果

The peak and average power in the 3 matches was similar in the 3 trials (Table 1). The power drop between match 2 and match 1, as well as between match 3 and match 1, were also similar in the 3 trials. Plasma glucose and insulin concentrations in the 3 trials were shown in Figures 9 and 10, respectively. After supplementations at the end of match 2, the CHO and CHO+AA trial showed significantly higher glucose concentration at 30 min, and significantly higher insulin concentration after 30, 60, and 90 min. Compared to the placebo trial, the CHO and CHO+AA trial also showed significantly higher AUC in glucose (Placebo: 428.69±24.80; CHO: 621.85±41.28; CHO+AA: 550.66±32.89 arbitrary unit; $p<0.01$) and insulin concentrations (Placebo: 368.99±68.24; CHO: 2947.01±665.08; CHO+AA: 2896.27±557.40 arbitrary unit; $p<0.01$) during the 2-hr recovery period after match 2. However, there was no significant difference between the CHO and CHO+AA trial in either glucose or insulin concentration at any time point. The AUC of plasma glucose and insulin concentrations were also similar between the CHO and CHO+AA trials.

The supplementation of CHO and CHO+AA resulted in significantly lower plasma concentrations of glycerol and NEFA at 90 and 120 min after match 2, as well as immediately after match 3 (Figures 11 and 12). In addition, the CHO and CHO+AA trials showed significantly higher carbohydrate oxidation rate at 60 and 90 min (Figure 13), and lower fat oxidation rate at 60, 90, and 120 min after the supplementation (Figure 14). Total fat oxidation was significantly lower in the CHO and CHO+AA trial during the 2-hr recovery period after match 2 (Placebo: 9.36±1.20; CHO: 6.04±1.01; CHO+AA: 5.09±1.41 g; $p<0.01$). Nevertheless, total carbohydrate oxidation were similar among the 3 trials during the same period (Placebo: 42.86±6.45; CHO: 51.04±4.84; CHO+AA: 49.42±8.21 g).

Plasma NOx concentrations in the 3 trials were shown in Figure 15. Despite the supplementation of arginine in the CHO+AA trial, there was no significant difference in NOx concentration among the 3 trials at any time point.

討論

To our knowledge, this is the first study that investigated the effect of supplementation during a short-term recovery period on the subsequent simulated match performance in combat sports. The results of this study suggested that the supplementation of carbohydrate, with or without additional BCAA and arginine, during the recovery period after two matches had no effect on the performance in the subsequent match in well-trained male college wrestlers.

The few available studies investigating the effect of carbohydrate and protein consumption during the post-exercise recovery period on the performance in the subsequent exercise have provided positive [15, 16] and negative [17, 18] results. The consumption of carbohydrate and protein during the 4-hr recovery period after glycogen-depleting exercise increased the time to exhaustion in the subsequently exercise at 70-85% $\dot{V}O_{2max}$, compared to a smaller or same amount of carbohydrate alone [15, 16]. The increase in performance may be attributed to higher glycogen resynthesis during the recovery period [15]. However, the carbohydrate-protein supplementation did not show any additional effect compared to isocaloric carbohydrate [16]. On the other hand, consumption of 0.6 g/kg/hr carbohydrate during the 2-hr recovery after a glycogen-depleting exercise resulted in similar time to exhaustion in the subsequent endurance exercise, compared to 1.0 g/kg/hr carbohydrate or 0.6 g/kg/h carbohydrate plus 0.4 g/kg/hr protein [18]. The authors concluded that the additional energy, either in carbohydrate or protein, did not provide additional effect above 0.6 g/kg/h carbohydrate during the 2-h recovery period [18]. With carbohydrate intake of 0.8 or 1.2 g/kg/hr during the 4-hr post-exercise recovery period, the additional protein showed no effect on the running time to exhaustion at 85% VO_{2max} in the subsequent exercise, despite higher insulinemic response [17]. One of the reasons that protein offered no additional benefit may be the higher carbohydrate oxidation rate and similar glycogen utilization rate during the subsequent endurance exercise [38, 39]. The aforementioned studies all focused on endurance exercise. For the first time, this study suggested that consumption of carbohydrate or carbohydrate plus BCAA and arginine during the recovery period had no effect on the performance in the subsequent intermittent high-intensity exercise in well-trained wrestlers.

The literature on the effects of BCAA on glucose uptake and glycogen synthesis in skeletal muscles has been equivocal [6, 40-42]. It has been reported that supplementation of leucine in combination with carbohydrate after exercise resulted in higher post-exercise insulin concentration and greater muscle glycogen recovery in athletes, compared to the same amount of carbohydrate [6]. In addition, oral supplementation of BCAA has been reported to increase glycogen synthase activity in rat skeletal muscles [41]. Leucine has also been shown to increase insulin-independent glucose uptake in isolated rat skeletal muscles through phosphatidylinositol 3-kinase (PI3K) pathway [43]. On the other hand, leucine infusion decreased glucose uptake in human forearm muscles in a dose-dependent manner despite the elevated plasma insulin levels [44]. Infusion of amino acid mixtures containing BCAA and arginine also impaired insulin-stimulated glucose disposal and glycogen synthesis in human skeletal muscles by increasing the inhibitory insulin receptor substrate-1 phosphorylation and decreasing PI3K activity [42, 45].

In conclusion, this study suggested that supplementation of carbohydrate with or without

BCAA and arginine during the post-match period did not provide additional effect on the performance in the following simulated match in well-trained male wrestlers when a carbohydrate-rich breakfast was eaten. It is possible that factors other than muscle glycogen content contribute to the performance in multiple bouts of high-intensity intermittent exercise. It is also possible that experienced wrestlers have the ability to recovery quickly from previous matches with or without supplementation. Furthermore, BCAA and arginine did not provide additional insulinemic effect when given after high-intensity intermittent exercise.

Year 3

研究方法

Cell culture

The cell culture will be performed according to the previously published methods [46-48]. C2C12 will be seeded in 6-well Petri culture dishes and grown in Dulbecco's modified eagle's medium (DMEM) containing 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin/streptomycin at 37℃ with 5% CO_2. At 95-100% confluence, the cells will be differentiated by replacing the original medium with DMEM containing 2% horse serum. The differentiation medium will be changed daily. The following experiments will always be carried out after 3 days of differentiation.

Treatments

The NO donor used in this study was sodium nitroprusside (SNP, 100 uM). Sodium pyruvate (50 mM) was used as the positive control for mitochondrial biogenesis. The hypoxia treatment was held at 1% oxygen.

RNA isolation, reverse transcription for cDNA, and real-time PCR

The mRNA levels of PGC-1α, NRF-1, mtTFA were analyzed. Total RNA was isolated using Cyclo-Prep Total RNA Purification Kit (Amresco, Solon, OH, USA). Complementary DNA will be synthesized with M-MMLV reverse transcriptase (Gibco-BRL, Gaithersburg, MD, USA) in PCR (PC818, ASTEC, Japan).

Real-Time PCR was performed using iQ5 and iCycler system (Bio-Rad). The primers used are presented in Table 2. Reaction volumes (20 μl) contained 2X SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), forward and reverse primers, and cDNA template (diluted 1:40). Samples were run in duplicate for 1 cycle (50℃ 2 min, 95℃ 10 min) followed by 40 cycles (95℃ 15 s, 60℃ 60s) and fluorescence emissions will be measured after each cycle.

Flow cytometry

The cells were incubated in 6-well plates for 3 days, then treated with different agents and/or hypoxia. The cells were cleaved with trypsin, centrifuged at 1800 rpm for 3 min, then stored in flow tubes. The cells were treated with 1% mitotracker for 30 min. After washing with 3 ml PBS, the cells were adjusted to 1x10-6/ml and analyzed with flow cytometer (FACSCalibur, Becton-Dickinson, Franklin Lakes, NJ, USA). The results will be analyzed by Cell Quest Software (BD).

結果

The mitochondria contents in C2C12 myotubes after various treatments are shown in Figure 16. SNP significantly increased mitochondria content after 4 hours of treatment. Moreover, this effect was even more obvious after 24 hours. The combination of SNP and hypoxia did not result in higher response compared to SNP alone. In addition, the positive effect of hypoxia and sodium pyruvate suggested that our model is adequate.

The expression levels of PGC-1a after various treatments are shown in Figure 17. The combination of 2 positive controls, hypoxia and sodium pyruvate, induced the largest response in PGC-1a. The treatment of SNP also significantly increased the expression level of PGC-1a, with the highest impact in 4 hr. The effect of all treatments disappeared after 24 hr. The expression levels of NRF-1 after various treatments are shown in Figure 17. SNP also significantly increased expression level of NRF-1, especially in 4 and 12 hr. Figure 18 showed the expression levels of mtTFA after various treatments. Similar to NRF-1, SNP also significantly increased expression level of mtTFA, especially in 4 and 12 hr.

討論

The results of this study suggested that NO may increase mitochondria biogenesis in C2C12 myotubes. The effect started to show after 4 hr of treatment and reached the peak after 24 hr. The genes involved in mitochondria biogenesis, PGC-1a, NRF-1, and mtTFA, were all up-regulated by NO. The time course of the expression levels of these proteins were proceeded from the mitochondria response. The NO-induced up-regulation of these protein were apparent after 4 hr of treatment and peaked at 12 hr.

Pyruvate has been shown to be able to induce mitochondria biogenesis [49]. Therefore, it is used as a positive control in this study, along with hypoxia. The fact that both pyruvate and hypoxia treatments induced mitochondria content and the expression levels of the 3 regulatory proteins indicated that the model we used in this study is suitable.

It has been shown that long-term exposure of cells to low concentrations of NO induced mitochondrial biogenesis through increased expression of PGC-1α in several cell types [28]. The process is mediated by cGMP as the activation of NO-cGMP pathway increased mitochondria content and oxidative phosphorylation in various cell lines [29]. Furthermore, inhibition of NOS by ingestion of L-NAME decreased expression levels of cytochrome c oxidase I and IV some in soleus in rats. [30]. In this study we suggested that a single treatment of SNP could increase mitochondria biogenesis in C2C12 myotubes after 24 hr.

Table 1. Peak and average power in 3 matches in the 3 trials[1]

	Placebo trial	CHO trial	CHO+AA trial
Peak power			
1st match (W/kg)	70.36±3.38	71.24±4.19	72.62±4.59
2nd match (W/kg)	69.45±5.40	69.05±5.42	72.08±6.14
3rd match (W/kg)	67.49±4.81	68.72±4.84	72.52±8.18
Average power			
1st match (W/kg)	61.97±3.33	63.90±3.82	64.24±4.14
2nd match (W/kg)	61.41±4.84	61.05±4.59	63.48±5.54
3rd match (W/kg)	59.27±4.15	60.89±4.42	63.85±7.09
Drop in peak power			
Match 1 – Match 2 (%)	1.93±5.07	3.35±4.36	1.49±4.14
Match 1 – Match 3 (%)	4.62±3.93	3.52±3.75	2.17±6.61
Drop in average power			
Match 1 – Match 2 (%)	1.28±5.18	4.58±4.23	2.00±4.14
Match 1 – Match 3 (%)	4.54±4.10	4.65±4.04	2.59±6.45

[1] All values are means±SEMs. Data were analyzed by using repeated measures ANOVA with time and group as factors. No significant main effect was observed for any of the variables.

Table 2. The primers used in Real-time PCR

genes	Primer
GADPH	Forward: ACCACAGTCCATGCCATCAC Reverse: TCCACCACCCTGTTGCTGTA
PGC-1a	Forward: AGACCTGACACACAACGCGG Reverse: GCAGGGTCAAAATCGTCTG
mtTFA	Forward: AAAGACCTCGTTCAGCATATAACATTTAT Reverse: CCAAGCCTCATTTACAAGCTTCA
NRF-1	Forward: TCCTCCAGGTTATAATCCCG Reverse: AGTGTAGAAAGTGGCTGGGCT

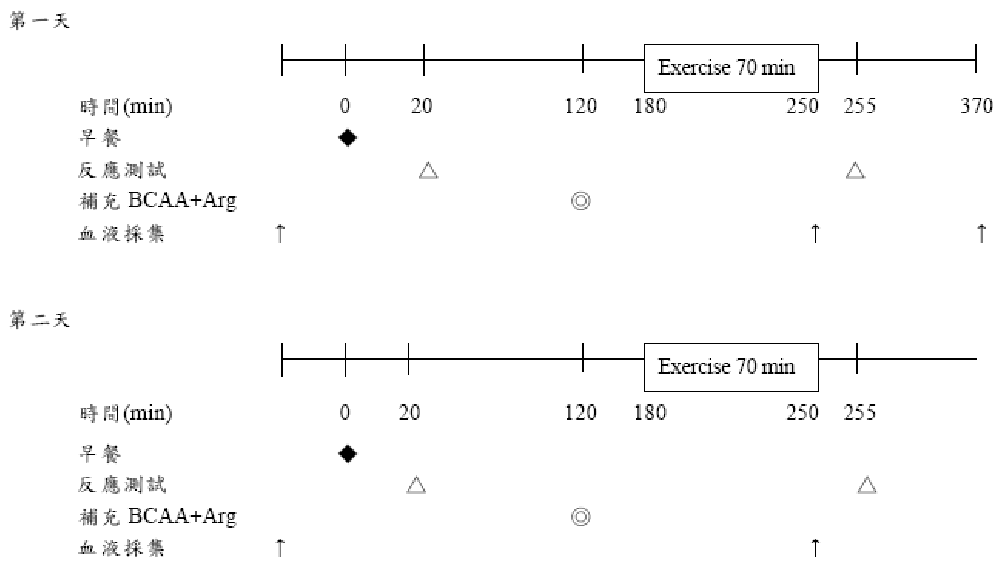

Figure 1. Experimental protocol of the study

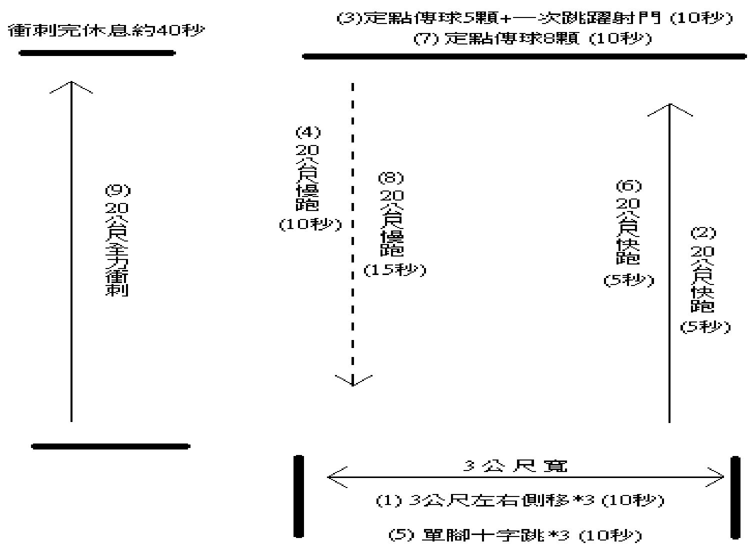

Figure 2. The exercise protocol for each trial

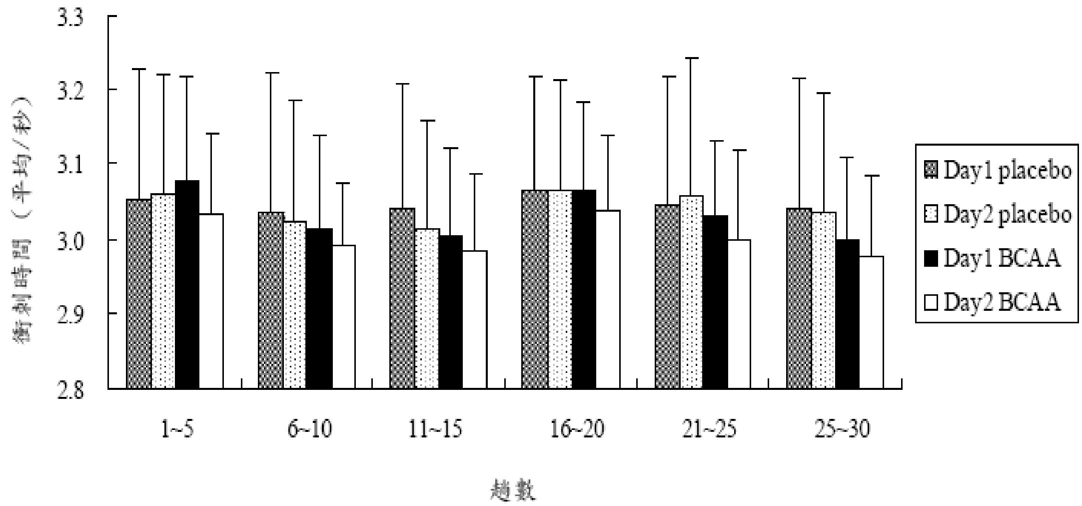

Figure 3. The average time of 5 sprints in the 2 trials (■：BCAA trial；□：placebo trial).

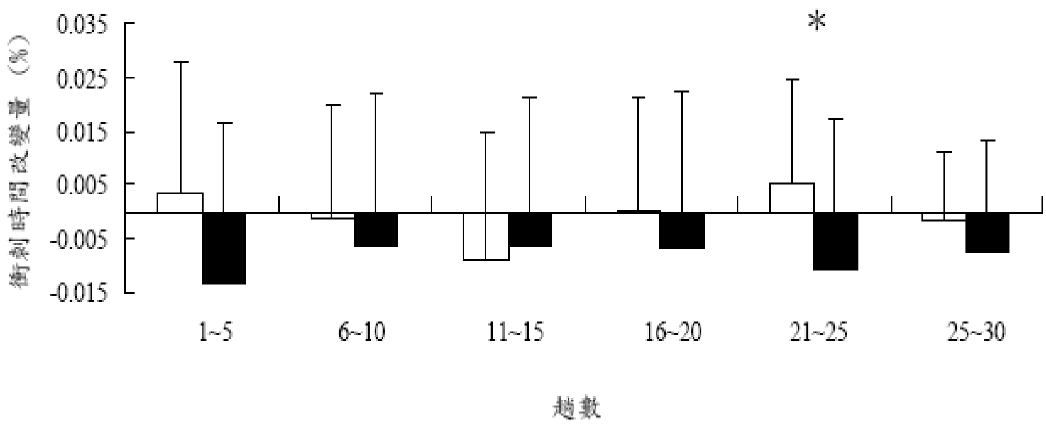

Figure 4. The percent change of sprint times between the 2 trials (■：BCAA trial；□：placebo trial).

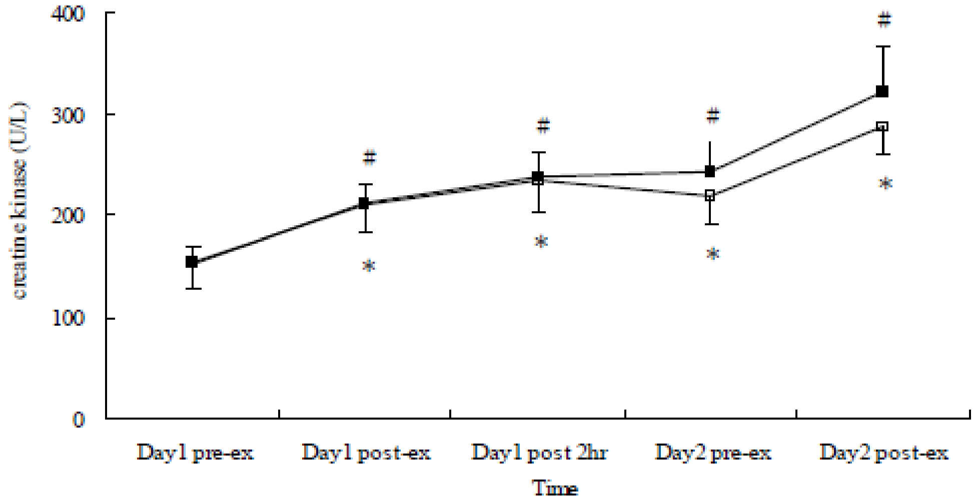

Figure 5. Plasma creatine kinase concentrations in the 2 trials (■：BCAA trial；□：placebo trial).

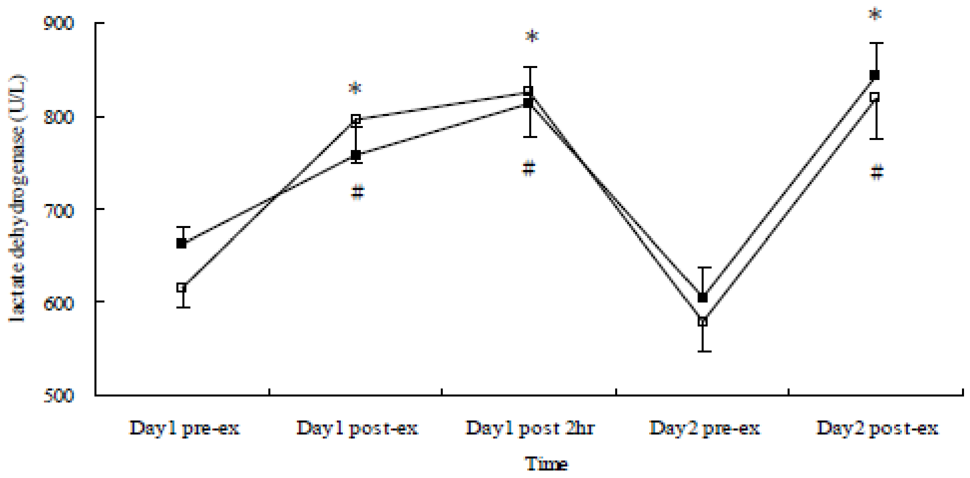

Figure 6. Plasma lactate dehydrogenase concentrations in the 2 trials (■：BCAA trial；□：placebo trial).

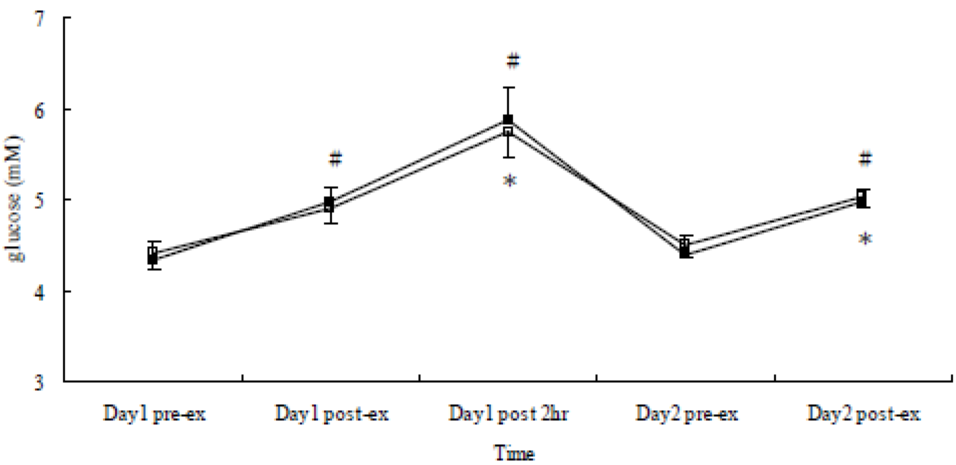

Figure 7. Plasma glucose concentrations in the 2 trials (■：BCAA trial；□：placebo trial).

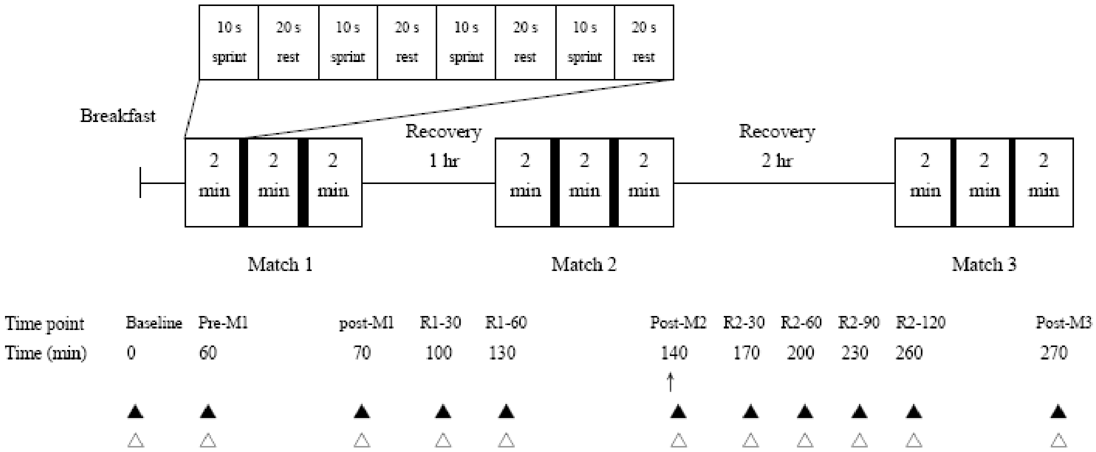

Fig. 8. Study protocol, Year 2.
■: 1-min rest; ↑: supplementation; ▲: blood sampling; △: gas analysis.

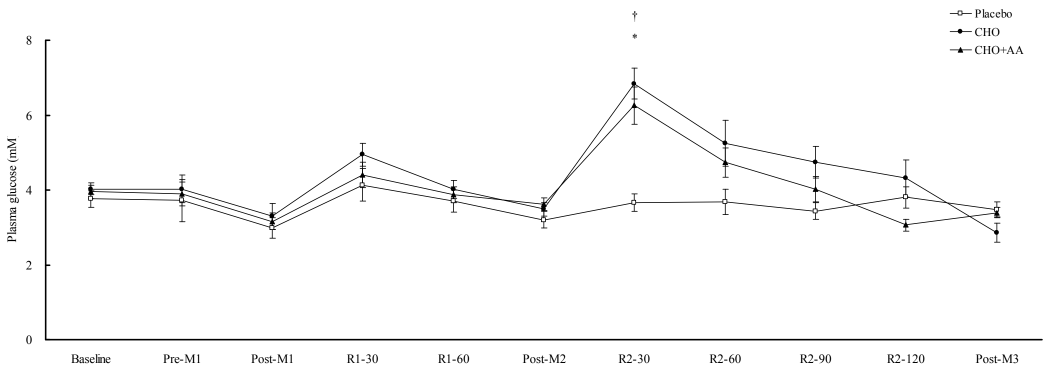

Fig. 9. Plasma glucose concentrations in the 3 trials.
Data were analyzed by using repeated measures ANOVA with time and group as factors. Treatment effect $p=0.006$; time effect $p<0.001$; interaction effect $p<0.001$.
*CHO trial significantly different from placebo trial at the same time point ($p<0.05$).
†CHO+AA trial significantly different from placebo trial at the same time point ($p<0.05$).

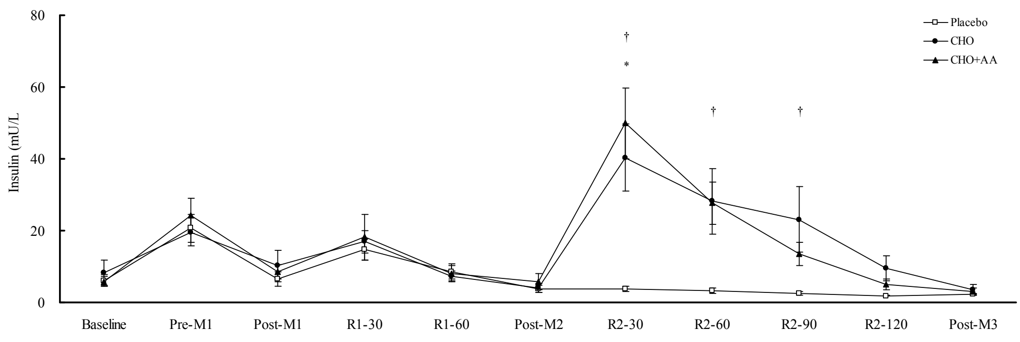

Fig. 10. Plasma insulin concentrations in the 3 trials.
Data were analyzed by using repeated measures ANOVA with time and group as factors. Treatment effect p=0.013; time effect p<0.001; interaction effect p<0.001.
*CHO trial significantly different from placebo trial at the same time point (p<0.05).
†CHO+AA trial significantly different from placebo trial at the same time point (p<0.05).

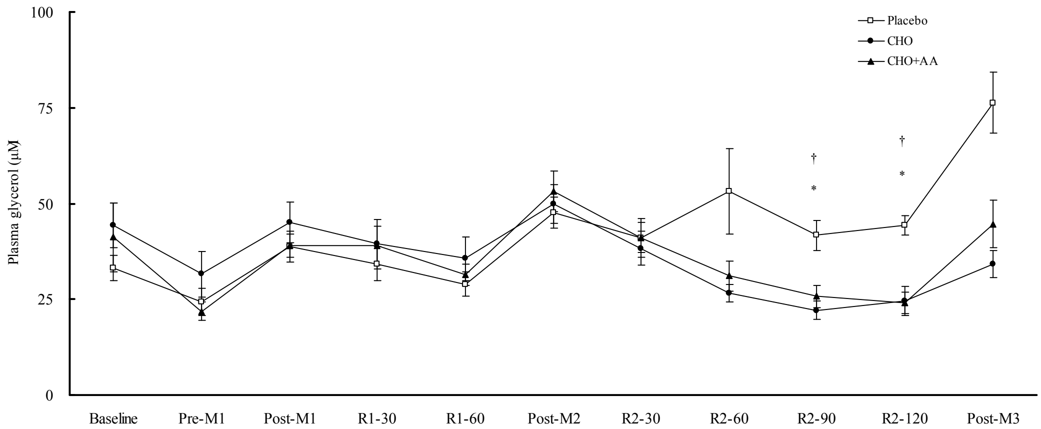

Fig. 11. Plasma glycerol concentrations in the 3 trials.
Data were analyzed by using repeated measures ANOVA with time and group as factors. Treatment effect p=0.262; time effect p<0.001; interaction effect p<0.001.
*CHO trial significantly different from placebo trial at the same time point (p<0.05).
†CHO+AA trial significantly different from placebo trial at the same time point (p<0.05).

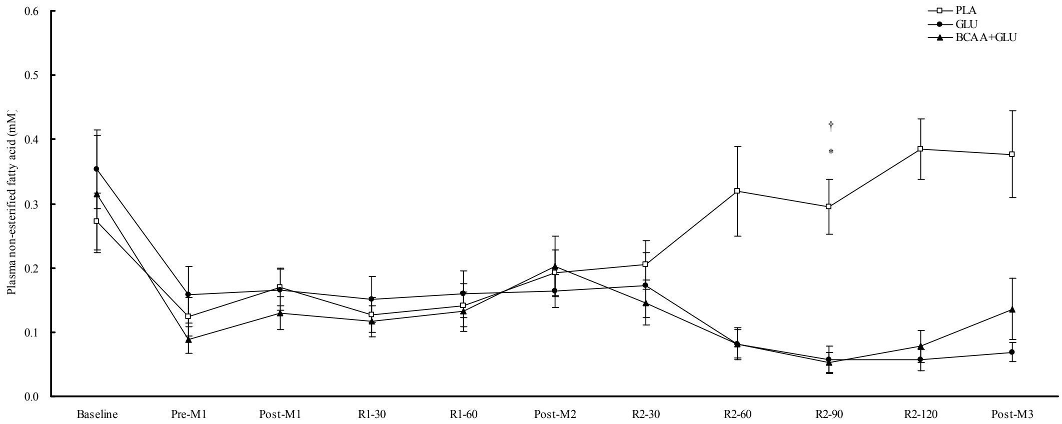

Fig. 12. Plasma non-esterified fatty acid concentrations in the 3 trials.
Data were analyzed by using repeated measures ANOVA with time and group as factors. Treatment effect p=0.017; time effect p<0.001; interaction effect p<0.001.
*CHO trial significantly different from placebo trial at the same time point (p<0.05).
†CHO+AA trial significantly different from placebo trial at the same time point (p<0.05).

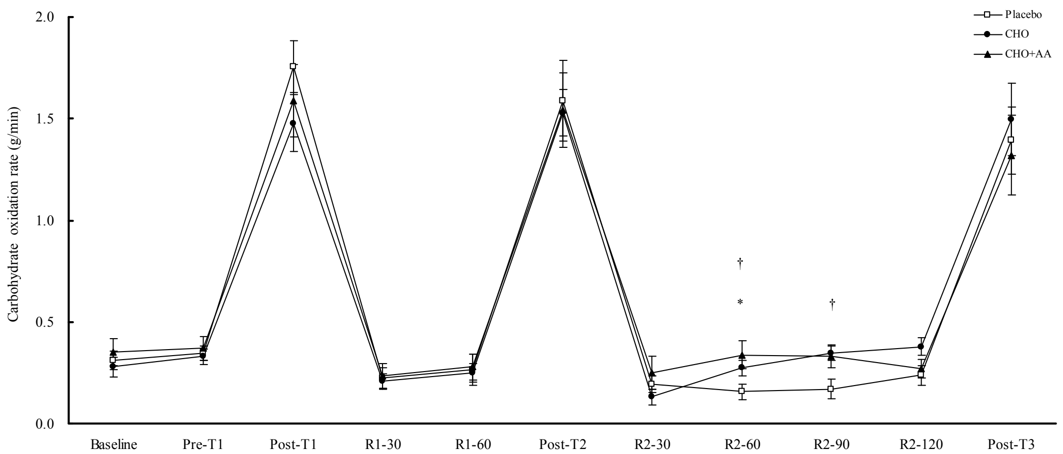

Fig. 13. Carbohydrate oxidation rate in the 3 trials.
Data were analyzed by using repeated measures ANOVA with time and group as factors. Treatment effect p=0.923; time effect p<0.001; interaction effect p=0.025.
*CHO trial significantly different from placebo trial at the same time point (p<0.05).
†CHO+AA trial significantly different from placebo trial at the same time point (p<0.05).

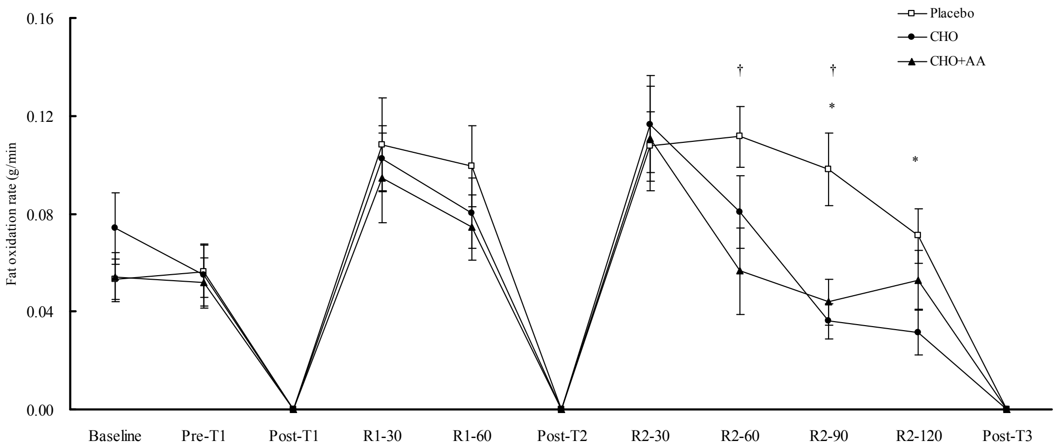

Fig. 14. Fat oxidation rate in the 3 trials.
Data were analyzed by using repeated measures ANOVA with time and group as factors. Treatment effect p=0.012; time effect p<0.001; interaction effect p<0.001.
*CHO trial significantly different from placebo trial at the same time point (p<0.05).
†CHO+AA trial significantly different from placebo trial at the same time point (p<0.05).

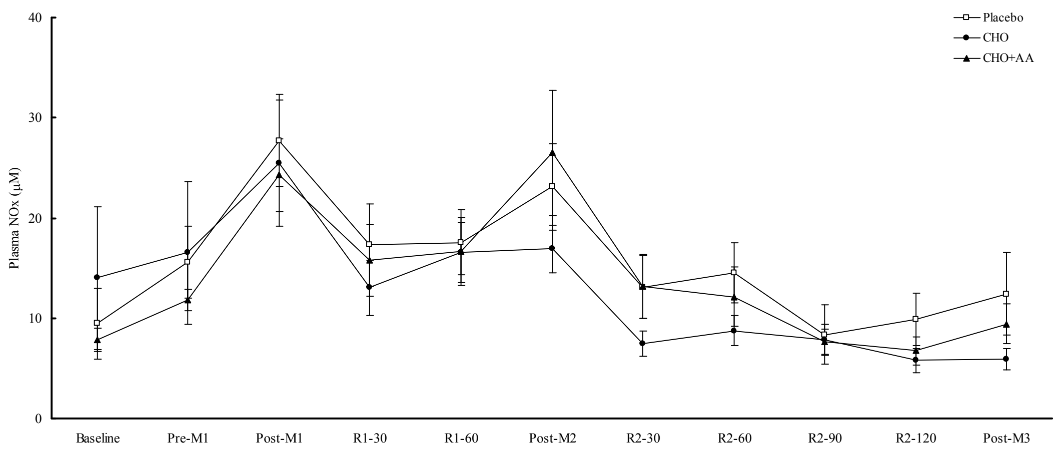

Fig. 15. Plasma NOx concentrations in the 3 trials.
Data were analyzed by using repeated measures ANOVA with time and group as factors. Treatment effect p=0.533; time effect p=0.002; interaction effect p<0.001.

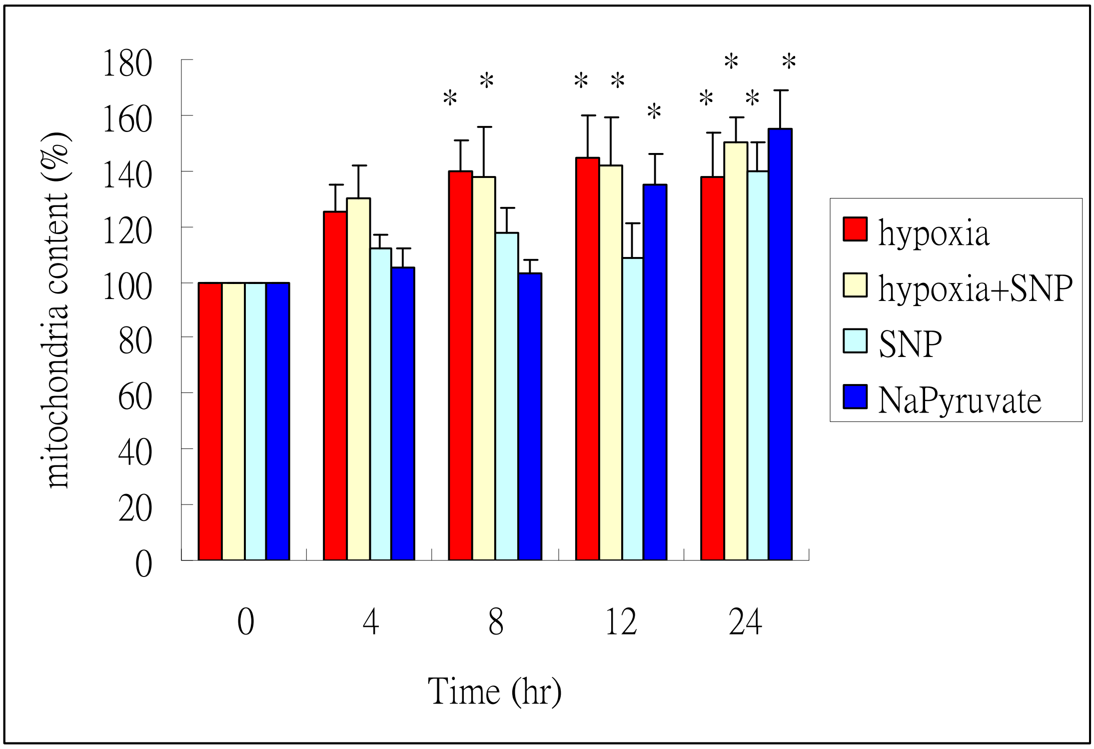

Figure 16. Relative mitochondria content in C2C12 myotubes after various treatments. *: significantly different from 0 hr.

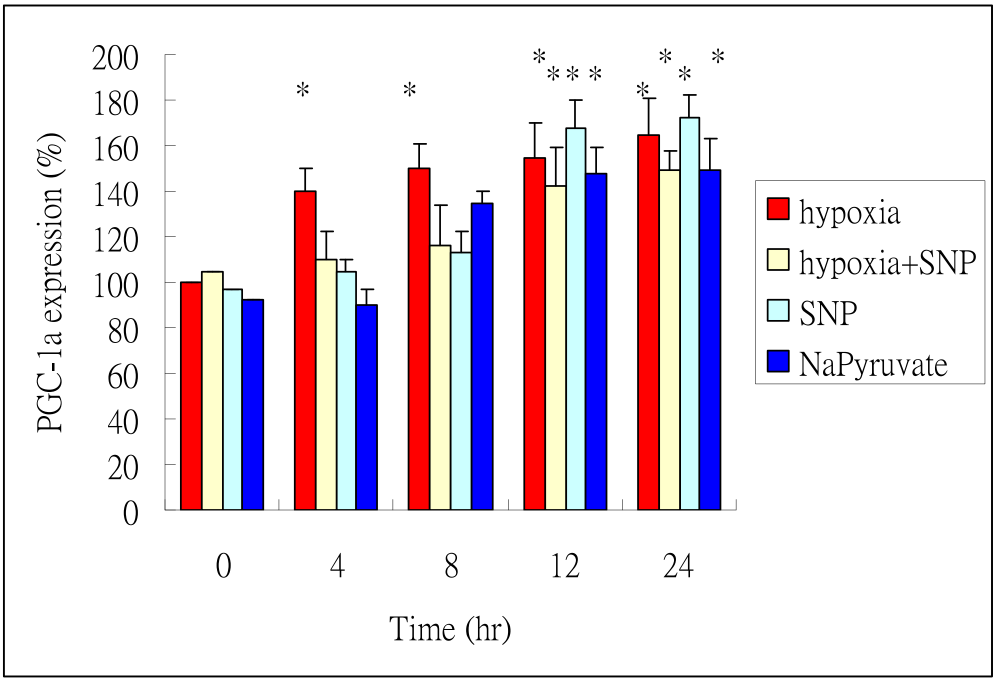

Figure 17. Relative expression levels of PGC-1a in C2C12 myotubes after various treatments. *: significantly different from 0 hr.

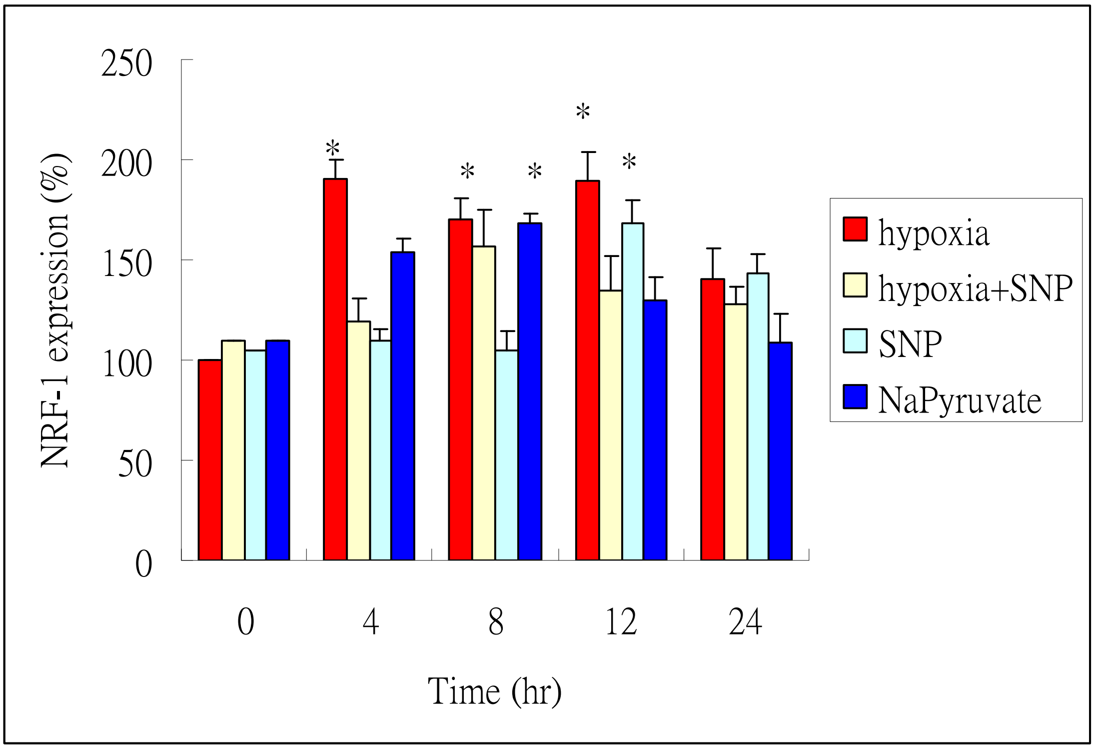

Figure 18. Relative expression levels of NRF-1 in C2C12 myotubes after various treatments. *: significantly different from 0 hr.

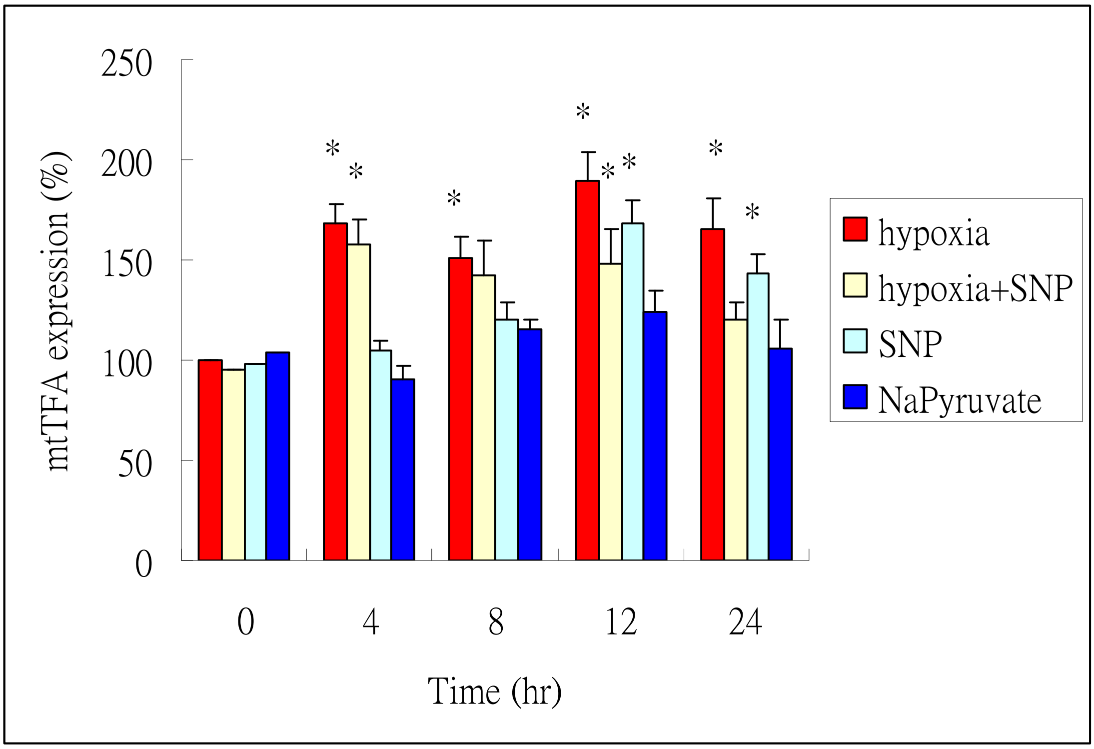

Figure 19. Relative expression levels of mtTFA in C2C12 myotubes after various treatments. *: significantly different from 0 hr.

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國科會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：
2. 研究成果在學術期刊發表或申請專利等情形： 論文：■已發表□未發表之文稿 ■撰寫中 □無 專利：□已獲得 □申請中 ■無 技轉：□已技轉 □洽談中 ■無 其他：（以 100 字為限） Jang TR, Wu CL, Chang CM, Hung W, Fang SH, **Chang CK***. (2011) Effects of carbohydrate, branched-chain amino acids, and arginine in recovery period on the subsequent performance in wrestlers. **Journal of the International Society of Sports Nutrition** 8:21 (SCI, IF: 2.675, 9/79 in Sport Science)

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）（以 500 字為限）

This 3-year study has fulfilled the proposed goal: to use supplementation of BCAA and Arg to improve performance and recovery in athletes. In addition, the effects of NO on mitochondria biogenesis in C2C12 myotubes, along with the potential mechanisms, are revealed. The exercise protocol developed in Year 1 can be applied to various ball games such as handball, basketball, volleyball, because these competitions are usually played on consecutive days. Very few studies have investigated the recovery on consecutive days. The results of Year 1 could help to enhance the performance in various ball sports. Year 2 is a novel study design which investigated the recovery from multiple bouts of wrestling competition. Very few studies have investigated the recovery on this type of competition. Although the performance was not changed after the supplementation, the protocol developed in this study can be used in further research in combat sports. Year 3 showed that NO, a product of Arg, is capable of inducing mitochondria biogenesis in myotubes. It has significant application value as it may enhance aerobic capacity. This effect would be very helpful in endurance athletes.

In conclusion, this 3-year study established protocols for performance measurements in consecutive days of team sports, and multiple bouts in combat sports. These 2 protocols are designed to mimic the real competition of these sports and are novel in scientific literature. The results are very useful to help the athletes in these sports worldwide. Year 3 provided further evidence that NO may help to induce mitochondria biogenesis in muscle cells. This could help endurance athletes to increase aerobic capacity, leading to better performance. This study covered a wide range of athletes. The supplementation of BCAA and Arg seemed to be promising in helping to improve the performance in various sports. Part of the results has been published in a SCI journal. Other results were also close to be submitted. This study provided solid contributions in both scientific and applied fields in sports nutrition and physiology. Further research on this topic is warranted.

國科會補助計畫衍生研發成果推廣資料表

日期:2011/10/29

<table>
<tr><td rowspan="3">國科會補助計畫</td><td>計畫名稱: 補充精胺酸與支鏈胺基酸對運動表現的影響(II)-分子機轉</td></tr>
<tr><td>計畫主持人: 張振崗</td></tr>
<tr><td>計畫編號: 98-2320-B-028-001-MY3　　　　學門領域: 保健營養</td></tr>
<tr><td colspan="2">無研發成果推廣資料</td></tr>
</table>

98 年度專題研究計畫研究成果彙整表

計畫主持人：張振崗	計畫編號：98-2320-B-028-001-MY3
計畫名稱：補充精胺酸與支鏈胺基酸對運動表現的影響(II)-分子機轉	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	1	1	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	3	3	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	3	3	100%		
國外	論文著作	期刊論文	1	1	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

其他成果 (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。)	無

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會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、主要發現或其他有關價值等，作一綜合評估。

<table>
<tr><td>
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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：
</td></tr>
<tr><td>
2. 研究成果在學術期刊發表或申請專利等情形：
論文：■已發表 □未發表之文稿 □撰寫中 □無
專利：□已獲得 □申請中 ■無
技轉：□已技轉 □洽談中 ■無
其他：（以 100 字為限）
Jang TR, Wu CL, Chang CM, Hung W, Fang SH, Chang CK*. (2011) Effects of carbohydrate, branched-chain amino acids, and arginine in recovery period on the subsequent performance in wrestlers. Journal of the International Society of Sports Nutrition 8:21
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</table>

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