Psycho-physiologic Effects of High Intensity Interval Trainings in Aged Ovariectomized Rats: a Pilot Study

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Introduction
Menopause is one of the most inevitable events in a woman’s life, which can lead to loss of stimulation of follicles and ovarian dysfunction with complete menstrual cessation (1). Hormonal changes, hot flashes and night sweats and other physiological changes (2) during this period can lead to weight gain and the development of metabolic syndrome (3), and then affect the physical and mental health of life (2). Reducing hormones such as follicular stimulating hormone (FSH) and estradiol (E2) has a significant relationship with increased calorie intake, decreased lipolysis of adipose tissue, and decreased physical activity, which increases the amount of visceral fat mass and increases the lipocyte lipid synthesis, change in levels of adipokines, triglyceride and pre-inflammatory agents, such as C-reactive protein, and result in increased stress hormones (4). Also, with regard to the relationship between body health and soul health, researchers believe that reducing social participation and unwillingness to communicate with others, postmenopausal women are prone to depression (1). On the other hand, today the role of physical activity in controlling weight, reducing fat mass and physical health has been proven. Exercise activity seems to modify metabolism, reduce visceral fat, and ultimately reduce weight (5). In this regard, studies have shown that long-term exercises reduce fat mass, height to waist ration and body mass index (BMI) (5), leptin, resistin, anthropometric characteristics (6) and improvement in memory, sleep quality (7), depression and anxiety (8) in postmenopausal...
women. However, a study by Courneya et al., showed that aerobic trainings for 300 minutes per week and 200 minutes per week had the same effects on quality of life and quality of sleep in postmenopausal women, but the duration of sleep after 300 minutes trainings per week in single women and postmenopause obese women was higher (9). On the other hand, recently adaptation to high intensity interval training (HIIT) has attracted the attention of researchers in the field of sport sciences. Regarding the effect of interval trainings, different results have been reported. For example, moderate intensity interval trainings (MIIT) reduced depression and anxiety as well as improved anthropometric characteristics of patients with myocardial infarction (10). Adjusted HIIT exercises had a significant effect on weight loss, body mass index, decrease in resting heart rate and non-significant improvement in mental health, but had no significant effect on anxiety and depression in patients with schizophrenia (11). Continuous training seems to have a long lasting effect on the physical health of postmenopausal women. However, given the rapid increase in risk factors in these individuals, it is necessary to conduct research on a variety of short-term trainings that have faster effects on the improvement and prevention of menopausal risks. Also, due to the contradiction in the effect of HIIT trainings and the observance of precautionary aspects in conducting this research in human samples and lack of sufficient information on the effect of this type of training on aging and menopause, this study aimed to investigate the effect of HIIT on body weight, visceral fat mass, aerobic power, anxiety, and depression in aged ovariecetomized rats.

Methods

In this experimental study, 12 female Sprague-Dawley rats, with an approximate age of 13-15 months, were purchased from the Laboratory and Animal Breeding Center of Marvdasht Islamic Azad University and transferred to the Animal Exercise Physiology Laboratory of this university. All rats were kept seven days in standard conditions in transparent polycarbonate cages with an autoclave capability, optimal temperature (20 to 24 °C), relative humidity of 55-65%, 12-hour darkness-lighting darkness cycle, and free access to water and food. On the eighth day, the rats ovariectomized. After ovariectomy, animals were kept under controlled conditions for 12 weeks with the aim of developing osteoporosis (12). Then all rats were randomly assigned to two groups of control and HIIT. For familiarity and compatibility with training, HIIT group were placed on treadmill for 2 weeks. In the first week the rats in HIIT group ran on treadmill at a speed of 10 m/min with a zero-degree gradient for 10 minutes and in the second week ran at a speed of 10 m/min with a zero-degree gradient for 30 minutes. Then HIIT group ran on treadmill for 8 weeks, three sessions per week, and each session with 9 intervals of one minute, with intensity of 90 to 95% VO$_{2}$max and one minute running between each interval with intensity of 50% VO$_{2}$max. It should be noted that the rats for warming up (at the beginning of the trainings) and cooling down (at the end of the trainings) ran 4 minutes with an intensity of 55% VO$_{2}$max (13). To measure the maximum oxygen consumption (VO$_{2}$max), rats ran 5m on the treadmill at a speed of 6m/min with a zero-degree gradient for 5 minutes (for warm up), and then every 3 minutes the speed of the treadmill, rises to 3 m/min until the animals reach the level of extinction and no longer able to continue. The criterion for reaching VO$_{2}$max was the inability of the rats to continue the exercise protocol with increasing speed and collision 3 times in one minute to the end of the treadmill, so VO$_{2}$max measured by using speed. To measure the weight of rats after anesthesia, a 10-g scale was used (Merk Company, Germany). To measure visceral fat mass, the abdominal cavity was cut longitudinally on the midline of the abdomen and mesenteric fats (under the peritoneum, around the kidneys, around the ovary and the
uterus, the gastrointestinal tract and the front of the lower back muscles) were isolated and measured by scale (Merk Company, Germany) (14). To measure anxiety, elevated plus-maze was used. This assessment was based on a model presented by Pellow et al. The rats were placed within the central area of the maze, in such a way that they were placed in an open arm. Within 5 minutes, when the animal freely moved in different parts of the maze, the number of times the animal entered the open arm, the number of times the animal entered the closed arm, the time the animal remained in the open arm, and ultimately, the time that the animal remained in the closed arm recorded. The mining of entering the open or closed arm was when all four feet of the animal were entered in arm. The time spent in each arm was calculated accordingly (15).

Forced swimming test (FST) is one of the most reliable and commonly used tests to measured depression in rodent. This test was carried out using an acrylic glass cylindrical vessel of 45 in diameter and a height of 79 cm, which was filled up to 30 cm with water with temperature of 23-24 °C. The rats were placed gently. The motionless in hands and feet as well as floating considered as immobile and its duration recorded as motionless. In present study we consider the duration of immobility as depression (16). To analyze the findings of this study, Shapiro- Wilk, paired sample t- test and independent sample t- test were used (p≤ 0.05).

### Results

Mean and standard deviation of weight and aerobic power presented in Table 1 and visceral fat mass, percentage of time spent in the open arm, percentage of number entered to open arm and depression presented in Table 2. The results of paired sample t- test showed that in the control group there was no significant difference in pre-test and post-test of weight (t = 0.76, p = 0.47). However, aerobic power in post-test time was significantly lower than the pre-test (t = 3.541, p = 0.01). Also in post-test of HIIT group in compare to pre- test weight significantly decreased (t = 7.38, p = 0.01) and aerobic power significantly increased (t = 3.541, p = 0.001) (Table 1). The results of independent sample t- test showed that in HIIT group reduction of weight (t=-4.168, p=0.002) and increase of aerobic power (t=9.37, p=0.001) were significantly higher than control group (Table 1). In HIIT group visceral fat mass (t=7.45, p=0.001) and duration of immobilization (t=6.82, p=0.001) were significantly lower than control group nevertheless percentage of time the animal spent in the open arm (t=-3.962, p=0.003) and percentage of number of times the animal entered the open arm (t=-2.376, p=0.03) were significantly higher than control group (Table 2).

### Table 1. The results of paired sample t- test and independent sample t- test for review the effect of HIIT on weight and aerobic power

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Time</th>
<th>Mean ± SD</th>
<th>Paired sample t-test</th>
<th>Independent sample t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>HIIT</td>
<td>Pre- test</td>
<td>235.72±12.47</td>
<td>7.387</td>
<td>0.001</td>
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<tr>
<td></td>
<td>Control</td>
<td>Post- test</td>
<td>202.0±2.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pre- test</td>
<td>246.66±14.61</td>
<td>0.769</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post- test</td>
<td>242.50±5.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIIT</td>
<td>Pre- test</td>
<td>34.35±3.12</td>
<td>14.33</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Post- test</td>
<td>34.35±5.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pre- test</td>
<td>26.16±4.99</td>
<td>-3.541</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post- test</td>
<td>20.50±3.13</td>
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</tbody>
</table>
Table 2. The results of independent sample t-test for review the effect of HIIT on visceral fat mass, percentage of time spent in the open arm, percentage of number entered to open arm and depression

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Mean± SD</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visceral fat mass</td>
<td>HIIT</td>
<td>0.775±0.28</td>
<td>7.451</td>
<td>0.001</td>
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<tr>
<td></td>
<td>Control</td>
<td>4.484±1.18</td>
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</tr>
<tr>
<td>Percentage of time spent in the open arm (Percentage)</td>
<td>HIIT</td>
<td>19.67±11.20</td>
<td>-3.962</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1.53±.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of number entered to open arm (Percentage)</td>
<td>HIIT</td>
<td>51.19±10.93</td>
<td>-2.376</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>36.11±11.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depression (second)</td>
<td>HIIT</td>
<td>106.49±20.73</td>
<td>6.820</td>
<td>0.001</td>
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<tr>
<td></td>
<td>Control</td>
<td>169.56±9.13</td>
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</table>

Discussion
The results of this study showed that eight weeks of HIIT significantly increased in aged ovariectomized rats. Menopause has always been reported with an increased risk of cardiovascular disease, cancer, and metabolic diseases in women (17). Also, increased calorie intake and unwillingness to perform regular exercise, resulted in losing physiological efficiency and capacity of body for oxygen transfer (18). In this regard, Bondarev et al. showed that the fat mass and the health-related physical fitness factors in postmenopausal women were significantly weaker than women before menopause (18); Earnest et al. Stated that the peak oxygen consumption (VO$_{2\text{peak}}$) reduces with age increases in postmenopausal women (17). On the other hand, the results of this study showed that HIIT has a significant effect on weight loss and reduction of visceral fat mass in aged ovariectomized rats. It seems that HIIT can enhance the mechanism of increasing the sensitivity of insulin and glucose transporters, and adaptation to this type of exercises increases the aerobic metabolism enzymes, which simultaneously by increase lipolysis of the adipose tissue and function of transfer the free fatty acids to the tricarboxylic acid cycle (TCA cycle ) reduces low density lipoprotein (LDL), very low density lipoprotein (VLDL), circulating triglycerides, and this increase in adipose tissue lipolysis leads to a decrease in fat mass and weight. On the other hand, there is a direct and significant relationship between weight loss and increase in VO$_{2\text{max}}$ in obese people (19). Several studies have been done on the effect of HIIT that reported different results. In line with the results of present study, the researchers showed that six weeks of HIIT improved plasma lipids, increased VO$_{2\text{max}}$, and improved body composition in obese overweight men (21-19). However, HIIT did not significantly affect the body fat loss changes in obese men (22). Also, the results of present study showed that eight weeks of HIIT had a significant effect on the reduction of anxiety-like behavior and depression in aged ovariectomized rats. The reduction of estrogen and other sex hormones in create the disorders such as vasomotor symptoms including hot flushes, night sweats and other distressing physiologic factors, cause mood changes, sleep disorders, chronic pain, anger and an increased risk of depression (23). On the other hand, exercise seems to affect the secretion of neurotransmitters that play an important role in menopausal symptoms. Also, regarding the effects of exercises on anxiety and depression, increasing levels of endorphins, decreasing levels of catecholamines and stress hormones like resting cortisol seems to be a justifiable mechanism for improving anxiety and
depression in menopause (25,24). Also, according to the results of present study, that HIIT improved physiological factors in aged ovariectomized rat; It seems that decreasing fat mass and increasing aerobic power with the mechanism of norepinephrine (NE) and serotonin increase can decrease depression (26). The results of the present study were consistent with the results of the Choi study that investigated the effect of HIIT on anxiety and depression in patients with myocardial infarction (10) and elderly people with depression (27). However, the results of the present study were inconsistent with study of Wu et al., who examined the effect of HIIT on depression and anxiety in patients with schizophrenia (11). Considering the many studies in past, reviewed the effects of interval trainings with low to moderate intensity, as well as long-term continued trainings in elderly people, in present study the researches could not compare the results of HIIT with the intensity of 90 to 95 % \( \text{Vo}_{2\text{max}} \) to reported studies; Therefore, it seems that conducting similar studies with the present study and comparing the results with the results of the present study would provide useful information for applied researches in future.

**Conclusion**

Regarding the results, it seems that HIIT have positive effects on anxiety-like behaviors, depression and improvement of physiological characteristics in aged ovariectomized rats.

**Ethical issues**

Not applicable.

**Authors contributions**

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

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