The effect of soy protein and soy isoflavones on calcium metabolism in postmenopausal women: a randomized crossover study

Lisa A. Spence, Elaine R. Lipscomb, Jo Cadogan, Berdine Martin, Meryl E. Wastney, Munro Peacock, and Connie M. Weaver

ABSTRACT

Background: Evidence suggests that soy isoflavones act as estrogen agonists and have beneficial skeletal effects, but the effects on calcium metabolism in humans are not known.

Objective: This study tested whether soybean isoflavones, soy protein, or both alter calcium metabolism in postmenopausal women.

Design: Calcium metabolism in 15 postmenopausal women was studied by using metabolic balance and kinetic modeling in a randomized, crossover design of three 1-mo controlled dietary interventions: soy protein isolate enriched with isoflavones (soy-plus diet), soy protein isolate devoid of isoflavones (soy-minus diet), and a casein-whey protein isolate (control diet).

Results: There was no significant difference between the diets in net acid excretion (P = 0.12). Urinary calcium excretion was significantly (P < 0.01) less with consumption of either of the soy diets (soy-plus diet: 85 ± 34 mg/d; soy-minus diet: 80 ± 34 mg/d) than with consumption of the control diet (121 ± 63 mg/d), but fractional calcium absorption was unaffected by treatment. Endogenous fecal calcium was significantly (P < 0.01) greater with consumption of the soy-minus diet than with consumption of the other diets. Total fecal calcium excretion, bone deposition and resorption, and calcium retention were not significantly affected by the dietary regimens.

Conclusions: The lower urinary calcium seen with the consumption of an isolated soy protein than with that of an isolated milk protein was not associated with improved calcium retention. This finding reinforces the importance of evaluating all aspects of calcium metabolism. Soy isoflavones did not significantly affect calcium metabolism.

KEY WORDS Postmenopausal women, soy protein, soy isoflavones, calcium absorption, calcium kinetics, urinary calcium

INTRODUCTION

The estrogenic properties of soy isoflavones (1, 2) and the efficacy of the synthetic isoflavone ipriflavone in reducing bone loss in rats (3) and humans (4) suggest that soy isoflavones may reduce bone loss in postmenopausal women. However, evidence of the efficacy of soy isoflavones as an alternative to postmenopausal estrogen replacement therapy is conflicting. Comparable bone-sparing effects of 17β-estradiol and soy protein isolate (5), genistein (6), and daidzein (6, 7) have been reported in a young ovariectomized rat model, whereas a study in adult ovariectomized rats (8) found no benefit of soy isoflavones in reversing established osteopenia. Moreover, soy protein had no effect on bone turnover or bone mineral density (BMD) in ovariectomized monkeys (9, 10).

Clinical studies of the effect of soy isoflavones have been of short duration, involved relatively few subjects, and examined markers of bone turnover and BMD. Studies in perimenopausal and postmenopausal women found no loss (11) or an increase (12) in lumbar spine BMD in women consuming soy protein at 40 g/d containing 80–90 mg isoflavones/d in comparison to women taking soy protein devoid of isoflavones (11), whey protein (11), or casein-based milk protein (12), all of which produced a decrease in lumbar spine BMD. In a randomized, double-blind, placebo-controlled, 12-mo trial, hormone replacement therapy and genistein (54 mg/d) were both effective in increasing femur and spine BMD in 90 healthy postmenopausal women (13). In contrast, a study in postmenopausal women supplemented with 150 mg isoflavones twice daily for 6 mo resulted in no significant changes in calcaneus BMD (14). Soy protein isolate supplemented with isoflavones showed no effect in early postmenopausal women after 9 mo (15) or in postmenopausal women after 12 mo (16). Furthermore, a landmark 4-y multicenter trial showed that ipriflavone had no effect on BMD in postmenopausal women (17).

The variable results in these studies and the lack of understanding of the mechanism by which soy isoflavones affect bone leave the relation between soy isoflavones and bone resorption unanswered. These varied clinical results may be related to the lack of control of the dietary factors that affect bone loss, ie, dietary sodium, calcium, and protein. Studies on the effect of isoflavones on calcium metabolism are necessary to determine whether calcium metabolism is perturbed by soy protein or soy isoflavones. This requires a controlled feeding study and calcium
kinetics. Because many subject characteristics and dietary factors affect bone loss, we thought it important to compare the effect of soy protein with and without soy isoflavones with that of milk protein in an otherwise constant diet on bone metabolism in the same postmenopausal women in a crossover study.

SUBJECTS AND METHODS

Subjects
Fifteen healthy, community-dwelling, postmenopausal white women were recruited through advertisements including flyers and posters. The exclusion criteria included existence of endocrine, gastrointestinal, bone, liver, or kidney disease; participation in energy-restricted diets; and sensitivity to soy or milk protein. Excluded medications included hormone replacement therapy, drugs for treatment of osteoporosis, thiazide diuretics, and steroids. At baseline, a fasting blood sample, urine and fecal samples, and height and weight data were obtained along with questionnaires on general health, nutrition, and physical activity practices and a 4-d diet record.

Study design
The study design was a blinded, randomized, crossover intervention to measure calcium balance and calcium kinetics along with urinary sulfate, net acid excretion, and renal function. Each subject, blinded to the intervention and serving as her own control, was studied 3 times under 3 different dietary interventions: soy protein enriched with isoflavones (soy-plus diet), soy protein void of isoflavones (soy-minus diet), and casein-whey (control diet). The 3-phase design allowed comparisons of soy isoflavones, soy protein, and casein-whey.

Under each phase of the study, subjects consumed a controlled diet containing the assigned protein for 28 d with a washout period of ≥4 wk between phases. The metabolic diets contained ≈1100 mg calcium/d, 40 g test protein isolates/d (protein isolates in powdered form were incorporated into baked goods and beverages as the only variable of diet), and 40–50 g protein/d from other sources (animal and vegetable protein) along with 1800–2000 kcal/d. Test proteins were supplied by The Solae Company (St Louis, MO); the soy protein products with isoflavones were made with SUPRO SOY, a soy protein isolate; the soy protein products with trace isoflavones were made with soy protein isolate that had undergone alcohol extraction; and the milk protein products were made with milk protein isolate. Test proteins were handled in the same manner in recipes used for each of the 3 dietary treatments. The energy needs of each subject were estimated to allow weight maintenance according to baseline food records. Macronutrient content was designed to approximate recommended guidelines of the American Dietetic Association and the American College of Sports Medicine.

Final diet composition included composition of protein isolates. Kilocalorie adjustments were made on an individual basis by using foods that would not contribute significant calcium, sodium, or protein (eg, fruit and hard candy) to the diet, so that each subject could maintain her weight during the intervention. Deionized water was allowed ad libitum. All foods and beverages were delivered to the free-living subjects at their home or workplace twice a week.

The first 7 d of each 28-d intervention was a period of equilibration to the diet. Days 8–28 made up the metabolic balance period, in which all urine and feces were collected. Completeness of collections was monitored and corrected for by using measurements of urinary creatinine concentrations and of a fecal marker, polyethylene glycol (PEG). Excreta sample collection containers were delivered to and collected from the subjects on a daily basis at their home or workplace. On days 8 and 15 of each phase, subjects were admitted to the General Clinical Research Center (GCRC) at the Indiana University School of Medicine in Indianapolis for the oral and intravenous administration of radioisotope, respectively. To determine calcium kinetics, 10 μCi $^{45}$Ca was given orally and intravenously, after an overnight fast, with a breakfast meal consisting of the test protein at approximately one-third of the daily intake (=13 g) and calcium at approximately one-third of the daily intake (=300 mg). Before isotopic administration of $^{45}$Ca, a catheter with a heparin lock was inserted into the forearm of the subject and a baseline blood sample was collected. Timed blood collections occurred at 60, 120, 180, 240, 300, 360, 420, 540, 720, and 1440 min after the oral isotopic administration and at 5, 10, 20, 60, 90, 120, 150, 180, 240, 300, 420, 600, and 1440 min and 36, 48, 72, 96 h, 6, 8, 10, 12, and 14 d after the intravenous administration.

Written informed consent was obtained from all subjects. The protocol was approved by the Human Institutional Review Boards and the Radiation Safety Committees at Purdue University, Indiana University-Purdue University Indianapolis, and Clarian IRB.

Chemical analysis
Daily fecal and urine samples were collected in acid-washed containers. Two 24-h urine samples collected at various times during days 15–28 of each metabolic period were used for measurement of net acid excretion (NAE) and sulfate concentrations. These samples were collected under mineral oil and a 5% (wt: vol) solution of thymol in isopropanol. Urine for mineral analysis was acidified with 1% (by vol) HCl and stored at −40 °C for future analysis. Nonacidified urine aliquots for measurement of NAE and sulfate were stored at −20 °C for future analyses. Fecal samples were homogenized with deionized water and concentrated HCl using a laboratory stomacher (Tekmar Co, Cincinnati, OH); they were then treated in a drying oven at 50 °C for a minimum of 24 h, ashed in a muffle furnace at 600 °C for 96 h, and diluted in 1N HCl for total calcium analysis and $^{45}$Ca. Both urine and fecal samples were further diluted with LaCl$_3$ (0.5%)-HCl (0.5N). Dietary composites for each day of the 7-d cycle were collected every 4 mo over the 2.5-year study. Diet, serum, urine, and feces were analyzed for total calcium by using atomic absorption spectrophotometry (5100 PC; Perkin-Elmer, Norwalk, CT). Serum, urine, and feces were analyzed for $^{45}$Ca activity by using beta scintillation counting (Beckman LS 6500;
Beckman Instruments Inc, Fullerton, CA). Counts were adjusted for decay.

Urine samples were analyzed for sulfate concentrations with the use of a turbidimetric technique (18). Urinary NAE was measured by titration (Model 290 Acid/Base Auto Titrator; Denver Instrument Co, Arvada, CO) (19).

Amino acid analysis was performed on the soy and milk protein isolates by using ion exchange chromatography with ninhydrin as a derivatization agent (Beckman System 7300; Beckman Coulter Inc, Fullerton, CA) (20).

Serum isoflavone concentrations were analyzed in the Comprehensive Cancer Center Mass Spectrometry Shared Facility at the University of Alabama at Birmingham with the use of reversed-phase HPLC-electrospray ionization and a PE-Sciex API III triple quadrupole mass spectrometer (Sciex, Concord, Canada). To measure isoflavones in dietary samples, methanol extraction of the isoflavones from the freeze-dried dietary sample was followed by HPLC analysis (21). The total isoflavone content was measured and then converted mathematically to aglycone units.

Radioimmunoassays, immunoradiometric assay, and enzyme immunoassays—specifically, enzyme-linked immunosorbent assays—were used to measure biochemical markers of bone turnover and hormonal concentrations in the serum and urine. Serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D were measured by using radioimmunoassays (DiaSorin Inc, Stillwater, MN), as were estrone-sulfate and sex hormone–binding globulin (Diagnostic Systems Laboratories Inc, Webster, TX). Serum osteocalcin was measured by using a radioimmunoassay developed at the Indiana University-Purdue University Indianapolis General Clinical Research Center. Parathyroid hormone was measured using an immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA). Both estradiol and estrone were measured by using enzyme immunoassays (Diagnostic Systems Laboratories Inc). Urinary crosslinked N-telopeptides of type I collagen, serum bone alkaline phosphatase (BAP), and serum follicle-stimulating hormone were measured by using an enzyme-linked immunosorbent assay, the OSTEOMARK test (Ostex International, Seattle, WA), the Metra test (Quidel Mountain View, Santa Clara, CA), and the AC-TIVE test (Diagnostic Systems Laboratories Inc), respectively.

Statistical analysis and kinetic data analysis

SAS software (version 6.0; SAS Institute Inc, Cary, NC) was used for all statistical analyses. Data were analyzed by accounting for dietary intervention, order of intervention by time, and subject based on the crossover design. Group mean differences were determined by using analysis of variance with Tukey’s grouping at \( P < 0.05 \). Calcium kinetic data were analyzed by using WinSAAM software (a Windows program of Simulation, Analysis, and Modeling; version 2.2.1; National Institutes of Health, Bethesda, MD) (22, 23) and a compartmental model (24) for all subjects under each dietary intervention.

RESULTS

All subjects completed all 3 phases of the study intervention. Subject characteristics are shown in Table 1. The study population was representative of the non-Hispanic white female US population in this age group with respect to age at menopause, height, total femur BMD, and lumbar spine BMD (25). Body weight and BMI were greater in the study population than in the reference population (26).

The average analytic nutrient content of the dietary composites is shown in Table 2. The diet was designed to approximate the recommended calcium intake for postmenopausal women, which is 1200 mg/d. Diets were designed to maintain constant nutrients in each treatment with the exception of the test protein and corresponding amino acid composition. The amount of total sulfur amino acid content in the milk was 18.7% greater than that in the soy protein isolate. Mean (± SD) cystine and methionine concentrations were 0 g/100 g protein and 3.48 ± 0.05 g/100 g.

### Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Study population ((n = 15))</th>
<th>Reference population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>57 ± 6(^1)</td>
<td></td>
</tr>
<tr>
<td>Time after menopause (y)(^2)</td>
<td>9.6 ± 7.0</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162.1 ± 6.6 (^3)</td>
<td>160.8–162.3 (^3)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.5 ± 20.7</td>
<td>70.6–74.3</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>29 ± 7</td>
<td>27.6–28.4</td>
</tr>
<tr>
<td>Bone mineral density (g/cm(^2))</td>
<td>1.13 ± 0.19</td>
<td>0.614–1.453</td>
</tr>
<tr>
<td>Total femur</td>
<td>0.94 ± 0.13</td>
<td>0.483–1.251</td>
</tr>
</tbody>
</table>

\(^1\) ± SD (all such values).
\(^2\) Subjects included women who had natural menopause \((n = 13)\) and those who had surgical menopause \((n = 2)\).
\(^3\) Ranges (all such values). The reference data for height, weight, and BMI were taken from the Third National Health and Nutrition Examination Survey database (1988–1994) representing non-Hispanic white women aged 50–69 y (26). The reference data for bone measurements were from reference 25.

### Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Soy-plus</th>
<th>Soy-minus</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mg)</td>
<td>1063 ± 61(^1)</td>
<td>1172 ± 46</td>
<td>1083 ± 78</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>96 ± 8</td>
<td>89 ± 7</td>
<td>91 ± 7</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>41 ± 8</td>
<td>33 ± 8</td>
<td>38 ± 8</td>
</tr>
<tr>
<td>Magnesium (mg)</td>
<td>259 ± 36</td>
<td>324 ± 44</td>
<td>432 ± 55</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>1879 ± 175</td>
<td>2208 ± 34</td>
<td>1762 ± 216</td>
</tr>
<tr>
<td>Potassium (mg)</td>
<td>2032 ± 346</td>
<td>2248 ± 352</td>
<td>2027 ± 308</td>
</tr>
<tr>
<td>Phosphorus (mg)</td>
<td>1073 ± 94</td>
<td>1125 ± 168</td>
<td>1040 ± 93</td>
</tr>
<tr>
<td>Total isoflavones (mg aglycone)(^2)</td>
<td>65 ± 0.5</td>
<td>3.1(^3)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Soy-plus, soy protein isolate enriched with isoflavones; soy-minus, soy protein isolate devoid of isoflavones; control, casein-whey protein isolate. Average nutrient values represent triplicate analysis of data from each day of the 7-d menu cycle with each of the 3 diet types. The individual protein isolates contained 67.15–69.6% protein, ≤1% fat, and moisture and ash including 583–658 mg sodium/100 g, 821 mg potassium/100 g, 583–658 mg calcium/100 g, 149–158 mg magnesium/100 g, 111 mg chloride/100 g, and 1030–1090 mg phosphorus/100 g for soy and 66.4–68.7% protein, ≤1% fat, and moisture and ash including 359–386 mg sodium/100 g, 639 mg potassium/100 g, 759–807 mg calcium/100 g, 369–382 mg magnesium/100 g, 111 mg chloride/100 g, and 910–980 mg phosphorus/100 g for milk.
\(^2\) ± SD (all such values).
\(^3\) Total isoflavones were 82 mg/d by a method of the Association of Official Analytical Chemists performed by Nestle Purina Analytical Lab (St Louis, MO).
\(^4\)
protein for the milk and 1.19 ± 0.07 g/100 g protein and 1.74 ± 0.22 g/100 g protein for the soy, respectively.

**Calcium kinetics**

The dietary interventions had no significant effect on calcium absorption, total fecal calcium, calcium retention, bone deposition, bone resorption, or bone balance. Urinary calcium was significantly lower with the soy-minus diet than with the control diet. Endogenous fecal calcium was significantly greater in the soy-minus diet than in the other 2 dietary interventions. Bone loss was significantly greater in the soy-minus diet than in the other 2 dietary interventions. The mean values for observed and model calculated variables under each dietary intervention are shown in **Table 3**.

**Urinary sulfate, net acid excretion, and renal function**

Urinary sulfate was 20% (P < 0.05) higher in subjects receiving the control diet than in those receiving the soy diets (4.5 ± 1.1 and 3.6 ± 0.97 mEq/d, respectively), which is reflective of the 18% greater sulfur amino acid content of the milk protein powder in the control diet. There was no significant treatment effect on net acid excretion (40.2 ± 19 mEq/d for milk and 38.6 ± 11 mEq/d for soy).

**Biomarkers of bone turnover and hormonal markers**

The baseline measures for the biochemical markers of bone turnover, serum BAP and osteocalcin, and urinary cross-linked N-telopeptides of type I collagen and the markers of calcium status, parathyroid hormone, 25-hydroxyvitamin D, and 1,25-dihydroxyvitamin D fell within normal ranges for postmenopausal women (27) and are shown in **Table 4**. The hormonal status of these subjects was representative of postmenopausal women with decreased concentrations of estradiol and estrone and increased concentrations of follicle-stimulating hormone. Follicle-stimulating hormone concentrations > 25 mIU/mL indicate stable menopausal status (28). The sex hormone–binding globulin concentrations in the study population were above normal ranges (29).

Dietary treatment had no significant effect on fasting biomarkers of bone turnover or hormonal markers with the exception of serum osteocalcin and serum estrone sulfate (Table 4). Serum osteocalcin was significantly greater (P < 0.05) with the soy-minus diet than with the control diet, whereas the concentrations of serum osteocalcin were intermediate with the soy-plus diet. Serum estrone sulfate was significantly greater (P < 0.05) with the soy-minus diet than with the other 2 dietary interventions. Serum isoflavones were significantly greater (P < 0.01) during the soy-plus diet than during the other 2 dietary treatments.

At day 28 of each dietary treatment, several variables differed significantly from their baseline values (P < 0.05) (Table 4). BAP was significantly lower at baseline than with the soy-minus diet, but baseline BAP did not differ significantly from concentrations with the other 2 dietary treatments. Serum 25-hydroxyvitamin D was significantly lower at baseline than with each dietary treatment because of supplementation with vitamin D during the intervention. Estrone sulfate was significantly greater at baseline than with the control diet and significantly lower at baseline than with the soy-minus diet, but baseline estrone sulfate did not differ significantly from that with the soy-plus diet.

**DISCUSSION**

Calcium absorption, fecal calcium, and calcium retention in postmenopausal women did not differ significantly with consumption of any of the 3 diets. The type and concentration of soy protein isolates and isoflavones used appears not to affect bone deposition, resorption, or balance in postmenopausal women who were beyond the phase of rapid bone loss. The negative calcium balance observed in this study is common in postmenopausal women. The study diets provided 1100 mg calcium/d, 1063 ± 55 mg calcium/d, and 1083 ± 78 mg calcium/d for the soy-plus, soy-minus, and control diets, respectively.  

**TABLE 3**

Calcium balance and kinetic modeling variables during each diet in postmenopausal women

<table>
<thead>
<tr>
<th>Variable</th>
<th>Soy-plus</th>
<th>Soy-minus</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium intake (mg/d)</td>
<td>1063 ± 61</td>
<td>1172 ± 46</td>
<td>1083 ± 78</td>
</tr>
<tr>
<td>Calcium urine excretion (mg/d)</td>
<td>85 ± 34</td>
<td>80 ± 39</td>
<td>121 ± 63</td>
</tr>
<tr>
<td>Calcium fecal excretion (mg/d)</td>
<td>1126 ± 201</td>
<td>1194 ± 265</td>
<td>1092 ± 256</td>
</tr>
<tr>
<td>Calcium retention (mg/d)</td>
<td>−160 ± 194</td>
<td>−70 ± 232</td>
<td>−159 ± 225</td>
</tr>
<tr>
<td>Fractional absorption from breakfast</td>
<td>0.29 ± 0.09</td>
<td>0.27 ± 0.06</td>
<td>0.26 ± 0.07</td>
</tr>
<tr>
<td>Fractional absorption from whole diet</td>
<td>0.21 ± 0.05</td>
<td>0.20 ± 0.06</td>
<td>0.20 ± 0.05</td>
</tr>
<tr>
<td>Calcium absorption, V_a (mg/d)</td>
<td>226 ± 52</td>
<td>239 ± 68</td>
<td>221 ± 58</td>
</tr>
<tr>
<td>Endogenous fecal excretion, V_e (mg/d)</td>
<td>150 ± 32</td>
<td>182 ± 52</td>
<td>152 ± 49</td>
</tr>
<tr>
<td>Bone deposition, V_{dep} (mg/d)</td>
<td>338 ± 106</td>
<td>272 ± 103</td>
<td>307 ± 138</td>
</tr>
<tr>
<td>Bone resorption, V_{res} (mg/d)</td>
<td>404 ± 129</td>
<td>348 ± 131</td>
<td>415 ± 192</td>
</tr>
<tr>
<td>Bone balance, V_{bal} (mg/d)</td>
<td>−86 ± 81</td>
<td>−76 ± 103</td>
<td>−108 ± 110</td>
</tr>
</tbody>
</table>

All values are x ± SD. Values in the same row with different superscript letters are significantly different, P < 0.01 (ANOVA with Tukey’s grouping).

Soy-plus, soy protein isolate enriched with isoflavones; soy-minus, soy protein isolate devoid of isoflavones; control, casein-whey protein isolate.

Direct analytical results. n = 15.

Derived results from kinetic modeling. Because of incomplete collection of tracer data, n = 14, 14, and 13 for the soy-plus, soy-minus, and control diets, respectively.
which was not sufficient to maintain calcium balance. The recommended calcium intake for this population is 1200 mg/d, whereas 1500 mg/d has been suggested for postmenopausal women who are not receiving hormone replacement therapy. According to the calcium retention of this study population, 1500 mg calcium/d may have been warranted to obtain a positive calcium balance.

Results from the analysis of hormones and biochemical markers of bone turnover were consistent with the results of the calcium kinetic analysis. We found no evidence of the effects of dietary treatment on hormones or biochemical markers of bone turnover, except for serum osteocalcin and estrone sulfate, and the effects on them likely have little clinical significance.

In designing this study, we postulated that fractional calcium absorption could have varied either because of differing bioavailability of calcium from meals containing different protein powders or because of an estrogen-like enhancement of calcium absorption due to adaptation to the soy isoflavones. Calcium fractional absorption was also similar between whole soybeans and milk (30) and between tofu and milk (31). In contrast, calcium fractional absorption from calcium-fortified soy milk was 75% of that from cow milk (32). Estrogen has been reported to increase calcium absorption, and estrogen replacement therapy administered to postmenopausal women can return calcium absorption to premenopausal amounts (33–35); however, in the present study, no estrogen-like enhancement from isoflavones occurred.

The 18% difference in the sulfur amino acid content of the protein isolate powders was reflected in the significantly greater urinary sulfate excretion with the control diet than with the soy protein diets. The higher urinary sulfate excretion with the control diet is an indication that the acid-generating potential of the milk protein was higher than that of the soy protein-powder. There was no significant difference in the effects of the milk (control) and soy diets on NAE in our study, possibly because of insufficient power to detect small differences, although others have reported increases in both urinary sulfate and NAE on high protein and animal protein diets in younger subjects (36–41).

Net endogenous acid production calculated by using the method of Sebastian et al (42) estimated that the net endogenous acid production of the soy isolate was approximately one-half that of the milk isolate, at 6.26 and 11.70 mEq/d, respectively. Urinary calcium with the soy diets, regardless of isoflavone content, was lower than that with the milk (control) diet. Reduced urinary calcium excretion was likely due to the lower content of sulfur-containing amino acids found in soy protein than in milk and to the effect of that lower content on the acid-base balance. This urinary conservation was not reflected in calcium retention, and the discrepancy may have been due to the high variation in fecal calcium, which drives the variation seen in calcium retention. One factor that can influence the calcium balance values is the degree of compliancy of free-living subjects with excreta collection. Laboratory analysis of weekly pooled fecal samples indicated an average recovery rate of 80%. Comparison of PEG recovery with chromium-51 chloride hexahydrate recovery in balance studies has indicated that stool recovery measured with PEG was 81%, whereas that measured with $^{51}$Cr was 95% (43). This suggests that PEG recovery may appear lower than the actual stool recovery, and thus collection compliancy may be greater than the sensitivity of the PEG assay can detect. Treatment effects tested with different PEG compliance percentages found no difference in calcium retention even when data below 80% compliance were eliminated. On the basis of 80% power with an $\alpha$ of 0.05 and an SD for calcium retention of 205 mg (the mean for all treatments taken from balance methods) in the present study, a sample size of 180 would have been needed to observe a difference of approximately 40 mg in calcium retention between dietary treatments, as was reported for urinary calcium.

### TABLE 4

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Soy-plus</th>
<th>Soy-minus</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum calcium (mg/dL)</td>
<td>10.3 ± 0.8*</td>
<td>10.2 ± 1.0</td>
<td>10.0 ± 0.9</td>
<td>10.0 ± 1.0</td>
</tr>
<tr>
<td>Serum BAP (ng/mL)</td>
<td>12.3 ± 2.8*</td>
<td>14.4 ± 4.6*</td>
<td>14.8 ± 4.5*</td>
<td>14.3 ± 4.0*</td>
</tr>
<tr>
<td>Serum osteocalcin (ng/mL)</td>
<td>8.5 ± 5.1 b</td>
<td>9.5 ± 4.9 b</td>
<td>10.2 ± 3.9 b</td>
<td>8.1 ± 3.8 b</td>
</tr>
<tr>
<td>Urinary NTx (nmol BCE/mmol creatinine)</td>
<td>52.3 ± 27.4</td>
<td>56.3 ± 23.1</td>
<td>48.0 ± 22.6</td>
<td>55.6 ± 29.0</td>
</tr>
<tr>
<td>Serum PTH (pg/mL)</td>
<td>46.9 ± 15.5</td>
<td>40.8 ± 14.9</td>
<td>40.1 ± 10.4</td>
<td>41.2 ± 13.1</td>
</tr>
<tr>
<td>Serum 25(OH)D (pg/mL)</td>
<td>23.6 ± 8.6*</td>
<td>29.7 ± 5.5*</td>
<td>30.6 ± 6.9 b</td>
<td>29.8 ± 7.1 b</td>
</tr>
<tr>
<td>Serum 1,25(OH)2D (pg/mL)</td>
<td>46.2 ± 11.3</td>
<td>57.1 ± 12.1</td>
<td>48.2 ± 11.2</td>
<td>46.5 ± 12.5</td>
</tr>
<tr>
<td>Serum estradiol (pg/mL)</td>
<td>&lt; 5.0*</td>
<td>&lt; 5.0*</td>
<td>&lt; 5.0*</td>
<td>&lt; 5.0*</td>
</tr>
<tr>
<td>Serum osteone (pg/mL)</td>
<td>3.8 ± 1.3</td>
<td>17.5 ± 12.4</td>
<td>20.6 ± 12.7</td>
<td>12.7 ± 11.2</td>
</tr>
<tr>
<td>Serum FSH (mIU/mL)</td>
<td>30.9 ± 17.8</td>
<td>33.2 ± 23.3</td>
<td>35.3 ± 23.1</td>
<td>37.6 ± 20.6</td>
</tr>
<tr>
<td>Serum estrone sulfate (ng/mL)</td>
<td>4.9 ± 0.65 b</td>
<td>0.78 ± 0.60 b</td>
<td>1.02 ± 0.79 b</td>
<td>0.69 ± 0.53 a</td>
</tr>
<tr>
<td>Serum SHBG (nmol/L)</td>
<td>113.3 ± 51.3</td>
<td>93 ± 3.81</td>
<td>94.9 ± 48.3</td>
<td>99.3 ± 38.4</td>
</tr>
<tr>
<td>Serum total isoflavones (nmol/L)</td>
<td>21 ± 13 b</td>
<td>21 ± 13 a</td>
<td>21 ± 13 a</td>
<td>21 ± 13 a</td>
</tr>
</tbody>
</table>

1 BAP, bone alkaline phosphatase; NTx, crosslinked N-telopeptides of type I collagen; BCE, bone collagen equivalents; PTH, parathyroid hormone; FSH, follicle-stimulating hormone; SHBG, sex hormone–binding globulin; 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)2D, 1,25-dihydroxyvitamin D. n = 15. Values in the same row with different superscript letters are significantly different (repeated-measures ANOVA with Tukey’s grouping): P < 0.05 or P < 0.01 (serum total isoflavones).

2 Soy-plus, soy protein isolate enriched with isoflavones; soy-minus, soy protein isolate devoid of isoflavones; control, casein-whhey protein isolate.

3 ± SD (all such values).

4 Assay cannot detect concentrations < 5 pg/mL.

5 ± (all such values).
Although we lacked the power to determine small differences in calcium retention by using the metabolic balance approach, given the large variation in fecal calcium, it is more likely that the soy treatments are not beneficial to bone, despite the difference in sulfur amino acid load. For no changes in calcium retention to occur when urinary calcium excretion is reduced, endogenous fecal calcium losses must increase, as was observed during the soy-minus treatment and as has been observed elsewhere with increases in dietary phosphorus (44, 45). Increasing dietary protein blunts the calciferic effect of the protein by increasing intestinal calcium excretion (46, 47) without an effect on calcium balance. Studies targeted only at understanding urinary calcium losses are unable to determine the benefits of a treatment. In support of our findings, whole-body $^{47}$Ca retention, a sensitive technique of whole-body retention that is not affected by fecal variation or complete collection, did not differ significantly between high- and low-meat diets in postmenopausal women (48).

Although these results may be affected by the nature of the proteins substituted for meat—i.e., high-sulfur acid casein proteins could also be calciuric (49)—these women’s diets achieved a 16% difference in urinary sulfate load. Urinary $^{47}$Ca excretion was initially greater on the high-meat diet, but it adapted over time.

Our study cannot exclude the possibility that soy isoflavones increase calcium retention at high doses or that soy isoflavones are site specific or more effective immediately after menopause, when bone turnover is higher. Other studies using similar or higher doses of isoflavones have produced mixed results. Wagen et al (28) provided dietary isoflavones at 65 mg/d and 132 mg/d and found no clinically relevant effects on bone biomarkers or hormones. Similarly, BMD was not significantly affected after supplementing postmenopausal women with 150 mg isoflavones twice daily for 6 mo (14) or with 99 mg isoflavones/d for 12 mo (16). On the other hand, isolated soy protein enriched with soy isoflavones increased BMD at the lumbar spine in postmenopausal and postmenopausal women after 9 and 6 mo, respectively (11, 12).

Controlled feeding studies that combine balance and kinetic tracer analysis are useful in quantitating changes in calcium metabolism in response to short-term treatment. To effect changes in bone mass, calcium balance must first be perturbed. In this study, calcium absorption, bone turnover, and bone balance were not affected by protein type or the presence of isoflavones. The greater calciuria with the control (milk) diet than with the soy diets and the associated changes in sulfate excretion are consistent with results of other studies using purified proteins to simulate protein from animal and vegetable sources. However, net calcium retention was not improved by the reduction in urinary calcium, which suggests that it is important to evaluate overall calcium metabolism rather than to rely exclusively on urinary calcium for predicting consequences to bone. Long-term studies to ascertain the effectiveness of soy isoflavones in reducing bone loss in postmenopausal women are not likely to be successful with the type and dose used in our study.

We thank Stephen Barnes at the University of Alabama at Birmingham, in whose laboratory and with the assistance of the laboratory research staff the isoflavone analysis was performed.

CMW was responsible for study design; LAS, ERL, JC, and MP were responsible for data collection; LAS, ERL, BM, and MEW were responsible for data analysis; and LAS, ERL, and CMW were responsible for writing the manuscript. None of the authors had any financial or personal conflicts of interest.

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