The effects of phytoestrogen isoflavones on bone density in women: a double-blind, randomized, placebo-controlled trial[1–3]

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ABSTRACT

Background: Isoflavone phytoestrogen therapy has been proposed as a natural alternative to hormone replacement therapy (HRT). HRT has a beneficial effect on bone, but few trials in humans have investigated the effects of isoflavones on bone.

Objective: The objective of the study was to determine the effect on bone density of a red clover–derived isoflavone supplement that provided a daily dose of 26 mg biochanin A, 16 mg formononetin, 1 mg genistein, and 0.5 mg daidzein for 1 y. Effects on biochemical markers of bone turnover and body composition were also studied.

Design: Women aged 49–65 y (n = 205) were enrolled in a double-blind, randomized, placebo-controlled trial; 177 completed the trial. Bone density, body composition, bone turnover markers, and diet were measured at baseline and after 12 mo.

Results: Loss of lumbar spine bone mineral content and bone mineral density was significantly ($P = 0.04$ and $P = 0.03$, respectively) lower in the women taking the isoflavone supplement than in those taking the placebo. There were no significant treatment effects on hip bone mineral content or bone mineral density, markers of bone resorption, or body composition, but bone formation markers were significantly increased ($P = 0.04$ and $P = 0.01$ for bone-specific alkaline phosphatase and *N*-propeptide of collagen type I, respectively) in the intervention group compared with placebo in postmenopausal women. Interactions between treatment group and menopausal status with respect to changes in other outcomes were not significant.

Conclusion: These data suggest that, through attenuation of bone loss, isoflavones have a potentially protective effect on the lumbar spine in women. *Am J Clin Nutr* 2004;79:326–33.

KEY WORDS Isoflavones, phytoestrogens, bone density, randomized controlled trial, body composition, estrogen and vitamin D receptor polymorphisms

INTRODUCTION

Estrogens play an important role in skeletal homeostasis, and ovarian hormone deficiency is one of the most important risk factors for osteoporosis. There are clear bone-related benefits of hormone replacement therapy (HRT; 1), but compliance with such regimens is generally very poor (2, 3), partly as a result of fears about the risks of cancer (3).

Isoflavones are compounds in plant foods, particularly soybeans (4, 5), that are structurally similar to the mammalian estrogens (6, 7) and that have received considerable attention for their potential bone-sparing properties. Rates of hip fracture in Asian populations, whose traditional diets are rich in soy, are substantially lower than those in whites residing in the United States (8). However, bone mineral density (BMD) in Asian populations is comparable to that in white populations after adjustment for height and weight (9). Nevertheless, data from animal experiments provided evidence that soy protein can attenuate menopausal bone loss (10, 11), and it was suggested that isoflavones in soy might be responsible for protective effects on bone (12). In humans, some (13–17) but not all (18) cross-sectional studies in Asian populations reported significant positive associations between soy protein or isoflavone intakes and BMD. Furthermore, intervention trials in humans using either soy protein or isoflavone extracts generally reported protective effects on bone, although sample sizes were small and trials often were relatively short. In postmenopausal women, consumption of soy protein providing 90 mg isoflavones/d for 24 wk resulted in a significant increase in lumbar spine bone mineral content (BMC) and BMD (19), and a red clover isoflavone supplement providing 57 or 85.5 mg isoflavones/d for 6 mo resulted in an increase in BMD of the proximal radius and ulna (20). In perimenopausal women, soy protein that provided 80.4 mg isoflavones/d for 24 wk did not increase lumbar spine BMC or BMD, but it did lower the extent of bone loss compared with that in the control group (21). Several intervention studies reported the effects of soy protein or isoflavone extracts on markers of bone turnover. In postmenopausal women, diets rich in soyfoods resulted in significant increases in serum osteocalcin concentrations (22, 23) and decreases in urinary *N*-telopeptide excretion (22). In perimenopausal women, a 4-wk intervention with an isoflavone extract resulted in a significant reduction in the bone

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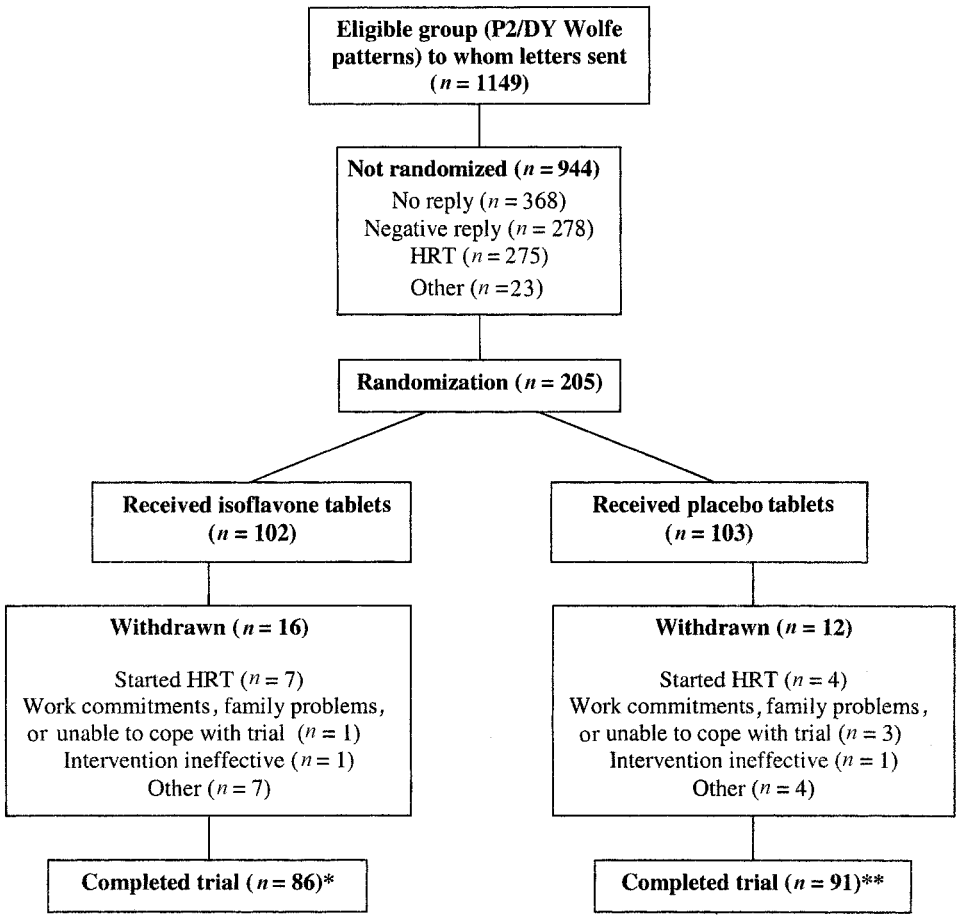

FIGURE 1. Flow chart describing the progress of the volunteers during the trial. HRT, hormone replacement therapy. *Includes 2 women who completed the trial but were excluded from all analyses because they had taken oral contraceptives or had been treated for alcoholism. **Includes 1 woman who completed the trial but was excluded from all analyses because she had taken oral contraceptives.

resorption marker pyridinoline (24), but other studies reported little or no effect of soy protein or isoflavone supplements on markers of bone turnover in humans (25–27).

We aimed to determine the effects of consuming a red clover–derived isoflavone supplement (in a daily dose providing 26 mg biochanin A, 16 mg formononetin, 1 mg genistein, and 0.5 mg daidzein) for 1 y on the BMC and BMD of the lumbar spine and hip, on biochemical markers of bone turnover, and on body composition.

SUBJECTS AND METHODS

Subjects

Between November 1997 and May 1999, women aged 49–65 y were recruited from the Breast Screening Unit of Addenbrooke's Hospital (Cambridge, United Kingdom). The primary outcome measure was breast density, and women were selected for the study according to the extent of dense tissue seen on their most recent mammogram; mammograms from 1908 women were classified according to their Wolfe pattern (28), and women with P2 or DY Wolfe breast patterns (ie, dense breast patterns; n = 1149, 60% of all mammograms classified) were sent a recruitment letter, which contained a short description of the study and a reply slip. The rate of response to the letter was 68% (n = 781); of the responders, 205 (18% of the women who received letters) were eligible and were randomly assigned to receive an isoflavone or placebo tablet (**Figure 1**). Women were not eligible to participate if they had a personal history of breast cancer or major breast surgery or if they were currently taking HRT. Women who expressed an interest in taking part were visited at home. During this initial home visit, the study was explained in detail, and all women who wished to participate were asked for written informed consent. A total of 205 women were randomly assigned to receive isoflavone or placebo tablets (Figure 1). Additional home visits were made after ≈5.5 and 11 mo on the study. All study procedures were approved by the Dunn Human Nutrition Unit Ethics Committee and the Cambridge Local Research Ethics Committee.

Participants were randomly assigned to receive daily either a red clover–derived isoflavone tablet that provided 26 mg biochanin A, 16 mg formononetin, 1 mg genistein, and 0.5 mg daidzein (Promensil; Novogen Ltd, Sydney, Australia) or a placebo of identical appearance. We did not measure the isoflavone content of the Promensil tablets in our laboratory, but an independent study showed that the manufacturer's statements as to the quantity and type of isoflavones in the tablets were correct (29). Randomization was performed by using random

number generation in MICROSOFT EXCEL (version 2.2; Microsoft Corp, Redmond, WA), and researchers and study participants remained blinded to the tablet allocation throughout the study. Participants were asked to take 1 tablet/d. The code identifying those who had been taking the isoflavone tablets and those who had been taking the placebo was broken when all participants had completed all stages of the study.

Urine samples

Women were asked to make 24-h urine collections at baseline and 12 mo. To check compliance, women also were asked to make a 24-h urine collection at 6 mo. The completeness of all urine collections was assessed by using the *p*-aminobenzoic acid (PABA)–check test (30). Samples containing 85–110% of the ingested PABA were designated satisfactory. For samples with PABA recoveries of between 70% and 85%, which indicated that all tablets had been taken but that the urine collection was incomplete, the urinary excretion of isoflavones was adjusted to 93% PABA recovery (31). Samples with <70% recovery were designated incomplete. Samples with >110% PABA recovery were considered unsatisfactory, because additional sources of PABA (eg, a multivitamin) may have been consumed, and an accurate determination of sample completeness could not be made.

Urinary excretion of genistein, daidzein, formononetin, and biochanin A was measured by using HPLC and a modification of the method of Setchell et al (32) and Franke et al (33). Briefly, samples were incubated with β-glucuronidase for 20–72 h at 37 °C. Phenolic components were extracted into an ethyl:acetate (6:4 by vol) solvent mixture. After vortex mixing for 30 s and subsequent 10-min centrifugation at 2320 $\times$ *g* and 20 °C, the organic phase was transferred to a 2-mL vial and evaporated to dryness under vacuum at 43 °C. Extraction residues were reconstituted in 100 μL 50% isopropanol solution and centrifuged for 10 min at 2320 $\times$ *g* and 20 °C. A 5-μL aliquot was then injected directly onto the HPLC column, which consisted of an Alltima 250 $\times$ 2.1-mm, 5 μmol/L, C-18 stationary phase (Alltech Associates, New South Wales, Australia), and a mobile phase (acetonitrile and water) containing 0.05% trifluoroacetic acid with a gradient of acetonitrile from 25–100%. Detection and quantification were performed via photo diode array detector. Flavone was used as the internal standard; the limit of detection (LOD) was 0.05 μg/mL, and the limit of quantitation (LOQ) was 0.1 μg/mL.

Total and free pyridinoline and deoxypyridinoline, markers of bone resorption, were measured in urine samples collected at baseline and when nearing completion at 12 mo with the use of a rapid automated assay technique according to the method of Pratt et al (34). Briefly, for total pyridinoline and deoxypyridinoline, 0.5 mL urine was hydrolyzed with an equal volume of 12 mmol/L HCl for 18 h at 107 °C. Hydrolysates were centrifuged for 2 min at 13 500 $\times$ *g* and room temperature, and hydroxypyridinium cross-links were extracted from 0.5 mL of the supernatant by solid-phase extraction (employing cellulose). Pyridinoline and deoxypyridinoline were separated and quantified by using HPLC. Free pyridinoline and deoxypyridinoline were measured without the hydrolysis step. The within-batch CV was <3% for pyridinoline and <5% for deoxypyridinoline measurements; baseline and 12-mo urine samples from individual participants were analyzed together in the same batch. All urine samples were analyzed for their creatinine content (Kone autoanalyser; Jaffe method), and excretion of pyridinoline and deoxypyridinoline was corrected for creatinine excretion.

Blood samples

Fasting blood samples were taken at baseline and 12 mo. Participants were asked to refrain from eating or drinking (except water) from midnight until after the sample had been taken the following morning. A total of 35.5 mL blood was drawn at each visit, including 9.0 mL blood that was drawn into a lithium heparin tube and 9.0 mL that was drawn into a serum tube. The serum tube was left at room temperature for $\geq$1 h before centrifugation to allow clotting. After centrifugation for 10 min at 2000 rpm and 5 °C, aliquots of plasma and serum were stored at −20 °C. Before centrifugation, ≈1 mL whole blood was removed from the lithium heparin tube, and DNA was extracted by using a Qiagen kit (Qiagen Ltd, Crawley, United Kingdom) and was used for genotyping of estrogen receptor and vitamin D receptor gene polymorphisms (*Pvu*II and *Bsm*I, respectively).

Plasma bone-specific alkaline phosphatase (bone ALP), a marker of bone formation, was measured by using the Metra kit (Quidel Ltd, Oxford, United Kingdom; 35). The intraassay and interassay CVs were 3.9% and 10.9%, respectively. Plasma concentrations of the *N*-propeptide of collagen type I (PINP), also a marker of bone formation, were measured by using the Orion Diagnostica radioimmunoassay kit (Espoo, Finland; 36), for which the intraassay and interassay CVs were 9.3% and 12.5%, respectively. For both assays, baseline and 12-mo samples from each participant were run together on the same plate to minimize variability.

Serum estradiol was measured by using a previously described method (37), and serum follicle-stimulating hormone (FSH) was measured by using an enzyme immunoassay on an Abbott AxSYM automated analyzer (Abbott Diagnostics, Maidenhead, United Kingdom). Menopausal status was determined by using baseline concentrations of estradiol and FSH as follows; women were classified as premenopausal if they had <30 IU FSH/L and >100 pmol estradiol/L, as postmenopausal if they had >30 IU FSH/L and <100 pmol estradiol/L, and as perimenopausal if they had >30 IU FSH/L and >100 pmol estradiol/L or <30 IU FSH/L and <100 pmol estradiol/L. However, if a woman had noted on the questionnaire completed at the initial home visit that she was currently menstruating, but her baseline hormone profile was that of a postmenopausal woman (ie, >30 IU FSH/L and <100 pmol estradiol/L), she was classified as perimenopausal.

Bone density, body composition, and calcium and vitamin D intakes

Bone density and body composition were assessed by dual-energy X-ray absorptiometry (DXA) at baseline and after ≈12 mo with the use of an Hologic QDR-4500A scanner (Hologic Inc, Waltham, MA). The precision of the in vivo measurement of BMC by DXA in the Metabolic Bone Unit at Addenbrooke's Hospital is 1% in the spine and 2–3% in the proximal femur.

Individual scans of the lumbar spine (L1–L4), hip (femoral neck, trochanter, and intertrochanteric region), and whole body were taken. If a scan revealed signs of osteoporosis (ie, a BMD T-score <−2.5), the participant was notified in writing and

TABLE 1
Baseline characteristics and time between measurements in subjects receiving isoflavone or placebo tablets[1]

	Isoflavone group (n = 84)	Placebo group (n = 90)
Age (y)	55.1 ± 4.7[2]	55.2 ± 4.9
Menopausal status [n (%)][3]		
Premenopausal	13 (16)	15 (17%)
Perimenopausal	12 (15)	14 (16%)
Postmenopausal	56 (69)	61 (68%)
Time between DXA scans (d)[4]	358 ± 23	359 ± 21
Calcium intake (mg/d)[5]	1007 ± 302	1013 ± 315
Vitamin D intake (μg/d)[5]	3.19 ± 1.79	3.37 ± 2.04
Spine bone density[6]		
BMC (g)	58.6 ± 10.1	59.7 ± 11.6
BMD (g/cm^2)	0.99 ± 0.14	1.00 ± 0.14
T score	−0.48 ± 1.30	−0.39 ± 1.30
Hip bone density[4]		
BMC (g)	32.8 ± 6.0	33.4 ± 5.6
BMD (g/cm^2)	0.92 ± 0.13	0.93 ± 0.12
T score	−0.15 ± 1.05	−0.06 ± 0.95
Markers of bone turnover[6]		
Bone ALP (U/L)	17.3 ± 7.3	17.0 ± 6.4
PINP (mg/L)	50.3 ± 22.2	56.0 ± 22.6
Total pyridinoline (nmol/mmol creatinine)	41.5 ± 11.7	40.3 ± 8.4
Total deoxypyridinoline (nmol/mmol creatinine)	9.9 ± 3.2	10.3 ± 4.3

[1] DXA, dual-energy X-ray absorptiometry; BMC, bone mineral content; BMD, bone mineral density; ALP, alkaline phosphatase; PINP, *N*-propeptide of collagen type I. Differences between treatment groups were nonsignificant ($P > 0.05$).

[2] $\bar{x}$ ± SD.

[3] Menopausal status unknown for 3 women in the isoflavone group; percentages may not add to 100% because of rounding.

[4] Isoflavone group, n = 78; Placebo group, n = 81.

[5] Isoflavone group, n = 81; Placebo group, n = 88.

[6] Isoflavone group, n = 77; Placebo group, n = 81.

advised to visit her general practitioner. Follow-up spine data were not available for one woman in the isoflavone group, and follow-up bone density and body-composition data were not available for one woman in the placebo group. Six women from the isoflavone group and 8 women from the placebo group were excluded from all analyses regarding bone, because they had taken medications during the study that can affect bone density (eg, bisphosphonates, statins, or calcium and vitamin D supplements). Participants' height and weight were measured at the time of the DXA scans, and body mass index (BMI) was calculated as [weight (kg)/height (m^2)]; baseline height was unavailable for one woman in the isoflavone group.

Participants were asked to complete a food-frequency questionnaire (FFQ) at baseline and after 12 mo on the study. Participants were given verbal and written instructions on how to complete the FFQ, and calculation of calcium and vitamin D intakes was based on published food-composition tables (38).

Sample size and data analysis

A sample of 100 in each treatment group gave 80% power to detect a ≥1.3% difference between groups with respect to the change in BMC over 1 y, assuming an SD of 3.2% and setting α at 0.05. Interactions between treatment group and polymorphisms in the estrogen receptor and vitamin D receptor genes are not reported because of a lack of power to detect significant associations. Results are available from the authors on request.

Statistical analyses were performed by using SAS statistical software (version 6.12; SAS Institute, Cary, NC) under the Windows operating system. A P value of <0.05 was considered significant. Changes in BMC, BMD, markers of bone turnover, body composition, and intakes of calcium and vitamin D were calculated as 12-mo data minus baseline data. We used Student's t tests to test for differences between treatment groups for changes in BMC, BMD, markers of bone turnover, and body composition. Data on changes in calcium and vitamin D intakes were skewed, and therefore the nonparametric Wilcoxon's rank-sum test was used to test for differences between treatment groups.

RESULTS

Baseline characteristics are shown in **Table 1**. Differences between treatment groups were nonsignificant ($P > 0.05$). Sixteen women withdrew from the isoflavone group and 12 withdrew from the placebo group (Figure 1). The principal reasons for withdrawal were commencement of HRT and work commitments or family problems that prevented completion of study activities. Other reasons included feeling no beneficial effects of the intervention or having no interest in continuing on the trial, heavy menstrual bleeding, or illnesses preventing completion of study activities (eg, severe hip pain, skin irritation and sores, vomiting, and diarrhea). One woman in the isoflavone group was diagnosed with an interval cancer of the breast (ie, a cancer detected in the interval after a negative mammographic result) 2 mo after the start of the intervention and was withdrawn from the study. The difference between

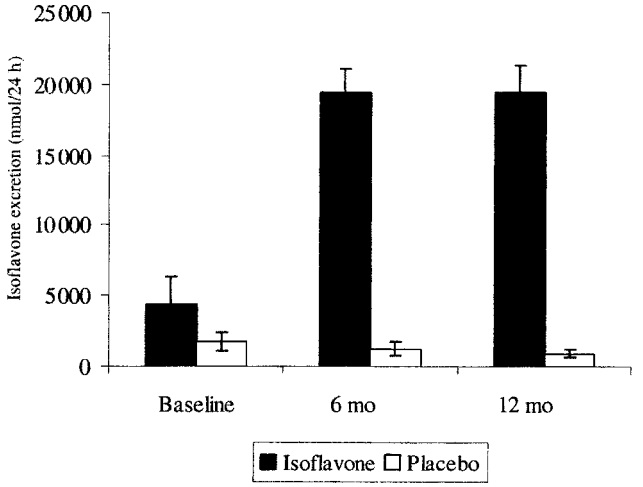

FIGURE 2. Mean (±SEM) urinary isoflavone excretion (sum of daidzein, genistein, formononetin, and biochanin A) by treatment group at baseline, 6 mo, and 12 mo, excluding samples with <70% or >110% *p*-aminobenzoic acid recovery. At baseline: isoflavone group, $n = 66$; placebo group, $n = 72$. At 6 mo: isoflavone group, $n = 70$; placebo group, $n = 73$. At 12 mo: isoflavone group, $n = 76$; placebo group, $n = 79$. The difference in isoflavone excretion between treatment groups was nonsignificant at baseline ($P = 0.23$), but differences between treatment groups at 6 and 12 mo were highly significant (both: $P < 0.001$).

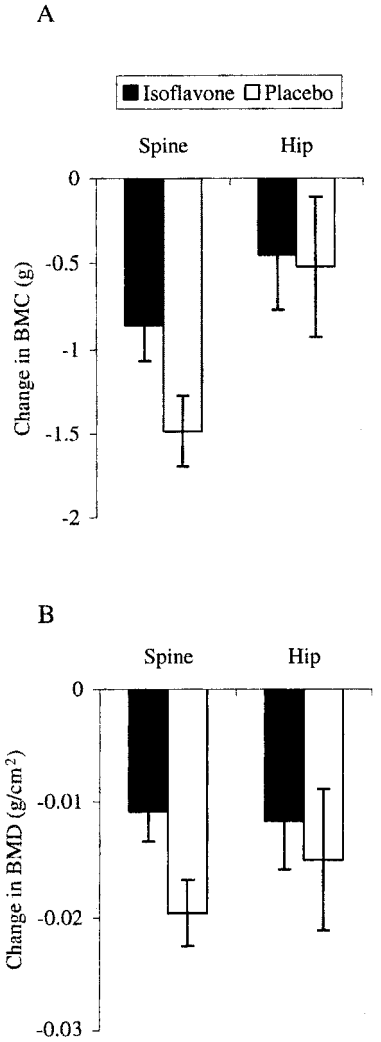

FIGURE 3. Mean (±SEM) changes in spine and hip bone mineral content (BMC) and bone mineral density (BMD) at 1 y by treatment group. Isoflavone group, $n = 77$ ($n = 78$ for hip); placebo group, $n = 81$. Interactions between menopausal status and treatment group for changes in BMC or BMD were nonsignificant. Differences between treatment groups with respect to changes in spine BMC and BMD were significant ($P = 0.04$ and $P = 0.03$, respectively); differences between treatment groups with respect to changes in hip BMC and BMD were nonsignificant ($P = 0.48$ and $P = 0.30$, respectively).

treatment groups in the number of withdrawals was not significant ($\chi^2 = 1.123$, $P = 0.29$).

According to the PABA-check method, 58%, 72%, and 77% of the women had complete urine collections at baseline, 6 mo, and 12 mo, respectively. A further 22%, 11%, and 13% had PABA recoveries between 70% and 85% at baseline, 6 mo, and 12 mo, respectively. Differences between treatment groups in sum isoflavone excretion (sum of daidzein, genistein, formononetin, and biochanin A) were nonsignificant at baseline, but differences were highly significant at 6 and 12 mo (**Figure 2**). The data shown do not include women with <70% or >110% PABA recovery. Inclusion of these women did not alter the results. Among women in the placebo group, isoflavone excretion did not change significantly from baseline to 6 mo ($P = 0.80$) or from baseline to 12 mo ($P = 0.15$; Figure 2).

There were no significant differences between treatment groups with respect to changes in dietary intakes of calcium and vitamin D from baseline to 12 mo; mean (±SD) changes in calcium intakes for women in the isoflavone and placebo groups, respectively, were – 43 ± 224 and – 54 ±165 mg/d ($P = 0.32$), and changes in vitamin D intakes for women in the isoflavone and placebo groups, respectively, were – 0.24 ± 1.6 and – 0.19 ± 2.3 μg/d ($P = 0.52$).

Changes in spine and hip BMC and BMD at 1 y by treatment group are shown in **Figure 3**. Interactions between treatment group and menopausal status for the changes in spine or hip BMC and BMD were not significant ($P > 0.05$). Differences between treatment groups for losses of spine BMC and BMD were significant. Percentage change in spine BMC and BMD among women in the isoflavone and placebo groups, respectively, showed similar trends: mean (±SEM) BMC: −1.42 ± 0.36% and −2.35 ± 0.37% ($P = 0.07$); BMD: −1.08 ± 0.27% and −1.86 ± 0.29% ($P = 0.05$). Decreases in BMC and BMD of the hip were generally greater among women in the placebo group than among women in the isoflavone group, but differences between treatment groups were not significant (Figure 3). Similar results were found when considering percentage changes (data not shown). In an analysis of covariance on changes from baseline to 12 mo, with baseline data (BMC or BMD) as a covariate, the effect of treatment group on the changes in spine BMC and BMD remained significant ($P = 0.05$, and $P = 0.03$, respectively). Similarly, for changes in hip BMC and BMD, the effect of treatment group remained nonsignificant ($P = 0.38$ and $P = 0.85$, respectively).

Markers of bone turnover at baseline and 12 mo by treatment group are shown in **Table 2**. The difference between treatment groups with respect to the change in PINP was of borderline significance. The interaction between treatment group and menopausal status was significant for the changes in bone ALP and PINP ($P = 0.05$ and $P = 0.03$, respectively). When grouped by menopausal status, differences between treatment

TABLE 2
Markers of bone turnover at baseline and at 12 mo according to treatment group[1]

	Isoflavone group ($n = 77$)		Placebo group ($n = 81$)[2]		
	Baseline	12 mo	Baseline	12 mo	P for change
Bone ALP (U/L)	17.3 ± 7.3	22.0 ± 9.2	17.0 ± 6.4	20.9 ± 7.1	0.26
PINP (mg/L)	50.3 ± 22.2	56.9 ± 26.0	56.0 ± 22.6	56.2 ± 23.6	0.06
Pyridinoline (nmol/mmol creatinine)	41.5 ± 11.7	42.3 ± 10.8	40.3 ± 8.4	42.3 ± 10.0	0.41
Deoxypyridinoline (nmol/mmol creatinine)	9.9 ± 3.2	10.4 ± 3.5	10.3 ± 4.3	10.7 ± 3.9	0.76

[1] $\bar{x}$ ± SD. ALP, alkaline phosphatase; PINP, *N*-propeptide of collagen type I.
[2] $n = 80$ for pyridinoline and deoxypyridinoline.

groups were significant among postmenopausal women; mean (±SD) changes in bone ALP among postmenopausal women in the isoflavone and placebo groups, respectively, were 5.65 ± 5.92 and 3.71 ± 2.92 U/L ($P = 0.04$), and changes in PINP among postmenopausal women in the isoflavone and placebo groups, respectively, were 9.72 ± 25.19 and −1.40 ± 19.12 mg/L ($P = 0.01$). No other changes differed significantly between treatment groups. Results similar to those for total pyridinoline and deoxypyridinoline were obtained for free pyridinoline and deoxypyridinoline (data not shown). There were no significant differences between treatment groups with respect to the changes in whole-body BMC or BMD or body-composition measures (**Table 3**), and interactions between treatment group and menopausal status were not significant ($P > 0.05$).

DISCUSSION

This is one of the longest and largest studies to date to examine the effects of an isoflavone supplement derived from red clover on spine and hip BMC and BMD, biochemical markers of bone turnover, and body composition. We observed an attenuation of lumbar spine bone loss among women taking the isoflavone supplement compared with that among women taking the placebo. Trends for losses of hip BMC and BMD were similar to those seen in the lumbar spine, but differences between treatments were not significant. However, this was not entirely surprising, given that the hip contains a relatively large proportion of cortical bone, which is metabolically less active than the trabecular bone of which the spine is primarily composed (39, 40). Furthermore, precision of the measurement of hip BMC is lower than that of the measurement of spine BMC (≈2–3% for the proximal femur compared with 1% for the spine; JE Compston, personal communications, 1997 and 2002), and therefore the power to detect significant differences between treatment groups was lower as a result of slow bone turnover and lower measurement precision.

Potter et al (19) previously reported an increase in lumbar spine BMC and BMD among postmenopausal women whose diets were supplemented daily for 24 wk with soy protein isolate providing 90 mg, but not 56 mg, isoflavones/d. In our study, we did not see an increase in BMC or BMD with the isoflavone supplement, but the daily dose of isoflavones was lower than that used by Potter et al (19). However, in a study of perimenopausal women, Alekel et al (20) did not see an increase in lumbar spine BMC or BMD with a daily dose of 80.4 mg isoflavones (as soy protein) for 24 wk, but, similar to our study, they saw a reduction in the extent of bone loss. In a recent study of 28 premenopausal women (mean age: 24 y) there were no significant effects of a soy protein supplement that provided either 0 or 90 mg isoflavones/d for 12 mo (41). This suggests that isoflavones may be beneficial in attenuating age-associated bone loss rather than in enhancing peak bone mass in younger premenopausal women.

Promensil tablets contain isoflavones derived from red clover and, unlike soy protein, relatively large proportions of biochanin A and formononetin, in addition to daidzein and

TABLE 3
BMI, body composition, and total-body bone mineral content (BMC) and bone mineral density (BMD) at baseline and at 12 mo by treatment group[1]

	Isoflavone group ($n = 83$)		Placebo group ($n = 88$)	
	Baseline	12 mo	Baseline	12 mo
BMI (kg/m^2)[2]	25.3 ± 3.9	25.7 ± 4.0	25.3 ± 3.5	25.8 ± 3.8
Total fat (kg)	24.36 ± 7.89	24.82 ± 7.97	24.19 ± 6.88	24.86 ± 7.16
Total lean (kg)	37.98 ± 4.14	38.16 ± 4.07	38.00 ± 4.12	38.22 ± 4.26
Percentage total fat (%)	37 ± 7	37 ± 7	37 ± 7	37 ± 7
Trunk fat (kg)	10.66 ± 4.59	11.02 ± 4.48	10.22 ± 3.69	10.74 ± 3.86
Trunk lean (kg)	19.37 ± 1.98	19.57 ± 2.01	19.49 ± 2.15	19.73 ± 2.25
Percentage trunk fat (%)	34 ± 9	34 ± 9	33 ± 8	34 ± 8
Total-body BMC (kg)[3]	2.17 ± 0.31	2.16 ± 0.31	2.21 ± 0.29	2.19 ± 0.29
Total-body BMD (g/cm^2)[3]	1.11 ± 0.10	1.10 ± 0.10	1.11 ± 0.09	1.10 ± 0.10

[1] $\bar{x}$ ± SD. Differences between treatment groups with respect to changes from baseline to 12 mo were nonsignificant ($P > 0.05$).
[2] Placebo group, $n = 90$.
[3] Isoflavone group, $n = 78$; placebo group, $n = 80$.

genistein (29). It has been observed that the use of isoflavone extracts does not always result in lipid-lowering effects (42, 43) that are the same as those seen with intact soy protein (44), and it has been suggested that the isoflavone extraction process may remove some component of the food that has synergistic effects in combination with isoflavones or that isoflavones may become inactivated during the isolation and purification process (45, 46). However, the combination of isoflavones in a food or dietary supplement also may be important in terms of their potential beneficial health effects. It was suggested that daidzein may be more important than other isoflavones in terms of relieving menopausal symptoms (47), and the tablets used in the present study were previously shown to produce beneficial effects on bone (20), but conflicting data have been presented with respect to blood lipid concentrations (42, 43).

Because of both the structural similarity of isoflavones to mammalian estrogens and their ability to bind to estrogen receptors (7), it is widely hypothesized that the actions of isoflavones are mediated via the estrogen receptors. However, the exact mechanism of action of isoflavones on bone remains to be fully elucidated. In our study, the 2 bone formation markers increased in postmenopausal women taking the isoflavone supplement. This is somewhat in agreement with studies in ovariectomized rats suggesting that soy or isoflavones stimulate bone formation (10, 48). However, we did not see overall increases in BMC or BMD, but such discord between markers of bone turnover and BMD adds to the ongoing controversy surrounding the use of biochemical markers as predictors of BMD (49).

Prospective and case-control studies showed positive associations between weight and the risk of breast cancer among postmenopausal women and negative associations among premenopausal women (reviewed in 50). Furthermore, increased abdominal fat has consistently been associated with increased mortality and with the incidence of cardiovascular disease, hypertension, and diabetes (51, 52). The menopause is associated with a potentially detrimental shift in the distribution of fat toward a more central location (53–55), but in users of HRT, this shift is minimized, and HRT users are more likely to have less abdominal fat than do nonusers (56–58). In the present study, we did not see a significant effect of the isoflavone supplement on the distributions of fat and lean tissue within the body after 1 y.

Despite being one of the longest intervention studies with an isoflavone supplement to date, a potential limitation of this study is that it was relatively short-term with respect to the outcome measures; longer-term studies are needed to determine the effects of isoflavones (both as intact soy protein and as dietary supplements) on hip BMC and BMD and also on longer-term outcome measures such as fracture rates. Nevertheless, our findings suggest that, through attenuation of bone loss, the isoflavone supplement has a potentially protective effect on the lumbar spine.

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All authors participated fully in the preparation of the manuscript. CA was responsible for subject recruitment and day-to-day running of the trial, sample analyses, and statistical analyses; JEC was responsible for bone densitometry; NED was responsible for the data analysis; MD was responsible for the hormone assays; and SAB was responsible for the design of the experiment, sample analysis, and significant advice and consultation regarding all aspects of the trial. None of the authors had conflicts of interest.

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