# Effect of 8-week Aerobic Exercise on Undercarboxylated Osteocalcin and Beta Cell Function in Postmenopausal Women

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## ABSTRACT

**Background and Objective:** The present study aims at investigating the possible effect of 8-week aerobic exercise on undercarboxylated osteocalcin and beta cell function in postmenopausal women.

**Methods:** The study included 20 postmenopausal women with mean weight, height and body mass index of 78.94 ± 5.72 kg, age 48.69 ± 3.21 years, 160.37 ± 4.12 cm and 30.72 ± 2.37 kg/m2. The participants were randomly selected and divided into experimental and control groups. Blood samples were taken 48 hours before the experiment and after eight weeks of exercise. Aerobic exercise was performed for eight weeks, three sessions per week with intensity of 65-70% of heart rate. Data analysis for intragroup and intergroup differences was done using paired and independent t-test, respectively. P-values less than 0.05 were considered statistically significant.

**Results:** The level of undercarboxylated osteocalcin in the experimental group decreased significantly compared to control group ($P<0.049$). The level of beta cell function index in the experimental group increased significantly after the 8-week exercise program compared to control group ($P<0.014$).

**Conclusion:** Exercise increases the level of undercarboxylated osteocalcin in postmenopausal women that has important consequences, especially for those at risk of developing diabetes.

**Keywords:** Exercise, Osteocalcin, Hemoglobin A, Glycosylated, Postmenopause.

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## INTRODUCTION

Age-related physiological and body composition changes such as decrease in lean body mass, fat accumulation, lack of exercise, reduced fitness, change in sex hormones and menopause are considered as main factors causing insulin resistance (IR) and metabolic syndrome (MS) in the elderly (1). These changes such as decreased level of sex steroids have great impact on women's quality of life and health after menopause. Normal levels of estrogen before menopause protect healthy women against IR, hypertension, cardiovascular disease and cancer. Obesity, impaired fasting glucose and impaired glucose tolerance (IGT) are related to increased risk of developing type 2 diabetes.IR in body tissues (such as liver, muscle and fat) and impaired insulin secretion from pancreatic beta cells trigger type 2 diabetes (2). Skeletal muscle is an endocrine organ that plays a significant role in energy metabolism and glucose homeostasis (3, 4). Studies on cells involved in bone formation identified a new mechanism of insulin secretion regulation stimulated by beta cells and IR (5). In both mice and humans, osteocalcin is one of the most abundant matrix proteins found in bones and blood (6). Osteocalcin contains 49 amino acid residues and undergoes γ-carboxylation of glutamyl residues at positions 17, 21 and 24, which facilitates binding of osteocalcin to hydroxyapatite in bone. Osteocalcin or bone Gla-protein produced by osteoblasts is a well-known organic and non-collagen bone protein and a marker of bone formation. In mice, undercarboxylated osteocalcin (unOC) stimulates beta cells and insulin secretion. Therefore, obese mice with osteocalcin deficiency exhibit features of IGT as well as type 2 diabetes (5). Loinger et al. stated that the decrease in glucose following a short-term exercise could be attributed to the elevated Gla-protein levels in bones (7). However, the result of a study indicated that 10-week aerobic exercise significantly decreases glucose and glycosylated hemoglobin, while serum concentrations of alkaline phosphatase, osteocalcin and insulin do not change significantly. The present study aimed to investigate the potential effect of 8-week aerobic exercise on unOC and beta cell function in postmenopausal women.

## MATERIAL AND METHODS

Given that the participants of the study were humans, controlling all factors influencing the study was not an easy task. Therefore, it can be claimed that the present study was conducted within the framework of quasi-experimental study.

The study included 20 healthy non-athlete postmenopausal women in Babol, Iran. The subjects had no history of disease, and did not receive any drugs during the study. They had not been exercising for at least 6 months before the start of the training program. The health status of participants was evaluated using a health questionnaire. A written consent was obtained from all the participants. They were asked to avoid any vigorous physical activity other than the training program given. The purpose and procedures of the study were fully explained for the participants. They were divided into an experimental and a control group. Height, weight and body mass index (BMI) of each subject were measured. Then, 10-ml blood samples were taken from the participants 48 hours before the exercise while resting. The same procedure was repeated 48 hours after the last training session.

After collecting the demographic information of the participants, the experimental group was subjected to 8 weeks of aerobic exercise consisting of 3 sessions of at least 45 min per week. All sessions included the following:

*Warm up*: 10-min walk, stretching, and jogging at 55-65% target heart rate

*Workout*: 30-min aerobic exercise and rhythmic footsteps at 65-70% target heart rate

*Cool down*: 5-min walk and stretching to reach a normal heartbeat.

The first two weeks of the workout included 30-min aerobic exercise at 65% heart rate reserve (HRR) per session. However, the intensity reached 70% HRR in the following weeks (Table 1).

Moreover, in order to determine the exercise intensity as a percentage of $VO_2$ max, each individual's target heart rate was calculated based on the Karvonen formula as follows:

Target Heart Rate = (max HR − resting HR) × %Intensity) + resting HR

Heart rate of the experimental group was controlled using a Beurer heart rate monitor (Made in Germany). The control group did

not perform any exercise and followed their routine physical activities. All participants were asked to avoid any other physical activities.

In order to evaluate biochemical variables, fasting blood test was performed twice, 48 hours before the first training session and 48 hours after the last training session. The participants were urged not to eat anything 12-14 hours prior to the testing. Briefly, 10-ml blood samples were drawn from participants' left forearm antecubital vein in resting and sitting position. After centrifugation and separation of serum, samples were stored at -80 °C until the time of training. In addition, blood test was performed at a specific time of day, between 8:30 and 9:30 to eliminate the effects of circadian rhythm. As mentioned earlier, the participants were strongly urged to avoid performing heavy physical activities 48 hours before blood testing. Serum levels of biochemical markers of unOC were measured and analyzed using ELISA kits and ELISA plate reader. Furthermore, levels of glycosylated hemoglobin, insulin and glucose were measured using commercial kits and standard methods. Beta cell function indices of all participants were evaluated using the following formula:

$$\text{HOMA-}\beta = \frac{360 \times \text{Insulin}}{\text{Glucose} - 63}\%$$

Data was analyzed using SPSS (version 16). Normality of data distribution was assessed by the Kolmogorov Smirnov test. Intragroup and intergroup differences were evaluated using paired and independent t-test, respectively

## RESULTS

Table 2 shows the descriptive characteristics of participants. There was no significant difference between the two groups regarding the age, height, weight, and BMI (Table 2).

The level of unOC decreased significantly in the experimental group compared to control group ($P<0.049$). The level of beta cell function index in the experimental group increased significantly after the 8-week exercise program compared to controls ($P<0.014$). Moreover, the level of glycosylated hemoglobin in the experimental group was significantly less than that of in the control group ($P<0.000$).

**Table 1- Intensity and duration of training**

| Exercise Duration | | Exercise Intensity (%HR) |
|---|---|---|
| Before training | Time (min) | Cardiovascular tests at the first level |
| First and second weeks | 30-45 | 65 |
| Third week up to last week | 45 | 65-70 |

**Table 2-Mean ± standard deviation (SD) of participants' demographic information**

| Variable | Control Group | Experimental Group |
|---|---|---|
| Age (year) | 48.25 ± 3.01 | 49.12 ± 3.56 |
| Weight (kg) | 79.62 ± 4.62 | 76.75 ± 6.92 |
| Height (cm) | 159.12 ± 3.68 | 161.62 ± 4.40 |
| BMI (kg/m2) | 31.43 ± 1.41 | 30.07 ± 2.98 |

**Figure 1- Comparison of changes in the level of unOC between the experimental and control groups**

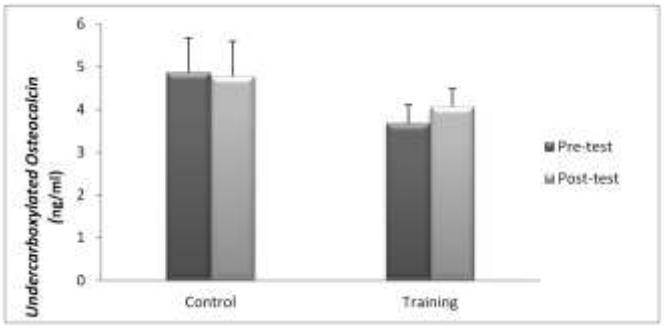
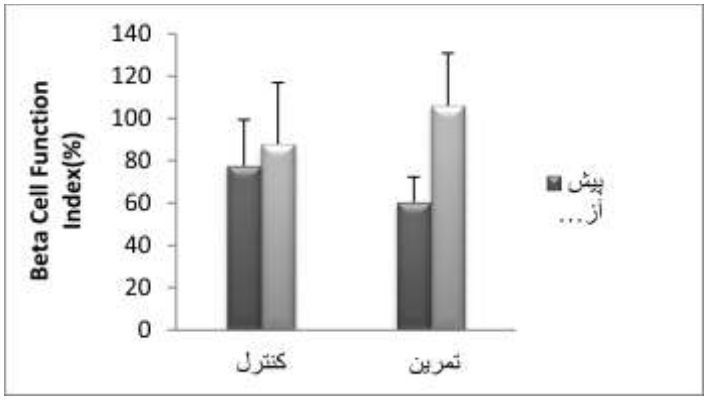


**Figure 2- Changes in beta cell function index of the experimental and control groups**




| Group | | Before Exercise | After Exercise | P-value |
|---|---|---|---|---|
| Experimental | Glycosylated | 4.72 ± 0.46 | 3.64 ± 0.70 | 0.001 |
| Control | Hemoglobin (%) | 5.92 ± 0.86 | 5.85 ± 0.77 | |
| Experimental | Insulin (IU/ml) | 3.75 ± 0.76 | 5.41 ± 1.68 | 0.00 |
| Control | | 7.21 ± 1.70 | 8.00 ± 1.88 | |
| Experimental | Glucose (mg/dl) | 89.41 ± 8.91 | 76.25 ± 6.70 | 0.001 |
| Control | | 96.75 ± 8.51 | 95.60 ± 7.22 | |

## DISCUSSION

Various studies have shown a relationship between menopause and increased risk of IGT. The incidence of MS increases with menopause, which is related to decreased level of estrogen. The risk of developing MS is affected by androgenic hormonal environment (8). There is a relationship between menopause and increased risk of developing diseases such as type 2 diabetes. Some of the diseases are closely related to changes in the body composition, distribution of body fat, and insulin sensitivity (9). In the present study, the level of unOC decreased significantly in the experimental group compared to the control group. Moreover, the level of unOC of the experimental group in the post-test (after 8 weeks of resistance training) was higher than that in the pre-test. However, the difference was not statistically significant. Moreover, the level of unOC did not change in control group. Osteocalcin is one of the best characterized organic and non-collagen bone proteins and a marker of bone formation. In mice, unOC stimulates beta cell function and insulin secretion. Therefore, osteocalcin–deficient obese mice may exhibit feature s of IGT and type 2 diabetes (5). Both forms of osteocalcin, i.e. carboxylate and undercarboxylated, can be detected in blood. Almost 50% of osteocalcin in healthy people is estimated to be undercarboxylated. Physical activity decreases serum glucose level and improves insulin sensitivity in obese individuals with type 2 diabetes (10-12). Furthermore, exercise improves insulin activity, insulin sensitivity and glucose intake. The amount of glucose intake increases during exercise and in overweight patients with poor glycemic control, which could be due to the effects of exercise on unOC (13). In addition, a study on adults with type 2 diabetes showed that unOC is negatively associated with fasting blood glucose and glycosylated hemoglobin (14). The level of glycosylated hemoglobin in the experimental group decreased significantly compared to control group. These findings were also observed in studies of Sigal et al. and Zanuso et al. that showed compound exercises lead to significant changes in the amount of glycosylated hemoglobin compared with each of the exercises alone (15,16). In this regard, Sigal et al. pointed out that compound exercises decrease the level of glycosylated hemoglobin. Previous studies showed that the insulin-like effect of muscle contraction transports a great amount of glucose to the cell in order to produce energy (17). Muscle contraction results in an increase in membrane permeability to glucose. The increase in membrane permeability may be attributed to increased number of glucose

transporters associated with the plasma membrane (Glut4). Exercise increases the amount of Glut4 in trained muscles, improves the mechanism of insulin action on glucose metabolism and decreases the amount of glycosylated hemoglobin (18). The results of the present study revealed that the insulin level of experimental group in the post-test (after 8 weeks of exercise) is higher than in the pre-test. However, the glucose level in the experimental group in post-test decreased compared to the pre-test. Therefore, beta cell function of the experimental group increased significantly compared to that of control group. Jorge et al. investigated the effect of 12-week compound exercise on postmenopausal women with type 2 diabetes and reported that training significantly decreases fasting blood glucose as well as 2-hour blood glucose (19).

The main purpose of improving the fasting glucose index in postmenopausal women through physical activities is to elevate glucose intake, which is accompanied with increased muscle blood flow and glucose uptake (20). Previous studies confirmed that exercise could decrease serum glucose through insulin secretion and stimulation of unOC (5). It also elevates glucose uptake in skeletal muscles, increasing insulin sensitivity. In addition, unOC may directly affect skeletal muscles and glucose transporters (Glu-4), and increase the capacity of the muscles for glucose uptake.

## CONCLUSION

Based on the results of this study, it can be concluded that exercise can increase the level of unOC in postmenopausal women. This increase has significant and serious consequences for all individuals, especially those susceptible to developing diabetes. Moreover, decreasing fasting blood sugar, as the best way of disease prevention, can be considered as a supplementary treatment.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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