The Effect of A Single Bout of Aerobic Exercise on Ergometer on Inflammatory and Hormonal Markers in Active Girls

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Abstract

Introduction: Exercise is an important strategy for reducing the risk of chronic disease, and recent research has focused on its role in the improvement of the inflammatory profile. The present study aimed to investigate the effect of a single bout of aerobic exercise on ergometer on inflammatory and hormonal markers in active girls.

Methods: 16 physical education students with average age of 20.25 ±0.9 years, maximal oxygen uptake of 42.14±9.8 ml/kg/m and body mass index of 22.11±2.66 kg/m$^2$ were randomly divided into experimental and control groups. The experimental group pedaled on ergometer with intensity of 75 % $\text{VO}_2\text{max}$ for 60 minutes and the control group had no activity during the test. Blood samples for measuring the levels of IL-10, TNF-α, CRP, cortisol, epinephrine were taken before, immediately and 2 hours after termination of the exercise. The data were analyzed by paired t-test, repeated measure ANOVA and Bonferroni post hoc at P<0.05.

Results: The results showed that IL-10, TNF-α, cortisol and epinephrine levels were not significantly different between experimental and control groups before, immediately and two hours after the end of aerobic exercise (P> 0.05). The mean values of CRP (P=0.002) and cortisol (P=0.001) were higher in the experimental group than the control group. In addition, the mean values of epinephrine in the control group were higher than the experimental group (P=0.009), while no significant difference was observed between the two experimental and control groups in the concentration of IL-10 (P> 0.05).

Conclusion: Based on the findings of study, it seems that an exercise session on an ergometer does not affect hormonal and inflammatory factors in active subjects.

Keywords: Exercise, Hormone- Immune Responses, Active Girls

Introduction

Inflammatory mechanisms play a significant role in the pathological processes of chronic disease (1). The increasing evidence suggests that inflammatory cytokines have a higher sensitivity and accuracy in predicting cardiovascular diseases and playing a critical role in the pathogenesis of atherosclerosis (2). The local release of cytokines such as C-reactive protein (CRP) and interleukin-10 (IL-10), as well as stress hormones (e.g., cortisol and epinephrine), are considered as modulating factors for the reinforcement and modulation of inflammatory cascades (3). In addition, Cytokines contribute to a large number of physiological functions such as bone tissue remodeling, immune regulation, and hematopoiesis, and their blood circulation is associated with various diseases such as atherosclerosis, cardiovascular diseases, and all chronic non-communicable diseases (4-6). Previous studies indicated that low-grade chronic inflammation is involved in atherosclerosis and the high levels of high-sensitivity CRP are a risk factor for coronary artery disease (7). As the most sensitive
inflammatory marker and independent predictor of cardiovascular risk, the increased CRP increases the risk of cardiovascular events by two-five times (8). Tumor necrosis factor alpha (TNF-α) and IL-10 are among the cytokines released from adipose tissue having many biological effects. The people with a high incidence of tumor necrosis factor and CRP are more likely to die from cardiovascular diseases regardless of age, sex, body mass index, and disease history (9). Physical activity is one of the effective interventions in modulating inflammatory mediators (10). Previous studies indicated the anti-inflammatory effect of exercise and physical activity leading to a decrease in the level of pre-arteriosclerotic inflammatory markers (11). Physical activity causes a lot of change in the parameters of the immune function and inflammation while the nature and amount of such changes depends on several factors. In this regard, it was reported that the acute exercise affects response of cytokines and inflammatory factors depend on the duration, severity, and type of exercise (e.g., acute resistance exercise against acute endurance exercise). Acute exercise can influence the immune system (12). Based on the findings of some studies, physical activity can reduce inflammatory markers (13, 14). However, some studies reported no change in inflammatory markers following different exercises (15-18). Exercise can cause major physiological, hormonal, metabolic and immunological changes (19). However, few studies were conducted on the effects of short-term and high-intensity exercise on inflammatory factors and stress hormones. Adipose tissue in women is typically higher than in men which may predispose women to chronic inflammation (20). This issue shows the importance of examining the effect of exercise on inflammatory conditions in women. On the other hand, recognizing the inflammatory and hormonal responses of women to exercise can help design appropriate training programs for this group. However, no study investigated the effects of short-term and high-intensity exercise on inflammatory factors and stress hormones in active women. Based on the above-mentioned research, the effect of acute exercise on inflammatory status is unknown. Therefore, the present study aimed at investigating the effect of aerobic exercise on ergometer on inflammatory and hormonal indices in active girls.

Methods
Sixteen healthy female physical education students (Table 1) participated in this study voluntarily (Mean±SD; Age: 20.25±0.9 years; Body Weight: 59.4±7.6 kg; Percentage body fat: 26.19±4.3%; Maximal aerobic capacity (VO\textsubscript{2max}): 42.14±9.8 ml/k/min; Body mass index (BMI): 22.11±2.66 Kg/m\textsuperscript{2}). Sampling was done according to available and purposeful method and the subjects were randomly and equally divided into two experimental (n: 8) and control (n: 8) groups. They consumed no drugs and narcotics during the study and performed regular aerobic exercise three days in a week. Strand test on ergometer was used to measure the subjects’ height, body mass, body composition, and maximal oxygen uptake. The experimental group entered with light and uniform covers after primary reading and warming body up by doing stretching exercises and accordingly closing rate meter to the environment with 18°C air temperature and 40% humidity and pedaling about 1 minute to warm up. The desired heartbeat for keeping the intensity of desired exercise was formerly calculated, was informed to all subjects, and asked them to keep the desired beating through regulating the speed of pedaling. They continued subject’s ergometer pedals until reaching the desired beating and keep this intensity for 60 minutes. The intensity of the exercise was controlled by heart rate at specific time intervals with the fingertip (Puls oximeter - Finger tip) A310 model of the German. Blood samples were taken immediately and 2 hours after the exercise of the subjects. Regarding the control
The samples were taken before entering the desired place and then sat in 18°C environment for 60 minutes without doing any activity, and left the controlled environment together when the exercise of the experimental group finished. Finally, the subjects' heartbeat was checked. In addition, the subjects were recommended not to participate in any match or heavy exercise and use the advised diets (including proteins) 48 hours before performing the test. Further, they were advised not to use vitamin C and carbohydrates because of their probable effect on immune system 12 hours before doing the test. Furthermore, they were restrained from using any kind of drinking and food except for mineral water while doing the exercise and 2 hours later. The tests were done between 9 am to 2 pm to control the effects of circadian rhythm on the dependent variables. Blood was collected in SS-T Vacutainer and serum was separated by centrifugation at 2500 rpm for 10 min at 22-24°C. Then, the serum was divided into aliquots and stored at -80°C until analyzing the inflammation related proteins. TNF-α, IL-10 was measured in serum by ELISA method (Diaclone, Bensacon, France). Cortisol, hs- CRPs were determined in serum by ELISA method (Diagnostics Biochem Canada, Ontario, Canada). In addition, the epinephrine level was measured in serum by ELISA method (IBL GmbH diagnostics, Hamburg, Germany). The coefficients of variation (inter assay precision) for TNF-α, IL-10, CRP, cortisol and epinephrine were 5.8%, 5%, 6.4%, 5.4%, and 7.3%, respectively. Further, the detection limits (sensitivity) for the analyses were 8pg/ml for TNF-α, 5pg/ml for IL-10, 10mg/ml for CRP, 0.4ug/dl for cortisol and 10pg/ml for epinephrine. All the collected samples were simultaneously analyzed at the end of the study in order to minimize the systematic variation. Descriptive statistics were used to determine the measured variables and paired sample t-test was used to compare the means and their differences in two groups. Finally, repeated measures ANOVA and Bonferroni test were utilized to evaluate the changes within a group. The collected data were analyzed by SPSS software version 23 and the significance level of 0.05 was considered for interpreting the data.

Results

Table 1 indicates the mean and standard deviations of subjects' demographic characteristics. The results of repeated measures ANOVA indicated that IL-10 level was not significantly different between experimental and control groups before, immediately and two hours after the aerobic exercise (P>0.05). In addition, no significant difference was observed between the two experimental and control groups in IL-10 level (P>0.05) (Figure 1). Further, the results of repeated measures ANOVA indicated that TNF-α level was not significantly different between experimental and control group before, immediately and two hours after the aerobic exercise (P> 0.05). However, a significant difference was observed between the experimental and control group two hours after the aerobic exercise (P=0.014). In fact, as illustrated in Figure 2, the mean of TNF-α was higher in the experimental group, compared to that of the control group. The mean of CRP immediately after the aerobic exercise was significantly different, compared to the two hours after the aerobic exercise (P=0.014). However, a significant difference was observed between the experimental and control group two hours after the aerobic exercise (P=0.005) although no significant difference was reported between before and immediately, and before and two hours after the aerobic exercise (P>0.05). In fact, the mean CRP was higher in two hours after the aerobic exercise than that of other periods. Finally, the results of t-test indicated that significant difference was observed between the experimental and control group in the level of CRP (P=0.002). In other words, the mean of CRP was higher in the experimental group, compared to that of the control group (Figure 3). As illustrated in Figure 4, the results of repeated measures ANOVA indicated that cortisol level was not
significantly different between experimental and control group before, immediately and two hours after the aerobic exercise (P> 0.05). However, the results of t-test indicated that significant difference was reported between the experimental and control group (P=0.001). In fact, the mean of cortisol was higher in the experimental group. As shown in Figure 5, the results of repeated measures ANOVA indicated that no significant difference was observed between the experimental and control group before, immediately and two hours after the aerobic exercise based on the epinephrine level (P> 0.05). However, the results of t-test indicated that there was a significant difference between the experimental and control group (P=0.009). The mean of epinephrine was lower in the experimental group, compared to the control group (Figure 5).

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Experimental group</th>
<th>Control group</th>
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<tbody>
<tr>
<td>Age (year)</td>
<td>20.25 ± 1.03</td>
<td>20.25 ± 0.88</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163 ± 5.75</td>
<td>165 ± 4.40</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>58.44 ± 7.55</td>
<td>60.44 ± 8.07</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>22.06 ± 2.98</td>
<td>22.16 ± 2.52</td>
</tr>
<tr>
<td>Percentage body fat</td>
<td>25.43 ± 4.60</td>
<td>26.95 ± 4.30</td>
</tr>
<tr>
<td>VO₂max (ml.kg.min)</td>
<td>47.01 ± 11.49</td>
<td>37.28 ± 4.49</td>
</tr>
</tbody>
</table>

**Figure 1.** IL10 changes in the subjects before, immediately and two hours after the end of aerobic exercise in the experimental and control group
* significant difference in the control group two hours after the intervention (P<0.05)

**Figure 2.** TNF-α changes in the subjects before, immediately and two hours after the end of aerobic exercise in the experimental and control group

¥ significant difference compared to immediately after the aerobic exercise (P<0.05)

* significant difference with the control group (P<0.05)

**Figure 3.** CRP changes in the subjects before, immediately and two hours after the end of aerobic exercise in experimental and control group
**Discussion**

The results of this study showed that there was no significant effect on the level of IL-10 and TNF-α in active girl. CRP and cortisol levels were higher in the experimental group than the control group. However, the mean values of epinephrine in the control group were higher than the experimental group. Consistent with this finding, some studies reported a lack of change in levels of IL-10 and TNF-α following an exercise (16-18, 21-24). The findings of this study were in line with Lasisi and Adeniyi (2016) indicating that 60 minutes of moderate-intensity soccer training had no
effect on the level of saliva IL-10 (16). Windsor et al. (2018) indicated that moderate intensity exercise and high intensity interval exercise had no effect on plasma IL-10 in healthy subjects (17). In addition, in a recent study on elite athletes compared to untrained adults, no significant difference was observed in IL-10 anti-inflammatory responses to one session of exercise (18). Most of the studies conducted on TNF-α reported no considerable change after exercise while the studies referring to the increase of TNF-α after exercise emphasized the effect of intensity and duration of exercise (23, 24). However, the results of this study are contrary to the findings of some researches. In a study, the researchers found that IL-10 concentration in plasma increased after the intense exercise (25). The reason for this difference is probably due to the intensity and duration of the exercise or the initial physical fitness of the subjects. The increased circulating concentrations of IGF-1 was observed with exercise, which may be one of the possible mechanisms for changing IL-10 due to exercise (25). During exercise, the IL-6 circulation level increases resulting in the creation of an inflammatory network producing IL-10, IL-1ra, and the prevention of TNF-α production (26, 27). The lack of IL-6 measurement in this study and its possible effects on inflammatory network and interpretation of results should be considered. Cortisol prevents the release of pro-inflammatory cytokines and cytokines produced by Th1 cells and stimulates the release of IL-10. Therefore, the cytokines released during exercise may mediate the immune responses (28). Catecholamines may prevent the synthesis of TNF-α during the exercise by increasing the synthesis of cyclic adenosine monophosphate (cAMP) and IL-6 or indirectly stimulating the synthesis of IL-1ra through IL-6 (29). Cortisol plays an important role in preventing the release of TNF-α (30). Accordingly, the increased cortisol concentration during exercise can be one of the reasons for not increasing TNF-α within 60 minutes of exercise. The production of TNF-α by stimulated mononuclear cells indicates the damage to muscle cells and the effects of exercise on the production of this cytokine in mononuclear cells may depend on the nature of exercise, the size of exercise, and the likely expansion of the tissue damage. Accordingly, the level of physical fitness of subjects in this research should be considered. CRP levels following a short-term session of exercise have been examined in some studies (15, 31, 32). In explaining the cause of increased CRP after exercise, the high levels of muscle damage induced by playing in tournaments and the maximum exercise cause a greater response to the acute phase (33). In interpreting the results of CRP, the factors such as the type of sport and possible damage should be considered. Furthermore, women respond differently to inflammation compared to men (34). Plaisance and Grandjean indicated that physical activity reduces CRP levels and the higher levels of cardiovascular activity and cardio respiration are associated with the reduction of 6 to 35% in CRP which is related to the base rate (35). However, there were few reports measuring the effects of one session of exercise on inflammatory markers (15, 31, 32). Exercise-induced CRP may reflect the effects of both intensity and duration of exercise. In other words, the time of blood transfusion, the level of exercise and the duration of exercise affect the CRP response. In addition, the changes in plasma volume, estrogen and body composition are effective (35). The results of the CRP research are contradictory as different intensities of combined exercise have no effect on the CRP level of active men indicating the significance of the volume of exercise relative to the intensity of exercise (15). On the other hand, the individuals who are physically more active and have higher physical fitness have lower levels of inflammatory markers (36). Accordingly, the level of physical fitness in girls should be considered. Perhaps, increasing
the levels of CRP from immediately to two hours after the exercise in the present study indicates a mild chronic inflammation state. Cortisol and epinephrine play mediating roles in changing the parameters of safety during exercise including the increase of white blood cells and increase cell toxicity of NK cells. During the muscular activity, cortisol is released by removing blood glucose by muscle and reducing blood glucose, and in severe exercise, more neutrophils are injected into damaged skeletal muscles. The penetration of neutrophils is related to skeletal muscles with hormones such as cortisol and IL-1β (30). The mechanisms by which these factors affect the invocation of immune cells during and after exercise are not yet known. Given the fact that the subjects had been banned from carbohydrates intake after 12 hours of exercise, the increase in cortisol up to 60 minutes of exercise was probably affected. At the onset of intense exercise, cortisol increases with a delay, which involves complex interaction between the receptor and the ligand with glucocorticoid transplantation sites in the target cell nucleus, gene expression changes, and synthesis of new proteins (30). As a result, 45 minutes of moderate intensity aerobic exercise is associated with the release of inflammatory and anti-inflammatory cytokines and increased catecholamines and cortisol (37). In this regard, it is necessary to pay attention to the athletes of the present study who had regular physical activity. The release of epinephrine during exercise is related to the entry of lymphocytes and NK cells, and to a lesser extent neutrophil to the blood circulation. and this may be related to the high proportion of high levels of β-adrenergic receptors in lymphocytes and NK cells (30). The central cerebrovascular hormones which change with exercise depend on the factors such as intensity of exercise, training period, age and gender. Thus, it is suggested that plasma catecholamines are higher in subjects with the higher age while the release of epinephrine in men is higher than that of women with similar intensity of exercise. Accordingly, the contradiction between some of the results of this research and other studies can be considered. In general, the results of various studies on the effects of exercise on the body, particularly controversial safety indices are controversial. In general, the immune system and cytokines cannot be considered from a single point of view because different internal and external factors can affect their function. On the other hand, the changes of cytokines cannot be attributed to just one factor because increasing or decreasing each one can affect the other factors in a cascade way. In addition to various sports parameters such as the duration, intensity and number of exercise sessions, some evidence suggests that the history of exercise, the location and methods of measuring cytokines, subjects' condition and physical fitness, age, gender, body mass, diet, lifestyle, stress, hormone intake, and even the exercise environment and laboratory are regarded as the factors affecting the results of the study. More empirical and controlled studies are required to evaluate the impact of these factors.

**Conclusion**

In general, the results of this study showed no significant effect of aerobic exercise on ergometer on IL10, TNF-α, cortisol and epinephrine levels in active girls. Based on the findings of this study, it seems that an exercise session on an ergometer does not affect hormonal and inflammatory factors in active subjects. However, due to few studies conducted in this regard, more studies are required on the relationship between acute exercise, inflammatory and hormonal indices.

**Ethical issues**

Not applicable.

**Authors’ contributions**

All authors equally contributed to the writing and revision of this paper.
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