Comparing the effects of two acute training methods of continuous and high-intensity interval on fibrinogen and high sensitivity C-reactive protein responses in sedentary women

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Abstract

Introduction:
Inflammatory markers such as fibrinogen and C-reactive protein are strongly associated with cardiovascular diseases and the type of exercise may influence these biomarker responses. The present study compared the effects of two acute training methods of continuous and high-intensity interval on fibrinogen and high sensitivity C-Reactive Protein (hs-CRP) responses in sedentary women.

Materials & Methods:
Twenty sedentary young women were randomly assigned to continuous exercise (CE) and high-intensity interval training (HIE) groups (n=10). CE group performed moderate-intensity continuous exercise at an intensity of 60 to 65% of Vo2max for 40 minute on treadmill. HIE group completed 36 minutes of treadmill running with alternating 60 s at 90-95% Vo2max and 240 s at 50% Vo2max. Venous blood samples were collected before, immediately after and 60 min after training.

Results:
Both Fibrinogen and hs-CRP levels significantly increased immediately after both acute exercises (P<0.05) and significantly decreased close to baseline levels after 1 hour of the passive recovery (P<0.05). However, fibrinogen levels remained higher than baseline levels during recovery in CE group (P=0.007).

Conclusion:
Fibrinogen and hs-CRP responses were similar in both acute training methods of continuous and high-intensity interval, but the continuous aerobic exercise may induce slightly higher levels of fibrinogen at recovery compared with high-intensity interval.

Keywords: C-Reactive Protein, Fibrinogen, Training

Introduction
Atherosclerosis is a disease of large blood vessels, which is considered a leading cause of hearth diseases, and brain stroke, and it accounts for half of deaths in industrialized countries (1). Atherosclerosis is not only an inevitable abnormal outcome of aging but it is mostly a chronic inflammatory disease that can turn into an acute clinical event through plaque rupture and thrombosis (2). These findings encouraged further studies on circulating biochemical markers that are
indicative of inflammatory activity in vessel wall (3). Besides fibrinogen that is one of the primary inflammatory markers associated with the risk of cardiovascular diseases (CVDs), C-reactive protein (CRP) is also considered a predictive factor of CVDs even in apparently healthy people (4, 5). CRP is formed in the liver in response to inflammatory mediators, and is part of natural immunity (6). This inflammatory marker protein is synthesized through inducing interleukin-6 and leptin in fat and arterial tissues, and it stimulates expression of inflammatory cytokines. Moreover, it activates complement system, regulates low-density lipoprotein, and decreases expression of nitric oxide synthase (7). On the other hand, any increase in fibrinogen is associated with inflammatory risks and clot formation (8). Plasma fibrinogen is an acute-phase reactive protein that boosts platelet degranulation in response to adenosine diphosphate. Its increase might be a secondary response to inflammation or infection, which is induced through platelet reactivity to some extent. Fibrinogen also plays a vital role in a few body processes such as inflammation, atherosclerosis, and clotting (9). Therefore, managing risk factors can play an important role in maintaining people’s health. Although nutritional intervention forms an important part of these programs, moderate-intensity physical activities are recommended as a non-medical intervention for primary and secondary prevention of cardiovascular diseases (10) and maintaining health (11). The type, intensity, duration, length, number of training sessions and graduality of the workouts are the main components of a regular individual exercise program (12). Clinical studies have shown that acute exercise can increase the risk of clotting in important arteries and temporarily cause heart attack (13). Therefore, despite the fact that physical activities protect people against cardiovascular diseases, exercising also induces platelets that might provoke serious vascular incidents (15) and increase the risk of sudden death (14). Previous studies have reported the following results: a significant increase in CRP, no changes in plasma fibrinogen in older adult men after circuit resistance exercise (16), no significant changes in fibrinogen after high-intensity exercise (Bruce Test Vo2max) in the morning and in the afternoon (17), further increase in fibrinogen following one session of resistance exercise in comparison with one session of moderate intensity exhaustive aerobic exercise (18). Yet, most results obtained from meta-analysis are indicative of 40-60% VO2max in moderate-intensity continuous exercises (19). High-intensity interval exercises are another type of sport activities which are employed occasionally in rehabilitation exercises. This type of training involves repetitive steps of 30-300 seconds of aerobic activities performed with intensity of 95-100 % of VO2max with equal, shorter or longer recovery periods than sport activities (20). Higher impacts of HIE trainings have been reported on health (21), especially in patients with coronary diseases. In fact, a training program that leads to targets such as health-based performance and physical readiness within a shorter period, is favorable for people who want to achieve more readiness faster (23), whereas traditional physical fitness programs require longer time to achieve training targets (24). Given the high prevalence of cardiovascular diseases in our society, the need for more effective exercise protocols for inflammatory markers, the need for improving sedentary lifestyle among women, and the few studies conducted on effectiveness of HIEs especially in women, the present study compared the response of fibrinogen and hs-CRP to two types of acute aerobic CE and HIE in sedentary young women.

Materials and Methods
The current quasi-experimental study recruited young healthy but sedentary
Comparing the effects of two acute

female students aged 22-25 years attending the Islamic Azad University-Saary Branch. The samples were selected through purposeful sampling technique following a notification call for volunteers and according to collected data from health questionnaire on age, history of having CVDs and orthopedic problems, and medicine use. In addition, the results of doctor’s checkup, height and weight measurement, body fat percentage, lean body mass, and VO2max were used to select matching subjects through convenience sampling technique. Then the 20 selected subjects were randomly divided into two groups of ten to perform continuous and interval exercises each.

**Exercise program**

Subjects were introduced to laboratory environment a week prior to start of exercises and their VO2max was recorded using Bruce incremental treadmill run test and Pollock formula (VO2max=4.38*time of training – 3.9). The body fat percentage was recorded by measuring skin fold thickness over triceps muscle, suprailiac and right thigh using Jackson-Pollock 3-site equation and body density (Siri equation) (25). The workout schedule of the continuous aerobic exercise group consisted of one moderate-intensity session in which subjects warmed up for 10 minutes by five minutes of stretching and five minutes of walking and jogging on treadmill, and then they ran for 40 minutes at an intensity of 60% to 65% of VO2max. Training schedule of interval exercise group consisted of 10 minutes of warm-up (five minutes stretching and five minutes of walking and jogging on treadmill), followed by 36 minutes of main exercise consisting of interval sets from 1 minute of running at intensity of 90-95% of VO2max and three minutes at intensity of 50% VO2max (20, 26). The subjects’ heartbeat rates were monitored using Polar heart rate monitor and maximum value was calculated using (=220-age).

**Venus blood collection and biochemical analysis**

Blood samples (5 cc) of participants were collected three times during luteal phase of the menstrual period following 12 hours of overnight fasting (light meal the night before) before the exercise (following a 30-minute rest in the laboratory), immediately after, and 60 minutes after both trainings. The blood samples were taken during passive recovery from left brachial vein at a sitting position 8-11 a.m. Then the blood samples were divided into two parts. The first part was added to bottles containing anti-coagulant citrate for fibrinogen measurement, while the second part was centrifuged for 15 minutes at 3000 rpm to separate plasma. The separated plasma was poured into microtubes and used to measure hs-CRP level. Fibrinogen was measured through coagulation laboratory test (Clauss method) using coagulometer and kit (Pars-Azmoon). The hs-CRP was measured using commercial kit (High Sensitive C-Reactive Protein ELISA Kit, Diagnostics Biochem, Canada) with sensitivity of 10 ng/ml and using ELISA method.

**Statistical tests**

The data were analyzed using SPSS 20. Shapiro-Wilk and Levene tests were used to confirm normality of data and homogeneity of variance, respectively. In addition, repeated measure ANOVA, post-hoc LSD, and independent t-test were also employed. The level of significance for all tests was set at P<0.05.

**Results**

The results obtained from Shapiro-Wilk test and Levene were indicative of normal distribution and homogeneity of variance in anthropometric characteristics of data, and level of fibrinogen and hs-CRP in the exercises. Moreover, independent t-test results showed no significant differences in anthropometric characteristics of subjects (Table 1) (p>0.05). Repeated measure ANOVA showed that time (before, immediately after, and 60 minutes after the test) had a significant impact on the level of fibrinogen or hs-
Comparing the effects of two acute

CRP in sedentary women regardless of the type of exercise (p<0.000). Also, the impact of the type of exercise (CE and IE) regardless of time and the interactive impact of exercise group and time were not significant on the level of these variables (p>0.05). The results of intragroup comparison showed that the level of fibrinogen increased significantly in CE group (19.74%, from 306.40±43.69 to 366.9±37.56 mg/dl) and in IE group (11.26%, from 319.10±47.44 to 359.60±38.41 mg/dl) immediately after the exercise (Table 2). Additionally, the level of fibrinogen of subjects in both CE group (335.30±41.80 mg/dl) and IE group (340.20±37.61 mg/dl) decreased significantly (8.61%, and 5.39% respectively) after one hour of recovery. Although despite this decrease, the level of fibrinogen in CE group remained significantly higher than baseline (Figure 1).

Also, hs-CRP level significantly increased immediately after both acute continuous exercise (21.54%, from 2.46±0.34 to 2.99±0.31 mg/dl) and interval training (18.49%, from 2.38±0.43 to 2.82±0.19 mg/dl). Following an hour of passive recovery, the level of hs-CRP significantly decreased to nearly baseline level (2.28±0.43 and 2.35±0.8546 mg/dl) (Table 2, Figure 2).

The results of the independent t-test showed no significant difference between levels of fibrinogen and hs-CRP in continuous and interval training groups with p=0.528 and p=0.624 at baseline levels, p=0.637 and p=0.173 immediately after the training, and p=0.876 and p=0.750 after an hours, respectively.

Table 1: The mean and standard deviation of indicators of body composition of samples

<table>
<thead>
<tr>
<th>Variable</th>
<th>Interval</th>
<th>Continuous</th>
<th>P value</th>
<th>Variable</th>
<th>Interval</th>
<th>Continuous</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>23.4±1.6</td>
<td>22.8±1.4</td>
<td>0.614</td>
<td>BMI (kg/m²)</td>
<td>21.9±2.2</td>
<td>21.7±2.1</td>
<td>0.759</td>
</tr>
<tr>
<td>Height (centimeter)</td>
<td>162.4±3.6</td>
<td>161.5±2.3</td>
<td>0.512</td>
<td>Fat percentage</td>
<td>27.6±2.3</td>
<td>26.9±3.9</td>
<td>0.598</td>
</tr>
<tr>
<td>Weight (kilogram)</td>
<td>57.8±7.1</td>
<td>57.7±5.0</td>
<td>0.972</td>
<td>VO2max ml/kg/m</td>
<td>40.2±2.5</td>
<td>39.6±2.3</td>
<td>0.575</td>
</tr>
</tbody>
</table>

P value: Independent t-test

Figure 1: Comparing fibrinogen before, immediately after, and one hour after one high-intensity interval and continuous exercise

*: significant compared to the value before and one hour after the exercise (for both groups)
Comparing the effects of two acute
Habibian M et al
Pars Journal of Medical Sciences, Vol. 13, No. 3, Fall 2015

#*: significant compared to the value before the exercise (in continuous exercise)

Figure 2. Comparing hs-CRP before, immediately after, and one hour after one high-intensity interval and continuous exercise
*:significant compared to the value before and one hour after the exercise (for both groups)

Table 2. LSD test results for comparing fibrinogen and hs-CRP of experimental groups in different stages of high-intensity continuous and interval exercise

<table>
<thead>
<tr>
<th>Variable</th>
<th>Continuous training group</th>
<th>Mean of score differences±standard error</th>
<th>P value</th>
<th>Internal training group</th>
<th>Mean of score differences±standard error</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>Before the exercise</td>
<td>60.50±9.48</td>
<td>0.000</td>
<td>Before the exercise</td>
<td>40.50±13.62</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>Immediately after the exercise</td>
<td>28.90±8.24</td>
<td>0.007</td>
<td></td>
<td>21.20±13.80</td>
<td>0.161</td>
</tr>
<tr>
<td></td>
<td>Before the exercise</td>
<td>-31.60±5.90</td>
<td>0.000</td>
<td></td>
<td>-19.40±4.94</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>One hour after the exercise</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hs-CRP</td>
<td>Before the exercise</td>
<td>0.52±0.09</td>
<td>0.000</td>
<td>Before the exercise</td>
<td>0.44±0.13</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>Immediately after the exercise</td>
<td>-0.18±0.15</td>
<td>0.259</td>
<td></td>
<td>-0.03±0.05</td>
<td>0.604</td>
</tr>
<tr>
<td></td>
<td>Before the exercise</td>
<td>-0.71±0.10</td>
<td>0.000</td>
<td></td>
<td>-0.47±0.15</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>One hour after the exercise</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P value: Using LSD post-hoc test

Discussion
Although regular aerobic activities are reported to decrease fibrinogen (27) and hs-CRP levels (28) through decreasing catecholamine stimulation and increasing blood circulation, the impact of different types of HIE or CE on these two inflammatory marker is controversial. According to the present research results, the level of fibrinogen and hs-CRP had a gradual increase immediately after both acute training methods with moderate intensity continuous exercise and HIE in sedentary women. The level of these
Comparing the effects of two acute inflammatory markers decreased in both groups following 60 minutes of passive recovery while fibrinogen level remained at higher values than baseline level in CE group. These results are indicative of activation of coagulation and inflammation following both CE and HIE thus lower risk of clotting and inflammation caused by fibrinogen during recovery phase is associated with HIE. Plasma fibrinogen is an important component of coagulation cascade and is a major determinant of blood viscosity and flow. Epidemiologic studies have shown that high levels of plasma fibrinogen are associated with higher risk of cardiovascular diseases such as ischemic heart diseases, strokes, and other arterial occlusive diseases (29). Moreover, fibrinogen level of resting heart rate has a direct relationship with age, gender, BMI, alcohol consumption, hormonal status, and even psychological health of people (9). Thus any changes in fibrinogen level after exercise is associated with its value at baseline level prior to physical activity (27). The present study observed no significant difference between initial level of fibrinogen in both groups, and it did not reach critical value though the level had significantly increased following continuous and interval training. Likewise, Mousavi and Habibian have shown that plasma fibrinogen concentration of active young women increase following one session of acute aerobic exercise (running on treadmill with 65-75% VO2max until exhaustion) or resistance training (including six training sessions with dumbbells in three sets of 5-7 repetitions and intensity of 80% of a one-repetition maximum) and it remains at higher values than baseline level until 60 minutes after recovery (18). Lim et al. reported that following both moderate and high intensity (60 and 80 VO2max) acute aerobic exercise of equal work output, the level of fibrinogen and hs-CRP in fat middle-aged women significantly increased regardless of the intensity (30).

Despite the fact that the level of fibrinogen has significantly decreased one hour after both types of exercise, its value remained higher than baseline only in CE group. So no significant differences were observed between levels of fibrinogen in different groups one hour after the exercise. Generally, HIE training devote longer time for high intensity exercises in comparison with continuous training in which exhaustion occurs suddenly. This could lead to higher output strength in interval stages and decreased pressure, increased perception of efforts despite higher serum lactate (28). Yet, Sabouri-Sarein et al. also mentioned no significant changes in fibrinogen level of young women following one session of acute activity (Bruce incremental run test to exhaustion) at different times of day during morning or afternoon (17). Moreover, Bije and Jafari observed no changes in fibrinogen of older adults following circuit resistance exercise (10 exercises with 35% intensity of one-repetition maximum) (16). The possible reason for this finding might be the exercise protocols, differences between subjects, inadequacy of intensity and duration of exercises for increasing fibrinogen, and inequality of baseline levels of fibrinogen in the subjects (16, 18). Also, the response of hs-CRP to one session of acute aerobic exercise in samples with less physical readiness was different (31) such that baseline level of fibrinogen increased with aging and decreased by regular sports activities (9) despite the fact that the subjects of the present study were young and sedentary. Nazarali and Hanachi have also reported increased level of fibrinogen in active young women following one session of aerobic exercise including six sets of 35-meter dash with 10 seconds of rest between each (32) which shows that exercises with maximum intensity increased coagulation and inflammatory...
Comparing the effects of two acute
Habibian M et al

Pars Journal of Medical Sciences, Vol. 13, No. 3, Fall 2015

response in active people similar to sedentary subjects of the present study. Aligned with the results of the present study, a significant increase in the level of hs-CRP in older adult men following one session of circuit resistance exercise (16), as well as more and insignificant increase in hs-CRP levels in higher intensity exercises following two sessions of aerobic exercises with different intensities of 65% and 85% VO2max (30 minutes), were observed (33). On the other hand, Needham et al. studied the response of hs-CRP following a 40-minute aerobic or resistance exercise with moderate-heavy and low intensity in sedentary men. Their results showed that the level of hs-CRP after one session of moderate-heavy resistance exercise was significantly higher than in acute resistance training or low-intensity aerobic exercise. Although no significant difference was observed between the response of hs-CRP to both types of training (34). In contrast, the present study revealed no significant difference between the level of hs-CRP in the two groups although the intensity of exercises was higher in IE than in CE group. This can be explained by intermittent training, lower intensity exercises between high-intensity phases, shorter exercise duration, and consequently, less pressure in comparison to continuous exercises. Moreover, the results of Taghian’s study showed that the levels of hs-CRP following one acute exercise session including 20 minutes of running on treadmill with 50-70% intensity of maximum heart rate increased significantly in both normal and fat women, while no difference was observed in hs-CRP between the two groups. These researchers believe that the intensity, duration, type of exercise, as well as muscle damage decrease in response to hs-CRP (35). Contrary to the present research results, Hoseini-Kakhak et al. observed no significant changes in the levels of hs-CRP plasma in overweight girls following one circuit resistance exercise with intensity of 40%, 50%, and 60% of repetition maximum (36). It seems that lower to moderate intensity resistance exercises were not associated with induced inflammation and CRP in these subjects.

With regard to the current research, the significant increase in the level of hs-CRP following both types of acute continuous and interval training and the significant decrease in their level following one hour of passive recovery show the similarity of intragroup changes of hs-CRP during different phases of both acute exercises and similar impact of 40 minutes of continuous activity and 36 minutes of interval exercises on the response of hs-CRP in sedentary women. This can be associated to similar mechanical strength, muscle damage and glycogen depletion in both exercises (31). Hs-CRP is an inflammatory marker in the body and risk factor of heart disease while in resting position. The exact functionality of hs-CRP increase following acute exercise is not clear but it seems that an increase in inflammatory cytokines such as interleukin 1, interleukin 6, and tumor necrosis factor alpha act as confounding mechanism in this process. Interleukin 6 is the most important and evident response of cytokines to sports as it stimulates liver cells for producing hs-CRP (37, 38) and is the key regulator of fibrinogen synthesis through other mediators such as glucocorticoid, interleukin-1, and tumor necrosis factor alpha (39). Although interleukin 6 was not measured in the present study which might be considered a limitation, other scientists have shown that muscle cell damage caused by exercise is the first stimulus for increasing interleukin-6 levels during exercise and shorter periods after that. Even signaling pathways within muscles may not only damage the cells but they also stimulate interleukin 6 and subsequently produce hs-CRP. On other hand, interleukin-6 develops prothrombotic state by increasing liver-produced fibrinogen (9). Fibrinogen
Comparing the effects of two acute
Habibian M et al

Pars Journal of Medical Sciences, Vol. 13, No. 3, Fall 2015

24

is an important coagulation factor though not all levels of increase in concentration is acceptable. In contrast, the level of CRP can increase hundreds of times within 24-48h hours after an inflammatory stimulus which is indicative of a closer association of this marker with inflammation (40). Thus in response to the intensity of exercise and induced by interleukin-6, CRP increases initially (16, 37) and higher intensity exercise also increases fibrinogen level (16). Previous studies have reported an increase in interleukin-6 levels immediately after interval running or cycling in trained samples, which was associated with less increase in comparison with long-term continuous exercise (41).

Other possible reasons for the increase in fibrinogen might be the decreased plasma volume and increased blood viscosity following acute sports activities (18, 42) such that no fibrinogen changes were reported after modifying plasma volume changes (42).

However, due to the importance of even a little impact of both types of moderate-intensity acute continuous exercise and high-intensity interval exercise on fibrinogen and hs-CRP inflammatory markers, further studies are required to determine the effective mechanism of the intensity and exercise duration on responses of fibrinogen and CRP inflammatory markers to acute exercise.

Conclusion
The results of the present study suggest a similar response of hs-CRP and fibrinogen following a moderate-intensity continuous exercise or high-intensity interval exercise, although, continuous exercise might induce a gentle increase in fibrinogen during passive recovery after the exercise. This can be an advantage of using high-intensity interval exercises in shorter times compared to continuous exercise. It can also be suggested as an effective and safer type of exercise for sick people or those at higher risk. Thus, it requires further investigation to confirm the effectiveness of such interval exercises.

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Conflict of Interest
The authors declare no conflicts of interest regarding the compilation/publication of this article.

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