

The Soy Isoflavones for Reducing Bone Loss (SIRBL) Study: a 3-y randomized controlled trial in postmenopausal women¹⁻⁴

D Lee Alekel, Marta D Van Loan, Kenneth J Koehler, Laura N Hanson, Jeanne W Stewart, Kathy B Hanson, Mindy S Kurzer, and C Theodore Peterson

ABSTRACT

Background: Our previous study indicated that soy protein with isoflavones lessened lumbar spine bone loss in midlife women.

Objective: We examined the efficacy of isoflavones (extracted from soy protein) on bone mineral density (BMD) in nonosteoporotic postmenopausal women. We hypothesized that isoflavone tablets would spare BMD, with biological (age, body weight, serum 25-hydroxyvitamin D) and lifestyle (physical activity, dietary intake) factors modulating BMD loss.

Design: Our double-blind, randomized controlled trial (36 mo) included healthy postmenopausal women (aged 45.8–65.0 y) with intent-to-treat ($n = 224$) and compliant ($n = 208$) analyses. Treatment groups consisted of a placebo control group and 2 soy isoflavone groups (80 compared with 120 mg/d); women received 500 mg calcium and 600 IU vitamin D₃. Outcomes included lumbar spine, total proximal femur, femoral neck, and whole-body BMD.

Results: Analysis of variance for intent-to-treat and compliant ($\geq 80\%$) models, respectively, showed no treatment effect for spine ($P = 0.46$, $P = 0.21$), femur ($P = 0.86$, $P = 0.46$), neck ($P = 0.17$, $P = 0.14$), or whole-body ($P = 0.86$, $P = 0.78$) BMD. From baseline to 36 mo, BMD declined regardless of treatment. In intent-to-treat and compliant models, respectively, BMD decreases were as follows: spine (-2.08% , -1.99%), femur (-1.43% , -1.38%), neck (-2.56% , -2.51%), and whole body (-1.66% , -1.62%). Regression analysis (compliant model) indicated that age, whole-body fat mass, and bone resorption were common predictors of BMD change. After adjustment for these factors, 120 mg (compared with placebo) was protective ($P = 0.024$) for neck BMD. We observed no treatment effect on adverse events, endometrial thickness, or bone markers.

Conclusion: Our results do not show a bone-sparing effect of extracted soy isoflavones, except for a modest effect at the femoral neck. This trial was registered at clinicaltrials.gov as NCT00043745. *Am J Clin Nutr* 2010;91:218–30.

INTRODUCTION

Estrogen deficiency plays a key role in osteoporosis and other menopause-related chronic diseases. Estrogen therapy alleviates vasomotor symptoms (1) and prevents bone loss (2) but also increases the risk of uterine cancer (3), may increase the risk of coronary heart disease (4) and invasive breast cancer (5), and is often accompanied by side effects (6). Studies have examined the potential benefits of isoflavones, which are structurally similar to estrogen, because they exert estrogenic activity in human tissue (7, 8). Isoflavones are hypothesized to protect against chronic

diseases, such as osteoporosis, breast cancer, and cardiovascular disease (9). Clinical studies in postmenopausal women worldwide have examined the effect of soy food, soy protein isolate, or isoflavone tablets on bone mineral content (BMC) and bone mineral density (BMD) or bone turnover markers. Results have been inconclusive because of various study designs, treatment dose or type (food compared with isolate compared with tablets), or subject characteristics, with few studies of sufficient duration to determine the long-term efficacy of isoflavones on bone. Support for a bone-protective effect of isoflavone-containing soy is intriguing but speculative at this time.

Our laboratory (10) showed that 6 mo of isoflavone-rich (80 mg/d), but not isoflavone-poor (4 mg/d), soy protein (40 g) isolate attenuated lumbar spine bone loss in 69 perimenopausal women. Lumbar spine BMD in the 80- or 4-mg/d group, respectively, did not change (-0.2% , $P = 0.7$, or -0.7% , $P = 0.1$), but loss occurred in controls (-1.3% , $P = 0.004$). Regression analysis revealed that isoflavones (80 mg/d), not soy protein, exerted the protective effect, given the effect on change in BMD (5.6%, $P = 0.023$) and BMC (10.1%, $P = 0.0032$). Some studies in postmenopausal

¹ From the Nutrition and Wellness Research Center, Department of Food Science and Human Nutrition (DLA, LNH, JWS, and KBH) and the Department of Statistics (KJK and CTP), Iowa State University, Ames, IA; the US Department of Agriculture, Agricultural Research Service, Western Human Nutrition Research Center, University of California, Davis, CA (MDVL); and the Department of Food Science and Nutrition, University of Minnesota, St Paul, MN (MSK).

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⁴ Address correspondence to DL Alekel, Nutrition and Wellness Research Center, Iowa State University, Research Park, Building 6, 2325 North Loop Drive, Suite 6100, Ames, IA 50010-8281. E-mail: alekel@iastate.edu.

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women have shown a modest bone-sparing effect with habitual soy food intake (11–13), isoflavone-rich soy protein isolate (10, 14), and isoflavone supplements (15, 16) or an effect on bone formation markers (15, 17), whereas others have reported no effects (18–21). The estrogen-like effect of soy isoflavones has also given rise to safety concerns (22, 23). Furthermore, researchers (24) have proposed that the greatest benefits may be in subjects whose intestinal bacteria degrade daidzein, which is a primary soy isoflavone, into equol, which is a highly active metabolite. Thus, we considered equol production in the examination of the effect of isoflavones.

We hypothesized that soy isoflavone tablets, taken for 36 mo, would decrease BMD loss at the lumbar spine and proximal femur in postmenopausal women. Furthermore, bone sparing would be modulated by biological (age, body weight, vitamin D status) and lifestyle (physical activity, dietary intake) factors and equol metabolism. We posited that bone sparing in isoflavone-treated groups might be reflected by preservation of bone formation [bone-specific alkaline phosphatase (BAP)] without change in bone resorption [cross-linked C-terminal telopeptide of type I collagen (CTX)], but treatment would not stimulate the endometrium or augment adverse events.

SUBJECTS AND METHODS

Study design

The primary objective of our study [Soy Isoflavones for Reducing Bone Loss (SIRBL)] was to determine the 3-y effect of 2 doses (80 and 120 mg/d) of isoflavones extracted from soybeans on lumbar spine and total proximal femur BMD in at-risk postmenopausal women. A secondary objective was to account for potential confounding factors (biological and lifestyle), as well as bone turnover via biochemical bone markers, in the modulation of bone loss in nonosteoporotic women. Healthy postmenopausal women aged 45.8–65.0 y were enrolled in our prospective, randomized, double-blind, placebo-controlled multicenter [Iowa State University (ISU), Ames, IA; University of California at Davis (UCD), Davis, CA; and University of Minnesota, St Paul, MN (analysis site)], National Institutes of Health–funded clinical trial. We collected data from subjects at baseline and at 6, 12, 24, and 36 mo from 2003 to 2008.

Our study protocol, consent form, and all subject-related materials were approved by the respective Institutional Review Boards at ISU (ID 02-199) and UCD (ID 200210884-2). Approvals for the dual-energy X-ray absorptiometry (DXA) procedures were obtained from each institution's Institutional Review Board and the State Department of Public Health in Iowa and California. At prebaseline, each woman was provided a written description and verbal explanation before we obtained signed informed consent.

Subject screening and selection

We recruited subjects (2003–2005) from the state of Iowa and the greater Sacramento and Bay Area regions in northern California, primarily through direct mailing lists, stories in local newspapers, and local/regional radio advertisements. Women who responded ($n = 5255$) to outreach materials were screened initially via telephone to identify healthy women aged ≤ 65 y who

had undergone natural menopause (cessation of menses 1 through 8 y), were not experiencing excessive vasomotor symptoms, were nonsmokers, and had a body mass index (BMI; in kg/m^2) from 18.5 through 29.9. We excluded vegans because they would likely be soy food consumers, and women who were high alcohol consumers (>7 servings/wk) because alcohol interferes with hormone metabolism (25). We excluded women diagnosed with chronic disease, who had a first-degree relative with breast cancer, or who used medication chronically (current: cholesterol-lowering or antihypertensive medications; past 12 mo: oral hormones/estrogen or selective estrogen receptor modulators; past 6 mo: estrogen/progestogen creams, calcitonin; past 3 mo: antibiotics; ever: bisphosphonates).

Women who met the initial screening criteria ($n = 677$) were invited to the clinic for further eligibility assessment (Figure 1). We measured height and weight to confirm BMI; to assess BMD eligibility, we used a Delphi-W QDR DXA bone densitometer (Hologic Inc, Bedford, MA). Because the SIRBL project focused on disease prevention rather than treatment, we excluded women with BMD lumbar spine and/or proximal femur T scores that were low (>1.5 SD below young adult mean) or high (>1.0 SD above mean) and those with evidence of previous or existing spinal fractures. Once the woman qualified on the basis of BMD, blood was drawn for a chemistry profile. We excluded women with evidence of diabetes mellitus [fasted blood glucose ≥ 6.93 mmol/L (126 mg/dL)]; abnormal renal, liver, and/or thyroid function; or abnormal lipid profile [LDL cholesterol >4.10 mmol/L (160 mg/dL); triacylglycerol >2.25 mmol/L (200 mg/dL)]. On the basis of our entry criteria, we randomly assigned 255 subjects to receive treatment.

Each subject obtained a signed medical release form from her personal physician; she was also required to complete an annual physical as well as a mammogram and breast and gynecologic examinations. We monitored endometrial thickness by using transvaginal ultrasonography at baseline and at 12 and 36 mo. We excluded women at baseline with endometrial thickness >5.0 mm, except for those with values of 5.0–6.0 mm who underwent an endometrial biopsy that proved normal. At baseline, 11 women at UCD who had endometrial thickness >5.0 mm were inadvertently started on treatment, but were instructed to cease treatment, because of the safety concern of isoflavone exposure. These women were invited to continue with follow-up safety examinations throughout the study. Nine women at UCD had BMI values beyond our inclusion criteria (1 woman: <18.5 ; 8 women: >30.0), 4 at UCD did not meet the criterion for time since last menstrual period [TLMP (current age – menopausal age)]; 1 woman: <1 y; 3 women: >8 y], and 2 (1 at UCD and 1 at ISU) had a lumbar spine BMD T score slightly below (-1.6) or above ($+1.1$) our cutoff (-1.5 to $\leq +1.0$). Our Data and Safety Monitoring Board (DSMB) granted waivers that sanctioned continued treatment of women with values beyond our criteria for BMI, TLMP, and BMD.

Subject randomization and treatment

To balance treatment allocation with respect to factors that may have influenced response to treatment, subjects at each location (ISU, UCD) were stratified according to initial total proximal femur BMD (high, medium, low) on the basis of the third National Health and Nutrition Examination Survey database population values (26): 1) high: greater than the mean (zero) but less than or equal to $+1.0$ SD above the mean; 2) medium: less than or equal to the

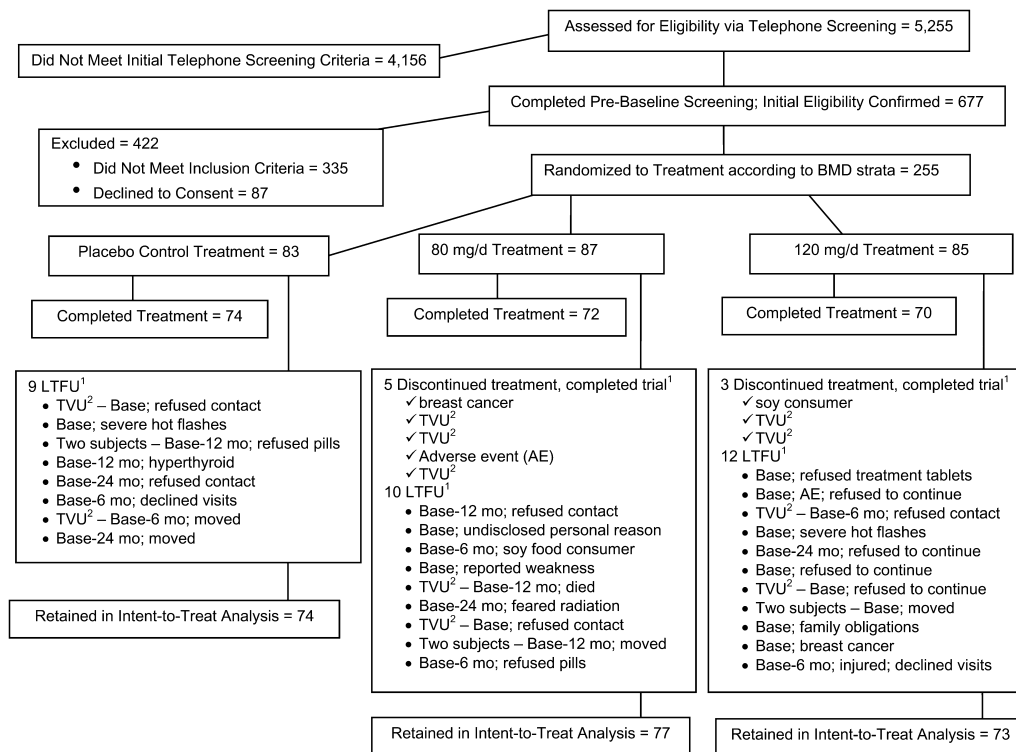


FIGURE 1. Study participant CONSORT (Consolidated Standards of Reporting Trials) diagram. ¹Altogether, 8 women discontinued treatment but completed the trial (1 at Iowa State University, 7 at University of California at Davis), and 31 women were lost to follow-up (LTFU; 2 at Iowa State University, 29 at University at California at Davis). Diagram indicates the last time point [baseline (base) or 6, 12, 24 mo] for each woman who was LTFU and reasons provided for discontinuing. ²Eleven subjects at University of California at Davis had thickened endometria [(determined by transvaginal ultrasound (TVU))] and thus did not meet inclusion criterion (≤ 5 mm). BMD, bone mineral density.

mean, but greater than -0.75 SD below the mean; or 3) low: less than -0.75 SD through -1.5 SD below the mean. Once subjects met inclusion/exclusion criteria, they were randomly assigned to 1 of 3 treatment groups within each BMD strata at each location: 1) placebo control, 2) 80 mg isoflavones, or 3) 120 mg isoflavones. Placebo material that was devoid of isoflavones consisted of maltodextrin (90%) and caramel color (10%), which were mixed and spray dried to produce a brown powder, which mimicked the isoflavone extract. Active tablets contained the same excipients as placebo tablets. All treatment tablets (identical in appearance) contained the same amounts of sorbitol, magnesium stearate, and dicalcium phosphate. The ratio of genistein to daidzein to glycitein (aglycone form) in these tablets was 1.3:1.0:0.3, which was similar to that shown in soybeans. An independent researcher (Patricia Murphy) at ISU confirmed that the actual and formulated isoflavone doses (mean \pm SD) were similar to those tested by the Archer Daniels Midland Co (Decatur, IL): control = 0 compared with 0.3 ± 0.4 mg; 80 mg = 89.5 ± 5.0 compared with 84.3 ± 4.5 mg; 120 mg = 124.0 ± 7.7 compared with 122.5 ± 3.4 mg for actual and formulated doses, respectively. Subjects in each group were instructed to take 3 compressed tablets/d. Bottles did not indicate treatment assignment, to preserve the double-blind nature of the study.

To minimize potential individual differences in dietary intake and in sun exposure across treatment groups, and to ensure adequate intake of calcium and vitamin D, we provided a daily calcium (500 mg) and vitamin D₃ (200 IU) supplement (GlaxoSmithKline, Moon Township, PA), with an additional

vitamin D₃ (400 IU) supplement (Pharmavite LLC, Northridge, CA). Subjects were instructed to consume one or more dietary sources of calcium, which provided a total of ≥ 600 mg from diet but not more than one calcium-fortified food/d. The intent was to approximate the Dietary Reference Intake guidelines (27) of 1200 mg for women aged 51–70 y.

Interviewer-administered, validated health-related questionnaires

To verify the health status of each subject and obtain health-related data, at prebaseline we used a health and medical history questionnaire (10, 28). We also gathered data on prescription and over-the-counter medications (indication, dose, unit, frequency, duration, route of administration) at each time point, as well as previous and/or present use of dietary supplements (which they were asked to discontinue). Each woman was asked questions about menstrual history (age at menarche and date of final period), previous use of estrogen or hormone therapy, and pregnancy and lactation history with a reproductive history questionnaire (29, 30). To account for habitual soy food consumption, as well as to verify avoidance of soy foods during the trial, we used a soy food questionnaire (31).

Monitoring adverse events

All outcomes and adverse events were monitored semi-annually by our National Institute of Arthritis and Musculoskeletal and Skin Diseases–appointed DSMB. Serious adverse

events were documented and details were provided to our DSMB and respective Institutional Review Board committee within 24 h of subject notification. We monitored adverse events by using a health status update form, chemistry profile, and complete blood count at each visit; transvaginal ultrasounds at 12 and 36 mo; and excessive bone loss (considered an adverse event if cumulative bone loss was $\geq 8\%$ at 12 mo, $\geq 10\%$ at 24 mo, or $\geq 12\%$ at 36 mo). Any abnormality noted on the woman's physical examination report constituted an adverse event. Adverse events were also defined as any self-reported health event that required treatment or resulted in a new diagnosis or new/worsened signs or symptoms, such as blood clots, vaginal bleeding, dizziness, headaches, insomnia, or fatigue. We required follow-up with our consulting physician if change was noted in her transvaginal ultrasound scan, such as a thickened endometrium (>5 mm) or 300% increase from baseline, or increase in size or development/appearance of a polyp, fibroid, or cyst.

Anthropometric measures

A trained research assistant assessed anthropometric measures for each subject. Body weight (to the nearest 0.1 kg) was measured with women wearing minimal clothing, with the use of a balance beam scale (abco Health-o-meter; Health-o-meter Inc, Bridgeview, IL) at ISU and an electronic scale (Circuits and Systems Inc, E. Rockaway, NY) at UCD. Standing height without shoes, and sitting height with women seated on a stool 44 cm high, were measured (both to the nearest 0.1 cm) with the use of a wall-mounted stadiometer (Ayrton stadiometer, model S100; Ayrton Corp, Prior Lake, MN). Weight and standing height measures were used to calculate BMI.

Bone mineral measurements

At each time point we assessed whole-body, posterior-anterior lumbar spine, and total proximal femur BMC (g) and areal BMD (g/cm^2) as well as body-composition (whole-body lean mass and fat mass) measurements. We used matching DXA instruments (Delphi W, Hologic) at each location to ensure that the instruments provided comparable results, and performed daily calibration with the use of a spine phantom. One certified DXA operator at ISU and another at UCD performed all DXA scans, with operator cross-training between locations to ensure comparable quality control. The within-subject in vivo CV at ISU and UCD, respectively, for areal BMD was 1.1% and 0.9% at the spine, 0.7% and 0.8% at the hip, and 0.8% and 1.0% for the whole body. We standardized subject placement for the scans and adhered to the manufacturer's recommendations. To assess lumbar spine and proximal femur BMD, as well as overall BMD and body composition from the whole-body DXA scans, the ISU DXA operator analyzed all DXA scans (software version 12.3:7) based on Hologic guidelines.

Biological samples

Phlebotomists collected fasted (9 h) blood samples between 0700 and 0800 h. We separated serum from whole blood, centrifuged for 15 min (4°C) at $1300 \times g$, and stored aliquots at -80°C until analyzed. We measured serum concentrations of 25-hydroxyvitamin D [25(OH)D] and markers of bone resorption (CTx) and bone formation (BAP), as well as urinary minerals (calcium, phosphorus, magnesium, sodium, potassium), as po-

tential covariates in modeling the bone-related outcomes. Certified clinical laboratories (LabCorp, Kansas City, KS, for ISU; UCD Medical Center, Sacramento, CA, for UCD) analyzed blood samples for general health markers, which included the complete blood count with differential, chemistry panel, and thyroid screen.

We measured serum analytes from ISU and UCD samples for each subject in duplicate in batch at ISU. We collected sufficient in-house serum as quality-control samples (frozen at -80°C) to run with each kit to calculate interassay CVs; we used duplicate serum samples to calculate intraassay CVs. The low-to-normal and normal-to-high controls for each kit [25(OH)D, CTx, BAP] were well within the acceptable ranges. The R^2 values were 0.9998 for serum 25(OH)D, 0.9983 for CTx, and 0.9990 for BAP. Serum 25(OH)D concentration was measured with radioimmunoassay kits [Diasorin 25(OH) vitamin D ^{125}I radioimmunoassay; Diasorin Inc, Stillwater, MN] with the use of a Cobra II series auto-gamma counting system (PerkinElmer Life and Analytic Sciences, Meriden, CT). Serum 25(OH)D intra- and interassay CVs were 2.71 and 2.69, respectively. Serum CTx concentration was measured with an enzyme-linked immunosorbent method (serum CrossLaps ELISA) in accordance with the manufacturer's guidelines (Nordic Bioscience Diagnostics, Herlev, Denmark). Serum CTx intra- and interassay CVs were 2.23 and 2.63, respectively. Serum BAP concentration was measured with the use of a monoclonal antibody by a solid phase enzyme-linked immunosorbent assay method (Metra BAP) in accordance with the manufacturer's (Quidel Corporation, Hanover, Germany) guidelines. Serum BAP intra- and interassay CVs were 1.33 and 1.50, respectively. Samples for CTx and BAP were read with the use of an automated microtiter plate reader (ELx808U with KC Junior software, version 1.14; BioTek Instruments, Winooski, VT).

Urine samples (24 h) were collected at each visit in polypropylene containers and kept cold (4°C) until each subject's sample was processed and volume recorded. Three aliquots were frozen for mineral, creatinine, and isoflavone analyses. For mineral analysis, 10 mL urine was acidified with 0.05 mL of concentrated trace metal grade HCl (SeaStar Chemicals Inc, Sidney, Canada). Urinary minerals from ISU and UCD samples were measured in batch at UCD. Acidified urine samples were centrifuged ($1000 \times g$ at 4°C) for 10 min with the use of Allegra 6R (Beckman Coulter Inc, Palo Alto, CA) to remove solid material, and diluted with 1.0 N nitric acid (trace metal grade; Fisher Scientific, Pittsburgh, PA). Urine was diluted 1500-fold for sodium, potassium, and phosphorus and 150-fold for calcium and magnesium. Concentrations of minerals were measured by inductively coupled plasma atomic emission spectroscopy (Varian Analytic Instruments, Walnut Creek, CA). Nonacidified urine aliquots were stored for creatinine analysis. Creatinine was measured with the use of the Hitachi 902 clinical chemistry analyzer (Hitachi, Tokyo, Japan). For the isoflavone aliquot, 1 mL 5% vol:vol of preservative sodium azide (FisherBiotech, Fair Lawn, NJ) was added per 50 mL urine and stored at -70°C .

From 24-h urine samples, the University of Minnesota measured daidzein and equol (and genistein, glycitein, *O*-desmethylangolensin, dihydrodaidzein reported elsewhere), analyzed for a given subject in the same batch, by liquid chromatography/mass spectrometry via a modified method (32). Liquid chromatography/tandem mass spectrometry analysis was performed on an Applied Biosystems (ABI, Foster City, CA) Qtrap 2000 triple quadrupole linear ion trap system with the use of a Phenomenex

Synergi Max-RP 80A column. Formononetin was added as an internal standard. Samples were hydrolyzed overnight at 37°C with 0.5 mol sodium acetate buffer/L and β -glucuronidase; samples were extracted thrice with ethyl ether and then evaporated to dryness at 40°C under nitrogen. For each isoflavone, the lowest detectable concentration was 0.47 ng/mL; intra- and interbatch CVs were 2.4–7.1 and 14.2–16.1, respectively.

Compliance

We evaluated treatment compliance by calculating the difference between the number of tablets provided and tablet counts returned by each woman at each visit. To classify subjects as either compliant ($\geq 80\%$) or noncompliant ($< 80\%$), we used the 36-month cumulative percentage compliance. Urinary isoflavone concentrations verified compliance but did not classify subjects into “compliant” and “noncompliant” groups on the basis of isoflavone excretion because of its relatively large interindividual variability. However, we used urinary equol to account for variability in isoflavone metabolism in modeling BMD outcomes.

Statistical power and analyses

Power analysis for the intent-to-treat model included 224 women (control: $n = 74$; 80 mg/d: $n = 77$; 120 mg/d: $n = 73$), with blocking by location (ISU, UCD) and prebaseline proximal femur BMD strata (high, medium, low). For lumbar spine BMD, our study had power of 0.94 to detect differences at the 0.05 level (F test), if the mean percentage decrease in spine BMD for the control group actually exceeded that for the high-dose group by 2 and exceeded that for the low-dose group by 1.5. Likewise, for total proximal femur BMD, our study had power of 0.99 to detect similar treatment effects at the 0.05 level (F test).

Statistical analyses were performed with the use of SAS software (version 9.1; SAS Inc, Cary, NC) with results considered statistically significant (2-sided) at $P \leq 0.05$. Descriptive statistics with 255 women at baseline included median (lower, upper quartile) values for all data, because the outcome data and most variables were not normally distributed. The primary analysis was intent-to-treat (33), which included all data from all women who had a follow-up BMD at 36 mo ($n = 224$), regardless of treatment compliance. Percentage change in BMD (primary outcomes) of the lumbar spine, total proximal femur, femoral neck, and whole body at 36 mo relative to baseline was determined for each woman. The effect of treatment on bone is typically greater in those with lower bone mass (34), and hence it is important to consider baseline BMD. Potential treatment effects on these 4 response variables were analyzed with repeated-measures analysis of variance with the use of the GLM and MIXED procedures. Baseline proximal femur BMD strata (high, medium, low) within each location (ISU, UCD) were incorporated as blocking variables in the statistical analyses. Tests for parallel profiles (based on Wilks' lambda criterion), to determine whether treatment differences are consistent across all time points, were run for percentage change in lumbar spine, total proximal femur, femoral neck, and whole-body BMD, as presented graphically.

Secondary analyses included all women from the intent-to-treat analysis who were protocol compliant ($\geq 80\%$, on the basis of her cumulative percentage compliance) and for whom we had complete data for variables included in the models. One subject

at UCD did not have a baseline blood sample for in-house assays and thus did not remain in the regression analysis for compliant women ($n = 208$). Secondary analyses assessed the effects of treatment after adjustment for covariates that might influence the response of BMD (percentage change from baseline to 36 mo) to treatment. Each model included as obligatory variables location, BMD strata, and treatment. The ISU location effect was part of each model intercept, whereas the UCD location effect was indicated separately. Classes of variables in modeling the outcomes of interest included independent variables that were biologically plausible that we hypothesized would be related to the outcomes of interest. These included age [or TLMP or estrogen exposure (age at menopause – age at menarche)] at baseline; mean values (across 5 time points for each subject) for weight (or whole-body lean or fat mass); serum concentrations of 25(OH)D, CTx, and BAP; physical activity; dietary intake (calcium, phosphorus, magnesium, protein); urinary minerals (calcium, phosphorus, magnesium, sodium, potassium); and mean values (across 4 time points, after baseline, for subjects in isoflavone groups) for urinary equol excretion. We used sequential multiple regression analyses to assess the combined contribution of these variables to percentage change in each BMD outcome. Model selections were guided by the all possible model selection option (Akaike's information criterion and Schwarz's information criterion) in the SAS regression procedure, which provided the best predictive model for each outcome. We also removed variables that exhibited multicollinearity as indicated by a variance inflation factor > 10 (35). Residual diagnostics revealed no serious violations of standard regression model assumptions.

RESULTS

Enrollment and retention of participants

Among the 677 women who completed prebaseline screening and met initial eligibility, 37.7% met the criteria that remained and were randomly assigned to treatment at each location (122 at ISU, 133 at UCD) within each proximal femur BMD strata (high, medium, low). Among the 255 women randomly assigned to treatment, the median (lower, upper quartile values) initial total proximal femur BMD T score was -0.3 ($-0.7, 0.2$); T score was 0.4 ($0.2, 0.7$) in high, -0.3 ($-0.6, -0.1$) in medium, and -1.0 ($-1.2, -0.8$) in low BMD strata. The median initial lumbar spine BMD T score was -0.6 ($-1.1, 0$). Altogether, 31 women (12.2%) were lost to follow-up (2 at ISU, 29 at UCD), and 8 women (3.1%) discontinued treatment but completed the trial (1 at ISU, 7 at UCD). The 11 subjects at UCD (8.3%) who had thickened endometria (> 5 mm, thus they did not meet inclusion criterion) were removed from treatment; 5 of these 11 women completed study visits. Altogether, 224 women (intent-to-treat) were retained (120 at ISU, 104 at UCD), and 216 remained on treatment (119 at ISU, 97 at UCD) for 36 mo. Among intent-to-treat subjects ($n = 224$), 68 were in the high (35 at ISU, 33 at UCD), 98 were in the medium (56 at ISU, 42 at UCD), and 58 were in the low (29 at ISU, 29 at UCD) BMD strata. Subjects in treatment groups for intent-to-treat and compliant models, respectively, were as follows: $n = 74$ and 72 in the control group; $n = 77$ and 67 in the 80-mg/d group; $n = 73$ and 69 in the 120-mg/d group.

TABLE 1
Characteristics of participants at baseline (*n* = 255)¹

Characteristic	Study group			Total percentage
	Control (<i>n</i> = 83)	80 mg (<i>n</i> = 87)	120 mg (<i>n</i> = 85)	
Age (y)	54.2 (51.8, 56.4) ²	54.3 (52.7, 56.8)	54.7 (52.0, 56.9)	—
Time since menopause (y) ³	2.7 (1.9, 5.3)	3.0 (1.8, 5.2)	2.8 (1.7, 4.8)	—
Race (<i>n</i>)				
Asian	3	1	1	2
Native Hawaiian or Pacific Islander	1	0	0	<1
American Indian or Alaskan	1	0	0	<1
Black or African American	0	1	2	1
White	76	79	79	92
More than one race	2	3	2	3
Unknown or not reported	0	3	1	2
Ethnicity (<i>n</i>)				
Hispanic or Latino	3	7	2	5
Not Hispanic or Latino	80	80	83	95
Highest level of education (<i>n</i>)				
High school	13	15	5	13
College	43	40	50	52
Post college	27	32	30	35
Family history of osteoporosis (<i>n</i>)				
No	51	59	54	64
Yes	27	25	26	31
Don't know	5	3	5	5
Previous bone fracture (<i>n</i>)				
No	55	54	56	65
Yes, impact related	27	29	23	31
Yes, not impact related	0	3	5	3
Yes, don't know if impact related	1	1	1	1
Weight (kg)	66.1 (61.0, 74.3)	68.7 (59.8, 74.7)	66.6 (60.4, 73.7)	—
Standing height (cm)	165.6 (161.3, 168.3)	164.6 (158.8, 167.7)	165.6 (161.8, 169.8)	—
BMI (kg/m ²) ⁴	24.3 (22.1, 26.8)	25.0 (23.0, 27.8)	24.6 (22.4, 26.9)	—
Sitting height (cm) ⁵	133.2 (130.9, 135.0)	131.9 (129.9, 134.0)	132.7 (130.5, 134.8)	—
Whole-body lean mass (kg) ⁶	42.7 (39.7, 46.0)	43.2 (40.0, 46.2)	43.3 (40.6, 46.2)	—
Whole-body fat mass (kg) ⁶	22.3 (18.1, 28.4)	23.6 (18.8, 27.2)	22.6 (18.0, 26.9)	—
Whole-body fat (%) ⁶	35.4 (30.9, 38.8)	34.8 (31.7, 38.0)	34.2 (29.2, 38.3)	—
Serum 25(OH)D (nmol/L) ^{7,8}	70.59 (53.04, 82.34)	66.10 (51.93, 83.77)	71.42 (54.11, 83.96)	—
Serum CTx (ng/mL) ^{7,9}	0.69 (0.51, 0.88)	0.80 (0.59, 1.04)	0.84 (0.58, 1.07)	—
Serum BAP (U/L) ^{7,10}	33.36 (28.53, 37.62)	34.01 (29.79, 39.20)	33.42 (27.71, 38.87)	—
Urinary calcium (mmol/d) ⁷	4.3 (2.9, 6.1)	4.5 (3.2, 5.8)	4.2 (2.9, 6.0)	—
Urinary magnesium (mmol/d) ⁷	3.3 (2.4, 4.4)	3.4 (2.8, 4.4)	3.6 (2.9, 4.3)	—
Urinary phosphorus (mmol/d) ⁷	23.5 (18.3, 28.0)	24.2 (19.6, 29.0)	24.3 (18.8, 30.1)	—
Urinary potassium (mmol/d) ⁷	49.3 (38.8, 63.2)	52.3 (40.6, 66.5)	51.7 (41.3, 65.4)	—
Urinary sodium (mmol/d) ⁷	112.8 (84.2, 147.3)	112.3 (83.6, 146.0)	109.3 (81.5, 142.3)	—

¹ 25(OH)D, 25-hydroxyvitamin D; CTx, cross-linked C-terminal telopeptide of type I collagen; BAP, bone-specific alkaline phosphatase. No statistically significant differences between treatment groups at baseline were noted.

² Median; upper, lower quartile values in parentheses (all such values).

³ Calculated for each woman by subtracting the date of her last menstrual period from her baseline test date. Four women at the University of California at Davis (UCD) did not meet the range (1–8 y) of the inclusion criterion: 1 in the control group, 2 in the 80-mg/d group, and 1 in the 120-mg/d group, with values of 0.8 y, 0.9 and 8.2 y, and 10.0 y, respectively.

⁴ At baseline, 9 women at UCD had BMI values (17.8–32.7) that did not meet the range of the inclusion criterion: 4 in the control group, 4 in the 80-mg/d group, and 1 in the 120-mg/d group.

⁵ One woman at UCD did not have sitting height measured at baseline.

⁶ Body composition [whole-body lean and fat mass (kg and %)] was determined by dual-energy X-ray absorptiometry.

⁷ One woman was missing a blood sample and another a urine sample at UCD at baseline.

⁸ The manufacturer of the 25(OH)D kit (Diasorin Inc, Stillwater, MN) indicated the expected range in the population to be 22.5–93.8 nmol/L (mean: 57.4 nmol/L).

⁹ The manufacturer of the CTx kit (Nordic Bioscience Diagnostics, Herlev, Denmark) indicated the expected range in postmenopausal women to be 0.14–1.35 ng/mL (mean: 0.44 ng/mL).

¹⁰ The manufacturer of the BAP kit (Metra BAP; Quidel Corporation, Hanover, Germany) indicated the expected range in postmenopausal women to be 14.2–42.7 U/L (median: 25.0 U/L).

Compliance

Compliance was excellent in women who remained on treatment ($n = 216$), with 209 [96.8% (117 at ISU and 92 at UCD)] of 216 women who achieved $\geq 80\%$ compliance (cumulative). We noted no difference in median compliance values across the 3 treatment groups ($P = 0.49$) or across strata within location ($P = 0.39$). Median (lower, upper quartile values) compliance was significantly ($P \leq 0.0001$) higher at ISU [98.0% (94.0, 99.6%)] than at UCD [95.2% (88.0, 98.2%)]. Median compliance for control ($n = 74$) was 97.0% (93.1, 99.2%), 80 mg/d ($n = 72$) was 96.1% (91.3, 98.6%), and 120 mg/d ($n = 70$) was 97.2% (91.8, 99.3%). Median values (baseline to 36 mo) for urinary daidzein (nmol/d) did not change (302–195) in the control group, increased from 208 to 8706 in the 80-mg/d group ($P \leq 0.0001$), and from 304 to 11,411 in the 120-mg/d group ($P \leq 0.0001$), consistent with excellent compliance. Similar patterns were noted in the excretion of other urinary isoflavone metabolites between the treatment groups.

Characteristics of participants

Descriptive characteristics and data are presented in **Table 1**. We showed no statistically significant differences between the treatment groups at baseline for any of these variables. Women ranged in age from 45.8 to 65.0 y, and TLMP ranged from 0.8 to 10.0 y. We enrolled predominantly white (92%) women, despite

our efforts to enroll women from all racial-ethnic backgrounds (self-reported as defined by the National Institutes of Health). At baseline, BMI values ranged from 17.8 to 32.7; approximately half of the women had values < 25 . Median weight and height remained stable over time, as did BMI, whole-body lean mass, and whole-body fat mass. Whole-body fat mass as assessed by DXA indicated wide variability among these women. Regardless of bone site, BMD declined from baseline through 36 mo in each treatment group (**Figure 2**).

Serum and urinary analytes

Serum analytes and urinary minerals are presented in Table 1, with no significant effect of treatment and no change over time for serum analytes [except 25(OH)D] or urinary minerals. Median values for CTx and BAP were within the acceptable range reported by the manufacturers, but minimal and maximal values were beyond the lower and upper limits for CTx and beyond the upper limit for BAP. We report total serum 25(OH)D [25(OH)D₂ + 25(OH)D₃] because clinical decisions are based on total values (36). The median value for 25(OH)D concentration at baseline was below the desirable level (≥ 75.0 nmol/L) (37), with many subjects being either insufficient or deficient at baseline (38), although values improved with supplementation. Urinary mineral excretion varied widely, particularly urinary potassium and sodium, which reflects the wide variability in dietary intake of these minerals.

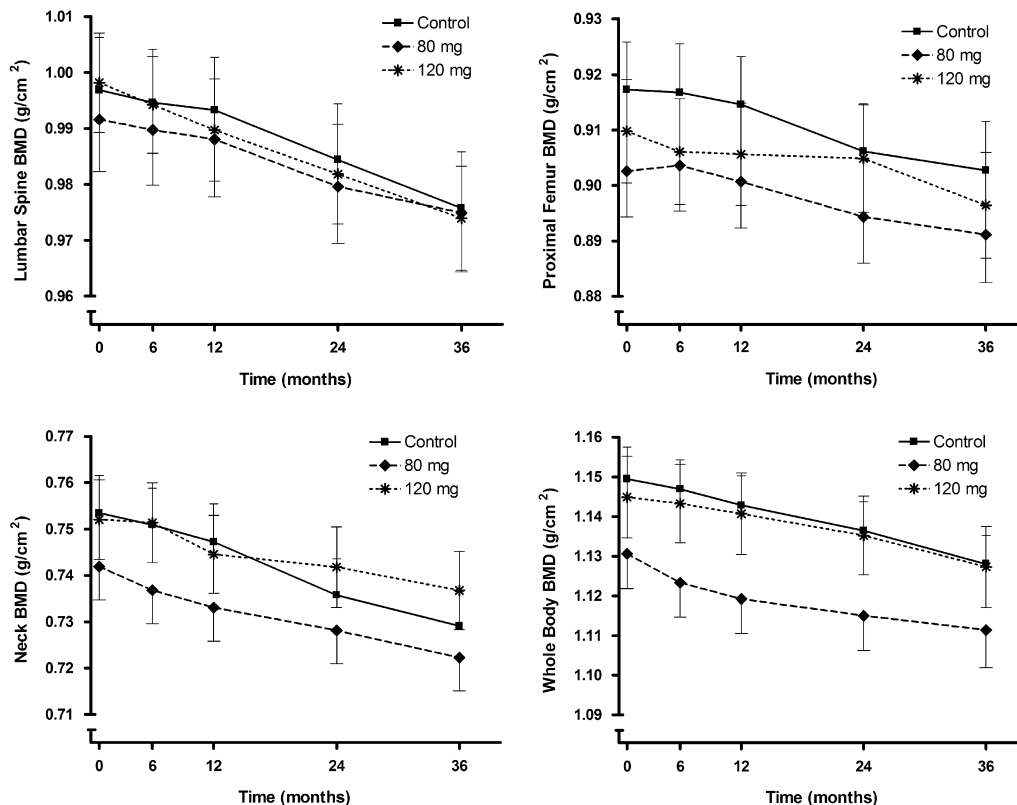


FIGURE 2. Parallel profile plot for intent-to-treat women ($n = 224$) with mean (\pm SEM) values for lumbar spine, total proximal femur, femoral neck, and whole-body bone mineral density (BMD) at each time point (baseline and 6, 12, 24, and 36 mo) for each treatment group: control, $n = 74$; 80 mg/d, $n = 77$; 120 mg/d, $n = 73$. Tests for parallel profiles for lumbar spine (Wilks' = 0.981, $P = 0.85$), neck (Wilks' = 0.951, $P = 0.21$), and whole-body (Wilks' = 0.962, $P = 0.38$) BMD indicated no interaction between treatment and time. A test for parallel profile for proximal femur BMD (Wilks' = 0.926, $P = 0.030$) indicated an interaction between treatment and time.

Effect of soy isoflavones and other factors on BMD

On the basis of the intent-to-treat ($n = 224$; **Table 2**) and compliant ($n = 208$) models, respectively, BMD declined, regardless of treatment, from baseline to 36 mo: lumbar spine (-2.08% , -1.99%), total proximal femur (-1.43% , -1.38%), femoral neck (-2.56% , -2.51%), and whole body (-1.66% , -1.62%). For the intent-to-treat analysis, tests for parallel profiles did not reveal any significant inconsistencies in treatment effects across time for lumbar spine (Wilks' = 0.981, $P = 0.85$), femoral neck (Wilks' = 0.951, $P = 0.21$), and whole-body (Wilks' = 0.962, $P = 0.38$) percentage change in BMD (Figure 2). The test for parallel profiles for total proximal femur BMD (Wilks' = 0.926, $P = 0.030$) indicated that there was an interaction between treatment and time, which signifies that the treatment differences were not consistent across all time points. Results from the compliant analysis were remarkably similar (graph not shown): tests for parallel profile plots and criteria for lumbar spine (Wilks' = 0.971, $P = 0.65$), femoral neck (Wilks' = 0.946, $P = 0.18$), and whole-body (Wilks' = 0.961, $P = 0.41$) BMD indicated that there was no interaction between treatment and time, which signifies that the treatment differences were consistent across all time points. The test for parallel profiles for total proximal femur BMD (Wilks' = 0.926, $P = 0.048$) indicated that there was an interaction between treatment and time, which signifies that the effect of treatment was not the same for all time points. However, the overall response (percentage change from baseline to 36 mo) of lumbar spine, total proximal femur, proximal neck, and whole-body BMD to soy isoflavone treatment in the intent-to-treat model was not significant, as indicated by P values that ranged from 0.17 to 0.86 (Table 2). Location (UCD) exerted a significant effect for lumbar spine ($P = 0.02$), femoral neck ($P = 0.02$), and whole-body ($P = 0.0003$) BMD in the intent-to-treat models.

Once other factors were taken into account in the compliant analysis (**Table 3**), location (UCD) exerted a significant effect for lumbar spine ($P = 0.047$) and whole-body ($P = 0.036$) BMD.

Regression analysis for compliant women indicated that age, whole-body fat mass, and serum CTx were common predictors for each bone outcome; the 120-mg (compared with placebo) dose exerted a protective effect ($P = 0.024$) on percentage decline for femoral neck BMD but not for other BMD outcomes. Urinary equol, either as a continuous or a categorical factor, dropped out of each model. Age was the most important predictor of lumbar spine BMD bone loss, whole-body fat mass was the most important predictor of total proximal femur and neck BMD bone loss, and bone resorption (CTx) was the most important predictor of whole-body BMD bone loss in these women. TLMP and estrogen exposure were also examined but did not improve the BMD models when both were substituted for age. Percentage loss in BMD at 36 mo tended to be greater for older women and for women further from TLMP. However, the relation was not as strong for TLMP as for age, because there were some relatively large losses in BMD for some women with shorter TLMP. Those women also tended to have longer estrogen exposure, so that both TLMP and estrogen exposure (weaker influence) were needed to replace age in the model. Thus, age emerged as the best single factor in these models, and the inclusion of one variable (age) in the models was more parsimonious. Women with proportionally higher fat mass had greater protection from bone loss, whereas those with higher bone resorption rates had greater bone loss. Higher urinary excretion of potassium ($P = 0.058$) and calcium ($P = 0.0048$) was associated with higher rates of BMD loss at the proximal femur and whole body, respectively.

Effect of soy isoflavones on adverse events and endometrial thickness

We observed no significant effect ($P > 0.05$) of treatment on any adverse event. We documented 18 serious adverse events among 16 women: 4 in the control group, 8 in the 80-mg/d group, and 6 in

TABLE 2

Overall response of bone mineral density (BMD) to soy isoflavone treatment in intent-to-treat women¹

BMD measure	Mean decrease	Source of variation	df	Sum of squares	Mean square	F value	P value
	%						
Lumbar spine ²	2.08	Location	1	58.544	58.544	5.46	0.02
		BMD strata (location)	4	54.193	13.548	1.26	0.29
		Treatment	2	16.671	8.335	0.78	0.46
Proximal femur ³	1.43	Location	1	1.833	1.833	0.25	0.62
		BMD strata (location)	4	34.372	8.593	1.16	0.33
		Treatment	2	2.176	1.088	0.15	0.86
Femoral neck ⁴	2.56	Location	1	73.859	73.859	5.69	0.02
		BMD strata (location)	4	140.818	35.205	2.71	0.03
		Treatment	2	46.192	23.096	1.78	0.17
Whole body ⁵	1.66	Location	1	119.158	119.158	13.25	0.0003
		BMD strata (location)	4	33.867	8.467	0.94	0.44
		Treatment	2	2.814	1.407	0.16	0.86

¹ ANOVA was used to measure treatment effect on percentage change in lumbar spine, proximal femur, femoral neck, and whole-body BMD, taking into account location (Iowa State University, University of California at Davis) and initial BMD strata within location. All women with a follow-up BMD at 36 mo were included, regardless of treatment compliance ($n = 224$).

² Overall model $R^2 = 5.3\%$, $P = 0.10$.

³ Overall model $R^2 = 2.3\%$, $P = 0.64$.

⁴ Overall model $R^2 = 8.5\%$, $P = 0.007$.

⁵ Overall model $R^2 = 7.4\%$, $P = 0.018$.

TABLE 3

Regression analyses of overall response of bone mineral density (BMD; % change from baseline to 36 mo) to soy isoflavone treatment in compliant women ($n = 208$)¹

Parameter	Parameter estimate	95% CI	Percentage variance ²	<i>P</i> value ³	VIF ⁴
Lumbar spine BMD⁵					
Intercept	20.477	13.545, 27.408	—	≤0.0001	—
Location (UCD)	-1.647	-3.274, -0.020	1.48	0.047	—
Treatment					
80 mg/d	-0.684	-1.662, 0.294	0.71	0.17	1.34
120 mg/d	-0.007	-0.973, 0.958	0.000085	0.99	1.34
Age (y)	-0.286	-0.411, -0.160	7.50	≤0.0001	1.06
Whole-body fat mass (kg)	-0.128	-0.195, -0.062	5.36	0.0002	1.07
CTx (ng/mL)	2.071	-0.596, 3.546	2.85	0.0062	1.16
Proximal femur BMD⁶					
Intercept	10.531	4.565, 16.498		0.0006	—
Location (UCD)	-0.528	-1.932, 0.876	0.22	0.46	—
Treatment					
80 mg/d	-0.472	-1.312, 0.367	0.49	0.27	1.35
120 mg/d	-0.169	-0.998, 0.659	0.065	0.69	1.34
Age (y)	-0.126	-0.234, -0.018	2.14	0.022	1.06
Whole-body fat mass (kg)	-0.143	-0.201, -0.086	9.84	≤0.0001	1.07
CTx (ng/mL)	1.474	0.209, 2.738	2.12	0.023	1.16
Urinary potassium (mmol/d)	0.006	-0.0002, 0.011	1.46	0.058	1.04
Femoral neck BMD⁷					
Intercept	18.758	11.161, 26.356		≤0.0001	—
Location (UCD)	-1.194	-2.977, 0.589	0.64	0.19	—
Treatment					
80 mg/d	-0.899	-1.971, 0.172	1.00	0.10	1.34
120 mg/d	-1.223	-2.281, -0.164	1.90	0.024	1.34
Age (y)	-0.231	-0.369, -0.094	4.03	0.0011	1.06
Whole-body fat mass (kg)	-0.183	-0.256, -0.110	8.93	≤0.0001	1.07
CTx (ng/mL)	1.799	0.183, 3.416	1.77	0.029	1.16
Whole-body BMD⁸					
Intercept	9.439	3.145, 15.732		0.0035	—
Location (UCD)	-1.575	-3.047, -0.104	1.53	0.036	—
Treatment					
80 mg/d	-0.435	-1.312, 0.442	0.33	0.33	1.34
120 mg/d	-0.715	-1.581, 0.151	0.91	0.11	1.34
Age (y)	-0.142	-0.255, -0.030	2.13	0.014	1.06
Whole-body fat mass (kg)	-0.124	-0.184, -0.063	5.60	≤0.0001	1.09
CTx (ng/mL)	3.140	1.786, 4.493	7.18	≤0.0001	1.22
Urinary calcium (mmol/d)	0.284	0.087, 0.480	2.79	0.0048	1.19

¹ VIF, variance inflation factor; UCD, University of California at Davis; CTx, cross-linked C-terminal telopeptide of type I collagen. Each BMD model includes location and initial BMD strata within location as obligatory variables to account for location differences, with Iowa State University and initial BMD strata folded into the model intercept and UCD indicated separately.

² Squared semipartial type II correlation coefficient; accounts for shared variance between variables.

³ Variables left in the model were significant at the $P \leq 0.10$ level.

⁴ Measures the effect of collinearity between independent variables in a regression equation and degree to which multicollinearity degrades the precision of the parameter estimate.

⁵ Overall model $R^2 = 26.7\%$ [$F = 7.18$, $df = (10, 207)$; $P \leq 0.0001$]; adjusted $R^2 = 23.0\%$.

⁶ Overall model $R^2 = 21.3\%$ [$F = 4.83$, $df = (11, 207)$; $P \leq 0.0001$]; adjusted $R^2 = 16.9\%$.

⁷ Overall model $R^2 = 27.8\%$ [$F = 7.59$, $df = (10, 207)$; $P \leq 0.0001$]; adjusted $R^2 = 24.1\%$.

⁸ Overall model $R^2 = 32.8\%$ [$F = 8.70$, $df = (11, 207)$; $P \leq 0.0001$]; adjusted $R^2 = 29.0\%$.

the 120-mg/d group. Each serious adverse event was reviewed by our DSMB and deemed not related to treatment. Median values for endometrial thickness (mm) declined from baseline through 36 mo at ISU (1.5 to 1.1) and at UCD (2.6 to 1.9), with no effect of treatment at 12 mo ($P = 0.76$) or 36 mo ($P = 0.64$). We noted one woman (120-mg/d group) with excessive lumbar spine bone loss at 24 mo (-8.3%) and 36 mo (-12.1% ; T score = -1.5) and another woman (control group) with excessive proximal femur

bone loss at 24 mo (-8.7% ; T score = -0.7). Each woman was notified and advised to report these results to her primary care physician for follow-up care.

DISCUSSION

Contrary to our hypothesis, these results did not indicate a bone-sparing effect of soy isoflavones in nonosteoporotic

women with either the intent-to-treat or compliant analysis. The exception was that the 120-mg dose exerted a modest protective effect on the decline in femoral neck BMD ($P = 0.024$) once other factors were taken into account in the compliant analysis. Because the soy isoflavone extract exerted a very modest effect on cortical bone (femoral neck), but had no effect on trabecular bone (lumbar spine) as we had predicted, or on biochemical markers of bone, we cannot conclude that soy isoflavones hold potential promise in the prevention of postmenopausal osteoporosis. The rationale for the examination of the effect of soy isoflavone extract on bone was that long-term efficacy and safety in vivo had heretofore not been shown convincingly. On the basis of our preliminary data, our study focused on soy isoflavone tablets and not soy protein per se because we desired an approach that would minimize inconvenience, not introduce dietary changes, and thus maximize compliance. Certainly, midlife women use complementary and alternative medicine at a high rate, and such treatments may be attractive alternatives because they are considered by women to be safer and more natural than conventional therapy (39). Thus, we designed our clinical trial to determine the effect of soy isoflavone extract on BMD, bone turnover markers, and key safety outcomes.

Observations that suggest that soybeans may contribute to bone health include the low rate of hip fracture in Asians who originate from the Pacific Rim (40, 41), the effectiveness of the isoflavone-derivative ipriflavone to prevent and treat postmenopausal osteoporosis (42, 43), the in vitro (44) and in vivo (45) estrogenic activity of soy isoflavones, and the lower urinary calcium losses in soy compared with animal protein diets (46). However, there are caveats to these findings that do not support the role of soy isoflavones, particularly without the soy protein matrix, in bone health.

Many studies during the past decade have examined the effect of soy isoflavones on bone in peri- and postmenopausal women. Because of heterogeneous study designs, treatment doses or types, and subject characteristics (ie, TLMP), it is clear why mixed results have emerged; however, the results serve to show why we must distinguish among the variety of isoflavone forms (type) used in these studies. Two studies used soy foods rich in isoflavones (47, 48), 2 used carbohydrate foods enriched with soy protein (17, 20), and 7 used soy protein isolate (10, 14, 18, 19, 21, 49, 50) as the isoflavone source. Two studies examined usual dietary soy isoflavone intake among US women (51, 52), whereas 4 examined usual soy food intake among Asians (12, 13, 53, 54), with 1 (54) focused on fermented compared with unfermented soybean intake. Two studies used soy isoflavones extracted from soy germ (16, 55), and 2 used genistein tablets (15, 56). The studies referred to in this paragraph used very different designs from our study, which makes comparisons impossible. We refer the reader to 2 instructive published reviews (57, 58), because an in-depth review is beyond the scope of this article.

Disparate results are likely due to differences in study design, which include various bone sites measured, type of product consumed (soy foods, soy foods rich in isoflavones, soy protein isolate, isoflavone tablets, soy germ tablets, genistein), dose of soy protein and/or isoflavones provided, length of intervention, sample size (often very limiting), as well as subject-related factors. Because our study also used soy isoflavone extract as a treatment, we have summarized results from 2 studies with a sufficient sample size that also used extracted soy isoflavones in

Chinese postmenopausal women (habitual soy consumers). Chen et al (55) conducted a double-blind, randomized clinical trial (1 y) to examine the effect of soy germ extract (40 or 80 mg isoflavones/d) compared with placebo (cornstarch) on bone loss in women aged 48–62 y ($n = 175$). Women in the high-dose group lost less total proximal femur and trochanteric BMC than did either the placebo or low-dose group, with the positive effect noted only among women with low baseline BMC values. Results suggest that isoflavones may have a significant effect on cortical (proximal femur) bone or that appendicular bone responds differently from the axial (ie, spine) skeleton, particularly in habitual soy food consumers. Likewise, Ye et al (16) reported that soy isoflavones (84 or 126 mg/d) from soy germ extract exerted a beneficial effect at 12 wk but not 24 wk, not only on the femoral neck ($P = 0.016$) but also on the lumbar spine ($P = 0.042$) BMD in women ($n = 84$). Still, short-term (≤ 1 y) studies cannot answer the question of whether such bone-sparing effects would be sustained over a longer period that encompasses a bone-remodeling cycle, which ranges from 30 to 80 wk (59). Thus, the reported bone sparing in short-term studies may be due to treatment or to an artifact of the bone-remodeling transient (59). However, soy germ (relatively rich in daidzein and glycitein, and low in genistein) used in these 2 studies is different from other treatments (products are typically relatively rich in genistein) in the literature, which makes comparisons difficult. In addition, these studies included women who consumed soy, and produced results similar to those of cross-sectional studies with habitual soy consumers. Studies that used genistein (15, 56) as the single isoflavone are difficult to compare with our study or others in the literature. In short, in studies that verified efficacy, the benefit was very modest.

It might be that soy isoflavones associated with soy protein (foods or isolate) exert a modest benefit on BMD, as evidenced by some (10, 14, 47, 48, 50) but not by other (17–21, 49) studies. Also, results may be affected by usual dietary soy isoflavone intake (from food), but in the United States (51, 52) any effect is difficult to discern because isoflavone intakes are very low (in the μg rather than mg range), except among Asians (51). Furthermore, it may be difficult to determine the relation of usual dietary soy isoflavone intake (from soy foods) to bone in Asian countries because Asians are life-long soy food consumers. Yet observational studies (12, 13, 53, 54), in general, indicate a more robust association with bone than intervention studies. One study with habitual soy consumers (12) examined the link between soy intake and risk of fracture (1770 incident fractures) in postmenopausal women and showed that the relative risk of fracture declined ($P < 0.001$) as soy protein intake increased. Certainly, epidemiologic studies are not randomized clinical trials and cannot determine cause and effect, but they do provide insights into very observable phenomena and can guide the design of controlled clinical trials.

As in previous reports, regression analysis (compliant models) in our study indicated that age (60), whole-body fat mass (61), and CTx (62) were common predictors of each BMD change outcome. Urinary equol (either as a continuous or a categorical variable) did not approach significance in any BMD model, but our study was not designed to focus on equol producers. Not surprisingly, age was the key predictor of lumbar spine BMD loss, with more rapid loss in younger than in older women, which reflects proximity to menopause. Similar to what has been shown

by some (63) but not all (64) researchers, women with higher fat mass had greater protection from bone loss, because fat mass was the key predictor of proximal femur and neck BMD bone loss. Likewise, similar to previous research (62), women with higher bone resorption rates had greater bone loss, with CTx the key predictor of whole-body BMD bone loss. Sodium, potassium, calcium, phosphorus, and magnesium are considered bone-related nutrients. Alkaline salts are released from bone to maintain acid-base balance (65), and excessive dietary sodium exerts a hypercalciuric effect (66). Because urinary excretion of these 4 minerals (excluding calcium) in the absence of disease may reflect dietary intake under steady state conditions (67), we posited that urinary minerals would be surrogate markers of dietary intake. Of interest, inclusion of urinary minerals improved 2 BMD models, because mineral excretion was associated with higher rates of BMD loss at the proximal femur (potassium) and whole body (calcium). The finding for urinary potassium is difficult to explain. Urinary calcium likely reflected the bone dynamic rather than dietary calcium intake, as reflected by its positive association with CTx ($r = 0.14$, $P = 0.04$), whereas the other urinary minerals apparently were not related to CTx.

The strength of our double-blind, randomized controlled trial, with the use of an intent-to-treat analysis, is that it was longer (36 mo) than previously published studies and had sufficient power to detect a measurable response to treatment. To encourage long-term compliance, we examined the effect of soy isoflavones extracted from soy protein, compressed into tablets, rather than that of soy isoflavones either from soy foods, integrated into foods, or as soy protein isolate. We showed excellent compliance among the 216 women (96.8% achieved 80% compliance) who remained on treatment throughout. It may be that soy isoflavones at higher doses, other bioactive components in soy protein not provided in our study, or soy as an integral part of its native protein matrix when consumed habitually may have beneficial skeletal effects. We cannot extrapolate the findings of our study, which used soy protein-extracted isoflavones, to those that used soy foods.

In conclusion, results from our 3-y multicenter clinical trial do not support that soy isoflavones extracted from protein exert a bone-sparing effect in postmenopausal women. Furthermore, our results do not substantiate the recommendation that soy isoflavones in tablet form should be used to treat or prevent osteoporosis. The modest bone-sparing effect for the femoral neck must be taken in context, given that whole-body fat mass, age, and bone resorption exerted an overriding effect on bone loss. Overall, some studies suggest that soy isoflavones may serve to maintain bone formation at the very least and indeed may dampen bone resorption. Yet, in our study, we did not show a treatment effect on biochemical markers of bone. However, we did not document any untoward effect of isoflavones on endometrial thickness or other adverse events, which indicates that these compounds were relatively benign at the doses we provided.

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The authors' responsibilities were as follows—DLA and MDVL: study concept and design and securing of funding; DLA, LNH, KBH, MSK, JWS, and MDVL: acquisition of data; DLA and MDVL: study supervision; DLA, LNH, KBH, MSK, CTP, JWS, and MDVL: administrative and technical support; KJK, CTP: statistical analysis and support; DLA, KJK, and CTP: analysis and interpretation of data; DLA and LNH: drafting of manuscript; DLA, KJK, MSK, CTP, JWS, and MDVL: critical revision of manuscript for important intellectual content; and DLA, LNH, KBH, KJK, MSK, CTP, JWS, and MDVL: final approval of manuscript. MSK is on the Scientific Advisory Board of the Soy Nutrition Institute (St Louis, MO). None of the other authors declared a conflict of interest.

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