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난소 절제한 동물모델에서 콩의 섭취가 콜레스테롤과 BMD와의 상관관계에 미치는 효과

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Effects of Legumes Consumption on the Association of Cholesterol and Bone Mineral Density in Ovariectomized Rats

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ABSTRACT : Soy isoflavones have been suggested to improve bone loss and lipid profile in postmenopausal women and ovariectomized rats. In present study, we investigated the hypothesis that consumption of soybean, mung bean, cowpea and azuki bean has a beneficial effect on lipid profile which associates with bone mass in ovariectomized rats. Forty two female Sprague-Dawley rats were either sham-operated (Sham) or surgically ovariectomized (OVX). Sham and OVX groups were fed a regular AIN-93M diet, but ovariectomized rats with soybean (OS), mung bean (OM), cowpea (OC) or adzuki bean (OA) were fed AIN-93M diet replacing 35% of corn starch with powdered OS, OM, OC or OA for 10 weeks. Total- and LDL-cholesterol concentrations were significantly lower in Sham and OC groups than other OVX groups. There was significant negative association between total cholesterol concentration and bone mineral density (BMD) of tibia in only OC group. In conclusion, total-cholesterol concentration was significantly and negatively correlated with BMD in rats consumed cowpea, suggesting that the reduced cholesterol concentration may have a beneficial effect on bone mass.

Key Words : Ovariectomized Rat, Legumes, Bone Mineral Density, Lipid Profile

INTRODUCTION

Osteoporosis is a widely prevalent public health problem in the elderly (Khosla *et al.,* 1997) and is well characterized by low bone mass and increased risk of fracture (Cooper *et al.*, 2006; Alexander *et al.*, 2001). Postmenopausal women are suffering from estrogen induced osteoporosis than men, since estrogen deficiency is one of major factors for pathological bone loss in postmenopausal women (McKane *et al.,* 1995).

Low bone mass is associated with increased risk of atherogenic lipid profile (Parhami *et al.*, 1999; Dang *et al.*, 2002). The relationships between bone formation and lipid profile have been performed through clinical studies, and the possible connection was appeared between them (Edwards *et al.*, 2000; Chan *et al.*, 2000). Intake of statin, a widely used lipid-lowering agent, has been reported to an increase of bone mineral density (BMD), and a prevention of osteoporotic fractures in postmenopausal women (Edwards *et al.*, 2000; Chan *et al.*, 2000). In animal model, high fat diets inhibited bone formation and resulted in osteoporosis (Parhami *et al.*, 1999), suggesting that the accumulation of oxidized lipids or hyperlipidemia inhibited osteoblastic differentiation (Parhami *et al.*, 1997, 1999).

Many countries, including Asian, have cultivated various legumes as excellent sources of diet because of containing not only nutrients such as starch, dietary fiber, protein, lipids, and minerals (Marathe *et al.*, 2011), but also bioactive components such as phenolic acids, flavonoids and lignins (Lin and Lai, 2006; Chung *et al.*, 1998). Particularly, phytoestrogens, rich in legumes exert an estrogen-like activity because of its structural similarities as that of estrogen (Cos *et al.*, 2003). Isoflavones, a type of phytoestrogens have beneficial effects against cardiovascular diseases, osteoporosis and menopausal symptoms

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(Albertazzi and Purdie, 2008; Lim *et al.*, 2007). In ovariectomized rat model, soy protein isolate decreased concentrations of triglyceride (Uesugi *et al.*, 2001), cholesterol (Uesugi *et al.*, 2001) and LDL-cholesterol, but increased HDL-cholesterol concentration (Uesugi *et al.*, 2001; Lucas *et al.*, 2003) and bone mass (Devareddy *et al.*, 2006). However, there has been no study to show the association between lipid profile and bone mass in osteoporotic animal model. Therefore, this study was investigated the hypothesis that consumption of soybean, mung bean, cowpea and azuki bean has beneficial effects on lipid profile, which positively associates with bone mass in ovariectomized rats.

MATERIALS AND METHODS

1. Animals and Diets

An animal protocol approved by the Institutional Animal Care and Use Committee of Hanyang University was used for all animal experiments (HY-IACUC-09-050). Nine week old female Sprague-Dawley rats (Orient Bio Inc., Seongnam-si, Korea) were housed in individual ventilated cages in an air-conditioned room maintained at 22 ± 2℃ with a 12-h light-dark cycle. After 1 week acclimation, rats were either sham-operated (Sham; n = 7) or surgically ovariectomized (OVX; n = 7). Sham and OVX control were fed an AIN-93M diet (Reeves *et al.*, 1993), containing 465.7 g corn starch, 140 g casein, 1.8 g L-cysteine, 100 g sucrose, 155 g dextrose, 40 g soybean oil, 0.008 g t-butylhydroquinone, 50 g cellulose, 35 g mineral mix, 10 g vitamin mix, and 2.5 g choline bitartrate. OVX rats with legumes (n = 7 per group) were randomly divided into soybean (OS, *Glycine max* var. Hwang-geumKong), mung bean (OM, *Vigna radiata* var. Geumseong Nokdu), cowpea (OC, *Vigna unguiculata* var. Huin Dongbu), or adzuki bean (OA, *Vigna angularis* var. Jungbu Pat). In these diets, 35% of corn starch of AIN-93M diet was replaced with powdered soybean, mung bean, cowpea, or adzuki bean. All legumes were provided by the Korea Rural Development Administration. All legumes were soaked for 24 h in water, boiled for 15 min, thoroughly dried, and ground into powder. During 10 weeks of feeding the diets, body weight was measured once a week and food intake was measured daily.

At the end of feeding, rats were fasted overnight and sacrificed under anesthesia with an intraperitoneal injection of tiletamine (25 ㎎/㎏) and zolazepam (25 ㎎/㎏) together with xylazine (10 ㎎/㎏). Blood was collected in serum separation tube by heart puncture and then centrifuged at 3000 g for 15 min (HA 1000-3, Hanil Sciences Industrial CO. Ltd., Incheon, Korea). The right tibia was dissected out, and subcutaneous fat and uterus were harvested and weighed. Blood and tissue samples were stored at −80℃ until use.

2. Analysis of Lipid Profile

Serum triglyceride and total cholesterol were determined by using a commercial kit (Asan Pharmaceutical Co., Seoul, Korea). Standards or samples of 20 ㎕ were added to 3 ㎖ of working reagent and incubated for 10 min or 5 min at 37℃, respectively. Optical density was read at 550 ㎚ or 500 ㎚ using a microplate reader, respectively (ELx 800 uv, BIO-TEK Instruments. INC, Vermont, USA). Serum HDL-cholesterol was also determined by using a commercial kit (Asan Pharmaceutical Co., Seoul, Korea). Samples of 200 ㎕ were added to 200 ㎕ of separation reagents, incubated for 10 min at room temperature, and then centrifuged at 3000 g for 10 min (HA 1000-3, Hanil Sciences Industrial CO. Ltd., Incheon, Korea) to obtain the supernatant. Standards or samples of 100 ㎕ were added to 3 ㎖ of working reagents, incubated for 5 min at 37℃, and then the OD was read at 500 ㎚ using a microplate reader (ELx 800 uv, BIO-TEK Instruments. INC, Vermont, USA). Serum LDL-cholesterol was estimated using Friedewald's equation (Reeves *et al.*, 1993).

3. Measurements of Bone Mass

Measurements of BMD on the experimental animals were executed at the end of the experiment. After the rats were anesthetized with an intraperitoneal injection of a combination of rompun (0.15 mL/㎏) and zoletil 50 (0.075 mL/㎏), their BMD of the right tibia was measured using dual-energy X-ray absorptiometry (DEXA, GE Lunar, Madison, USA). According to the DEXA system, BMC expressed in grams was divided by the area of the site being scanned to obtain BMD expressed in g/㎠.

4. Statistical Analyses

All data are expressed as mean ± standard error of the mean (SEM). Data were analyzed using the SPSS-PC+ statistical software package for Windows, version 17.0 (SPSS Inc., Chicago, USA). The differences among all groups were analyzed using a one-way analysis of variance (ANOVA), followed by Duncan's multiple-range test. The correlation between lipid profiles and the BMD was measured using Pearson's correlation analysis. Differences with $p < 0.05$ were considered statistically significant.

Table 1. Dietary intake, body weight and organ weight in rats consumed various legumes[*].

	Sham	OVX	OS	OM	OC	OA
Dietary intake (g/day)[**]	12.31±1.52	12.20±1.47	12.15±1.53	12.51±1.17	11.74±2.10	12.52±2.01
Initial body weight (g)	208.00±3.91	202.80±2.31	206.40±4.45	207.00±3.72	209.50±4.57	204.80±4.10
Final body weight (g)[***]	308.29±5.10^{b}	352.4±3.24^{a}	348.43±3.04^{a}	351.86±6.94^{a}	346.43±12.58^{a}	345.14±9.26^{a}
Subcutaneous fat weight (g)	6.73±0.45^{b}	9.63±0.70^{a}	9.33±0.31^{a}	10.32±0.28^{a}	9.29±0.78^{a}	9.76±1.36^{a}
Uterus weight (mg)	669.86±35.57^{a}	116.14±5.74^{b}	107.43±8.06^{b}	108.14±7.49^{b}	120.86±6.85^{b}	119.71±8.85^{b}

[*] Sham, sham-operated with AIN-93M diet; OVX, ovariectomized with AIN-93M diet; OS, ovariectomized with soybean; OM, ovariectomized with mung bean; OC, ovariectomized with cowpea; OA, ovariectomized with adzuki bean, [**]Values are expressed as mean ± SEM, n = 7, [***]Values with different superscripts within a row are significantly different at $P < 0.05$ by ANOVA with Duncan's multiple-range.

Table 2. Lipid profile of rats consumed various legumes[*].

	Sham	OVX	OS	OM	OC	OA
Triglyceride (mg/dℓ)[**]	53.59±6.39	49.96±2.11	46.65±3.06	43.21±2.46	53.87±4.39	49.96±4.09
Total cholesterol (mg/dℓ)[***]	52.05±4.19^{b}	64.97±1.76^{a}	64.48±5.02^{a}	62.44±3.74^{ab}	50.55±4.94^{b}	60.97±2.74^{ab}
HDL-cholesterol (mg/dℓ)	49.12±5.95^{a}	42.29±1.71	47.84±2.53	45.85±3.53	38.82±2.28	39.30±1.89
LDL-cholesterol (mg/dℓ)	7.32±3.83^{b}	16.43±2.96^{a}	17.18±2.27^{a}	11.64±2.89^{ab}	8.18±1.05^{b}	14.54±1.98^{ab}

[*] Sham, sham-operated with AIN-93M diet; OVX, ovariectomized with AIN-93M diet; OS, ovariectomized with soybean; OM, ovariectomized with mung bean; OC, ovariectomized with cowpea; OA, ovariectomized with adzuki bean, [**]Values are expressed as mean ± SEM, n = 7, [***]Values with different superscripts within a row are significantly different at $P < 0.05$.

RESULTS

1. Body Composition

Dietary intake, initial and final body weights, and subcutaneous fat and uterus weights are presented in Table 1. Dietary intake and initial body weight were not significantly different among groups. Final body weight and subcutaneous fat weight were significantly lower, but uterine weight was significantly higher in Sham than all OVX groups. However, there was no statistical significance in final body weight, subcutaneous fat weight, and uterus weight among OVX rats fed various legumes.

2. Lipid Profile

There was no statistical significance in serum concentrations of triglyceride and HDL-cholesterol among groups (Table 2). Total cholesterol concentration was significantly lower in Sham and OC groups than OVX control and OS group, which was due to the significant decrease in LDL-cholesterol concentration. However, soybean, mung bean, and adzuki beans had no significant effect on the concentrations of total- and LDL-cholesterol.

Table 3. Correlation between lipid profile and BMD of tibia in rats consumed various legumes[*].

	Triglyceride	Total Cholesterol	HDL-Cholesterol	LDL-Cholesterol
Sham				
BMD	0.165	–0.291	–0.171	0.071
OVX				
BMD	0.340	0.085	0.423	–0.045
OS				
BMD	0.192	–0.009	–0.014	–0.034
OM				
BMD	0.508	–0.312	–0.247	–0.303
OC				
BMD	0.535	–0.925[**]	–0.538	0.448
OA				
BMD	–0.201	–0.173	–0.081	–0.146

[*] BMD, bone mineral density; Sham, sham-operated with AIN-93M diet; OVX, ovariectomized with AIN-93M diet; OS, ovariectomized with soybean; OM, ovariectomized with mung bean; OC, ovariectomized with cowpea; OA, ovariectomized with adzuki bean, [**]Values are expressed as correlation coefficient in $P < 0.01$, n = 7.

3. Correlations between Lipid Profile and Bone Mass

The correlations between lipid profile and BMD of tibia are presented in Table 3. Tibia was measured because of the most sensitive bone affected by estrogen deficiency (Borah *et al.*, 2002). The significant negative correlation was shown between total cholesterol and BMD of tibia in OC group ($p < 0.001$), suggesting that the reduction of total cholesterol concentration induced by cowpea consumption may prevent bone loss. There were no significant correlations between BMD of tibia and triglyceride, HDL-cholesterol, or LDL-cholesterol in all groups. In addition, BMD of tibia were not significantly different among Sham (0.23 ± 0.01 g/㎠), OVX (0.21 ± 0.01 g/㎠), OS (0.21 ± 0.01 g/㎠), OM (0.19 ± 0.01 g/㎠), OC (0.20 ± 0.01 g/㎠), and OA (0.22 ± 0.01 g/㎠).

DISCUSSION

In present study, intake of various legumes did not direct affect on BMD, but the reduction of total-cholesterol concentration by cowpea consumption induced significant beneficial effect on bone loss. Previous study reported that postmenopausal women with hypercholesterolemia had about 10% lower BMD of various skeletal sites than those with normal lipid levels (Orozco, 2004). This observation could be explained by that total-cholesterol and its metabolites played an important role for osteoblast differentiation and activities (Parhami *et al*., 2000, 2002). We previously reported that intake of cowpea significantly increased bone formation markers and decreased bone resorption markers (Lee *et al.,* 2011). However, there were a few studies showing the contrast results; higher serum concentration of total-cholesterol (Adami *et al*., 2004; Brownbill and Ilich, 2006), LDL-cholesterol, and triglyceride (Adami *et al*., 2004) were positively associated with higher BMD in postmenopausal woman and men.

Effect of cowpea on lipid profile has been reported previously. Olivera *et al.* (2003) reported that raw cowpea seed reduced serum cholesterol concentration in rats. Frota *et al.* (2008) also showed that consumption of cowpea seed and its protein isolate significantly reduced plasma levels of total-cholesterol, non HDL-cholesterol, and fat accumulation in the liver of hypercholesterolemic hamsters. There was no study to investigate the dietary effect of mung bean and adzuki bean on lipid profile, but most legumes containing isoflavones and phenolic compounds had been suggested to have similar effect as compared with soybean (Uesugi *et al*., 2001; Lucas *et al*., 2003). In fact, Uesugi *et al.*(2001) found that administration of daidzein, genistein reduced serum levels of triglyceride and total-cholesterol in ovariectomized-induced rats. Lucas *et al.* (2003) also showed that supplement of soy isoflavones decreased plasma cholesterol concentration in a dose-dependent manner in ovariectomized hamster. On the contrary, Rios *et al.* (2008) and Gallo *et al.* (2005) reported that isoflavones and soy extract had no effects lipid profile in postmenopausal women and in OVX rats, respectively. This lack of agreement may be due to the study design, dosage of soy protein or isoflavones, and study duration.

In this study, there were no significant changes on triglyceride and HDL-cholesterol concentration between OVX and sham, but total- and LDL-cholesterol concentration were significantly higher in OVX than sham. Cui *et al.* (2005) also reported that concentrations of total- and LDL cholesterol, and triglyceride were significantly higher in post-menopausal women than pre-menopausal women. Total cholesterol and triglyceride concentrations were also significantly higher in OVX rats than sham (Uesugi *et al.*, 2001; Lucas *et al.,* 2003). Limitations of this study were that isoflavones and other phytoestrogen contents in the legumes were not measured. Second, duration of feeding was too short.

In conclusion, consumption of cowpea reduced total- and LDL- cholesterol concentration, which may have a beneficial effect on the prevention of bone mass. Future studies should examine mechanism controlling the association between cholesterol and bone loss.

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