Abstract: Cardiovascular disease (CVD) is discussed as the most important cause of death in developed and developing countries. HS-CRP and homocysteine have predictive agents for cardiovascular disease. This study investigated the effect of different menstrual cycle phases (mid follicular and luteal) on plasma homocysteine and serum HS-CRP after an aerobic exhaustive exercise in athlete and non-athlete females. Ten non-athlete females (age 24.2±1.61 years, weight 57.95±6.02, BMI 22.31±3.56 kg/m², fat 22.86±6.14) were selected for participating in two sessions of exhaustive exercise (Bruce protocol) at mid follicular and luteal phases. They had regular menstrual cycle and didn't use HT in the last 6 months. Estradiol, progesterone, LH, FSH (before), hs-CRP and homocysteine levels has been measured before and after exercise. Independent T test was used for statistical analysis ("p<0.05"). Results revealed that baseline homocysteine was higher at F phase. Exercise induced increase of homocysteine was 15% at both L and F phases. But baseline levels and exercise induced alterations of hs-CRP was higher at L phase. The reduction of homocysteine may be due to increased estradiol at L phase. But, hs-CRP increased with increasing amounts of progesterone at this phase.

Key words: Menstrual cycle phases %Plasma homocysteine %Serum hs-CRP %Exhaustive exercise

INTRODUCTION

It is predicted that Atherosclerosis is a dominate disease in 2020. Traditionally, lipid profile was as a standard tool was used for identifying individuals at risk for later cardiovascular events. But some studies have suggested that Heart disease is occurs in people with normal blood lipids and in some cases even less than normal therefore, researchers began to explore new indices. Such new risk factors are clotting disorders or Fibrinolysis, Homocysteine and inflammatory markers [1-3]. Meanwhile, the high sensitivity C reactive protein (HS-CRP) is known as the most sensitive indicator of inflammation and strong and independent predictors of cardiovascular risk [4,5]. In addition, the role of Homocysteine has been determined as a predictor of early atherosclerosis and sudden cardiac death [6]. Mac Kolley (1970) established Atherosclerosis Homocysteine theory and after many studies confirmed in Homocysteine metabolism as an important mediators of the disease [7]. Homocysteine levels in women is less than men But these values are increased in women after menopause. The likely reason is that estrogen is involved in Homocysteine metabolism. In hormone therapy, reduction in Homocysteine level has seen [8-13]. Female sex hormones at different stages of the Menstrual period are subject to fluctuations. The first five days of bleeding as the menstrual, 11 days later as the follicular phase, the last 12 days are considered Luteinizing period and Fourteenth day of menstruation is ovulation days [14].

De Cree (1999) stated that due to lower Homocysteine level in Luteinizing period, it be argued that This increase in estrogen level in this stage can reduce Homocysteine. In their study after one short period of training program, a Homocysteine level has increased significantly as an acute exercise session. He stated that increased activity following the same basic values in the follicular phase of the Luteinizing. Due to limited studies in this area, Cannot be surely insist about the effect of different exercise protocols and menstrual periods on stages of plasma...
Homocysteine levels [10]. Subjects previous activity reduces plasma Homocysteine in men and women [12,13,15] that this reduction is high in the subjects with strenuous trainings [15]. But some researchers believed that there wasn’t a relationship between reduced Homocysteine levels and previous activity [9,16]. On the other hand the amount of Homocysteine increases after strenuous activity [10,15,17,18] that this increasing was progressive after maximal activity [10] and in untrained subjects and subjects with low intensity trainings [15]. Also may be Folate, vitamins B6 and B12 consumption can reduce Homocysteine levels [12,15,19,20]. High sensitivity C reactive protein (HS-CRP) may be a piece of one acute inflammatory response that in cases of injury or infarction can rise to ten thousand times. This protein had a negative relationship with cardiovascular and respiratory fitness and provoked with increased fat mass and body mass index (BMI) [21]. Some studies showed that HS-CRP level Respectively, in elderly [22,23], males [22-24] and fatty persons [1,2,23] is higher than juveniles, females and inactive persons. In the other hand researcher opinion is that estrogen consumption (either alone or in combination with progesterone), resulted in CRP increasing While endogenous estrogen may reduce CRP. These proteins release during bacterial infections, surgeries, strokes, heart tissue and severe and prolonged sports [24]. Hinton and collegiate (2006) showed that CRP in hormone therapy group was significantly higher than the group with regular menstrual cycle. In This study, hormone consumption is a more important factor in raising the CRP compared with the irregularities menstrual cycles due to vigorous activity [25]. Souter (2005) also confirms these study results [26]. Although estrogen consumption as a drug increases CRP, but endogenous estrogen will be a decrease it [14,27].

Jilma el al. (1997) and Blum el al. (2005) also achieved similar results on the effect of menstrual stages cycle on CRP But none of them, did not included activity effect [28,29]. Church el al. (2002) reports a negative relationship between HS-CRP and subject’s physical fitness [1] this result was similar with some other studies [30]. However, some research also have been reported no relationship between HS-CRP and physical activity [31,32] and body weight [2]. In contrast, the results of some studies suggest that Hs-CRP levels increases after a session of intense aerobic activity such as marathon running [33] and intense anaerobic exercise [34].

As mentioned the effect of sex hormones, particularly estrogen, as well as different stages of the menstrual cycle on Homocysteine and CRP be somewhat clear but the effect of Stages results in the menstrual cycle are contradictory. And information was available about the effects of activity on these indicators. But the study on simultaneous period and menstrual cycle rarely done and is still uncertainty. Therefore this study investigated the effect of different menstrual cycle phases (mid follicular and luteinize period) on plasma Homocysteine and serum HS-CRP after an aerobic exhaustive exercise in athlete and non athlete females.

**MATERIAL AND METHODS**

This study was semi- experimental and sectional. For executing this study, 10 females were selected and consent form fulfilled via all subjects. Selected Persons did not experience painful menstrual periods in the last year and none of them not use specific drug within 6 months and have normal and regular menstrual periods and their period during were 26 to 32 days. After completing the questionnaire and provide comments to the disease records, consultant obstetrics and gynecology was conducted by experts.

The subjects were asked; they recorded their diet for one day before the test and in later stages are also trying to follow the same diet. 24 hours before the testing, also subjects were prohibited from eating any protein containing foods and folic acid and B vitamins and various drugs and strenuous activities. Before the main testing period, subjects randomly divided into two groups: mid-follicular and mid-luteinize period. To reduce the influence of circadian variations, all measurements were performed between 13.00 to 17.00 hours.

In the first stage of testing, the weight, height, subcutaneous fat was measured, then after 10 warm-ups, the blood sampling was done. After blood sampling, subjects placed in treadmill and the Bruce treadmill test was run. Bruce protocol continues to the exhaustion demand and immediately after the test (up to 10 seconds after the test), second stage blood sampling was performed. Blood samples up to an hour after taking the blood and with cold chain observance, were transferred to the laboratory. For this study, Serum HS-CRP, total plasma Homocysteine and Stradiol, progesterone, LH and FSH measured with isolate experiments.
Table 1: Changes in studied variables in mid-follicular phase in both groups

<table>
<thead>
<tr>
<th>Statistics</th>
<th>Durations</th>
<th>Mean Differences</th>
<th>SD</th>
<th>T</th>
<th>df</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma HCY (mid-follicle)</td>
<td>0.3100</td>
<td>0.5713</td>
<td>0.543</td>
<td>17</td>
<td>0.594</td>
<td></td>
</tr>
<tr>
<td>Plasma HCY (mid-luteinize)</td>
<td>0.8956</td>
<td>0.6995</td>
<td>1.280</td>
<td>0.218</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma HS-CRP(mid-follicle)</td>
<td>0.8778</td>
<td>0.7221</td>
<td>1.215</td>
<td>0.241</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma HS-CRP(mid-luteinize)</td>
<td>1.3856</td>
<td>0.9425</td>
<td>1.470</td>
<td>0.160</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma HCY(mid-follicle)</td>
<td>0.9511</td>
<td>0.6821</td>
<td>1.394</td>
<td>0.181</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serumic HS-CRP(mid-follicle)</td>
<td>0.1156</td>
<td>0.5284</td>
<td>0.219</td>
<td>0.830</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma HCY(mid-luteinize)</td>
<td>-0.3600</td>
<td>0.6432</td>
<td>-0.560</td>
<td>0.583</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serumic HS-CRP(mid-luteinize)</td>
<td>0.9800</td>
<td>0.7180</td>
<td>1.365</td>
<td>0.190</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Statistical Analysis:** The pre and post-test data’s were analyzed with a paired t-test and other data analyzed by sample t test. An alpha level of (0.05) was used in determining statistical significance using the SPSS program for Windows, version 18.0.

**RESULTS**

Results for changes in studied variables in mid-follicular phase in both groups are summarized in a Table 1.

**DISCUSSION**

Amounts of hormones in all subjects in both the test and after the activity were within normal limits but, there weren’t any significant differences. Also results showed that strenuous activity increases plasma Homocysteine and HS-CRP in both luteinize and follicular periods. Pre activity Homocysteine in follicular period 4% was higher than follicular period. Basic Homocysteine amount in both periods 15% increased after activity. On behalf of HS-CRP, results showed that after activity increasing in follicular period was lower than luteinize period and HS-CRP in follicular period increased 12% further than follicular period.

De Cree and coworkers (1999) indicated that there was a very light difference in Homocysteine concentration between follicular and follicular period. But totally, the basic Homocysteine level after subsequent activity in follicular period was higher than luteinize period. They were stating that lowering Homocysteine level in luteinize period is due to increased estradiol [10]. Guthrie et al. (2005) stated that Homocysteine levels in premenopausal women’s aged 45-55 was lower than postmenopausal women’s and this may be due to loss of estrogen at menopause [11]. Current research results revealed that Homocysteine levels in follicular period 4% was higher than follicular period that this results was similar with De Cree and coworkers results.

In this study the basic HS-CRP in follicular period was 3.16 mg/L that in luteinize period with 12% increasing, reached to 3.58 mg/L. this results is similar to Guthrie and coworkers (2005) [11]. Estrogen from mid-follicular period to mid-luteinizes period increase 12 times that this increase was correlation with HS-CRP increases (12%). These results have positive correlation with the results of Jilma el al. (1997) and Wander et al. (2008) [29,36]. They indicated that there are positive correlation between CRP in mid-luteinizes period with progesterone increasing.

Wunder et al. (2006) and Blum et al. (2005) expressed the contrary conclusion (negative correlation between estrogen and CRP) [27,28]. On the other hand, LH and FSH in non athlete group in mid-follicular period was 13.42 and 9.15 mIU/mL respectively that in luteinizes period 12% and 19% was decreased. Between serumic HS-CRP and LH and FSH in mid-luteinizes period seen negative correlation.

In the present study, this factor alone was used for homogenization and were measured in the mid-follicular phase or Luteinizing (depending on the person’s entry to the test period). Therefore they may be in the middle of Luteinizing are increased and increased subsequent HS-CRP. Many studies observed CRP increase following strenuous activity [38,39,40]. In this study CRP increase following strenuous activity was 22% and 31% respectively. But since CRP increase following strenuous activity follicular period was higher than luteinizes period, this seems that such increasing is correlated with amounts of estradiol and progesterone and also LH and FSH [28,29,36,37].

**CONCLUSION**

Levels of Homocysteine prior to exercise activity and its increases in the subsequent intense activity in the Luteinizing phase was lower than follicular phase, may Girls work on Luteinizing period, have lower risk of cardiovascular diseases Although our results are in
contrast to the findings on behalf of HS-CRP. However, it is confirmed that the activities have enhancing role as indicators for cardiovascular risks.

REFERENCES


