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The Soy Isoflavones for Reducing Bone Loss (SIRBL) Study: Three year effects on pQCT bone mineral density and strength measures in postmenopausal women

KRISTINE M. SHEDD-WISE¹, D. LEE ALEKEL², HEIKE HOFMANN³, KATHY B. HANSON², DAN J. SCHIFERL⁴, LAURA N. HANSON², and MARTA D. VAN LOAN⁵

¹ Nutrition Department, University of California, Davis (UCD)

² Department of Food Science & Human Nutrition, Iowa State University (ISU), Ames

³ Department of Statistics, Iowa State University (ISU), Ames

⁴ Bone Diagnostics, Inc.

⁵ USDA/ARS, Western Human Nutrition Research Center (WHNRC), Davis, CA

Abstract

Soy isoflavones exert inconsistent bone density-preserving effects, but the bone strength-preserving effects in humans are unknown. Our double-blind randomized controlled trial examined two soy isoflavone doses (80 or 120 mg/d) vs placebo tablets on volumetric bone mineral density (vBMD) and strength (via peripheral quantitative computed tomography) in healthy postmenopausal women (46–63 y). We measured 3 y change in cortical (Ct) BMD, cortical thickness (CtThk), periosteal circumference (PC), endosteal circumference (EC), and strength-strain index (SSI) at 1/3 midshaft femur (N=171) and trabecular (Tb) BMD, PC, and SSI at 4% distal tibia (N=162). We found no treatment effect on femur CtThk, PC, or EC, or tibia TbBMD or PC. Strongest predictors (negative) of tibia TbBMD and SSI and femur CtBMD were timepoint and bone resorption; whole body fat mass was protective of SSI. As time since last menstrual period (TLMP) increased (p=0.012), 120 mg/d was protective of CtBMD. Strongest predictors of femur SSI were timepoint, bone resorption, and TLMP (protective). Isoflavone tablets were negative predictors of SSI, but 80 mg/d became protective as bone turnover increased (p=0.011). Soy isoflavone treatment for 3 y was modestly beneficial for midshaft femur vBMD as TLMP increased, and for midshaft femur SSI as bone turnover increased.

Keywords

peripheral quantitative computed tomography (pQCT); soy isoflavone tablets; bone geometry; strength-strain index

Corresponding Author: Marta D. Van Loan, Ph.D., FACS; 430 West Health Sciences Drive, University of California, Davis, Davis, CA 95616. Tel: (530) 752-4160; Fax: (530) 754-4376. Marta.VanLoan@ARS.USDA.GOV.

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INTRODUCTION

The estrogen deficiency that accompanies menopause plays a key role in the development of osteoporosis among women, the consequence of which can lead to debilitating fractures. Isoflavones are structurally similar to estrogen, bind to estrogen receptors (1), and affect estrogen-regulated gene products (2). Thus, the estrogen-like effects have sparked considerable interest in the potential skeletal benefits of isoflavones. Although earlier studies in peri- and postmenopausal women demonstrated beneficial effects of soy isoflavones (3,4) on bone mineral density (BMD), more recent studies have not substantiated these favorable skeletal effects (5–8) in humans. Potter et al. (3), in a study designed to examine the lipid-related effects of soy protein, showed that isoflavone-containing (90 mg/d) soy protein given for six months prevented bone loss at the lumbar spine in hypercholesterolemic postmenopausal women. Alekel et al. (4), in a study designed to examine bone, also found that isoflavone-rich (80 mg/d) soy protein attenuated bone loss (BMD and bone mineral content [BMC]) at the lumbar spine after a six month treatment. Conversely, Kenny et al. (5) found no effect on BMD at the proximal femur, lumbar spine, wrist, or total body in women provided soy protein and isoflavones (either alone or together) for one year. Brink et al. (6) also found no effect of soy isoflavone-enriched foods on one year change in lumbar spine or total body BMD in postmenopausal women. Similarly, Wong et al. (7) found that daily supplementation with 120 mg soy hypocotyl (germ) isoflavones for two years reduced whole-body bone loss, but did not slow bone loss at common fracture sites in healthy postmenopausal women. Likewise, Alekel et al. (8) did not find a bone-sparing effect of extracted soy isoflavones—except for a modest effect at the femoral neck—in a three year multi-center clinical trial with postmenopausal women.

However, bone structure, independent of BMC and BMD, also contributes to fracture risk, making structural information about bone very valuable. Although dual energy x-ray absorptiometry (DXA) is considered the gold standard technique to measure BMD, it can only assess areal BMD, which limits its value as a tool to evaluate critical bone structural properties. Alternatively, quantitative computed tomography (QCT) measures volumetric BMD (vBMD) and also bone structural properties, making it an ideal technique to quantify important geometrical changes in bone that may not be elucidated with DXA alone.

A few studies have examined the bone geometry and strength effects of soy isoflavones (9) and soy protein plus isoflavones (10) in rats, as well as soy isoflavones in mice (11). Results from these studies have also been inconsistent, but suggest that isoflavones may exert a positive impact on bone geometry. The significance of this effect is yet unknown, and may also be dependent on other factors, such as calcium intake (9,11). However, no study has examined the three year effect of isoflavones on bone geometry and strength in postmenopausal women. With a growing body of literature suggesting that BMD alone is not predictive of fracture (12,13), treatments targeting other structural bone parameters may become increasingly important. Thus, we examined the three year effect of soy isoflavones on BMD and strength in postmenopausal women. We hypothesized that soy isoflavone tablets, taken for three years, would preferentially protect trabecular bone and would favorably affect bone strength in postmenopausal women.

MATERIALS AND METHODS

Study design

We enrolled healthy postmenopausal women (46.1 – 63.1 y) as part of a randomized, double-blind, placebo-controlled multi-center (Iowa State University [ISU], Ames, IA and University of California at Davis [UCD], Davis, CA) NIH-funded clinical trial. The parent study (Soy Isoflavones for Reducing Bone Loss; SIRBL) was designed to examine the

effect of two doses of isoflavones extracted from soy protein on bone loss assessed by DXA during the course of three years in at-risk postmenopausal women (8). The current study examined the effect of two doses of soy isoflavones on properties of bone density and strength during three years, as measured by peripheral QCT (pQCT), in a subset of women from SIRBL. Data were collected at baseline, 6, 12, 24, and 36 months. Our study protocol, consent form, and all subject-related materials were approved by the respective Institutional Review Boards (IRB) at ISU (ID# 02-199) and UCD (ID# 200210884-2) and approvals for pQCT procedures were obtained from each institution's IRB and appropriate radiation safety boards. Each woman at pre-baseline was provided a written description and verbal explanation before we obtained signed informed consent.

Subjects

Subjects were recruited from the state of Iowa and the greater Sacramento and Bay Area regions in northern California as previously described (8). Briefly, we screened subjects initially via telephone to identify women aged ≤ 65 years who had undergone natural menopause (cessation of menses 1 through 8 y), were not experiencing excessive vasomotor symptoms, were nonsmokers, and had a BMI from 18.5 through 29.9 kg/m². We excluded vegans because they would likely be soy food consumers, women diagnosed with chronic disease, who had a first-degree relative with breast cancer, who had evidence of previous or existing spinal fractures, or who chronically used medication. Based upon our entry criteria (8), we randomized 255 subjects to treatment; 224 eligible women remained on treatment for 3 y. Each subject obtained a signed Medical Release from her personal physician; she was also required to complete an annual physical, as well as mammogram, breast, and gynecological examinations.

Subject randomization and treatment

To balance treatment allocation with respect to factors that may influence response-to-treatment, subjects at each location (ISU, UCD) were stratified according to initial total proximal femur BMD (high, medium, low) based upon NHANES III database population values (14): (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 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 one of three treatment groups within each BMD strata at each location: 1) placebo control, 2) 80 mg of isoflavones, or 3) 120 mg of isoflavones. Placebo material, devoid of isoflavones, mimicked the isoflavone extract. Active tablets contained the same excipients as placebo tablets (identical in appearance). The ratio of genistein-to-daidzein-to-glycitein (aglycone form) in these tablets was 1.3-to-1-to-0.3, similar to what is found in soybeans. An independent researcher at ISU confirmed that the actual and formulated isoflavone doses (mean $\pm$ SD), respectively, were similar to those tested by The Archer Daniels Midland Co. (Decatur, IL): control = 0 compared with 0.3 $\pm$ 0.4 mg; 80 mg = 89.5 $\pm$ 5.0 compared with 84.3 $\pm$ 4.5 mg; 120 mg = 124.0 $\pm$ 7.7 compared with 122.5 $\pm$ 3.4 mg. Subjects in each group were instructed to take three compressed tablets/d. 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, to provide 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 (15) of 1,200 mg for women 51–70 y.

Interviewer-administered, validated health-related questionnaires

To verify the health status of each subject and obtain health-related data, at pre-baseline we used a Health and Medical History Questionnaire (4,16). 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 (17,18). 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 (19).

Anthropometric measurements

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

Bone measurements

At baseline, 12, 24, and 36 months, we used pQCT (XCT 3000; STRATEC Medizintechnik, Pforzheim, Germany) to assess volumetric cortical BMD (CtBMD; mg/cm^3) at the midshaft femur of the left leg for all subjects ($N=171$) (except 1 who had a surgical rod in her left leg due to traumatic fracture; her right leg was used instead). At baseline, 6, 12, 24, and 36 months, we assessed volumetric trabecular BMD (TbBMD; mg/cm^3) at the distal tibia in women ($N=162$). The midshaft femur was chosen to represent a skeletal site that is predominantly cortical bone, whereas the distal tibia represents a site that is predominantly trabecular bone.

Each subject was seated on a non-movable chair with her spine erect and feet flat on the floor with the tibia at a 90° angle to the floor. For the femur, the length of the femur from the apex of the lateral epicondyle to the inguinal crease was measured and then divided by three. The 33% site (moving proximally from the lateral condyle of the femur to the inguinal crease) was marked on the thigh; the mark was then superimposed by the laser once the subject was positioned in the gantry while seated on the pQCT chair. For the tibia, a scout scan was conducted to locate the distal end of the tibia, with the computer programmed to subsequently determine the 4% site proximal to the distal end. At the tibia, contour mode 3 at $169 \text{ mg}/\text{cm}^3$ was used to define the total bone. Peel mode 4 at $650 \text{ mg}/\text{cm}^3$ with a 10% peel was used to define the trabecular area. In addition, cort mode 4 with an outer threshold of $200 \text{ mg}/\text{cm}^3$ and inner of $-50 \text{ mg}/\text{cm}^3$ was used for SSI analysis. At the femur, contour mode 1 at $710 \text{ mg}/\text{cm}^3$ and cort mode 2 at $710 \text{ mg}/\text{cm}^3$ were used to measure CtBMD and SSI. Slice thickness was 2.2 mm; the voxel size was set at 0.6 mm at the femur and 0.5 mm at the tibia.

Bone at the 4% distal tibia is predominantly trabecular; thus, we assessed TbBMD and trabecular BMC (TbBMC; mg/mm) at this site. Conversely, the midshaft femur is predominantly cortical; thus, we assessed CtBMD and cortical BMC (CtBMC; mg/mm) at this site. The polar moment of inertia (PMI; mm^4), polar strength-strain index (SSI; mm^3), and periosteal circumference (PC; mm) were the strength indices measured at both skeletal sites. Additionally, trabecular area (TbA; mm^2) was measured at the distal tibia and cortical area (CtA; mm^2), cortical thickness (CtThk; mm), and endosteal circumference (EC; mm) were assessed at the midshaft femur. The SSI, which represents the torsional resistance of bone, takes into account both the structural and material properties of bone, the latter of which are represented by the quotient of cortical density and normal physiological density

(taken to be 1200 mg/cm³). pQCT cannot directly measure the material properties of bone, but the quotient of cortical density and normal physiological density has a very strong relationship with bone elastic modulus ($R^2 = 0.73 - 0.88$) (20), which is a material property (21). Therefore, SSI was determined using the following equation: $SSI = \sum ((Ad_z^2 [cortical\ density/normal\ density])/d_{max})$, where d_{max} is the maximum distance of a voxel from the neutral axis.

Matching instruments at each geographic site, cross-training for pQCT scanning between sites, and daily calibration prior to measurements ensured that the pQCT instruments across the two testing locations provided comparable results. One operator at each geographic site performed pQCT scans. Laboratory personnel at each site were trained by the manufacturers' technicians and received further training on pQCT software analysis (Bone Diagnostic, Inc.; Fort Atkinson, WI). One research assistant at UCD performed all pQCT scan analyses following guidelines provided by Bone Diagnostic, Inc. The within-subject *in vivo* precision error (coefficient of variation; CV) for trabecular vBMD and cortical vBMD using pQCT was 1.24% and 0.40% at the distal tibia and midshaft femur, respectively, at ISU (n = 10 per skeletal site) and 0.28% and 0.38%, respectively, at UCD (n = 7 per skeletal site).

Biological samples

Phlebotomists collected fasted (9 h) blood samples between 7:00 and 8:00 am. We separated serum from whole blood, centrifuged for 15 min (4°C) at 1300 × g, and stored aliquots at −80°C until analyzed. We measured serum concentrations of bone resorption markers (cross-linked C-terminal telopeptide of type I collagen [C-Tx]) and bone formation (bone-specific alkaline phosphatase [BAP]) markers, as well as urinary minerals (calcium, phosphorus, magnesium, sodium, potassium), as potential covariates in modeling the bone-related outcomes.

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 inter-assay CVs; we used duplicate serum samples to calculate intra-assay CVs. The low-to-normal and normal-to-high controls for each kit (C-Tx, BAP) were well within the acceptable ranges. The R^2 values were 0.9983 for C-Tx and 0.9990 for BAP. Serum C-Tx was determined with an enzyme-linked immunosorbent method (serum CrossLaps® ELISA) according to the manufacturer's (Nordic Bioscience Diagnostics; Herlev, Denmark) guidelines. Serum C-Tx intra- and inter-assay CVs (%) were 2.23 and 2.63, respectively. Serum BAP was determined using a monoclonal antibody by a solid phase ELISA method (Metra® BAP) according to the manufacturer's (Quidel Corporation; Hanover, Germany) guidelines. Serum BAP intra- and inter-assay CVs (%) were 1.33 and 1.50, respectively. Samples for C-Tx and BAP were read using an automated micro-titer plate reader (ELx808U with KC Junior software V 1.14, BIO-TEK INSTRUMENTS®, Inc.; 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. Urinary minerals from ISU and UCD samples were measured for each subject in batch at UCD. Acidified urine samples were centrifuged (1000 X g @ 4 °C) for 10 min using 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; 150-fold for calcium and magnesium. Concentrations of minerals were measured by inductively-coupled plasma-atomic emission spectroscopy (Varian Analytical Instruments; Walnut Creek, CA). Non-acidified urine aliquots were stored for creatinine

analysis. Creatinine was measured using the Hitachi 902 clinical chemistry analyzer (Roche Diagnostics; Germany). Isoflavone analysis methods have been described elsewhere (8).

Compliance

Our analyses only included women who were classified as compliant ($\geq 80\%$) based on their 36 mo cumulative percent 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. Urinary isoflavone concentrations verified compliance, but did not classify subjects into “compliant” and “noncompliant” groups based upon isoflavone excretion because of its relatively large inter-individual variability.

Statistical analyses

Statistical analyses were performed using SAS software (version 9.1; SAS Inc.; Cary, NC), with results considered statistically significant (two-sided) at $p \leq 0.05$. Descriptive statistics were used to characterize subjects at baseline including median (lower, upper quartile) values for all data, since the outcome data and most variables were not normally distributed. All analyses included a subsample of the women from SIRBL who were protocol compliant and for whom we had complete data for variables included in the models ($N=171$ for femur, $N=162$ for tibia). Percentage change in TbBMD, CtBMD, TbBMC, CtBMC, TbA, CtA, CtThk, femur and tibia PC and SSI, and femur EC at 36 mo relative to baseline was determined for each woman. At baseline, ANOVA was used to determine whether there were significant differences in these outcome variables among the treatment groups. Potential treatment effects on these response variables were analyzed using repeated measures ANOVA models. As blocking variables in the statistical analyses, baseline proximal femur BMD strata (high, medium, low) within each location (ISU, UCD) were considered as obligatory variables, as was treatment, in all models. Additionally, each model included as covariates: time since last menstrual period (TLMP; age at baseline –age at menopause); weight (or whole body lean mass or whole body fat mass); serum concentrations of C-Tx and BAP; and urinary minerals (calcium, phosphorus, magnesium, sodium, potassium). Restricted maximum likelihood (REML) estimation was used to obtain estimates of variances and correlations between repeated measures. Model selection was guided by model diagnostics (Akaike’s information criterion and Bayes information criterion). For an overall model fit, we used a likelihood ratio test based on maximum likelihood-fitted estimates of the full model and a more parsimonious model of only the obligatory variables. Thus, significance indicates that the full model with all of the variables included explains a greater proportion of the variability (random fashion) in the outcome than the parsimonious model.

RESULTS

Characteristics of participants

Descriptive characteristics of the 171 subjects are presented in Table 1. We found no statistically significant differences among the treatment groups at baseline for any of these variables. Women ranged in age from 46.1 to 63.1 y and TLMP ranged from 0.8 to 7.88 y. At baseline, BMI values ranged from 18.4 to 31.7, with approximately half of the women falling below 25 kg/m². Median weight and height remained stable over time, as did BMI, whole body lean mass, and whole body fat mass. Table 2 presents the baseline data for all tibia and femur pQCT variables studied. There were no differences among treatment groups at baseline for any tibia measure. At baseline, ANOVA indicated that femur CtBMD was significantly lower in the 80 mg/d group compared to the control group, and femur cortical thickness and BMC were significantly lower in the 120 mg/d group compared to the control group. There were no other statistically significant differences among groups at baseline.

Effect of treatment on bone parameters

Tibia and Femur ANOVA—The treatment effect on percentage change in pQCT tibia (4% distal) measurements (Table 3), as determined by ANOVA, indicated that treatment exerted a significant ($p=0.026$) effect on TbBMD (Figure 1) and a marginally significant effect ($p=0.095$) on TbSSI (Figure 2). The treatment effect on percentage change in pQCT femur (33% midshaft) measurements (Table 3), as determined by ANOVA, indicated that timepoint exerted a significant effect on CtBMD ($p=0.0055$; Figure 3) and on CtSSI ($p=0.029$; Figure 4). Thus, these bone outcomes were selected for further longitudinal modeling.

Tibia and Femur Linear Mixed Effects Longitudinal Models—At the 4% distal tibia, the models predicting PC, TbBMC, TbA, and PMI percentage change were not significant. The strongest predictors of TbBMD percentage change were timepoint (24 month, $p=0.046$; 36 month, $p=0.025$) and C-Tx ($p=0.028$), whereas whole body fat mass ($p=0.093$) remained in the model but was not significant (Table 4). Both timepoint and bone turnover, as reflected by C-Tx, were negative predictors of percentage change in TbBMD (loss increased with time and as C-Tx increased). The strongest predictors of tibia SSI were timepoint (12 month, $p=0.045$; 24 month, $p\leq 0.0001$; 36 month, $p=0.048$), C-Tx ($p\leq 0.0001$), and whole body fat mass ($p=0.0012$). Both timepoint and C-Tx were negatively associated with tibia SSI, whereas whole body fat mass had a protective effect on tibia SSI. The higher treatment dose (120 mg/d) showed a trend ($p=0.062$) for protecting tibia SSI, but this potential protective effect decreased as urinary excretion of phosphorus increased ($p=0.019$).

At the 33% midshaft femur, models predicting percentage change in femur CtBMC, CtA, CtThk, PC, EC, and PMI were not significant. The strongest (negative) predictors of femur CtBMD were timepoint (24 and 36 months, $p\leq 0.0001$) and bone turnover as reflected by serum BAP ($p=0.0004$) (Table 4). However, the higher isoflavone dose (120 mg/d) exerted a protective effect on CtBMD as TLMP increased ($p=0.012$). The strongest predictors of percentage change in femur SSI were timepoint (24 months, $p=0.0006$; 36 months, $p\leq 0.0001$), C-Tx ($p=0.012$) (both negative), and TLMP (positive predictor; $p=0.031$) (Table 4), whereas serum BAP did not reach significance. Both isoflavone treatments (80 mg/d, $p=0.0052$; 120 mg/d, $p=0.044$) exacerbated the decrease in SSI, but the 80 mg/d treatment became protective as bone turnover, reflected by BAP ($p=0.011$), increased. The higher dose (120 mg/d) showed a similar protective trend with BAP ($p=0.064$), although this did not reach statistical significance.

DISCUSSION

To our knowledge, this is the first study to examine the effect of soy isoflavone tablets consumed for three years on percentage change in BMD and strength as assessed by pQCT in postmenopausal women. We hypothesized that trabecular bone would be more responsive to isoflavone treatment than cortical or composite bone among our cohort of postmenopausal women, since trabecular bone is preferentially lost with estrogen deprivation. Contrary to our hypothesis, we did not find any treatment effect on TbBMD. Advancing time (timepoint) and bone turnover (serum C-Tx) were the only significant (negative) predictors of trabecular BMD in our model—consistent with the phenomenon of rapid bone loss that postmenopausal women experience. Likewise, advancing time and bone turnover (either serum C-Tx or BAP) were consistently negative predictors of bone outcome measures, whereas whole body fat mass was protective of distal tibia SSI ($p=0.0012$) and TLMP was protective of femur SSI ($p=0.031$). These findings are consistent with our expectations for these important predictors of bone loss.

Interestingly, the 120 mg/d treatment showed a trend toward protecting tibia SSI. However, this potential protective effect diminished as urinary phosphorus increased. It is possible that high dietary phosphorus may lessen any potential protective effect soy isoflavones might exert on trabecular BMD, in part because high phosphorus diets may stimulate bone resorption through increased parathyroid hormone secretion (22). This finding should be further investigated, since it may have important implications given that the average daily phosphorus intake of Americans is more than twice the dietary reference intake (23).

Although we did not find a significant impact of either isoflavone treatment on BMD at the tibia—and only a modest trend for protection of SSI—we cannot rule out a potential protective effect of isoflavones at other bone sites. It is important to recognize that the 4% distal tibia is not a clinically relevant fracture site, although it contains a high proportion of trabecular bone. The possibility remains that soy isoflavones may exert modest positive changes on the geometry of the vertebrae or hip, which theoretically might protect against fracture. Breitman et al. (9) found that soy isoflavones did not have an effect on biomechanical strength properties at the midshaft femur in ovariectomized rats, but did exert a positive effect at the vertebrae. It is unclear from our study whether or not findings from the distal tibia can be extrapolated to predict the response to isoflavones at more clinically relevant bone sites, such as the spine and hip. However, the results from our previously published DXA analyses (8) did not substantiate a biologically important impact of soy isoflavones on the lumbar spine or total proximal femur.

Contrary to our hypothesis that soy isoflavones would primarily impact trabecular bone, the 120 mg/d isoflavone treatment exerted a protective effect on femur CtBMD as TLMP increased. Although trabecular bone loss is initially greater than cortical bone loss, the loss of cortical bone does increase with age (24), and we also noted this in the present study. Since estrogen deficiency that accompanies menopause has a profound negative effect on the skeleton, particularly in the first 5–10 y following menopause (25), the modest protective benefit on CtBMD in the 120 mg/d group that we observed suggests that soy isoflavones may mitigate postmenopausal cortical bone loss. This may suggest an effect that is dissimilar to estrogen, which preferentially protects trabecular bone.

Inconsistent results have been found from studies examining the bone geometry and strength effects of soy isoflavones (9) and soy protein plus isoflavones (10) in rats, as well as soy isoflavones in mice (11). Devareddy et al. (10) reported that soy protein had positive effects on trabecular microarchitecture at the tibia in ovariectomized rats, but could not restore trabecular bone completely. Breitman (9) found that isoflavones were protective of vertebral biomechanical properties among ovariectomized rats receiving isoflavones and calcium. The SSI represents the torsional resistance of bone, taking into account both the structural and material properties of bone. In our study, we found that femur SSI was protected by the 80 mg/d (and marginally by the 120 mg/d dose) isoflavone dose as BAP increased ($p=0.011$), suggesting that isoflavones may protect bone strength when bone turnover is high, such as during the early postmenopausal years. However, we cannot explain why the higher dose (120 mg/d), as BAP increased ($p=0.064$), appeared to exert a lesser effect than the 80 mg/d dose. Our pQCT findings support the idea that soy isoflavones may modestly maintain bone strength, despite having negligible effects on areal BMD (8).

Another consideration is the effect of isoflavones both in the presence and absence of adequate calcium. Animal studies have found improved vertebral biomechanical strength in rats given isoflavone extract with calcium versus either nutrient alone (9). Likewise, Fonsesca et al. (11) showed that a combination of daidzein and high calcium in mice favorably affected femur and lumbar vertebrae BMD and strength, but that much of this effect was mediated by the high calcium diet. Because all women in our study were given

500 mg/d calcium plus 600 IU of vitamin D—in addition to their regular dietary intake—the potential additional benefit of soy isoflavones on bone may be minimal, as compared to the effect of isoflavones on bone in individuals who are lacking dietary calcium and vitamin D. However, this is speculative and would need to be verified in a human study. Additionally, the form of soy treatment—be it isoflavones, protein, soy foods, or some combination thereof—may also exert different effects on properties of BMD and strength. Further studies would be needed to evaluate these differences.

In conclusion, we found that soy isoflavone tablets taken for three years exerted a modest beneficial effect on percentage change in the midshaft femur vBMD as TLMP increased, as well as a modest beneficial effect on the midshaft femur SSI as bone turnover (reflected by serum BAP) increased. Contrary to our hypothesis, we could not document a significant treatment effect on the distal tibia TbBMD. Yet, we noted a trend toward a protective effect of treatment (120 mg/d) on tibia SSI, whereas this potential protective effect diminished as urinary phosphorus increased, suggesting the importance of dietary phosphorus intake. However, because this study did not examine fracture (we did not document any osteoporotic fractures during the course of the study), it is difficult to assess the clinical relevance of the modest effects we observed. Nonetheless, in light of a growing body of literature suggesting a negligible effect of soy isoflavones on areal BMD, it seems doubtful that isoflavones taken as dietary supplements would have any clinically relevant impact on fracture risk. Yet, the potential benefit of soy isoflavones in defending against osteoporotic fracture by means of protecting bone strength needs further elucidation, taking into account reproductive history (TLMP), bone turnover, and dietary intake.

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Figure 1. Percent change in distal tibia trabecular BMD over three years
 Treatment effects that are significantly different from the control group at the $p \leq 0.05$ level are indicated by shaded symbols.

Figure 2. Percent change in distal tibia strength-strain index over three years
Treatment effects that are significantly different from the control group at the $p \leq 0.05$ level are indicated by shaded symbols.

Figure 3. Percent change in midshaft femur cortical BMD over three years
Treatment effects that are significantly different from the control group at the $p \leq 0.05$ level are indicated by shaded symbols.

Figure 4. Percent change in midshaft femur strength-strain index over three years
Treatment effects that are significantly different from the control group at the $p \leq 0.05$ level are indicated by shaded symbols.

Table 1Descriptive Data: Characteristics of Participants at Baseline^a (N=171)

Characteristic	Control (n=61)	80 mg (n=56)	120 mg (n=56)
<i>Median</i> (lower, upper quartile value)			
Age (y)	54.2 (51.9, 56.2)	54.4 (52.9, 56.7)	54.5 (52.0, 57.2)
Time since last menstrual period ^b (y)	2.7 (2.0, 5.2)	3.1 (1.8, 4.8)	2.4 (1.7, 4.9)
Weight (kg)	65.7 (61.0, 74.3)	68.7 (60.0, 72.9)	65.0 (59.5, 73.7)
Standing Height (cm)	165.6 (162.1, 169.0)	165.0 (160.9, 167.9)	166.2 (161.9, 170.0)
BMI (kg/m ²)	24.2 (21.8, 26.6)	24.9 (23.1, 26.7)	24.4 (21.6, 27.7)
Whole Body Lean Mass ^c (kg)	42.7 (40.8, 46.2)	43.0 (40.1, 45.8)	43.4 (41.6, 46.1)
Whole Body Fat Mass ^c (kg)	21.8 (17.6, 27.9)	23.3 (18.8, 26.6)	22.0 (17.8, 27.5)
Whole Body Fat ^c (%)	33.7 (30.5, 38.5)	34.5 (31.8, 37.3)	34.3 (29.2, 38.6)
Serum C-Tx ^d (ng/mL)	0.67 (0.53, 0.88)	0.83 (0.62, 1.09)	0.84 (0.57, 1.07)
Serum BAP ^e (U/L)	32.89 (28.48, 35.13)	33.88 (28.72, 40.62)	31.94 (26.41, 38.04)
Urinary Calcium (mmol/day)	4.4 (2.8, 6.1)	5.0 (4.0, 6.2)	4.3 (3.0, 5.8)
Urinary Magnesium (mmol/day)	3.2 (2.5, 4.2)	3.5 (2.9, 4.5)	3.5 (2.9, 4.3)
Urinary Phosphorus (mmol/day)	23.5 (19.2, 27.9)	25.3 (20.3, 29.4)	25.0 (19.3, 29.9)
Urinary Potassium (mmol/day)	50.1 (38.4, 61.6)	59.7 (41.5, 69.7)	53.5 (42.0, 65.0)
Urinary Sodium (mmol/day)	113.8 (84.2, 147.0)	129.4 (85.7, 161.0)	117.2 (91.5, 142.0)

^aNo statistically significant differences among treatment groups at baseline were noted.^bCalculated for each woman by subtracting the date of her last menstrual period from her baseline test date.^cBody composition (whole body lean and fat mass [kg and %]) was determined using dual-energy X-ray absorptiometry (DXA)^dManufacturer of cross-linked C-terminal telopeptides of type-I-collagen (C-Tx; Nordic Bioscience Diagnostics) kit indicated expected range in postmenopausal women = 0.14–1.35 (mean = 0.44) ng/mL.^eManufacturer of bone-specific alkaline phosphatase (BAP; Metra) kit indicated expected range in postmenopausal women = 14.2–42.7 (median = 25.0) U/L.

Descriptive Data: Baseline Peripheral Quantitative Computed Tomography (pQCT) Measurements by Treatment Group (Median, Interquartile Range)

Table 2

pQCT Measurement	Control (n=59)	80 mg/d (n=54)	120 mg/d (n=49)
Distal Tibia (N=162)			
Trabecular BMD (mg/cm ³)	232.2 (216.9, 258.0)	230.7 (211.4, 252.0)	234.8 (214.2, 262.6)
Trabecular BMC (mg/mm)	172.5 (160.5, 203.8)	179.0 (165.6, 197.3)	180.3 (163.4, 201.6)
Trabecular Area (mm ²)	767.2 (704.5, 828.9)	761.4 (701.1, 860.4)	769.2 (698.5, 860.8)
Periosteal Circumference (mm)	109.0 (105.2, 113.3)	109.0 (105.3, 115.4)	109.9 (104.0, 115.4)
Polar Moment of Inertia (mm ⁴)	44919.3 (39350.0, 52390.0)	44201.4 (40340.0, 50570.0)	45619.7 (39560.0, 51470.0)
Strength-Strain Index (mm ³)	2168.7 (1944.0, 2439.0)	2112.3 (1982.0, 2338.0)	2097.6 (1958.0, 2404.0)
Midshaft Femur (N=171)	(n=61)	(n=56)	(n=56)
Cortical BMD (mg/cm ³)	1120.4 (1108.0, 1138.0)	1110.7 (1097.0, 1128.0) ^a	1118.7 (1100.0, 1134.0)
Cortical BMC (mg/mm)	329.5 (311.0, 362.0)	325.2 (304.8, 349.1)	322.7 (301.1, 340.8) ^a
Cortical Area (mm ²)	294.8 (277.9, 316.4)	289.8 (277.1, 316.5)	291.1 (266.9, 304.0)
Cortical Thickness (mm)	4.0 (3.7, 4.5)	3.9 (3.6, 4.5)	3.9 (3.6, 4.2) ^a
Periosteal Circumference (mm)	86.1 (82.3, 89.8)	87.1 (82.1, 90.8)	89.2 (83.5, 91.5)
Endosteal Circumference (mm)	60.9 (53.8, 66.3)	60.5 (55.7, 68.5)	64.9 (58.8, 69.3)
Polar Moment of Inertia (mm ⁴)	39146.5 (34280.0, 44770.0)	38058.4 (34490.0, 44510.0)	41310.7 (34910.0, 45680.0)
Strength-Strain Index (mm ³)	2548.4 (2279.0, 2719.0)	2503.1 (2211.0, 2708.0)	2525.6 (2299.0, 2774.0)

BMD: bone mineral density; BMC: bone mineral content

^aSignificantly different from control group (p<0.05).

Analysis of Variance^a; Response of Selected pQCT Distal Tibia and Midshaft Femur Measurements (percent change) to Soy Isoflavone Tablets in Postmenopausal Women^b

Table 3

Distal Tibia (N=162)	Source	DF	Mean Square	F Value	P value
Trabecular BMD^c Overall Model R ² =15.3% ($P \leq 0.0001$)	Location	1	1832	52.32	0.15
	Treatment	2	3281	3.68	0.026
	Timepoint	3	75	0.084	0.97
	Treatment*timepoint	6	8	0.0093	0.99
Periosteal Circumference Overall Model R ² =10.5% ($P \leq 0.0001$)	Location	1	3109	68.92	≤ 0.0001
	Treatment	2	77.72	1.72	0.18
	Timepoint	3	13.41	0.30	0.83
	Treatment*timepoint	6	0.51	0.011	0.99
Strength-Strain Index^c Overall Model R ² =8.4% ($P \leq 0.0001$)	Location	1	1451931	16.38	≤ 0.0001
	Treatment	2	209026	2.36	0.095
	Timepoint	3	11767	0.13	0.94
	Treatment*timepoint	6	1743	0.020	0.99
Midshaft Femur (N=171)					
Femur Cortical BMD^c Overall Model R ² =6.2% ($P = 0.0007$)	Location	1	20.96	19.85	≤ 0.0001
	Treatment	2	0.57	0.54	0.58
	Timepoint	2	5.56	5.26	0.0055
	Treatment*timepoint	4	0.19	0.18	0.95
Periosteal Circumference Overall Model R ² =2.7% ($P = 0.26$)	Location	1	28.84	4.61	0.032
	Treatment	2	2.76	0.44	0.64
	Timepoint	2	6.24	1.00	0.37
	Treatment*timepoint	4	2.33	0.37	0.83
Endosteal Circumference Overall Model R ² =1.3% ($P = 0.84$)	Location	1	72.82	1.57	0.21
	Treatment	2	17.37	0.38	0.69
	Timepoint	2	49.89	1.08	0.34
	Treatment*timepoint	4	9.48	0.20	0.94
Cortical Thickness Overall Model R ² =1.3% ($P = 0.81$)	Location	1	23.20	0.30	0.59
	Treatment	2	61.75	0.79	0.46

Distal Tibia (N=162)		Source	DF	Mean Square	F Value	P value
Strength-Strain Index^c	Overall Model R ² =5.9% (P=0.0011)	Timepoint	2	107.43	1.37	0.26
		Treatment*timepoint	4	18.18	0.23	0.92
		Location	1	224.78	15.22	≤0.0001
		Treatment	2	12.62	0.85	0.43
		Timepoint	2	52.85	3.58	0.029
		Treatment*timepoint	4	5.11	0.35	0.85

BMD, bone mineral density; pQCT, peripheral quantitative computed tomography

^aANOVA used to determine treatment effect on percentage change in pQCT measurements, taking into account location (Iowa State University [ISU], University of California at Davis [UCD]), as well as randomization to treatment at each site within BMD strata.

^bIncluded all women with a follow-up BMD at 36 months (N=162 for tibia; N=171 for femur), regardless of treatment compliance

^cModels of interest for further longitudinal analysis

Table 4

Linear Mixed Effects Longitudinal Models: Response of Peripheral Quantitative Computed Tomography Measurements to Soy Isoflavones in Postmenopausal Women

Distal Tibia Trabecular BMD (% change from baseline to 36 months) (N = 162)			
Overall Model^a vs. Model without Covariates: Likelihood Ratio^b = 9.280; df = 2; P = 0.0097			
Parameter	Parameter Estimate	95% CI	P value^c
Intercept	0.423	−0.668 to 1.513	0.45
Treatment (80 mg)	0.060	−0.679 to 0.799	0.87
Treatment (120 mg)	0.081	−0.675 to 0.836	0.84
Timepoint (24 mo)	−0.590	−0.170 to −0.011	0.046
Timepoint (36 mo)	−0.665	−1.243 to −0.086	0.025
Whole Body Fat Mass (kg)	0.028	−0.005 to 0.060	0.093
Serum C-Tx (ng/mL)	−0.595	−1.124 to −0.065	0.028
Distal Tibia Strength-Strain Index (% change from baseline to 36 months) (N = 162)			
Overall Model^a vs. Model without Covariates: Likelihood Ratio^b = 47.620; df = 5; P ≤ 0.0001			
Parameter	Parameter Estimate	95% CI	P value^c
Intercept	−0.144	−1.586 to 1.299	0.85
Treatment (80 mg)	0.548	−0.794 to 1.890	0.43
Treatment (120 mg)	1.388	−0.060 to 2.836	0.062
Timepoint (12 mo)	−0.810	−1.601 to −0.020	0.045
Timepoint (24 mo)	−1.815	−2.609 to −1.020	≤0.0001
Timepoint (36 mo)	−0.803	−1.596 to −0.010	0.048
Whole Body Fat Mass (kg)	0.069	0.027 to 0.111	0.0012
Serum C-Tx (ng/mL)	−1.835	−2.536 to −1.133	≤0.0001
Urinary P (mmol/d)	−0.001	−0.015 to 0.012	0.83
Treatment (120 mg) * Urinary P (mmol/d)	−0.048	−0.088 to −0.008	0.019
Midshaft Femur Cortical BMD (% change from baseline to 36 months) (N = 171)			
Overall Model^a vs. Model without Covariates: Likelihood Ratio^b = 48.798; df = 5; P ≤ 0.0001			
Parameter	Parameter Estimate	95% CI	P value^c
Intercept	0.773	0.239 to 1.306	0.0047
Treatment (80 mg)	0.033	−0.497 to 0.562	0.90
Treatment (120 mg)	−0.499	−1.016 to 1.019	0.061
Timepoint (12 mo)	−0.263	−0.526 to −0.00003	0.051
Timepoint (24 mo)	−0.613	−0.916 to −0.310	≤0.0001
Timepoint (36 mo)	−0.744	−1.106 to −0.383	≤0.0001
Serum BAP (U/L)	−0.020	−0.031 to −0.009	0.0004
TLMP (yr)	0.030	−0.057 to 0.118	0.50
Treatment (120 mg) * TLMP (yr)	0.159	0.035 to 0.282	0.012
Midshaft Femur Strength-Strain Index (% change from baseline to 36 months) (N = 171)			
Overall Model^a vs. Model without Covariates: Likelihood Ratio^b = 19.558; df = 5; P = 0.0015			

Distal Tibia Trabecular BMD (% change from baseline to 36 months) (N = 162)			
Overall Model ^a vs. Model without Covariates: Likelihood Ratio ^b = 9.280; df = 2; P = 0.0097			
Parameter	Parameter Estimate	95% CI	P value ^c
Parameter	Parameter Estimate	95% CI	P value ^c
Intercept	2.305	-1.334 to 5.944	0.22
Treatment (80 mg)	-6.790	-11.491 to -2.089	0.0052
Treatment (120 mg)	-4.753	-9.341 to -0.164	0.044
Timepoint (24 mo)	-1.617	-2.530 to -0.703	0.0006
Timepoint (36 mo)	-2.023	-3.042 to -1.004	≤0.0001
TLMP (y)	0.287	0.028 to 0.547	0.031
Serum C-Tx (ng/mL)	-1.723	-3.067 to -0.379	0.012
Serum BAP (U/L)	-0.091	-0.203 to 0.021	0.11
Treatment (80 mg) * Timepoint (24 mo)	1.255	-0.034 to 2.545	0.057
Treatment (80 mg) * Serum BAP (U/L)	0.191	0.045 to 0.336	0.011
Treatment (120 mg) * Serum BAP (U/L)	0.137	-0.008 to 0.282	0.064

BMD, bone mineral density; CI, confidence interval; C-Tx, cross-linked C-terminal telopeptides of type-I-collagen; BAP, bone-specific alkaline phosphatase; P, phosphorus; TLMP, time since last menstrual period

^aEach BMD model included obligatory variables: location (University of California at Davis [UCD]; Iowa State University [ISU]) and initial BMD strata within location to account for location differences, as well as initial BMD strata, folded into the model intercept and UCD indicated separately. Additional obligatory variables: treatment (vs. control), timepoint (vs. baseline), treatment * timepoint

^blikelihood ratio based upon maximum likelihood; estimates and significances are based upon restricted maximum likelihood.

^cVariables left in the model were significant at $P \leq 0.10$ level