

Effect of daidzein, a soy isoflavone, on bone metabolism in Cd-treated ovariectomized rats

Ae-Son Om[✉] and Jae-Young Shim

Department of Food and Nutrition, College of Human Ecology, Hanyang University, Seoul, South Korea

Received: 08 August, 2006; revised: 28 November, 2006; accepted: 01 August, 2007
available on-line: 24 August, 2007

This study compared the ability of daidzein, a soy isoflavone, with that of 17 β -estradiol to prevent bone loss in cadmium (Cd)-exposed ovariectomized (OVX) rats during growth. Four week-old female Wistar rats were randomly assigned to five treatment groups of 9 rats each, either (1) sham-operated (SH); (2) OVX and placed on experimental diets (OVX); (3) OVX fed 50 ppm of CdCl₂ (OVX-Cd); (4) OVX fed 50 ppm of CdCl₂ and 10 μ g of daidzein per kg of body mass (OVX-CD-D); or (5) OVX fed 50 ppm of CdCl₂ and 10 μ g of estrogen per kg of body mass (OVX-CD-E). All rats were given free access to AIN-76 modified diet and drinking water, with or without Cd, for 8 weeks. The OVX groups gained more ($P < 0.05$) body mass than the SH group. Femoral mass was increased by feeding daidzein and estradiol, whereas femoral length was not ($P > 0.05$) significantly different among groups. Femoral breaking force was not significantly different among groups, however, femoral BMD was significantly lower in OVX-Cd than in the SH and OVX groups. Morphologically proliferative cartilage and hypertrophic cells in femur showed normal distribution in OVX-CD-D and OVX-CD-E groups unlike those in OVX-Cd group. These findings suggest that Cd-OVX-induced osteopenia or osteoporosis probably results from an increase in bone turnover.

Keywords: daidzein, soy isoflavone, bone metabolism

INTRODUCTION

Osteoporosis is known as a skeletal disease mainly characterized by a reduction of bone mass (Hallworth, 1998) and impairment of microarchitecture (Riggs & Melton, 1986) with a consequent increase in bone fragility and susceptibility to fracture. The main risk parameters for osteoporosis are genetically influenced low peak bone mass, estrogen deficiency, and age. Women have a higher risk than men since women have a lower peak bone mass and a higher bone loss than man. Especially estrogen deficiency is regarded as a critical cause of osteoporosis, which can result from naturally or surgically induced menopause and endocrine disorders that reduce estrogen secretion in premenopausal women. It influences osteoclast which enhances bone loss by stimulating bone resorption. Other parameters that

can contribute to bone loss are exposure to heavy metals such as Cd and lead (Pb), excessive mass, poor nutrition, and disorders such as primary hyperparathyroidism and hyperthyroidism. Cadmium (Cd) is a very toxic contaminant that has a long biological half life in both humans and animals. This toxic metal can lead to itai-itai disease (Freiberg *et al.*, 1986), kidney tubular dysfunction (Freiberg *et al.*, 1984; 1986; Om *et al.*, 2002) and cancer (Itokawa *et al.*, 1978; Waalkers *et al.*, 1999). Moreover, Cd exposure can influence bone tissue leading to osteomalacia and (or) osteoporosis (Itokawa *et al.*, 1978). Several studies have shown that Cd affects the activity and metabolism of bone cell directly (Iwami & Moriyama, 1993; Wang & Bhattacharyya, 1993; Wilson & Bhattacharyya, 1997). Therefore, there has been an increased public awareness and concern about exposure of humans to Cd.

[✉]Corresponding author: Ae-Son Om, Department of Food and Nutrition, College of Human Ecology, Hanyang University, Seoul 133-791, South Korea; tel: (82 2) 2220 1203; fax: (82 2) 2292 1226; e-mail: aesonom@hanyang.ac.kr

Abbreviations: AIN, American Institute of Nutrition; BMD, bone mineral density; Cd, cadmium; Ca, calcium; OC, Serum osteocalcin; OVX, ovariectomized.

On the other hand, Asian women have lower rates of osteoporosis-related fractures than Western women (Ho *et al.*, 1993) because they consume more soy products such as miso and tofu that are rich in isoflavones, than Western women do (Adlercreutz *et al.*, 1995; de Kleijn *et al.*, 2001). Daidzein is one of the main soy isoflavones along with genistein and is a representative of a family of diphenolic compounds with structural similarities to estrogen. The estrogenic effects of daidzein as a phytoestrogen (Naim *et al.*, 1974; Eldridge, 1982; Nogowski *et al.*, 1993) are associated with prevention of bone resorption and augmentation of bone density (Picherit *et al.*, 2000; Sugimoto & Yamaguchi, 2000; Jia *et al.*, 2003). The current study examined the effects of daidzein on bone metabolism in ovariectomized (OVA) rats with Cd exposure.

MATERIALS AND METHODS

Experimental animals and treatments. Female Wistar rats (25 days old) were purchased from Samtako Inc. (Osan, Korea) and kept in a Hanyang University (Seoul, Korea) controlled environment animal facility at $22 \pm 2^\circ\text{C}$ with a 12-hour light/dark cycle. After 3 days of acclimation, the rats were assigned to either the sham-operated (SH) group or to groups to be operated on, anesthetized with an intraperitoneal injection of ketamine hydrochloride (Yahan Inc., Seoul, Korea) at doses of 50 mg/kg, and either OVX or subjected to the sham operation. One week after surgery, the OVX rats were randomly assigned to the following groups ($n=9/\text{group}$): OVX, OVX-Cd, OVX-Cd treated with daidzein at 10 $\mu\text{g/g}$ of body mass (OVX-Cd-D), or OVX-Cd treated with 17 β -estradiol at 10 $\mu\text{g/g}$ of body mass (OVX-Cd-E). Diets were prepared by mixing powdered daidzein (88.0% pure, BioSpectrum, Yongin, Korea) or 17 β -estradiol (Sigma-Aldrich Inc., Yongin, Korea) with AIN-76 powdered semipurified diet (Bifido, Seoul, Korea) (American Institute of Nutrition, 1997). The composition of the diet is shown in Table 1. The rats in the Cd-treated groups were given 50 ppm of Cd (CdCl_2 , Sigma-Aldrich Inc.) in drinking water for 8 weeks. After the experimental period, blood samples were collected by heart puncture, and the serum was centrifuged (3000 r.p.m., 20 min at 4°C), aliquoted, and stored at -70°C until measurements were made. Femurs were cleaned from adjacent tissues and stored at -70°C until measurements were made. Urine and feces were collected in separators in metabolic cages for 16 h. The urine samples were acidified with 2 ml of 1 M HCl and stored at -20°C until assayed.

Rats were maintained and utilized in accordance with Hanyang University Lab Animal Care Committee (HALACC) animal use protocols.

Measurement of breaking force and bone mineral density (BMD). Bones were kept in NaCl (9 g/l) at 4°C until femoral breaking force was determined 24 h later, using a three point bending test, with a Texture Analyzer (TAXT2i, Godalming, UK) (Ezawa & Ogata, 1979). BMD was assessed by dual-energy x-ray absorptiometry (Hologic France, Massy, France) (Omi *et al.*, 1994).

Femoral, fecal and urinary calcium (Ca) measurement. Bone and feces were dried at $60 \pm 10^\circ\text{C}$ for 24 h in a ceramic pot. The dry bones were weighed and then ashed at 550°C for 8 h to determine the ash mass. The ashed samples were dissolved in 1 M HCl and diluted with 1% lanthanum oxide (Association of Official Analytical Chemists, 1984). The Ca content was quantified with an atomic absorption spectrophotometer (Model 400, Perkin Elmer, Norwalk, CT, USA). The Ca concentrations in urine were measured by the same method as that of the bone samples after using trichloroacetic acid solution to remove protein (Yeager *et al.*, 1971; Zinterhofer, 1971).

Serum osteocalcin (OC). Serum OC was determined by an ELSA OC kit (CIS, France) using a gamma counter (Cobra[®], Hewlett-Packard, Palo Alto, CA, USA).

Histomorphometric measurement. The femur was dissected and fixed immediately in 0.1 M phosphate-buffered formalin for 24 h. Undecalcified sections for histomorphometric analyses were obtained by dehydrating the bone in acetone for 36 h, followed by immersion in xylene for 24 h. The bones

Table 1. Composition of soy-protein-free powdered semipurified diet

Ingredient	g/kg of diet
Protein	
Casein	200
Carbohydrate	
Cornstarch	600
Fiber	
Cellulose	50
Fat	
Corn oil	100
Vitamin mixture ^a	10
Mineral mixture ^a	35
DL-Methionine	3
Chlorine chloride	2

From Bifido (Seoul, Korea), containing: casein (Screma, Cludeville, France), cornstarch (Daesang, Seoul, Korea), vitamin mixture (Roche, Neuilly sur Seine, France), mineral mixture (Prolaba, Fontenay sous Bois, France), DL-methionine (Sigma-Aldrich), and chlorine chloride (Sigma-Aldrich). ^aPrepared according to AIN-76 formulation (Dyets Inc., Bethlehem, PA, USA).

Table 2. Effects of daidzein on body mass gain¹

Group	Body mass gain (g)
SH	144.57 ± 18.07 ^a
OVX	208.33 ± 26.62 ^b
OVX-Cd	216.38 ± 16.92 ^b
OVX-Cd-D	183.44 ± 25.04 ^b
OVX-Cd-E	178.86 ± 33.23 ^b

¹Data are mean ±S.D. values of nine rats per group. ^{a,b}Values with different letters are significantly different among groups at $P < 0.05$ by Duncan's multiple range test.

were then embedded in methyl methacrylate. Frontal sections (10 mm) were cut with a Reichert-Jung Microtome (Model 2050, Leica, Deerfield, IL, USA), and then stained with hematoxylin-eosin for histomorphometric analyses.

Statistical analysis. Means and S.D.s of all variables were computed for each of the groups. Analysis of variance (ANOVA) was first performed on the means to determine whether there were significant differences ($P < 0.05$). When ANOVA indicated statistical significance, Duncan's multiple range test (Duncan, 1957) was used to determine which means were significantly different. SPSS (Chicago, IL, USA) software was used for all statistical analyses.

RESULTS

Body mass gain and uterine mass

Table 2 shows the body mass gain. The OVX, OVX-Cd, OVX-Cd-D, and OVX-Cd-E rats had a significantly ($P < 0.05$) increased body mass compared with the SH groups. Uterine mass was strikingly decreased in OVX rats. Also, the OVX and OVX-Cd-D groups had a significantly lower uterine mass compared with the OVX-Cd-E group (not shown).

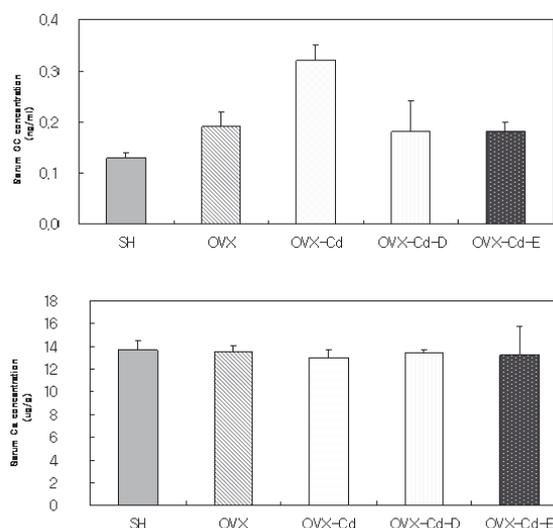
Femoral wet mass and length

Table 3 shows wet mass and length of the femoral bones. The femoral mass per 100 g of body

Table 3. Effects of daidzein on femoral wet mass, length, breaking force and BMD¹

Group	Mass (mg/100 g b.m.)	Length (mm)	Breaking force (kg)	BMD (mg/cm ²)
SH	532.67 ± 75.68 ^a	32.52 ± 2.31 ND	9.26 ± 1.29 ND	218.28 ± 7.86 ^a
OVX	399.14 ± 38.17 ^b	32.86 ± 1.58	9.59 ± 1.51	221.35 ± 6.37 ^a
OVX-Cd	400.42 ± 32.52 ^b	34.28 ± 0.53	9.35 ± 1.18	199.46 ± 1.36 ^b
OVX-Cd-D	455.01 ± 59.74 ^a	33.84 ± 1.15	9.47 ± 0.52	201.30 ± 4.08 ^b
OVX-Cd-E	491.58 ± 57.22 ^a	32.87 ± 1.55	8.64 ± 1.15	200.47 ± 8.34 ^b

¹Data are mean ±S.D. values of nine rats per group. ^{a,b}Values with different letters are significantly different among groups at $P < 0.05$ by Duncan's multiple range test.

**Figure 1. Effects of daidzein on serum OC and Ca concentrations.**

Data are expressed as mean ±S.D. values of nine rats per group. ^{a,b,c}Significantly different among groups at $P < 0.05$ by Duncan's multiple range test.

mass in the OVX and OVX-Cd group was significantly ($P < 0.05$) less than that in the SH, OVX-Cd-D, and OVX-Cd-E groups. The femoral mass was increased by feeding daidzein and estradiol, whereas femoral lengths among the groups were not ($P > 0.05$) significantly different.

Breaking force and BMD

The mean femoral breaking force was not ($P > 0.05$) significantly different among groups (Table 3). The BMD was significantly ($P < 0.05$) lower in the OVX-Cd group than in the SH and OVX groups, but there were no ($P > 0.05$) significant differences in femoral BMD among the OVX-Cd, OVX-Cd-D, and OVX-Cd groups.

Femoral ash and Ca contents

Femoral ash was not ($P > 0.05$) significantly different among groups (Table 4). Femoral Ca content

Table 4. Effects of daidzein on femoral ash and calcium and cadmium¹

Group	Ash/dry bone (%)	Ca (mg/g of dry bone)	Cd (mg/g of dry bone)
SH	62.15±0.94	401.42±13.61 ^b	1.61±0.55 ^b
OVX	65.11±1.87	391.54±24.49 ^b	1.91±0.73 ^b
OVX-Cd	66.99±0.82	385.48±16.47 ^b	2.17±0.19 ^a
OVX-Cd-D	66.24±2.02	412.44±7.35 ^b	2.09±0.52 ^a
OVX-Cd-E	66.01±1.32	458.47±21.94 ^a	2.32±0.83 ^a

¹Data are mean ±S.D. values of nine rats per group. ^{a,b}Values with different letters are significantly different among groups at $P<0.05$ by Duncan's multiple range test.

in the OVX-Cd-E group was significantly ($P<0.05$) higher than in the other groups.

Serum OC and Ca concentrations

Both serum OC and Ca concentrations were ($P<0.05$) significantly higher in the OVX-Cd group compared with the other groups (Fig. 1). Thus, osteopenia caused by OVX or Cd probably resulted from an increase in bone turnover.

Fecal and urinary Ca concentration

Fecal and urinary Ca concentrations were significantly ($P<0.05$) higher in the OVX-Cd group, showing that Cd may accelerate Ca excretion with urine and feces.

Histomorphological change

Femoral histomorphological changes in proliferative cartilage and hypertrophic cells are shown in Fig. 2. Both cells were decreased by feeding Cd and an irregular arrangement was also found in proliferative cells. However, both cells retained

normal distribution characteristics in the OVX-Cd-D and OVX-Cd-E groups.

DISCUSSION

We investigated in this study the possibility of a protective action of daidzein, an isoflavone, against bone loss in young OVX rats.

Body mass of all the rats increased over time. Unlike in a study of Picherit *et al.* (2000) who reported that ovariectomy did not influence body mass, OVX rats were significantly heavier than SH operated rats, conforming some other previous studies (Kalu, 1991; Ishimi *et al.*, 1999; Kim & Om, 2001; Paik *et al.*, 2003). Among the OVX groups, there were no significant differences in body mass. However, rats in the OVX-Cd-E group had a slight decrease in food consumption, suggesting a decrease in appetite from the use of estradiol (Picherit *et al.*, 2000). Osteoblasts and adipocytes are differentiated from the same mesenchymal stromal cells. Estrogen stimulates the differentiation from mesenchymal stromal cell to osteoblast, while it inhibits the differentiation to adipocytes. Hence, the body mass gain

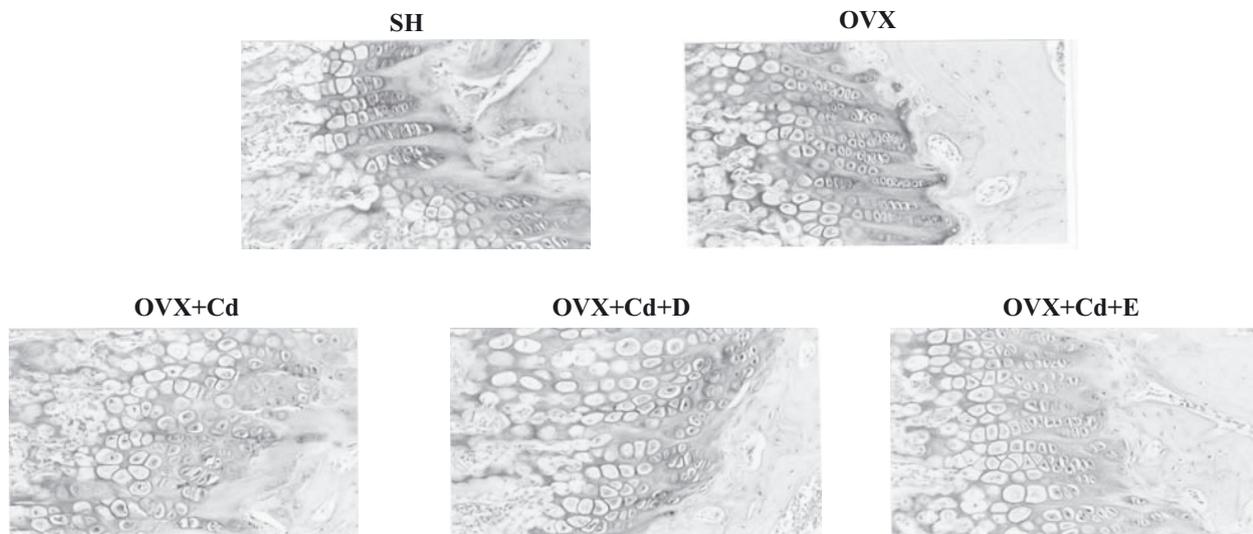


Figure 2. Morphological slides of femur. Hematoxylin-eosin staining, ×100.

after OVX could be caused by the accumulation of adipocytes due to estrogen deficiency (Grigoriadis *et al.*, 1988).

The BMD was lower in OVX-Cd rats than in SH rats. This trend of BMD was the same as in the study of Paik *et al.* (2003) who reported that the reduction of BMD could be partially attributed to a decrease in osteoblastic activity as shown by serum OC concentrations. They also reported that reduction of BMD in the OVX and OVX-Cd groups compared to the SH group was associated with a higher serum OC concentration and urinary Ca excretion. A decrease in efficiency of Ca absorption with age and an increase in fecal and urinary Ca excretion might result in the reduction of BMD (Avioli *et al.*, 1965; Gaumet *et al.*, 1997). Moreover, Wronski *et al.* (1985) demonstrated that bone modeling in rats was accelerated after the cessation of ovarian function. According to Picherit *et al.* (2000), only daidzein prevented the loss of lumbar and femur BMD but genistein did not.

OC is formed in the osteoblast and then deposited in bone matrix. OC is used as a biomarker of bone loss since serum OC concentration is increased after menopause. The osteoblast activity and synthesis of OC increase when BMD is decreased by an increase in bone resorption (Junqueira *et al.*, 1989). In the current study, serum OC in the OVX-Cd group was significantly higher compared with the SH, OVX, and OVX-Cd-E groups. Furthermore, OC as an indicator of bone formation, along with Ca excretion, was decreased by feeding daidzein and estradiol, suggesting that the bone turnover rate is not accelerated since bone formation occurs more slowly than bone resorption. Therefore, our findings demonstrate that daidzein may be effective in inhibiting fast bone turnover, especially bone resorption caused by ovariectomy or Cd.

The mechanisms of bone damage by Cd such as itai-itai disease have not been clearly elucidated, but one possible mechanism is damage to renal tubules by Cd accumulation leading to disturbance of 1,25-dihydroxycholecalciferol (vitamin D₃) activity, which increases the absorption of calcium from gut and mineralizes bone (Kjellstrom *et al.*, 1992; Jarup *et al.*, 1998). The other possible mechanism that has been widely suggested is that Cd directly influences bone metabolism, not by causing renal injury but by replacing Ca in the formation of hydroxyapatite crystals (Feldman, 1975; Hamilton & Smith, 1977; Wronski *et al.*, 1985; Bhattacharyya *et al.*, 1988; Junqueira *et al.*, 1989; Kjellstrom *et al.*, 1992; Wang & Bhattacharyya, 1993; Wilson & Bhattacharyya, 1997; Jarup *et al.*, 1998). In the current study, serum Ca concentration in the OVX-Cd group was significantly higher than in the SH and OVX groups. These results may demonstrate a Cd-stimulated Ca release from bone

increased serum Ca concentrations. This suggests an increased bone resorption, thereby accelerating osteoporosis or osteromalacia. When daidzein was provided, bone turnover occurred more slowly compared with the Cd-treated group. This may be due to formation of insoluble compounds of daidzein, containing hydroxyl groups, with Cd leading to an accelerated Cd excretion.

In conclusion, daidzein may play an important role in preventing Cd-induced bone loss in OVX rats by inhibiting bone resorption and decreasing serum Ca. Actually, the results and conclusion in the current study are very similar to those in the previous study of genistein performed by our laboratory. However, further work is needed to compare the effects of daidzein and genistein on the Cd-induced bone loss. Furthermore, there is a necessity to examine these effects in a longer study and elucidate the molecular mechanisms by which daidzein inhibits Cd-induced bone disease.

Acknowledgements

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (KRF-2003-C00149). And the authors thank Do Youn Kelly Kim, University of Wisconsin-Madison, for reviewing the manuscript.

REFERENCES

- Aldercreutz CH, Goldin BR, Gorbach SL, Höckerstedt KA, Watanabe S, Hämäläinen EK, Markkanen MH, Mäkelä TH, Wähälä KT, Adlercreutz T (1995) Soybean phytoestrogen intake and cancer risk. *J Nutr* **125**: 757S-770S.
- Avioli LV, McDonald JE, Lee SW (1965) The influence of age on the intestinal absorption of ⁴⁷Ca in postmenopausal osteoporosis. *J Clin Invest* **44**: 1960-1967.
- Bhattacharyya MH, Whelton BD, Stern PH, Peterson DP (1988) Cadmium accelerates bone loss in ovariectomized mice and fetal rat limb bones in culture. *Proc Natl Acad Sci USA* **85**: 8761-8765.
- de Kleijn MJJ, van der Schouw YT, Wilson PWF, Adlercreutz H, Mazur W, Grobbee DE, Jacques PF (2001) Intake of dietary phytoestrogens is low in postmenopausal women in the United States: the Framingham study 1-4. *J Nutr* **131**: 1826-1832.
- Eldridge AC (1982) Determination of isoflavones in soybean flours, protein concentrates and isolates. *J Agri Food Chem* **30**: 353-355.
- Ezawa I, Ogata E (1979) Ca²⁺-induced activation of succinate dehydrogenase and the regulation of mitochondrial oxidative reactions. *J Biochem (Tokyo)* **85**: 65-74.
- Feldman SL (1977) Induced of Cd on the metabolism of 25-hydroxycholecalciferol in chicks. *Nutr Rep Int* **8**: 251.
- Freiberg TA, Piscator M, Nordburg GF, Kjellstrom T (1984) Health effects of cadmium in the general environment in Japn. In *Cadmium in the Environment* (Freiberg L, ed) pp 137-195. CRC Press, Boca Ranton, FL.

- Freiberg TA, Elinder CG, Kgestrom T, Nordberg GF (1986) Itai-itai disease. In *Cadmium and Health* (Freiberg L, ed) pp 257–290. CRC Press, Boca Ranton, FL.
- Gaumet N, Seibel MJ, Coxam V, Davicco MJ, Lebecque P, Barlet JP (1997) Influence of ovariectomy and estradiol treatment on calcium homeostasis during aging in rats. *Arch Physiol Biochem* **105**: 435–444.
- Grigoriadis AE, Heersche JN, Aubin JE (1988) Differentiation of muscle, fat, cartilage, and bone from progenitor cells present in a bone-derived clonal cell population: effect of dexamethasone. *J Cell Biol* **106**: 2139–2151.
- Hallworth RB (1998) Prevention and treatment of postmenopausal osteoporosis. *Pharm World Sci* **20**: 198–205.
- Hamilton DL, Smith MW (1977) Cadmium inhibits calcium absorption by rat intestine. *J Physiol* **276**: 54–55.
- Ho SC, Bacon WE, Harris T, Looker A, Maggi S (1993) Hip fracture rates in Hong Kong and the United States, 1988 through 1989. *Am J Public Health* **83**: 694–697.
- Ishimi Y, Miyaura C, Ohmura M, Onoe Y, Sato T, Uchiyama Y, Ito M, Wang X, Suda T, Ikegami S (1999) Selective effects of genistein, a soybean isoflavone, on B-lymphopoiesis and bone loss caused by estrogen deficiency. *Endocrinology* **140**: 1893–1900.
- Itokawa Y, Nishino K, Takashima M, Nakata T, Kaito H, Okamoto E, Daijo K, Kawamura J (1978) Renal and skeletal lesions in experimental cadmium poisoning in rats. Histology and renal function. *Environ Res* **15**: 206–217.
- Iwami K, Moriyama T (1993) Comparative effect of cadmium on osteoblastic cells and osteoclastic cells. *Arch Toxicol* **67**: 352–357.
- Jarup L, Alfven T, Person B, Toss G, Elinder CG (1998) Cadmium may be a risk factor for osteoporosis. *Occup Environ Med* **55**: 435–439.
- Jia TL, Wang HZ, Xie LP, Wang KY, Zan RQ (2003) Daidzein enhances osteoblast growth that may be mediated by increased bone morphogenetic protein (BMP) production. *Biochem Pharmacol* **65**: 709–715.
- Junqueira LC, Carneiro J, Kelley RO (1989) *Basic Histology*, 6th ed, pp 136–159, Appleton and Lange, New York.
- Kalu DN (1991) The ovariectomized rat model of postmenopausal bone loss. *Bone Miner* **15**: 175–191.
- Kjellstrom T, Nordberg GF, Herber RF, Alessio L (1992) Cadmium. In *The Human Environment: Toxicity and Carcinogenicity*, International Agency for Research on Cancer. pp 301–310.
- Kim Y, Om AS (2001) Soy isoflavones in ovariectomized growing rats as bone disease prevention. In *International Symposium on Food, Nutrition, and Health for 21st Century*, Seoul, Korea. The Kor Soc of Food Sci Nutr **SIII-21**: 120–125.
- Kurzer MS, Ku X (1999) Dietary phytoestrogens. *Annu Rev Nutr* **17**: 353–381.
- Naim M, Gestener B, Zikah S, Bir Y, Bondi A (1974) Soybean isoflavones, characterization, determination and antifungal activity. *J Agric Food Chem* **22**: 806–810.
- Nogowski L, Maćkowiak P, Nowak KW (1993) Effect of a mycoestrogen, zearalenone, and a phytoestrogen, coumestrol on the liver membrane insulin receptor of ovariectomized rats. *Acta Biochim Polon* **40**: 147–150.
- Om AS, Chung KW, Chung HS (2002) Effect of cadmium accumulation on renal tissues in broilers. *Bull Environ Contam Toxicol* **68**: 297–301.
- Omi M, Morikawa M, Ezawa I (1994) The effect of voluntary exercise on bone mineral density and skeleton muscles in the rat model at ovariectomized and sham stages. *Bone Miner* **24**: 211–222.
- Paik MK, Lee HO, Chung HS, Yang SO, Kim JH, Om AS (2003) Genistein may prevent cadmium-induced bone loss in ovariectomized rats. *J Med Food* **6**: 337–343.
- Picherit C, Coxam V, Bennetau-Pelissero C, Kati-Coulibaly S, Davicco MJ, Lebecque P, Barlet JP (2000) Daidzein is more efficient than genistein in preventing ovariectomy-induced bone loss in rats. *J Nutr* **130**: 1675–1681.
- Riggs BL, Melton LJ (1986) Involutional osteoporosis. *N Engl J Med* **26**: 1676–1684.
- Sugimoto E, Yamaguchi M (2000) Stimulatory effect of daidzein in osteoblastic MC3T3–E1 cells. *Biochem Pharmacol* **59**: 471–475.
- Waalkes MP, Anver MR, Diwan BA (1999) Chronic toxic and carcinogenic effects of oral cadmium in the Noble (NBL/Cr) rat: induction of neoplastic and proliferative lesions of the adrenal, kidney, prostate, and testes. *J Toxicol Environ Health* **29**: 199–214.
- Wang C, Bhattacharyya MH (1993) Effect of cadmium on bone calcium and ⁴⁵Ca in nonpregnant mice on a calcium-deficient diet: evidence of direct effect of cadmium on bone. *Toxicol Appl Pharmacol* **120**: 228–239.
- Wilson AK, Bhattacharyya MH (1997) Effects of cadmium on bone: an *in vivo* model for early response. *Toxicol Appl Pharmacol* **45**: 68–73.
- Wronski TJ, Lowry PL, Walsh CC, Ignaszawski LA (1985) Skeletal alternations in ovariectomized rats. *Calcif Tissue Int* **37**: 324–328.
- Yeager H Jr, Massaro G, Massaro D (1971) Glycoprotein synthesis by the trachea. *Am rev Respir Dis* **103**: 188–97.
- Zinterhofer LJ, Jatlow PI, Fappiano A (1971) Atomic absorption determination of lead in blood and urine in the presence EDTA. *J Lab Clin Med* **78**: 664–674.