Effect of Four Weeks Plyometric Training with and without Blood Flow Restriction on Serum Bone Formation and Degeneration Markers in Inactive Girls

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Received: 2019/05/15
Revised: 2019/05/29
Accepted: 2019/06/1

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ABSTRACT

Background and Objectives: It has been shown that low intensity physical activity rarely increases bone density or renewal. Therefore, the purpose of this study was to investigate effects of four weeks of plyometric training with and without vascular occlusion on serum levels of bone alkaline phosphatase (BALP) and C-terminal telopeptide of type I collagen (CTX), as markers of bone formation and bone degeneration in inactive girls.

Methods: This was a semi-experimental study with a pretest-posttest design. The study population consisted of 36 inactive female students aged 23.84±1.096 years with a mean body mass index of 22.59±0.52 kg/m² who were randomly divided into a plyometric training group without blood flow restriction, a plyometric training group with blood flow restriction and a control group. The experimental groups performed four weeks of plyometric training (three sessions per week), while the control group did not perform any exercise. Blood samples were obtained 48 hours before the first training session and 48 hours after the last training session. Measurement of BALP and CTX was carried out using commercial enzyme-linked immunosorbent assay kits. Collected data were analyzed using t-test and one-way analysis of variance. All statistical analyses were performed using SPSS software (version 23) and at significance level of ≤ 0.05.

Results: The level of BALP was significantly higher in the low intensity exercise with blood flow restriction group compared to the control group (P=0.005) and the low intensity exercise group without blood flow restriction (P=0.003). The BALP/CTX ratio, as marker of bone metabolism, increased significantly following low intensity exercise with blood flow restriction compared with the other groups (P<0.05). However, low intensity exercise without blood flow restriction induced no significant change in the studied indices.

Conclusion: Plyometric training with blood flow restriction could be as effective as high intensity training for improving bone metabolism and turnover, particularly in inactive individuals.

Keywords: Alkaline Phosphatase, C-terminal telopeptide of type I collagen, Bone remodeling, Plyometric Exercise.

This paper should be cited as: Fakhri F, Habibi A, Ghanbarzadeh M, Ranjbar R. [Effect of Four Weeks Plyometric Training with and without Blood Flow Restriction on Serum Bone Formation and Degeneration Markers in Inactive Girls]. mljgoums. 2020; 14(3): 7-12.
INTRODUCTION
Osteoporosis and fracture are common among inactive people. It has been reported that inactivity has a negative correlation with bone mass density (1), while high physical activity is associated with increased bone density (2,3). Low mobility and inappropriate diet are changeable risk factors of osteoporosis (4). The short-term effects of interventions (physical activity, diet) on bone metabolism and remodeling are evaluated by markers of bone formation and absorption (5). Bone remodeling occurs through a coupled process of removing existing bone (resorption) and formation of new bone (6). An uncoupled increased concentration of bone formation markers such as bone alkaline phosphatase (BALP) indicates an increase in bone formation rate and is associated with increased bone mass density (7). Bone alkaline phosphatase is a tetrameric glycoprotein, which is predominantly produced by osteoblasts and its serum level reflects osteoblastic activity. Also, C-terminal telopeptide of type I collagen (CTX) is an indicator of bone loss and absorption, which reflects collagen type 1 degradation by osteoclasts and is used to measure bone remodeling (5). Bone markers’ response to training is more rapid than the bone marrow density’s response to training (8). In addition, they have been used to determine the bone turnover status and the effectiveness of training programs for improving bone metabolism. Among various exercise types, high-impact exercises are the most effective for increasing bone mass and bone metabolism (9). It has been shown that plyometric jump training, as a high-impact exercise, can increase expression of key transcription factors associated with osteoblasts differentiation (Runx2) in osteoblastic dyadic cells, increase expression of bone matrix proteins and stimulate osteoblasts differentiation in mice (10). The American College of Sports Medicine recommends moderate- and high-intensity exercise as well as high-intensity exercises such as jumps to increase bone health (11). However, many people do not tolerate such mechanical stress on their joints (12). Moreover, high-intensity exercise leads to early muscle exhaustion (13). On the other hand, low-intensity exercise (less than 2 times the body weight) rarely induces metabolic changes and increased bone density, while high-intensity physical activity more than 4 times the body weight improves bone metabolism (14). In order to resolve the potential drawback of low-intensity exercises in increasing bone density and bone adaptability, combined blood flow restriction exercises, known as Kaatsu exercises, are recommended (15). Kaatsu training utilizes pressure cuffs on each thigh, which when inflated, causes reduced femoral blood flow and pooling of venous blood in legs (16). These temporary alterations in leg circulation have implications for bone physiology. There is evidence suggesting that capillaries within the bone structural units and vascular endothelial cells may play a role in the coupling of bone resorption and formation (17) by secreting substances that inhibit osteoclast activity (18) and increase osteoblast recruitment (17). Therefore, even low-load blood flow restriction training is able to affect bone metabolism by altering vascular endothelial cell function. Bone health might be influenced by hypoxia, activity of vascular endothelial growth factor (15) or through increased intra-bone interstitial fluid intra-modular pressure (19). By analyzing BALP and CTx as bone formation and degenerative markers, respectively, it has been found that blood flow restriction can accelerate bone metabolism (20,21). In this study, we examined the effect of four weeks of plyometric training with and without blood flow restriction on serum levels of BALP and CTx, as markers of bone formation and degeneration in inactive girls.

MATERIALS AND METHODS
This was a semi-experimental applied study with a pretest posttest design. Study population included inactive students (aged 20-30 years) of Shahid Chamran University of Ahvaz, Iran. After completing the International Physical Activity Questionnaire (IPAQ), 36 people were selected (22). The mean age and body mass index (BMI) of the participants was 23.84 ± 1. 096 years and 22.59 ± 0.52 kg/m², respectively. A physician examined all subjects in terms of drug use, general health, cardiovascular health and blood pressure prior to participation in the study. Next, the subjects were randomly divided into three groups: traditional plyometric training without blood flow restriction (n=12), plyometric training with blood flow restriction (n=12) and controls (without blood flow...
restriction and exercise training) (n=12). The study protocol was approved by the ethics committee of the Shahid Chamran University of Ahvaz (code: EE/97.24.3.70342/scu.ac.ir) and written consent was obtained from all participants. First, the subjects completed a medical questionnaire and became familiar with the correct movements and proper breathing. Anthropometric measurements (height and weight) and body composition analysis were performed using a body composition analyzer (Olympia, model 3/3, Javan Company, South Korea). Also, the maximum oxygen consumed (VO\textsubscript{2max}) was measured using the Bruce protocol on treadmill and subjects were advised to avoid heavy physical activity before the assessments. The exercises were carried out for four weeks (three sessions a week). The training program consisted 10 minutes of warm-up, main training exercises and 10 minutes of cool down. The main training program consisted of four jump movements including deep jump (three sets of six repetitions), step jump (three sets of six repetitions), forward jump (two sets of eight repetitions) and side-to-side jump (two sets of eight repetitions). To overcome the principle of overload, two jumps were added to each training set every week. The rest period between each set was one minute. Exercise intensity was evaluated based on ground reaction force in a way that a force less than two times the body weight was considered a low intensity exercise for bone loading (14). Therefore, the subjects performed jumps on a force plate to determine the amount of force applied to the body. Subsequently, the perceived pressure was calculated based on the 10-point Borg scale (23,24). The experimental group performed their training on a step (height: 20 cm).

For the blood flow restriction group, the exercise intensity was determined during the initial stage of training and remained constant in the training period (the cuff pressure was considered 160 mmHg during exercise, which decreased to 50 mmHg at rest between sets). In the Kaatsu group, a specially designed elastic belt (Sato Sports Plaza Ltd., Tokyo, Japan) was placed around the most proximal portion of the legs during the exercise session (25). The belt contained a small pneumatic bag along its inner surface that was connected to an electronic pressure gauge that monitored the restriction pressure (MPS- 700, VINE, Tokyo, Japan).

In order to measure serum concentrations of BALP and CTX, fasting blood samples were taken by venipuncture by a nurse, 48 hours before the first session and 48 hours after the last training session. The samples were allowed to clot at room temperature and serum was separated by centrifugation at 3,000 rpm. The separated sera were transferred into polystyrene tubes and stored at -70 °C until analysis. Serum level of BALP was determined using an enzyme-linked immunosorbent assay (ELISA) kit (ZellBio GmbH, Germany). The minimum detection limit of the assay was 0.51 IU/L. The intra-assay and inter-assay coefficients of variation were less than 10% and 12%, respectively. Serum concentration of CTX was determined using an ELISA kit (ZellBio GmbH, Germany). The minimum detection limit of assay was 0.01 ng/mL. The intra-assay and inter-assay coefficients of variation were less than 10% and 12%, respectively.

Descriptive statistics were used to analyze the data. Normality of data was assessed using the Shapiro-Wilk test. Intra-group differences were assessed using the paired T-test. Intergroup differences were assessed using one-way ANOVA and LSD post hoc test. All statistical tests were performed in SPSS software (version 23) at a significance level of ≤ 0.05.

**RESULTS**

The results of the anthropometric measurements and body composition analysis in the pretest and posttest stages are presented in table 1. The BALP level increased significantly in the vascular restriction group (P=0.001). In addition, the CTX level reduced slightly in all training groups after the 4-week training period. The BALP/CTX ratio increased significantly in the low-intensity exercise group with blood flow restriction (P=0.008).

Based on the results of one-way ANOVA, the CTX level did not differ significantly between the study groups. Results of one-way ANOVA...
Moreover, the BALP/CTX ratio was significantly higher in the blood flow restriction group than in the control group (P=0.036) and the low-intensity exercise group (P=0.04) (Table 2).

Table 1. The results of the anthropometric and body composition measurements in the pretest and posttest stages

| Variable            | Group                                  | Pre-test     | Post-test    | Differences          |
|---------------------|----------------------------------------|--------------|--------------|----------------------|
| Age (Year)          | Training + vascular restriction         | 24.88±0.8    | ..............|                      |
|                     | Training                               | 22.64±1.15   | ..............|                      |
|                     | Control                                | 24±1.34      | ..............|                      |
| Height (cm)         | Training + Vascular restriction         | 159.88±1.33  | ..............|                      |
|                     | Training                               | 156.2±1.15   | ..............|                      |
|                     | Control                                | 157.66±1.20  | ..............|                      |
| Weight (Kg)         | Training + Vascular restriction         | 55.73±1.388  | 56.62±1.75   |                      |
|                     | Training                               | 57.02±0.25   | 56.38±0.68   |                      |
|                     | Control                                | 56.53±2.14   | 57.33±2.33   |                      |
| BMI (Kg/m²)         | Training + Vascular restriction         | 21.84±0.69   | 21.7±0.69    |                      |
|                     | Training                               | 23.22±0.18   | 22.9±0.24    |                      |
|                     | Control                                | 22.7±0.7     | 23.04±0.77   |                      |
| Body fat percentage | Training + Vascular restriction         | 26.27±2.11   | 25.61±1.94   |                      |
|                     | Training                               | 30.04±0.58   | 29±0.39      |                      |
|                     | Control                                | 27.76±1.73   | 27.70±1.96   |                      |

Results are presented as mean ± standard deviation.

*Significant difference between the pretest and the posttest stages (P≤0.05).

Table 2. Comparison of within-group and between-group variables of BALP, CTX and BALP/CTX ratio in the pre-and posttest stages

| Variables    | Groups                                  | Pre-test     | Post-test    | P-value Within group | P-value Between groups |
|--------------|-----------------------------------------|--------------|--------------|----------------------|------------------------|
| (BALP) (IU/L)| Training + Vascular restriction          | 127.9±1.83   | 110.04±2.32  | 0.001*               | 0.003* (a,b)           |
|              | Training                                | 106.01±2.11  | 105.63±3.19  | 0.84                 |                        |
|              | Control                                 | 104.5±2.53   | 104.72±2.76  | 0.96                 |                        |
| (CTX) (ng/mL)| Training + Vascular restriction          | 2.52±0.1     | 2.65±0.13    | 0.07                 | 0.41                   |
|              | Training                                | 2.50±0.06    | 2.55±0.08    | 0.45                 |                        |
|              | Control                                 | 2.56±0.2     | 2.53±0.16    | 0.83                 |                        |
| ALP/CTX      | Training + Vascular restriction          | 51.21±1.90   | 42.68±2.84   | 0.008*               | 0.043* (a,b)           |
|              | Training                                | 42.39±1.07   | 41.52±1.30   | 0.39                 |                        |
|              | Control                                 | 41.57±3.68   | 42±3.43      | 0.91                 |                        |

Results are presented as mean ± standard deviation.

* Statistically significant difference (P≤0.05).
a. Significant difference between the training + vascular restriction group and the training group.
b. Significant difference between the training + vascular restriction group and the control group.
DISCUSSION

Based on the results, four weeks of low intensity plyometric training with blood flow restriction resulted in a significant increase in BALP and BALP/CTX ratio (as the overall index of bone metabolism) compared to the low intensity exercise group and the control group. This is consistent with the results of Erickson et al., which showed that eight weeks of plyometric training can stimulate positive changes in bone turnover indices in young men (26). However, in a study by Kishimoto et al., a 2-week jump training at moderate or high intensity significantly decreased the level of BALP and CTX in young non-athletic women. Bone absorption markers can respond more quickly to stimulation than the bone formation marker (27). The inconsistency of the results could be due to the fact that CTX level may decrease by about 50% under the influence of diet or food intake. Moreover, Kishimoto et al. had not collected fasting blood samples. In a study by Vincent and Braith, ALP and ALP to pyridinoline ratio, as an indicator of bone renewal, increased significantly in the high intensity exercise group, while BALP did not change in the low-intensity exercise group (28), which are consistent with our findings. Similar to our results, Beeley et al. and Karabulut et al., reported a significant increase in BALP and CTX/ALP ratio as well as a significant reduction in CTX after six weeks of high intensity training with blood flow restriction (29). However, Young et al. demonstrated that walking with blood flow restriction does not alter bone markers in young women (30). Serum level of bone markers is higher in winter than in summer, which might be associated with variation of serum vitamin D levels (31). The present study was conducted in spring, while the study of Yang et al. was carried out in winter and spring, which may justify the disparity in results. Short-term caloric restriction and weight loss can alter bone formation markers. In a study on effect of eight days of caloric restriction and exercise on energy availability and bone turnover in young women, the marker of bone formation decreased significantly (32). Kim et al. indicated that high intensity resistance training results in a significant increase in the levels of BALP and bone formation marker to bone absorption marker ratio, while low intensity exercise with blood flow restriction cannot be as effective as the high intensity exercise (33). We found that exercise with vascular occlusion leads to a significant increase in BALP and a decrease in CTX. It seems that women are more sensitive to blood flow restriction exercise than men, hence they respond more quickly to these exercises. It is hypothesized that exercise with blood flow restriction changes bone metabolism, as the vascular endothelial cells inhibit osteoclast activity, thus stimulating the activity of osteoblasts and increasing bone turnover through the release of various molecules such as free radicals, local regulatory factors (IL-6, nitric oxide) and growth factors (34, 35). Therefore, exercise with blood flow restriction may affect the function of endothelial cells and improve bone metabolism. Even though the magnitude of mechanical load during blood flow restriction training is low, fluid shifts occurring during muscular contractions may stimulate piezoelectric potentials on osteocytes to induce remodeling (36, 37). Finally, endocrine responses to this training may either directly affect bone metabolism, or may alter the mechanosensitivity of bone cells, leading to decreased bone resorption (29). Given the limited number of studies on the effects of training with blood flow restriction on bone metabolism and incongruity of results, more research should be performed to assess the long-term impact of such trainings.

CONCLUSION

Performing low intensity plyometric training with blood flow restriction can increase bone metabolism. Therefore, engaging in plyometric training with vascular occlusion can prevent potential injuries caused by high intensity exercises and increase bone density in inactive people.

ACKNOWLEDGMENTS

This article is derived from a master’s thesis funded by the Shahid Chamran University of Ahvaz, Iran. The authors would like to express their deep gratitude to all people who have collaborated in this study, especially Dr Aliakbar Alizadeh and Miss Somaye Fakhri.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding publication of this article.
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