Isoflavone-rich soy protein isolate attenuates bone loss in the lumbar spine of perimenopausal women1–4

D Lee Alekel, Alison St Germain, Charles T Peterson, Kathy B Hanson, Jeanne W Stewart, and Toshiya Toda

ABSTRACT

Background: No published studies have directly examined the effect of soy protein with isoflavones on bone or bone turnover in perimenopausal women.

Objective: Our objective was to determine the effects of 24 wk of consumption of soy protein isolate with isoflavones (80.4 mg/d) in attenuating bone loss during the menopausal transition.

Design: Perimenopausal subjects were randomly assigned, double blind, to treatment: isoflavone-rich soy (SPI+; n = 24), isoflavone-poor soy (SPI−; n = 24), or whey (control; n = 21) protein. At baseline and posttreatment, lumbar spine bone mineral density (BMD) and bone mineral content (BMC) were measured by using dual-energy X-ray absorptiometry. At baseline, midtreatment, and posttreatment, urinary N-telopeptides and serum bone-specific alkaline phosphatase (BAP) were measured.

Results: The percentage change in lumbar spine BMD and BMC, respectively, did not differ from zero in the SPI+ or SPI− groups, but loss occurred in the control group (P ≤ 0.0036). By regression analysis, SPI+ treatment had a positive effect on change in BMD (5.6%; P = 0.0041; −1.73%, P = 0.0037). By regression analysis, SPI+ treatment had a positive effect on change in BMD (5.6%; P = 0.023) and BMC (10.1%; P = 0.0032). Baseline BMD and BMC (P ≤ 0.0001) negatively affected the percentage change in their respective models; baseline body weight (P = 0.0036) and bone-free lean weight (P = 0.016) contributed positively to percentage change in BMD and BMC, respectively. Serum BAP posttreatment was negatively related to percentage change in BMD (P = 0.0016) and BMC (P = 0.019). Contrast coding using analyses of covariance with BMD or BMC as the outcome showed that isoflavones, not soy protein, exerted the effect.


KEY WORDS Soy, isoflavones, bone density, lumbar vertebrae, biochemical markers, menopause, bone mineral content, perimenopausal women

INTRODUCTION

Current therapies for treating osteoporosis include estrogen and hormone replacement therapies (ERT and HRT), bisphosphonates, calcitonin, and raloxifene. Because of possible contraindications of ERT and HRT, such as breast cancer, endometrial adenocarcinoma, and undesirable side effects (1), compliance with hormonal therapy is poor (2), leading to loss of treatment efficacy (3). Continued uterine bleeding and other adverse side effects of HRT cause women to search for alternatives to traditional therapy. Isoflavone-containing soy may be a potential alternative for preventing bone loss during the menopausal transition.

Isoflavones, found predominantly in soy products, are estrogen-like substances structurally and functionally similar to 17β-estradiol (4). On the basis of evidence primarily from animal and in vitro studies, isoflavones are thought to exert both estrogenic and antiestrogenic effects, depending on the tissue in which they act (5). Isoflavones may exert a weak antagonistic effect on the estrogen receptor (5), thereby having an antiestrogenic effect on uterine and breast tissue (6), where excess estrogen may stimulate synthesis. Alternatively, isoflavones may combine with the estrogen receptor, albeit with lower affinity than 17β-estradiol (7), and stimulate estrogen activity, thus having an estrogenic effect on bone (5) and blood vessels (8). Recent observations indicate that soy or soy isoflavones have a positive effect on bone and other tissues, but the mechanism of action is unclear. Soybean milk–based diets were shown to increase calcium absorption in rats (9) and the isoflavones in soy protein isolate (10) were shown to prevent femoral (10, 11) and vertebral (11) bone loss in rats. In addition, soy protein isolate increased bone formation by stimulating insulin-like growth factor I messenger RNA synthesis in rats (12) or moderately increasing bone turnover, with formation being greater than resorption (10–12).

1From the Department of Food Science and Human Nutrition, Human Metabolic Unit, Center for Designing Foods to Improve Nutrition, the Department of Statistics, Iowa State University, Ames, and the Research and Development Laboratory, Fujico Co, Ltd, Kobe Hyogo, Japan.

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4Reprints not available. Address correspondence to DL Alekel, Human Metabolic Unit, 1127 Human Nutritional Sciences Building, Department of Food Science and Human Nutrition, Iowa State University, Ames, IA 50011-1120. E-mail: alekel@iastate.edu.
One published study designed to examine lipid-related effects also showed that soy protein isolate rich in isoflavones increased the lumbar spine bone mineral density (BMD) in a heterogeneous (with respect to time since menopause) group of postmenopausal women (13). That study was one of the first to examine the effect of isoflavone-rich soy protein on bone or bone turnover in humans. If studies indicate that isoflavone-rich soy attenuates bone loss, then soy supplementation could serve as an alternative or adjuvant treatment during menopause for women who either are poor candidates for HRT or choose not to receive it. The purpose of this study was to examine the hypothesized bone-sparing effect of isoflavone-rich soy protein isolate on BMD and bone mineral content (BMC) of the lumbar spine in perimenopausal women. Biochemical markers of bone resorption [urinary cross-linked N-telopeptides (N-Tx)] and of bone formation [serum bone-specific alkaline phosphatase (BAP)] were also measured.

SUBJECTS AND METHODS

Research design and treatment

This double-blind, 24-wk study examined the effects of isoflavone-rich soy protein (SPI+; Protein Technologies International, St Louis), isoflavone-poor soy protein (SPI−; Protein Technologies International), and whey protein (control; Ross Laboratories, Columbus, OH) on the attenuation of bone loss in perimenopausal women. Perimenopausal women were randomly assigned to 1 of 3 treatment groups: SPI+ (80.4 mg aglycone components/d; n = 24), SPI− (4.4 mg aglycone components/d; n = 24), or control (n = 21). The aglycone components are the unconjugated parent forms of the isoflavones. The subjects were free-living and were supplied with a total of 40 g protein/d, with one-half of this protein consumed in a jumbo muffin and the remainder as protein powder to be mixed with food or beverages. A recipe booklet providing suggestions for incorporating the powder was given to each subject. The muffins were baked in the Human Metabolic Unit of the Center for Designing Foods to Improve Nutrition at Iowa State University. Because the muffins and powder provided =2.09 MJ (500 kcal) and 650 mg Ca/d regardless of treatment, the subjects were instructed to consume these as a meal replacement and not a supplement. Women in each treatment group were instructed to avoid food items that contained soy isoflavones throughout the study. The subjects were required to cease taking any of their medications or illegal drugs, and women who were not experiencing regular hot flashes. The questionnaire also included information on smoking history, bone health, weight history, and overall health.

Lifetime use of cigarettes was expressed as pack-years, calculated by multiplying the average number of cigarette packs smoked/d by smoking history in y. The Paffenbarger physical activity recall (17) was used to obtain information on the previous year’s activity, including walking or climbing stairs, sports and recreational activity, rate of exertion with exercise, and the time spent daily on activities ranging from sleeping to aerobic activity. Each recreational activity was summed to provide an estimate of weekly energy expenditure. The nutrition history questionnaire (16) was used to elicit usual intake patterns. It included adherence to modified or specialized diets; intake of fats and oils; usual caffeine, alcohol, and sodium intakes; calcium...
intake from milk and yogurt throughout life; vitamin and mineral supplement use; and a typical 24-h dietary intake to clarify information obtained from the food records. Three-dimensional food models (Nasco, Fort Atkinson, WI) were used to approximate portion sizes reported in the 24-h intake. The subjects were required to complete a consecutive 5-d food record at baseline, midtreatment, and posttreatment, which each subject recorded in a different 5-d block of time to ensure inclusion of each day of the week twice during the study. Two-dimensional food portion visual aids (Morgan/Posner, 1981; Nutrition Consulting Enterprises, Framingham, MA) were provided to assist subjects in quantifying portion sizes. Diet records are considered the best method for assessing usual dietary intake (18) and were thus used to assess change in energy or nutrient intake throughout the study and to assess typical mean intakes of energy, macronutrients, dietary fiber, alcohol, caffeine, and a variety of vitamins and minerals (in addition to calcium) at baseline. The 5-d food records were analyzed by nutrition students using the NUTRITIONIST IV computerized nutrient database program (version 4.1, 1995; First DataBank, Inc, San Bruno, CA). These analyses did not include the vitamin and mineral supplements we provided the subjects.

Subjects collected 24-h urine samples in polyethylene containers the day before each visit. After the first morning void, all urine was collected, including the next morning’s first void. The total volume of urine samples was measured to an accuracy of 10 mL and recorded. A urinalysis was performed by using Multistix (Bayer Corp, Elkhart, IN) reagent strips. Aliquots were frozen at −80°C for subsequent analysis. Aliquots for each subject at each time point were shipped to Fujicco Co Ltd (Kobe, Japan) and analyzed for urinary isoflavones to monitor compliance. Urinary isoflavone (genistein and daidzein) concentrations were determined in each subject’s sample by using reversed-phase HPLC. Duplicate 1-mL samples of urine were incubated with sulfatase (EC 3.1.6.1; Sigma Chemical Co, St Louis) and β-glucuronidase (EC 3.2.1.31; Wako Pure Chemical Industries, Ltd, Osaka, Japan) at 37°C for 2 h to release the aglycones of the isoflavones; this was followed by purification of reactants using a Sep-Pak C18 cartridge (Waters Corp, Milford, MA).

Urinary isoflavones were separated at ambient temperature by reversed-phase HPLC on a 4.6 mm × 25 cm Zorbax octadecylsilane column (Hewlett-Packard Co, Palo Alto, CA) using a Waters 600 multisolvent delivery system with a WISP 710B autosampler and a model 490 programmable multiwavelength detector (Waters Corp). Elution was performed at a flow rate of 1 mL/min with a nonlinear gradient of acetonitrile solution (10–35%) containing a constant 0.1% acetic acid. The absorbance of urinary daidzein and genistein was measured at 254 nm. To estimate urinary genistein and daidzein concentrations, peak areas were compared with areas of corresponding isoflavone standards. Known concentrations of isoflavones were used to supplement some samples to determine recovery. For standards, commercially available daidzein and genistein (Fujicco Co Ltd) were used.

Quantitative, colorimetric determination of creatinine in urine was done at 500 nm according to the manufacturer’s guidelines (creatinine procedure no. 555; Sigma Diagnostics, St Louis). Urinary cross-linked N-Tx were measured at baseline, midtreatment, and posttreatment by using a competitive enzyme-linked immunosorbent assay according to the manufacturer’s guidelines (Osteomark; Ostex International, Inc, Seattle) and plates were read by using an automated microtiter plate reader (ELx800; Bio-Tek Instruments, Inc, Winooski, VT). Assay values were standardized to an equivalent amount of bone collagen, which is expressed as nmol bone collagen equivalents (BCE) of N-Tx immunoreactivity/mmol creatinine. The intra- and interassay variability was 7.5% and 5.7%, respectively, for N-Tx.

Fasting blood samples were collected at baseline, midtreatment, and posttreatment to measure serum BAP (Tandem-Microplate Ostase, Hybritech Inc) concentrations. This bone formation marker is measured by using a monoclonal antibody with a solid phase, immunoenzymetric method according to manufacturer’s guidelines. The Ostase kits were donated by Hybritech to our laboratory for this study. The plates were washed by using an ELx50 automated stripwasher (Bio-Tek Instruments, Inc) and read by using the automated microtiter plate reader. The intraassay variability was 3.2% and the interassay variability was 3.5%. Quest Diagnostics (St Louis), a certified clinical laboratory, performed the serum reproductive hormone analyses at baseline, midtreatment, and posttreatment.

Dual-energy X-ray absorptiometry via QDR 2000+ (Hologic, Inc, Waltham, MA) was used to assess overall body composition (lean and fat mass) and lumbar spine (vertebrae L1–L4) BMD and BMC at baseline and posttreatment. The two researchers, who were trained and certified by Hologic, Inc, performed the scans. A trained research assistant completed the spine analysis following guidelines from the Hologic QDR 2000+ operator’s manual (1992 version). The procedure involved selecting the vertebral region (L1–L4) and marking the space between each vertebra with a horizontal line. The posttreatment scans were analyzed by using the compare mode. Our laboratory’s short-term within-subject in vivo reproducibility CVs of lumbar spine BMD and BMC are 0.71% and 0.86%, respectively.

Power analysis and statistical analyses of data

This study focused on BMD of the lumbar spine and hence power analysis was based on this outcome in a multiple regression model. Because our sample size (n = 69) was smaller than desired, the effect size of 0.0895 was smaller than expected, and the power to detect a change in spine BMD in this study was 0.57. The power for the overall spine BMD regression model was 0.99 (19).

Statistical analyses were performed with PC SAS, version 6.12 (20). Descriptive statistics include means for normally distributed data (bone, BMI, and total dietary calcium intake), medians for data that were not normally distributed (biochemical markers of bone, serum reproductive hormone concentrations, reproductive history, calcium intake from dairy products throughout life, usual alcohol intake, and urinary isoflavone excretion), and frequencies (reproductive history and smoking history). Percentage change in lumbar spine BMD and BMC and body weight were calculated (((posttreatment − baseline values)/baseline values) × 100) for each group. To determine whether change over the course of treatment was significantly different from zero in each group, paired t tests were performed. Analysis of covariance (ANCOVA) was used to determine the effect of treatment on BMD and BMC, taking cohort and baseline measures of BMD and BMC, respectively, into account. To determine the effect of cohort, treatment, and time-by-treatment interactions on serum BAP and urinary N-Tx, repeated-measures ANCOVA was used. Residual analysis indicated nonconstancy of error variance (it increased with greater Ŷ values) for N-Tx as the outcome.
variable in a regression model (using the above-mentioned covariates as regressors); thus, N-Tx values were log-transformed for the repeated-measures ANCOVA.

Stepwise multiple regression analysis was used to determine the effect of treatment and contributors to percentage change in BMD and BMC from baseline to posttreatment. Classes of variables in modeling the outcomes of baseline BMD and BMC included the best index of baseline body size (weight or BMI), body composition (bone-free lean body mass or fat mass), estrogen exposure, posttreatment values of reproductive hormones (17 β-estradiol, estrone, or FSH), change in physical activity, posttreatment values of biochemical markers of bone turnover (serum BAP or urinary N-Tx), percentage change of dietary intake of selected nutrients (energy, protein, or calcium), typical weekly alcohol intake, and smoking history (pack-years). Residual analyses indicated that the model assumptions of independence of residuals, normality of error terms, and homogeneity of residual variance were satisfied for these regression models, indicating no violation of model assumptions. The variance inflation factor option in the regression analysis (19) did not reveal notable multicollinearities among independent variables.

RESULTS

Compliance with dietary treatment

Adherence to the dietary treatment was excellent, as reflected by the urinary excretion of isoflavones (genistein plus daidzein) and self-reported consumption of muffins and powder in each group. The one exception was for a control subject whose data were removed from all subsequent analyses. This decision was based on the subject’s urinary excretion of isoflavones during treatment, which was similar at all time points to that of the women in the SPI+ group. At baseline, urinary isoflavone excretion values were not significantly different among the groups (P = 0.62). At mid- and posttreatment, the distribution of urinary isoflavone excretion values were significantly (P ≤ 0.0001) different among the 3 groups when the Kruskal-Wallis chi-square approximation test was used. The control group had negligible urinary isoflavone excretion throughout the study. Midtreatment and posttreatment median values (mg/L), respectively, for each of the 3 groups were SPI+, 0.99 and 2.10; SPI−, 0.14 and 0.27; and control, 0.05 and 0.08.

Most subjects indicated that they perceived they were in the active treatment group (SPI+), suggesting that they could not distinguish by taste among the 3 treatments. Eighty-seven percent (60 of 69 women) reported consuming 100% of the muffins and 84% (58 of 69) reported consuming 100% of the powder throughout the study. During the 168 d of treatment in the SPI+ group, 4 women missed an average of 1 muffin and 7 d of powder; in the SPI− group, 3 women missed an average of 2 muffins and 5 women an average of 8 d of powder; in the control group, 2 women missed an average of 1 muffin and 2 d of powder. Sporadically, subjects neglected to consume their muffins or powder because of lack of convenience rather than because of any reported adverse effects. Compliance was not an issue in this study.

Subject characteristics

The median age of these perimenopausal women was 50.6 y. The highest level of education completed as reported by the subjects was high school for 26%, college for 49%, and postcollege for 25%. Three of the women (2 in the SPI+ group, 1 in the control group) were taking medication for hypothyroidism at the time of the study, but they were well controlled and euthyroid. Nonprescription medications used by these women included antacids by 10 (4 in the SPI+ group, 2 in the SPI− group, 4 in the control group), aspirin by 1 (in the SPI+ group), and ibuprofen by 2 (1 in the SPI+ group, 1 in the control group). These women were instructed and agreed to discontinue regular and continual use of these medications during the course of the study and to particularly avoid their use 1–2 d before blood draws. Prescription medications used by the women included albuterol inhalers by 2 (1 in the SPI+ group, 1 in the control group), a steroid nasal spray by 2 (1 in the SPI+ group, 1 in the control group), antidepressants by 2 (1 in the SPI− group, 1 in the control group), and a nonsteroidal antiinflammatory agent sporadically by 1 in the SPI− group. Additional baseline characteristics of the subjects are presented in Table 1.

The inclusion criterion for FSH concentration was ≥30 IU/L; however, 3 of the subjects with values <30 IU/L (22.2, 26.7, and 27.6 IU/L) at baseline were nonetheless included in the study because of recent menstruation and a presumed decline postovulation causing lower FSH values at the time of this blood draw. Baseline reproductive history and serum hormone concentrations among the groups were not significantly different; self-reported smoking history and current alcohol intake as assessed from the nutrition history were not significantly different. Calcium intake from milk and yogurt varied throughout life, with the highest intakes during childhood; calcium intake usually tapered off during adulthood. One woman had a weekly energy expenditure of 16.736 MJ (4000 kcal) from recreational activities because she managed a health club and engaged in extensive moderate activity. Each group experienced a significant gain in body weight (SPI+, 2.2%; SPI−, 1.5%; control, 2.5%) during the course of treatment but treatment per se was not related (P = 0.69) to weight gain as determined by ANCOVA, taking baseline weight (P = 0.17) and cohort (P = 0.37) into account. Moreover, dietary intake of energy or physical activity was not related to body weight gain on the basis of various regression analyses that included absolute values, percentage change, or change from baseline to posttreatment values for energy intake and physical activity.

Lumbar spine response to treatment

Mean (±SEM) percentage change in lumbar spine BMD or BMC (Figure 1) did not decline in the SPI+ or SPI− groups; however, significant losses occurred in the control group. Descriptive results for BMD and BMC are presented in Table 2. Absolute values for bone at baseline and posttreatment were not significantly different among the 3 treatment groups. Results of ANCOVA indicated that treatment had a significant effect on percentage change in BMC (P = 0.021) but not on percentage change in BMD (P = 0.25). However, when various contributing factors were taken into account by using multiple regression analyses (Table 3), the SPI+ treatment had a significant positive treatment effect on the percentage change (loss) in both BMD (5.6%; P = 0.023) and BMC (10.1%; P = 0.0032), whereas the other treatments had no effect. After adjustment for all covariates, there were significant differences in BMD between the SPI+ and the control group (P = 0.014), the SPI+ group and the SPI− plus control groups (P = 0.019), and the SPI+ plus SPI− groups and the control group (P = 0.046). Likewise, after adjustment for all covariates, there were significant
Calcium intake from milk and yogurt throughout life (mg/d)

<table>
<thead>
<tr>
<th></th>
<th>Isoflavone-rich soy (n = 24)</th>
<th>Isoflavone-poor soy (n = 24)</th>
<th>Whey control (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (y)</strong></td>
<td>50.2 (44.7–59.4)</td>
<td>50.9 (41.9–61.6)</td>
<td>49.4 (44.9–55.6)</td>
</tr>
<tr>
<td><strong>Age at menarche (y)</strong></td>
<td>13 (10–15)</td>
<td>13 (11–16)</td>
<td>13 (11–14)</td>
</tr>
<tr>
<td><strong>Estrogen exposure (y)</strong></td>
<td>37.2 (30.5–46.6)</td>
<td>37.0 (28.8–50.2)</td>
<td>37.1 (31.7–44.5)</td>
</tr>
<tr>
<td><strong>Time since last menstrual period (wk)</strong></td>
<td>19 (1–70)</td>
<td>17 (1–79)</td>
<td>14 (1–49)</td>
</tr>
<tr>
<td><strong>Time since hysterectomy (y)</strong></td>
<td>15.2 (2.9–18.3)</td>
<td>10.6 (6.5–25.1)</td>
<td>23.7 (17.9–27)</td>
</tr>
<tr>
<td><strong>Follicle-stimulating hormone (IU/L)</strong></td>
<td>68 (39–128)</td>
<td>65 (27–138)</td>
<td>67 (22–164)</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>24.0</td>
<td>24.2 ± 3.6</td>
<td>23.9 ± 3.0</td>
</tr>
<tr>
<td><strong>Smoking history</strong></td>
<td></td>
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<td></td>
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<tr>
<td><strong>Duration (y)</strong></td>
<td>6 (0.4–34)</td>
<td>12 (1–40)</td>
<td>8 (0.7–24)</td>
</tr>
<tr>
<td><strong>Time since stopping (y)</strong></td>
<td>22 (2–30)</td>
<td>25 (3–37)</td>
<td>9 (0.3–35)</td>
</tr>
<tr>
<td><strong>Cigarettes/d</strong></td>
<td>8 (4–50)</td>
<td>10 (1–60)</td>
<td>25 (5–60)</td>
</tr>
<tr>
<td><strong>Pack-years</strong></td>
<td>2.9 (0.1–34)</td>
<td>10 (0.2–90)</td>
<td>12 (0.5–30)</td>
</tr>
<tr>
<td><strong>Alcohol (g/wk)</strong></td>
<td>33.3 (1.3–113)</td>
<td>18.3 (1.7–295)</td>
<td>22.4 (1.1–281)</td>
</tr>
<tr>
<td><strong>Total dietary calcium (mg/d)</strong></td>
<td>790 ± 331 (218–1577)</td>
<td>883 ± 349 (354–1556)</td>
<td>761 ± 285 (310–1378)</td>
</tr>
<tr>
<td><strong>Calcium intake from milk and yogurt throughout life (mg/d)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Childhood (≤12 y)</strong></td>
<td>975 (0–3600)</td>
<td>900 (0–1800)</td>
<td>900 (0–1500)</td>
</tr>
<tr>
<td><strong>Adolescence (13–19 y)</strong></td>
<td>900 (0–3600)</td>
<td>600 (0–1500)</td>
<td>750 (0–1200)</td>
</tr>
<tr>
<td><strong>Young adulthood (20–29 y)</strong></td>
<td>704 (0–3600)</td>
<td>600 (0–1200)</td>
<td>600 (0–1200)</td>
</tr>
<tr>
<td><strong>Adulthood (30–45 y)</strong></td>
<td>479 (0–1650)</td>
<td>737 (0–1258)</td>
<td>507 (0–1066)</td>
</tr>
<tr>
<td><strong>Perimenopausal period (present)</strong></td>
<td>450 (0–1024)</td>
<td>670 (0–2145)</td>
<td>549 (0–1066)</td>
</tr>
</tbody>
</table>

1There were no significant differences among the 3 groups for any of these characteristics.
2Mean; range in parentheses.
3Exposure exposure = age − (time since last menses) − age at menarche.
4n in brackets.
5± SD.
6Pack-years = packs of cigarettes/d × years of smoking.
7Usual alcohol intake was assessed from the nutrition history in each group and included only women who reported a typical weekly intake.
8Calcium intake was assessed from 5-day food records and included only dietary intake, not intake from supplements.

Differences in BMC between the SPI+ group and the control group (P = 0.0027), the SPI+ group and the SPI− plus control groups (P = 0.0028), the SPI+ plus SPI− groups and the control group (P = 0.021), and the SPI+ group and the SPI− group (P = 0.028).

Baseline lumbar spine BMD and BMC, respectively, contributed negatively (P ≤ 0.0001) to percentage change in BMD and BMC (Table 3). In contrast, baseline body weight (P = 0.0036) and bone-free lean weight (P = 0.016) contributed positively to percentage change in BMD and BMC, respectively. Serum BAP posttreatment was negatively associated with percentage change in BMD (P = 0.0016) and BMC (P = 0.019), indicating that serum BAP was higher with greater loss in spine bone mass. Urinary N-Tx and serum reproductive hormones did not meet the entry criterion (P ≤ 0.15) in either lumbar spine regression model. The only dietary variable that remained was energy intake (P = 0.062) posttreatment for BMD, but it was not significant. In both models of percentage change, cohort was not significant and hence is not presented in the table; however, cohort is included in each bone model to provide correct error degrees of freedom, parameter estimates, percentage variances, P values, and variance inflation factors. Thus, initial bone mass, bone turnover, body mass or composition, and SPI+ treatment were significant contributors to percentage change in lumbar spine BMD and BMC.

Biochemical markers of bone

Given that only 4 of the 9 groups (3 treatments at 3 time points) of urinary N-Tx values whereas 7 of the 9 groups of serum BAP values were normally distributed, median values for these variables are presented in a scatter plot (Figure 2). Baseline values for serum BAP were significantly different (P = 0.036) among the groups; the SPI+ group had higher values than did the control group. Log-transformed N-Tx values were used for the repeated-measures ANCOVA, whereas residual analysis using serum BAP as the outcome in a regression model (and using the same covariates mentioned above) indicated constancy of error variance and thus BAP data were not log-transformed for the ANCOVA. Repeated-measures ANCOVA indicated that both time (P ≤ 0.005) and baseline value (P ≤ 0.0001) were significant, whereas treatment per se had no significant effect on either N-Tx (P = 0.12) or BAP (P = 0.32) values. Also, cohort had a significant effect on N-Tx (P = 0.0089) but not on BAP (P = 0.56) value, suggesting that cohort may reflect a seasonal effect on bone resorption.

DISCUSSION

Although recent animal experiments indicated that soy or soy isoflavones have a positive effect on bone, there are currently...
few short-term studies in perimenopausal or early postmenopausal women and no published long-term human studies on the effects of soy or their isoflavones on bone. To our knowledge, ours is the only published study designed specifically to examine bone in perimenopausal women, showing a positive effect of soy isoflavones on bone mass. Consistent with our hypothesis, our results indicated that SPI+ treatment attenuated bone loss from the lumbar spine in perimenopausal women, whereas the control group had significant loss. After we accounted for various contributing factors by using a regression approach, SPI+ treatment significantly affected both BMD and BMC. In contrast, SPI– treatment and the control had no effect on spinal bone loss. Body weight at baseline rather than weight gain or final weight was related to percentage change in BMD, suggesting that weight gain did not confound the effect of SPI+ treatment on bone. Contrary to our hypothesis, no effect of reproductive hormones or estrogen status on bone loss was found.

It was the bone-free lean body mass at baseline rather than gain in mass or final mass that contributed significantly to percentage change in BMC. Some studies showed that lean body mass is related to bone mass, whereas others showed that fat mass is more important. Glauber and et al (21) concluded that in postmenopausal women, the effect of weight on BMD at weight-bearing sites is a direct result of mass rather than adiposity per se; rather, at non-weight-bearing sites, adiposity is more important. In contrast, Reid et al (22) concluded that in postmenopausal women, fat mass was a factor in predicting BMD. Thus, the relative contribution of fat compared with lean body mass to BMD in peri- and postmenopausal women remains controversial.

Our positive results, in conjunction with those of ipriflavone studies, suggest that dietary isoflavones may be effective in preventing vertebral bone loss due to ovarian hormone deficiency. Several human studies showed that ipriflavone, a synthetic isoflavone, is effective in preventing and treating osteoporosis. Yet, the mechanisms of action of ipriflavone and isoflavones are unclear. Agnusdei et al (23) showed that ipriflavone prevented bone loss in postmenopausal women with established osteoporosis. Recently, Gennari et al (24) found that 600 mg ipriflavone/d prevented rapid bone loss and reduced bone turnover during early menopause in 28 women with low vertebral BMD. Ipriflavone, combined with estrogen, improves the therapeutic bone response, above that of estrogen or ipriflavone alone (25).

![Figure 1](image-url)  
**Table 2**  
Lumbar spine bone mineral density (BMD) and content (BMC) at baseline and posttreatment in perimenopausal women

<table>
<thead>
<tr>
<th>Measure and treatment group</th>
<th>Baseline</th>
<th>Posttreatment</th>
<th>P (ANCOVA)</th>
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<tbody>
<tr>
<td></td>
<td>Minimum</td>
<td>Maximum</td>
<td>Minimum</td>
</tr>
<tr>
<td>BMD (g/cm²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPI+</td>
<td>0.800</td>
<td>1.163</td>
<td>0.800</td>
</tr>
<tr>
<td>SPI–</td>
<td>0.683</td>
<td>1.170</td>
<td>0.685</td>
</tr>
<tr>
<td>Control</td>
<td>0.747</td>
<td>1.262</td>
<td>0.736</td>
</tr>
<tr>
<td>BMC (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPI+</td>
<td>37.729</td>
<td>78.488</td>
<td>37.097</td>
</tr>
<tr>
<td>SPI–</td>
<td>35.436</td>
<td>74.302</td>
<td>35.381</td>
</tr>
<tr>
<td>Control</td>
<td>35.653</td>
<td>79.422</td>
<td>35.349</td>
</tr>
</tbody>
</table>

1 SPI+, isoflavone-rich soy protein group (n = 24); SPI–, isoflavone-poor soy protein group (n = 24); control, whey protein control group (n = 21). There were no significant differences among treatment groups by ANOVA at baseline or posttreatment for BMD and BMC.

2 As a follow-up to ANCOVA, Tukey’s multiple comparison test identified that the SPI+ group was significantly different from the control group for BMC (P = 0.027), whereas the other group comparisons showed no significant differences.
Serum BAP posttreatment was negatively related to percentage change in both BMD and BMC, reflecting the fact that those with more lumbar spine loss during the study had higher BAP concentrations. Subjects with greater bone mass may have higher bone turnover, reflected by higher BAP, simply because of their greater skeletal mass. Notably, baseline spine BMD or BMC was a highly significant negative contributor to percentage change in each respective model, suggesting that those with higher initial bone mass had greater bone loss during the 24 wk, as reported previously (26). Accordingly, Ravn et al (27) concluded that high bone turnover is associated with low bone mass and spinal fractures in postmenopausal women. Our results may simply illustrate the fact that BAP is a marker of bone turnover rather than of bone formation per se. It may also be that BAP is not affected by seasonal variation in the same manner as N-Tx because bone resorption is greater during the months of minimal sun exposure (28). To lessen this seasonal variation, each of the 4 cohorts began at different times of the year, spanning 2 seasons. Assuming that cohort was a surrogate for seasonal effect, there was no effect of cohort on BAP, but there was a significant effect of cohort on N-Tx. Hence, seasonal variation may very well have been a confounding factor, clouding the relation between bone loss and N-Tx concentrations.

Repeated-measures ANCOVA of the data presented in the scatter plot (Figure 2) indicated an overall effect of time but not

**TABLE 3**
Regression analyses: contributors to percentage change in lumbar spine bone mineral density (BMD) and content (BMC) in perimenopausal women

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Parameter estimate</th>
<th>Overall mean</th>
<th>Percentage variance</th>
<th>P value</th>
<th>Variance inflation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>2.120569</td>
<td>—</td>
<td>0.43</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Spine BMD, baseline</td>
<td>8.620114</td>
<td>0.973</td>
<td>20.73</td>
<td>≤0.0001</td>
<td>1.21</td>
</tr>
<tr>
<td>Serum bone alkaline phosphatase, posttreatment</td>
<td>-0.159814</td>
<td>14.95</td>
<td>11.19</td>
<td>0.0016</td>
<td>1.38</td>
</tr>
<tr>
<td>Weight, baseline</td>
<td>0.081708</td>
<td>65.31</td>
<td>9.44</td>
<td>0.0036</td>
<td>1.17</td>
</tr>
<tr>
<td>SPI+ treatment</td>
<td>1.157615</td>
<td>1 if SPI+;</td>
<td>5.64</td>
<td>0.023</td>
<td>1.11</td>
</tr>
<tr>
<td>Energy intake, posttreatment</td>
<td>0.001410</td>
<td>1921.6</td>
<td>3.72</td>
<td>0.062</td>
<td>1.10</td>
</tr>
<tr>
<td><strong>BMC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.146438</td>
<td>—</td>
<td>0.97</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Spine BMC, baseline</td>
<td>-0.177058</td>
<td>53.346</td>
<td>19.72</td>
<td>≤0.0001</td>
<td>1.58</td>
</tr>
<tr>
<td>SPI+ treatment</td>
<td>2.356291</td>
<td>1 if SPI+;</td>
<td>10.13</td>
<td>0.0032</td>
<td>1.16</td>
</tr>
<tr>
<td>Bone-free lean body mass, baseline</td>
<td>0.000314</td>
<td>37711.41</td>
<td>6.62</td>
<td>0.016</td>
<td>1.65</td>
</tr>
<tr>
<td>Serum bone alkaline phosphatase, posttreatment</td>
<td>-0.169963</td>
<td>14.95</td>
<td>6.21</td>
<td>0.019</td>
<td>1.27</td>
</tr>
</tbody>
</table>

1 Overall BMD model $R^2 = 38.3\%$ (adjusted $R^2 = 30.1\%$), $F_{[8, 60]} = 4.66$, $P = 0.0002$; overall BMC model $R^2 = 34.3\%$ (adjusted $R^2 = 26.7\%$), $F_{[7, 61]} = 4.54$, $P = 0.0004$. SPI+, isoflavone-rich soy; SPI−, isoflavone-poor soy; whey, control.
2 Squared semipartial type II correlation coefficient; accounts for shared variance among variables.
3 Variables left in models are significant at $P \leq 0.10$.
4 Measures inflation in the variances of parameter estimates due to multicollinearities among regressors.
5 Other treatment effects (SPI− and whey) dropped out of both models.
6 Assessed from 5-d food records at baseline, midtreatment, and posttreatment.
7 Lean body mass without bone as determined by dual-energy X-ray absorptiometry.

**FIGURE 2.** Scatter plot (median values represented by —) of serum bone-specific alkaline phosphatase (BAP) and urinary cross-linked N-telopeptide (N-Tx) concentrations at baseline (base), midtreatment (mid), and posttreatment (post) in 3 treatment groups of perimenopausal women: isoflavone-rich soy (SPI+: x; $n = 24$), isoflavone-poor soy (SPI−: □; $n = 24$), and whey (control: ♦; $n = 21$) protein. BCE, bone collagen equivalents.
of treatment on BAP and N-Tx. It appears that serum BAP declined slightly in each group from baseline to posttreatment, whereas urinary N-Tx declined midtreatment but then returned to baseline posttreatment. These changes in bone markers reflect a relative increase in resorption compared with formation during the menopausal transition (29), suggesting that the overriding effect was due to the hormonal milieu. The failure of SPI+ treatment to affect bone resorption or formation implies that the reported bone-sparing effect of soy isoflavones may be different from that of estrogen and other antiresorptive agents.

A potential confounder in isoflavone feeding studies is the heterogeneity in bioavailability and metabolism; some subjects are high, some low, and some moderate metabolizers of soy isoflavones (30). Despite the fact that we provided the subjects in the active treatment group with the same dose of isoflavones, variable responses are probable (31). Although the subjects were instructed to consume the muffins and protein powder as a meal replacement and to decrease their protein intake from other sources, dietary intake changed from baseline to posttreatment in each group, with an overall increase in energy and protein and a decrease in dietary fiber. Nonetheless, treatment per se was not related to weight gain. Also, physical activity did not explain the variability in bone loss, which is not necessarily consistent with current research (32).

Compliance of our subjects was excellent, as reflected by isoflavone excretion and self-reported intake of muffins and powder. Yet, a limitation of the study was a smaller than desired sample size as a result of problems recruiting symptomatic women who met the inclusion criteria. Nonetheless, we detected a significant treatment effect in this homogenous group of white women. More subjects are needed to provide greater statistical power to substantiate the bone-sparing effect of soy isoflavones while accounting for confounding factors. Because a bone-remodeling cycle ranges from 30 to 80 wk (33), this 24-wk preliminary study cannot answer the question of whether these bone-sparing effects would be sustained over a longer period. From these results, we cannot determine whether the reported bone-sparing effect is due to treatment, is a transient artifact of bone remodeling, or is simply a result of physiologic changes during menopause (30). A longer study is necessary to determine whether soy isoflavones will affect the remodeling balance, tipping it in favor of bone formation rather than resorption.

In summary, the results of this study suggest that isoflavones attenuated bone loss from the lumbar spine in estrogen-deficient perimenopausal women, who may otherwise be expected to lose 2–3% of bone/y. This attenuation of loss, particularly if continued throughout the postmenopausal period, could translate into a decrease in lifetime risk of osteoporosis. The most important contributors to the percentage change in lumbar spine BMD and BMC were their respective baseline spine BMD and BMC measurements, with the isoflavone-rich treatment contributing significantly to bone mass. Thus, it is possible that the inclusion of isoflavone-containing soy products in the diets of perimenopausal women could serve as an alternative or adjunct treatment for women at risk of osteoporosis. This may be especially important for women who are poor candidates for HRT or choose not to receive it. To advance our basic knowledge and to pave the way for finding alternatives to steroid hormone therapy for postmenopausal women, a long-term dose-response study in humans designed to corroborate these findings and to examine potential mechanisms is needed.

ADDENDUM

Subsequent to the acceptance of the manuscript, additional supportive urinary data became available. The inclusion of urinary calcium excretion increased the explained variability in the percentage change in lumbar spine BMD and BMC (overall $R^2 = 41$%; $P \leq 0.0001$). Urinary calcium exerted a positive effect ($P = 0.011$) on bone and explained an additional 6.7% and 6.9% of the variability in BMD and BMC, respectively. When urinary calcium is included, the effect of SPI+ treatment on BMD (6.0%; $P = 0.016$) and BMC (10.3%; $P = 0.0019$) is slightly higher than what is reported in Table 3 for BMD (5.6%; $P = 0.023$) and BMC (10.13%; $P = 0.0032$).

We extend special thanks to all of our study participants; to the nutrition students in the Department of Food Science and Human Nutrition at Iowa State University, who helped with testing and muffin baking; and to Cindy Krueckenberg for phlebotomy.

REFERENCES