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Effect of Soy Protein and Exercise on Bone Mineral Density and Bone Mineral Content in Growing Male Rats

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ABSTRACT

The purpose of this study was to examine the effects of dietary protein and exercise on bone mineral density and bone mineral content of growing male rats. Forty male, Sprague-Dawley rats(age 21 days) were assigned to four groups that underwent 9 weeks of experimental treatment. Animals were assigned to one of two exercise treatments (treadmill running or sedentary). The exercise and nonexercise group were fed a diet containing casein or soy with rich isoflavones (3.4mg/g protein). The exercise group ran on a rodent treadmill(speed of 15m/min for 30min) three days per week during the 9-week study period. All rats were fed an experimental diet and deionized water ad libitum for 9 weeks. Total bone mineral density (BMD), total bone mineral content (BMC), total body calcium, spine BMD and BMC, and femur BMD and BMC were determined by using dual energy x-ray absorptiometry (FIXI-mus, GE Lunar Radiation Cooperation, Madison, WI, USA). The soy diet group appears to have a significantly higher total BMD/weight and total BMC/ weight, spine BMD/weight, spine BMC/weight, femur BMD/weight and femur BMC/weight compared to the casein group in nonexercise and exercise. The exercise group had significantly greater total BMD/weight and BMC/ weight, spine BMD/weight and BMC/weight, femur BMD/weight and BMC/weight compared to the nonexercise group when the protein source was casein. The exercise combined soy group had significantly greater total BMD/weight and BMC/weight, spine BMD/weight and BMC/weight, femur BMD/weight and BMC/weight, compared to the exercise combined casein group. The results indicate that exercise had a positive influence on bone mineral density and bone mineral content and soy significantly affect on bone mineral density and bone mineral content for the 9 weeks experimental period. It can be concluded that exercise combined with a soy diet is most beneficial for acquisition of spine bone mineral density in young growing male rats. This convincing evidence suggests that a change in life style such as increasing exercise and consumption of soy protein is a practical strategy for significantly reducing the incidence of osteoporosis. (*J Community Nutrition* 6(1) : 48~54, 2004)

KEY WORDS : soy · exercise · bone mineral density · growing male rat

Introduction

Osteoporosis, the loss of bone and degeneration of skeletal architecture with an increased risk of fragility fractures, is a major worldwide health problem, particularly for elderly women. Bone mineral status in later life is a function of the maximum bone mineral mass attained in young adult life and of subsequent age-related bone loss (Burckhardt et al. 1989), increasing peak bone mass in young persons could reduce the incidence of later osteoporotic fractures. Modification of diet and physical activity during childhood and adolescence may be an effective strategy for maximizing peak bone mass (Sabatier et al. 1996), which is attained by the third decade of life (Recker et al. 1992 ; Heaney et al. 2000), is an important determinant of osteoporosis risk.

Currently, in the United States 16.8 million (54%) postmenopausal white women have osteopenia and another 9.4 million (30%) have osteoporosis (Melton 1995). Total fracture load in any society depends not only on the incidence of the fractures, but also on the population size. Thus, 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 life expectancy has improved and continues to do so in Korea, the burden of ost-

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reduces the abdominal fat. This finding is of particular interest because it raises the question of whether soy protein stimulates the synthesis of growth hormone, which is known to decrease adipose tissue and to increase bone mass. Recently the effect of soy on growth hormone secretion was investigated in growing female Sprague-Dawley rats, and soy with rich isoflavones significantly increased growth hormones in growing rats (Choi 2002).

Effects of exercise and dietary protein on total bone mineral density, total bone mineral content, total body calcium in rats are presented in Table 4. The total BMD, total BMC were, and total calcium were significantly greater in the casein group to the soy groups. Weight is one of the main determinants of bone mass measured by DEXA in humans, and rats (Rico 1994). The BMD and BMC divided by the rats final body weight. The total BMD/weight, total BMC/weight, total calcium/weight were significantly greater in the soy group to the casein groups. The main effects of exercise were also significant on total BMD/weight, total BMC/weight, and total body calcium/weight. Total BMD/weight, total BMC/eight, and total body calcium/weight of the exercisers were significantly ($p < 0.05$) greater than nonexercisers.

Effects of exercise and dietary protein on spine bone mineral density and spine bone mineral content in rats are nted in Table 5. The spine BMD values were similar as reported in the earlier study. Choi (Choi 2003) reported that 14 week old female Sprague-Dawley rats (mean weighting 240g) had BMD mean values of 0.14g/cm^2. The main effects of dietary protein were significant on spine BMD. The mean value in spine BMD and spine BMC increased in the exercise soy group, however, significant losses occurred in the exercise casein group. And descriptive results for spine BMD and spine BMC of the casein exercise group were lower than the casein nonexercise group in Table 5, however, spine BMD/weight and spine BMC/weight of the exercisers were significantly ($p < 0.05$) greater than nonexercisers in the casein group. And the soy group had greater spine BMD/weight and spine BMC/weight compared to the casein groups in exercise and nonexercise groups. The main effects of exercise on spine BMD/weight and BMC/weight were also significant ($p < 0.05$). The exercise group had significantly greater spine BMD/weight and spine BMC/weight compared to nonexercisers.

Effects of exercise and dietary protein on femur bone mineral density and femur bone mineral content in rats are presented in Table 6. The effects of exercise on femur BMD/weight and BMC/weight were significant ($p < 0.05$). The exercise group had greater femur BMD/weight compared to nonexercise groups. The effects of diet on femur BMC/weight were not significant ($p < 0.05$). Compared to spine, bone mineral density and bone mineral content of the femur were not significantly affected by the weight-training program or soy protein. The different results for spine and femur between rats fed isoflavones and rats fed casein, or exercising or nonexercising may be the difference in type of bone that comprise the femur compared to the spine. The femur is primarily

Table 4. Total bone mineral density and spine bone mineral content in rats

	Non-exercise (n = 20)	Exercise (n = 20)
	Mean ± SD	Mean ± SD
Total BMD (g/cm^2)		
Casein (n = 10)	0.2862 ± 0.005$^{a1,2)}$	0.2834 ± 0.006^{a}
Soybean (n = 10)	0.2835 ± 0.011^{a}	0.2800 ± 0.006^{a}
Total BMD/wt (kg)		
Casein (n = 10)	0.6430 ± 0.06^{d}	0.8127 ± 0.04^{b}
Soybean (n = 10)	0.7101 ± 0.04^{c}	0.9499 ± 0.04^{a}
Total BMC (g)		
Casein (n = 10)	8.3365 ± 0.782^{a}	7.4428 ± 0.813^{b}
Soybean (n = 10)	7.7421 ± 0.728^{a}	7.1050 ± 0.498^{b}
Total BMC/wt (kg)		
Casein (n = 10)	1.8614 ± 0.0013^{c}	2.1280 ± 0.0018^{b}
Soybean (n = 10)	1.9334 ± 0.0012^{c}	2.4055 ± 0.0008^{a}

1) Mean ± SD
2) Values with different superscript within a row or a column are significantly different at $p < 0.05$ by Duncan's multiple range test

Table 5. Spine bone mineral density and spine bone mineral content in rats

	Non-exercise (n = 20)	Exercise (n = 20)
	Mean ± SD	Mean ± SD
Spine BMD (g/cm^2)		
Casein (n = 10)	0.1490 ± 0.011$^{a1,2)}$	0.1384 ± 0.007^{b}
Soybean (n = 10)	0.1441 ± 0.008^{a}	0.1527 ± 0.007^{c}
Spine BMD/wt (kg)		
Casein (n = 10)	0.3352 ± 0.04^{a}	0.39797 ± 0.04^{b}
Soybean (n = 10)	0.3612 ± 0.03^{c}	0.51775 ± 0.02^{d}
Spine BMC (g)		
Casein (n = 10)	0.5403 ± 0.051^{a}	0.4828 ± 0.030^{b}
Soybean (n = 10)	0.5098 ± 0.030^{c}	0.5314 ± 0.056^{c}
Spine BMC/wt (kg)		
Casein (n = 10)	1.2077 ± 0.0001^{a}	1.3876 ± 0.0001^{b}
Soybean (n = 10)	1.2779 ± 0.0001^{c}	1.7964 ± 0.0001^{d}

1) Mean ± SD
2) Values with different superscript within a row or a column are significantly different at $p < 0.05$ by Duncan's multiple range test

Table 6. Femur bone mineral density and femur bone mineral content in rats

	Non-exercise (n = 20)	Exercise (n = 20)
	Mean ± SD	Mean ± SD
Femur BMD (g/cm^2)		
Casein (n = 10)	0.2275 ± 0.014^a	0.2146 ± 0.007^b
Soybean (n = 10)	0.2189 ± 0.013^a	0.2110 ± 0.005^b
Femur BMD/wt (kg)		
Casein (n = 10)	0.5108 ± 0.05^a	0.6158 ± 0.04^b
Soybean (n = 10)	0.5474 ± 0.02^a	0.7158 ± 0.02^c
Femur BMC (g)		
Casein (n = 10)	0.5140 ± 0.041^a	0.4524 ± 0.022^b
Soybean (n = 10)	0.4604 ± 0.042^c	0.4166 ± 0.012^d
Femur BMC/wt (kg)		
Casein (n = 10)	1.1520 ± 0.1^a	1.2970 ± 0.08^b
Soybean (n = 10)	1.1551 ± 0.1^a	1.4125 ± 0.03^b

1) Mean ± SD

2) Values with different superscript within a row or a column are significantly different at $p < 0.05$ by Duncan's multiple range test

made up of cortical bone (55%) while the spine consists of mainly trabecular bone (95%) (Favus 1996). Cortical bone serves as the mechanical and protective functions of the skeleton, whereas trabecular bone is more metabolically active and has a higher rate of formation and resorption than cortical bone (Andreoli 1997). And 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. It is estimated that a 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 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 the lumbar spine BMD in the weight-trained group was significantly different from the control group. So they concluded that weight training may be a useful exercise modality for maintaining lumbar BMD in early postmenopausal women (Pruitt 1992).

No significant difference was observed between the dietary protein group in nonexercisers in the femur, and exercise had lower effect on BMD at the femur than at the spine 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. Therefore, cortical bone is much more slowly metabolized compared to trabecular bone (Bourrin 1995 ; Riggs 1986).

The soy group had greater femur BMD/weight compared to the casein groups in exercise and nonexercise groups. Exercisers had greater femur BMD/weight and femur BMC/weight compared to nonexercisers and soy groups had greater femur BMD/weight and femur BMC/weight compared to casein groups. This finding is in agreement with the study of Omi et al. (1994) who reported that soybean milk is an excellent source for increasing bone mineral density in the rat model with ovariectmized osteoporosis.

Actually, there are numerous reports of the positive effects of exercise on bone mass (Hatori et al. 1993 ; Yeh et al. 1993 ; Barvo et al. 1996). However, these effects are not necessarily consistent because the type, intensity, duration, frequency, and period of exercise described in these reports are not necessarily consistent (Marsh et al. 1983). The result indicates that exercise and soy had a positive influence on spine and femur bone mineral density and bone mineral content. This finding is in agreement with our previous study (Choi 2003). Swissa-Sivan and co-workers (Swissa-Sivan 1989) reported that exercise increased bone mineral density (7%) and bone mineral content (15%). Recently Kiuchi and co-workers (Kiuchi 1998) observed that training induced significant gain in bone mineral content. 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 20m/mim for 4 weeks (Yeh et al. 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 the sedentary animals. Steinberg and Treuta (Steinberg 1981) reported that bones of young active rats were heavier, longer, and had increased cortical thickness and trabecular. 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 (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 eoporotic fractures continues to rise. At present, there are somewhat in excess of 2 million individual and 50 to 100 thousand have a fracture(Sung 2002). Currently, in Korea, about 86.4% of the women, more than over 50 years of age in rural areas, have osteopenia or osteoporosis. About 90% of elderly Koreans more than over 60 years of age have osteopenia or osteoporosis(Kim 2000). And 11% 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 recommended by 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, therefore, 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.

Current therapies for treating osteoporosis include estrogen and hormone replacement therapies, bisphosphonates, calcitonin, and raloxifene. Because of possible side effects of hormone replacement therapies, such as breast cancer, endometrial adenocarcinoma, and undesirable side effects (Scharbo 1996), compliance with hormonal therapy is poor (Groeneveld 1994), leading to loss of treatment efficacy (Lignieres 1996). Isoflavones, found predominantly in soy products, are estrogen-like substances structurally and functionally similar to 17 β-estradiol(Knight 1996). Recent observations indicate that soy or soy isoflavones have a positive effect on bone. Soybean milk-based diets were shown to increase calcium absorption in rats(Omi et al. 1994) and the isoflavones in soy protein(Arjmandi et al. 1998) were shown to prevent femoral(Arjmandi et al. 1998) and vertebral (Arjmandi et al. 1996) bone loss in rats. The relation between dietary protein intake and bone metabolism is controversial, and questions about this relation are unresolved. Excess dietary protein was shown to cause urinary calcium loss, negative calcium balance, and bone loss in young and elderly men and women, and these effects are mainly attributable to a high acid load from metabolism of animal protein. Isoflavones, found predominantly in soy protein, are estrogen-like substances structurally and functionally similar to 17 β-estradiol. Isoflavones may combine with the estrogen receptor, albeit with lower affinity than 17 β-estradiol, and stimulate estrogen activity, thus having an estrogenic effect on bone. Recent observations indicate that soy or soy isoflavones have a positive effect on bone, but the mechanism of action is unclear. Soybean milk-based diets were shown to increase calcium absorption in rats and the isoflavones in soy protein isolate were shown to prevent femoral and vertebral bone loss in rats. Although recent animal experiments indicated that soy or soy isofalvones have a positive effect on bone, there are currently few-short term studies in growing rats. To our knowledge, a report designed specifically to examine bone in growing rats, showing a positive effect of soy isoflavones on bone mineral density was published(Choi 2003). 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. Exercise therapy that loads the mechanical stress to the bone is also effective in maintaining bone mineral density in early postmenopausal women(Pruitt 1992). 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). Mechanical loading of sufficient intensity promotes an increase in skeletal mass, especially during growth in the first 2 decades of life(Turner 1999). The purpose of this study was to examine the effect of isoflavone rich soy protein and exercise on BMD and BMC of the total, spine and femur in growing male rats.

Methods

Thirty male 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(n = 10) of four groups(casein, soy, soy + exercise, casein + exercise). The diets are based on AIN-76 rodent diet and the diet composition provided in Table 1. The first 2 weeks of treadmill running were a period of acclimation. During this time, the animals ran for 10 minutes with the 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 30min during the second week. And the exercise group ran on a rodent treadmill three days(Monday, Wednesday, and Friday) per week during the 9-week study period. At this time, the control group was fed a diet containing casein, and the experimental group was fed soy protein with rich isoflavones and the exercise group was fed a diet the same as casein and soy groups. Total bone mineral density (BMD), total bone mineral content (BMC), total body calcium, spine BMD and BMC, and femur BMD and BMC were determined by using dual energy x-ray absorptiometry (PIXI-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.

Table 1. Composition of experimental diet (g/100g diet)

Ingredients	Casein	Soy isolate
Corn starch	66.5	66.5
Casein	20.0	–
Soy protein$^{1)}$	–	20.0
a -Cellulose	3.8	3.8
Vitamin mixture$^{2)}$	1.0	1.0
Mineral mixture$^{3)}$	3.5	3.5
Corn oil	5.0	5.0
Choline	0.2	0.2

1) Soy isolate, supplied by PTI (Protein Technology Institute, St. Louis, USA)

2) Vitamin mixture, supplied by U.S. Corning Laboratory Services Company, Teklad Test diets, Madison, Wisconsin.

3) Mineral mixture, supplied U.S. Corning Laboratory Services Company, Teklad Test diets, Madison, Wisconsin

Table 2. The effects of exercise and soy protein on body weight during experimental period

	Non-exercise (n = 20)	Exercise (n = 20)
	Mean ± SD	Mean ± SD
Weight at beginning (g)		
Casein (n = 10)	80.70 ± 3.720$^{a1,2)}$	77.00 ± 2.979^{a}
Soybean (n = 10)	80.90 ± 3.580^{a}	76.90 ± 3.029^{a}
Weight at final (g)		
Casein (n = 10)	449.70 ± 49.40^{a}	349.40 ± 18.73^{c}
Soybean (n = 10)	400.75 ± 32.16^{b}	295.20 ± 14.51^{d}

1) Mean ± SD

2) Values with different superscript within a row or a column are significantly different at $p < 0.05$ by Duncan's multiple range test

Table 3. The effects of dietary protein and exercise on weigh gain, mean food intake and food efficiency radio (FER)

	Non-exercise (n = 20)	Exercise (n = 20)
	Mean ± SD	Mean ± SD
Food intake (g/d)		
Casein (n = 10)	22.39 ± 1.916$^{a1,2)}$	18.32 ± 1.053^{b}
Soybean (n = 10)	21.36 ± 0.887^{a}	17.74 ± 1.007^{b}
FER$^{3)}$		
Casein (n = 10)	0.262 ± 0.0169^{a}	0.239 ± 0.0071^{b}
Soybean (n = 10)	0.238 ± 0.0163^{b}	0.198 ± 0.0062^{c}
Weight gain (g)		
Casein (n = 10)	366.00 ± 48.28^{a}	272.40 ± 17.87^{c}
Soybean (n = 10)	316.85 ± 33.02^{b}	218.30 ± 13.77^{d}

1) Mean ± SD

2) Values with different superscript within a row or a column are significantly different at $p < 0.05$ by Duncan's

3) Food efficiency ratio (FER) = weight gain (g) / food intake (g)

1. Statistical Analyses

Statistical analysis were performed by using SAS ver 8.12. The effect of exercise and dietary protein source 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 reported as significant have p-values < 0.05.

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. At the end of the experiment, however, the exercising group was lighter (325g) than the nonexercisers (425g). This finding is in consistent with findings of other researchers. Studies have shown that sedentary animals have greater final body weights as compared to the exercising animals which is expected as a result of training (Nordsletten et al. 1994 ; Tamaki et al. 1998 ; Seco et al. 1998). Total food intake was significantly different between nonexercise and exercise groups. The mean food intake of exercisers was significantly lower than the values of nonexercisers.

Also, there were significant effects of protein source on incremental gain in body weight. It was observed that the casein group had greater body weights and food efficiency ratio (FER) compared to the soy groups, both nonexerciser and exerciser groups. The difference in the final body weights and food efficiency ratio (FER) of the dietary groups within exerciser or nonexerciser was not due to difference in amount of food ingested. In this study, the total food intake values of the soy groups were not lower than the values of casein groups, but food efficiency ratio (FER) of the soy groups were lower than the values of casein groups. It could be due to the type of protein ingested. The Soybean diet has been suggested to have a tissue-specific effect. Soybean diet significantly