Original Article

Relationship between Bone Formation Marker (BGLA-protein) with Adipokines and Insulin Resistance in Overweight Men after Aerobic Exercise

Mehdi Rostamizadeh¹, Alireza Elmieh², Farhad Rahmani Nia³

1. Department of physical education, Islamic Azad university of Ahar branch, Ahar, Iran
2. Department of physical education, Islamic Azad university of Rasht branch, Rasht, Iran (Corresponding Author)
3. Department of physical education, university of Guilan, Rasht, Iran

Received: 2020/01/25  Accepted: 2021/01/31

Abstract

Background and purpose: Osteocalcin (OC) as a small peptide secreted by osteoblasts has been recently described as a hormone involved in the regulation of energy metabolism. Moreover, the experimental data indicate regulation of adipocytes by bone. The aim of this study was to evaluate the relationship between bone formation marker (BGLA-protein) with adipokines and insulin resistance in overweight men after aerobic exercise.

Methods: Twenty-two male volunteers with a mean age of 28.50±2.23 years and body mass index (BMI) of 29.67±0.96 kg/m² were randomly assigned into control (n=11) and aerobic training (n=11) groups. Subjects in the aerobic training group participated in 8-week exercise training program, three sessions per week (60 minutes at 70-85% of heart rate reserve (HRR)). Weight, body fat and BMI were measured, and the fasting blood samples were collected before and after 8-week exercise program to determine serum OC, leptin, adiponectin, insulin and HOMA-IR. Data were analyzed using t-test.

Results: Findings showed that body fat, BMI, weight, insulin, glucose, HOMA-IR and leptin significantly reduced following aerobic training (P<0.05). However, the OC and adiponectin levels significantly increased (P<0.05, baseline vs. post exercise) in the exercise group compared to control group. The increase in OC levels had no correlation with leptin, adiponectin, HOMA-IR, glucose and insulin levels after 8-week aerobic exercises (all P>0.05).

Conclusion: According to these findings, it seems that the aerobic exercise through changing the body weight and BMI is considered as a non-prescriptive therapeutic approach to decrease the insulin levels and insulin resistance in overweight men. However, the increase in OC level is not associated with decreased leptin or increased adiponectin levels

Keywords: Osteocalcin, Adipokines, Insulin, Aerobic training, Overweight

1. Email: mehdirostami2352@gmail.com
2. Email: Email: elmieh@iaurasht.ac.ir
3. Email: frahmani2001@yahoo.com
Introduction

Many studies have demonstrated that there is a powerful relationship between bone mass and energy intake as well as have indicated that high regulation of energy metabolism is necessary for optimal bone health. In addition, it is found that several neuropeptides and hormones play important roles in the control of energy metabolism, adiponectin, leptin, NPY and insulin as well as influence bone remodeling (1, 2). There are cell communication pathways among adipose tissue, hypothalamus and bone marrow, leading to conjecture that there is a biological interaction between adipose tissue and bone (3). The osteocyte and adipose cell metabolism is modulated by these mechanisms via inter-regulated molecular pathways including skeletal system remodeling, sympathetic nervous system activity, energy balance and insulin-glucose axis. Insulin physiological effects and glucose metabolism are vital for metabolic alterations in the relationship between adipose tissue and bone (4).

Osteocalcin (OC) as the main noncollagen protein acts locally in the mineralization of bone. It is synthesized by the osteoblasts and has been utilized for bone turnover marker or bone formation (5). One of the OC’s functions in the body is stimulating β-cells to secrete more insulin; on the other hand, they force the fat cells to release adiponectin hormone, enhancing insulin sensitivity (6). Overweight and obese individuals have significantly higher leptin. Besides, adiponectin which is mainly secreted from adipose tissue significantly decreases in these people (7). Leptin is the most important cytokine secreted from adipose tissue, and its role is largely expressed in regulation of energy expenditure and energy homeostasis. In the recent years, it is found that leptin also plays a main role in the neuroendocrine regulation of bone metabolism (8,9). Leptin production has a positive correlation with BMI and fat mass (10). Leptin has an influence on bone homeostasis via peripheral and central pathways (Fig. 1) (8).
Fig1- central and peripheral pathways regulation of bone metabolism by leptin. VMH, ventromedial hypothalamus; Ob-Rb, hypothalamic receptors; BMSC, bone marrow mesenchymal stem cell; AdrB2, B2 adrenergic receptors (8).

In the central nervous system (CNS), the leptin regulates bone turnover via sympathetic nervous system (SNS) (11) and targeting osteoblasts. When leptin binds to its receptor on VMH neurons (Ob-Rb), signals are extended to osteoblasts and inhibit osteoblasts proliferation. Therefore, this action causes reduced OC production (12). Circulating adiponectin is correlated with decreased BMI in obese subjects (13). Adiponectin affects bone mass through two opposite mechanisms: 1) via osteoblasts in bone tissue, and 2) through influencing on CNS (Fig 2) (14).
In osteoblasts, the adiponectin directly increases receptor activator of nuclear factor kappa-B ligand (RANKL) expression and decreases forkhead box protein (FOXO1) activity. Indeed, adiponectin exerts its effect in the sympathetic tone and inhibits the activity of the sympathetic nervous system, followed by increasing bone formation in addition to decreasing energy expenditure and blood pressure (14). Exercise is defined as a series of physical activities whose objective is to improve or keep physical health and fitness. Exercise has a number of beneficial function such as decreasing leptin and increasing adiponectin levels (15). Recent studies have shown reciprocal functional interactions between organs including pancreas and adipose tissue, controlling intermediary metabolism and bone. The OC can promote insulin secretion through β-cells proliferation. Furthermore, measuring plasma OC concentration has been suggested as a potential biomarker of cardiometabolic risk together with its proved clinical significance as a bone turnover biomarker (16).

Exercise lessens leptin by enhancing energy expenditure and reducing adiposity. Adiponectin promotes insulin sensitivity and inhibits inflammation. Weight loss is correlated with a significant increase in adiponectin levels in obese subjects, suggesting that exercise may increase adiponectin levels via decreasing body weight. In addition, the mRNA expression of adiponectin is reportedly elevated after aerobic training in rats (17). Lately, growing attention has been directed toward the role of OC in modulating glucose and lipid metabolism. Lee et al. (2007) have demonstrated that the OC is a bone messenger affecting adipocytes and causes to increase insulin secretion, β-cell proliferation, insulin sensitivity, energy expenditure and adiponectin expression by upregulating the adiponectin gene expression in adipocytes. On the other hand, the adiponectin receptors are
expressed in osteoblasts. Adiponectin stimulates differentiation, proliferation and mineralization of osteoblastic cells (18). A positive feedback loop between insulin and OC has been described so that insulin signaling in osteoblasts enhances OC production and bioavailability (19). Both acute bouts of exercise and long-term training improve insulin sensitivity and increase serum OC in overweight men; therefore, insulin sensitivity enhanced with exercise is linked to a change in OC (20).

Chahla et al. (2015) indicated that people who participated in exercise interventions from one month to one year had significant increase in their OC levels. They believed that the significant increase of OC level in the study group was associated with the enhancement in the differentiation and activities of osteoblast which might increase the production of OC due to the mechanical loading from exercise (21). However, Abseyi et al. (2012) found no significant association between insulin resistance, metabolic syndrome parameters and OC levels in obese and overweight subjects (22). Therefore, these findings show the importance of OC in glucose metabolism which affects diabetic patients and increases this hormone with exercise; hence, the OC can be an important factor in controlling blood glucose and improving insulin sensitivity in the overweight or obese subject (6). In general, controversial findings of a study revealed that different exercises had effects on bone metabolism, and several factors such as the type of exercise activity (weight-bearing), gender and age might affect the response of bone metabolism (23).

Considering the importance of OC in regulating glucose metabolism and insulin secretion from beta cells as well as contradictory results of the studies on the effect of different physical activities in this field, it seems that finding a mechanism, by which the secretion of OC is increased, is effective in preventing metabolic diseases. Therefore, the aim of the present study was to evaluate relationship between bone formation marker (BGLA- protein) with adipokines and insulin resistance in overweight men after aerobic exercise.

**Methods**
This study was registered at the Iranian Center for Clinical Trials (IRCT) with the code of IRCT 20180226038876N1.

**Subjects**
Twenty-two overweight males (mean age: 28.50 ±2. 23 years; BMI: 29.67 ±0. 96 kg/m2) who met the criteria of overweight for adult Asians established by the World Health Organization (WHO) were recruited. All subjects had no systemic diseases, infections and physical disabilities undergo aerobic exercise. None of
them had a history of smoking, experiences of exercise training and medications for metabolic diseases. All participants gave written informed consent before entering into the present study. The subjects were randomly assigned into control (n = 11) and aerobic exercise (n = 11) groups. The baseline and follow-up characteristics of the subjects in each group are shown in table 1.

**Training Program**

The aerobic exercise group participated in an 8-week supervised exercise training program for three sessions (60 min per sessions) per week. Aerobic training was performed (10 min of warm-up and 25-40 min of main training and finally cool down for 10 min at 70–85% HRmax). Cardiorespiratory fitness (VO₂max) of the subjects was assessed using the Bruce protocol as introduced by the American College of Sports Medicine (ACSM) before the first session of the program (24). (Table 1).

The control group had normal lives.

**Table 1- Aerobic training details**

<table>
<thead>
<tr>
<th></th>
<th>Week 1-2</th>
<th>Week 3-6</th>
<th>Week 6-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity (HR max)</td>
<td>Duration</td>
<td>Intensity (HR max)</td>
<td>Duration</td>
</tr>
<tr>
<td>% 70</td>
<td>25 min</td>
<td>%70- %80</td>
<td>35 min</td>
</tr>
</tbody>
</table>

**Anthropometric Evaluation**

Body weight was measured by Digital Scale (Sahand Company, Iran, the nearest 0.1 kg), height was measured by the Seca Height Scale (Japan technology, 0.1 cm), and body mass index (BMI) was calculated as weight/height² (kg/m²).

**Biochemical Analyses**

Blood samples were collected from all participants after 8 h of fasting between 8:00 and 10:00 a.m. to minimize hormonal rhythmicity at the start of the study and 2 days after the termination of the study. Samples were immediately centrifuged at 4000 g for 10 min at 4 °C as well as serum and plasma samples were stored at -70 °C until analysis.

Insulin (IN) was measured by electrochemiluminescence immunoassay (LIAISON kits, England). The serum levels of OC, leptin and adiponectin were determined by Human ELISA Kit (Hangzhou eastbiopharm Co. LTD; CHINA) with the sensitivity of 0.026, 0.021 and 0.11 ng/ml, respectively as well as were measured by Sandwich ELISA based on the instructions given in the kit brochure. Whole body insulin resistance was obtained by calculating homeostasis model assessment of insulin resistance:

HOMA-IR= FBG (mg/dl) × FPI (µu/ml) / 405
Nutrition
Calorie intake was assessed using a 3-day food record and 24-h recall interview conducted at the beginning and end of the training period. Dietary intakes gained from the 3-day records and 24-h recalls were analyzed for calorie and macronutrient content using Food Processor Nutrition Analysis Software (Version 7.1, 1996, ESHA Research, Salem, OR), providing access to information on over 15,000 food items with data for 105 nutrient components. There was no confirmed significant variability between these two measures; hence, the two measures were combined to each other, and a mean energy intake was calculated over 4 discrete days at each time point (Table 2).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Saturday</th>
<th>Tuesday</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (Kcals)</td>
<td>2204.00 ± 122.9</td>
<td>2212.00 ± 113.8</td>
<td>0.48</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>14.6 ± 1.5</td>
<td>14.3 ± 3.2</td>
<td>0.84</td>
</tr>
<tr>
<td>Carbohydrates (%)</td>
<td>52.9 ± 4.2</td>
<td>52.2 ± 3.85</td>
<td>0.56</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>32.5 ± 3.9</td>
<td>33.5 ± 3.13</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Statistical Analyses
All data were analyzed using SPSS 25.0 (K; IBM, USA) and presented as mean ±SD.
Pearson’s bivariate correlation analysis was used to evaluate the associations between variables. The paired t-test was applied to examine the differences at the baseline and follow-up variables within the group. Comparison of variables between the groups was made using independent t-test. For variables not exhibiting a normal distribution, the Kolmogorov-Smirnov test was utilized. Levels of statistical significance were set at P < 0.05.

Results
The obtained results before and after 8-week exercise training interventions are presented in table 3. Table 3 shows that before the intervention, there is a significant difference between groups in body mass, weight and VO2max as well as no significant difference is observed in terms of other variables including BF% between groups.
The obtained results displayed that there were significant differences between control and exercise training groups with respect to changes of body mass index after 8-week intervention (aerobic exercise, pre=28.33±1.04 post=27.34±0.99 kg/m²). The BMI decreased in the exercise group after training. The changes in body fat percentage between groups were significantly different so that in the
exercise group, the body fat percentage decreased during 8-week period (aerobic exercise, pre=20.52±3.68 post=19.34±3.45 %). Additionally, the findings illustrated that there were significant differences in changes of OC, leptin, adiponectin, insulin level between intervention and control groups (P < 0.05). These changes were more in exercise group than control group.

Table 3- Baseline and follow-up characteristics of the subjects

<table>
<thead>
<tr>
<th></th>
<th>Aerobic exercise(N=31) Age: 29.54 ± 2.62</th>
<th>Control(N=31) Age: 28.57 ± 2.20</th>
<th>Independent T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Follow-up</td>
<td>P</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>83.01±4.65</td>
<td>83.91±4.31</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.33±1.04</td>
<td>27.34±0.99</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>%BF</td>
<td>20.32±3.68</td>
<td>19.34±3.45</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>82.63±5.85</td>
<td>79.18±4.46</td>
<td>0.008</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>7.49±1.46</td>
<td>6.45±1.58</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.51±0.28</td>
<td>1.25±0.26</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>OC (ng/ml)</td>
<td>23.11±3.01</td>
<td>26.49±3.38</td>
<td>0.004</td>
</tr>
<tr>
<td>Adiponectin (pg/ml)</td>
<td>52.83±9.32</td>
<td>56.38±8.81</td>
<td>0.004</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>6.71±1.49</td>
<td>6.02±1.50</td>
<td>&gt;0.001</td>
</tr>
</tbody>
</table>

Values: Mean ± SD
P: Difference between baseline and follow-up values in groups
P*: Difference between experimental and control groups
BMI: Body mass index; FBG: Fasting plasma glucose; HOMA-IR: Homeostatic model assessment for insulin resistance; QUlKI: Quantitative insulin sensitivity check index; osteocalcin: OC

In addition, the FBS, FPI, HOMA-IR (P< 0.05) decreased and OC levels had a significant increase (P< 0.05) in exercise group compared to control group. The correlations between changes were evaluated in terms of leptin, adiponectin, insulin homeostasis and circulating levels of OC. Pearson correlation showed that changes in leptin and adiponectin had no significant correlation with those in OC level of all subjects (P> 0.05).
Table 4- Linear associations between baseline OCs and measures of anthropometry and blood biochemical parameters

<table>
<thead>
<tr>
<th></th>
<th>Osteocalcin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Mean: 24.99±3.55 ng/ml; n= 22)</td>
</tr>
<tr>
<td></td>
<td>R</td>
</tr>
<tr>
<td>FBG</td>
<td>-0.420</td>
</tr>
<tr>
<td>Insulin</td>
<td>0.263</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.049</td>
</tr>
<tr>
<td>Leptin</td>
<td>-0.102</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>-0.341</td>
</tr>
<tr>
<td>P&gt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

Moreover, no significant correlation was found between insulin homeostasis and OC levels in all subjects (P > 0.05, Table 4).

Discussion
The current study examined the effect of exercise interventions (aerobic training) on OC, leptin, adiponectin and insulin resistance in overweight men. It was found that 8-week exercise training decreased body fat mass, weight, BMI, FBG, insulin and leptin, increased adiponectin and OC levels as well as improved insulin resistance in overweight young males. The bone regeneration process is significantly affected by mechanical loads. A previous study has suggested that the mechanical pressures based on weight-bearing exercise are the most important factors affecting the formation of new bones and increase of bone mineral density (25). Cross-sectional data indicate an inverse association between OC and leptin (26) in addition to positive association between OC and adiponectin (27). Adiponectin stimulates the differentiation and proliferation of osteoblastic cells (28). In the present study, serum leptin and adiponectin concentrations significantly changed in exercise group compared to control group. It was observed that serum leptin decreased with increasing of bone turnover biomarker (i.e. OC) in overweight young men. However, serum adiponectin increased with increasing of OC. Circulating adiponectin levels were lower in obese subjects, especially those with visceral fat accumulation in the ongoing study. Experimental data suggest that OC produced by osteoblasts increases insulin production, whereas enhancement in adiponectin (along with increase in OC) supports the effect of OC on insulin sensitivity (29).

In the current study, the serum concentrations of OC, leptin, adiponectin and insulin resistance were statistically different between experimental and control
groups. It was demonstrated that after 8-week exercise intervention, the OC and adiponectin levels had significant increase, and leptin, glucose, insulin and insulin resistance had reduction in the experimental group.

A study by Ghasemalipur et al. suggested that aerobic exercise increased OC levels in men (30). Besides, Akbarpour declared that aerobic exercise reduced leptin levels and increased adiponectin levels in obese and overweight men (31). Another study reported that exercise training increased OC levels (bone formation markers) and improved glycemic index and anthropometric indices in overweight women (32). Their findings are consistent with ours, indicating an increase in OC and adiponectin levels in addition to decrease in leptin levels. Furthermore, the result of the ongoing study is confirmed by many researchers who have stated that various exercises decline insulin and glucose levels, weight, body fat percentage and BMI in different individuals with different genders (33, 34). Further, these findings are inconsistent with those of Nouri et al (35) about the inactive men and Bizheh et al. (36) about the middle-aged women because they have declared no change or increase in OC and HOMA.

Furthermore, another aim of this study was to investigate the correlation between the OC level with insulin resistance, leptin and adiponectin in overweight men. Significant correlation was observed between changes in glucose and OC after exercise, and the associations were significant in the Ex group and all subjects and not in the control group. The findings of the ongoing study are similar to those of Kanazawa et al. (37) about men and Iki et al. (38) about young men who have reported a correlation between baseline OC and blood glucose. Relationship between OC with leptin, adiponectin and insulin was observed, which was not significant between groups.

The OC is generated from mature osteoblasts and activated in the low pH of the resorption lacunae. The OC bioactivity may be regulated by insulin through insulin receptors on osteoblasts. Conversely, the OC influences adiponectin expression, β-cell proliferation, insulin sensitivity and insulin secretion, possibly via GPCR6a receptors in β-cells (39).

Undercarboxylated bioactive osteocalcin (OCN) releasing in bloodstream influences glucose metabolism mainly in two ways. Firstly, the OCN directly impacts β-cell function through binding to the receptor GPRC6A and rising the capacity in order to proliferate as well as to synthesize and secrete insulin. Secondly, the OCN elevates energy expenditure and improves insulin sensitivity via multiple mechanisms. The OCN stimulates energy expenditure through regulating the expression of genes implicated in energy consumption in skeletal muscle and brown adipose tissue as well as via boosting mitochondrial biogenesis in muscle. In addition, the OCN affects insulin sensitivity, possibly through enhancing adiponectin expression in white fat and reducing inflammation and
lipid accumulation in the steatotic liver. A direct influence of OCN as an insulin-sensitizing hormone is speculative and remains to be established (40). Therefore, one of the mechanisms representing physical activity can improve insulin sensitivity and decline blood glucose in diabetic patients through effective increase in OC. First, the OC has an impact on pancreatic beta cells, enhances insulin secretion via improving glucose uptake in tissues, especially in muscle and augments glucose metabolism (3). On the other hand, the OC can rise adiponectin secretions via affecting the adipose tissue. It is clear that adiponectin is one of the most important adipokines in enhancing insulin sensitivity (4).

Long-term exercises can elevate glucose transporters to muscle cells and insulin receptor substrates, as well as its increase in muscle mass (more than 75% of glucose utilization due to insulin-induced muscle tissue stimulation) causes the response of body to insulin, enhances insulin sensitivity and prevents obesity in addition to its subsequent complications. The aerobic exercises increase insulin function by decreasing intracellular TG accumulation and rising the oxidation of fatty acids (41).

It seems that one of the major mechanisms is exercise which increases OC levels; firstly, the mechanical loads of exercise cause the response of bone cells to more activity, resulting in more OC secretion by the osteoblastic cells. Secondly, the mechanical loads of exercise disrupt the hemostasis of energy metabolism during physical activity in the body. Since bone is now considered as an active metabolic tissue during physical activity, the signals from insulin and glucose changes lead to more bone activity and stimulate OC secretion (6).

**Conclusion**

The results of the current study indicated that aerobic training significantly elevated the serum levels of OC, adiponectin and decrease leptin, insulin and insulin resistance in overweight young men. Comparing the means of two groups (control vs. aerobic exercise) illustrated that the changes have a greater effect in the aerobic exercise group than control group in the rate of change of the study factors. Thus, in summary, aerobic training by increasing mechanical load on bone mass causes changes in OC secretion, affects the energy metabolism and body weight as well as can be an important factor in increasing bone mass and weight loss in overweight and obese people.

**Acknowledgements**

This article was derived from the Ph.D. thesis in Exercise Physiology with the number of 11721404961011 in the Islamic Azad University of Rasht Branch. The
authors would like to thank the supervisors, advisors and all persons who cooperated with us in this field.

References