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The effects of coenzyme Q10 supplementation during footsal competition on serum lymphocyte and cortisol on the male players

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ABSTRACT

The aim of this study was to determin the effects of coenzyme Q10 Supplementation during Footsal competition on serum Lymphocyte and cortisol on the male players. Twenty four male footsal player of league(age23/5±1 year, height174/75±5.2cm,body mass 71/3±3.5 kg and BMI 22 /72±1) were selected and randomly assigned in four groups:(coenzyme Q10, competition + coenzyme Q10,competition + placebo and (placebo) . Based on research design, players took coenzyme supplement (100 mg coenzyme Q10) or placebo three times daily .The players accomplished three successive footsal competitions in time period of 4-6 p.m. The blood samples were collected for analysis of serum lymphocyte and cortisol at the rest status before, immediately and 24 hours after the final session of competitions .data were analyzed by repeated measure ANOVA,LSD post hoc test at P<0.05. Results showed that effect of supplementary consumption of coenzyme Q10 on lymphocyte levels during three consecutive matches in competition group with supplement was significantly (p=0.0001) different from other groups and there was no significant differences in blood levels of cortisol.The post hoc test indicated that lymphocyte level of significance were decreased in second stage and increased in third . Based on the results consumption of coenzyme Q10 supplement at the time of successive footsal competitions can decrease serum lymphocyte in second step and increase in third step of male footsal players , so can have a beneficial effect on players' immune system. It is suggested that, Footsal players use coenzyme Q10 supplement during consecutive matches.

Key words: Coenzyme Q10 Supplement,lymphocyte,cortisol, Footsal

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INTRODUCTION

Abnormal environments and sport activities are important sources for physiologic stress which can be resulted to normal disorders in immunity system [1]. Combination of sport activities and stressful environments can have more effects on changes of operation and calculation of immunity cells [2]. Research evidences supports the interchanges between hormone neural responses and immunity responses in sport activities [3,4]. It is determined that immunity cells have special receptors for stress hormones including Glico Corticoid. Based on this lots of changes made in immunity system after sport activity is attributed to adjusting effects of these hormones such as cortisol, epinephrine and norepinephrine [4,5]. Sport increases beta-adrenergic activity and in result it results in topical release of norepinephrine in blood vessels and spleen. This phenomenon may effect on while globule migration and exiting immunity cells from spleen.Lymphocytes are a group of white globules which are branched from prefabricated lymphoid in marrow and includes about 20 to 25% of white globules and incongruous groups of cells. Lymphocytes plays an important role in a wide range of immunity activities such as beginning of immunity responses, cytokines production, antibody production, killing exotic cells and tumors and especially recalling the previous infections. Two-stage increase in numbers of white globules are taken into consideration in reply to practices followed by heavy sports [6].It is shown that the number of lymphocytes immediately increases after sport activities and rapidly

decreases to the basic amount before activity or even become less than the basic amounts in the recovery period. This shall be resulted to a phenomenon known as open window which represents athlete's immunity system weakening after sport activity and prepares him to virus infection [7]. Today there is a consensus among researchers and clinicians that exercise have effects on various aspects of the immune function [8].The immune response can be divided into innate, natural-non-adaptive immunity and acquired-adaptive immunity.Innate immunity is the first response to physical or chemical foreign agents and it occurs naturally and immediately, providing the first line of defense in early stages of the infection. The innate immunity is comprised of phagocyte cells, natural killer cells, soluble factors as the complement and acute phase proteins, as well as the mucosal immune responses. The acquired immunity occurs after an adaptive, specific response to a pathogen and involves the antigen-antibody response. It includes B and T lymphocytes and the immunoglobulines [9]. Glucocorticoids are an important class of steroid hormones that modulate a diverse range of physiological effects. They are produced primarily by the adrenal cortex in response to the pituitary hormone ACTH which is in turn regulated by the hypothalamic peptide CRH [10].Together, these processes form the hypothalamic-pituitary-adrenal axis (HPA). While cortisol is the major glucocorticoid in humans and was first recognized for its role in glucose homeostasis, it is now known to have important anti-inflammatory and immunosuppressive effects on all tissues [11]. A basal concentration of cortisol is required at all times [10]. Although the onset of physical stress produces elevated concentrations [11], increased secretion of cortisol is also stimulated by perceived (i.e. before the actual threat occurs,[13] as well as actual threat. Cortisol has traditionally been assayed from serum, urine and saliva in various studies as ameasure of stress [14]. Footsal is the indoor version of soccer that is officially sanctioned by soccer's international governing body (Federation Internationale de Football Association).The game is growing in popularity all over the world, and since 1989, the world championships have been contested by 16 national teams every 4 years.Futsal is played 5-a-side (i.e., 4 outfield players and 1 goalkeeper), and during the competitions, unlimited substitutions are permitted. Consequently, physical demands of the game may result in being very high [15].It is likely that athletes confront inabilities of immune response and also it enhances physical and mental stress. Athletes who involved in heavy training and racing are more prone to upper respiratory tract infections, and their immune function may disrupt temporarily following intense exercise. Some athletes may start the next session before returning to initial state. If this process is repeated, it is likely that cause suppression of immune function and increasing risk of infection[16].Over the past 15 years a variety of studies have demonstrated that exercise induces considerable physiologicalchange in the immune system.The interactions between exercise stress and the immune system provide a unique opportunity to link basic and clinical physiology and to evaluate the role of underlying stress and immunophysiological mechanisms[17].Indeed, there is both scientific and anecdotal evidence that athletes suffer from an increased number of infections after a single intensive competitive event. It is well known that intensive exercise is also associated with alterations in several immunoregulatory hormones[18].It has been suggested that exercise represents a quantifiable model of physical stress. It is well known that intensive exercise is also associated with alterations in several immunoregulatory hormones.Responses of blood leukocyte subpopulations to an episode of acute exercise are highly stereotyped. Neutrophil concentrations increase during and after exercise, whereas lymphocyte concentrations increase during exercise and fall below pre-values after long-duration physical work.Acute, intense muscular exercise increasesthe concentrations of a number of stress hormones in theblood, including epinephrine, norepinephrine, growthhormone, β-endorphins, testosterone, estrogen, and cortisol,whereas the concentration of insulin slightly decreases.The plasma concentrations of cortisol increase onlyin relation to exercise of long duration. It has been shown that corticosteroids given intravenouslyto humans cause lymphocytopenia, monocytopenia,eosinopenia, and neutrophilia that reach theirmaximum 4 h after administration[17]. We hypothesize that cortisol likely has a role in maintaining the neutrophilia and lymphopenia after prolonged, intense exercise such as a marathon[17].Coenzyme Q10 (CoQ10), also known as ubiquinone, is a lipidsoluble,vitamin-like substance located in the hydrophobic interior of the phospholipid bilayer of the cellular membrane.CoQ10 increases mitochondrial activity related to the synthesis of ATP[19]. In addition, CoQ10 acts as an antioxidant in both the mitochondria and lipid membranes by scavenging reactive oxygen species (ROS), either directly or in conjunction with a-tocopherol[20, 21]. This antioxidant activity appears only with the reduced form (ubiquinol). The oxidized form (ubiquinone)is readily reduced to ubiquinol enzymically after dietary uptake[22]. Although CoQ10 is present in meat and fish, its content in such foods is very low[23]. Therefore, synthetic CoQ10 is used as a dietary supplement by both health-conscious individuals and those with ailments because of its important biological roles, such as mitochondrial energy metabolism and antioxidant activity[24].Nutrient availability has the potential to affect almost all aspects of the immune system, because many nutrients are involved in energy metabolism and protein synthesis. However it is likely that, athletes who are not on a balanced diet and not getting enough antioxidants, or given the high level of training, the amount of dietary antioxidants are insufficient[25]. In fact, a balanced diet has not often enough antioxidant substances to counter the effects of free radicals, especially during periods of high stress or during exercise. In such circumstances, it is possible that supplementary consumption with high antioxidant such as, vitamins A, C, E, beta-carotene and selenium are needed [26].Epidemiological evidence of clinical information indicates that, poor nutrition reduces the ability of the immune system and the risk of infection and infections that are medically safe and has an effect on your physical

activity performance[27,28].The question is whether there are special diets that can eliminate disorders in immune system that caused by exercise? The use of nutritional supplements during or after exercise or competition can reduce the impact of effects resulting from intense physical activity in immune system. Can supplement reduce the risk of infection after intense physical activity in immune system? Certainly, access to balance food can affect all aspects of immune system, because cells of immune system have a very high metabolic rate[29]. The important matter from viewpoint of scientists, trainers, and athletes is that knowing which type of exercise with how much intensity, duration and frequency could increase free radicals and may reduce the ability of immune system, and which type of supplement can modulate the effects of free radicals on immune system.The purpose of this study is to investigate the effects of supplementary consumption of coenzyme Q10 on percentage of lymphocyte and concentration of cortisol serum of male players during Footsal competition.

MATERIALS AND METHODS

Research design

The research is quasi-experiment, based on field research and research design is pretest-posttest with repeated measurement.

Subjects

And statistical population of this research is consist of all the players of Tehran's Footsal premier league, 24 subjects from eligible candidates were selected as samples, after selection, the research topic, purpose and method of implementation and its application as well as risk that involved in process of research were described. Then subjects that volunteered to participate in the study signed a written informed consent. Then through a questionnaire, their health status of last few months was studied. None of the subjects had a history of any disorders related to immune system and also was not under treatment at the time of the research.

Research protocol

Based on quasi-experimental study, subjects were randomly divided into four groups of six subjects, which include, experimental group1 (Q10 supplement), experimental group 2 (competition plus Q10 supplement), experimental group 3(experimental plus placebo) and control group (placebo). Groups of completion were implemented three consecutive matches in accordance with relevant rules. Three consecutive matches held on Saturday, Sunday and Monday between 4 to 6 p.m. basic data and blood samples before and immediately after the match and 24 hours after last match were collected. Each match consists of two halves of twenty minutes and fifteen minutes break time in between. Supplement was used for three days after every meal and during three matches 100mg of coenzyme Q10 in form of soft gel capsules were given to players. Also placebo group and competition group plus placebo have given the same amount of aspartame. Descriptive characteristics of subjects including, height, body weight, age and body mass were measured before the competition.

Laboratory methods

In each stage five cc of blood from a vein in the anterior forearm of subjects in sitting position was taken and split in two separate tubes to CBC. And one cc of it transferred to tubes which contain the E.D.T.A powder and four cc to clot tube. Then stirring C.C.B tube and after that freezing the clot tube and C.B.C tube. Then immediately put clot tube in centrifuge at low rpm for about 10 minutes, after that serum was divided to three eppendorf tube of one cc. one of them for an immunological tests, one for biochemical tests and last one was kept in the freezer at -70 degrees. Safety tests and separation of white blood cells were performed in laboratory.

Measurement of Lymphocyte and Cortisol

To measure the percentage of lymphocytes in blood we were used cell counter machine (EXCELL22) which manufactured in Germany.To determine the percentage of lymphocytes we made some blood smear on a slide and paint it with Giemsa. Then a specialist was separated the prepared slide with the aid of binocular microscope.To measure the concentration of cortisol in blood used ELISA kit with Sensitivity: 0.4 µg/dl which manufactured Diagnostics Biochem company in canada.

Statistical analysis

Normal distribution of data and homogeneity of variances were examined by kolmogorov-smirnov test and Levene's test. In data analysis to determine the mean difference in between group and within group variations, ANOVA analysis and post hoc LSD test at a significance level of ($p<0.05$) were used.

RESULTS

Table 1 details 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^2)
CO-Q_{10} group	24/41 ± 0/52	71/83 ± 6/11	175/00 ± 5/21	23/25 ± 0/41
Q10+Match group	22/74 ± 0/49	72/83 ± 3/53	174/33 ± 5/27	23/78 ± 0/68
Match group	24/06 ± 0/03	74/02 ± 3/86	172/30 ± 5/75	22/30 ± 1/05
Control group	23/54 ± 0/51	68/33 ± 6/53	178/00 ± 6/53	21/5 ± 0/86

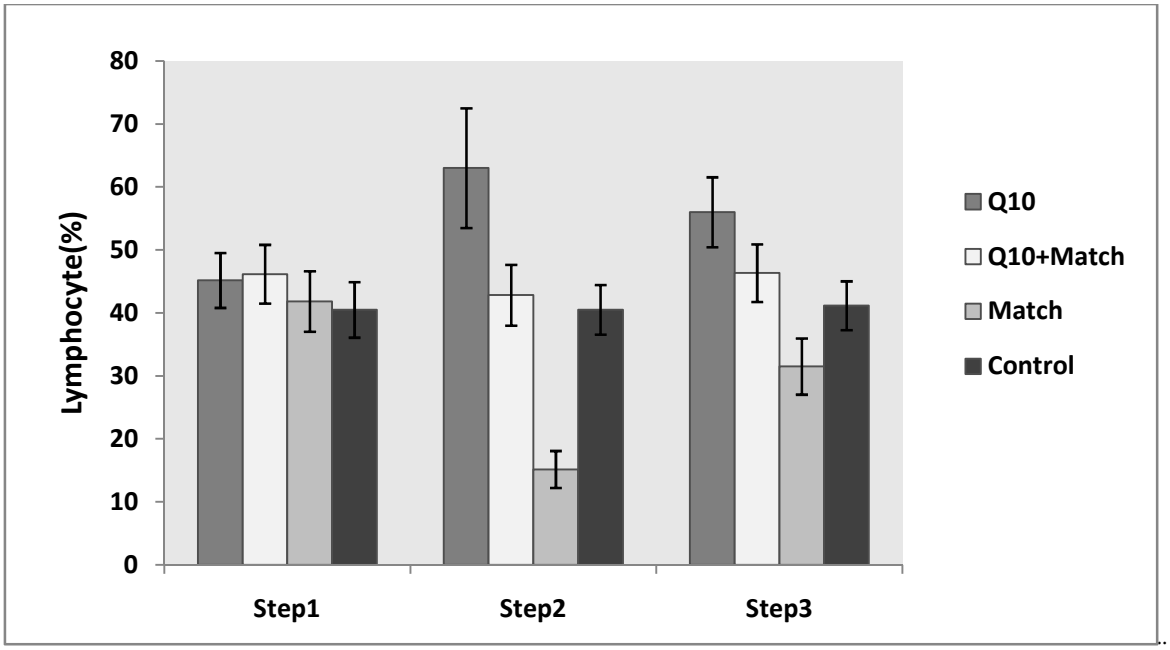

Figure 1: Measurement of lymphocytes serum levels from comparison in between group in three steps
(step 1=pre-competition,step2=immediately post-competition,step3=24 h post-competition)

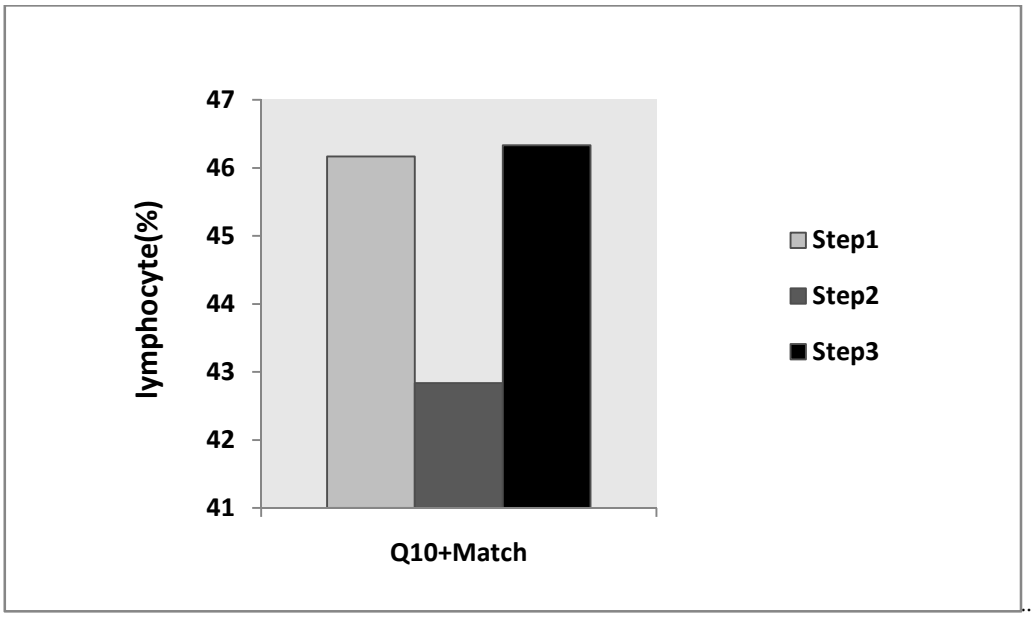

Figure 2: Measurement of lymphocyte serum levels of within group comparison in Q10 plus competition group

Figure 1 shows results from comparison in between group that calculated by ANOVA analysis. Results showed that effect of supplementary consumption of coenzyme Q10 on lymphocyte levels during three consecutive matches in

competition group with supplement was significantly (p=0.0001) different from other groups. Post hoc test revealed that, this difference was significant in the second and third stages.

Figure 2 shows results of within group comparison in ANOVA analysis showed that the level of lymphocyte in competition group with coenzyme Q10 was significant (p=0.029) in three stages of measurement. The post hoc test indicated that level of significance were decreased in second stage and increased in third.

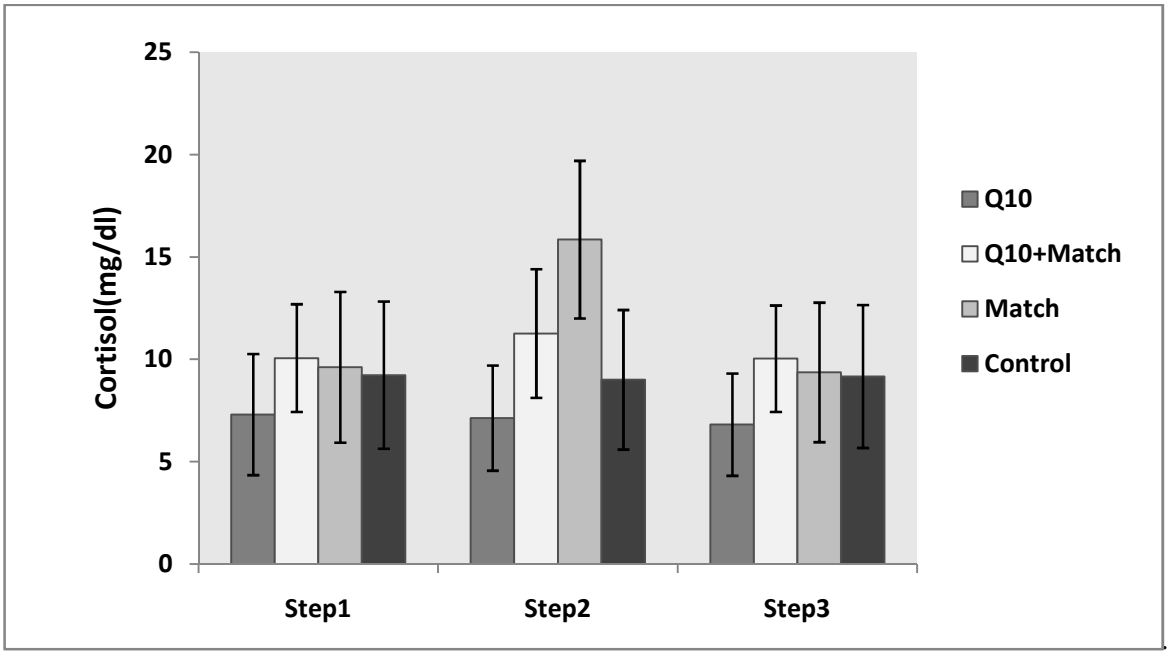

Figure 3: Measurement of cortisol serum levels from comparison in between group in three steps
(step1=pre-competition,step2=immediately post-competition,step3=24 h post-competition)

Figure 3 shows results from comparison in between group that calculated by ANOVA analysis. Results showed that effect of supplementary consumption of coenzyme Q10 on cortisol levels during three consecutive matches in competition group with supplement was no significantly (p=0.113) different from other groups.

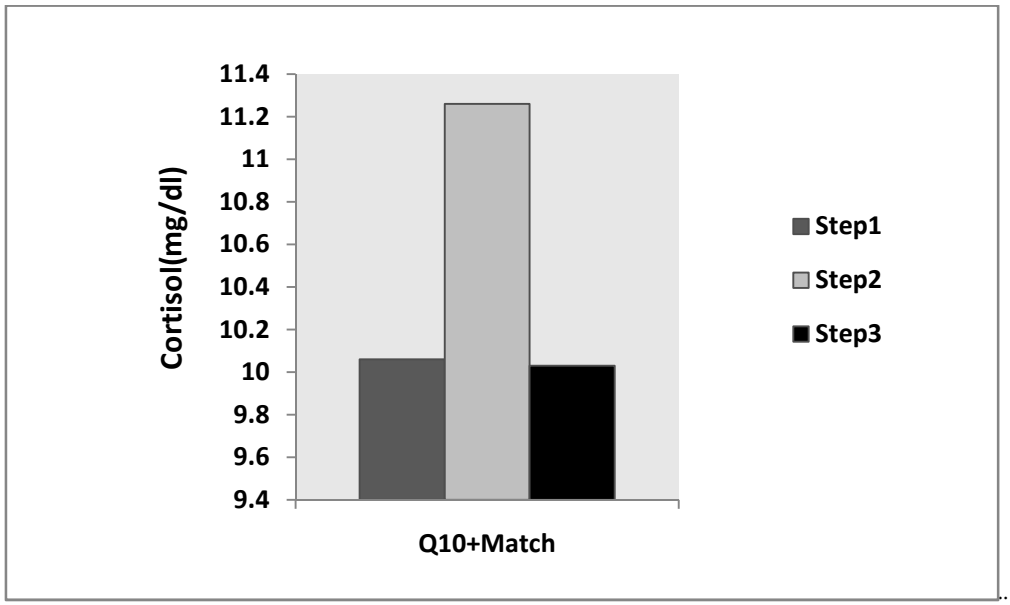

Figure 4: Measurement of cortisol serum levels of within group comparison in Q10 plus competition group

Figure 4 shows results of within group comparison in ANOVA analysis showed that the level of cortisol in competition group with coenzyme Q10 was significant (p=0.006) in three stages of measurement. The post hoc test indicated that level of significance were increased (p=0.030) in second stage and decreased in third (p=0.032).

DISCUSSION

These findings suggest that supplementary consumption of coenzyme Q10 during footsal tournament in competition group with supplement in lymphocytes levels was significant, and this significant was in second and third stages and there was no significanf in cortisol levels.

Sport training involves repeated bouts of exercise and high volume of physically demanding practice sessions and competitive games, which may lead to decline on performance, oxidative stress, and inflammation [30, 31].In fact, an exercise stimulus has been well recognized to induce the production of reactive oxygen and nitrogen species (RONS) [31, 32]. In spite of RONS having an essential role as signalling molecules in several cellular pathways,the imbalance in the pro- and antioxidant status can lead to detrimental effect on cellular loss of redox homeostasis and oxidative damage in lipids,proteins, and DNA [32].Most of the studies regarding exercise induced oxidative stress were carried out with exercise protocols including typical aerobic (running and cycling) [32,33] and anaerobic (resistance training and sprints) exercise [34,35].

Oxidative stress is a condition in which the delicate balance existing between prooxidant (free radicals) production and their subsequent amelioration via the antioxidant defense system becomes skewed in favor of free radical expression[36]. Both the radicals themselves as well as the non-radical species created via interaction with free radicals are collectively referred to as reactive oxygen/nitrogen species (RONS)[37] which arise as natural by products of normal cellular energy production or are generated in large amounts by exhaustive exercise or by chemical agents in the environment [38]. It has been shown that excess ROS generation may lead to oxidative damage to DNA, lipids and proteins [39].Leukocytes levels display different behaviors as exercises at morning and evening[40].Some studies showed that ingestion of carbohydrate with high or low glycemic index before endurance exercise had limited effects on circulating leucocytes[41]and may inhibit catabolic hormone (cortisol)[42].It is likely that antioxidant supplements such as coenzyme Q10 reduces oxidative stress and has an influence on cytokines production and also activation of exercise-induced immune and thus will influence the amount of lymphocytes. In this regard Shirvani et al was examined the effects of coenzyme Q10 supplementation during intense intermittent exercise on IL-6 serum levels in football players and as a result, coenzyme Q10 supplementation with anti-inflammatory effects could cause significant changes in cytokines[43].Moreover, the practice may result in changes in amount of adrenergic receptors of while globules and in result may change the cells' activity but the hormone level does not change in circulation [44].Catecholamines may have indirectly effects on white globules replacement by releasing Cytokine such as IL-1. This in turn results in adjustment of molecule cohesion [45].By prescribing Catecholamines at the time of sport, the amount of white globules shall be increased based on the amounts produced by sporting [46]. Increase in body temperature may help in changing white globules and distribute its subsets during and after practice. Increase of temperature during the practice by releasing Catecholamines may help in increasing total white globules and lymphocytes. Increase of temperature resulted from practice may have a deep effect on redistribution of subsets of white globules within the first hours after returning to the primary status after practice. It is said that the primary increase of white globules, neutrophils, lymphocytes, and NK cells is due to rapid increase in epinephrine and growth hormones. Moreover, this late releasing of cortisol during and after sport is responsible for permanent increase of numbers of neutrophils and decrease of NK cells and lymphocytes after sport[6]. The amount of lymphocytosis in sport depends on the counter effect of practice strength and individual readiness level. Meanwhile the number of lymphocytes may be unchanged in average or very short sports and or it may be increased up to 50% more than the rest time. The number of lymphocytes may be increased two or three times more than the rest time during the long term sports. Like number of lymphocytes the number of lymphocytes shall be increased by increasing the practice and its amount depends on sport strength. Anyway in spite of the findings related to the Leukocytosis duration of sport may not be a determining index [47].Lymphocytosis resulted from sport may be effected by amount of person's readiness. Such as the short term sports, in long term sports there is a two-step response in number of lymphocytes, e.g. during the sport the number of cells shall be increased and some hours later its amount shall be decreased less than the primary amount [47].Increase in cortisol secretion is subject to the practice intensity according to the individuals' practice capacity. The personal differences are more effective in reaction of Glucocorticoids to practice, because cortisol only release in hard free practices. In sport practices cortisol shall respond to a determined amount of practice. Cortisol shows a pause before increasing and after termination of the practice its increase shall be continued and or remain in a higher level. Moreover, cortisol hormone shows daily changes, e.g. it shows a primary high pitch at first and then a decrease during the morning[6]. In a research performed in 16 runners running 90 kilometers and consuming vitamin C supplement each day for 7 days before the competition and the day of competition and two days after competition, the serum cortisol show decrease [48].Probably more strength and mental and physical stresses are more other motivations for cortisol secretion. In general it seems that the duration and intensity of practice are effective in personal readiness level and daily changes of serum cortisol [6].In a research, 800 milligram C vitamin supplement were consumed respectively 2 and 14 hours before practice and 1500 milligrams were consumed during long term riding each 15 minutes once. Any meaningful

changes were not shown in cortisol concentration [49]. Sport activities more than 60%VO 2Max intensity increases cortisol hormone secretion. Hormone secretion increased by motivating adrenal hypothalamus-hypophsis axel is one mechanism existed in secretion of this matter which resulted to ACTH secretion from hypophsis and ACTH secretion increase is the most important factor in cortisol secretion motivation (Mehdi Vand & Sari Saraf, 2010). This hormone is basically secreted due to other conditions such as mental pressure, body practice and competition and plays an effective role in operation of some of immunity system cells [50].These responses are not observed in ordinary practices and one of its particulars in intensive primary increase in number of lymphocytes following that intensive decrease of the lymphocytes are observed. This condition shall be continued up to end of sport and or after stopping. Hansen et all analyzed white globule response in seven stable healthy men in three distances of 8.7, 4.1 and 10.5 kilometers. During the practice the number of lymphocytes became double and it returned to rest amount after passing 30 minutes of the practice and between 30 minutes and 4 hours after sport its decrease was continued and reached to 32-39% lower than the rest amount[51]. When resting less than half of body mature white globules are circulated in vessel system and the remaining are imprisoned in small vessels of spleen, liver and marrow. Very probably mechanical factors such as cardiac output increase and injection inside small vessels shall change the interchanges between white globules and endothelium (epithelial tissue) of vessels. Moreover, immature white globules may be intensively released by spleen and marrow. Some of these changes are resulted from stress hormones and inflammatory mediators such as cytokines and acute stage proteins [6]. There are some evidences showing that at the time of sport, stress hormones plays a mediator role in changing the number of white globules and redistribution of its cell subsets. It is determined that hormones such as epinephrine and cortisol are effective in redistribution of white globules between blood flood and various parts of body such as spleen, liver and marrow[6].

CONCLUSION

This study examines the effects of coenzyme Q10 supplementation on percentage of lymphocyte serum and concentration of cortisol on the male players during footsal competition.The results suggest that supplementary consumption of coenzyme Q10 during footsal tournament in competition group with supplement in lymphocyte levels was significant, and this significant was in second and third stages and there was no significant in cortisol levels. Coenzyme Q10 supplements significantly decrease the percentage of lymphocyte in second step and increase in third step in players during three consecutive matches,so can have a beneficial effect on players' immune system. It is suggested that, Footsal players use coenzyme Q10 supplement during consecutive matches.

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