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Soy Isoflavones Have a Favorable Effect on Bone Loss in Chinese Postmenopausal Women with Lower Bone Mass: A Double-Blind, Randomized, Controlled Trial

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Animal studies have shown that soy isoflavones have an effect in preventing estrogen-related bone loss, but few data are available in humans, especially in the Asian populations. This double-blind, placebo-controlled, randomized trial examines the effects of soy isoflavones on bone loss in postmenopausal Chinese women, aged 48–62 yr. Two hundred and three eligible subjects were randomly assigned to three treatment groups with daily doses of placebo (1 g starch; n = 67), mid-dose (0.5 g starch, 0.5 g soy extracts, and ~40 mg isoflavones; n = 68), and high dose (1.0 g soy extracts and ~80 mg isoflavones; n = 68). All were given 12.5 mmol (500 mg) calcium and 125 IU vitamin D_3. Bone mineral density (BMD) and bone mineral content (BMC) of the whole body, spine, and hip were measured using dual energy x-ray absorptiometry at baseline and 1 yr post treatment. Both univariate and multivariate analyses showed that women in the high dose group had mild, but statistically significantly, higher favorable change rate in BMC at the total hip and trochanter ($P < 0.05$) compared with the placebo and mid-dose groups, even after further adjustments for the potential confounding factors. Further stratified analyses revealed that the positive effects of soy isoflavone supplementation were observed only among women with lower initial baseline BMC (median or less). In conclusion, soy isoflavones have a mild, but significant, independent effect on the maintenance of hip BMC in postmenopausal women with low initial bone mass. (*J Clin Endocrinol Metab* 88: 4740–4747, 2003)

EPIDEMIOLOGICAL DATA indicate that Asian people have lower rates of osteoporotic fractures, cardiovascular diseases, postmenopausal symptoms, and certain cancers than Western populations (1). These health advantages are notably reduced when Asians adopt the Western lifestyle and eating habits (1). These observations have led researchers to look at the Asian diet for possible answers. Soy is part of the traditional diet in the Asian populations. An increasing number of studies are investigating the relation between soy intake and the above-mentioned diseases (1–3).

Several animal studies have provided convincing data on the significant improvement of bone mass or other end points after soy protein or isolated isoflavone-enriched soy extract supplementation (4–7). Increasing observational epidemiological studies have also examined the linkage between dietary intake of phytoestrogens and bone mass in humans and found soy protein or soy phytoestrogen intake beneficial in maintaining or modestly improving bone mass in postmenopausal women (8–10). Only a few randomized trials of relatively short durations and small sample sizes have been conducted in the Caucasian populations (5, 11–16). Some studies have revealed that isoflavone-rich soy protein had a modest effect in retarding bone loss in perimenopausal (11) and postmenopausal (13, 14) women, but other studies have not observed such effects (15, 16). Studies have also reported inconsistent effects of phytoestrogens (or soy protein) on bone markers in postmenopausal women (5). Arjmandi *et al.* (17) found enhancing effect of soy isoflavones on IGF-I synthesis, and the IGF-I concentration has been reported to be positively related to bone mass in women.

Therefore, the hypothesis of a beneficial effect of soy on bone mass is still speculative, and little is known about the effects of soy isoflavones in Asian populations where soy intake is part of the habitual diet. The optimal dosage and the components responsible for the favorable effects of soy or isoflavone-rich soy protein isolates on bone are still unclear. This study aims to investigate the effects of two different dosages of concentrated soy-derived isoflavones on bone mass in early postmenopausal women. It is hypothesized that isoflavone supplementation would be beneficial in the prevention of bone loss in early postmenopausal women.

Subjects and Methods

Subject recruitment

Subjects who met the following criteria were enrolled in the study. They were required to be Hong Kong residents of Chinese origin, aged 48–62 yr, and postmenopausal within 10 yr of natural menopause, which is defined as at least 12 months since the last menstrual cycle. Subjects with any detectable diseases or on medications, including current use or a history of 3-month (or more) use of exogenous estrogens, corticosteroids, thiazine, as well as any other medications known to affect bone mass were excluded.

Subjects were recruited from the community through advertisements, written invitations sent to selected housing estates in Shatin, Hong Kong, as well as health talks and subject referral. Potential subjects were screened for eligibility and were then invited to participate in the study. Written informed consent was obtained from all participants before enrollment. The ethical committee of the Chinese University of Hong

Abbreviations: BMC, Bone mineral content; BMD, bone mineral density; YSM, years since menopause.

Kong approved the study. A total of 232 volunteers who met the screening criteria were initially recruited.

Study design and treatment protocol

This double-blind, placebo-controlled, 1-yr randomized trial examined the effects of two doses of concentrated soy germ extracts of isoflavones (40 and 80 mg daily) and placebo (corn starch) on the maintenance of bone mass in Chinese early postmenopausal women. The subjects were randomly assigned to one of the three treatment groups with daily intakes of placebo [0 mg soy isoflavones, 12.5 mmol (500 mg) calcium, and 125 IU vitamin D], mid-dose (40 mg soy isoflavones, 12.5 mmol calcium, and 125 IU vitamin D), and high dose (80 mg soy isoflavones, 12.5 mmol calcium, and 125 IU vitamin D). Soy-derived isoflavones (8%, wt/wt) were supplied by Acatris Holding B.V. (Giessen, The Netherlands). The main isoflavone components of this product are daidzein (46.4%), glycetein (38.8%), and genistein (14.7%) when expressed in aglycone form. A coinvestigator was responsible for the quality control of capsule filling and tagging bottles containing different forms of capsules with different color tags (white, yellow, and orange). All capsules looked identical and were filled in one batch with half-daily doses of placebo material or soy isoflavones [0.5 g starch (placebo), 0.25 g starch and 0.25 g 8% soy isoflavone extracts (mid-dose), and 0.5 g 8% soy isoflavone extracts (high dose)]. Calcium carbonate (1.25 g; 12.5 mmol elemental calcium) plus 125 IU vitamin D were obtained from a commercial calcium tablet. The two supplements (isoflavones/placebo capsules and calcium tablets) were delivered to subjects at intervals of 2, 4, 4, and 2 months. The subjects were requested to record the residual supplements at the end of each of the delivery. Careful instruction was given to participants on the trial supplementation intake and to discontinue the use of other dietary supplements known to affect bone metabolism during the trial period. Continuous monitoring was conducted throughout the study.

To increase the compliance and to minimize the loss to follow-up, a 2-wk run-in period with the use of placebo supplements (two starch capsules and 12.5 mmol calcium/tablet) was carried out to familiarize the subjects with the trial requirements. Twenty-nine subjects dropped out or were excluded for reasons such as the inability to tolerate treatments, poor compliance (including poor adherence to take supplements and nonparticipation in subsequent interviews and measurements), side effects (such as dizziness, constipation, and other gastrointestinal symptoms), or loss of interest during the run-in period.

Randomization procedures

The remaining 203 subjects, who had good adherence to the study protocol and no detectable side effects, were formally recruited into the study at the end of the run-in period. They were randomly assigned to one of the three treatment groups according to a standard randomization procedure. Subjects were labeled continuously according to the sequence of their visits after the completion of the run-in period. Three groups were selected randomly from the 203 subjects using the procedure of random case of SPSS (18). The placebo, mid-dose, and high dose group each had 67, 68, and 68 subjects, respectively. Both the study subjects and investigators responsible for the day to day operation and data analyses were blinded to the group status.

Data collection

Questionnaire interview. Individual information was collected by trained interviewers with face to face interviews based on a structured and previously validated questionnaire on socio-demographic data; years since menopause (YSM); physical activities, including hours spent in sleep (Sleep), standing (Stand), walking (Walk), weight bearing (>2.5 kg; load), moderate (mild PA), and vigorous activities (vigorous PA); smoking and alcohol drinking; and other factors that may have possible confounding effects on the relation between dietary isoflavone consumption and bone mass. The dietary assessment of intakes of calcium, phosphorus, soy protein, and total protein was based on a quantitative food frequency questionnaire that included 60 food groups/items. The validity of the dietary questionnaire was demonstrated in our previous study (19). 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. Foods with intake frequency less than once per month were ignored. Food pictures and food models in the reference portion sizes were provided as visual aids. Soy protein intake was estimated based on 11 main soy food items/groups: soft tofu, firm tofu, tofu pudding, deep-fried tofu, soy vegetarian items, tofu-dried stick/sheet/skin, soy milk, soybean sprouts, soy bean, soy flour/soy protein isolate, and fermented soy products. Nutrients were calculated from food composition tables (20, 21).

Anthropometric and bone mass measurements. Height was measured to the nearest 0.5 cm, and weight to the nearest 0.1 kg in light clothing and without shoes. Body mass index was calculated as weight (kilograms)/height (meters) squared. The bone mineral content (BMC) and density (BMD) of the whole body, the lumber spine (L1–L4), as well as the left hip were measured by dual energy x-ray absorptiometry (QDR-4500, Hologic, Waltham, MA) at the initiation of the run-in period and 12 months post treatment. The same staff conducted the scans and analysis. The short-term within-subject *in vivo* precision error in our laboratory for BMD was 0.6% for the spine and 1.53 for the total hip. Long-term precision was 0.39% by daily testing the spine phantom over the previous 2.5 yr.

Statistical analysis

Baseline characteristics, dietary intake, and physical activities during the 12-month intervention period among the three treatment groups were compared by means of one-way ANOVA. Analyses of intervention effects on bone changes were conducted by including all subjects with intention to treat (good and bad compliance) and separately for subjects with good compliance (valid completer). Valid completers (or compliant subjects) were defined as those who consumed more than 480 isoflavone capsules and 240 calcium tablets (for full 8 months of use) during the intervention period and completed all questionnaire interviews and tests. Comparisons of rates of bone change over the 12-month intervention period among the three treatment groups were made using one-way ANOVA and analysis of covariance (univariate ANOVA, general linear model) adjusting for important factors known to affect bone loss. As only one subject was a smoker, and very few subjects (5.7%) drank alcohol (3.3 times/wk), these two factors were not included in the multivariate models. As a low initial bone mass might be a marker for a number of associated risk factors, stratified analyses by baseline bone mass (below and above the median) were conducted to investigate the role of soy isoflavone supplementation on bone changes. Stepwise multiple regression analysis was also used to examine the independent effects of soy isoflavones supplementation (method = enter) as well as the other main determinants (methods = stepwise, F to enter = 0.05, F to remove = 0.1) on the percent changes in BMD and BMC. SPSS for Windows (release 11, SPSS Inc., Chicago, IL) was used for the analysis.

Results

Baseline characteristics of study subjects and compliance

Subjects in the three intervention arms were similar in age, body weight, height, YSM, dietary intakes, physical activities, and bone mass ($P > 0.05$; Table 1). However, after excluding subjects lost to follow-up, the subjects in the placebo group had relatively higher mean intertrochanteric BMD and BMC than subjects in the high-dose group ($P < 0.05$; data not shown). The numbers of subjects lost to follow-up were eight, six, and 12, respectively, among the placebo, mid-dose, and high-dose groups. Reasons for lost to follow-up included loss of interest, loss of contact, gastrointestinal discomfort, menses-like bleeding, and suspected tumor in the breast or elsewhere. The remaining 175 subjects completed all tests at baseline and post treatment. Among them, 15 subjects had consumed less than 480 isoflavones/placebo capsules, 15 subjects had an intake ranging from 480–599 capsules, and 145 subjects consumed more than 600 supplementation capsules (mean, 686 capsules). A total of

TABLE 1. Characteristics of subjects at baseline by intervention groups

Items	Placebo		40 mg isoflavones/d		80 mg isoflavones/d	
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD
Age (yr)	67	54.1 ± 3.4	68	54.1 ± 2.8	68	54.4 ± 3.1
YSM (yr)	67	4.1 ± 2.4	68	3.9 ± 2.2	67	4.4 ± 2.5
Height (m)	66	1.54 ± 0.05	68	1.55 ± 0.06	68	1.55 ± 0.05
Weight (kg)	66	57.0 ± 9.3	68	57.3 ± 9.2	68	57.9 ± 8.2
BMI (kg/m^2)	66	24.1 ± 3.7	68	23.7 ± 3.3	68	24.2 ± 3.4
BMD measurement (g/cm^2)						
Whole body	67	0.968 ± 0.102	68	0.975 ± 0.087	68	0.973 ± 0.099
Spine, L1–L4	67	0.846 ± 0.125	68	0.874 ± 0.124	68	0.860 ± 0.150
Total hip	67	0.823 ± 0.118	68	0.824 ± 0.129	68	0.809 ± 0.113
Neck femur	67	0.679 ± 0.098	68	0.688 ± 0.111	68	0.680 ± 0.095
Trochanter	67	0.601 ± 0.096	68	0.613 ± 0.110	68	0.602 ± 0.101
Intertrochanteric	67	1.000 ± 0.141	68	0.992 ± 0.147	68	0.968 ± 0.134
BMC measurement (g)						
Whole body	67	1667 ± 287	68	1711 ± 258	68	1695 ± 279
Spine, L1–L4	67	45.39 ± 9.13	68	47.85 ± 10.10	68	46.84 ± 10.67
Total hip	67	24.68 ± 5.08	68	25.04 ± 4.78	68	24.33 ± 4.36
Neck femur	67	3.22 ± 0.58	68	3.29 ± 0.54	68	3.23 ± 0.48
Trochanter	67	5.67 ± 1.25	68	5.86 ± 1.18	68	5.53 ± 1.14
Intertrochanteric	67	15.79 ± 3.59	68	15.89 ± 3.35	68	15.57 ± 3.02

No significant difference was observed among the three groups (one-way ANOVA). BMI, Body mass index.

TABLE 2. Dietary intakes and physical activity over the 12-month intervention period by groups of intervention (intention to treat)

Dietary intakes/physical activities	Placebo		40 mg isoflavones/d		80 mg isoflavones/d	
	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n
Dietary intake						
Calorie (MJ/d)	5.44 ± 1.87	58	5.38 ± 1.43	62	5.49 ± 1.59	54
Protein (g/d)	74.6 ± 29.3	58	71.3 ± 24.7	62	68.2 ± 22.4	54
Protein from soy (g/d)	6.73 ± 6.66	58	5.89 ± 5.41	62	6.25 ± 6.11	54
Calcium from food (mmol/d)	17.85 ± 8.48	58	18.35 ± 6.63	62	16.63 ± 6.83	54
Calcium from supplementation (mmol/d)	10.80 ± 2.80	58	11.55 ± 2.58	62	11.55 ± 2.55	54
Total calcium intake (mmol/d)	28.65 ± 9.60	58	29.90 ± 7.13	62	28.18 ± 7.23	54
Phosphorus (mmol/d)	31.00 ± 12.06	58	30.52 ± 9.68	62	28.97 ± 9.35	54
Physical activity						
Sleep (h/d)	6.92 ± 1.29	58	7.03 ± 1.09	62	6.79 ± 1.23	54
Standing (h/d)	3.94 ± 1.86	58	3.98 ± 2.06	62	3.66 ± 1.94	54
Walking (h/d)	3.27 ± 1.75	58	3.52 ± 2.13	62	3.04 ± 1.36	54
Load (h/d)	0.60 ± 0.85	58	0.62 ± 0.54	62	0.62 ± 0.89	54
Mild PA (h/d)	5.92 ± 2.76	58	6.66 ± 3.27	62	5.76 ± 2.89	54
Vigorous PA (h/wk)	1.63 ± 5.96	58	2.20 ± 6.34	62	1.28 ± 3.75	54

Protein from soy: based on 11 items/groups of soy and soy products most frequently consumed by local Chinese. 1MJ = 4.18 kcal; 1 mmol Ca = 40 mg Ca; 1 mmol phosphorus = 31 mg phosphorus. No significant difference was observed among the three interventions [least significant difference test (LSD in this and following tables), one-way ANOVA]. PA, Physical activity.

160 subjects who completed all tests and consumed more than 480 isoflavones/placebo capsules and 240 calcium tablets were considered valid completers. The noncompliant subjects or those lost to follow-up had significantly higher BMD at all measured sites, including whole body ($P = 0.025$), lumbar spine ($P = 0.009$), and left hip ($P = 0.033$), than the compliant subjects, but the two groups were similar in age, body weight, height, YSM, dietary intakes, and physical activities at baseline. No statistically significant differences in dietary intakes and physical activities during the intervention period were observed among the three intervention groups ($P > 0.05$; Table 2).

Isoflavone supplementation and bone loss rate

The yearly rates of change in bone mass by the three intervention groups over the intervention period are shown in Table 3 (intention to treat) and Table 4 (valid completers). We did not observe any statistically significant difference in BMD at any of the studied sites among the three treatment groups. However, both univariate (ANOVA) and multivariate (analysis of covariance) analyses showed significantly higher positive rates of change in BMC at the total hip and trochanter in subjects in the high dose group compared with those in the placebo and mid dose groups ($P < 0.05$). A similar pattern was observed among the valid completers. We also observed similar changes in bone area at the total hip and trochanter (data not shown).

Isoflavone supplementation and BMC loss rate by baseline BMC

We also conducted stratified analysis to investigate whether soy isoflavones have similar effects on bone changes in women with the higher and lower levels of baseline BMC

TABLE 3. Yearly rate of change (percentage) in bone mass over the intervention period by intervention groups (intention to treat)

BMD and BMC	Placebo		40 mg isoflavones/d		80 mg isoflavones/d		*P* value[a]
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	
BMD measurement (g/cm^2)							
Whole body	58	−0.55 ± 1.74	62	−0.70 ± 1.70	55	−0.46 ± 1.58	0.781
Spine, L1–L4	58	−0.79 ± 2.56	62	−0.62 ± 2.64	55	−0.99 ± 2.01	0.665
Total hip	58	−0.63 ± 1.83	62	−0.44 ± 1.85	55	−0.41 ± 1.71	0.517
Neck femur	58	−0.12 ± 2.77	62	−0.50 ± 2.10	55	−0.22 ± 2.63	0.817
Trochanter	58	−0.34 ± 2.94	62	−0.51 ± 2.71	55	−0.12 ± 2.89	0.687
Intertrochanteric	58	−0.69 ± 2.26	62	−0.76 ± 2.34	55	−0.40 ± 2.10	0.496
BMC measurement (g)							
Whole body	58	−1.27 ± 2.14	62	−1.42 ± 1.76	55	−1.07 ± 1.65	0.590
Spine, L1–L4	58	−1.28 ± 4.02	62	−1.54 ± 3.28	55	−1.77 ± 3.08	0.454
Total hip	58	−0.48 ± 2.21	62	−0.48 ± 2.35	55	0.44 ± 2.46[b,c,d,e]	0.117
Neck femur	58	0.45 ± 3.84	62	−0.03 ± 4.06	55	0.16 ± 3.93	0.696
Trochanter	58	−0.99 ± 5.27	62	−1.80 ± 4.59	55	1.41 ± 5.88[b,d,f,g]	0.084
Intertrochanteric	58	−0.59 ± 2.82	62	0.01 ± 3.07	55	0.27 ± 3.04	0.432

[a] For χ^2 test for trend. Sequence code of the groups: placebo 1, mid-dose 2; and high-dose 3.

The *P* values for [b,c,g] are from LSD, one-way ANOVA. [b] $P < 0.05$ compared with the placebo group; [c] $P < 0.05$, $P < 0.01$ compared with the mid-dose group; [g] $P < 0.01$ compared with the mid-dose group.

The *P* values for [d,e,f] are from LSD; analysis of covariance controlling for YSM, baseline body weight and height, and BMC at the relevant sites; physical activity; total calcium intake; and dietary protein over intervention period. [d] $P < 0.05$ compared with the placebo group; [e] $P < 0.05$ compared with mid-dose group; [f] $P < 0.01$ compared with mid-dose group.

TABLE 4. Yearly rate change (percentage) in bone mass over the intervention period by intervention groups (valid completers)

BMD and BMC	Placebo		40 mg isoflavones/d		80 mg isoflavones/d		*P* value[a]
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	
BMD measurement (g/cm^2)							
Whole body	53	−0.58 ± 1.73	57	−0.76 ± 1.68	50	−0.39 ± 1.56	0.571
Spine, L1–L4	53	−0.63 ± 2.56	57	−0.47 ± 2.60	50	−0.89 ± 1.88	0.581
Total hip	53	−0.51 ± 1.82	57	−0.48 ± 1.81	50	−0.21 ± 1.62	0.398
Neck femur	53	0.04 ± 2.86	57	−0.47 ± 2.14	50	0.14 ± 2.19	0.839
Trochanter	53	−0.14 ± 2.92	57	−0.49 ± 2.73	50	0.03 ± 2.93	0.777
Intertrochanteric	53	−0.55 ± 2.25	57	−0.83 ± 2.28	50	−0.30 ± 2.04	0.577
BMC measurement (g)							
Whole body	53	−1.25 ± 2.08	57	−1.46 ± 1.73	50	−1.08 ± 1.57	0.645
Spine, L1–L4	53	−1.17 ± 4.16	57	−1.41 ± 3.32	50	−1.50 ± 2.27	0.612
Total hip	53	−0.17 ± 2.27	57	−0.45 ± 2.38	50	0.57 ± 2.44[b,c,d,e]	0.117
Neck femur	53	0.47 ± 3.99	57	0.07 ± 4.17	50	0.50 ± 3.83	0.978
Trochanter	53	−0.45 ± 6.03	57	−1.45 ± 6.19	50	1.08 ± 6.92[f,g]	0.234
Intertrochanteric	53	−0.16 ± 3.35	57	−0.15 ± 3.29	50	0.48 ± 2.87	0.316

P value for χ^2 test for trend. Sequence code of the groups: placebo 1, mid-dose 2, and high-dose 3.

The *P* values for [b,c,f] are from LSD, one-way ANOVA.

[b] $P = 0.070$ compared with the placebo group; [c] $P < 0.05$ compared with the mid-dose group; [f] $P < 0.01$ compared with the mid-dose group.

The *P* values for [d,e,g] are from LSD, analysis of covariance controlling for YSM, baseline body weight and height, and BMC at the relevant sites; physical activity; total calcium intake; and dietary protein over intervention period. [d] $P = 0.074$ compared with the placebo group; [e] $P = 0.035$ compared with the mid-dose group; [g] $P = 0.008$ compared with the mid-dose group.

values (Table 5). We observed that among women with lower initial BMC values, subjects in the high dose group had higher rates of change in BMC at the total hip and trochanter ($P < 0.01$) than the other two treatment groups. However, such a beneficial effect was not found among subjects with higher initial BMC values.

Main determinants of percentage BMC loss

Multiple regression analyses confirmed the independent favorable effects of soy isoflavones on the rates of change in BMC at the total hip ($P = 0.006$), trochanter ($P = 0.052$), and intertrochanter ($P = 0.017$) after adjusting for the covariates (Table 6). An increase of 10 mg isoflavones (supplementation)/d was associated with yearly increases of 0.18, 0.30, and 0.20% of BMC, respectively, at the three sites. The other main independent determinants of rate of change in BMC were YSM and body weight (Table 6). Dietary protein and physical activities also had independent beneficial effects on BMC at the total hip and/or some subregions.

Potential adverse or side effects

The most commonly self-reported adverse effects with the calcium and isoflavone/placebo supplementations included abdominal distention (14 cases), constipation (six cases), breast disorders (six cases), menses-like bleeding (three cases), and miscellaneous (including headache or swirl, diarrhea, bone or/and joint pain, hand itch, stomach tumor, *etc.*; 12 cases). We did not observe a statistically higher incidence of either one (or all) of the above adverse effects in the treatment groups compared with the placebo arm ($P > 0.05$).

TABLE 5. Baseline BMC stratified yearly rate of change (percentage) in BMC over the intervention period by intervention groups (intention to treat)

Rate of change (%) in BMC by baseline BMC	Placebo		40 mg isoflavones/d		80 mg isoflavones/d		*P* value[a]
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	
In women with baseline BMC <median[b]							
Whole body	26	−1.11 ± 2.49	29	−1.39 ± 1.64	30	−1.07 ± 2.00	0.979
Spine, L1–L4	26	−2.47 ± 4.34	28	−1.08 ± 3.43	31	−1.41 ± 2.72	0.158
Total hip	22	0.21 ± 2.48	28	−0.77 ± 2.51	33	0.97 ± 2.38[c]	0.064
Neck femur	26	−0.11 ± 3.96	29	0.15 ± 4.61	31	−0.11 ± 4.38	0.795
Trochanter	28	−0.79 ± 5.67	23	−2.99 ± 4.70	29	3.73 ± 6.53[d,e]	0.000
Intertrochanteric	22	−0.40 ± 3.38	29	−0.35 ± 3.27	29	0.83 ± 2.70	0.315
In women with baseline BMC ≥median							
Whole body	30	−1.46 ± 1.79	30	−1.39 ± 1.94	23	−1.07 ± 1.14	0.349
Spine, L1–L4	30	−0.33 ± 3.58	31	−2.02 ± 3.17	23	−2.36 ± 3.54	0.083
Total hip	31	−0.821 ± 1.93	31	−0.29 ± 2.19	20	−0.31 ± 2.41	0.406
Neck femur	30	0.85 ± 3.87	30	−1.75 ± 3.67	23	0.53 ± 3.38	0.540
Trochanter	25	−0.69 ± 4.69	33	−0.64 ± 4.20	22	−1.68 ± 3.09	0.942
Intertrochanteric	31	−0.74 ± 2.40	29	0.35 ± 3.00	24	−0.20 ± 3.29	0.340

[a] *P* value from F test (analysis of covariance).
[b] BMC medians were the medians at various sites.
[c] $P < 0.05$ compared with the mid dose group after controlling for baseline body weight, height, and BMC at the relevant sites; total calcium intake; and dietary protein over that intervention period (LSD, analysis of covariance).
[d] $P < 0.05$ compared with the placebo group.
[e] $P < 0.01$ compared with the mid dose group.

TABLE 6. Main determinants of rates of change in BMC at various sites by multivariate linear regression analysis (intention to treat)

Dependent variables: rate of change in BMC (%/y)	Independent variables in final model[a]	Total r^2	r^2 change[b]	β	SE of β	*P* value of β	Partial correlation
Whole body		0.139					
	Isof (g/d)		0.001	1.60	5.02	0.751	0.025
	YSM (yr)		0.044	0.121	0.057	0.036	0.163
	Weight (kg)		0.034	0.073	0.019	0.000	0.299
	BMC (g)		0.060	−0.0022	0.001	0.001	−0.245
Spine, L1–L4		0.025					
	Isof (g/d)		0.001	−3.00	9.82	0.760	−0.024
	Weight (kg)		0.024	0.063	0.031	0.045	0.154
Total hip		0.167					
	Isof (g/d)		0.035	17.7	6.33	0.006	0.216
	Protein (g/d)		0.065	0.026	0.007	0.000	0.294
	YSM (yr)		0.067	0.252	0.070	0.000	0.276
Neck		0.027					
	Isof (g/d)		0.000	1.43	11.2	0.899	0.010
	Standing (h/d)		0.027	0.337	0.159	0.035	0.163
Trochanter		0.063					
	Isof (g/d)		0.018	29.8	15.2	0.052	0.155
	Protein (g/d)		0.045	0.045	0.016	0.007	0.213
Intertrochanteric		0.128					
	Isof (g/d)		0.028	19.9	8.25	0.017	0.188
	Mild PA (h/d)		0.034	0.186	0.075	0.014	0.193
	YSM (yr)		0.039	0.261	0.092	0.005	0.219
	Protein (g/d)		0.027	0.0194	0.087	0.028	0.173

Isof, Isoflavones; PA, physical activity. β, beta coefficient, unit (%) change in the dependent variables (BMCs) per unit change in the independent variables. No significant association of BMD with isoflavone supplementation (data not shown).
[a] Independent variable: Intake of Isof from supplementation (Method = Enter), weight, age, YSM, height, body mass index, baseline BMC at the relevant sites, PA, total calcium intake, and dietary total protein, dietary soy protein intake, dietary phosphorus over intervention period (Method = Stepwise, *F*-to-enter = 0.05, *F*-to-remove = 0.1).
[b] r^2 changes in relation to the independent variables.

Discussion

Phytoestrogens have attracted increasing interests for their potential favorable effects on lipid profiles and bone (1–3, 5). Soy isoflavones are the main source of phytoestrogens found in natural foods. Although several animal experiments have consistently indicated the positive effects of soy or soy isoflavones in retarding bone loss caused by estrogen deficiency (4, 6, 7), only limited short-term data in humans are available (5). This 1-yr, placebo-controlled, randomized trial has examined the effect of isoflavone-enriched soy extracts on bone loss in Chinese postmenopausal women within the first 10 yr after menopause. To our knowledge this is the first study

designed specifically to test the relationship between soy isoflavones and bone loss in the Asian populations. We found a mild, but statistically significant, effect of daily supplementation of soy-derived isoflavones in attenuating BMC loss at the trochanter, intertrochanter, and total hip after controlling for the important confounding factors. These factors included YSM, body weight, height, baseline BMC at the relevant sites, total calcium intake, and dietary protein over the intervention period.

Previous studies (11, 13, 14) had used 100-mg isoflavone intake/d as the high dose group. Considering that a typical Chinese diet has an average daily intake of about 20 mg isoflavones (10, 22), daily supplementations of 40 and 80 mg were given, respectively, to the mid- and high-dose groups.

The current calcium intake (13.75 mmol at baseline) of this population was much lower than the recommended calcium intakes based on both the United States (23) as well as the Chinese recommended adequate intakes (24). Previous studies in the local populations indicated that adequate dietary calcium intake has a beneficial effect on bone loss or fractures (25, 26). Considering the threshold behavior of calcium on bone mass (27), calcium intake beyond the threshold would minimize the variations due to calcium related bone loss. A daily 12.5-mmol calcium supplementation was thus given to the trial subjects. In addition, 125 IU vitamin D were included in the daily calcium supplementation.

We observed that only the 80-mg isoflavone supplementation had a significant effect on BMC at the hip sites. The amount is consistent with the beneficial doses of 80–90 mg soy isoflavones observed to improve spinal BMD in the 6-month trials in Caucasian perimenopausal and postmenopausal women (11, 13, 14). Although local cross-sectional studies have noted that postmenopausal women in the top tertile or quartile of habitual intake of soy isoflavones (ranging from 40–53 mg/d) have significantly higher BMD at the spine or hip (10, 22), we observed no beneficial effect of mid-dose supplementation (40 mg isoflavones/d) on bone changes. Previous animal experimental studies suggest that a threshold dose of isoflavones needs to be consumed for a lengthy time period, such as months to years, before any measurable effect on bone mass can be observed (4, 28). It is possible that a relatively short duration of 40-mg supplementation may not be adequate to obtain a detectable effect on bone changes. A longer-term (*i.e.* 2–3 yr) study is required to confirm such a hypothesis.

We observed that BMC, but not BMD, increased with the isoflavone supplementation. The reasons for such differential responses of BMC and BMD to isoflavone supplementation are unclear. Arjmandi *et al.* reported that soy or its isoflavones promote IGF-I production in rats (29) and humans (17). Soy isoflavones may in part exert similar effects as GH on BMC by increasing IGF-I. In GH trials, the increase in BMC is much more substantial and stable than that in BMD (30, 31). As IGF-I is known to increase osteoblastic activities in humans (32, 33), the anabolic effect may result in appositional growth, resulting in an increase in bone area, as observed in this study (data not shown) as well as the GH study (31).

Initial bone mass might be a marker for other strong risk/protective factors, such as body weight and YSM (34–37); the effects of soy isoflavone supplementation might be masked or modified by these strong determinants. We conducted stratified analyses based on initial BMC values and observed a favorable effect of soy isoflavones on hip bone areas only among women with lower initial BMC levels. The nonsignificant effect among women with higher initial BMC values suggests that the presence of the many protective factors among these women renders them less sensitive to the relatively mild treatment effects of soy isoflavones. We observed a regression to the mean effect at the whole body BMC, in which baseline BMC was negatively associated with the rate of change in BMC. However, among the BMC-stratified subgroups, both the treatment and placebo groups had lower baseline BMC values with adjustment made. The benefit observed in the treatment group would thus not be due to regression to the mean.

Contrary to other studies that have reported positive soy effects on spine BMD (11, 13, 14), we did not observe any effect of soy isoflavone supplementation on spine BMC or BMD. Spine consists of mainly trabecular bone, which is markedly affected during estrogen deprivation. The soy isoflavones used in this study have a higher proportion of daidzein (46.4%) and glycetein (38.8%), but a lower percentage of genistein (14.5%), than those in natural soy products. Daidzein was reported to be more bioavailable than genistein in rats (38) and humans (39). It was also reported that daidzein is more efficient than genistein in preventing ovariectomy-induced cancellous bone loss in rats (7). The undetectable effect of isoflavone supplementation at the spine could be related to differences in racial responses at the spinal trabecular bone within the duration of intervention period, differences in physiological adaptations to soy intake, and individual ability to produce the metabolite, equol, to which the major phytoestrogen effect has been attributed.

It is well established that age, YSM, and body weight (and height for BMC) are the predominant determinants of BMD and BMC among postmenopausal women (34, 35, 37). In this study we observed similar findings that YSM and body weight were the main determinants of change in BMC at various bone sites. Loss of ovarian function plays a predominant role in bone loss within the first few menopausal years (40). Menopause-related bone loss would be less marked approximately 3–4 yr after the last menses when the body has become accustomed to a low level of estrogen (41).

In this study we observed a favorable association between higher protein intake and changes in BMC at multiple sites of the hip. Inconsistent findings on the association between protein intake and bone health have been observed (42). Although increased urinary calcium loss has been found in high protein intake populations, a positive association between protein intake and bone health has been observed in populations with moderate protein intakes (43, 44). As reported by Dawson-Hughes and Harris (42), the calcium supplementation might also contribute to the positive association between protein intake and changes in BMC in this study.

Although our study has the longest treatment period among the published studies, 1 yr of treatment may not be adequate to predict the long-term effects on bone mass. Bone remodeling is a relatively slow process, and the time required

to complete a cycle may increase with age. Normally 6–18 months are needed to reach a new equilibrium at a certain intervention (45, 46). Thus, caution needs to be exercised in applying these short-term trial results. Longer-term trials of at least 2 yr would be required to evaluate the effects of isoflavones on bone mass.

To date, although some beneficial effects of soy isoflavones on bone mass have been observed in limited short-term randomized trials in peri- or postmenopausal women, almost all significant effects were found in groups with about 80–90 mg daily isoflavone supplementation rather than among those with doses of 40–50 mg isoflavones/d. However, we cannot yet conclude that 80–90 mg/d is the optimal dose. The risks and benefits of the use of higher doses and for longer duration need to be carefully evaluated in future studies.

In conclusion, isoflavone-enriched soy extracts have a mild, but independent, effect in the maintenance of hip BMC in postmenopausal women with lower levels of baseline BMC values, even after controlling for the other important covariates, such as body weight and height, YSM, age, and dietary intakes of protein and calcium. Our findings add to the existing evidence that soy isoflavones may have a bone-sparing effect. Further studies are required to explore the optimal dosage of soy isoflavone intake as well as its long-term effect on maintenance of bone mass.

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