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The effect of one session intense anaerobic exercise (Bruce test) on serum level of IL-6 and IL-33 in volybalist athletes

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ABSTRACT

Sports activities can affect many components and functions of the immune system. The purpose of this study is to compare the effect of sports activities on serum level of interleukin 6 and 33 on athletes. In this study, exhaustive sports activity was carried out by 30 volleyball athletes, (each group 15 people) using aerobic exercise (Bruce test) by treadmill. Blood samples were taken before, after and 2 hours after doing sports exercise. IL-33 and IL-6 serum levels of peripheral blood were measured by ELISA (Bioscience-America. For data analysis, repeated measurements and one way variance tests were used. Serum level of interleukin 6 and 33 had a significant increase after exercise than before exercise in both male and female groups ($P<0.01$). On the other hand the level of these two interleukins had a significant decrease after exercise than 2 hours after exercise ($P<0.01$). Although the level of interleukin 6 and 33 in men were higher than female after exercise but this difference was not significant ($P>0.01$). Physical activities had effect on the function of immune system of athletes and it seems that these activities lead to increase the serum level of interleukin 6 and 33.

Key words: Intense aerobic activity, interleukin 6, interleukin 33

INTRODUCTION

Sports activities can affect many of components and functions of immune system [1]. Recently exercise immunology studies focused on main components of immune functions like immune cells, Immunoglobulins, glutamine and soluble messenger molecules (cytokines) and the effect of environmental factors, nutrition [2] and exercise [4 , 5]. Among the different components of immune system, cytokines are form of soluble factors in this system.

Cytokines, are peptides or proteins that produce and release by the cells of immune system and mediate generating of immune responses. In the general case, cytokines are divided in two major groups, pre and anti-inflammatory. Pre inflammatory cytokines are involved in creating and progression of inflammation. IL-6, IL-18, IL-1β and TNF-α are pro inflammatory cytokines. Anti-inflammatory cytokines are secreted in response to inflammation and limiting factors and reserve inflammation progressive process. Cytokines like interleukins 5, 13, 3, 4, 10, 6 are allocated in this group of cytokines that release from immune cells. Interleukin 6 is a cytokine that shows has pre and anti-inflammatory effects [6].

The main sources of IL-6 serum are activated macrophages, fibroblasts and endothelial cells. However, other cell sources are also known for IL-6 that are included as neutrophils, eosinophils, B and T lymphocytes, osteoblasts, keratinocytes and monocytes [7, 6].

In exercise production of IL-6 in response to contraction of skeletal muscle, has been reported [8]. But it is not clear what type of cell in the muscle is responsible for production of IL-6. However myoblasts have the capacity of IL-6 production. In special occasions, endothelial cells, fibroblasts and also smooth muscle cells produce IL-6 [7].

In 2008, Handschin and Espigmen showed that skeletal muscles have the capacity to express several cytokines including, interleukin 8, 6 and 15 that in overall names called myokine and these myokines cause facilitating several cellular responses to exercise like suppression of proteolysis, angiogenesis and regulation of muscle glycogen [9]. Increase in the circulating concentration of pre inflammatory cytokines like IL-1β and TNF-a anti-inflammatory like IL-6, IL-10 after endurance exercise has been reported [6, 10]. Serum concentrations of IL-6 increases during muscular activity [11] which may reach up to 100 times than its base.

Ali Nejad and his colleagues in a study in 1388 showed that increase more levels of IL-10 and IL-6 has been associated with active recovery after extroverted exercise in comparison with passive recovery to anti-inflammatory effects of IL-6 [12, 13]. While exercising, skeletal muscles contraction causes release of IL-6 from muscles to the blood stream [7, 8]. There is a hypothecs that IL -6 production from muscle cells has metabolic roles. IL-6 responses may indicate a critical reduction of muscle glycogen stores and greater reliance of skeletal muscle on glucose as an energy source. IL-33 is a new member of IL-1 family that its biological action is similar to IL-1β and IL-18 as regulators of immune and inflammatory responses and this cause increase production of Th2-type cytokines and immunoglobulins [15]. IL-33 is expressed widely in various organs. IL-33 expression in these organs is limited to certain types of epithelial cells in the bronchi, fibroblasts and smooth muscle cells [16].

As the role of IL-33 activity has not been examined in athletes, so the aim of this study was to investigate serum level of this cytokine and IL-6 in athlete individuals and also after intense exhaustive activity (Bruce test).

MATERIALS AND METHODS

Method of study:
The method of present study was semi-experimental. The population study was male and female volleyballist athletes of Zahedan city that were invited purposefully to the study. Among of them, 30 people (each group 15 people) with age average of male: 20±80 and female: 21±50) were chosen as sample.

Training Program:
In this research an aerobic exercise program (Bruce test) by treadmill, was used in two separated sessions for gentlemen and ladies. Training session lasted in 3 hours. Bruce test that was included ten stages, was used to measure aerobic power. In each stage tilt and the speed of treadmill increased, and the time of every stage was taken 3 minutes. At the first, athletes warmed up themselves for 5 minutes, and then they started to running on treadmill. When they could not continue the activity and got tired, training program stopped.

Blood samples before each training session and immediately after each individual test and two hours after testing was collected by specialist and transferred to the laboratory for analyzing. This sampling was carried out using special equipment. To measure the amount of IL-33 and IL-6, ELISA method (eBIOscince Company, America's BENDRMED), was used.

Statistical Analysis:
The obtained results from blood samples were analyzed by using SPSS software version 18. For classification and data adjustment, descriptive statistics method was used. For determination the central index, the average and distribution index of standard deviation was used. To determine the normal data distribution, one side Kolmogorov – Smirnov (KS) method, was used.

For comparing between the results of pre and post tests intergroup, repeated measure test was used and for comparison intergroup together, one way variance analysis test was also used. A significant statistically correlation in this study was considered as ($P \leq 01$).

RESULTS

Physical indices and maximal oxygen consumption (Vo2max) of all studied subjects are shown in table 1.

Table 1: Anthropometric characteristics of all studied subjects

Sex	Male	Female
Age	20/80±2/89	21/50±2/22
Height	182/34±12/04	174/22±9/44
Weight	67/24±10/71	59/35±8/11
BMI	22/07±1/55	21/27±2/19
Vo2max	48/20±4/70	35/0±2/70
Resting beat	85/70±12/04	91/20±22/24
Activity Beat	198/70±4/29	191/80±7/68
Waist circumference	81/80±4/87	73/20±5/26
Maximum time for Bruce test	13/80±1/07	9/40±1/53

Note: Values for each group was expressed as average ± standard deviation

Another finding of this research was a significant increase in serum level of interleukin 6 and 33 in female after training and its decrease after 2 hours of training (P≤01) (table 2).

Table 2: Shows changes in serum level of IL-33 and IL-6 of female individuals before, after and 2 hours after training

Variable	IL-33 (M± SD)	IL-6 (M±SD)
Before training	6.3240±3.22468	13.8340±1.62093
After training	20.4270±9.78768	19.1100±2.12667
2 hours after training	5.7600±1.12318	14.2470±2.88648
P value	000	000

Also another finding of this research was a significant increase is serum level of interleukin 6 and 33 in men after training and its decrease after 2 hours of training (P≤05) (table 3).

Table 3: Shows changes in serum level of IL-33 and IL-6 of male individuals before, after and 2 hours after training

Variable	IL-33 (M±SD)	IL-6 (M±SD)
Before training	5.9830±1.32017	14.4990±0.86969
After training	22.3050±11.49112	23.5380±3.45577
2 hours after training	7.6720±3.72914	13.7290±1.36832
P value	000	000

On the other hand comparing the average serum level of interleukin 6 and 33 between male and female showed that the level of interleukin 6 and 33 in male after sports activity was higher than its amount in female although this difference wasn't significant (Diagram 1 and 2).

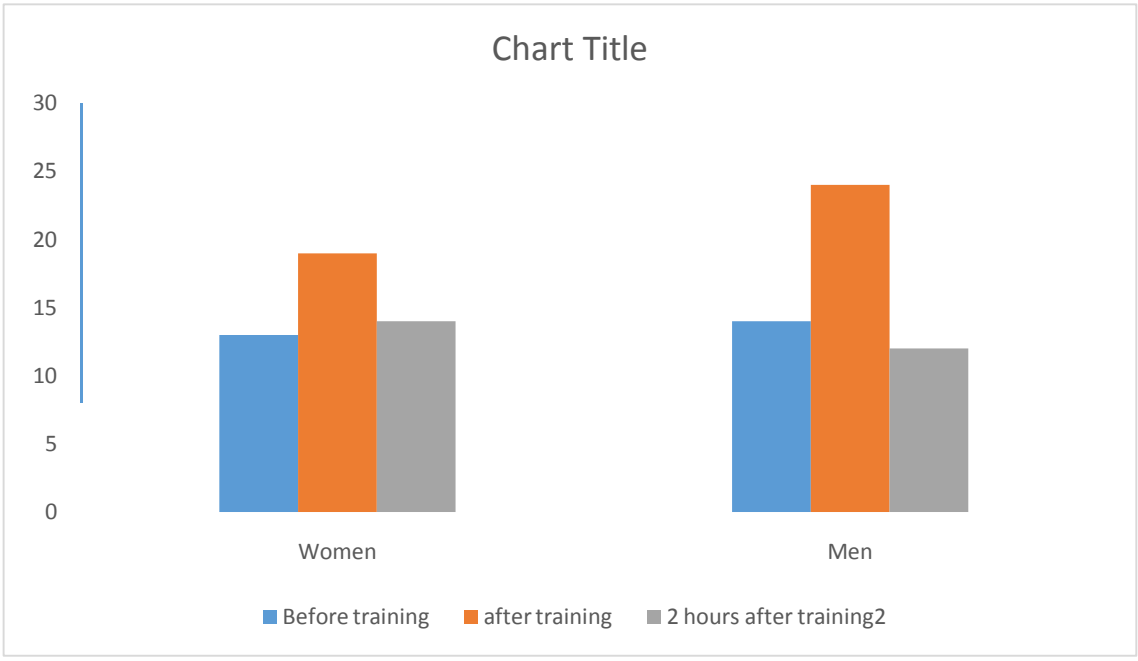

Diagram 1: Shows the serum level of interleukin 6 in men and women before, after and 2 hours after training

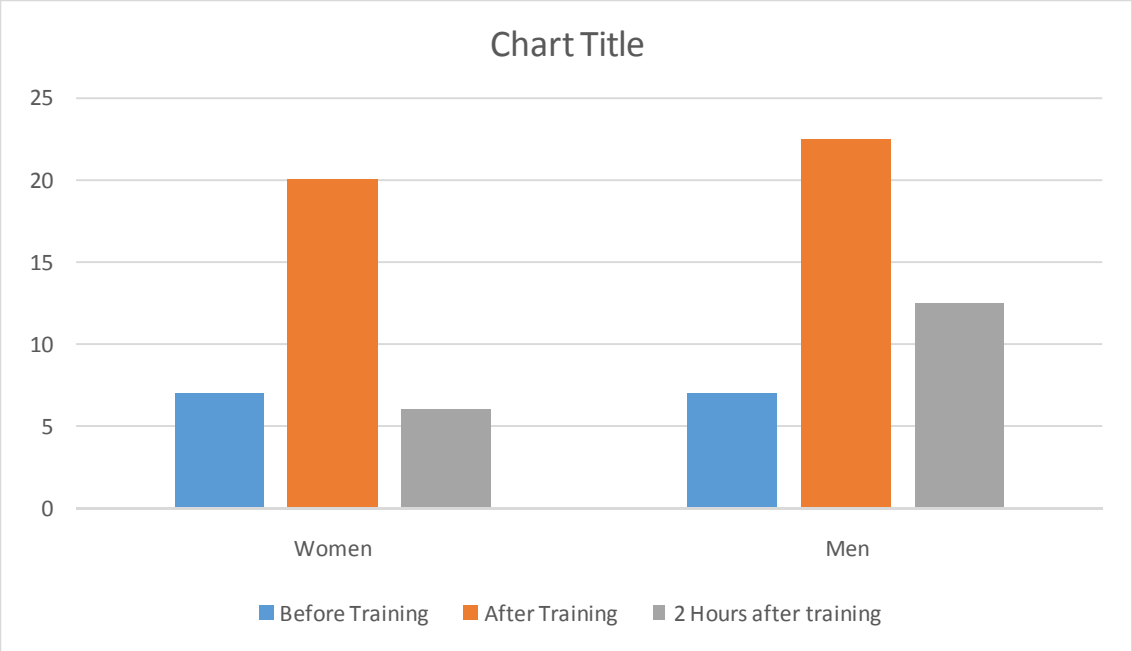

Diagram 2: Shows the serum level of interleukin 33 in men and women before, after and 2 hours after training

DISCUSSION

One of the results of this study is a significant increases in the serum level of interleukin 6, after sports activity than before.

Consecutive measurements of IL-6 during exercise have been shown that rapid increase level of IL-6 serum is more exponentially. In addition, peak of serum at the end of exercise or a little after one, is seen followed by a rapid decline in subsequent periods. Ahmadi et al (1388) have reported that the level of IL-6 and kreatine kinase after two kinds of introverted and extroverted exercise with the intensity of 70 to 75% of introverted heart beat significant increase in the serum level of IL-6 followed by both types of activities. They also revealed that, increase level of serum IL-6 has been dependent on the duration and the intensity of activity, involved muscle mass and endurance capacity. Response of serum IL-6 was more sensitive to the intensity of exercise [17]. Even if, Fischer in 2006 showed that the duration of exercise was most important factor in increasing capacity of this cytokines after exercise [18].

Recently this theory has been proposed that, contraction can cause transcription of IL-6 gene by calcium released from sarchoplasmy network terminal tank and thus led to increase the transcription of IL-6 gene [19]. However, the mediation of this process is not well understood. Study in human samples showed that the production of nitric oxide in contracting skeletal muscles is the key regulator of pre translation signaling pathway that causes the production of muscular IL-6 [20].

Protein combine NF-kB, is one of the most important active signaling pathways while eccentric exercise that may be a probable mediator of synthesized muscular IL-6 [21]. This theory has been proposed while doing long term exercise, decrease activation of content of muscle glycogen P38, MAPK and actives transcription of IL-6 in the working muscles. In addition, decrease in content of glycogen inside cell, causes increase of P38 MAPK phosphorylation in cores [22].

IL-6 cytokine production during exercise may play a role in providing energy by activating the lipolysis in adipose tissue [24]. Released cytokines from muscles, are separated differently from pre inflammatory process and take part as anti-inflammatory processes instead of inflammatory processes [25]. Researchers believe that the level of serum IL-6 can simulate the adrenaline- pituitary- hypothalamus axis and increases secretion of cortisol that has anti-inflammatory effects [26].

The serum level of interleukin 33 in athletes has not been investigated yet. So in this study, for the first time we showed significant increase of this cytokine after exercise. Connecting IL-33 to its receptor leads to the start of IRAK 4, IRAK1 and MyD88 in the receptor complex and causes activation of NF-KbIkBα and MAP kinase and

causes cytokines release of IL- 3, IL-5, IL-6, IL-13 and calcium [16] that this calcium release causes release of histamine to the tissue and increases the production of acid nitric from macrophages [27]. So it can be said that one of the reason for increasing interleukin 6, is due to the increase of interleukin 33.

Also in this study, serum level of interleukin 6 and 33 in men was shown higher after sports activity but this increase wasn't significant. Serum concentration of IL-6 increases during muscular activity [11] that may reach up to 100 times than its base level. On the other hand this theory exists that released IL-6 from muscle has metabolic roles. IL-6 response may indicate a critical reduction of muscular glycogen stores and greater reliance of skeletal muscles on blood glucose as an energy source. The finding of a lot of studies has pointed to the role of release of IL-6 from muscular muscle in metabolism [8].

On the other hand we know the differences in the level of interleukin 6 and 33 that is due to the involvement of sex hormone secretion and this can affect mechanisms of immune system [28]. It is possible that observed sex difference in our study, was due to modulated innate immune response by sex hormones. Sex hormones including estrogen can affect the activity of natural killer (NK) cells and macrophages. Also the difference between the secretion of cytokines in individuals can be emanated from different level of sex hormones and distribution and proportion of fat mass in body because adipose tissues, secretes inflammatory markers that is accompanied with blood pressure, syndrome metabolic and insulin resistance. The main mechanisms for sex differences is related with fat distribution in body and this can be difference in mobility, oxidation, storage of fatty acids among men and women [28]. Also the studies of Diodato and colleagues (2001) showed that the amount of pre inflammatory cytokines like IL-6 and TNF-α in men is higher than women [29].

So the result of this study showed that increase level of interleukin 6 and 33 after sports activity in men is probably emanated from more size of muscular mass, increase of metabolic activity during sports activity and also the effect of sex hormones.

CONCLUSION

Our results showed that the serum level of interleukin 6 and 33 after training had a significant difference than before training and 2 hours after training but a significant difference in the level of these two interleukins in men and women didn't observe.

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REFERENCES

[1] Agha Alinejad H., *Olympic Journal*, **1998**, 11: 3- 18.
[2] Ashtarani B, Agha Alinejad H, Gharakhanlou R, Rajabi H, Rajabi Z, Kardar Gh. *Olympic Journal*, **2003**, 29: 41-54.
[3] Agha Alinejad H, Sarafnejad A, Gharakhanlou R, Memari A, Mirshafie A, Nikbin B. *Olympic Journal*, **2001**, 22: 83-7.
[4] Rajabi Z, Agha Alinejad H, Salami F, Ashtarani B, Shahsavani M, Saghafi Sh. *Olympic Journal*, **2005**, 32:31-40.
[5] Farzanegi P, Azarbayjani M.A, Resai M.G, Agha Alinejad H. *Movement Journal*, **2006**, 29: 57-69.
[6] Gleeson M, editor. *Philadelphia: ELSEVIER*; **2006**.
[7] Yan SF, Tritto I, Pinsky D, Liao H, Huang J, Fuller G, et al. *J Biol Chem*. **1995**.
[8] Pedersen BK, Akerstrom TC, Nielsen AR, Fischer CP. *J Appl. Physiol.* **2007**, 103:1093-8.
[9] Handschin C, Spiegelman BM. *Nature*, **2008**, 454: 463-9.
[10] Mogharnasi M, Gaeini A.A,Sheikholeslami Vatani D. *Journal of Endocrinology and Metabolism*, **2009**, 11: 191-198.
[11] Cappelli, K, Felicetti M, Capomaccio S, Pieramati C, Silvestrelli M, Verini-Supplizi A. *BMC Physiol*, **2009**, 24; 9: 12.
[12] Agha Alinejad H, Molanouri Shamsi M. Azarbayjani M.A, Asghari Jafarabadi M, Tofighi L.S, Mirani SM. *Iranian Journal of Endocrinology and Metabolism*, In press, **2009**.
[13] Suzuki K, Nakaji S, Yamada M, Totsuka M, Sato K, Sugawara K, *Cytokine kinetics. Exerc Immunol Rev*, **2002**, 8: 6-48.
[14] Steensberg A, van Hall G, Osada T, Sacchetti M, Saltin B, Klarlund Pedersen B. *J Physiol*, **2000**, 529 (Pt 1):237-42.
[15] Küchler, A.M., Pollheimer, J., Balogh, J., Sponheim, J., *The American Journal of Pathology*, **2008**, Vol. 173, pp. 1229-1242.

[16] Akdis, M., Burgler, S., Crameri, R., Eiwegger, T., *J Allergy Clin Immunol*, **2011**, Vol. 127, pp. 701-721.
[17] Ahmadi A, Agha Alinezhad H, Gharakhanlou R, Zarifi A. *Olympic Journal*, **2009**, 17: 63-72.
[18] Steensberg A, Fischer CP, Keller C, Moller K, Pedersen BK. *Am J Physiol Endocrinol Metab*, **2003**, 285: E433-7.
[19] Banzet S, Koulmann N, Sanchez H, Serrurier B, Peinnequin A, Alonso A, *J Cell Physiol*, **2007**, 210: 596-601 .
[20] Al-Khalili L, Bouzakri K, Glund S, Lonnqvist F, Koistinen HA, Krook A. *Mol Endocrinol*, **2006**, 20 : 75-3364.
[21] Pritts TA, Hungness ES, Hershko DD, Robb BW, Sun X, Luo GJ, *Am J Physiol Regul Integr Comp Physiol*, **2002**, 282: R1016-26.
[22] Chan MH, McGee SL, Watt MJ, Hargreaves M, Febbraio MA. *FASEB J.* **2004**, 18:1785-7.
[23] Leveille SG, Guralnik JM, Hochberg M, Hirsch R, Ferrucci L, Langlois J, *J Gerontol A Biol Sci Med Sci*, **1999**, 54:M487-93.
[24] Yan SF, Tritto I, Pinsky D, Liao H, Huang J, Fuller G, *J. Biol. Chem.* **1995**, 270:11463-71.
[25] Steensberg A, Fischer CP, Keller C, Moller K, Pedersen BK. *Am. J. Physiol. Endocrinol. Metab.* **2003**, 285:E433-7.
[26] Steensberg A, Toft AD, Bruunsgaard H, Sandmand M, Halkjaer-Kristensen J, Pedersen Bk. *J. Appl. Physiol.* **2001**, 91:1708-12.
[27] Valent, P., A regulator of basophils, Blood, **2009**, 113(7): 1396-1397.
[28] Andreas, T., Kristina, M., Hans-Jörg, G., Uwe, S., Andreas, H. and Dieter, L., *Critical. Care*, **2001**, 5(6): 343-348.
[29] Diodato, M.D., Knoferl, M.W., Schwacha, M.G., Bland, K.I., Chaudry, I.H., *Cytokine*, **2001**, No. 14, p.p. 162–169.