The effects of the six week high intensity interval training (HIIT) on resting plasma levels of adiponectin and fat loss in sedentary young women

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Abstract:
Introduction:
High intensity interval training (HIIT) enhances the capacity for fat oxidation and mitochondrial enzyme activity, but the effect of HIIT on plasma adiponectin levels is not yet clear. The aim of this study was to determine the effects of the six week HIIT on resting plasma levels of adiponectin, insulin sensitivity and resistance and fat loss in sedentary young women.

Materials and Methods:
Twenty two students voluntarily participated in this study and were randomly divided into two groups of experimental and control. The experimental group performed three HIIE sessions per week for 6 weeks. Each session consisted of either four to six repeats of maximal sprint running within a 20 m area with 20–30 sec recovery. Fasting blood samples were collected 24 hours before and after the exercise protocol. Data were analyzed using dependent t-test and paired t-test.

Results:
Statistical analysis of the data showed that resting adiponectin levels post-intervention in the experimental group significantly increased (p=0.047). Significant improvements (P<0.05) were found in body fat percentage, body mass index (BMI) and waist to hip ratio (WHR) post-intervention (experimental group).

Conclusion:
The findings of present study demonstrated that high-intensity interval training is an appropriate method to reduce body fat and improve anthropometric indices in sedentary young women. Also, the intensity of exercise as an important factor in the increase of adiponectin levels could be noted.

Keywords: Training, Exercise, Adiponectin

Introduction
The relationship between physical activities and health has a long history. Sedentary lifestyle leads to a dramatic reduction in physical activities that increases the risk of chronic diseases in women and men. In sedentary people, obesity and weight gain are strongly related to development of chronic diseases such as hypertension, hyperlipidemia and insulin resistance which all are risk factors for cardiovascular diseases (1). Compared with women, men are more afflicted with cardiovascular diseases. This disease is the...
main cause of death in men and women (1). Obesity and its related diseases is one of the major causes of death in industrialized and developing countries. Obesity is considered as a part of a disease called metabolic syndrome which in fact is related to increase in body fat percentage, particularly in visceral areas (2).

In recent years, in order to better understand the pathophysiologic mechanisms of obesity that is the main cause of metabolic disorders, central regulatory elements in energy homeostasis such as food intake behavior and energy cost have been noted (3). Although, at first glance, the process of changes in energy intake and expenditure to a positive balance is considered a major factor, it seems that there are complex mechanisms beyond this simple principle which are very important from cellular and molecular aspects. In studies related to the mechanisms of obesity and incidence of cardiovascular diseases, increased body fat has been introduced as the most influential factor (4). For a long time adipose tissue was wrongly considered as the fat storage original tissue, but in recent studies adipose tissue is known as an active endocrine and paracrine organ with synthesis and secretion of a set of adipocytokines and bioactive mediators such as leptin, resistin, visfatin and adiponectin. It not only is involved in body weight balance control, but also by affecting lipid, metabolic, and inflammatory profile justifies the relationship between overweight and obesity with insulin resistance, diabetes and atherogenic cardiovascular diseases (5).

Adiponectin, also known as Adipo Q, apM-1, GBp28, and Acrp30 is one of the adipocytokines whose gene expression and secretion is only done by adipose tissue (6). In the mid-1990s, several independent research groups described adiponectin as a new 30-kDa protein containing 244 amino acids that is produced by apoMI gene and is primarily secreted by white adipose tissue (7). Adiponectin concentration is 5 to 30 microgram per milliliter and constitute approximately one percent of total plasma protein. There are three forms of adiponectin with high, medium and low molecular weight in blood circulation (8). Adiponectin has different physiological roles. Experimental findings show that this protein increases insulin sensitivity and has anti-atherogenic and anti-inflammatory properties (9).

There is an inverse correlation between circulating adiponectin levels with body fat percentage and glucose tolerance (10). Moreover, in vitro and in vivo studies show that glucose metabolism and insulin sensitivity are regulated by adiponectin through activating AMP kinase (11). In addition to regulating glucose homeostasis and lipid metabolism, adiponectin is related to cardiovascular health. Available evidence suggests that decrease in circulating adiponectin levels is related to increase in prevalence and severity of atherosclerosis (12). Unlike other adipocytokines, mRNA expression and adiponectin levels are less in people with obesity, diabetes and coronary heart disease, and body weight loss increases it (13). Hypoadiponectinemia can cause insulin resistance and increased risk of type 2 diabetes and it is likely a risk factor for coronary heart disease (14). Therefore, the mentioned indicator has attracted the attention of many researchers of the medical and sports community and the effect of various exercise protocols on it is being studied.

Although most exercise programs designed for weight loss are focused on steady state exercise activities for 30 minutes and a moderate intensity in most days of the week, recently it has been demonstrated that this type of exercise programs will lead to little or no fat loss (15). Therefore, a type of exercise protocol is required that can both be tolerated by sedentary overweight people and lead to more effective fat loss.
One of the exercise protocols that have been recently of interest to exercise physiology researchers is high intensity interval training. High intensity interval training includes very high intensity exercise intervals and resting active recoveries with very low intensity (15). Previous studies suggest that high intensity interval training increases fat oxidation capacity and mitochondrial enzyme activity (16). However, the previous few studies on the effect of high intensity interval training on plasma adiponectin levels have obtained quite contradictory results (increase, decrease and no change). The effect of exercise is not evident on it and requires further studies. According to the available information, there is a relationship between reduced levels of adipose tissue and increased adiponectin levels, and high intensity interval training reduces body fat, but few studies have been conducted in this field. For the above reasons, it is necessary to study changes in adiponectin concentration due to high intensity interval training. It is possible that overweight and sedentary people have problems to do high intensity interval training protocols and adhere to them, but capability of this training method to reduce body fat has led researchers to seek a type of high intensity interval training protocol that in addition to reducing body fat is acceptable by sedentary people.

The aim of the current study is to determine the effect of six-week high intensity interval training on resting plasma levels of adiponectin, fat loss, insulin resistance and sensitivity in sedentary young women.

**Materials and Methods**

In this quasi-experimental study, 22 sedentary female students voluntarily participated. At first, the necessary knowledge about the research conditions and its stages were given to the subjects. Then, information about the physical activity level and health condition of the subjects was obtained by questionnaires and finally they gave their written consent to participate in the research. Subjects were randomly divided into two experimental and control groups of 11 people. None of the subjects had done high intensity interval trainings at least in the past three months. Two weeks before the start of training, initial assessments including height, weight, body fat, waist to hip ratio (WHR) and body mass index (BMI) were measured. Harpenden skinfold caliper and three-site method (triceps, suprailiac, and thigh) were used to measure body fat percentage.

The subjects of experimental group performed the training protocol at a distance of 20 meters which was marked by three cones in three sessions per week for six weeks as follows (Figure 1). In the exercise protocol of 40-meter maximal shuttle run test, subjects at first sprinted from starting point (cone 1) toward cone 2 on route A, then returned in the opposite direction on route B, sprinted 20 meters to the third cone and then finally sprinted back on route C to the starting point (cone 1) sprinted again so that distance of 40 meters was completed. The subjects continued to do so with maximum speed so that the exercise protocol time period of 30 seconds was completed and after 30 seconds of recovery, they repeated the exercise protocol. The exercises progressed by increasing the number of exercise repetitions for 30 seconds from four times in the first and second weeks to five times in the third and fourth weeks and then six times in the fifth and sixth weeks. Before starting the exercise protocol, the subjects in each session warmed themselves for five minutes and at the end of each workout session, they cooled down themselves for five minutes, as well. This protocol is a valid test to assess anaerobic performance (17). In the six weeks of performing the workout protocol, subjects in the control group had no regular exercise.
Figure 1: Schematic design of exercise protocol

Fasting blood samples (10cc) were drawn from the antecubital veins of all subjects in both groups 24 hours before the first workout session and 48 hours after the last session (at 8:30 am). Blood samples were poured into tubes containing EDTA anticoagulant immediately and then they were centrifuged at 3000 rpm for 10 minutes at a temperature of 4 °C. The plasma obtained was maintained at -80°C for subsequent measurements. Adiponectin serum level was measured by ELISA method by using total adiponectin-specific kit (Mediagnost, Germany; with the sensitivity of 0.6 ng/ml and intra-assay and inter-assay coefficients of variation, 6.7% and 4.7%, respectively) and the fasting blood glucose level was measured by enzymatic method of glucose oxidase by using glucose kit (BioSystem, Spain; with the sensitivity of 0.23 mg/dL, and intra-assay and inter-assay coefficients of variation are 1.2% and 0.9%, respectively). Insulin serum level was also measured by a kit (Diasorin, Italy; with the sensitivity 0.5 micro units per ml and the coefficients of variation 20%). Values of pancreatic beta-cell function indicators, insulin resistance (HOMA-IR) and insulin sensitivity (QUIKI) were calculated by the following formulas and by using serum glucose and fasting insulin concentrations (18-19):

\[ \frac{22.5 \div \text{plasma glucose concentration (mmol/L)} \times \text{plasma insulin concentration (micro unit per ml)}}{\text{plasma glucose concentration (mmol/L)} \times \text{plasma insulin concentration (μIU / ml)}} = \text{insulin resistance index} \]

\[ 3.5 - \text{plasma glucose concentration (mmol/L)} \div \text{plasma insulin concentration (μIU / ml)} \times 20 = \text{beta cell function index} \]

\[ \log \text{Fasting glucose (mmol/L)} + \log \text{fasting insulin (μIU / ml)} / 1 = \text{insulin sensitivity index} \]

The data were analyzed by SPSS-16 statistical software. Kolmogorov Smirnov test was used to determine normality of data and given that the results of this test showed normality in distribution of data, parametric statistical tests were used. First, independent t-test was used to ensure consistency of both groups before starting workout and then intragroup comparison was performed by paired t-test and intergroup comparison was performed by independent t-test. In all statistical tests the significance level was considered \( \alpha = 0.05 \).

**Results**

The results showed that after exercise intervention, resting adiponectin concentration increased significantly in the experimental group (from 6.92 ± 2.68 to 7.56 ± 2.97) (P=0.024). Also a significant
The effects of the six week exercise intervention on adiponectin concentration in sedentary young women. Also the body fat percentage, BMI and WHR were significantly decreased after training exercise intervention. Although, after six weeks of high intensity interval training, the insulin sensitivity levels were increased and the insulin resistance levels, beta cell function and weight were decreased in all subjects, these changes were not statistically significant.

The effect of exercise on plasma adiponectin concentration is unknown. Some researchers have reported the increase (20-21), and others have reported no changes (15, 22) and decrease (23) of adiponectin levels in response to exercise. These inconsistencies may be due to the differences in the severity, duration, exercise type, presence or absence of diabetes and cardiovascular disease, weight, age and gender of the subjects.

### Discussion

The results of the current study showed that six-week high intensity interval training led to a significant increase in plasma adiponectin concentration in male rats. After exercise intervention, insulin sensitivity increased and insulin resistance, weight, insulin, glucose and beta cell function decreased, but these changes were not statistically significant (P<0.05). In the control group, no significant changes were observed in any of the research variables (Tables 1 and 2).

### Table 1: Subjects’ anthropometric variables (mean ± standard deviation) before and after exercise intervention

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Pre-test</th>
<th>Post-test</th>
<th>Significance Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>control</td>
<td>18.27±1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>18.54±0.67</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>Control</td>
<td>166.2±6.88</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>163.8±4.72</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>Control</td>
<td>68.55±7.23</td>
<td>68.47±6.90</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>66.91±6.98</td>
<td>66.18±7.10</td>
<td>0.059</td>
</tr>
<tr>
<td>Body mass index (kg/m2)</td>
<td>Control</td>
<td>24.88±2.03</td>
<td>24.85±2.00</td>
<td>0.179</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>25.00±1.99</td>
<td>24.62±2.24</td>
<td>0.042*</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>Control</td>
<td>0.85±0.03</td>
<td>0.85±0.05</td>
<td>0.741</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>0.85±0.04</td>
<td>0.84±0.03</td>
<td>0.044*</td>
</tr>
<tr>
<td>Body fat (percentage)</td>
<td>Control</td>
<td>36.34±3.19</td>
<td>36.02±3.11</td>
<td>0.583</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>37.75±3.91</td>
<td>33.30±2.22</td>
<td>0.013*</td>
</tr>
</tbody>
</table>

* P≤ 0.05 significance of pre-test vs. post-test

The result of the current study related to significant increase in plasma adiponectin concentration is consistent with findings of Garekani et al., Zeng et al. (20, 21). Garekani et al. investigated the effects of different intensities of exercise (low intensity from 50 to 55%, medium intensity from 70 to 75% and high intensity from 80 to 85% maximal oxygen uptake) on adiponectin concentration in male rats (20). The results of their study showed that after exercise the total serum adiponectin concentration with high molecular weight increased significantly in both groups with high and moderate intensity exercise, but the decrease in serum glucose and insulin concentrations and insulin resistance in all three groups was not statistically significant. As a result, they reported that the intensity of training as an important factor in increasing serum adiponectin...
The effects of the six week exercise (20). Zeng et al. also showed that changes in total plasma adiponectin levels in response to exercise activities were dependent on the intensity and duration of exercise (21). In this regard, Kraemer et al. in a review article noted the intensity and duration of workout protocol as the key factors affecting the adiponectin response to exercise (24).

Given that all three studies performed by Garekani, Zeng and Kraemer have noted that the intensity of workout program as an important and effective factor in response of plasma adiponectin concentration to exercise activities (20, 21, 24), it seems that high intensity exercise intervention used in the present study is one of the possible reasons of the significant increase in adiponectin. Therefore, it can be said that the intensity of workout is the most effective factor for adiponectin release from adipose tissue into the bloodstream. The significant adiponectin increase in the current study is inconsistent with the results of studies performed by Buchan et al., Trapp et al., Richards et al. and Numaa et al. (15, 22, 23, and 25). It seems that the type and volume of exercise (intensity, duration and frequency) as well as differences in subject types (animal or human and subject’s gender) are of the main reasons for the differences in findings of the current study and other studies related to changes in adiponectin concentration.

In the present study, body fat percentage decreased significantly after high intensity interval training, which is consistent with the results of Trapp et al. (15). Trapp et al. reported that high intensity interval training compared with steady state endurance exercise resulted in a significant decrease in body fat percentage in sedentary young women (15). Generally, the results of previous studies suggest that high intensity interval training increases the fat oxidation capacity and mitochondrial enzyme activity (16). On the other hand, due to the significant increase in adiponectin in this study and based on the results of previous studies it has become clear that adiponectin removes free fatty acids from plasma by stimulating free fatty acid consumption and oxidation in muscle (26). This finding can be explained as high intensity interval training increases fatty acid oxidation by increasing circulating adiponectin levels in skeletal muscle, which ultimately reduces body fat percentage. Also, due to the inverse relationship of adiponectin with

Table 2: Changes in adiponectin and other research variables (standard deviation± mean) in control and experimental groups before and after 6 weeks of workout

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Pre-test</th>
<th>Post-test</th>
<th>Significance level (P-Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>Control</td>
<td>5.83±2.56</td>
<td>5.89±2.80</td>
<td>0.880</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>6.92±2.68</td>
<td>7.56±2.97</td>
<td>0.024*</td>
</tr>
<tr>
<td>Insulin (micro unit per ml)</td>
<td>Control</td>
<td>12.17±7.73</td>
<td>12.27±7.04</td>
<td>0.472</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>11.90±5.22</td>
<td>9.37±3.58</td>
<td>0.092</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>Control</td>
<td>95.88±9.71</td>
<td>95.22±8.26</td>
<td>0.306</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>92.36±8.06</td>
<td>89.00±7.97</td>
<td>0.166</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>Control</td>
<td>2.88±2.20</td>
<td>2.88±1.74</td>
<td>0.990</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>2.75±1.26</td>
<td>2.14±0.89</td>
<td>0.062</td>
</tr>
<tr>
<td>Insulin sensitivity</td>
<td>Control</td>
<td>0.330±0.023</td>
<td>0.339±0.03</td>
<td>0.774</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>0.336±0.03</td>
<td>0.349±0.02</td>
<td>0.142</td>
</tr>
<tr>
<td>beta cell function</td>
<td>Control</td>
<td>136.91±68.39</td>
<td>138.03±77.24</td>
<td>0.362</td>
</tr>
<tr>
<td></td>
<td>Experimental</td>
<td>146.69±62.30</td>
<td>131.19±42.16</td>
<td>0.388</td>
</tr>
</tbody>
</table>

*P≤ 0.05 significance of pre-test vs. post-test
body fat percentage, it seems that one of the other possible reasons for adiponectin increase in the present study is decrease in body fat percentage. Although in the present study insulin resistance, plasma insulin levels, fasting glucose and beta cell function decreased and insulin sensitivity increased, these changes were not statistically significant. These findings are consistent with the results of Garekani et al. study (20). There are few studies on the effects of high intensity interval training on resting adiponectin levels and different findings of these studies and failure to provide or suggest any specific mechanism by the researchers makes it difficult to interpret the results obtained in the present study. However, some studies can be referred to for interpretation of these results, which have shown insulin resistance improves despite no changes in adiponectin concentration in response to prolonged exercise (13). Many researchers reported that increase in insulin sensitivity due to exercise occurs independent of changes in plasma adiponectin levels (6). These studies have not referred to a direct causal relationship between insulin resistance and adiponectin. On the other hand, most studies have shown that increase in insulin sensitivity due to exercise in individuals with normal weight is lower than those who are overweight (27). Given that the subjects who participated in the current study had no chronic diseases and their average weight was normal, small changes in insulin resistance and sensitivity seem natural. Due to the positive changes in insulin sensitivity and resistance, though insignificant in the present study, it seems that high intensity interval training is an effective factor in prevention of metabolic disorders in sedentary young people.

In this study, despite increased adiponectin, decrease in WHR and BMI in the experimental group, no significant decrease was observed in body weight. Although most studies have shown that increased circulating adiponectin is associated with weight loss or fat loss and there is some evidence that adiponectin concentration has an inverse relationship with adipocytes size and body weight (7), some studies have not confirmed this result. It has been reported that abdominal fat reduction due to exercise is associated with no change in serum adiponectin concentration in men with type 2 diabetes (28). Also in other studies no change was seen in circulating adiponectin levels despite weight loss due to exercise (15). A possible explanation for this finding is increased adiponectin due to physical activity as a result of stimulation of mitochondrial biogenesis in adipocytes. The mitochondrial function in adipocyte is important for adiponectin synthesis. Due to mitochondrial dysfunction of adipocytes, adiponectin synthesis reduces and by increasing mitochondrial biogenesis in adipocytes, adiponectin synthesis increases (29). In addition, reports suggest that exercise stimulates mitochondrial biogenesis in white adipose tissue properly. According to these data and also the findings of the current study, it seems that performing high intensity interval training stimulates mitochondrial biogenesis in adipocytes and this is associated with an increase in mitochondrial function that ultimately will lead to increased plasma adiponectin concentration. This is currently suggested only as a hypothesis and further studies are needed for general conclusion.

**Conclusion**

In summary, based on findings of the present research, it seems that high intensity interval training is a good workout method to reduce body fat percentage and improve anthropometric indices (BMI and WHR) in sedentary young women with normal weight range. Also, the intensity of exercise can be noted as the crucial factor in increasing adiponectin concentration in response to exercise. Consequently, it seems that high intensity interval training in terms of time


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