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## The Effects of Soccer Specific Exercise and Taurine Supplementation on Serum Cytokine Response in Male Elite Soccer Players

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### ABSTRACT

The purpose of this study the effects of Taurine supplementation for two weeks and three sessions of 90minutes of intense exercise on serum cytokines responses in well-trained soccer players. Therefore, Eighteen football players under 21 years of league were selected and divided randomly to Taurine (TG=6), placebo (PG=6) and control (CG=6) groups. TG Daily supplements of 15 mg Taurine per kg of body weight and PG groups received the same amount of aspartame and both groups performed three times a soccer specific exercise protocol. The CG group received no supplementation and follow-up was just your ordinary Program. Blood samples were taken in the sixth stage (48 h before Period, before and immediately after first & third exercise protocol and 48 h after the end of the Period) of the 5cc of the anterior forearm venous in sitting position. Then the samples to determine changes in cytokine (IL-6, TNF- $\alpha$ ) in the central endocrine laboratory by special kits were tested. The ANOVA with repeated measures and Tukey's post hoc test with Spss.18 software was used to compare the means in each group. Results showed that the six-stage measure, significant differences ( $P=0/035$ ) in levels of serum IL-6 has emerged and the difference between control group and the placebo group ( $P=0/049$ ) has been observed. The serum TNF-levels no change significantly ( $P=0/937$ ) in any group during the measurement. It appears that the three 90-minute soccer specific protocol to enter stress on the inflammatory system in soccer players such pressure may again be repeated during the competition season. The other hand, results showed that Taurine supplementation before and during this period have anti-inflammatory effects and the noticeable changes to these cytokines are prevented. Thus, short-term use of Taurine supplementation during pressure-filled week of competition and training to be advised in elite soccer players.

**Keywords:** Taurine supplementation, High-intensity intermittent exercise, well-trained soccer, Interleukin-6, Tumor necrosis factor- $\alpha$

### INTRODUCTION

In soccer, players have to perform weekly in comparison with some individual sports comprising only a few large competitions per year. A competitive soccer season includes one to two games per week in addition to several training sessions [1]. Today, can be seen the average of 2.5 match per week in some soccer league. Sometimes in

football tournaments, the games will be held within 48 hours. The high volume of training/ competition and lack of adequate recovery is great stress to immune and muscular system and cause inflammation.

Cytokines are glycosylated polypeptides that are secreted by, and influence the action of, most cells of the body [2]. The pro-inflammatory cytokines, including interleukin (IL) -6 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), modulate immune cell function and migration, initiating and amplifying the acute-phase and stress responses, and Pyrogenesis [3, 4, 5]. The local production of these molecules coordinates the function of innate and adaptive immune cells. In certain pathological states such as trauma, sepsis, and thermal injury, pro-inflammatory cytokines are released into the circulation [6]. Therefore, cytokines are released to the location of inflammation induced by pathogens or tissue damage and facilitating the arrival of neutrophils, monocytes and other cells involved in antigen clearance and healing of the tissue [7, 2]. It is known that muscular exercise increases some plasma cytokines [8, 9].

Andersson and colleagues (2008) showed that changes in enzyme levels of creatine kinase (CK) and muscle soreness to more than 69 hours after the first match is continued. The findings show that several physiological parameters are impaired after a football match. As this can trigger a systemic immune response that leads to the secretion of cytokines and drop of the immune cells [4]. Several studies examined the cytokine response following a football match and increase the number of leukocytes, IL-6 and TNF- $\alpha$  in male soccer players have reported [3, 10, 11]

Competitive soccer affects the musculoskeletal, nervous, immune and metabolic systems [12]. Andersson (2008) reported that several neuromuscular parameters are affected for up to 69 h after an elite female soccer game [3]. For instance, the isometric knee strength was reduced up to 45 h after a first game and jump performance did not recover before the start of a second game played 72 h after the first one. Furthermore, changes in CK levels and perceived muscle soreness lasted up to 69 h after the first soccer game [3]. These findings show that several physiological parameters are disturbed after a soccer game. This, in turn, may trigger a systemic immune response that leads to the secretion of cytokines by inflammatory cells [8]. Few studies are available on the cytokine response following soccer games [13, 14, 10]. An increased leukocyte count and increased levels of interleukin (IL)-6 and tumor necrosis factor (TNF- $\alpha$ ) have been shown in male soccer players [13, 14].

It has also been shown that 5 days of consecutive soccer training in youth male players decreased T and B cell numbers, possibly affecting their capability to activate the immune system and resist infections [15]. Cytokines are potent intercellular signaling molecules that regulate the inflammation response [7]. It has been shown that the plasma pro-inflammatory cytokine response after endurance exercise can be balanced by the production of anti-inflammatory cytokines [8].

Ascensão and colleagues (2010) Effect Loughborough Intermittent Shuttle Test (LIST) against a soccer match on muscle damage and inflammation indicators measure and understand an increasing impact on both activity levels of CK, myoglobin, IL-6, uric acid and blood leukocytes of 30 Minutes after they, therefore concluded that the study of biochemical and physiological changes resulting from football match can be used the same test soccer such as LIST [13].

In recent years, various strategies for antioxidant and anti-inflammatory supplementation have been adopted in an attempt to attenuate the damage and inflammation caused by exercise [1]. Taurine "2-aminoethanesulphonic acid" is one of the most abundant free amino acids in mammalian cells [16].

Taurine has cytoprotective and enantiostatic properties conferred by its antioxidation, detoxification, osmoregulation and membrane-stabilization actions, as well as intracellular calcium flux regulation, due to its molecular structure [16]. Numerous studies have reported the cytoprotective actions of Taurine against ischemia, hypoxia and reperfusion injury in various tissues [17]. Taurine supplementation ability to prime leukocytes and to regulate the release of pro-inflammatory cytokines. It's also conferred immune benefits by down regulating TNF- $\alpha$  release and unregulating antibacterial capacity, as assessed by peritoneal macrophage superoxide generation. In the neutrophils Taurine is a potent modulator of Myeloperoxidase as a key enzyme in the respiratory burst chain [18].

Overall, the literature shows that few studies to evaluate the cytokine response to one or two football match or are similar tests but so far, any study cytokine responses to the special 90-minute soccer tests at intervals of 48 hours has not investigated. Short or long term effects of Taurine supplementation on cytokine changes in soccer players had

not been found. Hence in this context there is knowledge void. Therefore the aim of this research 1- Three times the effect of soccer specific exercise in a week on cytokine response. 2- Effect of Taurine supplements two weeks before and during the three sessions of soccer specific intermittent exercise on the cytokine response of male soccer players. Obviously, the results of this research, provides a new insights into the physiological changes in footballer during high pressure a week.

## MATERIALS AND METHODS

### Research design

Research design is Quasi-experimental with pretest-posttest study with repeated measurements.

### Subjects

The study population included footballers in first division under 21 years league of Tehran and Eighteen players, one of the top teams in this category were selected as subjects. Players, before doing tests were informed how to do research, procedures and objectives of the study and written consent was signed by all of them. Their clinical status information was obtained through questionnaires and any signs of infection or drug treatment for 4 weeks before participating in this study did not report. Then, according to research design, subjects were divided equally and randomly to 3 groups including Taurine (TG), placebo (PG) and control (CG) groups.

### Maximum oxygen consumption

For the measurement of maximum oxygen consumption ( $VO_2\max$ ) subjects, two weeks before the implementation period from Hoff-Helgerud Football Endurance Test was used.

### Body mass index

BMI subjects, divided by body weight (kg) by height squared (meter) was calculated.

### Supplementation

During the two-week research project, experimental subjects were found 15 mg/kg body weight per day aspartame in placebo group and 15 mg/kg body weight per day Taurine in Taurine group after the main meal as oral capsules. The control group received no supplementation and follow-up was just your ordinary Program. To estimate the total energy consumed per day, subjects completed Food frequency questionnaires (FFQ).

### Soccer specific exercise protocol

Experimental subjects in addition found to supplements and placebo, on the tenth, twelfth and fourteenth in the 18-16 afternoon in Lawn football stadium began to perform the exercise protocol. Each exercise protocol comprised two periods of intermittent exercise (45-min each) that simulated the activity patterns of a soccer match, using the activity profile described by Bangsbo *et al* (1991) and modified by Bishop *et al* (2002). The two periods were separated by a 15 min half-time interval. Each 45-min period was divided into three bouts of exercise. Each bout was separated by 1.5 min of rest. The bouts comprised seven circuits of 2 min each: 50 m dribbling the ball through cones placed 5 m apart, 50 m back-wards running, 25 m cruising, 25 m sprinting and 50 m walking. Any remaining time at the end of each 2 min circuits was a rest period. A total distance of approximately 9.7 km was covered during the 90-min exercise protocol. This distance is similar to that reported to be covered during English first division matches by midfield players [14].

### Blood Sampling

In order to measure the dependent variables of the subjects in six steps (48 h before Period, before and immediately after first & third exercise protocol and 48 h after the end of the Period), blood samples were taken. At each stage, 5 ml of blood from the anterior forearm vein in the sitting position of the subjects were divided into two separate tubes. 1 cc was transferred to tube containing EDTA powder was taken for CBC and 4 ml test tube to clot. Then Clot tube was transferred in the centrifuge machine with a low around and serum was separated after 10 minutes. Serum separations storage in frozen was analyzed in further. All experiments in the endocrine laboratory of Shahid Beheshti University in Tehran.

### Measurement of cytokines

Boster's human IL-6 and TNF- $\alpha$  ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human IL-6 specific-specific monoclonal antibodies (clone No. 6708.111) were percolated onto 96-well

plates. The human specific detection polyclonal antibodies were biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxides Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human IL-6 amount of sample captured in plate.

**Statistical analysis**

The results were presented as mean values and standard deviation. Paired t-tests were used for comparing any significant difference of each group on the before and after protocol. Analyses of variance with repeated measures were used for determination of any significant difference in any variables in the six stage of sampling. The Tukey's HSD Post-hoc Test was used to assess significant differences between groups.

**RESULTS**

Table 1 details some characteristics of anthropometry and psychological subject's research shows.

**Table 1: Mean ± SD Characteristics of subjects in different groups**

specification group	Age (year)	Weight (kg)	Height (cm)	BMI (kg/m <sup>2</sup> )	Vo <sub>2</sub> max (ml/kg.min <sup>-1</sup> )
Control group	19.54 ± 0.51	66.33 ± 6.53	178.00 ± 6.51	19.96 ± 0.30	55.24 ± 2.51
Placebo group	19.41 ± 0.51	64.83 ± 6.11	175.00 ± 5.21	20.10 ± 0.48	58.20 ± 3.01
Taurine group	19.74 ± 0.49	62.83 ± 3.43	171.33 ± 5.27	21.04 ± 0.81	60.04 ± 4.41

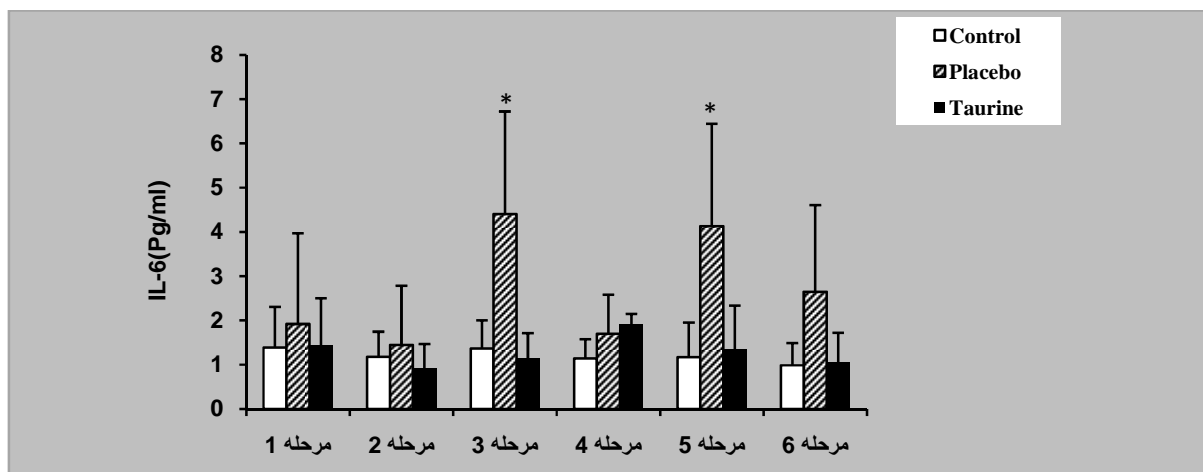


Figure 1: Measurement of serum levels of IL-6 in six different research groups. \* Significantly different (P<0.05).

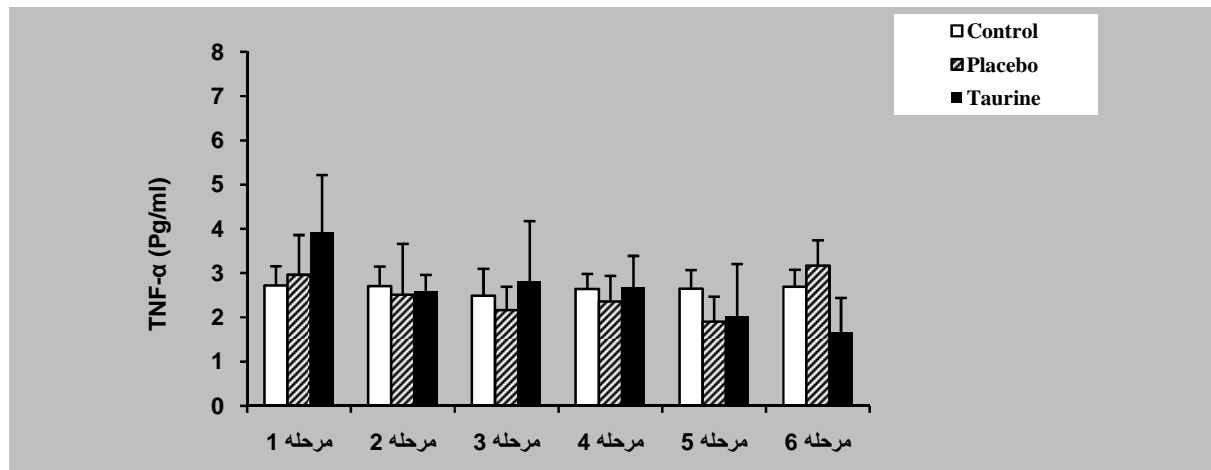


Figure 2: Measurement of serum TNF- $\alpha$  levels in six different research groups

Results showed that the six-stage measure, significant differences ( $P=0/035$ ) in levels of serum IL-6 has emerged and the difference between control group and the placebo group ( $P=0/049$ ) has been observed. The serum TNF- $\alpha$  levels no change significantly ( $P=0/937$ ) in any group during the measurement.

## DISCUSSION

The present study provides a thorough evaluation of the serum inflammatory cytokine response in elite soccer players and establishes a time course of cytokine changes during a period consisting of three games separated by a 48-h recovery and Taurine supplementation. Our results showed that the six-stage measure, significant differences ( $P=0/035$ ) in levels of serum IL-6 has emerged and the difference between control group and the placebo group ( $P=0/049$ ) has been observed. The serum TNF- $\alpha$  levels no change significantly ( $P=0/937$ ) in any group during the measurement. It seems that this type of especially football exercise could also lead to the release of IL-6 in circulation of soccer players and Taurine supplementation also prevented the occurrence of this phenomenon.

We now know that the cytokine response to exercise [8, 6, 9]. Cytokines are polypeptides, originally discovered within the immune system. However, it appears that many cell type produce cytokines and that the biological roles of cytokines go beyond immune regulation. Recent data suggest that several cytokines have important metabolic functions and that they exert their effects locally or work in a hormone-like fashion [19].

Until now, pro- and anti-inflammatory cytokines have been considered part of the acute (local or systemic) phase response to infection or tissue injuries. Thus, cytokines are released at the site of inflammation (caused by an infectious pathogen or traumatic injury) and facilitate an influx of lymphocytes, neutrophils, monocytes and other cells that participate in the clearance of the antigen and healing. The local inflammatory response is accompanied by a systemic response known as the acute phase response [9, 19]. Interleukin-6 is produced in larger amounts than any other cytokine in relation to exercise. The finding of increased levels of IL-6 after exercise is remarkably consistent [8]. A twofold increase in plasma IL-6 was demonstrated after 6-min intense exercise. In treadmill running, the IL-6 level in blood was significantly enhanced 30 min after the start of running, with the IL-6 peaking at the start of running [6].

Andersson et al, reported for the first time that a female soccer game induces a robust, but transient, increase in a large number of both pro- and anti-inflammatory cytokines and inflammatory cells, and that a dampened cytokine response is observed when a second game is played 72 h after the first game. Moreover, IL-6, IL-12, IL-8, MCP-1 and MIG increased following both games, indicating a more pronounced pro-inflammatory response by the end of the second game [1]. Apart from exercise, intensity duration, and mode, it has also been suggested that the exercise-induced increase in plasma IL-6 is related to the Sympatho-adrenal response [9]. Previous studies have also shown that increased epinephrine plays only a minor role in the exercise-induced increase in plasma IL-6. It was previously demonstrated with peak plasma IL-6 during exercise correlated with plasma lactate [8].

High-intensity exercise induces a cardiovascular stress that increases the levels of stress hormones. The increase in catecholamine and Cortisol, together with changes in metabolic activities, the occurrence of membrane disruptions in muscle cells and increased free radical production [20], can lead to the activation of the immune system, such as the release of several pro-inflammatory cytokines and chemokines.

The pro-inflammatory cytokines and chemokines are believed to activate neutrophils and anti-inflammatory cytokines. The anti-inflammatory cytokines would attenuate inflammation by restricting inflammatory cytokine production, up-regulating their soluble antagonist-binding proteins and suppressing inflammatory cell activity [1].

The increase in the total number of circulating leukocytes occurring by the end of both games was expected, and similar to what has been reported in male soccer players [11, 13, 10].

We observed an increase in the mixed cytokine IL-6 following the performed first and three sessions of the soccer specific protocol. Altogether, these results clearly illustrate a response pattern where increases in pro-inflammatory cytokines are accompanied by increases in anti-inflammatory cytokines following the first soccer specific protocol. This observation is in agreement with the hypothesis suggesting a balance between the pro- and the anti-inflammatory response following exercise [8, 21].

Andersson *et al* reported the all pro- and anti-inflammatory cytokines reverted to baseline values within 21 h following the first game. The robust change in the cytokine response within 15–20 min after the game is similar to what has been shown in different exercise protocols. A normalization of the cytokine response 1–2 h after endurance exercise has been reported previously [21]. This fast counter-regulation might indicate that the normalization of the cytokine levels after exercise occurs within a few hours after the exercise. Likewise, Weinstock *et al.* (1997) showed a fast counter-regulation of the cytokine response within a few hours after the exercise and a normalization of cytokine levels at 20 h [23]. Unexpectedly, the cytokine response following the second game clearly indicates a dampened response that markedly differed from that seen in the first game. There are several possible hypotheses behind the dampened cytokine response following the second game. First, it has been shown that the cytokine response during exercise is dependent on the exercise intensity and duration [9]. However, our results show that the physical load placed on the players after the first and second games is comparable.

It has previously been reported that a second bout of exercise would cause a dampening of the inflammatory response [23], the CK and delayed onset muscle soreness levels and blood oxidative stress markers [24], a concept called a “repeated bout effect” [25]. However, this phenomenon is reported to occur mostly in untrained subjects [23, 16]. Because the participants in our study were well-trained soccer players, it is unlikely that the dampened cytokine response is related to the training status of the subjects.

Our results showed that non-significant changes in serum TNF- $\alpha$  level in different groups of soccer player. This can be due to the intensity of this type of exercise or inhibition of Taurine supplementation on production inflammatory cytokines such as TNF- $\alpha$ . Some studies have shown that regular exercise can reduce TNF- $\alpha$  production. Also, Andersson *et al* has shown that long-term adaptation with training and match soccer lead to down regulation of the production of TNF- $\alpha$  from leukocytes in well-trained football player [1].

Damage to skeletal muscle cell membranes by ROS, specifically due to lipid peroxidation, can impair cell viability and lead to necrosis and an acute-phase inflammatory response [16]. Eccentric actions are characterized by a loading profile that combines high force and low fiber recruitment (i.e. high force per fiber ratio), which places a substantial mechanical stress on the associated structures, resulting in oxidative stress. These actions are done in a lot of football games and lead to damage muscle and inflammation. Taurine may affect cellular hyper-excitability by increasing the membrane conductivity of potassium and chloride ions; possibly by modulating the intracellular availability of calcium [17] another possibility is that Taurine reduces protein carbonyls, providing an excellent scavenger for HOCl, produced by MPO [18]. This effect is mimicked by sulphasalazine, the NF- $\kappa$ B blocker, and Taurine is known to inhibit the actions of NF- $\kappa$ B. This effect of Taurine on NF- $\kappa$ B appears to be mediated by Taurine chloramines [16]. In conclusion, it can be said that Taurine supplementation by limiting inflammatory responses to moderately intense soccer specific exercise can be helpful, therefore can be recommended that elite football players to get Taurine supplements during high volume of training and competition weeks.



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