

## Original Research

# Beneficial Effects of Soybean Isoflavone Supplementation on Bone Metabolism and Serum Lipids in Postmenopausal Japanese Women: A Four-Week Study

Takehiko Uesugi, MS, Yutaka Fukui, MS, and Yukio Yamori, MD, PhD

*Fujicco Co., Ltd., Kobe (T.U., Y.F.), WHO Collaborating Center for Research on Primary Prevention of Cardiovascular Diseases, Department of Environmental Preservation and Development, Graduate School of Human and Environment Studies, Kyoto University, Kyoto (Y.Y.), JAPAN*

**Key words:** isoflavone, bone, pyridinoline, deoxypyridinoline, cholesterol, perimenopausal women

**Objective:** The aim of this study was to determine the effects of soy isoflavones with weak estrogen-like activities both on bone metabolism and on serum lipids in perimenopausal women.

**Methods:** Twenty-three healthy perimenopausal women were assigned randomly to either isoflavone or placebo groups. The isoflavone group (n = 12) received daily capsules of soy isoflavone extract (61.8 mg of isoflavones) and the placebo group (n = 11) received daily placebo capsules for four weeks. Urinary excretion of isoflavone was measured at weeks 0, 2 and 4. Urinary excretion of pyridinoline and deoxypyridinoline, bone stiffness and levels of serum cholesterol, triglyceride and cholesterol fractions were measured at weeks 0 and 4.

**Results:** As compared to the placebo group, urinary isoflavone, primarily daidzein, excretion was increased at weeks 2 and 4 in the isoflavone group. Excretion of bone resorption markers was reduced significantly in the isoflavone group. Both total serum cholesterol and LDL cholesterol were decreased significantly in the isoflavone group. Other serum biochemical parameters were not changed in either group.

**Conclusion:** Soy isoflavone supplementation for four weeks showed potentially beneficial effects on bone metabolism and on serum lipids in perimenopausal women. These effects could have the potential to reduce the risks of postmenopausal osteoporosis and of cardiovascular diseases in such women.

## INTRODUCTION

Postmenopausal osteoporosis is associated with estrogen deficiency [1], and this can be prevented by estrogen replacement therapy (ERT) [2]. Estrogens are known to have effects not only on bone metabolism, but also on cholesterol metabolism and blood pressure [3]. Indeed, ERT is considered the most effective method of reducing bone loss and of preventing other climacteric symptoms in postmenopausal women. However such therapy may have serious side-effects [4]. For this reason, it would be desirable to identify selective estrogen receptor modulators which, while providing benefit to bone metabolism, do not increase the risk of breast cancer.

Isoflavones present one such class of estrogenic molecule.

Soybean has a high isoflavone content, and it is widely consumed as a foodstuff in many Asian countries. The soybean-derived isoflavones, daidzein and genistein, and their glycosides, daidzin and genistin, have been reported to possess both estrogenic [5] and anticarcinogenic activities [6, 7].

We have shown previously that treatment with daidzin and genistin reduces bone loss and inhibits the reduction of bone strength in ovariectomized rats [8]. Epidemiological studies by our group in both native Japanese and Japanese Hawaiians have demonstrated that urinary isoflavone excretion is associated negatively with bone resorption and positively with bone density [unpublished]. Finally, an intervention study in Brazilians of Japanese ancestry, who consumed only low levels of soybean products, showed that increased ingestion of isoflavone-rich foods

Address reprint requests to: Yukio Yamori, MD, PhD, Director, WHO Collaborating Center for Research on Primary Prevention of Cardiovascular Diseases, Kokusaikenju Bldg, 86-2, Shimobanba-cho, Jodoji, Sakyo-ku, Kyoto 606-8413 JAPAN. Email: Yukio.Yamori@ma3.seikyoku.ne.jp

reduced bone loss significantly, based on the analysis of bone resorption markers in the urine [unpublished].

Native Japanese in Japan have been shown to have a much higher mean dietary intake of isoflavones than that observed in Europeans or Americans [9]. In this study, we aimed to examine the possibility that supplemental isoflavone intake would both decrease bone turnover and alter blood lipids in postmenopausal Japanese women who already have high daily dietary intakes of soybean products.

## MATERIALS AND METHODS

### Isoflavone Extraction

Crude isoflavone extract was prepared by extracting soybean hypocotyls (100 kg) in boiling water (1000 L) for 30 minutes. The extract was poured over a synthetic resin (SP-850, Mitsubishi Chemical Industry Ltd., 70 L) for batch absorption, and the resin was then washed three times with 140 L water. After addition of 70 L 60% ethanol, the resin was stirred for 30 minutes to elute isoflavones. The elution process was repeated three times, and the combined eluate was concentrated and dried to obtain the partly-purified isoflavone extract. The extract was spray-dried with dextrin (Fujiflavone P10; Fujicco Co. Ltd., Japan) and made up into capsules, each containing 30.9 mg of isoflavones, 8.2 mg of protein, 3.3 mg of ash and 6.6 mg of water. No lipids were detectable. The isoflavone content of the extract is listed in Table 1.

### Subjects

This randomized double-blind, placebo-controlled clinical trial was conducted at the food processing factory of Fujicco Co., Ltd., at Nishinomiya Hyogo, Japan, under the supervision of the WHO Collaborating Center for Research on Primary Prevention of Cardiovascular Diseases. Informed consent was obtained from all subjects on the basis that the substance to be

administered was safe, that the subjects had freely taken part in the study and that there would be no ill effects. Subjects were considered eligible for the study if they were disease-free as confirmed by routine regular annual health checks and were taking no anti-osteoporotic, lipid-lowering, hypotensive or hormonal drugs.

Twenty-five women were recruited from among the employees of the food processing factory; of these, twenty-three subjects entered and completed the trial. Subjects were healthy perimenopausal Japanese women in the age range 40 to 62 years. For each subject, body height and weight were recorded at the beginning of the study. Twelve subjects were assigned randomly to receive soybean isoflavone extract, two capsules daily (61.8 mg of isoflavones, Table 1), and the remaining 11 subjects were assigned to receive placebo capsules containing of only dextrin for four weeks.

### Isoflavone Intake

All subjects were required to keep quantitative records of all meals during the test period, in terms both of food constituents and of quantities. Based on these records, daily isoflavone intakes were calculated for each subject. Published data by Toda *et al.* [10] were used to determine the isoflavone content of each food item.

### Bone Stiffness

Right calcaneal stiffness was measured at the beginning of the study and four weeks later, using an ultrasound bone densitometer (Achilles A-1000, Lunar Corp., Madison, WI).

### Urine Analysis

Twenty-four-hour urine specimens were collected to determine isoflavone excretion at weeks 0, 2 and 4, and to determine pyridinoline and deoxypyridinoline excretion at weeks 0 and 4. After measurement of the volume, a portion of each sample (10 mL × 2 tubes) was stored at -30°C until analysis. Urinary creatinine (CRE) was determined by the Jaffe method using a commercial creatinine test kit (Wako Pure Chemical Industry, Osaka, Japan). Samples for which the CRE coefficient (mg/day/kg body weight) fell within a range of 10.8 to 25.2 were considered to represent complete urine collection and were analyzed further.

To produce free isoflavones from conjugates, urine samples were treated with sulfatase (EC 3.1.6.1 Type H-1, Sigma, St Louis, MO) and  $\gamma$ -glucuronidase (EC 3.2.1.31, Wako Pure Chemical Industries, Ltd., Japan). The reactants and urine samples were purified by Sep-pak cartridge before HPLC analysis. Quantitative HPLC analysis of urinary isoflavones was carried out in a Zorbax ODS column (4.6 mm × 25 cm; Waters) using a Waters 600 multisolvent delivery system with a WISP 710B autosampler and a Waters 490 programmable multiwavelength detector. Step gradient elution was achieved

**Table 1.** Isoflavone Contents of the Sample

	Isoflavone Extract	
	(mg/g)	(mg/capsule)
Daidzin	47.5	15.6
Genistin	10.4	3.4
Glycitin	32.4	10.6
6'-O-malonyldaidzin	0.9	0.3
6'-O-malonylgenistin	n.d.	n.d.
6'-O-malonylglycitin	n.d.	n.d.
6'-O-acetyldaidzin	1.5	0.5
6'-O-acetylgenistin	n.d.	n.d.
6'-O-acetylglycitin	0.9	0.3
Daidzein	n.d.	n.d.
Genistein	n.d.	n.d.
Glycitein	0.6	0.2
Total	94.1	30.9

**Table 2.** Baseline Data

	Isoflavone (n = 12)	Placebo (n = 11)	p-value
Age (years)	51.8 ± 1.9	51.0 ± 1.6	0.76
Time since menopause (years)	4.4 ± 1.4	3.5 ± 1.1	0.33
BMI (kg/m <sup>2</sup> )	22.4 ± 0.8	22.9 ± 0.9	0.69

Values are mean ± S.E.

at a flow-rate of 1 mL/min with acetonitrile (A.N.) solution containing constant 0.1% acetic acid: 10% to 18% (v/v) A.N. for the first 20 minutes, 18% to 20% A.N. for another 20 minutes, and 20 to 35% A.N. for 37.5 minutes, then 35% A.N. at constant for the last seven minutes. The absorptions of urinary De and Ge were measured at 254 nm. Commercially available daidzein and genistein (Fujicco Co., Ltd.) were used for standard chemicals.

Urinary pyridinoline was determined as described by Takahashi *et al.* [11]. Deoxypyridinoline was determined using a commercial ELISA kit (Pyrilinks-D, Metra Biosystems, Mountain View, CA).

**Blood Analyses**

Venous blood (5 mL) was collected by using vacuum syringes (XX-VP010hg, Terumo Co. Ltd.) at weeks 0 and 4 and after four weeks. Immediately after collection, blood samples were centrifuged for 30 minutes at 3,000 rpm and 4°C (Model KN-30F, Kubota Co. Ltd.). Sera were stored at -30°C until analysis.

Blood tests were carried out by Aijinkai Healthcare Corp. AST, ALT and γ-GTP were determined spectrophotometrically [12]. Hemoglobin A1 and A1c were determined by HPLC analysis [13]. The bone formation marker osteocalcin was measured by EIA [14]. Serum triglycerides were analyzed using the GPO DAOS glycerol method [15]. Total cholesterol was determined using the cholesterol oxidase DAOS method [16]. Cholesterol fractionation was carried out by agarose gel electrophoresis [17].

**Statistical Methods**

StatView-j Version 4.11 (Abacus Concepts, Inc., Berkeley, CA) was utilized for statistical analysis. Differences between

the means for the two groups were evaluated by using Student’s *t* test. Changes within each group were evaluated by using Student’s paired *t* test, and differences between the changes observed in each groups were evaluated by using repeated-ANOVA; *p*-values of less than 0.05 were considered to indicate statistically significant differences.

**RESULTS**

There were no significant differences in age, body mass index (BMI) and time after menopause, between the test (isoflavone supplemented) and placebo groups (Table 2).

Calculated isoflavone intake in daily food intake and actual urinary excretion are shown in Table 3. There was no statistically significant difference between the two groups in daily daidzin and genistin intakes at the base line. Urinary daidzein excretion was increased significantly at weeks 2 and 4 in the test group, both in absolute terms and also relative to the placebo group. Genistein excretion was not increased by the supplement.

Bone metabolism parameters are shown in Table 4. Excretion of pyridinoline and deoxypyridinoline, markers of bone resorption in the test group, was decreased significantly at four weeks. A significant difference from the base line levels, between the test and placebo groups, was also noted in pyridinoline excretion at four weeks, but no significance in deoxypyridinoline excretion. Levels of intact osteocalcin, a marker of osteogenesis, were not significantly different between the two groups either at the base line or at the end of the study. Bone stiffness did not change within or between the two groups during the four weeks of the study (Table 4).

The results of the serum biochemical parameters are presented in Table 5. Serum total and LDL cholesterol levels fell significantly from the base line only in the isoflavone supplemented group and were lower, but not significantly, from those in the placebo group. No changes in HDL, VLDL cholesterol and triglyceride levels were observed during the study in either the test or the placebo group. AST, ALT, γ-GTP and hemoglobin A1 and A1c levels (laboratory indicators of liver cell damage or of a tendency to diabetes) were normal, and they displayed no changes over time in either the test or placebo group. No side effects were detected.

**Table 3.** Isoflavone Intake and Urinary Excretion

	Intake in Food (mg/28 days)		Urinary Excretion (mg/day)					
			Baseline		2 Weeks		4 Weeks	
	Placebo	Isoflavone	Placebo	Isoflavone	Placebo	Isoflavone	Placebo	Isoflavone
Daidzein	299 ± 21	341 ± 36	3.3 ± 0.8	4.8 ± 1.0	4.6 ± 1.0	9.2 ± 1.3* <sup>#</sup>	5.1 ± 1.3	9.3 ± 1.3* <sup>#</sup>
Genistein	446 ± 38	499 ± 61	1.1 ± 0.3	2.2 ± 0.5	1.4 ± 0.3	1.9 ± 0.5	2.2 ± 0.5	2.4 ± 0.3

Values are means ± S.E.

\* Significant differences from the baseline (*p* < 0.05).

<sup>#</sup> Significant differences from the placebo group (*p* < 0.05).

**Table 4.** Effect of Isoflavone Supplementation on Bone Markers and Bone Stiffness

	Placebo		Isoflavone	
	Baseline	4 Weeks	Baseline	4 Weeks
Serum intact-osteocalcin ( $\mu\text{g/mL}$ )	7.2 $\pm$ 0.7	7.1 $\pm$ 0.6	8.5 $\pm$ 1.2	7.4 $\pm$ 1.1
Urinary pyridinoline excretion (nmol/mmol of CRE)	16.2 $\pm$ 1.6	17.6 $\pm$ 1.3	19.7 $\pm$ 2.3	13.1 $\pm$ 1.7* <sup>#</sup>
Urinary deoxypyridinoline excretion (nmol/mmol of CRE)	10.1 $\pm$ 0.9	9.8 $\pm$ 0.8	11.7 $\pm$ 1.6	9.8 $\pm$ 1.2*
Bone stiffness	73.0 $\pm$ 3.3	73.0 $\pm$ 3.6	71.0 $\pm$ 4.9	71.0 $\pm$ 4.9

Values are mean  $\pm$  S.E.

\* Significant differences from the baseline ( $p < 0.05$ ).

<sup>#</sup> Significant differences from the placebo group ( $p < 0.05$ ).

## DISCUSSION

This experiment was designed to observe the effects of four-week isoflavone supplementation of 61.8 mg/day. During the experiment, all subjects were free to take any additional soybean products. The isoflavone intake during the study was therefore in addition to the common dietary intake among Japanese. From the results of the dietary survey and the urinary isoflavone excretion, it appears that the supplemental isoflavone intake was of the same order of magnitude as the existing dietary intake.

Daidzein and genistein, like estrogen, are thought to suppress bone resorption by attenuating the activity of osteoclasts [18, 19]. Glycitein has a weak estrogenic activity comparable to that of daidzein and genistein [20]. Genistin and daidzin have been shown to prevent bone loss in ovariectomized rats [8]. Four weeks of isoflavone supplementation appear to be effective in suppressing bone resorption in perimenopausal women (a previous report showed that estrogenic effects appeared in a short term treatment [21]). Bone metabolic parameters were changed by estrogen administration after two weeks [21]. However, bone mineral density and content change only very slowly, and a long term study would be necessary for the demonstration of the effect on bone stiffness.

Our previous epidemiological research in Japanese women has indicated a negative association between bone density and

bone resorption markers such as pyridinoline [unpublished], and Tsuchida *et al.* reported that soybean intake was associated positively and significantly with bone mineral density in Japanese middle-aged women [29]. Higher levels of urinary pyridinoline and deoxypyridinoline excretion are associated with faster bone loss at the hip [22], and urinary deoxypyridinoline levels are reported to predict the subsequent risk of hip fracture in elderly women [23]. Inhibition of bone resorption, when not associated with reduction in bone formation, has the net effect of reducing the rate of bone loss [24]. Dalais *et al.* reported that diets including 45 g of soy grits containing 52.64 mg of isoflavones were associated with increased bone mineral content within 12 weeks [28]. Potter *et al.* reported that a diet including a high isoflavone content soy protein containing 90 mg/day of isoflavones was associated with increased bone mineral density and content within six months [25].

Based on these data, we conclude that isoflavone supplementation appears to be effective in inhibiting reduction in bone density and/or in promoting bone strength. Isoflavone supplementation in Japanese subjects, who already consume relatively high levels of soybean products, thus appears to provide further benefit to bone metabolism.

In addition to beneficial effects on bone metabolism, isoflavones lowered total serum cholesterol levels as well as LDL cholesterol. High LDL cholesterol levels are known to be associated with atherosclerosis [26]. Estrogen increases LDL

**Table 5.** Effect of Isoflavones on Serum Parameters in Perimenopausal Women

	Placebo			Isoflavone		
	Baseline	4 Weeks	After 4 Weeks	Baseline	4 Weeks	After 4 Weeks
T-Chol	237.6 $\pm$ 31.2	240.9 $\pm$ 25.4		226.3 $\pm$ 39.7	215.4 $\pm$ 44.4*	
LDL-Chol	162.3 $\pm$ 24.5	163.9 $\pm$ 21.6		148.1 $\pm$ 34.7	138.4 $\pm$ 36.1*	
HDL-Chol	64.9 $\pm$ 15.2	68.1 $\pm$ 14.2		66.2 $\pm$ 15.8	65.1 $\pm$ 16.8	
VLDL-Chol	9.2 $\pm$ 4.0	9.9 $\pm$ 3.2		11.9 $\pm$ 9.7	11.9 $\pm$ 10.7	
TG	105.2 $\pm$ 40.0	97.5 $\pm$ 27.5		94.8 $\pm$ 43.9	106.1 $\pm$ 51.0	
AST	24.8 $\pm$ 13.6	21.0 $\pm$ 4.8		19.6 $\pm$ 6.2	16.2 $\pm$ 3.8	
ALT	25.8 $\pm$ 17.3	21.7 $\pm$ 9.5		17.1 $\pm$ 7.0	16.2 $\pm$ 3.8	
$\gamma$ -GTP	27.8 $\pm$ 19.9	16.8 $\pm$ 5.2		23.9 $\pm$ 17.7	19.8 $\pm$ 13.4	
Hemoglobin A1	6.6 $\pm$ 0.4	6.8 $\pm$ 0.4	6.5 $\pm$ 0.4	6.6 $\pm$ 0.4	6.8 $\pm$ 0.3	6.4 $\pm$ 0.3
Hemoglobin A1c	5.2 $\pm$ 0.3	5.2 $\pm$ 0.3	4.9 $\pm$ 0.3	5.2 $\pm$ 0.3	5.2 $\pm$ 0.2	4.8 $\pm$ 0.2

Values were mean  $\pm$  S.E.

\* Significant differences from the baseline ( $p < 0.05$ ).

catabolism by increasing the number of LDL receptors, as shown in hepatocytes from estrogen-treated rats [27]. Isoflavones may reduce LDL level by increasing the number of LDL receptors in a similar manner. Our data thus confirmed that isoflavones had beneficial effects on bone and lipid metabolisms, suggesting they may be useful for preventing both osteoporosis and coronary heart diseases. However, Potter *et al.* reported that isoflavones provided effective benefit to mineral density and content, but provided little benefit to blood lipids [25]. The estrogen threshold hypothesis is now widely accepted, and the positive effective dose of estrogen for the reduction of bone resorption is as low as 30 pg/mL, while for benefit to serum cholesterol much higher doses are necessary [30]. Our observations support this hypothesis. Since the isoflavone extract used in this study included large amounts of daidzin and glycitin, a small amount of genistin, but little soy protein, the observed benefits appear to be attributable to isoflavones and mainly to daidzin and glycitin.

Isoflavone supplementation at this level over four weeks appears to be safe, as judged by clinical laboratory blood tests, physical examinations and on the basis of subjective symptoms. No side effects were detected.

## CONCLUSIONS

Daily supplementation of soybean isoflavones (61.8 mg) for four weeks was associated with a significant reduction in the excretion of bone resorption markers (pyridinoline and deoxy-pyridinoline), concomitantly with marked increases in urinary daidzein excretion and with lowered serum total and LDL cholesterol levels in postmenopausal Japanese women.

Isoflavone supplementation at this daily dose for four weeks did not affect liver function, and the benefits of soy isoflavone supplementation for reducing risks of osteoporosis and coronary heart disease appear not to be associated with any deleterious metabolic effects.

## REFERENCES

1. Gibaldi M: Prevention and treatment of osteoporosis: Does the future belong to hormone replacement therapy? *J Clin Pharmacol* 37:1087–1099, 1997.
2. Lindsay R, Hart DM, Clark DM: The minimum effective dose of estrogen for prevention of postmenopausal bone loss. *Obstet Gynecol* 63:759–763, 1984.
3. Walsh BW, Schiff I, Rosner B, Greenberg L, Ravnikar V, Sacks FM: Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. *N Engl J Med* 325:1196–1204, 1991.
4. Colditz GA, Stampfer MJ, Willett WC, Hennekens C, Rosner B, Speizer FD: Prospective study of estrogen replacement therapy and risk of breast cancer in postmenopausal women. *JAMA* 264:2648–2653, 1990.

5. Bickoff EM, Livingston AL, Hendrickson AP, Booth AN: Relative potencies of several estrogen-like compounds found in forages. *J Agric Food Chem* 10:410–412, 1962.
6. Peterson G, Barnes S: Genistein inhibition of the growth of human breast cancer cells: Independence from estrogen receptors and the multi-drug resistance gene. *Biochem Biophys Res Commun* 179:661–667, 1991.
7. Mousavi Y, Adlercreutz H: Genistein is an effective stimulator of sex hormone-binding globulin production in hepatocarcinoma human liver cancer cells and suppresses proliferation of these cells in culture. *Steroids* 58:301–304, 1993.
8. Ishida H, Uesugi T, Hirai K, Toda T, Nukaya H, Yokotsuka K, Tsuji K: Preventive effects of the plant isoflavones, daidzin and genistin, on bone loss in ovariectomized rats fed a calcium-deficient diet. *Biol Pharm Bull* 21:62–66, 1998.
9. Adlercreutz H, Honjo H, Higashi A, Fotsis T, Hamalainen E, Hasegawa T, Okada H: Urinary excretion of lignans and isoflavonoid phytoestrogens in Japanese men and women consuming a traditional Japanese diet. *Am J Clin Nutr* 54:1093–1100, 1991.
10. Toda T, Tamura J, Okuhira T: Isoflavone contents of commercial soy foods. *FFI J Jpn* 172:83–89, 1997.
11. Takahashi M, Ohishi T, Aoshima H, Kushida K, Inoue T, Horiuchi K: Pre-fractionation with cation exchanger for determination of intermolecular crosslinks, pyridinoline, and pentosidine, in hydrolysate. *J Liquid Chromatogr* 16:1355–1370, 1993.
12. Shaw LM, Stromme JH, London JL, Theodorsen L: International Federation of Clinical Chemistry, (IFCC), Scientific committee, Analytical section. IFCC methods for the measurement of catalytic concentration of enzymes. Part 4. IFCC method for gamma-glutamyltransferase [(gamma-glutamyl)-peptide: amino acid gamma-glutamyltransferase, EC 2.3.2.2]. *J Clin Chem Biochem* 21:633–646, 1983.
13. Goldstein DE, Peth SB, England JD, Hess RL, Da Costa J: Effects of acute changes in blood glucose on Hb A1c. *Diabetes* 29:623–627, 1980.
14. Hosoda K, Eguchi H, Nakamoto T, Kubota T, Honda H, Jindai S, Hasegawa R, Kiyoki M, Yamaji T, Shiraki M: Sandwich immunoassay for intact human osteocalcin. *Clin Chem* 38:2233–2238, 1992.
15. Spayd RW, Bruschi B, Burdick BA, Dappen GM, Eikenberry JN, Esders TW, Figueras J, Goodhue CT, LaRossa DD, Nelson RW, Rand RN, Wu TW: Multilayer film elements for clinical analysis: applications to representative chemical determinations. *Clin Chem* 24:1343–1350, 1978.
16. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC: Enzymatic determination of total serum cholesterol. *Clin Chem* 20:470–475, 1974.
17. Phillips GB, Wille LE: The phospholipid composition of human serum lipoprotein fractions separated by electrophoresis on agarose gel. Demonstration of a fraction with high lysolecithin content. *Clin Chim Acta* 49:153–160, 1973.
18. Gao YH, Yamaguchi M: Anabolic effect of daidzein on cortical bone in tissue culture: Comparison with genistein effect. *Mol Cell Biochem* 194:93–98, 1999.
19. Yamaguchi M, Gau YH: Inhibitory effect of genistein on bone resorption in tissue culture. *Biochem Pharm* 55:71–76, 1998.
20. Song TT, Hendrich S, Murphy PA: Estrogenic activity of glycitein, a soy isoflavone. *J Agric Food Chem* 47:1607–1610, 1999.

21. Stock JL, Coderre JA, Mallette LE: Effects of a short course of estrogen on mineral metabolism in post menopausal women. *Endocrinol Metab* 61:595–600, 1985.
22. Bauer DC, Sklarin PM, Stone KL, Black DM, Nevitt MC, Ensrud KE, Arnaud CD, Genant HK, Garnero P, Delmas PD, Lawaetz H, Cummings SR: Biochemical markers of bone turnover and prediction of hip bone loss in older women: the study of osteoporotic fractures. *J Bone Miner Res* 14:1404–1410, 1999.
23. Garnero P, Hausherr E, Chapuy MC, Marcelli C, Grandjean H, Muller C, Cormier C, Breart G, Meunier PJ, Delmas PD: Markers of bone resorption predict hip fracture in elderly women: the EPIDOS prospective study. *J Bone Miner Res* 11:1531–1538, 1996.
24. Garnero P, Shih WJ, Gineyts E, Karpf DB, Delmas PD: Comparison of new biochemical markers of bone turnover in late postmenopausal osteoporotic women in response to alendronate treatment. *J Clin Endocrinol Metab* 79:1693–1700, 1994.
25. Potter SM, Baum JA, Teng H, Stillman RJ, Shay NF, Erdman Jr JW: Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. *Am J Clin Nutr* 68:1375–1379, 1998.
26. Steinberg D: Antioxidants and atherosclerosis: a current assessment. *Circulation* 84:1421–1425, 1991.
27. Windler E, Kovanen PT, Chao YS, Brown MS, Havel RJ, Goldstein JL: The estradiol-stimulated lipoprotein receptor of rat liver. *J Biol Chem* 255:10464–10471, 1980.
28. Dalais FS, Rice GE, Wahlqvist ML, Grehan M, Murkies AL, Medley G, Ayton R, Strauss BJG: Effects of dietary phytoestrogens in postmenopausal women. *Climacteric* 1:124–129, 1998.
29. Tsuchida K, Mizushima S, Toba M, Soda K: Dietary soybeans intake and bone mineral density among 995 middle-aged women in Yokohama. *J Epidemiol* 9:14–19, 1999.
30. Barbieri RL: Hormone treatment of endometriosis: The estrogen threshold hypothesis. *Am J Obstet Gynecol* 166:740–745, 1992.

*Received May 31, 2000; revision accepted July 31, 2001*