

## Effects of 9-Weeks High Intensity Aerobic Exercises on Hormones and Marker of Metabolism of Bone Formation in Young Women

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**Abstract:** Physical activity is an important factor in attaining bone mass. However, the basic mechanisms of this effect are not fully understood. The present study was performed to investigate the effects of 9-weeks high intensity (70-80% of maximal heart rate) aerobic exercises on hormonal concentrations and Marker of metabolism of bone formation in young women. A total of 22 healthy, sedentary female volunteers aged  $27.13 \pm 3.69$  years divided in to 2 groups control ( $n = 11$ ) and experimental ( $n = 11$ ) using random sampling. The experimental group performed an activity for 9 weeks, 3 session per week and each session lasted 45 min with the intensity 70-80% of maximal heart rate, but the control group did not participate in any intervention. For measuring enzymatic and hormonal factors, from each two groups of experimental and control blood samples were taken just before and after exercise program. PTH and  $E_2$  activity measured by ELISA and ALP activity measured DGKC methods respectively. We also used Paired t-test and ANCOVA to analyze the data ( $p < 0.05$ ). The results showed that 9 weeks high intensity aerobic exercises increased significantly in both PTH and ALP ( $p < 0.001$ ) and  $E_2$  ( $p < 0.018$ ) in the experimental group in comparison with the control group. Research findings shows that 9 weeks aerobic exercises causes considerable changes in PTH,  $E_2$  and ALP. Result this study suggest physical activity programs with specific intensities utilized in present research, can be used as a prevention factor of osteoporosis in young women.

**Key words:** Parathyroid hormone, estradiol, marker of bone formation, aerobic exercises, young women

### INTRODUCTION

Osteoporosis affects nearly 45 million women worldwide with fracture rates that far exceed the combined incidence of breast cancer, stroke and heart attacks (Stepnick, 2004). The disease results from a disruption of the fine balance between osteoblastic bone formation and osteoclastic bone resorption (Manolagas and Jilka, 1995). After menopause, resorption significantly exceeds formation and this imbalance results in net bone loss (Lindsay, 2004). Although, genetic factors may be the main determinants of bone mass and BMD, environmental factors also influence the quality and durability of bone. Several cross-sectional studies have shown that high impact weight-bearing activity is beneficial for the load-bearing sites of the skeleton (Dyson *et al.*, 1997). Parathyroid Hormone (PTH) is important for the regulation of bone metabolism with catabolic as well as anabolic properties (Eriksen and Charles, 1995). Its major physiological function is the maintenance of plasma ionized calcium ( $Ca^{2+}$ )/inorganic phosphate homeostasis

via the PTH/PTH-related protein receptor complex in kidney and bone (Barrett *et al.*, 1997) and is some evidence to believe that exercise disturbed calcium homeostasis and PTH secretion (Maimoun *et al.*, 2005).

The female sex steroid, estrogen, is a critical hormone for the maintenance of bone mass in women and an important regulator of bone metabolism, affecting the bone remodeling process via direct and indirect mechanisms. In addition, estrogen plays a major role in the adaptive response of bone to load bearing by increasing the osteogenic response to loading. Estrogen also affects calcium homeostasis by increasing calcium absorption (Lanyon, 1996). And by decreasing urinary calcium excretion (Mekane *et al.*, 1995). Physical activity causes that Bone alkaline phosphatase, as a tetrameric glycoprotein, has an anabolic effect in bone mineralization. Rudberg *et al.* (2000) reported an increase in bone ALP, after a moderate jogging exercise of 30-40 min. The peak of bone mass achieved before the third decade of life and factors that influence bone loss determine the amount of bone mass present at a

given time in adult age (National Institutes of Health Consensus Development Conference Statement. Osteoporosis Prevention, Diagnosis and Therapy. Bethesda, USA 2000, <http://consensus.nih.gov>). Physical activity can reduce fracture risk by increasing bone mass during youth, by preventing bone loss and by improving muscular and articular fitness that decreases the risk of falling (Kemper *et al.*, 2000). However, the mechanisms by which exercise leads to changes in bone metabolism are not fully understood. The purpose of this study was to determine whether 9 weeks high-intensity physical activity would affect hormones bone metabolism and marker of bone formation as measured PTH,  $E_2$ , ALP in young females.

## MATERIALS AND METHODS

**Subjects:** Twenty-two healthy and sedentary young females with regular menstruation aged (27.13±3.69) years (mean±SD) were participated in this study. The controls were randomly selected from the population to match the age and sex of the experimental group. The experimental group consisted of 11 sedentary females and the control group consisted of 11 females who had not exercise regularly for the past year (Table 1). The age, height, weight, BMI and body fat were not statistically significant between the groups. Informed consent was given to all subjects and prior to the study. Subjects were told to kept their daily routine. None of the subjects had any disease or had been consuming any drugs that could affect bone metabolism.

**Preliminary tests:** Before the test session, each subjects was given the following tests:

Medical history, physical and blood examinations. Height was measured to the nearest 0.1 cm with a fixed stadiometer (Harpenden stadiometer, Holtain, Crymych, UK) and weight was measured with a regularly calibrated electronic scale (EKS Exclusive, EKS International, Sweden). BMI ( $\text{kg m}^{-2}$ ) and Body fat percentage were assessed at baseline and after 9 weeks using a (Body logic/body fat analyzer, OMRON, Fanland).

**Exercise protocol:** The exercise intervention consisted of at least 45 min of high-intensity exercise 3 days/week for 9 weeks. The training program started at 40% of maximal heart rate for 16 min per session and gradually increased to 70-80% of maximal heart rate for 45 min per session by week 9. The weight bearing aerobic training include running, climbing stairs, skipping the rope, jumping (volleyball, basketball). Participants heart rate were monitored by Polar heart rate devices during exercise

Table 1: Physical characteristics of subjects

Characteristics	Exercise group (n = 11)	Control group (n = 11)
Age (Years)	27.00±4.20	27.27±3.32
Height (cm)	160.14±6.17	157.73±5.02
Weight (Kg)	57.23±11.18	54.18±6.31
Body fat(%)	24.95±6.12	24.58±3.92
BMI( $\text{Kg m}^{-2}$ )	22.35±4.30	21.67±2.27

Values are means±SEM

Table 2: Parathyroid hormone values ( $\text{pg mL}^{-1}$ )

	Baseline	Week 9	Paired t-test	
	(Mean±SD)	(Mean±SD)	(Sig)	ANCOVA
Exercise (n = 11)	75.70±17.44	87.75±20.39*	0.001*	0.001*
Control (n = 11)	71.32±16.14	71.33±16.23	0.942	

Comparison mean (SD) changes in serum concentration of Parathyroid Hormone (PTH) in experimental and control group pre and after 9-weeks aerobic exercises program in young women were determined by using Paired t-test. Comparison different significance between groups after 9-weeks aerobic exercise program in young women was determined by using the ANCOVA test. \*p<0.05

session. Intensity of aerobic exercise was defined in terms of the individual's heart rate and maximum Heart Rates ( $\text{HR}_{\text{max}}$ ) of subjects were calculated by using formula:  $\text{HR}_{\text{max}} = 220 - \text{age}$ . Targeted heart rate for aerobic exercise at maximal intensity was 70-80% of  $\text{HR}_{\text{max}}$  (Moldover and Bartels, 2000). Exercisers and control participants were asked to maintain their usual diet.

**Blood sampling and analysis:** Fasting blood samples were collected from the antecubital vein in the morning at scheduled intervals and centrifuged instantly at room temperature. Plasma obtained was frozen at  $-80^\circ \text{C}$  for subsequent analysis. To prevent interpretation errors, all blood were analyzed in the same run by the same investigator. Serum PTH was measured by an Enzyme-Linked Immunosorbent Assay (ELISA) (BioSource hPTH-ELISA Kit, Biosource Europe S.A. B-1400 Nivelles, Belgium). Plasma Estradiol was measured by an Enzyme-Linked Immunosorbent Assay (ELISA) (DRG Estradiol ELISA Kit,  $G_m$  bH.Germany) and serum B-ALP activity was measured with a colorimetric method that utilizes P- nitrophenyl phosphate as a substrate (Monotest ALPopt and Iso ALP, Boehringer Mannheim, Mannheim, Germany) (Table 2).

**Statistical analysis:** Data are presented as mean±SEM in the text, tables and figure. All data were analyzed for significance of differences between the control group and the experimental group using a paired t-test. The difference between before and after exercise was also compared. A two-way ANCOVA was used to determined if significant ( $p < 0.05$ ) differences existed among the group for PTH,  $E_2$  and ALP variables between before and after exercise testing. The statistical software program SPSS for windows, Version 15 was used for all data analysis.

**RESULTS**

Physical characteristics of the subjects are shown in Table 1. There were no significant differences between the 2 groups for any of the variables listed. Significant increases were found in PTH serum (Paired t-test,  $p = 0.001$ ) (Fig. 1 and Table 2).  $E_2$  Serum (Paired t-test,  $p = 0.018$ ) (Fig. 2 and Table 3) and B-ALP (Paired t-test,  $p = 0.001$ ) (Fig. 3 and Table 4) in exercise group after 9- weeks training. But in control group none of those factors had significant changes ( $p > 0.05$ ).

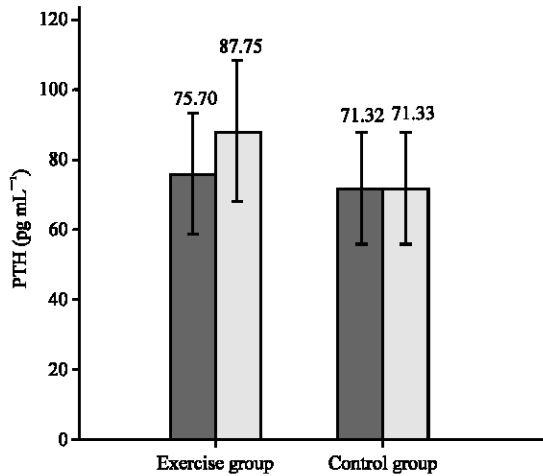


Fig. 1: Mean (SD) changes in serum concentration of Parathyroid Hormone (PTH) in experimental and control group pre and after 9-weeks aerobic exercises program in young women

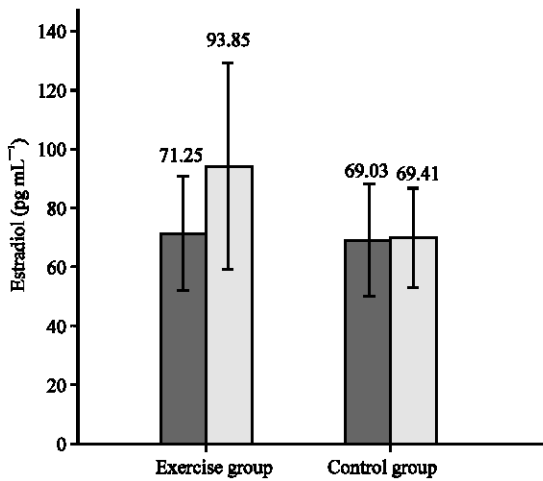


Fig. 2: Mean (SD) changes in serum concentration of Estradiol ( $E_2$ ) in experimental and control group pre and after 9-weeks aerobic exercises program in young women

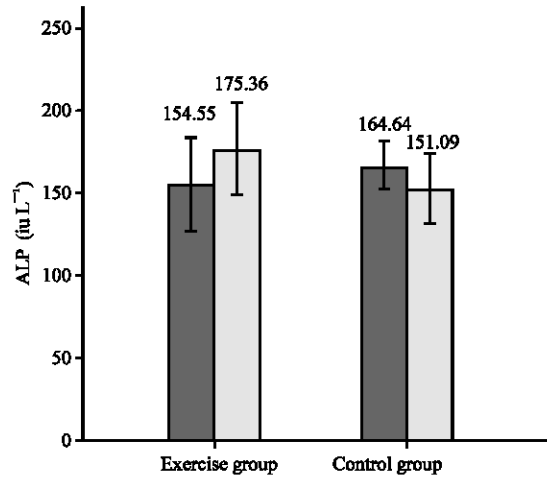


Fig. 3: Mean (SD) changes in serum concentration of Alkaline Phosphatase (ALP) in experimental and control group pre and after 9-weeks aerobic exercises program in young women

Table 3: Estradiol hormone values (pg ml<sup>-1</sup>)

	Baseline (Mean±SD)	Week 9 (Mean±SD)	Paired t-test (Sig)	ANCOVA
Exercise (n = 11)	71.25±18.95	93.85±35.43*	0.018*	0.011*
Control (n=11)	69.03±18.61	69.41± 16.69	0.867	

Comparison mean (SD) changes in serum concentration of Estradiol hormone ( $E_2$ ) in experimental and control group pre and after 9-weeks aerobic exercises program in young women were determined by using Paired t-test. Comparison different significance between groups after 9-weeks aerobic exercise program in young women was determined by using the ANCOVA test. \* $p < 0.05$

Table 4: B-ALP values (iu L<sup>-1</sup>)

	Baseline (Mean±SD)	Week 9 (Mean±SD)	Paired t-test (Sig)	ANCOVA
Exercise (n = 11)	154.55±27.90	175.36±28.16*	0.001*	0.001*
Control (n = 11)	164.64±15.28	151.09±22.03	0.095	

Comparison mean (SD) changes in serum concentration of Alkaline Phosphatase (B-ALP) in experimental and control group pre and after 9-weeks aerobic exercises program in young women were determined by using Paired t-test. Comparison different significance between groups after 9-weeks aerobic exercise program in young women was determined by using the ANCOVA test. \* $p < 0.05$

**DISCUSSION**

Understanding the reaction of bone to physical exercise is important for the development of strategies to achieve high peak bone mass and prevent bone loss. This study aimed to investigate the relationship among exercise duration, intensity, physical fitness, hormonal and the enzymatic changes of bone metabolism in healthy young females. In our study, PTH significantly increased after 9 weeks high intensity exercise (70-80% of maximal heart rate). As the increase was equaled 13.73% and so, concentration of PTH didn't have any significant changes in the control group. In compared with experimental group, the PTH changes in exercise group was significant

in the comparison with the control group. As results the 18.71% increased was experimented in PTH concentration. Parathyroid hormone has both anabolic and catabolic effects on bone metabolism. Chronic hypersecretion of PTH causes a bone loss where as intermittent PTH secretion has anabolic properties and enhances bone formation (Bouassida *et al.*, 2003). In our study, the significant increase of PTH observed in the exercise group suggests an anabolic effects of exercise on bone metabolism. The results of this study is matched with other investigations results (Tosun *et al.*, 2006; Bouassida *et al.*, 2003; Maimoun *et al.*, 2006). There are also differing results in the literature. Brahm *et al.* (1996) did not find any changes in PTH values after a long-distance run. Thorsen *et al.* (1996) did not find any alteration in PTH values in response to brisk walking at 50% HR<sub>max</sub> in postmenopausal women and also Linda *et al.* (2005) did not find any alteration in PTH values after strength and endurance exercise. Kim *et al.* (2004) reported a little different in the PTH values in response to 12 weeks weight-bearing exercise in healthy college women. Another finding in this study was shown that 9 weeks high intensity aerobic exercises increased significantly in serum Estradiol ( $p = 0.018$ ). So that the increase was equaled 24.08% and concentration of E<sub>2</sub> didn't have any significant changes in the control group. In compared with experimental group, the E<sub>2</sub> changes in exercise group was significant in the comparison with the control group. As results the 26.04% increased was experimented in the E<sub>2</sub> concentration. these finding adapted with (Consitt *et al.*, 2001; Copeland *et al.*, 2002). But Jasienka *et al.* (2006) reported the decrease in estradiol levels in response to habitual physical activity in women of the reproductive age. Tieman *et al.* (2004) reported that a 12 month moderate- intensity exercise intervention in postmenopausal women resulted in significant decreases in serum estrogens. Atkinson *et al.* (2004) did not find significantly alter urinary excretion of 2-OH E<sub>1</sub>, 16 $\alpha$ -OH E<sub>1</sub>, in response to moderate intensity exercise in postmenopausal women. It is evident that estrogen has a critical role in bone metabolism; however, the exact mechanism by which estrogen influences bone is not clear. *In vitro* study documented that estrogen and mechanical loading have independent effects on bone cells (Damien *et al.*, 1998). In our experimental study, healthy young women were included. The results would probably differ in postmenopausal osteoporotic women since there are studies stating that high-intensity exercises do not have positive effects on bone mass in postmenopausal women; which recalls that estrogen receptors might have a role on the response of osteoblasts to exercise (Sugiyama *et al.*, 2002). And also

results of the present investigation showed that ALP significant increased after 9-weeks high intensity exercise (70-80% of maximal heart rate). In the form that the increase was equaled 11.86% and so, concentration of ALP didn't have any significant changes in the control group. In compared with the experimental group, the ALP changes in exercise group was significant in the comparison with the control group. As results the 13.84% increased was experimented in ALP concentration. This finding adapted with (Tosun *et al.*, 2006. Rudberg *et al.*, 2000. Ashizawa *et al.*, 1998). Alkaline phosphatase is a marker of bone formation and our results may be interpreted as that 9-weeks high intensity exercise has anabolic effects on bone metabolism. It seems that training intensity positively correlated with circulating level of serum ALP activity. One proposed hypothesis to explain changes in bone metabolism is that mechanical stress itself is translated into biomechanical signals. (Brighton *et al.*, 1985). These findings might support the idea that different markers reflect different stages in osteoblastic cell proliferation and function (Stein *et al.*, 1993). The results of the present in investigation shows that concentrations PTH, E<sub>2</sub> and ALP in young women is increased by 9-weeks high intensity (70-80% of maximal heart rate) aerobic exercises that implies the effect of exercise type, intensity and duration of activity in young women.

## CONCLUSION

In conclusion, results this study suggested that in youth, adequate weight-bearing exercise is essential to build peak bone mass and reduce the risk of later fracture later in life. However, following the changes of bone formation markers and recognition of effective metabolism of physical activities with different intensity in young women seems serious.

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