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The Influence of Nine-Week Intensive Aerobic Exercises, Calcium and Vitamin D Supplementation on the Metabolic Response of Bone Formation Biomarkers

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Abstract

***Background*:** The purpose of the study is to analyze the impact of 9-week intensive aerobic exercises and the calcium and vitamin D supplement intake on the indicators which show bone formation in young women.

***Materials and Methods*:** 44 non-athletic women in their thirties (27.18±3.65) were studied randomly in four groups: 1) Exercise, 2) Calcium and vitamin D supplement, 3) Exercise and supplement, and 4) control. The subjects exercised three sessions a week for 9 weeks, each session lasted 30-45 minutes. The blood and urinary samples were collected from the experimental and control groups while they fasted either before or after exercise program. The concentration of alkaline phosphates and procollagen I, C terminal (PICP) had been measured using DGKC and HPLC techniques, respectively. The collected data had been combined with the statistical t-tests and were analyzed by ANCOVA (p<0.05).

***Results*:** Significant increasing of ALP and PICP was observed in exercise group (p=0.001) and exercise + supplement group (p=0.001). In supplement group, alkaline phosphate and procollagen type 1, C-terminal show a significant decrease (p=0.001), while the difference was not significant in the control group.

***Conclusion*:** Generally, the research results show that the intensive aerobic exercise and adequate intake of calcium and vitamin D affect positively the bone mass development during youth and they reduce fracture risk; however, such claim needs further investigations.

Introduction

According to the reports of World Health Organization, osteoporosis has been introduced as the third health problem and also is known as the silent epidemic. According to the recent studies, about 40% of the young women's lifetime is threatened by osteoporosis [1]. In present century, the importance of osteoporosis has become clearer, because the situation will begin from 20 to 35 years old with 1 to 5 percent intensity per year and will be increased remarkably after menopause [2, 3].

One of the most important biological markers of the bone tissue is procollagen I, C-Terminal Propetide (PCIP). This synthetic index is produced out of procollagen fibrils and constitutes 20 to 25 percent of the human being's proteins [4]. Various researches reported that short-term exercises have little impact on PCIP of blood circulation [5, 6]. However, intensive aerobic exercises have favorable impacts on keeping the balance between bone absorption and development of bone tissue during losing weight [5].

Alkaline phosphate is another indicator of bone formation which is a tetramericgilcoprotein. In bone tissue, steoblasts are great sources of alkaline phosphate which their serum levels reflect steoblastic activity [7, 8]. On the other hand, in bone tissue, calcium storage will be increased as the intercellular regulator in responding to sport exercises. Likewise, the role of vitamin D is evident in skeleton system; it increases the intestinal absorption and mineralization of bone matrix. Biologically, calcium supplements and vitamin D will reduce the speed of bone mass breakdown and danger of bone fracture. Ian Reid et al. reported a significant decrease in alkaline phosphate and PCIP after 5 years of using one gram of calcium per day in post menopausal women. Baecker et al. also reported decrease of PCIP in two groups of men in age of 23 and 25 after using 1000 and 2000 mg of calcium per day [9, 10].

This is while most of our society's women do not carry out regular physical exercises. Statistics indicates that only 11% of 16-year-old girls do regular exercises. Likewise, based on Iran's Nutrition Researches Institute, 90% of Iranian families receive calcium less than standard level which is resulted in early osteoporosis at the age of 35. In one year period, it is reported that approximately 36761 individuals were died as a result of this disease [1]. Thus, the present study tries to develop prognosis and to predict early bone fractures, while it investigates the effects of 9-week period of aerobic exercises, use of calcium supplement and vitamin D on biological factors of bone metabolism in young women.

Materials and Methods

The present paper is half experimental. Regarding the statistical criteria and standard methods in analyzing the bone tissue, supplements and exercising, we have used a pilot plan with four groups including three experimental and one control group. The present research has been applied in Urumiyeh in 2009 in which healthy 20 to 35-year-old non-athletic women who were qualified and have no history of effective disease on bone metabolism and are under no medical control in research period and no back ground of regular physical (at least 6 month before participating in the research) participated in the present study after filling the consent form.

The experimental group 1 has been considered as practice group which is constituted of 11 individuals. The participant exercised regularly trice a week, each 30 to 45 minutes along with 70-80% of intensity for 9 weeks.

The experimental group 2 has been considered as practice group with supplements which consists of 11 individuals, beside regular physical trice a week, each 30 to 45 minutes along with 70-80% of intensity for 9 weeks used calcium supplements and vitamin D based on international agreements, 19 to 50 years old, 1000 milligrams of calcium per day and for 1 to 50 years old, 200 IU of vitamin D.

The experimental group 3 is considered as supplement group including 11 testing individuals who used calcium supplements and vitamin D according to the international standard unit, for age of 19 to 50, 1000 milligrams per day and for age of 1 to 50, 200 IU of vitamin D for nine weeks. Participant in this group did not attend in physical exercises. The control group including 11 individuals neither participated in any physical exercise nor received calcium supplement and Vitamin D.

To control relatively the participants' nutrition, dietary remembrance method has been used in three days [11]. Health condition and family history of those under the test had been evaluated by standard health questionnaires [13]. The field variables of length (cm) and weight (kg) had been respectively measured with height gauge device and Seca digital scale made in Germany and also fat percentage and body mass index had been measured by Omron body logic/body fat analyzer made in Finland and blood pressure and heart beat by pressure indicator and digital beat counter (model Digimad 16, MBO made in England). Before and after the test we measured the blood and urine sample participants for measuring the factors of alkaline phosphates and Procollagen I, C-Terminal Propetide in fasting conditions.

In the present study, in order to determine the level of heartbeat exercises, pilot test was conducted on 15 individuals and the range of exercises of heart beat in participants had been analyzed with Karvonen Formulain work load of 70 to 80 percent of heartbeat equals 136 to 180 beat per minute. The program contains morning exercises, three times a week, each 30 to 45 minutes for 9 weeks. The heartbeat of rest and blood pressure of rest is measured and recorded before any kind of exercise. The heart beat and blood pressure is also recorded during the exercise. Two weeks before main exercises, the participants attended in intermediate exercises to be gradually adapted with the load of main exercise which is 70 to 80 percent of the highest level of heartbeat. Then, the main part of exercises with 70 or 80 percent of the highest level of heartbeat had been applied.

The schedule of exercises included the following stages: One minute of slow walking, 5 minutes of exercise, 10 minutes of professional exerciseand about 35 minutes of intensive aerobic exercises as the main exercise with 70-80 % of the highest heart beat, 7 to 10 minutes of running on rotating surface, Quinton track 3 model made in U.S with specific intensity and speed, 4 minutes of jugging, 4 minutes of height jump as well as various jumping of steps, 5 minutes of mixed exercise including jugging and running, 7 minute of playing with balls, fast two-players-games and finally 5 minutes of exercise to return to the primary condition. Calcium supplements and vitamin D had been compiled in 15 bottles, each containing 100 Caltrex calcium tablets in 500 milligrams and also 200 IU of vitamin D made in USA from pharmaceutical company of distribution of medicines. Based on standard principles, the second and third experimental groups received specific doses of such medicines during the research.

The amount of alkaline phosphate had been determined by experts of plasma alkaline phosphates of Pars Company due to DGKC (photometric kinetic) which is accorded with standard method of biochemical organization of Germany. To analyze urine samples, HPLC equipped with Florence indicator had been used. This method recently developed and had been recorded in United States patent in 1997 and is able to analyze PICP of urine sample. Adding internal synthetic standards (pyridinoline) adds to the accuracy of this method. The time of analyze is 30 minutes for each sample and it has special advantageous comparing Radioimmunoassay and ELISA.

To analyze data, descriptive statistics and statistical paired *t*-test (intergroup) and ANCOVA (comparison of the groups through balancing time and basic amounts) had been used significantly. In order to homogenize and distribute data normally, Kolmogorov-Smirnov had been used and data had been analyzed through SPSS-17.

Results

In table 1, physiologic and anthropometric features of youth have been illustrated. The result showed that after nine weeks of aerobic exercises, alkaline phosphates has been increased in the practice group (p=0.001). Moreover, considering the significance, PICP of young women had been increased significantly after nine weeks of severe physical activity with 70 to 80 percent of the highest level of heartbeat (p=0.001), (Table 1 &2, Fig. 1&2).

Alkaline phosphates of the individuals in the practice group as well as the supplement group had been increased severally with the intensity of 70-80% of the highest level of heartbeat after nine weeks (p=0.001) in addition the levels of PICP had been considerably increased after nine weeks of exercise (p=0.001) (Table 2, Fig. 1&2). On the other side, after nine weeks of using calcium supplement

Table1. Physiologic and anthropometric features of testing cases

Variable / Group	Exercise Mean±SD	Exercise & Supplement Mean±SD	Supplement Mean±SD	Control Mean±SD	Total Mean±SD
Age (year)	27±4.2	28.45±4.23	26±2.72	27.27±3.32	27.18±3.65
Height (cm)	160.14±6.17	159.64±4.32	162.73±7.73	157.73±5.02	160.06±6.02
Weight (kg)	57.23±11.18	59.27±7.08	59.18±6.24	54.18±6.31	57.47±7.96
Fat (%)	24.95±6.12	27.45±2.46	25.01±6.84	24.58±3.92	25.50±5.10
Body Mass Index (kg/m^2)	22.35±4.30	23.17±2.16	22.47±2.99	21.67±2.27	22.42±2.99
Resting Systolic Blood Pressure (mm Hg)	106.55±5.39	108.27±4.96	110.73±9.22	110.55±5.32	109.02±6.47
Resting Diastolic Blood Pressure (mm Hg)	67.91±4.37	67.27±3.50	70.27±7.64	70.45±5.20	92.50±25.60
Resting heart rate (beat /min)	76.09±3.70	75.64±5.26	73.64±7.09	72.27±3.55	74.41±5.16

Table 2. The average changes in alkaline phosphatase and procollagen type 1 C-terminal propeptide in the participants of the exercise group, exercise & supplement group and supplement group, before and after a nine-week aerobic program

Group	Variable	Before exercise Mean±SD	After exercise Mean±SD	*p*-Value
Exercise	ALP (IU/L)	154.55±27.90	175.36±28.16	0.001
	PICP (ng/ml)	160±53	184±49	0.001
Exercise & Supplement	ALP (IU/L)	134.64±10.52	153.18±12.95	0.001
	PICP (ng/ml)	158±41	170±55	0.001
Supplement Group	ALP (IU/L)	168.18±17	166.18±16.99	0.001
	PICP (ng/ml)	184.7±11.4	144.2±12.5	0.001

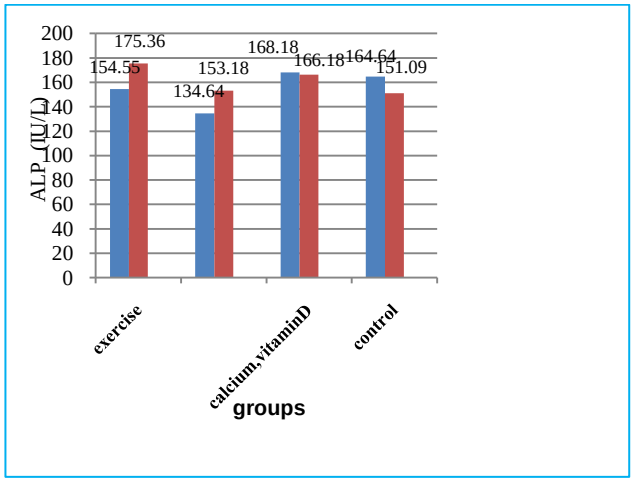

Figure 1. The average changes of alkaline phosphatase (IU/l) in participants serum in 4 group before and after a nine week aerobic exercise

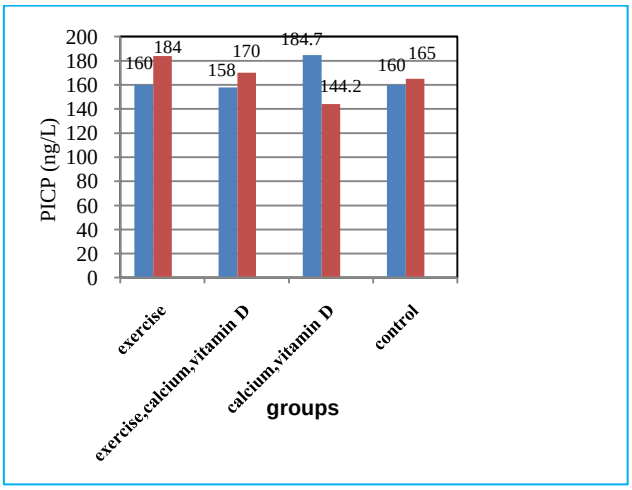

Figure 2. The average changes of procollagen type 1 C- terminal (IU/l) in participants serum in 4 group before and after a nine week aerobic exercise

and vitamin D alkaline phosphates had been reduced in the supplement group members (p=0.001). Also the level of PICP in young women of the supplement group had been decreased after nine weeks of using calcium and vitamin D (p=0.001) (Table 2, Fig. 1&2).

Discussion

The result of the present paper showed that the alkaline phosphates (IU/1) had increased significantly after nine weeks of aerobic exercises with the intensity of 70 to 80 percent of the highest level of heart beat, so that it had been increased 11.86 %, but viscosity of alkaline phosphates did not have significant change in the control group.

Comparing the groups, there had been significant discrepancy in the level of alkaline phosphates of the practice group compared to the supplement and control group and the only case with no discrepancy was between practice group and practice-supplement group. Alkaline phosphates had been increased 5.26% comparing the supplement group and 13.85% compared to the control group. Hinton et al. reported that the viscosity of alkaline phosphates increased significantly after 45 minutes walk with 75% of the highest level of heart beat activity for six weeks.

In another research, whipple et al. reported significant increase in the amount of alkaline phosphates after one session of resistance activity for 45 minutes. In the

present study, it seems that increase of alkaline phosphates has positive effect on the surface of alkaline phosphates of the blood circulation, so that increase of alkaline leads to transformation of mechanical pressures into biochemical signals and will increase bone mineralization (the hypothesis of transforming mechanical forces). In addition, the result showed that it supports the hypothesis of biological signs of bone which reflect different stages of cell proliferation of osteoblasts and their functions. The present paper's result accorded with what Tosun et al. and Maimoun et al. figured out. On the other side, the impact of calcium supplement and vitamin D had been reported as reducing factor of fracture in laps and spines.

In the present study, based on international unit, use of calcium supplements and vitamin D had been suggested for 19 to 50 years old in 1000 mg calcium per day for age of 1 to 50 and 200 IU vitamin D per day. Thus, the result of the present paper based on these standards showed that changes in the amount of alkaline phosphates in supplement group for nine weeks, 1000 mg calcium per day, 200 IU vitamin D reduced significantly, so that this reduction was 1.2%, despite all these, there had been significant difference in the amount of alkaline phosphate of the supplement group compared to the practice and control group. The only difference was between supplement group, supplement and practice groups.

The amount of alkaline phosphates had 7.82% increase compared to supplement and practice group and also 9.08% increase compared to the control group, it will also had 5.52% reduction comparing the practice test. Eskiyurt et al. demonstrated that using 1000 milligrams calcium, 300 mg citrate, and 800 IU vitamin D per day will reduce biological signs of bone metabolism and also increase in bone density during a 12-month period. Short-term use of calcium supplement and vitamin D will reduce absorption process as well as bone formation, this temporary effect will occur when changing unit' activity and bone change had been destroyed and those units in process of completion will expand their adjusting program. Such temporary imbalance between newer and older units will make special increase in bone units to reach a steady point.

The results of the present paper and results of Reid et al. and Meunier et al. in terms of significant reduction in alkaline phosphates were similar. Doyle et al., found significant change in the levels of indicators of bone formation (osteocalcin, alkaline phosphate) as a result of using supplementary linoleic acid in men of 39 to 64 years old. Apalacious et al. also did not found any significant change of alkaline phosphate after 6 months using saturated milk with 1200 mg calcium per day in Postmenopausal women.

Perhaps the reason of contrast between the above results and the present study is the type, duration and dose of consumption as well as gender. In addition, the results of this research showed that conspicuous increase was observed in the level of alkaline phosphates in practice group that had aerobic exercises of 70-80% of heart beat for nine weeks and also used 1000 mg calcium and 200 IU of vitamin D per day (12.1%). Comparing the groups, there is no remarkable difference in alkaline phosphates of the practice and supplement group toward practice and supplement groups, the only significant discrepant had been with the control group.

So that the level of alkaline phosphate of the practice and supplement group had 14.47%, 8.48%, 1.36% decrease compared to the practice, supplement and control groups, respectively. Wagner et al. reported increase in alkaline phosphates resulted from 12 weeks supplementary calcium diet including 750 mg calcium per day along with physical exercise trice a week in young women [25].

Also the present paper shows that type 1 collagen had remarkably increased less than nine weeks of intensive aerobic exercises with 70 to 80 percent of the highest level of heartbeat activity (15%). But type 1 collagen did not have any significant changes in control group. In comparison of the groups, significant difference was observed in the levels of type 1 collagen of the practice group compared to control and supplement group so that level of type 1 collagen of the practice group had increased 21.63% compared to the supplement group. Braham et al. reported significant difference of biological signs of bone formation on rotating surface [26]. Whipple et al. also figured out conspicuous increase in non-sport men after 45 minutes of resistance training [27]. However, the significant mechanism in type I collagen, is enzyme adjusting by Procollagen C proteinases (PCP) which divides PICP from procollagen to insolublecollagen, also it is reported that mechanisms of physical pressures lead to PCP genes and respectively procollagen syntheses and process will be increased by mechanical pressures. Another mechanism is the role of derived factors out of plasma including Interleukin 1, TGF-Beta, Plasma-derived growth factor (PDGE) in collagen syntheses and their impacts on fibroblasts' activity [29].Therefore, due to mechanical pressures on tendons, these factors will increase. Such changes lead to signaling in order to express procollagen gens and processing in fibroblasts. Also, the results of the present study showed that due to change in the levels of type I collagen in supplement group for nine weeks, each day 1000 mg of calcium and 200 IU of vitamin D reduced conspicuously (21.92%).

Comparison of different groups indicated conspicuous difference in the level of type I collagen of supplement group and practice group, practice and supplement and also control group. I-type collagen in supplement group has 18% reduction comparing supplement and practice group, 14.6 % reduction compared with control group, 24.6% reduction comparing to practice group. Marlena et al. reported remarkable reduction of procollagen I C-Terminal propetide after 16 weeks of using 1000 mg of calcium in women (20 to 35 years old). Baecker et al. also reported reduction of Procollagen I C-Terminal propetide in 2 groups of men (23 to 25 years old) after using 1000 and 2000 mg of calcium per day. However, Zittermann et al. reported no change in density of PICP of women who used wheat and flour per day for 4 weeks.

However, the most indicating metabolism of such changes is that short-term consumption of calcium supplements and vitamin D reduce absorption and formation of bone. Considering mechanostat hypothesis by Forest which acts as a strong stimulus for protection of bones are affected by calcium. Thus, that body immobility leads to bone absorption and consequently increase calcium absorption, in later steps, such process prevents emitting Procollagen I C-Terminal Propetide at the end of C terminal in bone matrix and cause balance between new units of bone forming by osteoblasts and older units which are going to be destroyed by osteoclasts in each bone units to reach a steady condition.

Generally, the results of the present paper shows that nine weeks of intensive aerobic exercises, consumption of calcium supplements and vitamin D in young women made a remarkable change in biological indicators of bone metabolism in mineral bone density in youth as well as decrease in danger of fracture in later years which indicates the satisfying impact of physical exercise, its intensity, duration and enough use of supplement dose of calcium and vitamin D based on international organization of women. Nevertheless, investigations on effective physical exercises with different intensity as well as consumption of different supplements with different doses on bone metabolism in young women need further studies.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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