The Effects of Taurine Supplementation on Inflammatory Cytokines and Physical Performance to Simulated Taekwondo-Specific Protocol in Elite Male Athletes: A Double-Blinded, Placebo-Controlled, Crossover Study

Ahmad Samandari
Shahrekord University
Fatemeh Sanjideh
Shahrekord University
Mohammad Faramarzi (m.faramarzi@spi.ui.ac.ir)
University of Isfahan https://orcid.org/0000-0003-3729-7568
Ebrahim Banitalebi
Shahrekord University
Majid Mardaniyan Ghahfarrokhi
Shahrekord University

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Abstract

Background: The main purpose of this study was to investigate the effects of taurine supplementation as a countermeasure against decrement in athletic performance to enhance metabolic inflammation status, circulating lactate levels, and functional capacity following a simulated taekwondo competition day with the same physiological pressure in elite male taekwondo athletes.

Methods: A double-blinded, placebo-controlled, crossover design was utilized in the present trial. A total number of ten healthy, highly-trained, elite male taekwondo athletes (age=20.60±3.5 years) were accordingly recruited to receive taurine (a dose of 15 mg/ body weight, approximately three servings per day) or the same doses of a placebo for 10 days, separated by a 10-day washout period. The simulated taekwondo competition day included five exhaustive single competition sessions using a special time framework of taekwondo test with the same physiological pressure. Five competition taekwondo stations were also done with a time-course order.

Results: The results showed significant time-placebo interaction effect for IL-15 levels (0.001). Using post-hoc comparisons, a significant increase was also found between supplemental and placebo occasions 24 h after the simulated taekwondo competition day (0.001). However, no significant changes were observed in IL-8 (0.19), IL-17 (0.808), hs-CRP (0.766), and TNF-α (p=0.970) levels. In addition, there were no significant changes in final heart rate (p=0.413), countermovement jump (p=0.884), and turning kick (p=0.790) following the simulated taekwondo competition day and taurine supplementation.

Conclusions: The results of the present trial did not support the ergogenic effect of short-term (10-day) taurine supplementation on performance and inflammation status of elite male athletes.

Introduction

Taekwondo (TKD) is currently one of the most popular forms of combat/martial art sports a well as modern Olympic combat-related ones, consisting of three two-min competition rounds separated with a one-min rest in between [1]. It has been shown that TKD requires high levels of both aerobic and anaerobic capacities, so athletes train and combat at near 100% of maximum heart rate (HR) (>90% of HR_{max}) and lactate responses of 7.0-12.2 mmol.L-1 [2]. A TKD athlete may thus have more than 5-7 competitions to the final match during a single day with varied time schedules. Such repeated exposures to extremely high-intensity combats may alter physiological and metabolic demands once compared with a single fight (based on the Wingate Anaerobic test) [3]. Therefore, athletes may experience immunological and physiological changes such as accumulation of some metabolites e.g. reactive oxygen species (ROS), lactate, and a temporary suppression of the immune system function, which can be associated with an elevated susceptibility to systemic inflammation [3, 4].

Although a balanced diet contains sufficient essential macronutrients, adequate supply of essential amino acids is also required for the production of some immunoregulatory factors such as immunoglobulins, cytokines, and acute-phase proteins[5]. In this regard, taurine (TAU) as a sulfuric semi-

essential amino acid, an ergogenic aid, and an immunonutrition [6], can prevent muscle damage and decrease oxidative stress in order to improve the immune system deficiency [7], produced by intense exercise training [8].

However, findings from previous trials regarding the effects of TAU intake on muscle cell damage, catabolic/anabolic status, oxidative stress, athletic performance, and the immune system function are conflicting. For example, growing evidence has suggested that such an immunonutrition can reduce oxidative stress markers[8] and superoxide radical production [9], eccentric exercise-induced muscle damage biomarkers [10], and exercise-induced delayed onset muscle soreness (DOMS) [11, 12]. On the other hand, other trials have failed to confirm the effects of TAU supplementation on enhanced physiological parameters such as aerobic performance[8], anaerobic capacity, perceived exertion, and HR response [8, 13], as well as serum interleukin (IL)-6 and tumor necrosis factor alpha (TNF-α) inflammatory markers in triathletes[13]. Recently, two different studies have shown that acute administration of low-dose (1.66 g) [14] and high-dose(6.00 g) [15]TAU have failed to improve physical performance in endurance-trained cyclists and healthy males; respectively.

It seems that non-specific TKD tests are not appropriate to study the physiological response of this sport, since they do not have external validity [16, 17]. So, the use of simulated competitive TKD protocols with the same physiological pressure can be more efficient to reflect on physiological, immunological, and hormonal responses to dietary supplementation in TKD athletes, compared with graded exercise testing [18, 19]. The simulated protocols of TKD with equal physiological pressure can thus simulate the most common movements performed in real competitions to permit the study of responses of different interventions [20].

On the other hand, it can be hypothesized that biomarkers such as cytokines (e.g. IL-8, -15, -17, and high-sensitivity C-reactive protein (hs-CRP), and TNF-α), as well as blood lactate concentrations would be improved from the baseline within immediately and 24 h after the last bout of simulated competition plus TAU supplemental consumption compared with the same subjects after 10-day wash out period in the placebo group in elite TKD athletes. Accordingly, it is hypothesized that a specific simulated TKD protocol can fulfil a better assessment of physiological pressure in comparison with non-specific tests such as graded exercise testing. TAU supplement was thus selected because it had been recommended as a performance/immune-boosting supplement, prescribed for optimal immune [21] and performance[22]benefits in elite TKD athletes. Secondly, it was assumed that dietary supplemental TAU would be associated with improved blood lactate responses and physical performance in TKD athletes. To the best of authors' knowledge, there has been no study examining the effects of TAU supplementation on physiological and physical responses and circulating biomarkers, determining inflammation status and blood lactate concentrations to a specific simulated protocol in elite TKD athletes. Given this latter point, the present trail was to evaluate the effects of TAU supplementation on inflammation status, circulating lactate levels, and functional capacity in elite male TKD athletes. The main objective was to shed light on the effects of TAU supplementation combined with the simulated

taekwondo competition day (SCTD) with the same physiological pressure in shaping the inflammation status, circulating lactate levels, and functional capacity in TKD athletes.

Therefore, the main purpose of this study was to compare the effects of TAU supplementation and the SCTD with the same physiological pressure on improved performance parameters (i.e., countermovement jump (CMJ), skipping (SK), and turning kick (TK)), profiles of serum cytokine: IL-8, -15, and -17, as well as hs-CRP, TNF-α, and blood lactate concentrations in elite male TKD athletes.

Methods

Participants

A total number of ten elite male TKD athletes (age=20.60±3.5 years, height=174.50±0.60 cm, body mass=67.10±10.47 kg, body mass index (BMI)=21.97±2.60, and competition experience=6±2 years) competing in Iranian TKD professional league as members of Iranian national team volunteered to take part in this trial. These subjects were regularly competing in various national and international events (e.g. Olympic Games, Asian Championships, and Iranian TKD Professional League). The study was performed within the preparation phase of a training program.

All the participants were more than brown and black belt holders according to the International Taekwondo Federation (ITF). They were also in the following weight categories based on the Olympic Weight Categories: 58-68 kg (n=5), 68-80 kg (n=4), and >80 kg (n=1). The subjects who agreed to participate were thus asked to provide a written consent. The approval for this study was additionally granted by the Human Ethics Committee of Shahrekord University.

Permission was also obtained from the organizers of the events and the coaches of the Iranian national team to make the measurements. These subjects had not injured over the past six months and had not even taken anti-inflammatory drugs or nutritional supplements in the past three months.

Supplementation

A double-blinded, placebo-controlled, crossover research design was used in this study. All the participants also received three capsules of a dose of 15 mg/body weight Aspartame or TAU supplementation three servings per day (each capsule containing 1000 mg TAU, >99% pure, KAREN Pharma and Food Supplement Co., Iran. https://en.karenpharma.com), one after each main meal for 10 days in placebo and intervention occasions, separated by 10-day washout period in which the subjects were following a regular daily diet [23]. Supplemental and placebo TAU were prepared in capsule forms and packaged in bottles for double-blinded administration. Each capsule was then assigned a randomized alphanumerical code until data analysis was complete to ensure the study had remained double-blinded. During the first and the second conditions, the participants completed the SCTD with the same physiological pressure plus placebo and TAU ingestion (Figure 1).

Experimental procedures

Testing protocol

Immediately after blood sample collection, a 20-min warm-up and a pre-designed SCTD with the same physiological pressure for TKD were administered. All the subjects were also asked to use standard protective equipment such as gloves, HUGO chest protection, helmets, foot protective gears, and mouth guards, normally used for TKD, during the trials. The pre-designed SCTD included five simulated competition stages, consisting of three 45-sec stations with different movements as 15-sec CMJ training, 15-sec SK, and15-sec TK as fast as they could (Figure 2). There were also variable rest intervals between the stages as 40, 30, 20, and 10 min; respectively.

A TK was further executed via throwing kicks on the target pad and the SK movements were considered valid when the TKD athletes touched a pad with their knees located at pelvic height.

HR was controlled and recorded at a 5-sec frequency (using Polar Electro Oy, Kempele, Finland) to adjust the load to achieve the desired rate. The highest HR value recorded during each SCTD station was also considered as the individual HR peak.

All the participants visited the testing room (temperature: 19.5^{o}C; pressure: 794.4±3.2 Pascal, humidity 35.7±4.2%) on two conditions (namely, preliminary placebo and supplemental occasions) at a similar time of day, i.e.,10.00 am).

At the initial occasion, all the subjects had their body mass, height, and BMI measured according to standard protocols. Then, 10 cc blood samples were taken from an antecubital vein at baseline, pre-SCTD, immediately after the SCTD, and 24 h after the SCTD using standard procedures (Figure 3).

The blood samples were immediately centrifuged at 3000 g using a standard bench top centrifuge for 15 min at room temperature. The serum samples were also transferred in Eppendorf tubes and stored at -80^{o}C until analysis. These serum samples were then analysed for circulating biomarkers determining inflammation status (cytokines: IL-8, -15, -17, hs-CRP, and TNF-α) and blood lactate concentrations.

All the defined adverse events that occurred during or up to 48 h after resistance training were subsequently recorded every session and reported to the local Ethics Committee. To evaluate the athletes' overall SCTD, CMJ (cm), SK (n), and TK (n) were considered.

Statistical analysis

Statistical analyses were performed using the IBM SPSS Statistics software (version 22) (IBM, USA). Two-way analysis of variance (ANOVA) (time×supplement) was also used to determine the responses of biochemical, physiological and functional factors to exercise and supplementation. Bonferroni test was further employed as a post-hoc test where appropriate. An alpha level of 0.05 was considered significant. The data were presented as mean±standard deviation (SD).

Results

The demographic characteristics of the subjects are illustrated in Table 1. In this respect, the results of two-way ANOVA showed significant interaction effect (p=0.001), but not time (P=0.16) and supplement effect (P=0.16) for the IL-15 levels. Upon post-hoc comparisons, a significant increase was also found between supplemental and placebo occasions 24 h after exercise (p=0.001). On the other hand, there were no significant changes in IL-8 levels (time: p=0.57, supplement: p=0.55, and interaction: p=0.19), IL-17 (time: p=0.492, supplement: p=0.323, and interaction: p=0.808), hs-CRP (time: p=0.508, supplement: p=0.298, and interaction: p=0.766), and TNF-α (time: p=0.797, supplement: p=0.999, and interaction: p=0.970) (Figure 4).

In addition, there was a significant time effect (p=0.019), but not supplement (P=0.114) and interaction effect (p=0.880) for HR base. Post-hoc comparisons also suggested a significant rise in HR base in phase 3 (p=0.023), phase 4 (p=0.019), and phase 5 (p=0.002) compared with phase 1. Significant supplement effect (p=0.001), but not time (p=0.153) and interaction effect (p=0.389), were additionally found in SK. Post-hoc comparisons similarly revealed a significant growth in SK in supplement occasion compared with placebo (p=0.001). On the other hand, there were no significant changes in HR final (time: p=0.105, supplement: p=0.173, and interaction: p=0.413), CMJ (time: p=0.076, supplement: p=0.211, and interaction: p=0.884), and TK (time: p=0.266, supplement: p=0.219, and interaction: p=0.790) (Figure 5).

Significant time effect (p=0.0001), but not supplement (p=0.88) and interaction effect (p=0.388) was found for blood lactate concentrations. Post-hoc comparisons also demonstrated a significant increase in lactate after phase 1 (p=0.001), phase 4 (p=0.001), after phase 5 (p=0.033) compared with baseline, before phase 2, and before phase 5; respectively (Figure 6).

No significant adverse events were reported by investigators, not blinded to group assignment during the 10-day intervention and performing the testing protocol. However, a few participants reported muscle soreness following two testing occasions.

Discussion

The main purpose of this study was to examine the ergogenic effect of a 10-day TAU vs. placebo supplementation on inflammation and performance indices in TKD athletes. To the best of authors' knowledge, this research was unique as the first attempt, rigorously comparing the inflammatory and functional responses of TKD athletes to a SCTD, with an emphasis on TAU supplementation as a nutrient gaining popularity for its protective effect against inflammation-induced exercise training. Exercise performance was also assessed using the SCTD with the same physiological pressure in elite male TKD athletes.

Regarding inflammatory disorders, moderate physical activity seems to benefit health since exercise training is necessary to improve the immune system [24]; however, strenuous exercise training such as combat competition can attenuate the immune system and may cause inflammation if the innate immune system is not effective [25, 26], thereby compromising athletic performance [27]. Therefore,

utilization of immunonutrition aims to prevent the immune system disorders and possibly enhance athletic performance [28].

Accordingly, TAU supplement has been considered as an immunonutrition compound due to its various beneficial effects on the immune system [6] such as inflammation associated with oxidative stress[29], inhibition of cytokine production [30], and microbicidal activity [31].

Numerous studies have also confirmed the effectiveness of TAU supplementation on inflammation process [13, 32]. However, the attenuating effects of TAU on inflammatory disorders have been occasionally limited, especially in case of intensive exercise training [13, 33, 34]. Consequently, further effective nutritional strategies need to be discovered.

In the present study, the effects of TAU supplementation on the SCTD-induced inflammation were investigated via a placebo-controlled and double-blinded trial, because TAU was illustrated to improve the immune system following exercise-induced metabolic stress. The study results suggested that supplementation with TAU or a placebo for a10-day period did not improve cytokines of IL-8, -17, hs-CRP, and TNF-α in TAU vs. placebo occasions.

The supplementation with TAU together with the SCTD did not also improve the immune system parameters in comparison with the SCTD alone, but significantly increased IL-15 levels after 24 h. Besides, the study observations did not provide support for the capacity of TAU supplement (a dose of 15 mg/ body weight, approximately three servings per day, one after each main meal) to moderate acute inflammation in TKD. Contrary to the hypotheses raised in this study, non-significant changes in pro-inflammatory cytokines (i.e., IL-8, -17, hs-CRP, and TNF-α) between the two occasions (placebo vs. TAU) might be relevant to the simulated protocol used in present study, which was extremely intense for TKD athletes, leading them to increase the inflammatory factors following this protocol even in TAU occasion. Not only intense training such as this protocol could alter inflammatory responses, but eccentric-type training observed in this protocol also resulted in acute muscle damage and inflammation. The study participants were TKD athletes with an average age lower than that in other studies with TAU treatment, in which improvements had been observed in inflammatory status [8]. In addition, these subjects were healthy and physically fit compared with others with significant improvements following TAU supplementation [35-38].

While no effect with orally administered TAU was detected, it was demonstrated in the present study that TAU supplementation did not exhibit anti-inflammatory effects when the type of administration was ingestion (15 mg/ body weight, approximately three servings per day). This may be due to the rapid clearance of TAU, resulting in low mean blood levels [39]. Moreover, TAU possesses anti-inflammatory effects in-vivo only when administered through intracerebroventricular injection (ICVI) [40]. In addition, it could be concluded that the differences observed on the effects of TAU in this trial with exogenous TAU might be the result of the more rapid tissue distribution or clearance of the natural product and prolonged plasma half-life of the TAU in-vivo.

Thus, it was concluded that TAU ingestion could not alleviate inflammation biomarkers, but may improve anti-inflammatory cytokine IL-15. In this respect, Shirvani et al. had reported that TAU administered in the same way had significantly attenuated the severity of inflammation during pressure-filled week of competition and training in elite soccer players[41].

In conflict with the study findings, Zhang et al. had found that a 7-day TAU supplementation (6 g/day) had decreased the levels of lipid peroxidation biomarkers. In an animal study, Silva et al. had further evaluated the effects of a 15-day high dose TAU supplementation on oxidative stress biomarkers following a long-term eccentric exercise and had concluded that TAU had mitigated oxidative stress [9].

As precise mechanisms, underlying TAU inhibition of inflammation, have not been fully understood and demand further studies, the above data did not support the proposal that TAU may be a useful candidate for complementary treatment for elite TKD athletes following a competition day. However, the stability of TAU should be increased in body to improve TAU efficiency. It has been shown that C-methylated derivatives of TAU with better stability [42] and a synthetic form of TAU (namely, taurolidine: TRD) can be a potential candidate as an immunonutrition [43].

In contrast to the research hypotheses, this type of supplement could change systemic IL-15 levels compared with the placebo 24 h after the SCTD, suggesting that these elevations might be related to damage to fibers [44] following such an intense exercise training protocol.

This discrepancy between studies might be due to differences in testing protocols as well as duration and dose of TAU supplementation. These findings based on this higher intensity TKD performance testing suggested that taking TAU in a short term before the SCTD protocol might not prevent severe inflammation. In addition to protocol intensity, the amount of oral TAU intake and administration time can be of important factors for preventing exercise-induced inflammation. According to Ghandforoush-Sattari et al. [45], TAU dose needed to be adjusted according to body mass and maximum circulating TAU concentrations may be reached 90 min at the post-ingestion stage, because TAU administration is expected to increase the availability and the utilization of TAU after exercise, stimulate glycogen synthesis, and improve protein synthesis.

In support of the given hypotheses, the study findings revealed no effect of oral TAU ingestion (15 mg/ body weight) on performance and blood lactate responses compared with the placebo trial. This study was also unable to assess the circulating TAU levels during this protocol, which may provide a more complete evaluation of the dynamics of TAU metabolism. It seems that any potential ergogenic effect of TAU could only occur through interactions with receptors on the muscle membrane or by affecting other organs such as the liver and the adipose tissues during the exercise training protocol, and not within the muscle itself[14].

At odds with this study, other investigations had not shown any ergogenic effects of chronic TAU supplementation in blood lactate responses and athletic performance following intense exercise training upon using TAU-containing energy drinks [10, 46-48]. Variables such as time, period, amounts of TAU

intake as well as testing protocols may thus contribute to whether or not TAU ingestion enhances performance capacity and to what extent [47]. For example, chronic TAU supplementation improves muscle TAU content and contributes to maintenance of performance improvement [49]. However, the absence of measuring intracellular TAU content after chronic TAU supplementation was a limitation of present study. Further studies should accordingly consider the effects of different TAU supplementation periods in individuals with various physical fitness levels using sophisticated techniques for evaluation of bimolecular mechanisms.

From a practical perspective, the results of the present trial did not support others in terms of demonstrating the ergogenic effect of TAU ingestion (15 mg/ body weight of TAU for a 10-day period) on performance and inflammation status. There have been also no adverse side effects reported across studies using doses of 15 mg/ body weight, inferring the safety and tolerability of this supplement. The effects of TAU on endurance performance are fairly well established among TKD athletes, but, to date, there has been no work carried out on elite TKD athletes, which makes it necessary to develop an understanding of the scope of ergogenic effects induced by TAU.

Conclusions

In conclusion, this trial provides novel findings, as it was demonstrated that chronic ingestion of a 15 mg/ body weight of TAU for a 10-day period did not enhance performance in TKD following the SCTD protocol. The present study also indicated that the ingestion of 15 mg/ body weight TAU failed to improve HR responses, inflammatory biomarkers, blood lactate levels, as well as performance of trained TKD athletes during and after the SCTD protocol.

Abbreviations

TKD: Taekwondo, HR: Maximum Heart Rate, ROS: Reactive Oxygen Species, TAU: Taurine, DOMS: Delayed onset muscle soreness, IL-6: Interleukin-6, TNF-α: Tumor Necrosis Factor Alpha, hs-CRP: high-sensitivity C-Reactive Protein, SCTD: Simulated taekwondo competition day, CMJ: Countermovement jump, SK: Skipping, TK: Turning kick, BMI: Body mass index, ITF: International Taekwondo Federation.

Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Shahrekord University, Shahrekord, Iran (SKU.REC.1396.456). All participants were informed about the procedures and signed an informed consent form prior to commencement of the research.

Consent for publication

Not applicable.

Availability of data and materials

Data and publication materials are available from the corresponding author

on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors contribution

Mohammad Faramarzi, Ebrahim Banitalebi, Ahmad Samandari, and FatemehSanjieh designed the study. Ebrahim Banitalebi, Ahmad Samandari, and FatemehSanjieh supervised the exercise training protocols. Ebrahim Banitalebi and Mohammad Faramarzi supervised the laboratory tests and data collection. Mohammad Faramarzi, Ebrahim Banitalebi, and Majid Mardaniyan Ghahfarrokhi interpreted the data. Mohammad Faramarzi, Ebrahim Banitalebi and Majid Mardaniyan Ghahfarrokhi wrote the first draft of the manuscript. Ebrahim Banitalebi and Mohammad Faramarzi edited the paper. All the authors contributed to the writing of the paper, they also read and approved the final manuscript.

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Tables

Table 1. Demographic Characteristics

BMI (kg/m^2)	Weight (kg)	Height (cm)	Age (years)	Factor
21.97±2.60	67.10±10.47	174.50±0.60	20.60±3.50	Mean ± SD

Figures

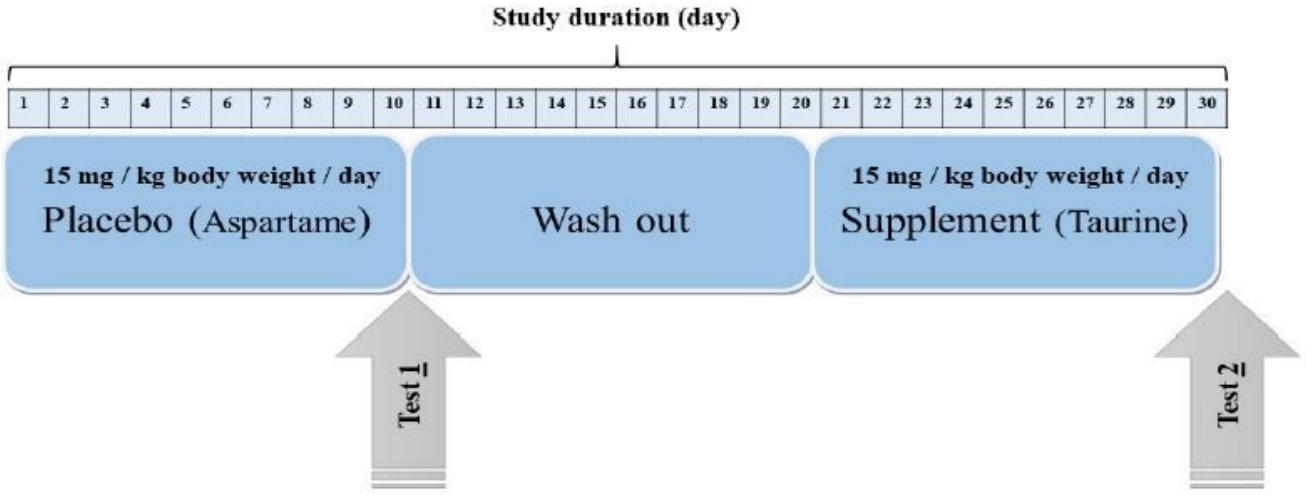

Figure 1

Time course of supplementation period.

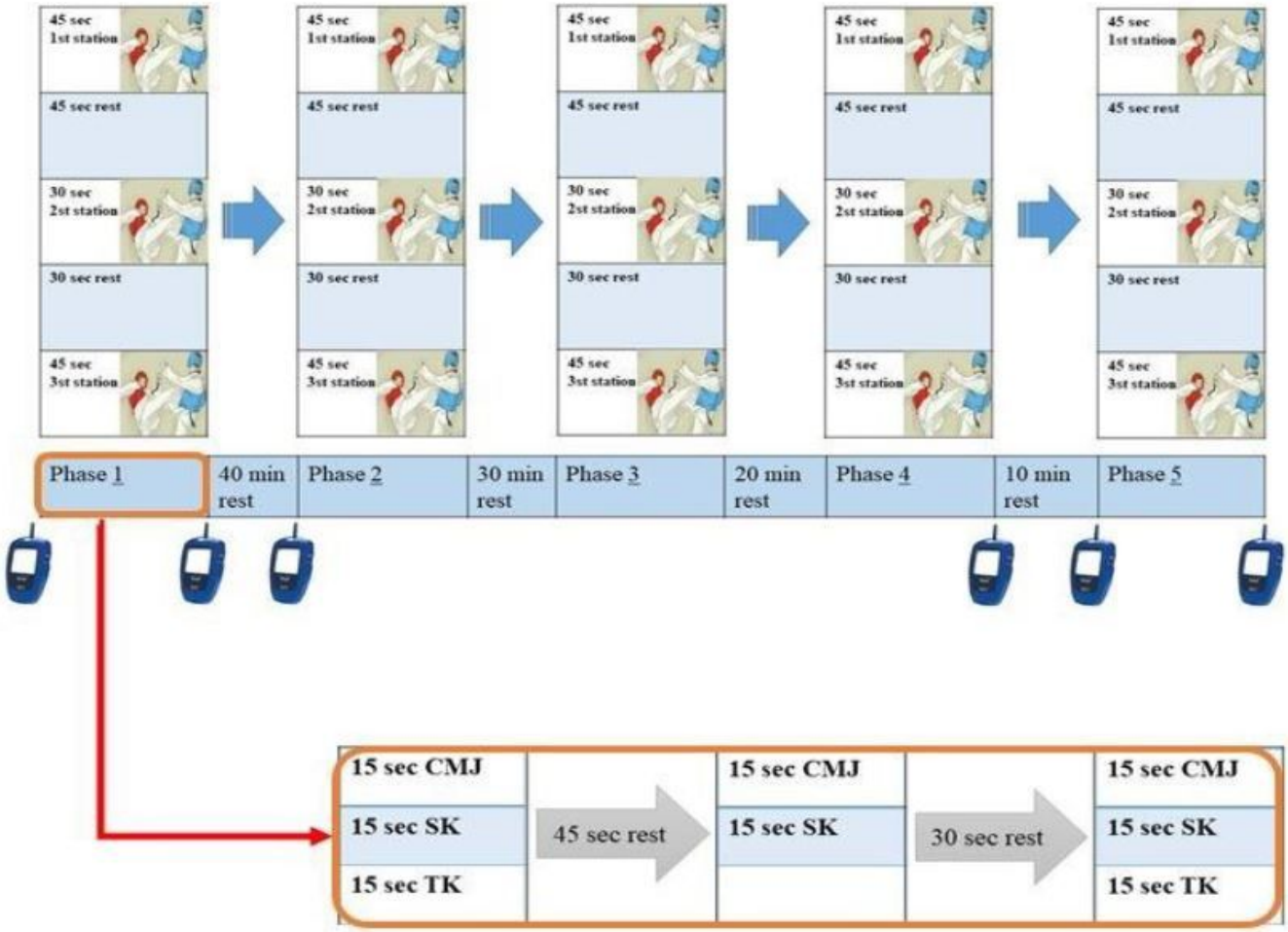

Figure 2

STCD with the same physiological pressure in elite athletes and blood lactate measurement time-

Figure 3

Blood sampling during STCD

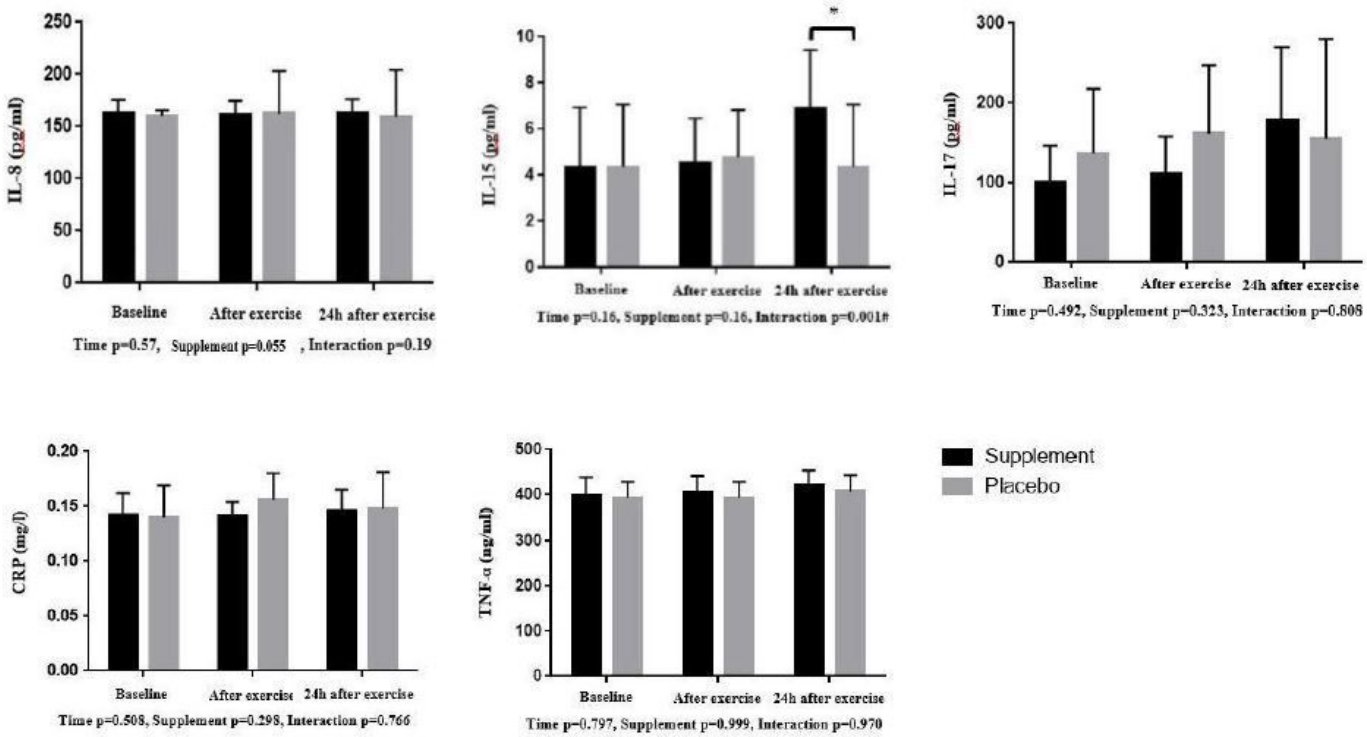

Figure 4

Cytokine responses to SCTD with the same physiological pressure in elite TKD athletes following TAU supplementation Legend: *Significant increase in TAU occasion compared with placebo

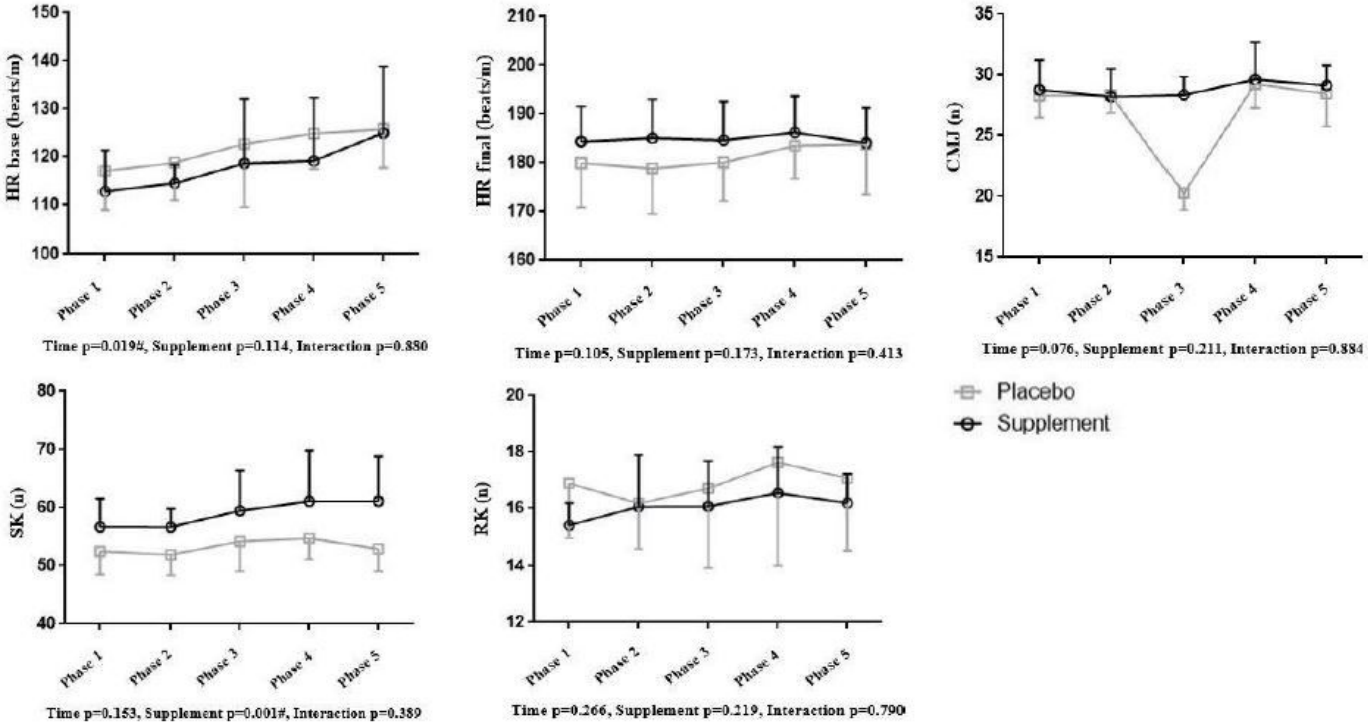

Figure 5

HR, CMJ, SK, and TK responses to SCTD with the same physiological pressure in elite TKD athletes

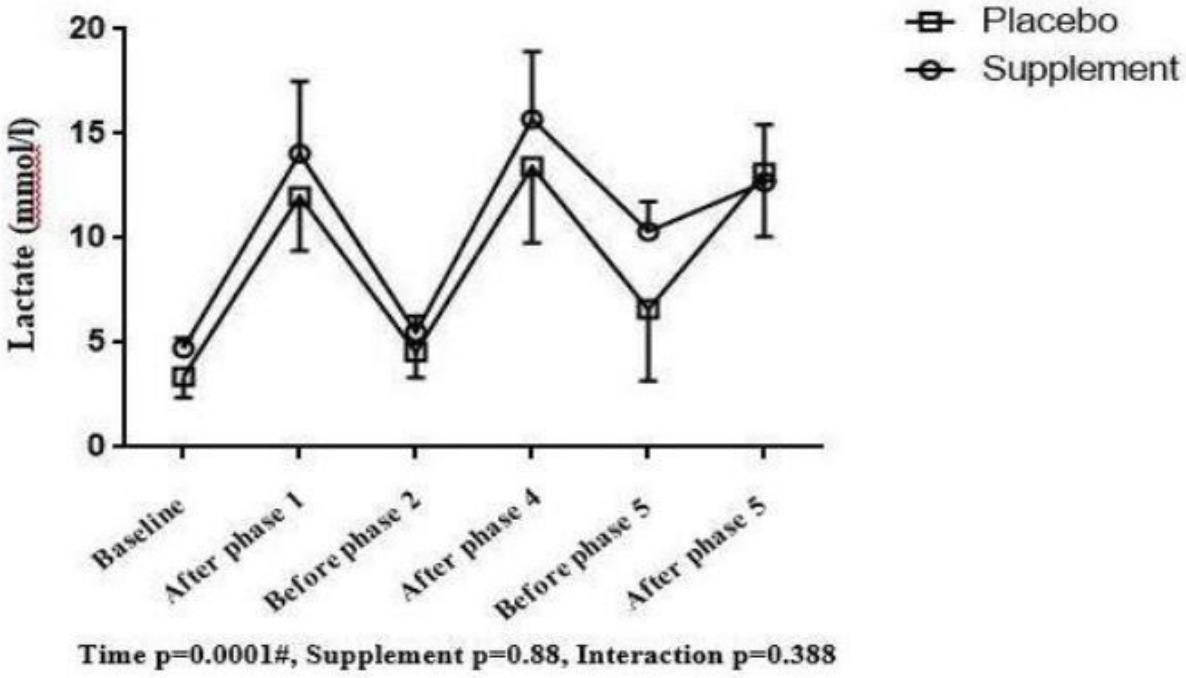

Figure 6

Blood lactate responses to SCTD with the same physiological pressure in elite TKD athletes following TAU supplementation Legend: *Significant increase in TAU occasion compared with placebo