

Comparing the Serum Levels of IL-2, IL-6, TNF α , IFN- γ and CRP between Sprinter Athletes and Non-athletes

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ABSTRACT

The aim of this study was to compare changes in serum levels of IL-2, IL-6, TNF α , IFN- γ and CRP in athletes of speed runners and non-athletes. The statistical sample of this study included 70 young men aged 20 to 25 years old. Twenty participants were sprinter and 50 non-athletes. Then, they were divided into two groups: speed runners (20 persons) with mean (78 ± 41.4 kg; height: 181.2 ± 13.2 cm; age: 21.2 ± 14 years; body mass index: 1.94 ± 1 ; 23) and non-athletic group (50 persons) with mean (weight: 77 ± 6.26 kg; height: 180 ± 3.22 cm; $22 \pm 2/45$; body mass index $23 \pm 2/02$). Blood samples were prepared to measure the IL-2, IL-6, TNF α , IFN- γ and CRP variables and were determined by ELISA method. In order to analyze the data, inferential statistical test of independent t test was used for comparison between groups. Independent t-test showed a significant difference between the groups in IL-2 ($p = 0.0225$, IL-6 ($p = 0.028$), TNF α ($p = 0.1010$) and CRP ($p = 0.016$); however, there was no significant difference in the level of IFN- γ between the two groups. Considering that cytokine changes were observed in speed runners. It is recommended that the trainers make a significant contribution to strengthening the safety system during the preparation period.

1. Introduction

It is known that performing a series of activities leads to improving and maintaining health, promoting short-term and long-term sports adaptations and improving metabolic diseases (Kaya, 2016); Sports activities also result in improvement of physiological changes following the disruption of homeostasis. The resulting changes are achieved until the low workout pressure is not cut. Therefore, exercise and sports activities are an excellent way to study the physiological stress and body adaptations capacity (Neto et al, 2009; Lira et al, 2009). Cytokines are protein molecules involved in the immune system that play a mediation role. Cytokines can include chemokines, interferons, interleukins, lymphokines, and tumor necrosis factors, but not hormones or growth factors. Cytokines are released into the circulation in response to various stimuli, such as antigens, physical activity, mental pressures and inflammation (Vijayaraghava & Doreswamy, 2017). Although sports activity leads to structural lacerations in muscle-skeletal tissue, the body is faced with reactions of the immune system (Kara et al, 2011). Studies have shown that, following sports activities, the response of cytokines can vary considering the type of exercise activity and workout protocol (Kara et al, 2010). The determining diagnostic factor to increase the cytokines during the reaction depends on the rate of infection by immune responses after exercise activities due to intensity and type of activity. IL-2, IL-6, TNF α , IFN- γ cytokines play a significant role in acute or chronic inflammation. IL-2 is a glycoprotein with a molecular weight of 15 kDa, which has a growth and activation effect on T cells. IL-2 is often produced from TCD_4^+ and TCD_8^+ cells that applies its role through paracrine and autocrine pathways. IL-2 is a type of cytokine signaling molecule in the immune system that regulates the activity of leukocytes and often lymphocytes that are responsible for cell-mediated immunity (Zhang et al, 2007; Arenas-Ramirez et al, 2015). Several studies examined the acute and chronic effects of exercise on serum IL-2. Hence, some studies have reported a decrease of serum IL-2, and other studies reported no changes of serum IL-2, but most studies have reported significant increases, especially serum IL-2 concentrations (Suzuki et al, 2003; Fuente et al, 2005; Sellar et al, 2006; Romeo et al 2008). IL-2 also plays an important role in immune responses as a result of the regeneration of damaged muscle through activity (Neto et al, 2009), while IL-6 is coded with a molecular weight of 21 to 28 kDa of the IL6 gene that has cytokine pro-inflammatory and myokine anti-inflammatory roles. IL-6 is released from macrophages and T cells in response to infection and trauma, resulting in the incidence of inflammation. Also, skeletal muscle is released from osteoblasts, lining muscle cells of the vessel wall (Reihmane & Dela, 2014). Most studies have focused on IL-6 as an energy sensor in skeletal muscle during the recent years, which plays a role in energy supply. It is reported that the value of IL-6 increases up to 100 times after the marathon. However, the type of muscle contraction plays a crucial role in IL-6 changes in connection with the contraction of the muscle (Pedersen et al, 2001). It has been demonstrated that maximum intensity activity leads to an increase in serum IL-6 concentration, which is influenced by neutrophil activity during maximum activity (Reihmane et al, 2012), although it seems that white blood cells in circulation are not the main source of IL-6 plasma in prolonged activity (Reihmane et al, 2013).

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Interferon-gamma or type II interferon is the main pro-inflammatory cytokines belonging to the interferon families, which plays a vital role in the inherent and adaptive immunity against bacterial and viral infections. An important activator of macrophages as well as the stimulant of the main series is type II tissue adaptation. TNF α is a multi-functional cytokine that is involved in the regulation of inflammation and tissue damage. TNF α has usually pro-inflammatory role in tissue decomposition (Pedersen et al, 2001). TNF α can be synthesized through neuron and immune cells and is quickly released into the bloodstream through monocytes and into skeletal muscle by macrophages following exercise-induced muscle damage (Zaldivar et al, 2006). Moreover, TNF α can lead to the expression of both myocyte necrosis and apoptosis through intracellular signaling pathways (Hardin et al, 2008). Intense activity often leads to significant muscle damage that can affect the performance of athletes (Sellar et al, 2006). (Bernecker et al., 2013) reported that TNF α levels have increased after a marathon (Suzuki et al, 2003). IFN- γ is predominantly produced from natural killer and T natural killer cells in response to innate immunity (Schoenborn & Wilson, 2007). IFN- γ is secreted from the most of immune cells including lymphocytes, granulocytes, and macrophages; as well as, it is produced from other tissues of the body such as adipose tissue, brain tissue, and skeletal muscle mass, (Heidarianpour et al, 2016). IFN- γ is considered as anti-inflammatory cytokines (Vijayaraghava & Radhika, 2014) and pro-inflammatory cytokines (Kohara et al, 2011) at low concentrations. It was shown in a study that IFN- γ levels increase after an exercise session with moderate intensity and decrease following an intense course of workouts, and it significantly increases with regular moderate exercise after four weeks of regular exercise in young men compared with pre-workout levels (Vijayaraghava & Radhika, 2014). (Heidarianpour et al., 2016) showed that the eight weeks of training increase the IFN- γ levels (Zaldivar et al, 2006). Also, (Heidarianpour et al., 2016) reported that the eight weeks of training for 30 minutes for 5 days significantly increase the IFN- γ levels. The C-reactive protein is an acute reaction agent that is produced from the liver in response to an increase in IL6 and is associated with systemic inflammation (Heikkilä et al, 2007; Michigan et al, 2011), which its role is to remove necrotic and infectious materials; Its level in plasma increases up to 1000 times, and is referred to as diagnosis and disease progression index (Johnson et al, 2010). The effects of different types of exercise protocols on the CRP levels have been identified. (Balagopal et al. 2005) and (Kadoglou et al. ,2007) pointed out that performing 45 to 60 minutes of aerobic exercise per week led to a reduction of 30 to 40% in CRP (Balagopal et al, 2005; Kadoglou et al, 2007). Dangz also noted that 10-week resistance training reduces the CRP by 33% (Donges et al, 2010). It seems that exercise is effective in reducing CRP levels. Considering that the role of cytokines in incidence of inflammation is clear and given that the damage caused by exercise activities leads to the occurrence of this response, the aim of this study was to determine the effect of adaptations created after long-term exercise training on these cytokine factors. Thus, the main objective of the present study was to compare serum levels of IL-2, IL-6, TNF α , IFN- γ and CRP between sprinter athletes and non-athletes.

2. Research Methodology

This was a causal-comparative study and the statistical population of the research consisted of all sprinter athletes in the city of Mashhad. A total of 70 people participated in this study voluntarily based on inclusion criteria, including age range of 20 to 25 years, history of at least five years of regular exercise, enjoying physical health, body mass index (BMI) of 20 to 25, and eventually the number of 20 sprinters was selected purposefully. As well as, 50 non-athletes were selected and divided into two groups of men sprinter athletes (20 people) and non-athletes (50 people). Participants were given additional information about the research in the first session. Anthropometric indices such as age, height, weight, body mass index (BMI) were measured and recorded in the second session. Their diet was also controlled every other day during a week before blood sampling, and there was no significant difference between meals and all had a normal diet. For blood sampling, all subjects were asked to abstain from exercise for 48 to 72 hours prior to blood sampling, and they were asked to be in a state of fasting at the night before the day of blood sampling (8 to 10 hours before blood sampling). At eight o'clock of the next morning, the blood samples were taken from the right arm's brachial vein of the participant at a dose of 10 cc in the sitting position. The prepared blood was poured in dry or non-anticoagulant tubes. After clotting the blood for 20 minutes, the serum of them was discarded using a centrifuge with 3000 rpm for 15 minutes and frozen at temperature of -35 ° C. To measure the variables IL-2, IL-6, TNF α , IFN- γ and CRP, the standard kits of Bender-Med Company were used by ELISA method.

2.1. Statistical Method

In this study, the mean and standard deviation were used to analyze the data obtained in the descriptive statistics section, and the independent t-test was used in the inferential statistics. The significance level in all test procedures was considered to be $P < 0.05$.

3. Statistical findings of the research

Findings associated with physiological and anthropometric characteristics are given in Table 1, which indicates that there is a normal distribution.

Table1. Anthropometric characteristics of participants by separation of each group

Attribute	Sprinter Athletes Group (20)	Non-athletes Group (50)
Age (years)	14.2 \pm 21.8	22.6 \pm 45.2
Height (cm)	181.1 \pm 2.13	180.8 \pm 3.22
Weight (kg)	78.2 \pm 414	77.1 \pm 6.26
Body mass index (BMI) (Kg square meters)	23.86 \pm 1.94	23.80 \pm 2.02

Table 2 shows data related to the variables IL-2, IL-6, TNF α , IFN- γ and CRP, which enjoy normal distribution. The results of independent t-test showed that there was a significant difference between the groups in variables of IL-2 ($p = 0.225$, significant decrease), IL-6 ($p = 0.028$, significant increase), TNF α ($p = 0.010$ significant increase) and CRP ($p = 0.016$); however, there was no significant difference in IFN- γ levels between the two groups ($p = 0.089$).

Table2. Data of variables IL-2, IL-6, TNF α , IFN- γ and CRP for participants by group separation

Variable	Group	M SD	Kolmogorov-Smirnov Test		Independent t-test	
			Z	Sig	T	Sig
	Sprinter Athletes Group	21.24 \pm 1.71				

IL-2 (ng/ml)			0.405	0.997	-2.441	*0.025
	Non-athletes Group	22.98 ± 1.46	0.590	0.878		
IL-6 (ng/ml)	Sprinter Athletes Group	3.84 ± 1.22	0.358	1.000	2.385	*0.028
	Non-athletes Group	2.73 ± 0.80	0.562	0.911		
TNF- α (ng/ml)	Sprinter Athletes Group	2.64 ± 27.14	0.420	0.995	2.89	*0.010
	Non-athletes Group	2.25 ± 23.95	0.430	0.993		
CRP (ng/ml)	Sprinter Athletes Group	3.98 ± 0.72	0.437	0.991	2.66	*0.016
	Non-athletes Group	3.230 ± 0.53	0.933	0.348		
IFN- γ (ng/ml)	Sprinter Athletes Group	2.68 ± 0.38	0.884	0.416	-1.801	0.089
	Non-athletes Group	3.09 ± 0.60	0.742	0.640		

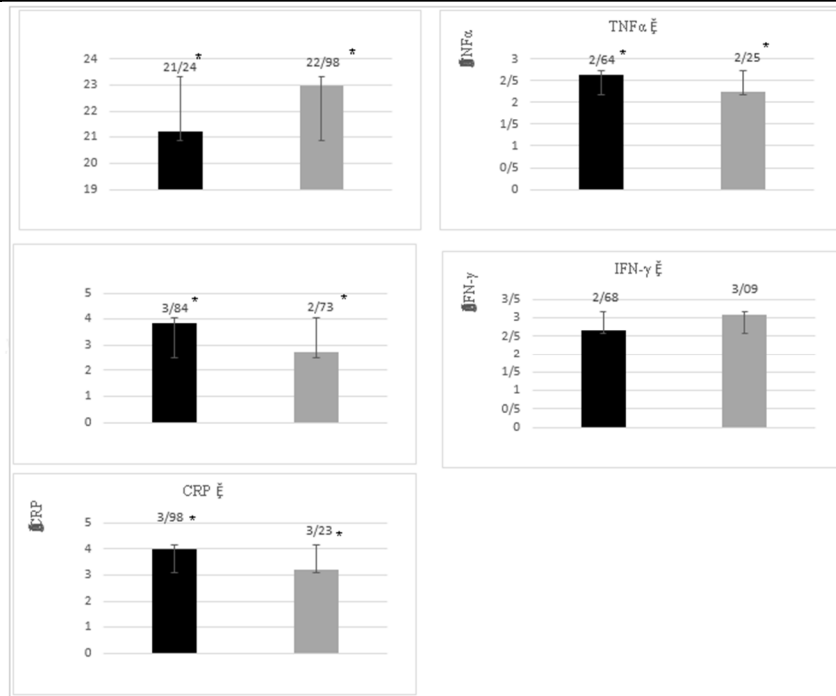


Figure1. Data related to variables of IL-2, IL-6, TNF α , IFN- γ and CRP by separation of groups (significance between groups *)

4. Discussion & Conclusion

Many studies have demonstrated that physical activities lead to the release of inflammatory cytokines and CRP (C-reactive protein) from immune cells and changes of their levels in the blood. Of course, the time of this increase or decrease or reaching them at the base level is not the same and depends on the type and duration of exercise and the time of blood sampling, and may affect the physiology of the body for several days (Kohut et al., 2006).

The results obtained from serum IL-2 levels showed that IL-2 levels were significantly lower in sprinter athletes group than non-athlete group. The findings of the research were consistent with the studies by (Bagherabadi et al., 2009) and inconsistent with the findings of (Zaldivar et al., 2006) and (Zhang et al., 2007). (Bagherabadi et al., 2009) reported a significant decrease in IL-2 during a course of aerobic exercise on IL-2 levels in men (Bagherabadi et al., 2009). While (Zaldivar et al., 2006) reported those 30 minutes of cycling anaerobic exercise on 11 healthy men showed a significant increase in IL-2 concentration (Reihmane & Dela, 2014). (Zhang et al., 2007) did not report a significant change in serum IL-2 levels during a course of physical activity (Zhang et al., 2007). Tevada also reported that levels of IL-2 in mononuclear cells secreted by PHA decreased during and two hours after intense exercise in untrained men. Results of the present study on reducing IL-2 in the trained group showed that this reduction can be attributed to the decline in CD4 cells, which may represent a decrease in the ability of lymphocytes in response to external factors, as well as a reduction in the proliferative response of lymphocytes after short-term intense exercises. On the other hand, this decline can be attributed to increased expression of IL-2 receptor in activated lymphocytes; In this case, the number of cells that contain more receptors quickly removes IL-2 in circulation, thus reducing the levels of IL-2 (Heikkilä et al., 2007).

In relation to changes in serum IL-6 levels, the results showed that IL-6 level in the sprint training group is higher than the non-athlete group by 40%. The results of this study were in line with the research of (Shojaei, 2011), who reported an increase of IL-6 in a 45-minute activity of moderate-intensity

aerobic exercise (Shojaei ,2011). In a research, Richard Scott also compared the effect of exercise intensity on the response of cytokines in the race competition. They reported that concentration of IL-6 increased in activity with an intensity of 75% of oxygen consumed at a duration of 60 compared to the intensities of 55% and 65% of the maximum oxygen consumed (Zaldivar et al, 2006). While (Haqiqi et al. 2005) showed that sprint training for 13 weeks had a decreasing effect on the amount of il-6 (Haqiqi et al. 2005). It has been known that IL-6 increases in response to exercise activity under the influence of intensity and duration. In other words, IL-6 is a cytokine sensitive to cellular energy (Pedersen et al, 2001). The long-term activity at maximum or prolonged intensities seems to have an effect on increasing serum concentrations of IL-6 among the sprinter athletes' group in the present study.

The results obtained from changes of TNF α showed that the amount of it in the sprinter athlete group was higher than non-athlete group. In this regard, (Sprenger et al., 1992) showed that running with long distances causes a sharp increase in the release of cytokines, including TNF α , in comparison with the control group (Sprenger et al., 1992). In a study, (Starkie et al.,2005) examined the effect of two 90-minute sessions of cycling exercise with 70% VO₂max intensity in seven healthy men on the production of cytokines. Finally, the measurements showed that concentration of the mentioned cytokine did not show any significant increase at the end of the training sessions (Starkie et al.,2005). Also, (Bernecker et al., 2013) reported a significant increase in the amount of TNF α after a marathon (Bernecker et al., 2013). Tartibian also showed that a session of incremental exercise may increase inflammatory cytokines especially TNF α in female athletes. However, Rihmaneh did not find any significant increase in the amount of TNF α (Michigan et al, 2011). It seems that the level of physical fitness, gender, type and intensity of exercise, genetic differences, nutritional background and age of subjects have been the main and crucial reasons for these changes. It also appears that one of the major reasons for this increase in the current study is due to the activity of immune cells caused by muscle rupture during their activity (Heikkilä et al, 2007).

The results of IFN- γ changes in sprinters group showed a decrease compared to the control group, but the change was not statistically significant. In this regard, (Nickolas et al., 2004) investigated the effect of sprint training on the level of IFN-gamma in blood culture sampling. The results of the experiment were in such a way that that the number of IFN- γ was significantly lower after 30 minutes of boring exercise, (Nickolas et al., 2004). On the other hand, (Zaldivar et al., 2006) in a study showed that the amount of IFN- γ significantly increased after exercise. As well as, (Heidarianpour et al., 2016) reported that eight weeks of training leads to increase of IFN- γ . Given that IFN- γ is an anti-inflammatory cytokine for health and cellular immunity, adaptations induced following the exercise, as well as adaptation to sports injuries, lower inflammation in athletes has been impressive on IFN- γ production. Considering that the participants in the present study were trained athletes, it can be inferred that exercise adaptation had an effect on IFN- γ serum level changes.

Finally, changes in serum levels of CRP showed a significant increase in sprinters compared to non-athlete groups that the increase is 23%. In this regard, (Soheili et al., 2009) examined the effect of a course of endurance and sprint training on inflammatory indices among men. The results of this study showed that sprinting exercises increase CRP inflammation index (Soheili et al., 2009). (Akbari et al. 2009) also reported that intense and prolonged swimming exercises significantly increase the concentration of CRP levels (Akbari et al, 2009). In a study, (Goldhammer et al. ,2005) examined the effect of 12 weeks of aerobic exercise on activity of cytokines. The results indicated that aerobic exercise significantly reduced CRP (Crameri et al, 2009). On the other hand, Friedenreich in a study examined the effect of changes in inflammatory indices and the interventional effect of aerobic exercise activity during a year among women. The results showed that the CRP level of the exercise group was significantly lower than the control group (Goldhammer et al, 2005). Dangz also pointed out that 10 weeks of resistance training led to a reduction in the CRP by 33%. Due to the fact that IL-6 and CRP are two physiological indices that are sensitive to chronic systemic inflammation, it seems that sprint training or high intensity exercise is associated with an increase in IL-6, which is a liver stimulant for the synthesis of CRP, and it has been reported that it increases up to 100-fold after exercise, which creates in response to muscle damage or tissue infections. It appears that the type of exercise, the intensity and duration of the exercise are effective on changes in CRP among athletes. The findings of this research showed that serum levels of inflammatory cytokines are different in sprinter athletes than non-athletes. Due to the fact that exercise protocol variables are effective on these changes and sprinters are no exception, it is therefore recommended to trainers to adopt the necessary measures to strengthen the immune system when designing intense and painful exercises.

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