The Effect of a Period of Selective Aerobic Exercise on Serum Level of Leptin and Some Hormones in Obese Men

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ABSTRACT

Aims and Background: The purpose of the present study was to examine the effect of 12-week aerobic exercising on the serum levels of leptin, testosterone, insulin, glucose, cortisol and growth hormone in obese men. Methodology: A quasi-experimental research method was used. A sample of thirty males (30-45 years old, BMI of 31.8±2.1 kg.m⁻²) was randomly divided into experimental (n=15) and control groups (n=15). The experimental group performed an aerobic training for 3 days per week which lasted for 12 weeks. Leptin, testosterone, insulin, glucose, cortisol and growth hormone were measured before and 48 h after the training program. Results: There were no significant differences in the serum levels of insulin, glucose, cortisol and growth hormone after the training. However, leptin and testosterone were significantly lower and higher after the training, respectively. Body weight, body mass index (BMI) and body fat percentage significantly decreased after the training (P<0.05). Concurrently, VO₂max (P<0.001) increased following the training. After 12 weeks, changes in the control group were not statistically significant (P>0.05). Conclusion: In obese men, regular exercising can decrease BMI, weight, body fat and serum leptin levels and increase serum testosterone levels. Apparently, increase in the serum testosterone level is not a reason for the decrease in the serum leptin. The levels of cortisol, insulin resistance and growth hormone are not affected by exercising.

Key words: Aerobic training, Leptin, Testosterone, Insulin resistance, Cortisol, Growth hormone, Obese men.

INTRODUCTION

Obesity has been related to several conditions of diseases including type-2 diabetes, cardiovascular diseases and some types of cancer and has been introduced as a main health
problem. Although increase in calorie absorption and decrease in energy consumption as a result of doing physical activities may be responsible for the obesity prevalence, some genetic factors have been identified as its main determinants. Despite many studies on the genetic and non-genetic (psychological and psycho-social) determinants, there are relatively few studies related to the regulation of body weight (30).

Leptin is an ob gene product, is secreted from fat tissues and indicates obesity by absorbing and consuming energy and regulating appetite, metabolic domain and body mass. In humans, the level of leptin decreases and increases as a result of weight loss and weight gain or high-calorie diet, respectively. Blood leptin concentration is positively related to body fat mass and is regulated by means of nutritional conditions (33). Nutritional index can be categorized as a short-term and long-term factor which may be related to hunger or satiety, obesity or thinness and central or environmental activities. Environmental hunger index includes glucose, cortisol and guerlain while environmental satiety index includes insulin, leptin, clucagon, somatostatin and cholecystokinin (24). Therefore, nutrition disorder which happens mostly as a result of obesity results in excessive hunger or satiety; this leads to decrease or increase in the leptin level of blood circulation (3).

The hormonal changes observed in obese people can be an explanation for the change in the hypothalamic-pituitary-adrenal (HPA) axis and cortisol secretion. It is conceived that disorder in the HPA axis due to lack of central glucocorticoid receptors in obesity and the relationship between glucocorticoid receptors and central obesity can have an important role in the development of obesity (35).

It has been shown that obesity is related to the decrease in growth hormone and testosterone levels in men and increase in testosterone level and decrease in progesterone levels in females (35). Regular physical activities are accompanied by increase in the energy requirement and body composition changes which can affect plasma leptin concentration (20). Moreover, it has been reported that exercising leads to the increase in testosterone and cortisol secretion (19); however, there is no complete agreement on this finding. Some authors have demonstrated that, although exercising increases the level of serum testosterone (4), it does not affect cortisol secretion (27). Thus, the aim of this study was to investigate the effect of 12 weeks of aerobic training on leptin, testosterone, cortisol and growth hormone in obese men.

**MATERIALS AND METHODS**

**Subjects**

In a quasi-experimental trial, 30 volunteer obese men were randomly selected for participation in a training program. A pretest and a posttest were done on experimental (n=15) and control (n=15) groups. The entry condition to the study was the age domain between 30 and 40 years old, body mass index of higher than 25 kg.m\(^{-2}\), no history of regular exercise, no change of body weight more than 2 kg and lack of specific diseases and no smoking for at least 6 recent months. The rejection criteria included having body mass index of less than 25 kg.m\(^{-2}\) and acute diseases which interfere with exercising. Also, no drugs should be used during the recent years and no entry conditions should be violated while doing the research. The participants got informed about the aim, advantages and possible dangers of the experimental design and filled in a testimonial before beginning the experiments. Participant characteristics are given in Table 1.
Table 1: Physical and physiological characteristics in two experimental and control groups (standard deviation ± mean)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Experimental group</th>
<th></th>
<th>Control group</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st week (base)</td>
<td>12th week (base)</td>
<td>Significance</td>
<td>1st week (base)</td>
</tr>
<tr>
<td>Age (year)</td>
<td>35.2±3.1</td>
<td>-</td>
<td>-</td>
<td>35.5±2.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176.1±4.2</td>
<td>176.6±7.2</td>
<td>94.8±6.8</td>
<td>175.4±3.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>97.66±7.2</td>
<td>31.51±2.2</td>
<td>31.5±2.1</td>
<td>31.8±2.1</td>
</tr>
<tr>
<td>Body mass index (kg.m^-2)</td>
<td>28.03±2.1</td>
<td>31.8±2.1</td>
<td>0.000</td>
<td>32.5±2.1</td>
</tr>
<tr>
<td>Fat percent</td>
<td>25.8±4.2</td>
<td>31.26±5.6*</td>
<td>0.000</td>
<td>25.43±3.5</td>
</tr>
</tbody>
</table>

* denotes significance (p<0.05)

Designing the Experiment
The participants attended the laboratory from 8 to 10 a.m. while being fast in order to measure their body composition. Body weight, height, body mass index, fat percent and fat-free body mass were measured, too. To omit individual error, all the measurements were done by one person.

After that, the participants were taken to a gym in order to get familiar with the training program and sports equipment. They started activities after 10 min of special warm up in order to estimate their maximum oxygen consumption (VO₂max) via YMCA test (1). Three days after determining the above-mentioned tests for doing physical activities, the participants came to the laboratory at 8 a.m. while being fast and their blood factors were measured. After blood sampling, they had equal types of breakfast and, after one hour, they started performing their physical activities. This training program continued for 12 weeks, three sessions per week. Principle of overload was designed so that, after every three weeks of training, the considered intensity could be regulated. After the 12th week, the participants came to the laboratory and their blood factors were determined and their body composition was measured.

Aerobic Training Program
The training program applied in this study lasted for 12 weeks, three sessions of aerobic training per week. The training program of each session included a general warm up (10 min), the considered training and stretching exercises and a cool-down (10 min).

The aerobic training was done every three weeks with the intensities of 55, 60, 65 and 70% heart rate reserve for 25, 30, 35 and 40 min and continued until the 12th week. The heart rate of the participants in the experimental group while doing exercises was controlled by a Polar heart rate meter. During the training period, the participants of the control group did not participate in any physical exercises and only performed their routine physical activities.

Diet
The information related to the participants’ diet was recorded by the 24-hour food recall questionnaire in three days (two first days of the week and one last day of the week) by the participants in a sheet which was specifically used for diets (25). The participants were asked to mention all the foods and beverages which were consumed during 24 previous hours. To analyze the data, first, the consumed foods were converted to gram and then the Dorosty Food Processor (NIII, FP2) was used for analyzing the information related to dieting and macronutrients. On the day of the activity, the participants used a standard diet (Dietry Reference Intake-DRI) (25). Basic metabolic energy requirements were calculated based on age, gender and weight according
to Harris & Benedict formula and the total required energy was computed after adjusting the activity factor (16).

**Physiological Measurement Tool**
The height of people was measured by a wall height gauge (44440 made in Kaveh Company) with the accuracy of ± 0.1 cm while the participants were standing by the wall bare feet and the shoulders were in normal condition. Weight and body composition of the participants were measured by a body composition analyzer (Omron made in Finland) while the participants had the least pieces of clothes and no shoes on. Body mass index was calculated by dividing the person’s weight (kg) by the square of height (m) (SM, 2006). To omit individual error, all the measurements were done by one person. The maximum consumed oxygen (VO$_{2\text{max}}$) of the participants was estimated using an aerobic YMCA test (1) on a stationary bicycle in a laboratory (Tentori type, E604 Model). To determine the intensity as the percentage of VO$_{2\text{max}}$, the maximum heart rate while reaching the stage of failure and using the Kauronen formula (1957) (1).

$$(\text{indicator heart rate}) = (\text{maximum heart rate}) - (\text{resting heart rate}) \times (\text{intensity training}) + (\text{resting heart rate})$$

It should be mentioned that required instructions were given before doing the experiments in order to maximize the efforts of the participants who participated in the experiment in a competitive way.

**Biochemical Measurement Tool**
After 8 to 10 h of being fast in two stages, i.e. before starting the activities and 48 h after the 12 weeks of doing physical activities, 10 ml of venous blood was collected from each participant while they were seated and resting; immediately after that, the serums were separated by a 3000 rpm centrifuge and were maintained at a fridge with the temperature of -70 degrees Centigrade until the day of the experiment. To take blood from the participants, they were asked to stop physical activities for 2 days before the experiment.

The amount of glucose was measured while being fast by the enzyme glucose oxidase method (kit of Pars Azmoon Company, Tehran, Iran) by the Coobas autoanalyzer system (Germany). The amount of serum insulin was calculated while being fast using the Eliza method, competitive sandwich type (kit of demeditec company, Germany, sensitivity of 0.5 µU/I/ml, within and external assessment variation coefficient of 1.79 and 2.88%, respectively). Insulin resistance index (Homeostasis Model Assessment Insulin Resistance-HOMA-IR) was calculated based on the multiplication of the fasting blood glucose concentration (mM/l) by the fasting insulin concentration (microinternational units/ml) divided by the constant of 22.5 (Matues et al., 1985). Fasting serum leptin was calculated using the Eliza method, competitive sandwich type (kit of Biovendor Company, Czech, within and external assessment variation coefficient of 4.2 and 6.7%, respectively). Fasting testosterone serum was also measured by the Eliza method, competitive sandwich type (kit of DRG Company, Germany, within and external assessment variation coefficient of 3.28 and 6.71%, respectively). The level of fasting cortisol serum was measured by the Eliza Method, competitive sandwich type (kit of Diagnostic Company, England, sensitivity of 5 nanogram/ml and within and external assessment variation coefficient of 4.6 and 4.3%, respectively). Moreover, the level of growth hormone of the fasting serum was calculated by the Eliza method, competitive sandwich type (kit of Diagnostic Company, Canada, sensitivity of 0.2 nanogram/ml and within and external assessment variation coefficient of 5.5 and 4.4%, respectively).
Statistical Analysis
First, all the data were tested by the Kolmogorov-Smirnov test in order to determine the normality of distribution. A paired t-test was applied for examining the differences before and after training in the experimental group and an independent t-test was used for determining the differences between two experimental and control groups. In all these cases, the alpha level of less than 0.05 was considered for indicating statistical differences. All the data were analyzed using the SPSS 15 software.

RESULTS
The results obtained from this study showed that 12 weeks of aerobic training may significantly decrease body weight ($97.66 \pm 7.2$ versus $94.6 \pm 6.8$ kg and P-0.000). Body mass index was $31.51 \pm 2.1$ versus $30.58 \pm 1.9$ kg (P-0.000) and body fat percent was $30.73 \pm 2.5$ versus $28.03 \pm 2.1$ (P-0.000) while the level of maximum oxygen consumption (VO$_{2\text{max}}$) of the participants significantly increased from $25.8 \pm 4.2$ to $31.26 \pm 5.6$ ml/kg min (P-0.000) after 12 weeks of aerobic training.

Moreover, the results of the present study demonstrated significant decrease in the level of serum leptin ($17.6 \pm 7.9$ versus $9.94 \pm 4.4$ nanogram/ml and P-0.001) and significant increase in the level of testosterone ($5.12 \pm 1.2$ versus $5.88 \pm 1.6$ nanogram/ml and P-0.001) from the pretest to the posttest. Therefore, doing 12 weeks of aerobic training led to the significant decrease in serum leptin (P-0.002) and significant increase in testosterone (P-0.003) and a significant difference was observed between the level of leptin and testosterone in the experimental and control groups in the pretest stage (Table 2); thus, in the experimental group, the level of serum leptin after 12 weeks of training was lower than that in the control group (P-0.001) while the concentration of testosterone in the experimental group was higher than that in the control group (P-0.002). Furthermore, the results of this study indicated no within group and between group differences in the amounts of insulin, glucose, insulin resistance and growth hormone after the training (P<0.05).

Table 2: Biochemical Variables in the two experimental and control groups (standard deviation)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Groups</th>
<th>1\textsuperscript{st} week (base)</th>
<th>2\textsuperscript{nd} week (base)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>Experimental group</td>
<td>93.7±5.5</td>
<td>92.5±6.3</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>90.7±7.5</td>
<td>98.5±8.3</td>
</tr>
<tr>
<td>Insulin (microinternational units/ml)</td>
<td>Experimental group</td>
<td>7.97±2.4</td>
<td>8.38±2.3</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>8.18±2.3</td>
<td>8.27±2.3</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>Experimental group</td>
<td>1.91±0.6</td>
<td>1.85±0.6</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>1.95±0.6</td>
<td>1.99±0.5</td>
</tr>
<tr>
<td>Cortisol (nanogram/ml)</td>
<td>Experimental group</td>
<td>92.86±24.4</td>
<td>100.81±22.3</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>96.25±25.4</td>
<td>94.86±26.6</td>
</tr>
<tr>
<td>Growth hormone (nanogram/ml)</td>
<td>Experimental group</td>
<td>2.18±0.7</td>
<td>1.94±0.5</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2.04±0.7</td>
<td>1.93±0.7</td>
</tr>
<tr>
<td>Testestrone (ng/ml)</td>
<td>Experimental group</td>
<td>5.02±1.65</td>
<td>6.12±0.81</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>4.95±0.84</td>
<td>4.67±0.73</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>Experimental group</td>
<td>17.6±4.65</td>
<td>10.12±4.81</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>18.2±5.11</td>
<td>19.25±4.87</td>
</tr>
</tbody>
</table>

† denotes significance in the 1\textsuperscript{st} week (p<0.05)
‡ denotes significance between the experimental and control groups (p<0.05)

DISCUSSION
Leptin hormone regulates energy balance by increasing energy consumption during exercising and decreases total body fat. It has been assumed that the effects of physical activities on levels of leptin are mediated by a smpato adrenregic system (34). In the present study, level of leptin
significantly decreased in the training group. As was demonstrated, levels of serum leptin changed with the intensity of training and amount of consumed energy (34). In addition, glucose, fatty acids, sympathetic nervous system, insulin, glucocorticoid, growth hormone and catecholamines affected the synthesis and secretion of leptin (17). Decrease of leptin serum in the present study was related to the decrease of weight and body mass index. Also, it was shown that irregular and short-term exercising was not a factor for determining BMI change and leptin level (11). The results of the present study were in line with those of other studies. Esij et al. (2000) found that leptin concentration did not change immediately or 24 h after the training in 70% maximum oxygen consumption; however, 48 h after the training, it decreased by 30% (9). Similarly, Olive and Miller (2001) reported that leptin concentration did not have any changes immediately after exercising (60 min in 70% VO\textsubscript{2max}) however, after 24 and 48 h, it decreased by 18% and 40%, respectively (29). Gutin et al. (1999) demonstrated that, in obese children, plasma leptin concentration decreased after 4 months of training program (40 min per day for 5 days per week) and increased after 4 months of no training. They concluded that leptin levels reflected the changes in energy balance (13).

This study did not report any changes in the level of serum cortisol after 12 weeks of incremental aerobic activity which was in line with the study by Konsil et al. (2002) (5); they found that long-term endurance training did not make any changes in the resting cortisol levels (5). Daguco et al. studied the effect of endurance training using a bicycle ergometer test and stated that glucocorticoids had an important role in physiological regulation of leptin and cortisol was simultaneously effective on the production and storage of leptin (6). Noland et al. (2001) showed that, although fat mass decreased, no changes were observed in the leptin concentration of collegiate female swimmers in the competition season and they stated that change in leptin may be a reason for the increase of cortisol (28). Cortisol stimulates leptin gene expression and it has been demonstrated that resting cortisol levels has a direct relationship with leptin (19). Conversely, leptin directly inhibits cortisol secretion from adrenocorticotropic cells (2). It also has a feedback mechanism with hypothalamic-pituitary-adrenal (HPA) axis and decreases the corticotropin releasing hormone. Therefore, leptin responds to stress by affecting HPA axis. It is obvious then that resistance to leptin or inability to secrete leptin increases cortisol by increasing corticotropsecretion (31). On the other hand, stabilization of cortisol levels may be the reason for the lack of change in leptin concentration.

In the present study, there were no significant changes in the insulin level of the experimental group compared with the control one. French and Qutin stated that 12 weeks of swimming endurance training led to leptin decrease and quick increase in the insulin level (12). It seems that three parameters of insulin resistance, weight and negative balance of energy were related to regulating leptin levels. Physical activities may increase insulin response by increasing glucose carrier inside the muscle cells (Glut-4) and insulin receptor substrates (IRS) and also increasing the muscular mass. Fatty acids produced in the fat tissue disturb the transfer of Glut-4 to the cell surface by being aggregated in the muscle cells. Physical activities prevent from their aggregation in the muscle cells by increasing the oxidation of fatty acids (10); however, it has been proved that endurance training along with dieting increases insulin sensitivity (8). Weak diets including high-fat diet can neutralize this positive process (8). Decrease in the leptin sensitivity in the skeletal muscle which happens as a result of high-fat diet may lead to the increase of fatty acid transfer in the cell membrane and this transfer can be considered as a potential factor for decreasing insulin sensitivity (8). Additionally, inhibition of 3 kinase Inositol phosphatidyl choline an important enzyme in the insulin signal, destroyed the ability of leptin in the redistribution of fatty acids for the oxidation route (8). In other words, glucose which is the main stimulus for insulin secretion (15) caused no significant changes in this study. It should be
mentioned that changes in the leptin level were important in terms of ability to control hepatic glucose output and resistance to leptin affected resistance to insulin (22). Possible mechanism of leptin action is to directly and indirectly activate AMPK enzyme in the muscle by the mediated responses of hypothalamus and increase of fatty acid oxidation which leads to the glucose absorption. Therefore, leptin has a role in controlling, absorbing and consuming energy (31). Leptin has a positive effect on the metabolism of fatty acids in the skeletal muscle and, because of this increase, triacylglycerol storage occurred in skeletal muscle. Therefore, decrease of sensitivity to leptin in the skeletal muscle may lead to the sensitivity decrease to insulin due to the aggregation of lipids inside the muscle cells and creation of disorder in the insulin signaling route (8). Then, lifestyle which includes weak dieting and lack of physical activity may omit sensitivity to leptin and insulin.

The present investigation did not demonstrate a significant difference in growth hormones as a result of 12 weeks of aerobic training which was in line with the findings of some other studies (23). The results of the studies showed that physical activity is a stimulus or releasing factor for the growth hormone. Some studies have investigated factors like intensity, duration and type of activity and environmental conditions and have concluded that these factors may influence the effect of physical activities on the secretion of growth hormone (22, 23). Therefore, the data of this study revealed that probably one of the effective factors on the changes of growth hormone was the intensity of the applied activities and obesity percent of the participants. So, it is likely that decrease in obesity and increase in the intensity of exercises and change in the type of activities increase the response of growth hormone to the exercises.

This study showed significant increase in the testosterone of the training group. Previous studies have demonstrated that androgens inhibit leptin (22, 26). The present research demonstrated increase in the testosterone level and decrease in the serum leptin as a result of 12 weeks of aerobic training. Considering the lack of a significant relationship between testosterone and leptin concentration during exercising and recovery, the data of this study suggested that testosterone had no acute effect on the levels of leptin during exercising and 48 h after that. Therefore, the difference in the response of these hormones may be due to individual differences in terms of intensity of activities. Previous studies have demonstrated that testosterone levels increase with the resistance training (21, 20) and with physical activity (36, 14) with sufficient volume and intensity. Testosterone increase in the present study was probably due to the intensity of training program. According to the present study and other investigations (18), possible mechanisms for increased testosterone were increase in blood concentration, decrease in metabolic clearance and increase in blood lactate concentration.

CONCLUSION

In general, the findings of this study showed that incremental aerobic training can affect serum leptin; however, considering lack of change in the hormones which affect leptin, the reason of the leptin decrease can be attributed to other hormones like epinephrine, norepinephrine, thyroid hormone and so on. Probably, if the intensity and duration of activities were higher than their present levels, significant results would be obtained and would highly affect the discussed indexes. In addition, a high volume of samples may help the validity of the results.

Acknowledgements
This investigation is the result of the research plan approved by the Department of Research, Islamic Azad University, Saveh Branch. Hereby, we appreciate the efforts of all the respected colleagues in that department and in the Council of Research.
REFERENCES