Soy proteins and isoflavones affect bone mineral density in older women: a randomized controlled trial[1-3]

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ABSTRACT

Background: Soy foods contain several components (isoflavones and amino acids) that potentially affect bone. Few long-term, large clinical trials of soy as a means of improving bone mineral density (BMD) in late postmenopausal women have been conducted.

Objective: Our goal was to evaluate the long-term effect of dietary soy protein and/or soy isoflavone consumption on skeletal health in late postmenopausal women.

Design: We conducted a randomized, double-blind, placebo-controlled clinical trial in 131 healthy ambulatory women aged >60 y. Ninety-seven women completed the trial. After a 1-mo baseline period, subjects were randomly assigned into 1 of 4 intervention groups: soy protein (18 g) + isoflavone tablets (105 mg isoflavone aglycone equivalents), soy protein + placebo tablets, control protein + isoflavone tablets, and control protein + placebo tablets.

Results: Consumption of protein powder and isoflavone pills did not differ between groups, and compliance with the study powder and pills was 80–90%. No significant differences in BMD were observed between groups from baseline to 1 y after the intervention or in BMD change between equol and non-equol producers. However, there were significant negative correlations between total dietary protein (per kg) and markers of bone turnover ($P < 0.05$).

Conclusions: Because soy protein and isoflavones (either alone or together) did not affect BMD, they should not be considered as effective interventions for preserving skeletal health in older women. The negative correlation between dietary protein and bone turnover suggests that increasing protein intakes may suppress skeletal turnover. This trial was registered at clinicaltrials.gov as NCT00668447. *Am J Clin Nutr* 2009;90:234–42.

INTRODUCTION

Osteoporosis is a disease that primarily affects older women. Because of the risks of using hormone replacement therapy (1), many postmenopausal women are insisting on natural treatments (eg, soy foods) for chronic diseases (eg, osteoporosis). Soy contains several components that could potentially benefit skeletal health, such as isoflavones and low sulfur amino acid composition.

Soy foods contain isoflavones, which are naturally occurring plant compounds similar to mammalian estrogens. The major isoflavones in soy are daidzin and genistin (as glycosides) and their corresponding aglycone forms, daidzein and genistein. The phenolic ring is a critical structural element of these compounds that binds to estrogen receptors and exerts estrogen-like effects in cells (2). Isoflavones selectively bind to and activate estrogen receptor-β more so than estrogen receptor-α (3, 4). Thus, the clinical effects of isoflavones may be similar to selective estrogen receptor modulators, ie, beneficial effects on bone and heart without detrimental effects on breast and uterine tissue. Soy isoflavones may be helpful for older women because the pathogenesis of osteoporosis involves not only increased bone resorption, because of estrogen deficiency and decreased calcium absorption, but also age-related decreases in bone formation because of decreased osteoblast renewal (5). Furthermore, Prestwood et al (6) suggested that the skeleton of women older than 70 y is more sensitive to low-dose estrogen treatment than is that of younger women.

Although there are studies showing potential beneficial effects of soy isoflavones on bone mineral density (BMD) and/or markers of bone turnover in peri- or postmenopausal women (7–14), there are no known publications addressing this question in older postmenopausal women. Interestingly, many human intervention trials have failed to find beneficial effects of soy isoflavones and/or protein on bone (15–22). In a recent meta analysis, Liu et al (23) did not find significant beneficial effects of soy isoflavones on BMD changes in women from 10 nutrition trials of 1 y duration. These authors identified only a slight trend toward improvement at the spine ($P = 0.08$) in those receiving the high doses of isoflavones. Accordingly, we report here a 1-y nutrition intervention study to evaluate the effect of soy protein and soy isoflavone alone and in combination on BMD and bone turnover in late postmenopausal women.

SUBJECTS AND METHODS

Study overview

We conducted a 1-y, double-blind, randomized, placebo controlled, prospective, 2×2 factorial intervention trial. Although 131 women older than 60 y began the clinical trial, only 97 women successfully completed the trial. After a 1-mo lead in period (designed to stabilize calcium intake), the subjects were randomly assigned into 1 of 4 intervention groups: soy protein + isoflavone tablets, soy protein + placebo tablets, control protein + isoflavone tablets, and control protein + placebo tablets. We subsequently counseled the subjects every 3 mo on incorporating the protein into their diet while maintaining their total dietary calcium close to the Recommended Dietary Allowance. The primary outcome was BMD at the beginning and end of 1 y of intervention; secondary outcomes included markers of bone turnover and equol production. We tested the following hypotheses: *1*) soy protein or soy isoflavones alone will have a beneficial effect on bone in older women compared with the control and placebo treatment, *2*) soy protein plus isoflavones will have an additional effect on bone turnover and BMD compared with the other interventions, and *3*) within the group receiving the isoflavones, equol producers would have more positive effects on BMD than would the non-equol producers.

Study subjects

The institutional review board at the University of Connecticut Health Center approved the study, and all women gave written informed consent before their screening evaluation. Participants in this study included women older than 60 y (mean ± SD: 73.1 ± 5.9 y; range at entrance: 60–93 y). Women were excluded if they had any disease that could affect bone metabolism (including Paget disease, primary hyperparathyroidism, osteomalacia, untreated hyperthyroidism, or multiple myeloma); had cancer of any kind (except basal or squamous cell of skin) in past 5 y; had used of any of the following medications within the past 2 y (calcitonin, calcitriol, heparin, phenytoin, or phenobarbital); had ever used bisphosphonates, corticosteroids for a long time (>6 mo), methotrexate, or fluoride; had an estimated creatinine clearance <50 mL/min; had a history of chronic liver disease or evidence of liver disease on screening; had a history of hip fracture; had known vertebral fracture within the past year; and were vegans. Subjects initially underwent a screening visit that included BMD testing. Those women with a BMD T score < -3.0 at the hip or spine during the screening visit were excluded. The screening visit was followed 1 mo later by the baseline visit, at which time they received the intervention products (protein powders and tablets). After the second visit, the subjects were seen every 3 mo during the 1-y intervention period. Transvaginal ultrasound was conducted at the beginning and end of the 1-y intervention to assess endometrial thickness (a potential consequence of isoflavone administration).

Nutrition intervention

At the screening visit, we instructed the women on how to achieve a total dietary calcium intake of 1200–1500 mg/d (from food and supplements). For those women who did not consume enough dairy foods to achieve this level, we provided calcium citrate caplets that included vitamin D (315 mg Ca and 200 IU vitamin D; Mission Pharmacal Company, San Antonio, TX). The calcium and vitamin D contents of the calcium citrate caplets approximated one serving of dairy products. One month later, the subjects returned for their baseline visit, at which point they were assigned randomly to 1 of 4 treatment groups: soy protein + isoflavone tablets, soy protein + placebo tablets, control protein + isoflavone tablets, and control protein + placebo tablets.

All subjects received dietary counseling every 3 mo at the University of Connecticut's General Clinical Research Center from the research dietitian; we carefully assessed their nutrient intake and monitored their compliance with the protocol. The subjects completed 4-d dietary records every 3 mo during the 1-y intervention. These records were carefully reviewed with each subject for completeness and accuracy and were then analyzed with the Food Processor II Nutrient Analysis Program (ESHA Research Inc, Salem, OR). We asked each subject to suspend taking any nutritional or herbal supplement that would interfere with our primary outcomes. Other soy foods were prohibited during the clinical trial. At each 3-mo visit, the subject returned her unused protein powders and tablets, we accounted for the amount she consumed, and a new 3-mo supply was dispensed. All subjects and investigators were blinded to the protein and the tablet intervention.

Soy and placebo products

Both control and soy proteins were isolates, meaning that they were of the highest concentration of protein (85–90% by wt) to minimize the volume that each woman ingested. The soy protein was an alcohol-washed, soy protein isolate containing 90% protein and negligible isoflavone (0.2 mg/g product; Pro Fam 930, 066–930). The soy protein and isoflavones were kindly provided to us by Archer Daniels Midland, Co, Decatur, IL. The control protein was a mix consisting of 50% protein from sodium caseinate, 25% from whey protein, and 25% from egg white protein (Century Foods International, Sparta, WI). The use of a mix of proteins as a control provides a more balanced level of amino acids and better reflects the mix of proteins that humans typically consume, while avoiding the unique characteristics of one source of protein. The calculated amino acid composition of the soy and the control protein is provided in **Table 1**. Subjects consumed 20 g protein powder (containing 18 g protein) daily by incorporating it into their commonly consumed beverages or foods. To maintain the dietary protein intake constant, the subject was counseled to decrease her intake of other sources of

TABLE 1
Essential amino acid content of soy protein and control mix protein

	Soy isolate	Control mix
	mg/20 g protein	*mg/20 g protein*
Isoleucine	980	1085
Leucine	1600	1894
Lysine	1320	1527
Threonine	760	915
Valine	940	1232
Methionine + cystine	520	892
Tryptophan	240	261
Phenylalanine + tyrosine	1880	1451

protein from primarily animal sources by ≈3 oz/d (90 g/d)—the approximate equivalent of the protein powder.

All subjects ingested 3 study tablets daily that contained soy isoflavones or placebo, which were both formulated to appear identical (kindly provided by Archer Daniels Midland, Co). Each soy isoflavone tablet contained 35 mg isoflavone aglycone equivalents from primarily genistein, glycitein, and daidzein and their β-glycosides. The aglycone equivalent value was determined by multiplying the total isoflavones by 0.61. The placebo pills contained a blend of 10-DE maltodextrin (90%) and caramel color (10%) to match the color of the isoflavones; both components were food-grade. The composition of the tablets is shown in **Table 2**.

Biochemical assessment

During the screening visit, we collected blood and urine for measurement of screening chemistries (serum total or ionized calcium, albumin, thyroid-stimulating hormone, alkaline phosphatase, complete blood count with differential, urinary calcium, and creatinine) measured by routine clinical chemistry. The women completed a brief physical examination that included BMD testing.

Women who were eligible after the screening visit returned for the baseline visit 1 mo later. We then measured serum markers of bone formation [bone-specific alkaline phosphatase (BAP)] and markers of bone resorption [urine N-telopeptide crosslinks of collagen normalized for creatinine (NTX/CRT)]. During the 1-mo period between the screen and the baseline visit, the subjects were instructed to consume between 1200 and 1500 mg Ca (from both food and supplemental sources) to help stabilize bone and calcium homeostasis. Blood and urine samples were collected between 0700 and 1000 after the subjects fasted for 10 to 12 h and divided into 0.5-mL aliquots and stored at –70°C. Ionized calcium was measured within 2 h of collection. Urinary NTX measurements were performed by using the Osteomark NTX Direct Response assay (Ostex International, Seattle, WA); the intraassay variability was <10% for measures of bone resorption. BAP was measured by enzyme-linked immunosorbent assay (CIS Bio International, Bagnols/Cèze, France); the average intraassay variability was <5%.

Bone density measurement

Areal bone mineral content (g/cm^2) of the proximal femur, lumbar spine, wrist, and total body were measured by dual-energy X-ray absorptiometry by using a Lunar DPX-IQ (Lunar Radiation Inc, Madison, WI). The accuracy for bone mineral content measurement reported by the manufacturer has an SEE of 0.8%, with a correlation of bone mineral content to known calcium hydroxyapatite of 0.99. The long-term stability of this instrument is 0.5%, as determined by daily measurement of a Hologic spine phantom. The CVs of bone density measures analyzed with 4.6f software were as follows: femoral neck, 1.5%; femoral total, 1.1%; trochanter, 1.7%; ultradistal radius, 3.4%; 33% radius, 1.6%; total radius, 1.3%; lumbar spine, 1.8%; total body, 0.7%.

Serum isoflavones and equol

Serum isoflavones and equol were measured by using HPLC-Coularray as described previously (24). In brief, serum (500 μL) was buffered with 500 μL of 100 mmol/L ammonium acetate (pH 4.6), mixed with 50 μL internal standard (4-hydroxybenzophenone; 110 μmol/L), and subjected to enzymatic hydrolysis (overnight, 37°C) using 200 U β-glucuronidase and 15 U sulfatase prepared in 100 μL ammonium acetate (pH 4.6). After incubation, proteins were precipitated by using acetonitrile (1 mL), the sample was delipidated by using hexane (3 mL), and the isoflavones were extracted 3 times by using 3 mL methyl tert-butyl ether. Extracts were dried under nitrogen gas, reconstituted in mobile phase A, and injected on the HPLC system. The sample was separated at 1 mL/min on a C$_{18}$ Luna (2), 250 × 4.6 mm (5 μm; Phenomenex; Torrance, CA) by using 25 mmol potassium phosphate buffer/L (pH 2.7) as mobile phase A and methanol:acetonitrile:mobile phase A (50:30:20) as mobile phase B. The gradient was delivered as follows: 50% B to 65% B from 0 to 20 min, a linear gradient to 75% B from 20 to 30 min, and a linear gradient to 100% from 30 to 35 min. Initial conditions were returned over 2 min, and the system was equilibrated

TABLE 2
Analyzed isoflavone content of the isoflavone and placebo tablets used in this study

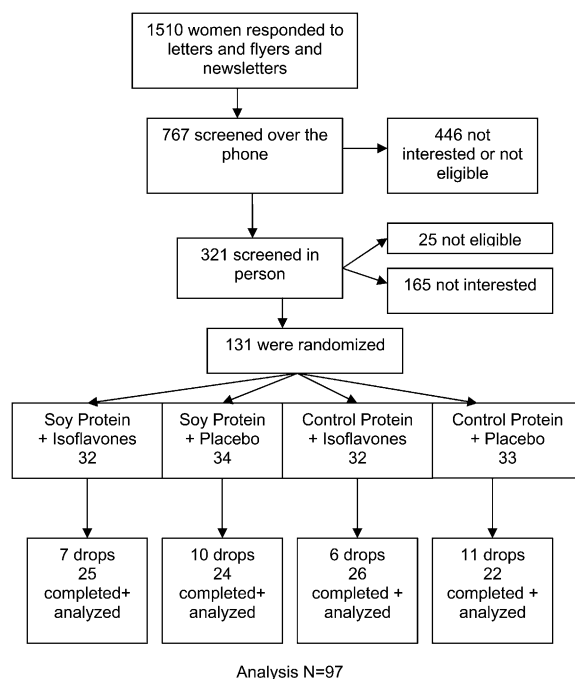|	Isoflavones	Placebo
	mg/tablet	*mg/tablet*
Daidzin	22.5	0
Glycitin	3.7	0
Genistin	28.5	0
Daidzein	0.5	0
Glycitein	0.3	0
Genistein	0.3	0
Other	1.2	0
Total	57.0	0

FIGURE 1. Inclusion and follow-up of the study population. Analysis *n* = 97.

TABLE 3
Baseline characteristics of the study participants[1]

	Soy protein + isoflavones (n = 32)	Soy protein + placebo (n = 34)	Control protein + isoflavones (n = 32)	Control protein + placebo (n = 33)	P (ANOVA)
Age (y)	73.0 ± 5.7[2]	74.0 ± 6.2	72.3 ± 5.7	72.9 ± 6.1	0.71
Height (cm)	157.7 ± 6.4	159.9 ± 6.1	159.6 ± 6.1	159.1 ± 5.2	0.44
Weight (kg)	72.3 ± 14.8	70.5 ± 10.8	70.3 ± 12.2	71.8 ± 12.6	0.90
BMI (kg/m²)	29.3 ± 6.9	27.6 ± 4.3	27.8 ± 5.4	28.4 ± 4.8	0.59
Smokers [n (%)]					0.63
Never	12 (40)	19 (61)	15 (50)	16 (52)	
Current/previous	18 (60)	12 (39)	15 (50)	15 (48)	
Drinks alcohol (%)	22 (76)	21 (68)	21 (70)	13 (43)	0.05
Time since last menses (y)	23.5 ± 9.9	24.3 ± 10.8	22.6 ± 9.3	22.8 ± 8.1	0.91
Endometrial thickness (mm)	2.97 ± 0.81	3.14 ± 0.81	2.82 ± 0.73	3.32 ± 1.25	0.50
Total no. of observations	16	11	17	13	
No. of observations >6	0	0	0	0	
Nutrient intake[3]					
Protein (g)	62.5 ± 13.7	65.4 ± 16.7	67.7 ± 25.5	57.0 ± 21.9	0.30
Calcium (mmol)	19.3 ± 7.7	19.3 ± 6.7	19.2 ± 8.0	17.8 ± 8.1	0.88
Vitamin D (IU)	183 ± 162	119 ± 69	153 ± 104	157 ± 86	0.22
Energy (MJ)	5.96 ± 1.41	6.73 ± 1.96	5.80 ± 1.71	5.40 ± 1.71	0.06
Bone variables					
Femoral neck BMD	0.809 ± 0.098	0.861 ± 0.088	0.810 ± 0.085	0.800 ± 0.108	0.05
Femoral neck T score	−1.4 ± 0.8	−1.0 ± 0.7	−1.4 ± 0.7	−1.5 ± 0.9	0.05
Total femur BMD	0.888 ± 0.137	0.922 ± 0.093	0.869 ± 0.112	0.872 ± 0.144	0.30
Total femur T score	−0.9 ± 1.1	−0.6 ± 0.8	−1.1 ± 0.9	−1.1 ± 1.2	0.30
Trochanter BMD	0.747 ± 0.148	0.769 ± 0.093	0.733 ± 0.116	0.729 ± 0.135	0.56
Trochanter T score	−0.4 ± 1.6	−0.2 ± 0.8	−0.5 ± 1.1	−0.6 ± 1.21	0.56
Ward triangle BMD	0.641 ± 0.123	0.700 ± 0.096	0.674 ± 0.114	0.647 ± 0.114	0.13
Ward triangle T score	−2.1 ± 0.9	−1.6 ± 0.7	−1.8 ± 0.9	−2.0 ± 0.9	0.13
Lumbar spine L2-L4 BMD	1.127 ± 0.160	1.218 ± 0.196	1.102 ± 0.207	1.110 ± 0.208	0.07
Lumbar spine L2-L4 T score	−0.6 ± 1.3	0.1 ± 1.6	−0.8 ± 1.7	−0.7 ± 1.7	0.07
Forearm 33% radius BMD	0.585 ± 0.073	0.596 ± 0.068	0.583 ± 0.089	0.579 ± 0.086	0.85
Forearm 33% radius T score	−1.8 ± 1.0	−1.6 ± 0.9	−1.8 ± 1.2	−1.9 ± 1.2	0.85
Ultradistal radius BMD	0.311 ± 0.062	0.319 ± 0.050	0.306 ± 0.048	0.311 ± 0.060	0.80
Ultradistal radius T score	−1.8 ± 1.7	−1.6 ± 1.4	−2.0 ± 1.3	−1.8 ± 1.6	0.80
Total radius BMD	0.461 ± 0.067	0.465 ± 0.054	0.461 ± 0.068	0.458 ± 0.075	0.98
Total radius T score	−1.8 ± 1.4	−1.7 ± 1.1	−1.8 ± 1.4	−1.9 ± 1.5	0.98
Total-body BMD	1.106 ± 0.085	1.129 ± 0.071	1.092 ± 0.075	1.086 ± 0.103	0.18
Total-body T score	−0.2 ± 1.1	0.05 ± 0.8	−0.4 ± 0.9	−0.5 ± 1.3	0.18
Markers of bone turnover					
Serum BAP (U/L)	24.7 ± 8.8	21.2 ± 4.7	24.1 ± 7.5	26.8 ± 8.6	0.09
Urinary NTX/CRT (nmol BCE · mmol⁻¹ · L creatinine⁻¹)	37.5 ± 16.8	33.1 ± 14.8	40.0 ± 19.6	37.5 ± 14.9	0.54

[1] NTX/CRT, urinary *N*-telopeptide crosslinks of collagen normalized for creatinine; BAP, bone-specific alkaline phosphatase; BMD, bone mineral density; BCE, bone collagen equivalents.

[2] Mean ± SD (all such values).

[3] Nutrient intake from food sources only; supplements not included.

at 50% B for 12 min before subsequent injection. Analytes were detected by using potential settings of 325, 450, 575, and 700 mV and were quantitated on their dominant channel. Serum isoflavone concentrations were calculated by using area ratios for standards and the internal standard, and the lower limit of quantification was ≈20 nmol/L for each analyte. Equol producers were defined as those individuals who had a 12-mo serum concentration of *S*-equol >20 nmol/L (5 μg/L) according to the method of Setchell and Cole (25). The intraassay and interassay CVs were <8% for all analytes.

Statistical analysis

Baseline and clinical characteristics are reported as means ± SDs stratified by treatment group. All subsequent data are pre-sented as means ± SEMs. One-factor analysis of variance was used to test the difference in baseline characteristics between the treatment groups and to also test nutrient intake, serum iso-flavones, serum equol, and endometrial thickness at 12 mo. Then, a 2-factor analysis of variance was used to evaluate dif-ferences between groups in BMD change from baseline to 12 mo. The changes in bone markers were evaluated at baseline, 3 mo, and 12 mo by using the same statistical procedure. In those subjects who received the isoflavone supplement, an in-dependent *t* test was used to evaluate the difference in BMD change between equol and non-equol producers. Data from all subjects were pooled, and partial correlations examined the re-lation between total protein intake and percentage change in bone markers from baseline to 12 mo. We also assessed the

association between serum isoflavones and BMD (change from baseline) by using Pearson's correlation statistic in those participants receiving isoflavones. All analyses were done by using SPSS version 14.0 (SPSS Inc, Chicago, IL). A P value <0.05 was considered statistically significant.

RESULTS

Subjects

There were 1510 individuals who responded to the advertisements for this study, of whom 767 passed the initial telephone screen. The profile of the study subjects and their treatment assignment are presented in **Figure 1**. After randomization, 34 women voluntarily withdrew from the study. One-third of the withdrawals were due to nonstudy-related issues (eg, hospitalization for other medical conditions and change in family situation or lifestyle), one-third were due to study-related issues (eg, time commitment and gastrointestinal changes), and approximately one-third were due to other or unspecified reasons. The subjects' baseline characteristics (age, anthropometric measures, lifestyle, nutrition, BMD, and markers of bone turnover) are presented in **Table 3**. No differences in subject characteristics at baseline were observed between groups, as expected.

Intervention compliance

Consumption of protein powder and isoflavone pills did not differ between the 4 groups. On average, the participants consumed 16.1 ± 0.4 g soy (or control) protein supplement daily containing 14.3 ± 3.6 g protein (79% compliance). The actual average isoflavone pill intake was 2.7 ± 0.35 (containing 94 ± 12 mg aglycone equivalents), which reflected 90% compliance.

As anticipated, genistein and daidzein were present in the serum from the women supplemented with isoflavones (**Table 4**). Of the 51 total women who received isoflavones tablets, all but 3 had measurable concentrations of isoflavones in their fasting serum. In the 46 women who received the placebo tablets, only 4 women had measurable serum concentrations of isoflavones. Of the 51 subjects supplemented with isoflavones, 25 were equol producers (49%) and 26 were non-equol producers (51%).

Overall, our subjects gained 0.5 kg, on average, during the intervention. No differences in initial or ending body weight were observed between groups. Average calcium and protein intakes (from diet and supplements) also did not differ between groups (Table 4).

Bone variables

No significant differences in BMD from baseline to 1 y after intervention were observed between groups (**Table 5**). No differences in the markers of bone turnover were observed between the 4 groups over the duration of the study (expressed as a percentage of baseline), as evident in **Figure 2**. In the women who received isoflavones, no significant differences in BMD were observed between equol and non-equol producers (**Figure 3**). However, there was a significant correlation between change in NTX/CRT ($P = 0.023$, $r = -0.23$) and dietary protein per kilogram and change in BAP ($P = 0.005$, $r = -0.28$) and dietary protein per kilogram (**Figure 4**), which suggests that protein intake was related to bone turnover. No significant associations

TABLE 4
Nutrient intake, serum isoflavones, serum equol, and endometrial thickness at 12 mo by treatment group

	Soy protein + isoflavones ($n = 25$)	Soy protein + placebo ($n = 24$)	Control protein + isoflavones ($n = 26$)	Control protein + placebo ($n = 22$)	P (ANOVA)
Nutrient intake					
Protein (g/d)					
Food sources	65.3 ± 2.2[1]	65.3 ± 2.4	65.0 ± 3.2	67.9 ± 2.9	0.87
Supplement	15.1 ± 0.5	14.5 ± 0.8	13.9 ± 0.8	13.6 ± 0.7	0.46
Total	80.4 ± 2.3	79.8 ± 2.7	78.9 ± 3.6	81.5 ± 3.0	0.94
Calcium (mmol/d)					
Food sources	20.6 ± 1.5	21.3 ± 1.5	20.4 ± 1.5	21.0 ± 1.0	0.96
Supplement	14.4 ± 1.3	12.0 ± 1.2	13.4 ± 1.4	12.1 ± 0.8	0.48
Total	35.0 ± 1.2	33.3 ± 1.4	34.0 ± 1.4	33.1 ± 1.0	0.72
Vitamin D (IU)					
Food sources	137 ± 13	143 ± 20	157 ± 19	161 ± 15	0.71
Supplement	366 ± 34	305 ± 32	340 ± 35	308 ± 21	0.48
Total	503 ± 27	448 ± 36	502 ± 32	469 ± 26	0.52
Energy (MJ)[2]	6.08 ± 0.22	6.52 ± 0.24	6.30 ± 0.27	6.32 ± 0.27	0.66
Serum isoflavones (μmol/L)					
Daidzein	1.18 ± 0.23	0.21 ± 0.11	0.87 ± 0.17	0.13 ± 0.13	0.01
Genistein	0.90 ± 0.20	0.03 ± 0.02	0.88 ± 0.18	0.01 ± 0.04	0.01
Equol from producers ($n = 25$)	0.145 ± 0.046	—	0.191 ± 0.061	—	0.72
Endometrial thickness (mm)	3.38 ± 0.32	4.00 ± 0.54	2.88 ± 0.21	3.19 ± 0.33	0.16
No. of observations >6	0	1	0	0	
Endometrial thickness change (mm)	0.41 ± 0.22	0.86 ± 0.54	0.06 ± 0.23	−0.16 ± 0.43	0.24
No. of observations	16	11	17	13	

[1] Mean ± SD (all such values).

[2] Energy from food sources only, not supplement.

TABLE 5
Bone characteristics at 12 mo by treatment group[1]

	Soy protein + isoflavones (n = 25)	Soy protein + placebo (n = 24)	Control protein + isoflavones (n = 26)	Control protein + placebo (n = 22)	P for main effects (ANOVA)		P for interaction
					Soy protein	Isoflavone	
Femoral neck BMD	0.821 ± 0.018[2]	0.842 ± 0.020	0.804 ± 0.020	0.795 ± 0.025	0.130	0.775	0.490
BMD change[3]	0.001 ± 0.008	0.001 ± 0.005	−0.006 ± 0.005	−0.003 ± 0.005	0.317	0.835	0.818
Femoral neck T score	−1.3 ± 0.2	−1.2 ± 0.8	−1.5 ± 0.2	−1.5 ± 0.4	0.130	0.775	0.490
Total femur BMD	0.901 ± 0.025	0.892 ± 0.022	0.860 ± 0.022	0.866 ± 0.032	0.188	0.948	0.776
BMD change[3]	0.003 ± 0.006	−0.011 ± 0.006	−0.003 ± 0.004	−0.008 ± 0.006	0.830	0.093	0.430
Total femur T score	−0.8 ± 0.2	−0.9 ± 0.2	−1.2 ± 0.2	−1.1 ± 0.3	0.188	0.948	0.776
Trochanter BMD	0.751 ± 0.030	0.734 ± 0.022	0.720 ± 0.021	0.720 ± 0.031	0.407	0.744	0.743
BMD change[3]	−0.004 ± 0.010	−0.018 ± 0.008	−0.002 ± 0.008	−0.016 ± 0.009	0.819	0.102	0.946
Trochanter T score	−0.4 ± 0.3	−0.5 ± 0.2	−0.6 ± 0.2	−0.6 ± 0.3	0.407	0.744	0.743
Ward triangle BMD	0.660 ± 0.025	0.681 ± 0.023	0.674 ± 0.024	0.635 ± 0.024	0.501	0.695	0.205
BMD change[3]	0.009 ± 0.012	−0.003 ± 0.010	0.004 ± 0.008	−0.012 ± 0.007	0.478	0.129	0.813
Ward triangle T score	−1.9 ± 0.2	−1.8 ± 0.2	−1.8 ± 0.2	−2.1 ± 0.2	0.501	0.695	0.205
Lumbar spine L2-L4 BMD	1.111 ± 0.032	1.191 ± 0.045	1.140 ± 0.051	1.103 ± 0.045	0.489	0.611	0.170
BMD change[3]	0.004 ± 0.009	0.001 ± 0.008	0.018 ± 0.009	0.010 ± 0.007	0.181	0.500	0.826
Lumbar spine L2-L4 T score	−0.7 ± 0.3	−0.8 ± 0.3	−0.5 ± 0.4	−0.8 ± 0.4	0.489	0.611	0.170
Forearm 33% radius BMD	0.565 ± 0.015	0.591 ± 0.016	0.565 ± 0.017	0.579 ± 0.018	0.722	0.242	0.720
BMD change[3]	−0.004 ± 0.004	−0.004 ± 0.003	−0.008 ± 0.003	−0.006 ± 0.003	0.383	0.690	0.653
Forearm 33% radius T score	−2.1 ± 0.2	−1.7 ± 0.2	−2.1 ± 0.2	−1.9 ± 0.3	0.722	0.242	0.720
Ultradistal radius BMD	0.306 ± 0.013	0.312 ± 0.012	0.301 ± 0.010	0.310 ± 0.012	0.770	0.504	0.907
BMD change[3]	−0.002 ± 0.002	−0.004 ± 0.003	−0.004 ± 0.003	−0.003 ± 0.003	0.836	0.864	0.582
Ultradistal radius T score	−2.0 ± 0.4	−1.8 ± 0.3	−2.1 ± 0.3	−1.8 ± 0.3	0.770	0.504	0.907
Total radius BMD	0.449 ± 0.014	0.462 ± 0.013	0.451 ± 0.013	0.458 ± 0.015	0.940	0.480	0.840
BMD change[3]	−0.001 ± 0.002	−0.002 ± 0.002	−0.006 ± 0.002	−0.005 ± 0.002	0.056	0.848	0.808
Total radius T score	−2.0 ± 0.3	−1.8 ± 0.3	−2.0 ± 0.3	−1.9 ± 0.3	0.940	0.480	0.840
Total-body BMD	1.094 ± 0.019	1.121 ± 0.014	1.085 ± 0.014	1.085 ± 0.019	0.183	0.423	0.419
BMD change[3]	−0.007 ± 0.004	−0.003 ± 0.003	−0.004 ± 0.003	−0.005 ± 0.003	0.969	0.678	0.510
Total-body T score	−0.4 ± 0.2	−0.1 ± 0.2	−0.5 ± 0.2	−0.5 ± 0.2	0.183	0.423	0.419
Markers of bone turnover							
Serum BAP (U/L)	23.7 ± 1.65	18.8 ± 1.07	23.3 ± 1.27	25.2 ± 2.03	0.051	0.333	0.030
Urinary NTX/CRT (nmol BCE · mmol⁻¹ · L creatinine⁻¹)	35.9 ± 2.44	30.2 ± 2.74	35.3 ± 3.61	35.0 ± 3.21	0.497	0.320	0.367

[1] NTX/CRT, urinary *N*-telopeptide crosslinks of collagen normalized for creatinine; BAP, bone-specific alkaline phosphatase; BMD, bone mineral density; BCE, bone collagen equivalents.
[2] Mean ± SEM (all such values).
[3] Raw change in BMD from baseline in the 97 valid completers.

between serum isoflavones and change in BMD were observed between groups.

Adverse events and subject safety

No significant differences in endometrial thickness at baseline or 1 y after the intervention were observed between groups. One woman had an endometrial thickness >6 mm. Other complaints included gastrointestinal disturbance (*n* = 10), differences in mammogram or breast tenderness (*n* = 2), new-onset cardiac symptoms (*n* = 2), increases in blood pressure (*n* = 4), respiratory infections (*n* = 3), and unrelated medical conditions (*n* = 4). The rates of adverse events did not differ between groups.

DISCUSSION

The primary purpose of this prospective intervention trial was to identify the potential skeletal benefit of incorporating soy protein and/or soy isoflavones into the diets of healthy late

postmenopausal women. After 1 y of intervention, in comparison with the control group, we observed no measurable improvement in BMD or in markers of bone turnover between the women consuming soy protein or isoflavones alone or in combination. Likewise, the ability to synthesize equol from dietary isoflavone precursors was unrelated to BMD. The subjects' compliance with both the protein and isoflavone intervention in this study was excellent, as assessed by serum isoflavone concentrations at 12 mo.

There is tremendous interest in the use of soy foods to protect the skeleton. Much of the enthusiasm is based on the cross-sectional studies that suggest that high consumption of soy products is associated with increased bone mass or decreased bone resorption or fractures (26–33). Only a few epidemiologic studies failed to find a positive association with bone health (34, 35). Whereas population studies have shown an association, they are limited in that they cannot establish causality, which requires an intervention trial.

Overall, the intervention trials that examined the effect of soy isoflavones on BMD or markers of bone turnover are less
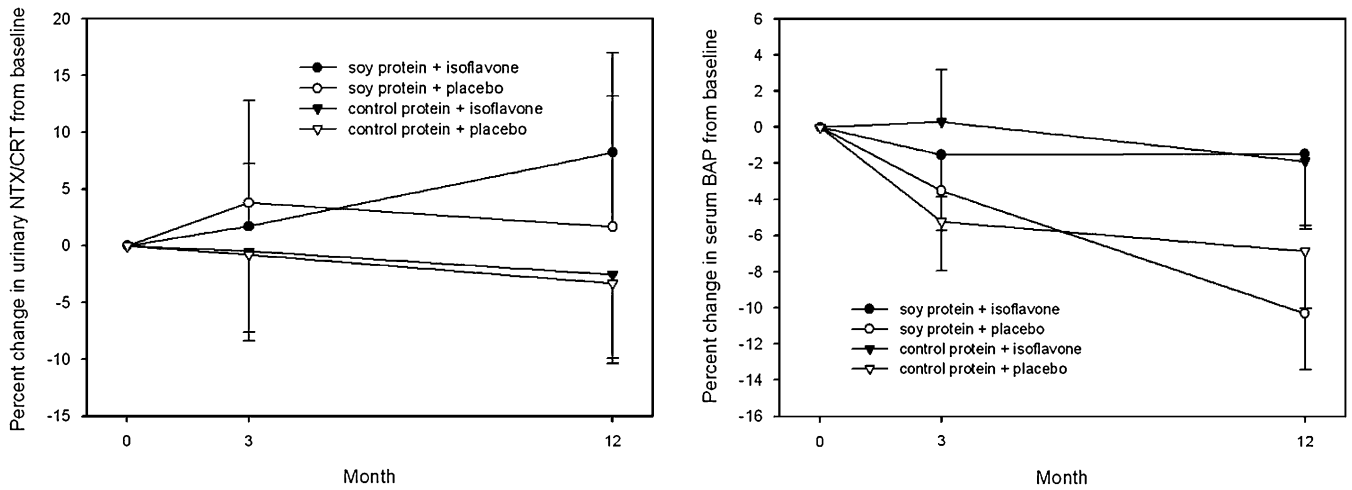
FIGURE 2. Mean (±SEM) changes in markers of bone turnover [urinary *N*-telopeptide crosslinks of collagen normalized for creatinine (NTX/CRT) and bone-specific alkaline phosphatase (BAP)] expressed as a percentage change from baseline in the 97 women who completed the study. There were no significant differences from baseline or between the 4 groups by ANOVA.

conclusive than the epidemiologic studies. Potter et al (7) initially showed a significant increase in both bone mineral content and density in the lumbar spine in postmenopausal women who consumed 90 mg isoflavones for 6 mo. Subsequently, a second 6-mo intervention trial by Alekel et al (8) also showed that 80 mg isoflavones/d attenuated bone loss at the lumbar spine in perimenopausal women. A series of short-term human intervention trials (≤6 mo) reported positive changes (13, 36–38), as did longer-term studies (9, 10, 12, 14). Most recently, Atteritano et al (39), in a study of young postmenopausal women selected for osteopenia, found an increase in spine and hip BMD with genistein compared with a loss in the placebo group.

However other human trials with skeletal-related outcomes have not been supportive (15–22, 40–43). Most recently, Brink et al (22) found in a 1-y randomized, double-blind, placebo-controlled,

clinical trial that the consumption of isoflavone-enriched food did not affect BMD in a group of early postmenopausal women. In a recent meta-analysis of 10 randomized controlled clinical trials (23), isoflavones at a dosage similar to ours, produced no change in BMD over a 1-y period in women.

To attempt to explain the divergent results, we hypothesized that those individuals who were able to produce the bioactive compounds (eg, equol) from isoflavones would show the largest benefit. After oral ingestion, isoflavone glucosides are hydrolyzed by small-intestinal β-glucosidases to produce aglycones. Aglycones may be absorbed intact or further fermented by intestinal microflora in the large intestine to yield equol (44). Equol is readily absorbed, enters the circulation, and is excreted in the urine (45). It is estimated that 30–50% of humans produce equol from soy isoflavones (46); a relatively high proportion of
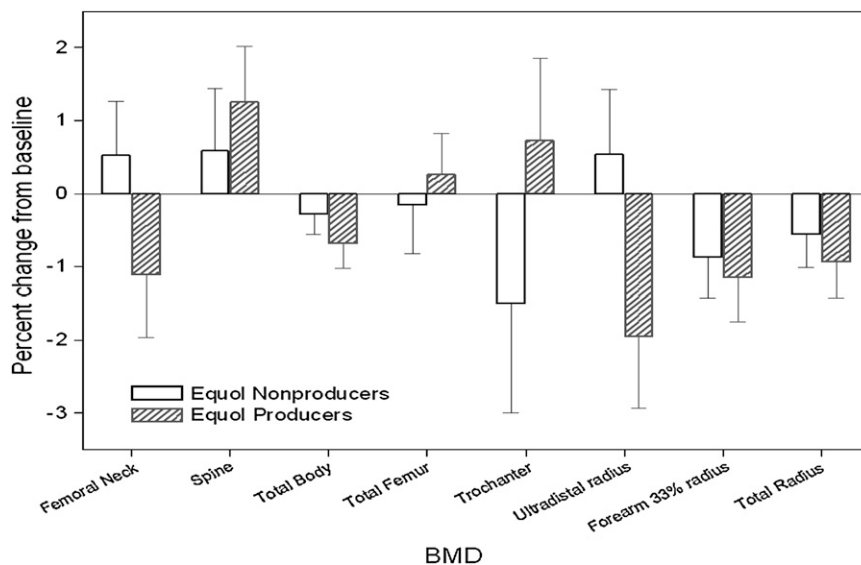

FIGURE 3. Mean (±SEM) percentage change in bone mineral density (BMD) between equol (*n* = 25) and non-equol (*n* = 26) producers among women who received the isoflavone tablets. There were no significant differences between equol and non-equol producers at any of the bone sites by an independent *t* test.
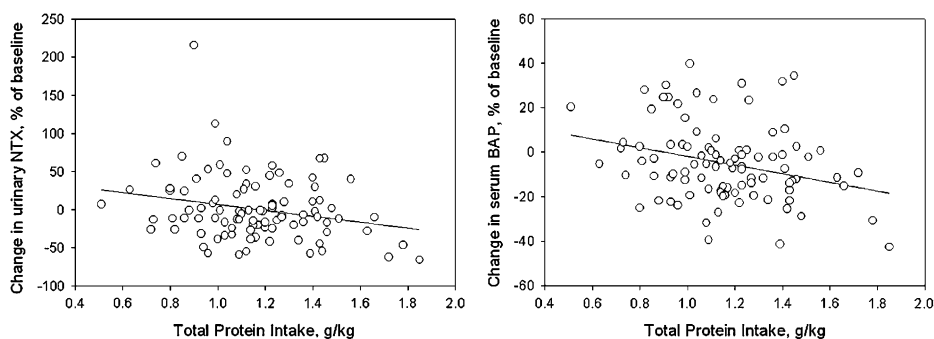
FIGURE 4. Significant correlations between total dietary protein and urinary *N*-telopeptide crosslinks of collagen normalized for creatinine (NTX) at 12 mo ($P = 0.023$, $r = -0.23$) and bone-specific alkaline phosphatase (BAP) ($P = 0.005$, $r = -0.28$) expressed as a percentage change from baseline in the 97 women who completed the study.

Chinese are equol producers. Setchell et al (47) showed that, in humans, *S*-equol has a high affinity for estrogen receptor β [$K(i) = 0.73$ nmol/L], whereas *R*-equol is far less active. Equol production may be associated with a reduced risk of chronic diseases (46). However, we were unable to find differences in BMD dependant on equol production (Figure 3). Perhaps the dosage of oral isoflavone was not high enough to raise serum isoflavones or equol to the level needed for a biological effect. Our oral dose of isoflavone was selected because it is at the higher range of what can be obtained from food alone.

Soy proteins are relatively low in the sulfur-containing amino acids (compared with meat proteins) and, as such, are hypothesized to be potentially beneficial to the calcium economy and the skeleton (48). Because fewer sulfur-containing amino acids are found in soy foods, the result is a slight decrease in urinary calcium (49), which was assumed to occur because of better retention of calcium in bone. However, our data do not support this contention because we did not observe differences in bone variables between the control protein mix from animal sources and the soy protein.

The data from all groups were pooled to evaluate the association between total dietary protein (from all sources) and changes in the markers of bone turnover. Indeed, inverse correlations were observed between total dietary protein and change in NTX/CRT and BAP (Figure 4). Regardless of the source of protein, higher protein intakes were associated with lower bone turnover—a process generally considered favorable to bone. Our cross-sectional observation is entirely consistent with intervention trials. For example, in a short-term dual-stable calcium isotope kinetic study, we (50) showed that increasing dietary protein does not increase bone resorption. In fact, during the high-protein diet, there was a significant reduction in the fraction of urinary calcium of bone origin and a trend toward a reduction in the rate of bone turnover. The trend toward a reduced bone turnover observed in the short term highly controlled clinical trial (50) may be applicable to free-living subjects under less-stringent experimental conditions. In any case, there was no evidence of an increase in bone turnover or skeletal catabolism in our subjects because they increased their overall protein intake from 63 g/d (baseline mean) to 80 g/d (12-mo mean).

This study had limitations. We experienced a 25% dropout rate, similar to other trials (16). The duration of the intervention was limited to 1 y. To address the adequacy of our sample size, we calculated a 95% CI for the variable where the *P* value of the 12-

mo change in BMD was the lowest. From Table 5, this happens for the main effect of soy protein on the change in total-radius BMD, $P = 0.056$. The 95% CI for the true effect of soy protein at this site is -0.00005 g/cm^2, 0.0082 g/cm^2. Thus, it is unlikely that soy protein increases total-radius BMD (or averts its decline) by >0.0082 g/cm^2. This represents a change of $\approx2\%$, which is well within the error of the measurement and would generally not be considered clinically significant. Therefore, it is unlikely, even with a larger sample size, that we could find a clinically meaningful effect of soy on BMD. Furthermore, we did not see any hints of change in the markers of bone turnover, which corroborates the stability of BMD. This study also had its strengths. We enrolled older women who are rarely studied in long-term nutrition trials and who are at high risk of bone loss. The 2×2 design allowed us to separate the potential effect of soy protein from that of soy isoflavones.

In summary, the addition of dietary soy protein and or isoflavone did not affect the change in BMD (or markers of bone turnover) over a 1-y period in a group of late postmenopausal women.

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