

Full Length Research Paper

Some cellular immune components and C-reactive protein monitoring in female handball players during a competitive period

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The aim of the study was to determine the effect of the competitive period training on leukocytes, some leukocyte subgroups and C-reactive protein (CRP) in sportswomen playing in the professional handball league. The average age of the group was 20.6 ± 3.37 years, height average was 163 ± 6.30 cm, weight average was 58.73 ± 4.92 kg. Blood samples were taken before and after the training period of the competition. Subjects rested for 48 h until taking blood samples. Subsequently, leukocytes, some leukocyte subgroups and CRP were determined. Leukocytes and leukocyte subgroups were determined using a Roche Sismex 2000XL device. CRP was determined using Beckman immunochemistry systems *in-vitro* diagnostic kits and nephelometric methods (Beckman Coulter Array 360 System, USA). Statistical analysis was performed using a paired t-test. p values <0.05 were considered statistically significant. In this study no significant difference was found on WBC and granulocyte values between pre and post training period. However, significant differences in lymphocyte values were measured. After training period, lymphocytes declined ($p < 0.05$), but no significant differences in monocyte levels were determined. CRP values after the training period were significantly decreased ($p < 0.05$), as compared to pre training values. We can conclude that competition period training did not significantly affect the cellular immune elements, but caused a significant decrease on CRP values, which are markers in chronic inflammation.

Key words: Training, immune component, C-reactive protein, female athlete.

INTRODUCTION

Intense exercise is one of the major stresses exposed to organisms. Organisms respond to stress via a set of physiological changes in its metabolic, hormonal and immunological systems. Optimum intensive regular exercise has been reported to strengthen the immune system (Özdengül et al., 1999). There are some studies showing that the function of immune system is restrained in spite of intensive training period and intense exercise (Gleeson, 2002). Some studies reported that long-term exercise reduces infection rates. (Nieman et al., 1990). Some animal studies have reported that chronic exercise can prevent or slow down the spread or progress of experimental tumors and provide a protective effect

against infection during exercise in animals (Shephard et al., 1995). Throughout the training period, the amount of leukocytes was between the normal rates at rest (Hooper et al., 1993) clinically, whilst during the intensive endurance exercise period, the amount of leukocytes decreased (Mackinnon et al., 1997). This situation is associated with overtraining and usually emerged after repeated intensive exercise programs (Lakier, 2003). Besides, some studies suggested that, exercise leads to an increase in the amount of leukocytes (Brian et al., 2003).

Furthermore, the increase in the number of circulating leukocytes in the circulation pool may result from demargination together with the increased blood flow by acute exercise. Demargination with the effects of stress hormones, by a reduction in the endothelial wall leukocyte accumulation, increases the total number of

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leukocytes in blood (Pyne, 1994). Systemic inflammation gives rise to an increase in inflammation markers. C-reactive protein (CRP) is an acute phase reactant that reflects low-grade systemic inflammation. It is a commonly used inflammation marker owing to its advantages such as cost-effectiveness, ease in application, rate of being affected by the environmental factors, ease of storage, not being affected by diet and long half life (Rifai et al., 2001, Kondo et al., 2005).

Previous studies indicated that, skeleton muscle produces interleukin-6 (IL- 6), inflammatory protein and the biological sign of CRP excessively, in order to help organize homeostasis during the exercise period. This increase may have resulted from intensive exercise or a significant mechanical load on the muscle. It is informed that regular exercise changes this response, related to its strength or intensity. (Noriyasu et al., 2005; King et al., 2003).

Since competition period trainings are intensive and exhausting training periods, thanks to the competitions required maximal load, in addition to the preparatory trainings. Considering this fact, studying the effects of the competition period training on systematical inflammation and some immunity markers of the athletes, has an important role to ensure the effectiveness of the training period and to prevent overtraining. This study investigated the changes which occurred on the aforementioned parameters, belonging to the 15 women athletes playing in the professional female handball league during the 8-week competition training period.

The aim of this study was to investigate changes which occurred on some cellular immune components and C - reactive protein in female handball players during a competitive period

METHODS

Subjects in this study were fifteen volunteer female athletes, chosen from the handball team of Kayseri Erciyes University in 1st League. The competitive training program included mainly technical-tactical stages and general condition training throughout 12 weeks. The average age of subjects was 20.6 ± 3.37 years, height average was 163 ± 6.30 cm, weight average was 58.73 ± 4.92 kg. The blood parameters of the subjects were drawn from the arm veins and leukocyte levels ($\times 10^3/\mu\text{l}$) were measured. The whole blood parameters were determined using Roshe Sismex 2000 XL kit and device. Blood samples collected for CRP were put into the biochemistry tubes and centrifuged at 3.000 rpm for 5 min. Serum and plasma were determined by Beckman Immunochemistry Systems CRP PN *in vitro* diagnostic kit, using nephelometric method (Beckman Coulter Array 360 System, USA). Data were statistically tested using the t-test for paired data in SPSS for Windows. Kolmogorov-Smirnov test was used to test normality of data. The alpha level was set at 0.05 for all analyses.

RESULTS

It was found that, a significant decrease of lymphocyte

and C – reactive protein occurred after the training period ($p < 0.05$). But the changes of the remaining data were not statically significant ($p > 0.05$).

DISCUSSION

Leukocytes have an active role especially on exercise-related inflammations. Three important mechanisms take place in this change. The initial one is the change on the leukocyte concentration. The second one is the filtration of the leukocytes to the damaged tissue, and the last one is the functional change of the leukocytes. The functional change of the leukocytes after exercise is basically focused on the phagocytic activities of neutrophils and the cytokine production capability of neutrophils (Mackinnon et al., 2000). In the study, no significant change was determined on leukocyte, monocyte and granulocyte levels, whereas there was a relatively significant decrease on the lymphocyte levels in the post-training period. Some studies in the literature showed that, exercise causes an increase on the immunity markers (Miles et al., 2002; Gleson, 2007). The common opinion is that, exercise increases the leukocyte level related to its difficulty and intensity. In general, during a moderate exercise, immunity cells are stimulated as a result of chemical toxin, oxidative burst and phagocytic conditions (Brines et al., 1996). Increase on acute exercise is explained by some mechanisms. The first is the joining of the immune component in the margination pool to the circulation, by means of demargination, thanks to increased amount of epinephrine by exercise (Pyne, 1994).

The other mechanism is the acceleration of immune component release in the bone marrow from the storage pool, as a result of exercise-induced stress, muscle damage and heat increase (Severs et al., 1996). In some studies, it was mentioned that at the end of the exercise training period, the immunity values had normal levels (Gleeson, 2007). This result is explained by the training level. Stress level during the first moments of the exercise is lessened in some time and after enough time it disappears. Not finding a significant change in the leukocyte levels in the study can be explained through this fact. C-reactive protein is one of the acute phase reactants, indicating low grade systemic inflammation and is secreted from the liver (Rifai and Ridker, 2001)

Previous studies indicated that, skeleton muscle produces interleukin-6 (IL- 6), inflammatory protein and the biological sign of CRP excessively, in order to help organize homeostasis during the exercise period. This increase may have resulted from dense exercise or a significant mechanical press on the muscle. It is informed that regular exercise changes this response related to its strength or intensity (Noriyasu et al., 2005; King et al., 2003).

In this study, it was found that the competitive training

Table 1. Leucocytes and CRP value.

Variables		N	Mean	SD	t	p
WBC(x10 ³ /μl)	Before training period	15	6.765	1.231	-1.391	0.186
	After training period	15	7.111	1.433		
MO (x10 ³ /μl)	Before training period	15	0.500	0.101	0.523	0.609
	After training period	15	0.477	0.177		
MO %	Before training period	15	6.537	1.044	0.242	0.816
	After training period	15	6.345	2.420		
GR (x10 ³ /μl)	Before training period	15	3.793	1.035	-1.885	0.056
	After training period	15	4.190	1.197		
GR %	Before training period	15	54.112	7.675	-2.074	0.077
	After training period	15	59.325	4.947		
LY (x10 ³ /μl)	Before training period	15	2.691	0.492	2.648	0.019*
	After training period	15	2.462	0.401		
LY %	Before training period	15	39.600	6.827	1.848	0.107
	After training period	15	34.237	4.357		
CRP (mg/dl)	Before training period	15	0.661	0.784	2.272	0.039*
	After training period	15	0.316	0.212		

*p < 0.05. WBC: White blood cell. MO: Monocyte GR: Granulocyte, LY: Lymphocyte, CRP. C - reactive protein.

period causes a significant decline on the CRP level, in that regular exercise decreases basal CRP concentration. Though this mechanism was not clarified exactly, it was asserted to have caused the effects of exercise on TNF and IL-6 mechanism (Church et al., 2002). However, there is a close relationship between CRP and cytokine concentration. While CRP synthesis from the liver is intensively carried out by IL-6 (Yudkin et al., 2002), TNF induces IL-6 (Mackiewicz et al., 1991). It is a general view that CRP increases are related to the cytokine production, with the inflammatory effect emerged at a single exercise declines, thanks to the antiinflammatory effect resulted from regular exercise (Aronson et al., 2004; Petersen and Pedersen, 2005). In this research, the decrease on CRP values after training period can be explained through this view.

In conclusion, the competition period training, significantly decreased both lymphocyte and CRP values (Table 1). This study demonstrated that high level handball players are used to intensive training and training processes including competitions and during the process chronic inflammation were not observed.

REFERENCES

Özdengül F, Uysal H, Gökbel H, Çelik , Altıdı M (1999). Akut

Submaksimal Egzersizin mmün Sisteme Etkisi., Genel Tıp Dergisi., 9(3): 99-104.

Gleeson M (2002). Biochemical and Immunological Markers of Over Training, J. Sports Sci. Med., 1: 31-41.

Nieman DC, Nehlsen-Cannarella SL, Markoff PA (1990). The Effects of Moderate Exercise Training on Natural Killers Cells and Acute Upper Respiratory Tract Infections. Int. J. Sports Med., 11: 467-473.

Shephard RJ, Kavanagh T, Mertens DJ, Qureshi S, Clark M (1995). Personal Health Benefits of Masters Athletics Competition. Br. J. Sports Med., 29: 35-40.

Hooper SL, MacKinnon LT, Gordon RD, Bachmann AW (1993). Hormonal Responses of Elite Swimmers to Overtraining. Med. Sci. Sports Exerc. Jun., 25(6): 741-747.

Mackinnon LT, Hooper SL, Jones S, Gordon RD, Bachmann AW (1997). Hormonal, Immunological, and Hematological Responses to Intensified Training in Elite Swimmers. Med. Sci. Sports Exerc., 29(12): 1637-1645.

Lakier Smith L (2003). Overtraining, Excessive Exercise and Altered Immunity: Is This a T Helper-1 Versus T Helper-2 Lymphocyte Response? Sports Med., 33(5): 347-364.

Brian K, McFarlin JB, Michel MA, Steinhoff GM (2003). Repeated Endurance Exercise Affects Leukocyte Number But Not NK Cell Activity, Medicine And Science In Sports And Exercise, 35(7): 1130-1138.

Pyne DB (1994). Regulation of Neutrophil Function During Exercise., Sports Med., 17: 245-258.

Rifai N, Ridker PM (2001). High-sensitivity C-reactive protein: a Novel and Promising Marker of Coronary Heart Disease. Clin Chem., 47(3): 403-411.

Kondo N, Nomura M, Nakaya Y, Ito S, Ohguro T (2005). Association of Inflammatory Marker and Highly Sensitive C-Reactive Protein With Aerobic Exercise Capacity, Maximum Oxygen Uptake and Insulin

- Resistance in Healthy Middle-Aged Volunteers *Circulation J.*, 69(4): 452-457.
- King DE, Carek P, Mainous AG 3rd, Pearson WS (2003). Inflammatory Markers and Exercise: Differences Related to Exercise Type. *Med. Sci. Sports Exerc.*, 35(4): 575-581.
- Mackinnon LT, Hooper SL (2000). Overtraining and Overreaching: Causes, Effects and Prevention. *Exercise And Sport Science* (William E., Garrett JR., Ed.) USA, p. 492.
- Miles MP, Kraemer WJ, Grove DS, Leach SK, Dohi K, Bush JA, Marx JO, Nindl BC, Volek JS, Mastro AM (2002). Effects of Resistance Training On Resting Immune Parameters in Women. *Eur. J. Appl. Physiol.*, 87(6): 506-508.
- Gleeson M (2007). Immune Function in Sport and Exercise. *J. Appl. Physiol.*, 103(2): 693-699.
- Brines R, Hoffman-Goetz L, Pedersen BK (1996). Can You Exercise to Make Your Immune System Fitter? *Immunol Today*, 17: 252-254.
- Severs Y, Brenner I, Shek PN, Shephard RJ (1996). Effects of Heat And Intermittent Exercise On Leukocyte And Sub-Population Cell Counts. *Eur. J. Appl. Physiol. Occup. Physiol.*, 74(3): 234-245.
- Church TS, Barlow CE, Earnest CP, Kampert JB, Priest EL, Blair SN (2002). Associations Between Cardiorespiratory Fitness and C-Reactive Protein in Men. *Arterioscler. Thromb. Vasc. Biol.*, 22(11): 1869-1876.
- Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW (1999). C - reactive protein In Healthy Subjects: Associations with Obesity, Insulin Resistance And Endothelial Dysfunction: A Potential Role for Cytokines Originating from Adipose Tissue? *Arterioscler Thromb. Vasc. Biol.*, 19(4): 972-978.
- Mackiewicz A, Speroff T, Ganapathi MK, Kusner I (1991). Effects of Cytokine Combination On Acute Protein Production In Two Human Hepatoma Cell Lines. *J. Immunol.*, 146: 3032-3037.
- Aronson D, Sheikh-Ahmad M, Avizohar O, Kerner A, Sella R, Bartha P, Markiewicz W, Levy Y, Brook G (2004). C-Reactive protein Is Inversely Related To Physical Fitness In Middle-Aged Subjects. *Atherosclerosis*, 176(1): 173-179.
- Petersen AM, Pedersen BK (2005). The Anti -Inflammatory Effect of Exercise. *J. Appl. Physiol.*, 98(4): 1154-1162.