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# Effect of Exercise and Calcium Supplementation on Bone Mineral Density and Bone Mineral Content in Growing Female Rats

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## ABSTRACT

The purpose of this study was to examine the effects of dietary calcium supplementation and exercise on bone mineral density and bone mineral content of growing female rats. The exercise and control group were fed a diet containing 0.5% calcium and Ca supplementation group were fed a diet containing 1.0% calcium diet. The exercise group ran on a rodent treadmill (speed of 15m/min for 30 min) three days per week during the 3-week study period. Bone mineral density (BMD) and bone mineral content (BMC) of spine and femur were determined by using dual energy x-ray absorptiometry (FIXI-mus, GE Lunar Radiation Cooperation, Madison, WI, USA). The exercise group had significantly greater (6.25%) spine BMD compared to the nonexercise group and the exercise group had but not significantly greater spine BMC (7.1%) compared to nonexercisers. Femur BMD and BMC divided by the rats final body weight appears to have a higher BMD (7.5%) and BMC (4.5%) in the exercise group, which indicates that exercise had a positive influence on femur bone mineral density and bone mineral content. The supplementation of calcium did not significantly affect spine and femoral BMC and BMD for the 3 weeks experimental period. It can be concluded that when calcium intake meets the recommended, exercise is beneficial for acquisition of spine bone mineral density in young growing female rats. (*J Community Nutrition* 4(3) : 195~201, 2002)

KEY WORDS : exercise · calcium · bone mineral density · growing female rat.

## Introduction

The annual costs to the U.S. health care system for the treatment of osteoporosis is an estimated $10 billion – $20 billion (Lindsay 1995) Currently, in the United States 16.8 million (54%) postmenopausal white women have osteopenia and another 9.4 million (30%) have osteoporosis. About 4.8 million women (51% of the osteoporotic women and 16% of all white women more than 50 years of age) are estimated to have established osteoporosis (Melton 1995). Total fracture load in any society depends not only on the incidence of the fractures, but also on the population size. Although there is a lower incidence of hip fracture among Asians than among Caucasians, 88% of the hip fractures in the world occurs in Asia (Coaper 1992).

Since the average life expectancy is increasing in Korea, the burden of osteoporotic fractures continues to rise. At present, it is estimated that more than 2 million individuals have fractures (Sung 2002). In Korea, about 86.4% of the women more than 50 years of age, in rural areas, currently have osteopenia or osteoporosis. About 90% of the elderly Koreans of more than 60 years of age are estimated having osteopenia or osteo-porosis (Kim 2000). Eleven percent of young women aged 25 – 35 years of age have osteopenia (Lee 1999). Unfortunately, calcium intake by many Koreans, especially females, is less than that of the Korean Recommended Dietary Allowances (Choi 2001 ; Choi 2002).

Understanding the factors that influence bone mass accumulation in childhood and maximize peak bone mass in young adulthood is important for designing preventative strategies to combat osteoporosis. Nutrition may be an important determinant of peak bone mass, with calcium as the most important nutrient. A few reports have indicated some beneficial effects of calcium supplementation on the skeleton of children (Johnstone 1992 ; Dibba 2000). Evidence for less bone formation caused by insufficiency of dietary calcium have been reported (Black 2002). Bonjour (1997) reported

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that Milk supplementation augmented bone gain in children, at least in the short term. In growing children, long-term avoidance of cow milk is associated with small stature and poor bone health (Black 2002). Several studies have shown a significant effect of a calcium supplement on bone mineral acquisition (Heaney 2000). The results of many studies indicated that a high habitual calcium intake is associated with higher peak bone mass values ; the relationship is not supported by strong evidence because nearly all studies have ecologic, cross-sectional, or retrospective designs. Ecologic and cross-sectional studies are particularly prone to bias as a result of confounding. However, a 15 year longitudinal study (age 13 – 28) evaluated the effect of daily calcium intake and physical activity during adolescence and young adulthood on the development of peak bone mass (Welton 1994). Calcium intake was not a significant predictor of bone mineral density, but a trend was demonstrated toward a higher bone mineral density in subjects in the highest quartile of calcium intake (Welton 1994). Welton et al did not find an calcium effect. They gave one of the possible explanations that the mean calcium intake was high in their subjects. However, Recker et al. (1992) demonstrated in a longitudinal study in young women that increased self-selected calcium intake enhanced the gain in spinal bone mass. Riss et al. (1987) showed that early post-menopausal women calcium supplementation may have had an effect only on the loss of cortical bone, but it had no effect on trabecular bone.

Skeletal modeling and maturation are influenced by changes in physical activity and nutrition. Weight-bearing exercise exerts many forces (or stress) on bone and therefore can ultimately increase bone mass. Several studies have shown that skeletal unloading caused by immobilization (Chen 1992), space-flight, and bed rest (Mazess 1983), results in osteopenia. Nordsletten (Nordsletten 1994) found a 40% decrease in ash weight in both the ovariectomized sedentary and exercising groups, indicating that training had a negative effect on the growth changes induced by an ovariectomy. Exercise therapy that loads the mechanical stress to the bone is also effective in maintaining bone mineral density in early postmenopausal women (Pruitt 1992). Weight bearing exercises, therefore, have been recommended, especially for young women, in order to develop strong bones and to possibly obtain a greater peak bone mass. Exercise, such as resistance training or weight-bearing activities like running or walking, seems to have an osteogenic effect on developing and maintaining bone mineral density (Going 1991).

A greater bone mass gained early in life is considered a critical factor in protecting against osteoporotic fractures later in life. The critical years for skeletal growth and accumulation of bone mass lie in the prepubertal and pubertal decade beginning at about 10 years in girls (Bonjour 1991). Mechanical loading of sufficient intensity promotes an increase in skeletal mass, especially during growth in the first 2 decades of life (Turner 1999). Diet and exercise are two lifestyle choices that can be altered by individuals to benefit health. Dietary calcium and physical activity have been independently associated with the development of increased peak bone mass and reduced bone loss later in life. Data is scarce with respect to calcium and exercise and bone health in growing children. A few reports have indicated some beneficial affects of calcium supplementation on the skeleton of children (Bonjour 1997). So the purpose of this study was to examine the effects of dietary calcium supplementation and exercise on bone mineral density of growing female rats.

## Methods

Thirty female Sprague-Dawley rats were obtained from KLEC (Korea Life Engineering Corporation, Seoul, Korea). The animals were housed individually in stainless steel wire bottom metabolic cages in an environmentally controlled room. The room was maintained at 24℃ on a 12-hour light-dark cycle. Those animals were randomly assigned to one of three groups (control, exercise, Ca supplementation). The first 2 weeks of treadmill running were a period of acclimation. During this time the animals ran for 10 minutes with time increasing 5 minutes every day until all animals were running 30 minutes continuously. The exercise running speed was increased gradually to a final speed of 15m/min for 30 minutes during the second week. The exercise group ran on a rodent treadmill three days (Monday, Wednesday, and Friday) per week during the 3-week study period. The control group was fed a diet containing 0.5% calcium and the Ca supplementation group was fed a diet containing 1.0% calcium (Table 1). Bone mineral density (BMD) and bone mineral content (BMC) of the spine and femur were determined by using dual energy x-ray absorptiometry method (FIXI-mus, GE Lunar Radiation Cooperation, Madison, WI, USA). Throughout the study period, daily quality assurance tests were performed to ensure the effectiveness of the lights, beam, mechanics, and tissue value of the scanner. The results are expressed in grams (BMC), and $g/cm^2$ (BMD). Body weight was measured weekly by using a scale.

### Statistical Analyses

Statistical analysis was performed by using SAS ver 8.12. The effect of exercise and calcium level were evaluated by using analysis of variance (ANOVA). After ANOVA, the Duncan's multiple procedure was performed for multiple comparisons. Results are expressed as means ± SDs. Values were reported as significant have p-values <0.05.

**Table 1.** Composition of experimental diets (g/kg of diet)

| Ingredients | Control Diet |
|---|---|
| Casein[1] | 200 |
| Corn starch | 530 |
| Sucrose | 100 |
| Soybean oil | 70 |
| Cellulose | 50 |
| Min-mix[2] | 35 |
| Vit-mix[3] | 10 |
| L-cystine | 3 |
| Choline | 2.5 |
| Tert-butyl hydroquinone | 0.014 |

1 : Casein high protein (total protein 85%), Teklad Test Diets, Medison, Wisconsin, USA, 2 : AIN-93G-MX, Teklad Test Diets, Medison, Wisconsin, USA, 3 : AIN-93G-VM, Teklad Test Diets, Medison, Wisconsin, USA. ※ Calorie % of diet-CHO : protein : fat = 64 : 19 : 17

## Results and Discussion

The body weight change and food intakes during the experimental period of the experimental groups are presented in Table 2 and Table 3. Rats in the experimental groups had similar mean initial body weights and final body weight. There were no significant effects of exercise or calcium supplement at either on incremental gain in body weight or food intake. However, in the present study, weight was not significantly impacted by exercise effect and calcium supplementation.

Effects of exercise and calcium supplementation on spine bone mineral density and spine bone mineral content in rats are presented in Table 4. The exercise group had significantly greater (6.25%) spine BMD compared to nonexercisers and the exercise group had tendancy of greater spine BMC (7.1%) compared to nonexercisers. This finding is in agreement with other studies. Swissa-Sivan and co-workers (1989) reported that exercise increased bone mineral density (7%) and bone mineral content (15%). Recently Kiuchi and co-

**Table 2.** The effects of exercise and calcium supplementation on body weight change during experimental period

| Week \ Group | Control | Exercise | High Ca[1] | p value |
|---|---|---|---|---|
| Baseline (g) | 196.9 ± 16.7[2] | 195.6 ± 13.6 | 196.7 + 12.6 | NS[3] |
| 1 week (g) | 212.0 ± 19.6 | 210.1 ± 13.4 | 209.3 ± 14.7 | NS |
| 2 week (g) | 227.5 ± 23.6 | 221.3 ± 15.6 | 222.6 ± 14.2 | NS |
| 3 week (g) | 240.0 ± 23.4 | 234.5 ± 19.6 | 240.7 ± 17.3 | NS |

1 : High Ca : calcium content is 1.0% of diet, 2 : Mean ± SD, 3 : NS : Not significantly different at p<0.05

**Table 3.** The effects of exercise on weigh gain, mean food intake and food efficiency radio (FER) during experimental period

| | Control | Exercise | High Ca | p value |
|---|---|---|---|---|
| Weight Gain (g) | 40.13 ± 10.5[1] | 34.13 + 5.99 | 43.83 ± 6.3 | NS[2] |
| Food intake (g/d) | 12.9 ± 0.56 | 11.9 ± 0.43 | 12.3 + 0.65 | NS |
| FER | 0.19 ± 0.05 | 0.17 ± 0.03 | 0.22 + 0.04 | NS |

1 : Mean ± SD, 2 : NS : Not significantly different at p<0.05

**Table 4.** Spine bone mineral density and spine bone mineral content in rats

| | Control | Exercise | High Ca |
|---|---|---|---|
| Spine BMD ($g/cm^2$) | 0.144 ± 0.003[1)a2)] | 0.156 ± 0.002[b] | 0.148 + 0.002[a] |
| Spine BMC (g) | 0.422 ± 0.019[a] | 0.452 + 0.055[a] | 0.442 ± 0.034[a] |
| Spine BMD/wt (kg) | 0.599 ± 0.012[a] | 0.664 ± 0.015[a] | 0.619 ± 0.021[a] |
| Spine BMC/wt (kg) | 1.753 ± 0.190[a] | 1.899 + 0.200[a] | 1.841 ± 0.211[a] |

1 : Mean ± SD, 2 : Values with different superscripts within the column are significantly different at p < 0.05 by Duncan's multiple range test

workers (1998) observed that training induced significant gain in bone mineral content. Steinberg and Treuta (Steinberg 1981) reported that bones of young active rats were heavier, longer, and had increased cortical and trabecular thickness. The weight is one of the main determinants of bone mass measured by DEXA in humans, and rats (Rico 1994). Spine BMD and BMC divided by the rats final body weight were not significantly greater in the exercise group. However, when spine BMD and BMC divided by the rats final body weight, a trend was demonstrated toward a higher spine BMD (10.8%) and BMC (8.3%) in the exercise group, which indicates that exercise had a positive influence on spine bone mineral density and bone mineral content. Weight-bearing activities to the youths, beginning at the age of 12y, were shown to have a significant influence on the gain of lumbar bone mineral density, which was not significantly affected by calcium intake (Turner 1999). In addition, the athletes had already achieved maximal bone mineral density values of sedentary control subjects of comparable body weight (Anderson 2000). Physical activity and, to a lesser extent, diet (particularly calcium intake) during adolescence and early adulthood have been implicated as determinants of peak bone mass (Welton 1994, Valmaki 1994). Physical activity such as running or weight lifting stimulates the secretion of sex hormones, growth hormones and catecholamines (Hakkinen 1993), and increases the bone formation resulting from an increase osteoblastic recruitment and in the activity of individuals osteoblasts in rats and humans (Barengolts 1994 ; Bourrin 1995 ; Eliakim 1997 ; Tamaki 1998). Because we did not see any effect of exercise on such hormones and bone formation indexes, this must be evaluated in the further studies.

In the present study, increased calcium intake didn't lead to increased spine bone mineral density or spine bone mineral content. Small, but not significant, increase in spine BMD and BMC was increased. These explanations could be attributed to calcium intakes approaching to required levels in both groups.

Effects of exercise and calcium supplementation on femur bone mineral density and femur bone mineral content in rats are presented in Table 5. No significant difference was observed among all groups. In the present study, there was no significant difference between normal (100%) and high (200%) calcium intake in the femur BMD except the calcium supplementation group appears to have a slightly higher femur BMD in the control group. No significant differences may be attributed to the fact that calcium intake above a certain threshold does not contribute much further to bone mineralization. Many of the supplement studies showing positive results were conducted in populations consuming a relatively low background calcium intake (Merrilees 2000 ; Dibbia 2000). Ovariectomized rats fed a high calcium diet (200%) for 8 weeks had a significantly higher femur bone mineral content (Kim 1996). So the effect of calcium intake may be different by hormonal status or the intervention period. Thus, the impact of calcium intake on bone warrants further exploration.

Femur BMD and BMC divided by the rats final body weight were not significantly greater in the exercise group. However, when femur BMD and BMC were divided by the rats final body weight, a trend was demonstrated toward a higher BMD (7.5%) and BMC (4.5%) in the exercise group, which indicates that exercise had a positive influence on femur bone mineral density and bone mineral content. The result of this study confirmed that at intakes approaching calcium requirement, physical activity is a more important predictor of bone mineral density than is calcium intake (Weaver 2000). The effects of exercise on strength and chemical composition of rat femur bone was examined in ten 5-week-old rats forced to exercise on a treadmill with a running speed of 20 m/mim for 4 weeks (Yeh 1993). The chemical composition of bone varies with the degree of exercise, and that the bone becomes strengthened at the same time. Yeh et al. (Yeh 1993) reported that after 4 weeks of treadmill running, femur calcium content was significantly greater than

**Table 5.** Femur bone mineral density and femur bone mineral content in rats

| | Control | Exercise | High Ca | p value |
|---|---|---|---|---|
| Femur BMD ($g/cm^2$) | 0.188 ± 0.015[1)] | 0.201 ± 0.066 | 0.199 ± 0.034 | NS[2)] |
| Femur BMC (g) | 0.341 ± 0.015 | 0.353 ± 0.030 | 0.349 ± 0.027 | NS |
| Femur BMD/wt (kg) | 0.785 ± 0.069 | 0.844 ± 0.020 | 0.828 ± 0.045 | NS |
| Femur BMC/wt (kg) | 1.419 ± 0.220 | 1.482 ± 0.112 | 1.453 ± 0.221 | NS |

1 : Mean ± SD, 2 : NS : Not significantly different at $p < 0.05$

the sedentary animals.

It has been suggested that exercise increases BMD by improving mechanical stress on the bone and including hypertrophy of the cortical bone in experimental animals (Barvo 1996). In the present study, femoral BMD and BMC were not significantly greater in the exercisers. But a trend was demonstrated toward a higher femur BMD and BMC divided by the rats final body weight in the exercise group.

Compared to spine, bone mineral density and bone mineral content of the femur were not significantly affected by the weight-training program. It was hypothesized that skeletal regions with a predominance of trabecular bone would be the first to respond to a mechanical stimulus like weight lifting (Pruitt 1992). Trabecular bone is more metabolically active than cortical bone, the other type of bone present in the skeleton. It is estimated that vertebral body consists of greater than 66% trabecular bone, and the lumbar spine was influenced by the weight-training program. The femur has proportionally less trabecular bone. The estimated composition of bone at the femur is 75% cortical and 25% trabecular (Pruitt 1992). An earlier study tested the effect of weight training on bone mineral density with 17 women for 9 months. They found no significant weight training effect was detected at the femoral neck but mean change in lumbar spine BMD in the weight-trained group was significantly different from the control group. They concluded that weight training may be a useful exercise modality for maintaining lumbar BMD in early postmenopausal women (Pruitt 1992). Exercise did not effect BMD at the femur in our study, perhaps as a result of a proportionally greater amount of cortical bone, which is less metabolically active than trabecular bone, the primary component of the vertebral body. Cortical bone is more slowly metabolized compared to trabecular bone (Riggs 1986 ; Bourrin 1995). Twenty-five percent of all trabecular bone is remodeled annually compared with 2 – 3% of cortical bone (Riggs 1986 ; Bourrin 1995).

Consequently, this result is not in agreement with the study conducted by Seco and co-workers (Seco 1998). The rats exposed to strenuous exercise (treadmill running) showed that BMC and BMD of the femur were less in the exercising group than in the sedentary group. However the intensity of exercise was very high compared to other studies. On the other hand, when the young rats trained on a rodent treadmill 5 days/wk for 10 weeks, the training increased femur bone mass and breaking forces (Raab 1990). Therefore, further longitudinal studies are needed to approve effect of calcium and exercise on bone in growing rats.

## Summary and Conclusion

Skeletal modeling and maturation are influenced by the changes in physical activity and nutrition. Understanding the factors that influence bone mass accumulation in childhood, and to maximize peak bone mass in young adulthood, is important for designing preventive strategies to combat osteoporosis. The purpose of this study was to examine the effects of dietary calcium supplementation and exercise on bone mineral density of growing female rats. The results are as follows :

1. There were no significant effects of exercise or calcium supplement either on body weight gain or the increase of food intake.

2. The exercise group had significantly greater (6.25%) spine BMD compared to the control group.

3. Exercise did not significantly affect femoral BMC and BMD.

4. There were no significant differences, between the normal and calcium supplementation group in the spine and femur BMD and BMC.

In conclusion, our findings indicate that in young growing female rats running exercise leads to increased spine bone mineral density. A higher than recommended calcium intake afford no additional advantage in increasing BMD. Therefore, it can be concluded that when calcium intake is adequate, exercise is beneficial for acquisition of spine bone mineral density in young growing female rats. For the femur bone, additional studies are necessary. The exercise load employed in the present study might not exceed the minimum stimulus level to increase bone formation during our 3-wk exercise period or more exercise duration is need to increase bone formation.

Further longitudinal studies are needed to approve the effect of calcium and exercise on bone in growing rats.

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