Eur J Nutr (2006) 45:327–334
DOI 10.1007/s00394-006-0602-2

ORIGINAL CONTRIBUTION

Yan-Bin Ye
Xing-Yi Tang
Marian A. Verbruggen
Yi-Xiang Su

Soy isoflavones attenuate bone loss in early postmenopausal Chinese women

A single-blind randomized, placebo-controlled trial

Abstract *Background* Previous studies show that daily doses of 40–99 mg soy isoflavones produce inconsistent effects on preventing estrogen-related bone loss in postmenopausal women. *Aim of the study* To examined the bone-sparing effect of isoflavones at a higher dose in early Chinese postmenopausal women. *Methods* A total of 90 eligible women aged 45–60 years were randomly assigned to three treatment groups (30 subjects/group) with daily dosages of 0 (placebo), 84 and 126 mg isoflavones for 6 months. Further inclusion criteria included body mass index <30 kg/m^2 and Kuppermann Climacteric Scale >15. Bone mineral density (BMD) of the spine and hip were measured using dual- energy X-ray absorptiometry at 0 and 6 months. Serum osteocalcin, bone-specific alkaline phosphatase (BAP) and urinary deoxypyridinoline were examined at 0, 3 and 6 months. *Results* Mean percent changes in BMD at the lumbar spine ($p = 0.114$) and femoral neck ($p = 0.053$) increased with the supplementations of soy isoflavones after adjusting for age, years since menopause, body weight and height, dietary intakes of isoflavones, calcium and protein, physical activities and baseline BMD at the relevant sites. We observed significantly dose-dependent linear relationship between the supplemental isoflavones and percent changes of BMD at the spine ($p = 0.042$) and femoral neck ($p = 0.016$) post-treatment, and urinary total deoxypyridinoline ($p = 0.014$) at 12 weeks but not at 24 weeks after adjusting for the above factors. No significant difference in percent changes in serum osteocalcin ($p > 0.05$) and BAP ($p > 0.05$) was found among the three treatment groups at 12-week and 24-week post-treatment. *Conclusion* There is a significantly dose-dependent effect of soy isoflavones on attenuating bone loss at the spine and femoral neck possibly via the inhibition of bone resorption in non-obese postmenopausal Chinese women with high Kuppermann Scale.

Key words soy isoflavones – bone mineral density – bone markers – Postmenopausal women – Chinese

Received: 8 November 2005
Accepted: 28 April 2006
Published online: 8 June 2006

Y.-B. Ye, MPhil · Prof. Y.-X. Su, MD (✉)
Dept. of Nutrition
School of public Health
Sun Yat-sen University
74 Zhongshan Rd 2
Guangzhou 510080, P.R. China
Tel.: +86-20/8733-3166
Fax: +86-20/8127-9121
E-Mail: suyx@mail.sysu.edu.cn

Y.-B. Ye, MPhil
Dept. of Nutrition
The First Affiliated Hospital
Sun Yat-sen University
Guangzhou, P.R. China

X.-Y. Tang, MD
Dept. of Paediatrics
The Third Affiliated Hospital
Sun Yat-sen University
Guangzhou, P.R. China

M.A. Verbruggen, PhD
Acatris Holding B.V.
ZG Giessen, The Netherlands

Introduction

Osteoporosis is a health problem experienced by about one third of postmenopausal women [1, 2]. Estrogen deficiency is a major cause of postmenopausal osteoporosis [3]. There is a series of physiological changes in bone metabolism after menopause. Yearly bone loss rate, especially in the lumbar spine, reaches 4~5% during the first 5 years of menopause [4]. Hormone replacement therapy (HRT) is so far the most effective therapy for maintaining bone density. However, its acceptability and compliance have been poor due to its

potential adverse effects, which include an increased risk of endometrial cancer and breast cancer [5]. Therefore, a more acceptable alternative therapy that might offer the benefits of HRT, but without the undesirable side effects, needs to be identified.

Isoflavones are found predominantly in soy products. Their structure and functions are similar to estrogen [6]. Epidemiological studies have shown that higher intakes of soy and soy products are associated with lower prevalence of osteoporosis [7, 8]. A number of animal studies have almost consistently indicated that isoflavone-enriched soy protein, or concentrated soy isoflavones have a bone-sparing effect in estrogen-deprived animals [9, 10]. Our previous study also showed that soy protein with isoflavones attenuated bone loss and increased calcium retention while decreasing calcium excretion in ovariectomized rats [11]. In vitro, studies have also reported that soy protein with isoflavones or soybean isoflavone extracts can improve the reproduction and differentiation of osteoblasts [12, 13]. A few short-term human clinical trials have been reported, but the findings are less convincing, showing either a weak effect or no benefit to bone health [14]. So far, few interventional studies have examined the effects of either soy or isoflavones on bone density and biomarkers of bone metabolism in humans [15–20], especially in Asian populations [17].

In most human studies, the test dose of soy isoflavones given ranged from 40 to 99 mg/day [10, 14, 20, 21]. Many clinical trials showed that doses between 40 and 60 mg/d did not have any significant benefit on bone health; a weak effect was found using even higher doses of 80–90 mg/d [10, 14, 21]. It remains unclear whether soy isoflavones cannot efficiently prevent postmenopusal bone loss, or the previously tested doses were too low to consistently produce positive results.

This study aims to examine the hypothesis that a high-dose supplementation of soy isoflavones would retard bone loss in early postmenopausal women. We tested the effects of soy isoflavones on bone loss at two higher doses of 84 and 126 mg isoflavones/day in early postmenopausal Chinese women.

Materials and methods

Subjects

Subjects were recruited by advertisements in the local media. They were required to be Chinese, aged between 45 and 60 years, and be within 5 years postnatural menopause defined as at least 12 months since the last menstrual cycle and serum follicle-stimulating hormone (FSH) >30 IU/l. None of them had any detectable diseases or were taking medications known to affect bone health, including chronic diseases of the kidney, liver, heart, endocrine system, and diabetes. Women who had used exogenous estrogens, corticosteroids, thiazine diuretics, drugs or other treatments for osteoporosis, as well as any other medications known to affect bone mass, for 1 year prior to recruitment, were also excluded.

Potential eligible subjects were invited to the First Affiliated Hospital of Sun Yat-sen University. The eligibility of subjects was determined via face-to-face-interviews using a structured questionnaire including sociodemographic data, medical history, and reproductive and menopausal history. A total of 110 subjects who met the above requirements were given further examinations including bone scanning, measurement of body weight and height, fasting serum follicle stimulate hormone (FSH) and menopausal symptoms. Only those who met the following additional criteria were enrolled in the study: body mass index (BMI) <30 kg/m^2, serum follicle-stimulating hormone (FSH) >30 IU/l, the Kuppermann Climacteric Scale >15. Written informed consent was obtained from all the participants before enrollment. The Ethical Committee of the First Affiliated Hospital of Sun Yat-sen University approved this study. A total of 90 volunteers were met all the criteria and finally enrolled in the study.

Study design

This study was a half-year, single blind, randomized, placebo-controlled trial. The 90 eligible subjects were randomly assigned to receive 0 mg soy isoflavones (Isof.) (Placebo, starch) (Control Group), 84 mg Isof. (Low-dose Group) or 126 mg Isof. (High-dose Group) on a daily basis for 6 months. The 30 subjects were randomly selected for the first group from the 90 subjects using the SPSS procedure of "Data-Select cases-Random sample of cases" (SPSS for Windows, V10, SPSS Inc., Chicago, IL, USA); another 30 subjects were then selected for the second group from the remaining 60 subjects in the same way; and the residual 30 subjects were the third group. All subjects were blinded to their group status.

The soy isoflavones were soy germ isoflavone extract powder (SoyLife® EXTRA) provided by Acatris Holding B.V., Giessen, The Netherlands. The product contained 7.05% isoflavones (aglycone equivalent). The isoflavones contained 52, 15 and 33% daidz(e)in, genist(e)in and glycit(e)in, respectively (data provided by the manufacture). A daily dose of placebo and isoflavones was filled into six capsules, which were given in the morning and the evening after meals (three capsules each time). All capsules looked identical and were filled in one batch. The capsules were delivered to subjects every month by the project

coordinator (research staff). The residual capsules were collected at the time of next delivery, and the net consumption was calculated.

All participants were given detailed written instructions on how to take the supplement. They were asked to withdraw any other dietary supplement (such as vitamin D, vitamin K, complex vitamins, and calcium tablets, etc.) known to affect bone metabolism, and consume more calcium-enriched foods but less soy foods during the treatment period. They were closely monitored throughout the study.

Measurements

Questionnaire interview

The dietary assessment of intakes of calcium, protein and soy isoflavones at baseline and post-treatment was based on a quantitative food frequency questionnaire that included 95 food groups/items mainly focused on soy and calcium intakes. The questionnaire included cereal (14 items), animal foods (14 items), milk and milk products (5 items), soy and soy products (28 items), vegetable (15 items), fruit (10 items), snack and drink (9 items). The mean intake of food per day, week, or month was reported at the face-to-face interviews, using the past 12 months before the interview as the reference period. The content of soy isoflavones was calculated based on the food composition data reported by Murphy et al. [22] and Huang et al. [23]. Other nutrients were estimated using the *Foods and Nutrition Software* (version II, 2000; the 301 Hospital, Beijing, China) based on a Chinese food composition table [24]. Three 24-h food intake diaries were completed for dietary energy intake.

Physical activities were assessed using the questionnaire reported by Liu et al. [25] at baseline and post-treatment. Energy expenditure from the physical activities was calculated according to the indices indicated in the questionnaire [25].

Bone mineral density and biomarker measurements

Bone mineral density (BMD) at the lumber spine (L1–L4), and the left hip was measured using dual-energy X-ray absorptiometry (DXA, Hologic QDR-2000+, Waltham, MA) at baseline and post-treatment after 6 months. The same scanner-operator who blinded to the subjects' grouping status conducted the scans and analysis. Long-term precision was 0.45% by daily testing the spine phantom over the previous 5 years, and the in vivo precision error in our laboratory for BMD were 1.0% for the spine and 1.48% for the total hip.

Fasting blood samples and the next morning urine samples were collected at 0, 12 and 24 weeks of treatment to test biomarkers of bone metabolism and hormones as described below. The serum and urine samples were stored at −80°C until analyzed.

Serum *osteocalcin* (OC) concentration was measured with the IMMULITE Analyzer system (Diagnostic Products Corporation-DPC, Ltd, USA). The intra- and inter-assay coefficients of variation (CVs) were 4.7% and 6.5%, respectively. Serum *bone-specific alkaline phosphates* (BAP) concentration was measured using the method of indirect precipitation with wheat-germ lectin, as described by Behr and Barnert [26]. The intra and inter-assay CVs were 4.1% and 5.3%. Urinary total *Deoxypyridinoline* (Dpd) concentration was measured with a commercial enzyme-linked immunosorbent assay kit (Metra Biosystems, Inc, Mountain View, CA, USA), and the values were adjusted for urinary creatinine. The intra- and inter-assay CVs for Dpd were 4.8% and 6.5%, respectively. Urinary creatinine was determined with a colorimetric method (Metra Biosystems, Inc, Mountain View, CA, USA).

Statistical analyses

Baseline characteristics, dietary intake, and energy expenditure in physical activities among the three treatment groups were compared by means of one-way ANOVA or nonparametric test when equal variance not assumed. Percent changes in BMDs and bone markers were calculated as "[(follow-up value−baseline value) × 100%]/baseline value". Comparison of the mean differences in BMDs, bone markers, and their percent changes among intervention groups were conducted using one-way ANOVA and analysis of covariance (ANCOVA) by including all subjects with intention-to-treat (ITT). Potential confounders, such as age, years since menopause (YSM), body weight and height, dietary intakes of isoflavones, calcium, and protein, energy expenditure in physical activities and the relevant baseline values of BMD or bone markers, were included in the ANCOVA models. *Bonferroni* test was used for *Post-Hoc Multiple Comparisons* in the ANOVA and ANCOVA analyses. Dose-dependent linear trends were examined using polynomial test in the ANOVA and ANCOVA analyses. Only the covariates which remained significant were retained in the final model. Manual backward stepwise method was used in removing the potential covariates, with education level forced into the models. *F*-to-entry and remove criteria were 0.05 and 0.10. A two-tailed *p*-value of less than 0.05 was considered statistically significant for all analyses. The values of serum OC, BAP and urinary Dpd were logarithmic transformed prior to all analyses. SPSS for Windows (release 10, SPSS Inc., Chicago, IL, USA) was used for the analysis.

Table 1 Baseline and follow-up characteristics of subjects in the group (means ± SD)

Items	Placebo	Low-dose	High-dose	*p*-value*
Baseline (n)	30	30	30	
Age (y)	52.7 ± 3.7	52.5 ± 3.0	51.6 ± 3.2	0.398
Years since menopause (y)	2.9 ± 1.6	2.6 ± 1.4	2.3 ± 1.5	0.395
Body weight (kg)	56.6 ± 7.1	54.7 ± 7.9	56.2 ± 6.4	0.650
Body mass index (kg/m^2)	23.3 ± 2.4	22.3 ± 2.5	22.3 ± 2.1	0.173
Follicle stimulate hormone (IU/l)	77.2 ± 31.7	74.2 ± 37.8	74.8 ± 37.2	0.913
Energy intake (kJ/d)[a]	7283 ± 1273	7293 ± 722	7269 ± 1335	0.981
Protein intake (g/d)[b]	77.1 ± 18.0	74.3 ± 15.7	77.5 ± 14.8	0.791
Dietary Isof. Intake (mg/d)[b]	28.0 ± 23.0	20.8 ± 15.4	33.0 ± 28.8	0.208
Total calcium intake (mg/d)[b]	619 ± 377	651 ± 238	770 ± 265	0.061
P.A. expenditure energy (kJ/d)[c]	5960 ± 1558	6174 ± 1340	5901 ± 1357	0.057
Follow-up (n)	30	28	26	
Energy intake (kJ/d)[a]	7279 ± 974	7430 ± 634	7358 ± 920	0.796
Protein intake (g/d)[b]	75.9 ± 9.6	76.4 ± 8.5	78.1 ± 10.0	0.667
Dietary soy isoflavone intake (mg/d)[b]	5.7 ± 4.8	5.3 ± 4.4	7.0 ± 5.1	0.384
Total calcium intake (mg/d)[b]	752 ± 229	794 ± 169	855 ± 189	0.158
Physical activity expenditure energy (kJ/d)[c]	6376 ± 920	6607 ± 764	6292 ± 899	0.383
Changes of follow-up and baseline (n)	30	28	26	
Energy intake (kJ/d)[a]	−0.6 ± 98.4	29.9 ± 90.1	30.8 ± 125.7	0.437
Protein intake (g/d)[b]	0.43 ± 7.30	0.012 ± 6.54	−0.46 ± 7.53	0.897
Dietary soy isoflavone intake (mg/d)[b]	−22.3 ± 18.5	−15.9 ± 11.5	−28.6 ± 25.5	0.158[d]
Total calcium intake (mg/d)[b]	55.6 ± 102.1	75.8 ± 88.6	38.2 ± 81.1	0.324
Physical activity expenditure energy (kJ/d)[c]	99.1 ± 181.9	80.4 ± 165.3	101.1 ± 164.5	0.884

* From ANOVA, [a]Obtained from 3-d dietary intake records, [b]Assessed using food frequency questionnaire; total calcium included dietary calcium and supplemental, [c]Assessed using physical activity frequency questionnaire, [d]From Kruskal–Wallis Test

Results

Characteristics of total subjects and compliance

The baseline characteristics of age, body weight and BMI, years since menopause (YSM), serum FSH, dietary energy intake, dietary protein intake, dietary calcium and isoflavone intakes, physical activities, and bone mass were very similar among the treatment groups ($p > 0.05$). No significant differences in dietary intakes and physical activities during the intervention period or their changes between the follow-up and baseline values were observed among the three treatment groups ($p > 0.05$). Dietary Calcium intake increased moderately, but soy isoflavone intake decreased greatly during the intervention period as compared with the baseline values as a result of our advice (Table 1).

Among the 90 subjects at beginning, six subjects (two in low-dose group and four in high-dose group) withdrew, due to suspected breast cancer; confirmed diabetes mellitus, traffic accident, emigration, and a skin allergy (two cases), which the etiology of which could not be confirmed. Another six subjects (three in placebo, one in high-dose group and two in low-dose group) completed bone scanning, but refused to collect blood and urine samples at 12-week and 24-week post-treatment and were excluded from the relevant analyses.

The 84 subjects who completed bone scanning consumed more than 95% of the assigned capsules. Adherence and compliance of the treatment in our subjects were excellent. At the beginning of the trial, a few subjects in the isoflavone treatment groups reported thirst, but the symptom disappeared after about 6 weeks. There were no further side effects from the treatment during the intervention period.

Soy isoflavone treatment and bone loss

Mean BMD loss at the lumbar spine and femoral neck tended to be attenuated by supplementation with soy isoflavones after adjusting for age, years since menopause, bone weight and height, dietary intakes of isoflavones, calcium and protein, physical activities and baseline BMD at the relevant sites ($p = 0.114$ and 0.053, respectively). We observed significantly dose-dependent linear associations between the intervention of isoflavones and percent changes of BMD femur neck (p-for linear trend = 0.042 and 0.016, respectively) in the multivariate analyses. No significant differences in mean BMDs and percent changes of BMDs were observed among the treatment groups at the total hip, trochanter, and intertrochanter (Table 2).

Soy isoflavone treatment and bone turnover during the treatment period

Both one-way ANOVA and ANCOVA analyses showed that the Dpd was significantly lower in the high-dose group than in the other groups ($p < 0.05$) at 12 week. Percent changes in urinary Dpd between baseline and 12 weeks post-treatment was significantly decreased in the high-dose group as compared

Table 2 BMD (g/cm^2) at post-treatment and percent changes (%) by treatment groups[a]

BMDs	Placebo (n = 30)	Low-dose (n = 28)	High-dose (n = 26)	p-values			
				ANOVA	Linear trend	ANCOVA[b,c]	Linear trend
Spine, L1–L4							
Baseline	0.864 ± 0.103	0.839 ± 0.111	0.892 ± 0.151	0.272	0.346		
Post-treatment	0.851 ± 0.102	0.841 ± 0.108	0.904 ± 0.149	0.126	0.105	0.180	0.077
Percent changes	−1.424 ± 3.220	−0.052 ± 2.885	0.357 ± 3.233[d]	0.081	0.035	0.114	0.042
Total Hip							
Baseline	0.792 ± 0.065	0.796 ± 0.102	0.813 ± 0.118	0.628	0.338		
Post-treatment	0.794 ± 0.071	0.801 ± 0.100	0.827 ± 0.112	0.414	0.205	0.718	0.431
Percent changes	0.273 ± 3.528	0.195 ± 3.373	0.911 ± 4.220	0.743	0.523	0.554	0.293
Femoral Neck							
Baseline	0.690 ± 0.068	0.692 ± 0.089	0.725 ± 0.102	0.202	0.104		
Post-treatment	0.686 ± 0.076	0.692 ± 0.085	0.742 ± 0.091[e]	0.031	0.015	0.123	0.043
Percent changes	−0.590 ± 4.794	0.854 ± 4.341	1.57 ± 6.705[f]	0.306	0.135	0.053	0.016
Trochanter							
Baseline	0.579 ± 0.053	0.587 ± 0.077	0.600 ± 0.094	0.617	0.333		
Post-treatment	0.579 ± 0.056	0.587 ± 0.072	0.605 ± 0.089	0.384	0.191	0.486	0.426
Percent changes	0.095 ± 3.902	−0.154 ± 4.822	0.742 ± 3.746	0.585	0.580	0.428	0.368
Intertrochanter							
Baseline	0.936 ± 0.081	0.935 ± 0.128	0.959 ± 0.146	0.639	0.414		
Post-treatment	0.937 ± 0.089	0.931 ± 0.127	0.972 ± 0.143	0.334	0.292	0.547	0.777
Percent changes	0.216 ± 4.535	−0.224 ± 5.053	0.526 ± 4.539	0.642	0.813	0.538	0.679

[a]Values are means ± SD
[b]Analysis of ANCOVA was conducted for BMDs of post-treatment and percent changes of BMDs
[c]Covariates: baseline value, age, YSM, body weight, height, dietary intakes during the follow-up period included isoflavone intake, calcium intake, protein intake and physical activities during the follow-up period
[d]Compared with placebo group, p = 0.042 (Bonferroni, pairwise compairson of ANCOVA)
[e]Compared with placebo group, p = 0.044 (Bonferroni, pairwise compairson of ANCOVA)
[f]Compared with placebo group, p = 0.016 (Bonferroni, pairwise compairson of ANCOVA)

with those of the placebo and low-dose group even after adjusting for age, YSM, body weight and height, dietary intakes of isoflavones, calcium, and protein, energy expenditure in physical activities and baseline Dpd ($p < 0.05$). Such a difference disappeared at 24 weeks after treatment. There was no significant difference in serum OC and BAP among the treatment groups during the intervention period (Table 3).

Discussion

Animal and in vitro studies have consistently revealed a favorable effect of soy isoflavones or other phytoestrogens on the prevention of bone loss and decreases of bone turnover due to estrogen deficiency [21]. However, evidences from some human intervention studies were inconsistent [15–20]. In this 6-month randomized, placebo-controlled trial, we examined the effects of soy isoflavones on bone loss and bone turnover in early postmenopausal Chinese women between 1 and 5 years since menopause. Our findings showed that the high-dose supplementation of soy isoflavones had a significantly positive effect on retarding bone loss at the lumbar spine (L1–L4) and femoral neck, and bone resorption, even after the adjustment for age, body weight and height, years since menopause and baseline values of Dpd or baseline values of BMD at the same sites being modeled in this population.

Although similar trend was also observed at the total hip and other sub-sites of the hip, but we did not observe any statistical significance at these bone sites. Possible reasons might include the weak effect of soy isoflavones on bone loss and/or short treatment duration. Since lumbar spine and femoral neck primarily compose trabecular and cortical bone, respectively, the differential effects of isoflavone on bone loss at various bone sites were unlikely to be related to the proportion of trabecular or cortical bone as proposed in previous studies [17, 27]. In this study, BMI was not included in the multivariate models since BMD were more closely correlated with body weight than BMI, and there is a strong association between body weight and BMI in this population. Due to a negative association between baseline BMD and BMD change, we adjusted for the baseline values of BMD or bone markers to avoid the effect of regression to the mean.

Most of previous human interventional studies had examined the effects of soy isoflavones at doses between 40 and 90 mg isoflavones/d [15, 17]. In general, a daily supplementation of around 40 mg isoflavones per day showed no significantly favorable effect, where 80~99 mg isoflavones/d presented an inconsistent beneficial impact on the prevention of estro-

Table 3 Bone markers at 0, 12, and 24 weeks and percent changes by treatment groups[a]

				p-values			
Biomarkers	Placebo (n = 27)	Low-dose (n = 26)	High-dose (n = 25)	ANOVA	Linear trend	ANCOVA[b,c]	Linear trend
Serum osteocalcin (log_{10}-transformed, μg/l)							
Baseline	0.784 ± 0.186	0.760 ± 0.242	0.655 ± 0.233	0.077	0.039		
12 week	0.926 ± 0.195	0.789 ± 0.240	0.791 ± 0.207	0.034	0.022	0.286	0.212
% change[d]	24.2 ± 45.3	23.4 ± 66.2	44.0 ± 120.3	0.634	0.407	0.688	0.389
24 week	0.705 ± 0.257	0.726 ± 0.276	0.680 ± 0.239	0.810	0.775	0.503	0.455
% change[d]	−6.0 ± 40.7	15.5 ± 68.7	63.2 ± 195.4	0.123	0.044	0.699	0.412
Serum bone special alkaline phosphatase (log_{10}-transformed, IU/l)							
Baseline	1.515 ± 0.245	1.556 ± 0.212	1.444 ± 0.160	0.202	0.215		
12 week	1.702 ± 0.169	1.681 ± 0.154	1.588 ± 0.206	0.074	0.031	0.120	0.046
% change[d]	16.1 ± 18.9	13.3 ± 18.6	10.7 ± 16.0	0.586	0.304	0.056	0.021
24 week	1.597 ± 0.203	1.597 ± 0.174	1.535 ± 0.155	0.420	0.251	0.785	0.491
% change[d]	6.4 ± 13.9	4.5 ± 13.9	7.3 ± 12.4	0.771	0.826	0.682	0.384
Urinary total deoxypyridinoline(log_{10}-transformed, nmol/mmolCr)							
Baseline	0.792 ± 0.232	0.896 ± 0.26	0.913 ± 0.202	0.154	0.151		
12 week	0.795 ± 0.212	0.845 ± 0.220	0.695 ± 0.226[e]	0.038	0.076	0.043	0.013
% change[d]	7.3 ± 34.8	3.5 ± 49.4	−26.0 ± 18.5[e,f]	0.004	0.002	0.014	0.037
24 week	0.952 ± 0.239	0.823 ± 0.219	0.835 ± 0.182[g]	0.070	0.072	0.007	0.01
% change[d]	30.0 ± 51.7	1.6 ± 51.4	−3.8 ± 32.0	0.024	0.012	0.180	0.089

[a]Values are mean ± SD
[b]Analyses of ANCOVA was conducted for bone markers of post-treatment and percent changes of bone markers
[c]Covariates: baseline value, age, YSM, body weight, height, dietary intakes during the follow-up period included isoflavone intake, calcium intake, protein intake and physical activities during the follow-up period
[d]As compared with baseline values
[e]Compared with low-dose group, $p < 0.05$
[f]Compared with placebo group, $p < 0.01$
[g]Compared with Placebo group, $p < 0.05$

gen-related bone loss over a short treatment period in human clinical trials [10, 14, 20, 21].

Two reasons might account for such results observed in the low-dose treatment (~40 mg). One is because the short treatment durations had not persisted long enough to produce a detectable favorable difference in the percentage change of bone mass; another potential reason is due to the low doses of interventions, which might not be as effective as the high dose. The first reason might be demonstrated from observational studies [7, 8, 28–30]. Observational studies found that a habitual daily intake of 30~40 mg (top quartile) isoflavones was associated with a better peak bone mass in young female adults [28] and better bone mass in postmenopausal Chinese women [8, 30]. Previous animal experimental studies also suggested that a threshold dose of isoflavones needs to be consumed for a lengthy time period (i.e. months to years) before any detectable impact on BMD can be observed [29].

However, previous studies have not established if the inconsistent effects of isoflavone supplementation on bone mass are due to a real weak positive effect, low tested dosages, short treatment period, or a combination of these factors [14]. Based on the existing evidence, we might conclude that the dosage of ~40 mg isoflavones/d is unlikely to be an efficacious dosage for the prevention of bone loss. Since few studies have examined the effect of isoflavones at a dose over 100 mg/d [18], it is unclear whether a dosage over 90 mg/d would be more effective.

In this study, we examined the effect of isoflavone supplementation at two higher dosages (84, 126 mg/d) in postmenopausal Chinese women, and found that there was a significant linear dose-dependent beneficial effect on bone loss. The higher dose of isoflavones had a significant effect on retarding bone loss at the femoral neck. The higher dose was much more effective than the 84 mg/d dose. Our findings suggested that the optimal intervention dose might be larger than 80–90 mg isoflavones/d for the prevention of bone loss in this population. Bone remodeling is a relatively slow process, and the time required to complete a cycle may be increased with age. Normally 6–18 months are needed to reach a new equilibrium [31]. Due to the limitation of the short duration of this study, a long-term study would be required to confirm the long-term effect of isoflavones on bone health.

Consistent with the bone changes, we observed that the bone-specific biomarker of bone resorption, Dpd excretion, was significantly decreased in subjects with isoflavone supplementations at daily doses of 84 and 126 mg. Several human studies had also observed the anti-resorptive properties of soy protein or soy isoflavones [14, 32, 33]. Some [32, 34], but not all [33], human studies reported that bone formation markers (such as serum OC, insulin-like growth factor-I, and BAP) were enhanced concomitantly with decreased bone resorption. We did not find a significant difference in serum OC and BAP among the three treatment groups possibly due to the inconsistent effect on bone

formation and/or the short duration of the intervention. In our study, OC and BAP significantly increased in a parallel way at 12 weeks among all treatment groups. The samples collected at 0, 12, and 24 weeks were examined separately. We observed relatively higher values (5–9%) of OC and BAP of the control samples at 12 weeks than those at 0 and 24 weeks, although their values were within the reference range given by the suppliers. Between-run error in the measurements of OC and BAP might be a possible reason of such unexpected increases in OC and BAP.

Barnes [35] has concluded in his review that isoflavones consumed orally and in doses below 2 mg/kg body weight/d should be considered safe for most population groups. Our study also showed that this population had a good tolerance on the supplementation of soy isoflavones. We found that a daily oral dose of 126 mg soy isoflavones did not have any adverse effect except that a few subjects reported thirst at the beginning of the treatment.

In this study, natural menopause was defined as at least 12 months since the last menstrual cycle and serum follicle-stimulating hormone (FSH) > 30 IU/l. We thus excluded subjects with serum follicle stimulate hormone ≤30 IU/l. Since heavy subjects might be insensitive to isoflavone treatment than light subjects [36], only those with BMI < 30 kg/m^2 were included in the study. Therefore, it would be inappropriate to generalize the results to obese postmenopausal women. We also eliminated women with the Kuppermann climacteric scale ≤ 15 in order that we could examine the effect of isoflavone supplementation on menopausal symptoms (reported elsewhere). Previous study showed that subjects with more menopausal symptoms typically had lower estrogen level than those with fewer symptoms [37]. So far, no study has examined the interaction of serum estrogen and isoflavones or soy protein to bone loss. A meta-analysis by Zhan and Ho [38] found that isoflavone supplementation was more effective on serum lipid profile in postmenopausal women than in pre- or perimenopausal women. Since isoflavones might play a role in bone mass via a similar mechanism to that in lipid profile, it was possible that the isoflavone effect on bone mass might be more pronounced in women with a lower than a higher estrogen level. Thus, caution needs to be exercised in applying the results obtained from women with more menopausal symptoms to general postmenopausal women.

We recognize that the relatively small sample size of this study might limit the generalization of the results. In addition, the short duration of treatment might have prevented us from observing positive changes in the subjects on mid-dose supplementation, as a relatively low dose of isoflavones might require a longer time to produce a detectable difference in BMD compared to the higher dose. Due to limited human studies available at present, our finding adds to the existing evidence that soy isoflavones may have a bone sparing effect in postmenopausal women.

Although the single blinding technique might introduce some observational biases, however, the main outcomes of BMD and bone markers were objective parameters, and BMD measurements were done by the same scanner operator who blinded to the subject's grouping status. Moreover, there were no significant changes in dietary intakes and physical activities among the treatment groups. It was unlikely to introduce severe information bias in this study. Since many subjects enquired us how to improve bone mass, a standardized general guideline on diet and physical activities were given to each subjects. Also, to minimize the effects of dietary isoflavones, subjects were instructed to avoid large consumption of soy foods. As expected, we observed increases in dietary calcium and physical activities, and a decrease in soy foods. However, such changes in dietary habits and physical activities were not significantly different among the three groups, and thus be unlikely to contribute the group differences in the changes of BMD or bone marker among the treatment groups.

In summary, our findings suggested that soy isoflavones had a significantly positive dose-dependent effect on attenuating bone loss at the spine and femur neck possibly via the inhibition of bone resorption in postmenopausal Chinese women with more menopausal symptoms; the high-dose of 126 mg isoflavones/d is an effective dose for the prevention of postmenopausal bone loss over a short duration. Further long-term large studies are required to confirm the efficacious dose for bone mass maintenance.

Acknowledgments This study was jointly supported by Guangzhou Sciences and Technology Bureau (2002J1-C0081), and Acatris Holding B.V. The soy isoflavone extract was donated by Acatris Holding B.V., Giessen, The Netherlands.

References

1. Wasnich RD (1997) Epidemiology of osteoporosis in the United States of America. Osteoporos Int Suppl 3:S68–S72
2. Ho SC, Lau EM, Woo J, Sham A, Chan KM, Lee S, Leung PC (1999) The prevalence of osteoporosis in the Hong Kong Chinese female population. Maturitas 32:171–178
3. Hodgson SF, Watts NB, Bilezikian JP, Clarke BL, Gray TK, Harris DW, Johnston CC, Kleerekoper M, Lindsay R, Luckey MM, McClung MR, Nankin HR, Petak SM, Recker RR (2001)

American association of clinical endocrinologists 2001 medical guidelines for clinical practice for the prevention and management of postmenopausal osteoporosis. Endocr Pract 7:293–312
4. Xu H, Wu Y, Yan Y (1998) Preliminary study on bone loss rate in early and late stages of postmenopausal women. Chinese J Obstet Gynecol 33:542–545
5. Garg P, Kerlikowske K, Subak L, Grady D (1998) Hormone replacement therapy and the risk of epithelial ovarian carcinoma: a meta-analysis. Obstet Gynecol 92:472–479
6. Murkies AL, Wilcox G, Davis SR (1998) Clinical review 92: Phytoestrogens. J Clin Endocrinol Metab 83:297–303
7. Greendale GA, FitzGerald G, Huang MH, Sternfeld B, Gold E, Seeman T, Sherman S, Sowers M (2002) Dietary soy isoflavones and bone mineral density: results from the study of women's health across the nation. Am J Epidemiol 155:746–754
8. Mei J, Yeung SS, Kung AW (2001) High dietary phytoestrogen intake is associated with higher bone mineral density in postmenopausal but not premenopausal women. J Clin Endocrinol Metab 86:5217–5221
9. Blum SC, Heaton SN, Bowman BM, Hegsted M, Miller SC (2003) Dietary soy protein maintains some indices of bone mineral density and bone formation in aged ovariectomized rats. J Nutr 133:1244–1249
10. Anderson JJ, Ambrose WW, Garner SC, Ohue T (2003) The effects of phytoestrogens on bone. Nutr Res 17:1617–1631
11. Shi LN, Su YX (2000) The effect of soybean isoflavones on bone loss in ovariectomized rat. Acta Nutrimenta Sinica 22:113–118
12. Anderson JJ, Anthony M, Messina MJ, Garner SC (1999) Effects of phyto-oestrogens on tissues. Nutr Res Rev 12:75–116
13. Anderson JJ, Ambrose WW, Garner SC (1998) Biphasic effects of genistein on bone tissue in the ovariectomized, lactating rat model. Proc Soc Exp Biol Med 217:345–350
14. Arjmandi BH, Smith BJ (2002) Soy isoflavones' osteoprotective role in postmenopausal women: mechanism of action. J Nutr Biochem 13:130–137
15. Alekel DL, Germain AS, Peterson CT, Hanson KB, Stewart JW, Toda T (2000) Isoflavone-rich soy protein isolate attenuates bone loss in the lumbar spine of perimenopausal women. Am J Clin Nutr 72:844–852
16. Anderson JJ, Chen X, Boass A, Symons M, Kohlmeier M, Renner JB, Garner SC (2002) Soy isoflavones: no effects on bone mineral content and bone mineral density in healthy, menstruating young adult women after one year. J Am Coll Nutr 21:388–393
17. Chen YM, Ho SC, Lam SSH, Ho SSS, Woo JLF (2003) Soy isoflavones have a favorable effect on bone loss in Chinese postmenopausal women with lower bone mass: a double-blind, randomized, controlled trial. J Clin Endocrinol Metab 88:4740–4747
18. Hsu CS, Shen WW, Hsueh YM, Yeh SL (2001) Soy isoflavone supplementation in postmenopausal women. Effects on plasma lipids, antioxidant enzyme activities and bone density. J Reprod Med 46:221–226
19. Potter SM, Baum JA, Teng H, Stillman RJ, Shay NF, Erdman JW (1998) Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. Am J Clin Nutr 68:1375S–1379S
20. Kreijkamp-Kaspers S, Kok L, Grobbee DE, Haan EHF, Aleman A, Lampe JW, Schouw YT (2004) Effect of soy protein containing isoflavones on cognitive function, bone mineral density, and plasma lipids in postmenopausal women: a randomized controlled trial. JAMA 292:65–74
21. Setchell KD, Lydeking-Olsen E (2003) Dietary phytoestrogens and their effect on bone: evidence from in vitro and in vivo, human observational, and dietary intervention studies. Am J Clin Nutr 78:593S–609S
22. Wang HJ, Murphy PA (1994) Isoflavone content in commercial soybean foods. J Agric Food Chem 42:1666–1673
23. Huang MH, Harrison GC, Mohamed MM, Gombein JA, Henning SM, Go VL, Greendale GA (2000) Assessing the accuracy of a food frequency questionnaire for estimating usual intake of phytoestrogens. Nutr Cancer 37:145–154
24. Institute of Nutrition, Food Safety, China CDC (2002) China food composition 2002, 1st edn. Peking University Medical Press, Beijing, pp 24–219
25. Liu B, Woo J, Tang N, Ng K, Ip R, Yu A (2001) Assessment of total energy expenditure in a Chinese population by a physical activity questionnaire: examination of validity. Int J Food Sci Nutr 52:269–282
26. Behr W, Barnert J (1986) Quantification of bone alkaline phosphatase in serum by precipitation with wheat-germ lectin: a simplified method and its clinical plausibility. Clin Chem 32:1960–1966
27. Atkinson C, Compston JE, Day NE, Dowsett M, Bingham SA (2004) The effects of phytoestrogen isoflavones on bone density in women: a double-blind, randomized, placebo-controlled trial. Am J Clin Nutr 79(2):326–333
28. Ho SC, Chan SG, Yi Q, Wong E, Leung PC (2001) Soy intake and the maintenance of peak bone mass in Hong Kong Chinese women. J Bone Miner Res 16:1363–1369
29. Anderson JJ, Garner SC (1998) Phytoestrogens and bone. Baillieres Clin Endocrinol Metab 12:543–557
30. Ho SC, Woo J, Lam SSH, Chen YM, Sham A, Lau J (2003) Soy protein consumption and bone mass in early postmenopausal Chinese women. Osteoporos Int 14:835–842
31. Springfield IL, Charles C, Thomas H (1973) Remodeling as a determinant of envelope physiology. In: Frost HM (ed) Bone remodeling and its relationship to metabolic bone disease. Orthopedic Lectures 28–53
32. Arjmandi BH, Khalil DA, Smith BJ, Lucas EA, Juma S, Payton ME, Wild RA (2003) Soy protein has a greater effect on bone in postmenopausal women not on hormone replacement therapy, as evidenced by reducing bone resorption and urinary calcium excretion. J Clin Endocrinol Metab 88:1048–1054
33. Horiuchi T, Onouchi T, Takahashi M, Ito H, Orimo H (2000) Effect of soy protein on bone metabolism in postmenopausal Japanese women. Osteoporos Int 11:721–724
34. Wangen KE, Duncan AM, Merz-Demlow BE, Xu X, Marcus R, Phipps WR, Kurzer M S (2000) Effects of soy isoflavones on markers of bone turnover in premenopausal and postmenopausal women. J Clin Endocrinol Metab 85:3043–3048
35. Barnes S (2003) Phyto-oestrogens and osteoporosis: what is a safe dose? Br J Nutr 89:S101–S108
36. Chen YM, Ho SC, Lam SS, Ho SS, Woo JL (2004) Beneficial effect of soy isoflavones on bone mineral content was modified by years since menopause, body weight, and calcium intake: a double-blind, randomized, controlled trial. Menopause 11:246–254
37. Zhao G, Wang L, Yan R, Dennerstein L (2000) Menopausal symptoms: experience of Chinese women. Climacteric 3:135–44
38. Zhan S, Ho SC (2005) Meta-analysis of the effects of soy protein containing isoflavones on the lipid profile. Am J Clin Nutr 81:397–408