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The Effect of Running Exercise and Calcium Supplementation on Femoral Bone Strength in Ovariectomized Rats

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Abstract: Osteoporosis may occur due to unbalanced feeding and lack of physical activity. While the role of calcium supplementation is of great importance, we planned this study to determine the effect of running exercise and calcium supplementation on femoral bone strength in experimental ovariectomized rats. Sixty female Sprague Dawley rats were divided into two groups. Group one (10 rats) was considered as the control and did not undergo any intervention (ovariectomy) and were followed for 3 months. Group two (50 rats) was considered as experimental group and all underwent ovariectomy and was divided into 5 equal subgroups. Subgroup 1 was left without any intervention and was followed for 3 months, Subgroup 2 underwent exercise including incremental running on treadmill for 2 months, Subgroup 3 received calcium supplementation for 2 months, Subgroup 4 received calcium supplementation together with incremental running for 2 months. Subgroup 5 just underwent ovariectomy but without any intervention and was followed up for 5 months. After euthanasia, both femoral bones of animals were removed for further studies. Femoral bone strength was measured by Hounsfield system. Deposition of calcium was measured by atomic absorption device. A 10.44% increase of weight was noticed in group 2 subgroup 1. This finding has been 12.40% in subgroup 2, 12.35% in subgroup 3, 12.80 in subgroup 4 and 12.78 in subgroup 5. The bone strength in subgroup 2 was 9.89±0.919 in pre test state and 11.22±1.129 kg force in post test state. These figures in subgroup 3 were 9.89±0.919 and 10.96±0.533, in subgroup 4 were 9.89±0.919 and 11.55±0.8 and in subgroup 5 were 9.89±0.919 and 9.86±0.689 kg force respectively denoting to a 14% increase in bone strength in subgroups 2-4. Regarding calcium deposition, a 3.7% increase was seen in subgroup 2 when pre and post tests were compared. This figure was 0.9% in subgroup 3 and 10.4% in subgroup 4 showing a 0.4% decline in calcium deposition. In ovariectomized rats, calcium supplementation could significantly increase the bone strength in comparison to running exercise and when a combination of them was implemented, the difference was more significant. Therefore, a combination of running exercise and calcium supplementation seems more beneficial in osteoporosis.

Key words: Running · Exercise · Calcium · Bone Strength · Ovariectomy · Osteoporosis · Rat

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INTRODUCTION

Osteoprosis is a disorder that can be prevented by physical activities. In childhood, inadequate mineral and vitamine supplementation in food and lack of physical activity would result into insufficient calcium deposit in bones and in adulthood would lead into reduction of bone minerals and exposure to bone fractures [1, 2].

Although the disorder has been noticed in both men and women, it is more prevalent in old women who are related to hormonal changes in old age [3, 4]. Female hormones were shown to have a protective role in re-absorption of bony tissue. In osteoporosis, reduction in estrogen level, lack of calcium consumption and decrease in physical activities are the main causes of the disease. Therefore, any strategy to prevent the disease would be of great importance to reduce the therapeutic costs of the patients [1-10].

It was shown that the mineral concentration of bony structures is less in Asian and Caucasian accounting for the smaller body size of these populations causing more pelvis fracture including 30% of whole fractures in Asia [4]. Furthermore World Health Organization has estimated that about 530 million women and men older than 65 years will live in Asian countries by 2050 [4]. So it is estimated that in these populations, osteoporosis would have an increasing trend and would be one of the most important health problems in these countries. Lack of calcium and vitamin D and absence of physical activity in these populations denote to the importance of preventing measures to control osteoporosis [4].

Calcium and estrogen levels are important in this relation. Estrogen can increase the bone strength as during menopause, the estrogen level falls down and the absorption of bone minerals has a decreasing trend [3]. Thereby, the use of calcium or vitamin D supplementation would increase calcium absorption which is essential during menopause period [3]. Chang and colleagues [11] also have considered the changes in bone mass during exercise in 50-55 years menopause women after 8 years from their last menses. They showed that exercise (6 sessions/week, each session 2 times for 12 month) had a positive effect on mineral density in upper thigh bone and lumbar vertebra and caused enhancing of the bone minerals in the exercise group.

Tukanon *et al*. considered physical exercise and calcium supplement when testosterone secretion was stopped by castrating male rats (3 months old, weighted 264±4 g) after 4 and 8 weeks. Rats in the exercise group ran on a rolling band without slope (speed of 10 m/min, one hour daily during 8 weeks) and showed that castration decreased the growth of bones and led to osteoporosis although exercise had a positive effect on osteoblast, osteoclast and calcium amounts [12]. So this study was undertaken in Sprague Dawley rats to determine the effect of running exercise and calcium supplementation on femoral bone strength.

MATERIALS AND METHODS

From February to June 2009, sixty female Sprague Dawley rats (225±25 g) were provided from Shiraz University of Medical Sciences Laboratory Animal Center. Animals were housed individually in one cage in an ambient temperature of 21±2°C and a 65-70% relative humidity. They received a balanced diet and had free access to water. They were divided into two groups. Group one (G1,n=10) was considered as the control (Non-ovariectomized without any intervention and was followed up for 3 months for 3 months). Group two (50 rats) was considered as experimental group, all underwent ovariectomy and left for 3 months before start of treatment. Rat in the second group (G2) was divided into 5 equal subgroups). After ovariectomy, the first subgroup (G2a) was considered as control ovarictomized group and were followed up for 3 months (n=20) subgroup 2 (G2b, n=10) underwent exercise using an incremental running on treadmill (7 channels, Iran) for 8 weeks (5 times/week). All exercises were done in the morning with a speed of 12 m/min for 3 min (Table 1). Exercise duration was first 10 min (incremental running on treadmill) and then reached 59 min. The speed was first 12 m/min and then reached to 20 m/min equal to the intensity of 70% of maximum oxygen consumption. In subgroup 3(G2c, n=10) received calcium supplementation for 2 months (5 times/week) by gavage using 6 ml calcium syrup (Osteocare Liquid, Vitabiotics, UK; Each 200 ml contained 300 mg calcium, 150 mg Mg, 6 mg Zn, 3.8 µg Vitamin D) (Table 1). In subgroup 4(G2d) underwent the exercise plan and calcium supplementation similar to subgroup 2 and 3 for a period of 2 months (Table 1). Subgroup 5(G2e) which were ovariectomized animals was just followed up for 5 months without any exercise and calcium supplementation (Table 1). Rats of all G1 and half G2a were euthanized after 3 months while the other subgroups were euthanized after 5 months of starting the experiment. Femoral bones were removed to investigate the bone strength by Hounsfield system, Deposition of calcium was determined by atomic absorption device.

Table 1: Running exercise on the tread mill in different sessions and speeds during the 2 months period.

		Duration							
Session/ week	Speed (m/min) Time (min)	1st week	2nd week	3rd week	4th week	5th week	6th week	7th week	8th week
First	Speed	12	13	14	15	16	17	18	19
	Time	10	15	20	25	30	38	45	53
Second	Speed	12	13	14	15	16	17	18	19
	Time	11	16	21	26	32	39	47	54
Third	Speed	12	13	14	15	16	17	18	20
	Time	12	17	22	27	33	40	48	56
Forth	Speed	12	13	14	15	16	17	18	20
	Time	13	18	23	28	35	42	50	57
Fifth	Speed	12	13	14	15	16	17	19	20
	Time	14	19	24	29	36	44	50	59

Electrical shock or any other stimulation was not applied throughout the study. The enrolled rats were all in estrus cycle (proestrus, estrus, metestrus, diestrus).

Hounsfield tool (H50KS, UK) was applied to measure the bone strength using the right femoral bone and the other femoral bone was used to determine the calcium deposition by atomic absorption instrument (Spectra Plus 2, USA). After burning the bones, they were placed in electrical heater in 600°c for 36 hours to be converted into ashes to determine the calcium amount.

In the animal selection, subsequent care and the sacrifice procedure were all adhered to the guidelines and were under the supervision of the Animal Care Committee of Iran Veterinary Organization. The study was approved by the Ethics Committee of the University.

SPSS Software (version 15, Chicago, IL, USA) was used for statistical analysis. T test, one-way analysis of variance and Tokay test were used to analyze the results. A significant level was considered at $p \leq 0.05$.

RESULTS

A 10.44% increase of weight was noticed in group and subgroup 1 after 5 months. This figure has been 12.40% in subgroup 2, 12.35% in subgroup 3, 12.80 in subgroup 4 and 12.78 in subgroup 5. The highest bone strength was noticed in subgroup 4 (11.55±0.8 kg; p=0.001) and the lowest in subgroup 5 (9.86±0.689 kg; p=0.95) (Table 2). These findings denote to a 14% increase in bone strength in subgroups 2-4 while in subgroup 5, a decrease in bone strength was observed. There was a significant difference for thigh bone strength between ovariectomized and non-ovariectomized rats. The difference was also significant between subgroups 2-4 and the controls but the difference between the subgroups 2 to 4 was not statistically significant.

Regarding calcium deposition, the highest value was noticed in subgroup 4 (89.15±0.63; p=0.001) and the lowest in subgroup 5 (80.47±2.22; p=0.95) (Table 2).

DISCUSSION

Our findings showed that the mean of bone strength was 11.3 kg before ovariectomy and was 9.89 kg after ovariectomy. So primary results showed that 3 month after ovariectomy, the bone strength decreased significantly due to a decline in bone density. Several authors demonstrated the correlation between ovariectomy in rats and the bone density [13-20]. It was shown that ovariectomy could affect biomechanical and structural features of the bone in rats 6 weeks after surgery [20]. The ovariectomized rats were a very suitable model for menopause women. Similar changes were observed in

Table 2: The changes in weight, bone strength and calcium in different groups in pre and post- test stages.

Groups	Weight (g)	Age (week)	Bone strength (kg)	Bone calcium	T	P value
G1	225±25	24	11.28±0.794	-	-3.63	0.002
G2a	261±10.92	24	9.89±0.919	80.77±1.7		
G2b	280.13±30	32	11.22±1.129	83.74±1.6	-0.28	0.017
G2c	278.63±24.88	32	10.96±0.533	81.54±1.776	-2.93	0.01
G2d	288±26.79	32	11.55±0.8	89.15±0.63	-4.03	0.001
G2e	287.63±25.58	32	9.86±0.689	80.47±2.22	0.06	0.954

these animals. Ovariectomy caused a decline or stop of estrogen secretion while this hormone has a critical role in body calcium regulation so that its level remains stable. Estrogen can increase the bone strength by saving calcium deposits. After menopause, the estrogen level falls down, the absorption of bone components would be less than its excretion. By the begging of menopause in women and the stop of estrogen secretion, various complications may happen while the most important one is osteoporosis. As one of the most important actions of estrogen on bone is the aggravation of osteoblasts and stopping the osteoclast activity, so ovariectomy may cause a decrease in bone strength, mineral density, mineral content and an increase in re-absorption of calcium [20].

We showed that 8 weeks running exercise on treadmill caused a significant increase in bone strength in female ovariectomized rats in comparison to the control group. These findings are identical to several reports performing running exercise on treadmill [13, 20-24]. Barongotles *et al.* [19] showed the positive effect of endurance exercise on bone mass. They showed a correlation between primary bone mass and the effect of exercise. Exercise was shown to have more effects on bone mass and osteoporosis when compared with bones in healthy subjects. In other words, exercise led to more mineral absorption in bones with osteoporosis than normal bones [22].

In a similar study, running on treadmill could cause an increase in bone strength in rats after ovariectomy due to a decrease in re-absorption of bony tissue and an increase in bone formation. In this study, endurance exercise could prevent the decrease in bone mass in ovarieectomized rats and that bone mass and mechanical strength of bone especially in long bones bearing the body weight, increased in osteoporosis condition [13].

The reports revealed that running exercise on treadmill during an 8 and 18 weeks period of time could significantly prevent the decline in the bone mass and increased the bone strength [20]. It seems that during exercise course, the intensity of exercise was important in maintenance of bone strength. NordSelton *et al*. [25] demonstrated that intensity and strength of bone decreased in an intense running exercise on treadmill. So the decrease in bone strength in intense exercise condition was 26% and the negative effect of intense exercise on bone strength was more than non-exercise group.

In another study by Peng *et al*. [26], bone density using a low intense running exercise on treadmill (10 m/min) resulted into an increase in bone strength in comparison to a high intense running exercise (18 m/min).

Gero *et al.* [24] reported that an increase in mineral density of bone during an intense exercise was much more than a low intense exercise program. In Nordstelon and Peng study, the kind of exercise was endurance and running on treadmill and subjects were ovariectomized rats [26], while in Gero study, the exercise plan was aerobic running and endurance exercise and subjects were menopause women [24]. So it seems that the kind of exercise had more effect on bone density as high intensity exercises had more effects.

The other important issue is the duration of exercise course. Gala *et al*. [23] revealed that bone mineral density increased during the 13 weeks running exercise on treadmill, while 28 weeks running exercise on treadmill had no effect on bone density. Therefore, more passing of time from menopause beginning, exercise may have less effect on bone density and strength.

Our findings showed that 8 weeks consumption of calcium supplement had a significant effect on bone strength in ovariectomized rats when compared to the control group. Many studies demonstrated similar results [18, 20, 27-30]. Rid *et al*. [29] reported that long-term daily consumption of 1000 mg calcium supplement could decrease the possibility of bone fracture in menopause women by approximately 30%. Prestod *et al*. (2002) also noticed the positive effect of calcium consumption on bone density in menopause women and showed that calcium and estrogen together had more effect on bone density in comparison to the group of consumption of calcium or just estrogen [19].

Jiang *et al*. [28] reported that bone minerals, strength and its mechanical features decreased in a low-calcium diet in groups of ovariectomized rats and healthy group, but estrogen consumption only could prevent this decrease in menopause group. Previous studies and our findings denote to the effect of calcium or estrogen absence in decrease of bone strength even their mixture had a more effect on bone mass.

The results of our study showed that 8 weeks running exercise on treadmill simultaneous with calcium consumption had a significant effect on bone strength in ovariectomized rats comparing to the control group. Several other studies have also confirmed our findings and showed that by passing of more time from the beginning of menopause, exercise would have less effect on bone density [23, 31-35].

It was shown that running exercise on treadmill and estrogen consumption could both cause an increase in content and density of bone minerals. Also, the effect of a combination of those would cause a significant increase in the bone mass. Estrogen could lead to a decrease in destruction of bone in both thigh and lumbar vertebrae bones, as running exercise on treadmill could cause a decrease in bone destruction mainly in the thigh bone. It was shown that the effects of estrogen consumption and running exercise on treadmill in thigh and lumbar vertebrae bone mass increased independently [36].

Although different effects of endurance exercise and calcium consumption on lumbar vertebrae and bone density were studied showing that high intense exercises could decrease the bone mineral density in lumbar vertebrae but increased the density in the thigh bone, while low intense exercises increased the density in both thigh and lumbar vertebrae bones [31]. The difference of their results with our findings may be due to difference in anatomy of bone used and the performed exercise. In our study, running on treadmill increased the calcium deposit in bones and consequently increased the bone strength. Fonseca *et al*. (2011) showed that voluntary exercise prevented osteocyte death and that this protective effect was associated with increases in femur ultimate stress [37]. Marques *et al*. (2011) suggested that 8 months of resistance exercise may be more effective than aerobic exercise for inducing favorable changes in BMD and muscle strength, whilst both interventions demonstrate to protect against the functional balance control that is strongly related to fall risk [38]. Beck *et al*. (2011) demonstrated that more active women had geometrically stronger femurs. So exercise could improve the strength of the femur largely by adding bone to the outer cortical surface and improved resistance to bending [39]. Isaksson *et al*. (2009) reported that an improvement of the properties of mineralized bone after exercise [39].

Therefore, applying any of interferences (running, calcium supplementary or both together) was identically effective in prevention of osteoporosis. So it can be advised to people suffering from osteoporosis and fracture, that they can prevent the progress by running exercises, consumption of calcium supplement and a combination of them.

As the results from animal studies can be extended to human studies too, it is advised to consider the effects of these kinds of exercises on bone strength in old men and women. The endurance of load pressure, minerals concentration, minerals and calcium absorption are different in various anatomical conditions, so the consideration of exercise effects on other bones especially bones which endure body weight seems necessary when comparing with non-body weight bearing bones.

We showed that in ovariectomized rats, calcium supplementation could significantly increase the bone strength in comparison to running exercise and when a combination of them was implemented, the difference was more significant. Therefore, a combination of running exercise and calcium supplementation seems more beneficial in osteoporosis.

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