Effects of Combined Exercise Training on Osteocalcin, Body Weight, and Inflammatory Markers Among Pre-Obese Women

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Abstract

Background: Osteocalcin is a bone-related protein, recently found to correlate with body weight and metabolic syndrome in adult. Exercise stimulates bone remodeling and improves inflammatory markers, even without associated weight loss. The association between serum osteocalcin and physical activity has not been well characterized.

Objective: This study assessed changes in osteocalcin and inflammatory markers (interleukin-6 and tumor necrosis factors) in healthy young women by 12-weeks of aerobic training.

Methods: Convenience sample of 40 young women aged 17-24 years were recruited to participate in this randomized control study. Participants were randomized to either intervention (N=20) or control groups. (N=20) Participants undergoing the intervention completed three sessions per week of moderate intensity endurance and resistance exercise training for 12 weeks. The control group was instructed not to change their lifestyle. Osteocalcin bone gamma-carboxyglutamate, body mass index, and inflammatory function (interleukin-6 and tumor necrosis factors) were measured at baseline and 12-week.

Results: The results showed that the 12-weeks of moderate aerobic training produced a significant improvement in serum osteocalcin, a significant decrease in inflammatory markers (interleukin-6 and tumor necrosis factors). These advances are significantly negatively correlated with a decrease of body weight index.

Conclusion: A structured combined endurance and resistance exercise training exerts significant positive effects on osteocalcin and inflammatory markers that could aid in risk factor modification of obesity and overweight young women.

Key Words: Aerobic exercise – Osteocalcin – Inflammatory markers.

Introduction

SEDENTARY behavior is widely prevalent across all age groups, sexes, and ethnicities [1]. Sedentary behavior is defined by sitting or reclining activities expending less than 1.5 metabolic equivalents (METs) [2]. Research suggests sedentary behavior and lack of physical activity are associated with overweight and obesity and is an independent risk factor for chronic diseases, including metabolic and cardiovascular disease [3,4].

Osteocalcin, a marker of bone formation, is also known as a regulator of glucose and fat mass [5]. Osteocalcin is released by osteoblasts during bone formation and binds with the mineralized bone matrix its precise function in bone metabolism has not been fully clarified. Several experimental studies demonstrated that osteocalcin promotes the recruitment and differentiation of circulating monocytes and osteoclast precursors, suggesting its role on osteoblast-osteoclast interaction and bone resorption. In humans, lower circulating osteocalcin levels were found in overweight and diabetic subjects compared with normal weight individuals. Moreover, osteocalcin concentrations increase after weight loss in overweight individuals and after improvement in glycemic control in subjects with type 2 diabetes [6,7].

Obesity and impairments in glucose homeostasis are associated with increased inflammation in white adipose tissue subsequently increased the expression of inflammatory cytokines such as IL-6, TNF-(x, etc.) [8]. Adipose tissue has been shown to affect bone metabolism by direct and indirect mechanisms including body weight-dependent effects on skeletal loading and the release of bone active hormones from the pancreatic beta cell (e.g., insulin, amylin, and preptin), and the adipocyte (e.g., leptin and, in some circumstances, estrogen). However, the previously proposed mechanisms do not fully explain [9]. The aims of this study were to determine the effects of structured exercise training on chang-
es of osteocalcin level and its relationships with inflammatory markers for young obese and overweight female.

Material and Methods

Convenience sample of 65 pre-obese young women aged 17-24 years were recruited from different secondary schools and colleges to participate in this randomized control study as shown in Fig. (1). Eighteen subjects were excluded, and a further seven of them did not respond to preliminary contact. Therefore, 40 participants enrolled. Participants were randomized equally to either intervention (N=20) or control groups (N=20) (using block randomization method from www.randomization.com). The study was conducted between June 2013 and Sept 2013. Participants were required to be sedentary to lightly active, participating in less than One hour of moderate-intensity physical activity each week over the previous three months. Participants were excluded from the study if they reported any of the following: diagnosed cardiovascular, diabetes mellitus (DM), or systemic disease; hyperthyroidism, hypoparathyroidism; irregular menstrual cycles. Approval from the Ethical Committee of scientific researches at faculty of Physical Therapy was obtained before conducting the study informed written consent was obtained from all participants.

Study design:

The study was a randomized control design which examining the effects of structured exercise training on osteocalcin and the inflammatory markers (IL-6 and TNF-α) in pre-obese individuals [10] compared to no exercise. Participants in the intervention group were undertaking the intervention completed three supervised exercise sessions per week for 12-weeks. The supervised sessions consisted of a five minutes of general warm-up, endurance exercise training in form of jogging on a motorized treadmill up to 30 minutes. Pulse meter was used to monitor subjects HR during training session under careful supervision.

The intensity of endurance exercise training was set between (65 to 75) % heart rate reserve (HRR) with HRR estimated using the Karvonen equation, which is:

\[
\text{THR} = [(\text{HR}_{\text{max}} - \text{HR}_{\text{rest}}) \times \% \text{ intensity}] + \text{HR}_{\text{rest}}[11].
\]

Whereas \(\text{HR}_{\text{max}} = 220\text{-age}\); \(\text{HR}_{\text{rest}} = \text{Resting heart rate}\); \(\% = \text{The desired intensity}\).
The resistance training was consists of abdominal curl, shoulder press, seated row, bicep curl and triceps extension. A rest period between resistances training muscle group was taken to ensure the heart rate was fallen between the desired intensity which was of 60% of one repetition maximum (I-RM) \[12\]. The program was progressive in linear manner moving from 3 sets of 8 repetitions to 4 sets of 12 repetitions. Finally, the session ended by ten minutes of cooling down in form of stretching and breathing exercises. Every session duration lasted approximately 60min. the control group was instructed to do not change their life style. Participants in both groups were encouraged neither to alter their habitual diet nor to change their physical activities.

**Measurements:**

A self-administered lifestyle survey was completed to provide data on (i) health status and medical conditions, (ii) nutrition, and (iii) current physical activity habits.

At baseline, a complete physical examination was performed and personal medical history and lifestyle factors were determined using a standardized questionnaire \[13\]. Blood pressure was measured using a mercury sphygmomanometer on the right arm with subjects in a sitting position after a 5-min rest.

**Anthropometric measurements:**

Height was measured to the nearest centimeter using a rigid stadiometer. Body weight was measured to an accuracy of 0.1kg. Weight and height were measured in the morning with subjects wearing light clothing but no shoes, and the BMI was calculated as the weight in kilograms divided by the square of the height in meters (kg/m\(^2\)) \[14\].

**Biochemical analyses:**

Fasting blood sample were taken for determination of Osteocalcin bone gamma-carboxyglutamate, and inflammatory markers (IL-6 and TNF-\(\alpha\)) at baseline and 12-week. Osteocalcin was measured by ELISA (enzyme-linked immunosorbent assay) ELISA kits according to the manufacturer’s instructions (BioSource, USA). Kits have estimated minimum detectable concentrations of 3pg/ml for TNF-\(\alpha\) and 2pg/ml for IL-6.

**Statistical analysis:**

Analyses were performed using SPSS version 20.0 (SPSS, Chicago, IL, USA). Statistical significance was set at an alpha level of 0.05 for all analysis. All data are presented as the means and stander deviations (±SDs). Kolmogorov-Smirnov test for normality was used to determine whether the variables for the study groups were normally distributed. Independent t-test to assess each dependent variable between both groups. Person’s correlation test was used to determine the correlation between osteocalcin and BMI, and inflammatory markers (IL-6 and TNF-\(\alpha\)).

**Results**

Participants who completed the study (N=38) included (N=20) for the intervention group and (N=18) for the control group. Two participants withdrew from the study after bassline due to personal reason or lost of interest.

**Baseline characteristics:**

Demographic data (Table 1) did not show significantly differ between the two groups at a baseline assessment.

<table>
<thead>
<tr>
<th></th>
<th>Intervention group</th>
<th>Control group</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>17.50 (3.63)</td>
<td>18.222 (4.30)</td>
<td>0.5</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>158.05 (4.68)</td>
<td>157.55 (5.09)</td>
<td>0.75</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72.50 (7.62)</td>
<td>72.22 (8.39)</td>
<td>0.9</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>74 (5.6)</td>
<td>75 (6.4)</td>
<td>.06</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>115 (7.8)</td>
<td>118 (8.9)</td>
<td>0.2</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>78 (8.4)</td>
<td>81 (6.2)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Data presented as mean and stander deviation (±SD). Significant level \(p<0.05\).

**Osteocalcin level:**

The value of osteocalcin level increased after 12-week for intervention group indicated a statistical significant level. There was no significant change in the control group at the end of study compared to bassline as shown in Table (2).

**Inflammatory markers:**

Levels of inflammatory markers were significantly lower in the intervention group compared to the control group after 12-weeks of exercise.
training. There was a significant statistical difference for the intervention group at the end of the study comparing with pre-study value. In the control group, there were no statistical significance difference of levels of IL-6 and TNF-α respectively at the end of the study when comparing to pre study values.

As shown in Table (2). The improvement of IL-6 was (−23%) and the improvement of TNF-α was (−24%).

Table (2): Mean value of all variables of both groups at the end of the study.

<table>
<thead>
<tr>
<th></th>
<th>Intervention group</th>
<th>Control group</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Osteocalcin (ng/ml):</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>16.16 (4.67)</td>
<td>15.20 (4.60)</td>
<td>0.47</td>
</tr>
<tr>
<td>Post</td>
<td>19.34 (5.02)</td>
<td>14.98 (2.66)</td>
<td>0.001</td>
</tr>
<tr>
<td>p-value</td>
<td>0.001</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td><strong>IL-6 (pg/mL):</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>3.07 (1.13)</td>
<td>2.95 (1.12)</td>
<td>0.75</td>
</tr>
<tr>
<td>Post</td>
<td>2.34 (1.16)</td>
<td>3.11 (1.21)</td>
<td>0.05</td>
</tr>
<tr>
<td>p-value</td>
<td>0.004</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td><strong>TNF-α (pg/mL):</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>1.17 (0.39)</td>
<td>1.06 (0.64)</td>
<td>0.5</td>
</tr>
<tr>
<td>Post</td>
<td>0.73 (0.64)</td>
<td>1.32 (0.48)</td>
<td>0.003</td>
</tr>
<tr>
<td>p-value</td>
<td>0.012</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td><strong>BMI (kg/m²):</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>29.54 (1.78)</td>
<td>30 (2.24)</td>
<td>0.9</td>
</tr>
<tr>
<td>Post</td>
<td>28.17 (1.64)</td>
<td>29.73 (2.03)</td>
<td>0.013</td>
</tr>
<tr>
<td>p-value</td>
<td>0.001</td>
<td>0.15</td>
<td></td>
</tr>
</tbody>
</table>

Data presented as mean and standard deviation (±SD). Significant level p<0.05.

**Abbreviations:**
- BMI = Body mass index.
- IL6 = Interleukin-6.
- TNF-α = Tumor necrosis factor alpha.

**Body mass index:**

After 12-week, BMI in the intervention group showed a statistical significance difference compared to the control group. There was a decrease in BMI without statistical significant at the end of the study in the control group when comparing to baseline assessment as shown in Table (2).

**Correlation between osteocalcin body mass index, and inflammatory markers:**

The correlation between change in osteocalcin and BMI and inflammatory function (IL-6 and TNF-α) at 12-week for the intervention group. There were statistical significant negative correlations between change level of osteocalcin and BMI (r=−0.7, p<0.01), Figs. (1,2), shows the correlation between osteocalcin and IL-6 that was ( r=−0.54, p<0.01). Finally, correlations between chang level of osteocalcin and TNF-α was (r=7, p<0.001), Fig. (3).
Discussion

The current study provides new information on associations of osteocalcin, particularly carboxylated osteocalcin, with BMI and inflammatory markers in young women (age range 17-24). The results of present study demonstrated that combined exercise training (endurance and resisted) were associated with an elevated level of osteocalcin. Furthermore, the present study showed that increase osteocalcin level was correlate negatively with BMI and inflammatory markers.

Dubnov-Raz et al. [15] found that higher plasma osteocalcin levels were associated with a significantly lower BMI, compared with females with lower osteocalcin concentrations. Previous studies showed that moderate to high levels of unstructured physical activity were associated with an elevated level of osteocalcin compared to a low level of physical activity [16]. Another study showed that Children and adults who complete exercise interventions, ranging from one month to one year, all demonstrate significant increases in osteocalcin levels [17]. Weight loss may be due to osteocalcin stimulates pancreatic beta cell to produce more insulin and, increases sensitivity to insulin by stimulating the secretion of adiponectin subsequently osteocalcin prevents weight gain [18].

Lee et al., documented that osteocalcin affects adiposity and glucose homeostasis in mice. His study concluded the skeleton influences on energy metabolism subsequently, osteocalcin-deficient leads to obesity, hyperglycemia and insulin resistance [19].

The present study pointed out the improvement of osteocalcin level after exercise training and a reduction of BMI and inflammatory markers. Different studies showed the anti-inflammatory effects of regular exercise which may be mediated via both a reduction in visceral fat mass (with a subsequent decreased release of adipokines) and the induction of an anti-inflammatory environment with each bout of exercise [20,21]. Recent study concluded that in obese subjects, physical activity may lower levels and thus reduce pro-inflammatory effects of cytokines that may link obesity, insulin resistance and diabetes [22]. The decrease in TNF-α in response to exercise training observed in this study may be due to a number of factors. IL-6 has inhibitory effects on TNF-α production and circulating levels of IL-6 are involved in the regulation of TNF-α [23].

References


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الملخص العربي

المقدمة: الأستيوكالسين يعد من بروتينات العظام المرتبطة بوزن الجسم والمتابعة الغذائية. تُعد التمارين بمثابة منبه للحفاظ على الغذاء، و أيضًا تستطيع أن تحتسب دلالات الإجهاد حتى بدون تأثيرها على انخفاض الوزن. العلاقة بين الأستيوكالسين والنشاط الرياضي لم يتم دراستها بشكل كاف.

الهدف من الدراسة: دراسة التغيرات التي تحدث لمستوى الأستيوكالسين وعلاقته بدلاليات الإجهاد لدى النساء اللاتي قبل مرحلة البداية بعد اثنتي عشر أسبوع من تمارين التحمل وتمارين القدرة.

الإرشادات: أخترت عينة عشوائية واختير أربعين شخصًا من الدراسة. تم تقسيم العينة عشوائياً إلى مجموعتين المجموعة العلاجية والمجموعة الضابطة. المجموعة العلاجية تلقى اثنتي عشر أسبوعًا من تمارين التحمل على جهاز السير الكهربائي وتمارين تقوية بواقع ثلاثة جلسات أسبوعياً. بينما المجموعة الضابطة لم تتلقى هذه التمارين كما تلقى تعليمات بعد تغير أسلوب حياتها خلال فترة الدراسة. تم قياس معدل الأستيوكالسين ومعدل الوزن ودلاليات الإجهاد قبل الدراسة وبعد الدراسة مباشرة.

النتائج: لوحظ تحسن ذا دلالة إحصائية على معدل الأستيوكالسين ومعدل الوزن ودلاليات الإجهاد لدى المجموعة العلاجية. كما أظهرت النتائج ارتباط سلبي قوي ذا دلالة إحصائية بين معدل الأستيوكالسين ودلاليات الإجهاد لدى المجموعة العلاجية بعد النهاية من الدراسة مباشرة. كما أظهرت النتائج عدم وجود أي تحسن ذو دلالة إحصائية على المجموعة الضابطة.

المستخلص: تمارين التحمل وتمارين القدرة المجموعة أظهرت تحسنتين ايجابية في معدل مستوى الأستيوكالسين ومعدل الوزن ودلاليات الإجهاد لدى النساء اللاتي قبل مرحلة البداية.