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Original Article

Comparison of the acute effects of an incremental exhaustive aerobic exercise session by upper-body and lower-body on the NK and T cells response

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Abstract
The purpose of the present study was to compare the acute effects of an incremental exhaustive aerobic exercise session by arm and leg on the response of NK (CD16, CD56 and CD16/CD56) and T (CD4, CD8 and CD4/CD8) immune cells of athlete male students. Twenty male students with mean age 22.4±1.8 years, maximal oxygen uptake 41.7±71 $ml.kg^{-1}.min^{-1}$, BMI 23.3±1.87 kg/m^2 and BF% 19.83±3.3 selected and randomly assigned into two experimental (n=10) and control (n=10) groups. Experimental group carried out two incremental exhaustive aerobic protocols by foot and hands on ergometer cycles in two different days. The control group didn't participate in any physical activity program. Blood samples of both experimental and control groups taken before, immediately after and two hours after the end of the exercise tests then collected and evaluated by Flowcytometry analysis. Paired t-test, independent t-test, ANOVA, Tukey and HSD tests at significant level of 0.05 were used to statistical analyses. The results didn't show any significant differences between the two types of training protocols in response of NK and T cells in both arm and leg, so that both protocols caused significant increasing in CD8, CD16/56 cells, significant decreasing in CD4 and CD4/CD8 ratio and no significant change in CD16 and CD56 cells.
Key words: arm and leg exhaustive aerobic exercise, immune cells

Introduction

In the last two decades several studies have been shown that sport activities result in significant physiological changes on the immune system. The interaction effects between exercise pressures and immune system have provided unique opportunity to link between basic physiology and clinical physiology and also evaluation the hidden roles of these underlying pressures and immunological mechanisms. It has been suggested that physical activities provide an evaluable pattern of physical pressures [1,2].
Studies have been shown that regular exercise can increase infectious diseases tolerance [3,4,5]. However, epidemiologic data show that elite endurance athletes compared with their inactive counterparts, are more exposed to upper respiratory tract infections (URTI) two weeks after intense exercise or competition [5,6,7]. This relationship between level of physical activity and risk of URTI is known as a J-shaped curve [8], suggests that the risk of URTI in those who participate in regular exercise with moderate intensity may be lesser compared to inactive individuals, while the amount of excessive severe exercise training may increase URTI risks [5].

However, the effects of exercise on infections and immune system is not fully known, but it can be said that exercise temporarily affects various immune system parameters including leukocytes [9], leukocyte subsets, lymphocytes, T and NK cells [10]. It has been shown that following intense exercise, blood lymphocytes levels drops into below normal levels, and duration of this suppression depends on exercise intensity and duration [1]. The exercise-induced mobilization of leukocyte subsets is related to increased plasma concentrations of hormones [5]. In connection with the exercise on lymphocytic subsets, Vander Pompe et al (2001) a showed significant increasing in NK (CD16/56), CD4 and CD8 cells after a graded exercise in women after menopause period [11].

Ramel, et al (2003) showed that submaximal resistance exercise with 75% intensity of a maximum repetition, leads to a significant decreasing in CD4 and CD4/CD8 cells ratio in resistance trained male athletes than untrained resistance males [12]. Also, Scharhag et al (2005) found a significant reduction in NK cells following 4 hours pedaling at 75% of intensity anaerobic threshold [13]. In another study, Buyukyazi et al (2004) investigated effect of prolonged exercise training with different intensity and duration in 12 elite male athletes (high exercise intensity and duration), 11 recreational male athletes (moderate exercise intensity and duration) and 12 inactive men and showed that the percentage of NK, CD4, CD8 and CD4/CD8 ratio cells in the three groups did not change significantly [14].

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Since, intense sports activities can change severely immune system factors and sometimes causing falling in immune function and because so far in comparison the effect of exercise training by hand and foot on the performance of the immune system, few researches has been done, so the aim of the present study was to comparing the acute effects of an incremental exhaustive aerobic exercise session by hand and foot on the response of NK and T cells in athletes male students.

Methodology

Participants: Twenty male participants (mean age 23.49±1.8 years, mean VO_{2max} 41.75± 7.1 $ml.kg^{-1}.min^{-1}$, mean BMI 23.36±1.8 kg/m^2.mean BF% 19.8±3.3%) were selected and were randomly assigned into two experimental (n=10) and control (n=10) groups.

Measurements: Astrand test was used to measuring participants' Vo_{2max}. For measuring BMI and BF% a Body Composition Analyzer apparatus, model Olympia by Jawon Company in Korea was used. Measurement of lymphocytic subsets (CD4, CD8, CD16, CD56 and CD16/56) with flowcytometry method was performed

Methods: In this study Astrand protocol [15] was used to the lower-body testing and the *Sawka* et al protocol [16] was used for the upper-body testing. All the participants were asked to avoiding any exercise training or medication for two days before performing the tests. To performing both tests, at first the height of the ergometer cycles were set according to participants' height. Afterwards, the participants were asked to stabilize the place on the chair and ready to begin their training program.

The method was similar for both arm and leg protocols at starting in this way that first 2 minutes the participants began to worm ping with zero watt loads. After warming, the initial load of 50 watts for leg protocol and 25 watts for arm protocol were considered. Afterward, for both protocols, 25 W were added every 2 minutes, and the pedaling rate speed was considered 50 rpm. The participants would continue to pedaling until they would reach at exhaustion or they cannot maintain the speed of 50 rpm for 15 seconds. At this time, the test was ended and the time was recorded.

Statistical analyses: Paired samples t-tests were used for determining within-group differences and independent t-test was used for assessing between-groups differences. One way analyze of variance and followed up Tuky (HSD) testes were used to comparing means between three groups. Data are shown as mean and standard deviation. Values of $p<0.05$ were found significant. Statistical analyses were performed using the SPSS version 17 for Windows.

Results

the results are as follows:An incremental exhaustive aerobic exercise session by arms and legs has no significant effect on CD16 and CD56 cells.There aren't any significant differences between effects of an incremental exhaustive aerobic exercise session by arms and legs on CD16/56, CD4, CD8 cells and CD4/CD8 ratio responses .

Table1. Mean and standard deviation of NK and CD cells at different phases of exhaustive aerobic exercise by arm and leg.

	Phase	Before		After		2hours after	
Variable	Group	n	M± SD	n	M± SD	n	M± SD
CD4	Leg	10	44.84±9.36	10	35.3±6.56	10	45.79±5.15
	Arm	10	45.03±6.04	10	33.86±6.04	10	45.08±8
	Control	10	49.59±9.69	10	47.85±9.41	10	48.12±9.13
CD8	Leg	10	34.5±5.24	10	35.3±6.56	10	34.57±4.62
	Arm	10	34.43±5.44	10	33.86±6.04	10	32.99±6.03
	Control	10	31.81±3.37	10	47.85±9.41	10	33.08±4.31
CD16	Leg	10	3.78±2.54	10	6.01±5.48	10	4.23±3.14
	Arm	10	3.61±2.07	10	4.63±3.23	10	3.04±1.31
	Control	10	3.77±4.74	10	4.59±5.21	10	5.42±5.68
CD56	Leg	10	3.96±1.77	10	3.87±2.02	10	3.33±1.16
	Arm	10	3.44±1.53	10	4.01±2.48	10	3.54±1.71
	Control	10	2.64±1.56	10	3.16±1.96	10	3.69±2.37
CD16/CD56	Leg	10	11.51±6.18	10	22.12±10.56	10	11.13±5.21
	Arm	10	13.17±6.50	10	25.70±11.14	10	13.12±7.35
	Control	10	10.30±3.44	10	9.33±3.07	10	8.37±2.70
CD4/CD8	Leg	10	1.35±0.44	10	0.67±0.27	10	1.35±0.29
	Arm	10	1.35±0.36	10	0.87±0.22	10	1.43±0.47
	Control	10	1.49±0.27	10	1.47±0.27	10	1.46±0.28

Discussion

As was noted in Table 1, the results of both types of exercise training were similar, such that both exercise training protocols increased both cell CD8, CD16/56, reducing cell CD4, CD4/CD8 ratio and had no significant changing in CD16 and CD56 cells. It also was shown that there was no significant difference between both types of exercise training protocols.

CD16 and CD56 cells

NK Cells (CD16 and CD56) are identified but heterogeneity subtypes of lymphocyte cells that are capable to detection and removing infected by virus cells, especially tumor and some microorganisms without previous collision [17]. The results showed that an intensive training session by arms and feet had no significant effect on these cells. These findings are consistent with the results of *Haq* and colleagues [18], but are inconsistent with the findings of *Nielsen* et al [19,20,21], Gabriel and colleagues [22] and Tvede et al [23]. In explaining these results it can be said that no significant changes of these cells may be due to inhibitory effect of some hormones such as cortisol and prostaglandins [24]. Mechanisms associated with hormone cortisol it should be said that released cytokines by the activated T cells may be associated with NK cells mobilization. T cells realize type I of cytokines such as interferon gamma (INF-γ) and interleukin 2 (IL-2) which can increase NK cell levels [25]. Various studies have been shown that cortisol can suppress cytokines of type I of T cells [26] and can show its inhibitory role through this way. Furthermore, reduction of amino acid of Glutamine and Alanine can also lead to inhibition of lymphocyte subtypes [24].

CD16/CD56 cells

These cells are NK or natural killer cells are that involve almost 15% of the peripheral circulation lymphocytes and they have this ability to kill some tumor cells and natural cells are infected with virus [17]. The results showed significant increasing in these cells after exercise in both types of protocol by leg ($P<0.001$) and arm ($P<0.001$). The result of this study are supported by *Timmons* and colleagues [27], *McFarlin* et al [25] and *Gannon* and colleagues [28] findings. Among reasons for this increasing in NK cells it can mention the effect of epinephrine on Beta-adrenergic receptors on surface of these cells [2]. In addition, the secreted cytokines by the type I of T cells, such as INF-γ and IL-2 can also result in this increasing [25]. Furthermore, increasing in blood flow and dehydration [6], increased Beta-endorphins [2] and central body temperature [29] can lead to increasing in the number of NK cells.

CD4 cells

The CD4 (helper T cells) are the most frequent type of T cells and usually they involve three quarters of the all T cells [30]. The results of the present study showed significant reduction in these cells immediately after both types of protocol by leg ($P<0.003$) and arm ($P<0.001$). In this case, *Yukie* et al [10], *Steensberg* [26] and *Nielsen* and colleagues [19,20] achieved similar results. Among possible reasons for these changes can point to reduction in the plasma glutamine concentration after intense exercise [30]. In addition, it has been proven that the immune cells get their energy from glucose metabolism [2]. In this case, it has been suggested that decreased plasma glucose levels following intense exercise leads to increasing in levels of stress hormones and it affects immune function in this way [30]. Also, decreased CD4 cells can due to the effects of cortisol [31], Beta-endorphins and testosterone on these cells.

TCD8 cells

CD8 cells are regarded as the most important defense mechanism against within cell microbes [24]. The results of the present study showed significant increasing in these cells immediately after both types of protocol by leg ($P<0.016$) and arm ($P<0.005$). These results support *McFarlin* et al [25], *Simpson* and colleagues and *Nielsen* and colleagues [19,20] results who have also showed increasing in the CD8 cells immediately after exercise**.** The effect of epinephrine on Beta-adrenergic receptors on surface of CD8 cells may be related to this change-reason . In addition, some cytokines such as IL-1, IL-2 and tumor necrosis factor (TNF-α) can also cause to CD8 increasing [17].

CD4/CD8 ratio

CD4/CD8 ratio is as the clinical indicators of immune disorders [24]. The results showed significant reducing in these cells immediately after both types of protocol by leg ($P<0.002$) and arm ($P<0.002$). This result is consistent with the findings of *Yukie* et al [10] and *Nielsen* and colleagues [21] who have found similar results. Decreasing in CD4/CD8 ratio may be because of inhibitory effect of stress hormones such as cortisol [2], epinephrine [17], prostaglandins [23] and also reduced levels of glutamine and glucose on CD4 cells. On the other hand, some researchers consider further increasing of CD8 cells than CD4 as the reason of reducing in CD4/CD8 ratio [2]. Decreased CD4/CD8 ratio weakened the immune system and can increase susceptibility to infection [12].

As shown in the results of the study, there were no significant differences between the two training protocols by hands and feet in response of NK and T cells. Since the intensity of the exercise is one of the main factors affecting the immune system response to exercise, so, maybe it can be said that in both exercise protocols, the intensity was similar and thus have led to similar response. In general, it can be concluded that a short-term intense exercise session can increase or decrease the responses of NK and T cells of immune system, but this change are temporary and soon dates back to the normal status in recovery period. In addition, the results of this study showed that between short-term intensive training session by arm and by leg, there was no significant difference. Considering these findings, it's recommended to those athletes who need practice more than one session per day that if they kept the appropriate time between the exercise sessions, they will achieve to their training objectives and will not threaten from clinical risks.

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