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# The Effect of Intense Short-Term Training on Serum Levels of Homocysteine and C-Reactive Protein in Elite High School Volleyball Players

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Short-term intensive training

**Abstract**

The aim of this study was to investigate the effect of an intense short-term training course on concentration of serum of selected cardiovascular and inflammation markers (CK, CRP, and Hcys) in male elite volleyball players of high school in Golestan province during the training period. The experimental group consisted of 12 elite volleyball players in the premier league (age 17.08 ± 0.66 years, height 190.33 ± 6.02 cm, weight 65.12 ± 4.52 kg) and a control group including 12 healthy inactive students (age 17.00 ± 0.73 years, height 182.58 ± 10.24 cm, weight 64.12 ± 4.82 kg). The experimental group experienced 6 weeks of preparation training, whereas the control group did not exercise regularly. In order to measure the changes of selected biochemical variables in two stages (before training and after 6 weeks of preparation training), subjects were invited to the laboratory and their blood sampling was done in a fasting state. T-Test results were as follows: Homocysteine in experimental group had a non-significant increase (P= 0.945). But, the C-reactive protein and creatine kinase levels in both groups did not change significantly (respectively P= 0.991, P= 0.061). It seems that 6 weeks of short-term intense training had not any negative effects on cardiovascular health, and can be used in the course of preparation by coaches and players.

## 1. Introduction

Cardiovascular disease is known as one of the main causes of death in the world (1). According to the America Heart Association, cardiovascular diseases have an inflammatory background while general inflammation plays a pivotal role in the development of atherosclerosis (2). For a long time, lipid profiles were considered as an indicator of cardiovascular disease, but reports suggest that some people with normal HDL and LDL cholesterol suffer from cardiovascular disease (2, 3). Therefore, researchers' attention was focused on indicators that accurately predict cardiovascular disease risk (4). Increased levels of serum biomarkers such as homocysteine (Hcy) and C-reactive protein (CRP) are independently associated with an increased risk of cardiovascular disease (5). Abnormal levels of Hcy causes many complications, including atherosclerosis, thrombosis and many cardiovascular problems (6, 7). Hcy is a non-essential amino acid and a natural metabolite of methionine, an essential amino acid that is mainly derived from cellular methionine. Structurally, Hcy is very similar to methionine and cysteine, and the three of them are sulfur-containing amino acids (8). On the other hand, recent studies have shown that CRP is an indicator stronger than LDL-C in the prediction of cardiovascular events (8, 9); hence, an increase in this index predicts the future risk of plaque rupture (10). CRP is an acute phase protein that dramatically increases during infection, inflammation and tissue damage. CRP is often made in response to inflammatory mediators of blood and is released into the blood by the liver (11). Exercise and physical activity is one of the factors affecting homocysteine and CRP serum concentration. Physical activity causes many biochemical changes which can affect the metabolism of homocysteine. In this regard, oxidative stress may have a key role. Intense long-term exercise reduces the body's capacity to fight free radicals and reactive oxygen and they can cause oxidative stress (12, 13). Intense physical activity with a strong eccentric part and mechanical stress can cause muscle damage and cytokine release (14). CRP level is reduced in sports that have less mechanical stress (15).

Coaches and athletes are always seeking to employ the best training methods that have a scientific support in order to achieve valuable results and victories. The elite volleyball players in the world applied a combination of several methods of training (speedy, repetitive, periodic, etc.) in a season of training and in preparation camps. Today, the time of preparing students for athletic sports teams is very limited; so, the training and preparation are done in short-term and highly intense conditions. Since that students' competition is concurrent with their education and schooling time, therefore, athletic students are invited to training camp for a short time. From one hand, high intensity and volume of training imposes a high physical and mental pressure on the players which can bring changes in some of their biochemical parameters like enzymes involved in inflammation and pressure, such as lactate dehydrogenase (LDH) and creatine phosphate kinase (CPK) or creatine kinase (CK). These enzymes play a key role in speeding up the chemical reactions (glycolysis process in heavy and extreme sports) (16). Muscle stress that occurs in volleyball training (especially preparations) is sometimes associated with muscle injuries. In most studies which examined muscle injury, serum CK enzyme had been measured as a muscle activity reaction (17).

With this introduction, this study is intended to investigate the effect of intense short-term exercise on one of the indicators of muscle damage (creatine kinase) and some inflammatory markers and predictors of cardiovascular disease (homocysteine, C-reactive protein) in male volleyball elite players in high school.

## 2. Materials & Method

By simple random sampling, 12 boys have been selected as the statistical sample (as the training or experimental group) from among the elite players in the high school volleyball league of Golestan province who have reached the first place and will be sent to the France tournament as the elected representatives of the Islamic Republic of Iran. Among high school students, another 12 healthy students were selected randomly as the control group.

In order to carry out the research, the researchers took the permission of the university and the Education Department of Golestan province. Also, after sampling, the written consent of parents of selected students was obtained in a consent form. To start the research plan, the details and stages of tests and blood sampling was explained by the researcher. Then subjects were divided into two experimental and control groups. Before the implementation of the exercise program in both groups, they provided demographic information in a form for this purpose.

**Table 1.** Training intensity during 6 weeks' preparation

| Intensity / Week | 50-60% | 60-65% | 70-75% | 80-85% | Repetition num. |
|---|---|---|---|---|---|
| 1 | * | | | | 1 |
| 2 | | * | | | 1 |
| 3 | | | * | | 1 |
| 4 | | | | * | 1 |
| 5 | | | * | | 1 |
| 6 | | * | | | 1 |

**Table 2.** Training program in first week.

| First week with 50-60% | 8 - 9:30 | 10:30 -12 | 16-18 |
|---|---|---|---|
| Sat | Warm up- jogging - stretching – warm-up specialized moves with the ball - claw - forearm - serve - receive - flexibility- recovery | Warm up - theory of training - strength training - tiller - serve - forearm - a multiplayer game - recovery | Warm up - theory of training - exercises with balls - receivers areas - defense on tour - serve areas - receive areas- group play |
| Sun | Warm up- jogging - stretching - claw - forearm – serve- combined training (spoke- defense, simulation, support from defense, attack speed)- recovery | Warm up- jogging - stretching - claw - forearm – serve- combined passes-receiving- serve, receive and attack power- recovery | Warm up- jogging - stretching- claw - forearm – serve- defense on tour- repetitive jumping- explosiveness exercises- recovery |
| Mon | Warm up- jogging - stretching- support from attacker- serve receive- claw- attack power- recovery | Warm up- jogging – stretching- resistance training (endurance)- combined passes- flexibility- recovery | Warm up- jogging – receive specialized ball - serve and defense forearm and defense claw- recovery |
| Tue | Warm up- jogging – stretching- serve- receive- defense on tour– match three against four - triple defense simultaneously- recovery | Warm up- jogging – stretching- tactical training-receive- jump and hammer serve- short starting- short diving- recovery | Warm up- jogging – stretching- passing two to two- combination of claws and forearm- recovery |
| Wed | Warm up- jogging - stretching- training theoretical- general defects elimination- practical implementation- friendly games - combined practice- recovery | Warm up- jogging - stretching- serve receive drills- claw- forearm- defense on tour- defense of 4, 3 and 1 player- recovery | Warm up- jogging - stretching- long and short serve- forearm- combined passes- recovery |
| Thu | Warm up- jogging - stretching- serve- claw- spike- combined defense-receive- simultaneous jumps and two against four- recovery | Warm up- jogging - stretching- -procurement game- short starting- jogging around the volleyball ground- claw- serve- recovery | Warm up- jogging - stretching- recreational training with ball- short throws and get two hands- claw- forearm- recovery |
| Fri | Rest | Rest | Rest |

### 2.1 Blood sampling and analysis of blood markers

Blood samples were collected from the anticoagulant vein in the pre-test and post-test (after 6 weeks of training) to determine the CRP, CK and Hcy serum concentrations after 12 hours of fasting. The venous blood samples were taken at rest for at least 48 hours after the activity and were poured into pre-cooled serum tubes, allowed to be clogged at room temperature for one hour. Then the samples were centrifuged at 1300 g for 12 minutes and at 4 ° C. The collected serum was evacuated in Eppendorf tubes and stored at -80 ° C until analysis. The serum Hcy value was determined using an ELISA kit, based on the kit manufacturer's instructions (AXIS-SHIELD, UK).

The minimum and maximum measurable value of the Hcy kit was 2 and 50 μmol / L, respectively. CRP was measured by immunoturbidimetry and kit method (Bionic Company, Iran) with a range of 2 to 6 mg / L and a sensitivity of 0.01. The results of the experiment were evaluated by the reader-ELISA device (Ststfax, USA). In addition, for the evaluation of the activity of the creatine kinase enzyme from the CKNAC-activated kit, the creation of the RANDOX factory in the United Kingdom was done automatically by Ependrof's autoanalyzer.

### 2.2 Statistical method

The results of pre-test (before training) and post-test (after training) of each group were correlated by T-test. Independent T-test was used to examine and compare the mean variations of the variables in the experimental group with the control group.

(Pre-test values) – (Post-test values) = variables difference

From now on, the values are expressed in terms of SE ± Mean (standard deviation error ± mean), and the difference is significant at $P \leq 0.05$. The calculations and statistical analysis were performed using SPSS 16 software.

## 3. Results

The mean and standard deviation of the general characteristics of the subjects in the experimental and control groups are presented in Table 3. It shows that there was only a significant difference between the experimental group and control group in body mass index, so that the BMI of the experimental group was significantly reduced compared to the control group ($P = 0.0001$).

The mean and standard deviation of homocysteine, CRP and creatine kinase (CK) changes for the study groups before and after the training period are shown in Table 4. The paired T-test showed no significant changes in variables before and after training ($P > 0.05$).

The mean and standard deviation of homocysteine, CRP and CK changes for the study groups are shown in Table 5. Independent T-test showed no significant changes in variables level before and after training ($P > 0.05$).

***Table 3***. General characteristics of subjects

| variable | experimental group (n= 12) | | control group (n= 12) | |
|---|---|---|---|---|
| | **Pre-test** | **Post- test** | **Pre-test** | **Post- test** |
| Age (year) | 17.08± 0.66 | - | 17.00± 0.73 | - |
| Height (cm) | 190.33± 6.02 | - | 182.58± 10.24 | - |
| Weight (kg) | 65.56± 4.61 | 64.88± 4.43 | 63.91± 4.92 | 64.33± 4.72 |
| BMI (kg/m$^2$) | 18.14± 1.35 | 17.85± 1.35 * | 19.39± 3.02 | 19.52± 3.03 |

***Table 4***. Changes in Hcy, CRP and Ck in the study groups before and after training period

| groups | variable | Pre-test | Post-test | P value |
|---|---|---|---|---|
| control | Hcy (µmol/L) | 16.04± 7.31 | 15.97± 7.40 | 0.978 |
| | CRP (Mg/l) | 3.12± 1.26 | 3.11± 1.25 | 0.99 |
| | CK (U/L) | 140.00± 18.48 | 144.91± 19.77 | 0.081 |
| experimental | Hcy (µmol/L) | 16.08± 5.26 | 16.20± 3.76 | 0.895 |
| | CRP (Mg/l) | 1.91± 0.99 | 1.98± 0.89 | 0.989 |
| | CK (U/L) | 292.58± 40.63 | 399.00± 83.90 | 0.072 |

***Table 5.*** Changes in Hcy, CRP and Ck in control and experimental groups

| variable | Control group | Experimental group | P value |
|---|---|---|---|
| **Hcy (µmol/L)** | 0.073± 0.09 | 0.122± 1.50 | 0.945 |
| **CRP (Mg/l)** | 0.01± 0.01 | 0.07± 0.10 | 0.99 |
| **CK (U/L)** | 4.91± 1.29 | 106.42± 43.27 | 0.061 |

## 4. Discussion and conclusion

The results of present study showed that Hcy increased in the experimental group, while in the control group it slightly decreased. Statistical analysis showed that changes in Hcy levels were not significant within ($P \leq 0.978$, $P=0.98$) and between groups ($P \leq 0.945$). Although there is no information about the impact of participation in short-term volleyball camps on the different variables of this study, it seems that exercise training does not have sustained effects on the Hcy levels (18-20). The reasons for this heterogeneity are cited by factors such as risk factors, assessment method, sample size, gender, age range, baseline concentration, exercise intensity and volume (21). The results of this study showed that the pre-training value of Hcy was pathologically slightly higher than optimal level (16 µm versus 15 µm), and physical training cannot lead to any significant changes in that until the baseline values are low. (22) reported that serum homocysteine levels were higher in passive patients. (18) reported that serum homocysteine was significantly lower in trained men compared to untrained men. On the other hand, its significant reduction following exercise in hyperhomocysteinemia patients indicates that the initial levels of homocysteine and physical fitness are effective on changes from training (23). Some findings indicate that there is both a significant decrease (23) and no significant difference (24) in this index in active people compared to passive people. In this regard, (25) found decreased Hcy level after 6 months of resistance training in healthy elderly people (26). The probable mechanism of these reductions may be increased remethylation homocysteine and, consequently, elevated levels of S-adenosylmethionine (SAM), plus an increase in antioxidant capacity (13). One of the possible reasons for the lack of consistency between the results of these studies with the present study may be the type of exercise, the intensity of exercise, and the age range of the subjects. Statistical analysis indicated that changes in CRP levels were not significant within ($P = 0.99$) and between groups ($P = 0.991$), and CRP level remained almost unchanged in both groups. Contrary to the present study, Gaeini et al. (2008) investigated the effect of 12-weeks aerobic training protocol on hs-CRP levels in obese rats. The results showed that regular aerobic training decreased the hs-CRP and reduce the atherogenesis process (27). Church and colleagues (2002) investigated the relationship between fitness and CRP, which showed a reciprocal relationship between these two cardiopulmonary and respiratory variables (28). (29) investigated the effect of eight weeks of strength training on C-reactive protein and plasma fibrinogen in passive students and reported that strength training did not significantly reduce serum CRP and plasma fibrinogen in untrained students which is contrary to the results of this study; this can be attributed to increased fitness of the subjects, which means that six weeks of regular volleyball exercises lead to compatibility of the subjects and prevented the increase of muscle damage and inflammation caused by training, which is justifiable. No significant change in CK (muscle injury index) was observed in the present study. Several studies have reported that there is an inverse relationship between serum CRP levels and physical fitness. These studies suggest that regular exercise training can reduce CRP levels (30, 31). Fallah Mohammadi and Nazari (2013) showed that four weeks of plyometric training did not significantly change the serum levels of CRP (32). They stated that one of the reasons for not changing serum CRP levels (as in the present study) was the base values of the subjects. Studies have shown that, if the CRP baseline is high, the effect of the exercise is more pronounced (33, 34), which means that it is more perceptible to reduce CRP when studying on subjects with overweight or obese subjects. The subjects of this study were students with CRP levels in their natural range. In addition, a strong association between CRP and BMI has been reported in several studies (35, 36), which indicate the potential role of adipose tissue in increasing CRP levels. In the present study, BMI in the experimental group decreased significantly which may be one of the factors influencing the unchanged CRP after short-term severe training. Therefore, observing such results is not beyond expectation. In relation to the adaptive effect of aerobic exercises on the levels of CRP, several mechanisms have been proposed. One of these explanations is the increase in nitric oxide from endothelial cells, resulting in a reduction in systemic inflammation, thereby reducing the production of inflammatory cytokines, which ultimately reduces the production and release of CRP from the liver (37). Another justification is the effect of regular activity on decreasing the sympathetic stimuli, which in turn leads to a decrease in the secretion of TNF-α (a strong stimulant for IL-6 production) and IL-6 (a strong stimulant of production CRP) (34); while the mechanism of the effect of resistance training on modulation of inflammation is not well known (30). Overall, it seems that six weeks of intense short-term exercises to prepare volleyball players may lead to physical fitness, and no significant tissue damage or inflammatory responses was created. Therefore, coaches and players can use this preparation method without concern.

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