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Controlled Substitution of Soy Protein for Meat Protein: Effects on Calcium Retention, Bone, and Cardiovascular Health Indices in Postmenopausal Women

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In a controlled feeding study, the effects of substituting 25 g soy protein for meat on calcium retention and bone biomarkers were determined. Postmenopausal women (n = 13) ate two diets that were similar, except that, in one diet, 25 g high-isoflavone soy protein (SOY) was substituted for an equivalent amount of meat protein (control diet), for 7 wk each in a randomized crossover design. After 3 wk of equilibration, calcium retention was measured by labeling the 2-d menu with ^{47}Ca, followed by whole-body counting for 28 d. Urinary calcium and renal acid excretion were measured at wk 3, 5, and 7. Biomarkers of bone and cardiovascular health were measured at the beginning and end of each diet. Calcium was similarly retained during the control and SOY diets (d 28, percent dose, mean ± pooled SD: 14.1 and 14.0 ± 1.6, respectively). Despite a 15–20% lower renal acid excretion during the SOY diet, urinary calcium loss was unaffected by diet. Diet also did not affect any of the indicators of bone or cardiovascular health. Substitution of 25 g high isoflavone soy protein for meat, in the presence of typical calcium intakes, did not improve or impair calcium retention or indicators of bone and cardiovascular health in postmenopausal women. (*J Clin Endocrinol Metab* 90: 181–189, 2005)

IN ADDITION TO providing structural framework and locomotion, the skeletal system serves as a buffering reservoir and aids the kidneys and lungs in the tight regulation of the systemic hydrogen ion concentration. Dietary practices that lead to chronic production of acid ash, such as diets high in meat, are hypothesized to tap into this alkali reservoir and cause a gradual dissolution of bone mineral (1, 2) and, as such, are considered a risk for hypercalciuria and osteoporosis (3–5). Although the sulfur amino acids in animal proteins, such as meat, are thought to cause hypercalciuria, the high phosphorus content of these proteins has been found to negate this effect (6). Many staple plant proteins, such as wheat and rice, have sulfur amino acid contents that are similar to or higher than those in meats (7), but the coexisting alkalis are thought to reduce the dietary acid load (8). Furthermore, the increased ammoniagenesis observed with higher protein intake may partly neutralize the acid production (9). Therefore, the net effect of a protein source on calcium balance is determined by many coexisting factors in the protein and in the whole diet and is therefore difficult to predict. Observational studies have indicated positive (10–12), negative (5, 13–15), or no association (16) between animal protein intake and bone health.

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Abbreviations: CONTROL, Control diet; GFR, glomerular filtration rate; HDL, high-density lipoprotein; IGF-IBP3, IGF-I binding protein-3; iPTH, intact PTH; LDL, low-density lipoprotein; NS, not significant; SOY, 25-g high-isoflavone soy protein (diet).

Very few controlled feeding studies have tested the relative calciuric effect of animal *vs.* plant protein food sources, in the context of a mixed diet (17–19). In a short-term feeding study (12 d), under conditions of equalized phosphorus and low calcium (400 mg), higher net renal acid excretion and urinary calcium loss were observed from diets containing meat *vs.* soy protein (19). However, in a recent controlled feeding study of high- *vs.* low-meat diets of much longer duration (8 wk), in which phosphorus content of the diets was not manipulated and calcium content mimicked typical intakes (~600 mg/d), no increase in urinary calcium excretion or change in whole-body calcium retention was observed in postmenopausal women (20). Similar calcium retention occurred despite the higher sulfur amino acid intake and the higher urinary sulfate and renal acid excretion during the high-meat diet. Although these findings (20), combined with those from earlier controlled feeding studies (17, 18), indicate that a moderate increase in meat intake does not impair calcium retention, three short-term (3- to 6-month) supplementation studies suggest that incorporation of soy protein isolate in the diet may improve calcium homeostasis (21–23). Although these studies point to a component in soy protein isolate, such as isoflavones, as an enhancer of calcium absorption and/or utilization, the phytate and oxalate content of soy may reduce the intestinal absorption of calcium (24) and other minerals (25, 26); thus, the net effect of regular consumption of soy protein on calcium homeostasis is not known.

The primary objective of this carefully controlled feeding study was to determine the effects of daily substitution of

high-isoflavone, intact soy protein for meat protein, in a typical mixed diet, on calcium retention and bone metabolism in healthy postmenopausal women. Because the amount of soy protein (25 g) was selected to conform with the Food and Drug Administration (FDA)-approved health claim (27), the secondary objective was to test the effects of this dietary practice on indicators of cardiovascular health.

Subjects and Methods

Subjects

Postmenopausal women were recruited through public advertising (television, newspapers) and by direct mailings. The women were selected after an interview and blood analysis and qualified to enter the study if they were age 50–75 yr; at least 3 yr since last menses; had FSH more than 40 IU/liter; were nonsmokers; had no apparent underlying disease; had normal bone mineral density (femoral neck T score ≥ -2.5) as determined by dual-energy x-ray absorptiometry (Hologic Delphi QDR, Bedford, MA); had normal thyroid, liver, and kidney functions; were willing to discontinue any nutritional supplements as soon as their applications were received; and did not regularly use any medications (except for hormone replacement therapy).

The study was approved by the University of North Dakota Radioactive Drug Research Committee and Institutional Review Board, and by the United States Department of Agriculture Radiological Safety Office. The study was explained verbally and in writing by the investigators, and written informed consent was given by each woman.

Of 15 women enrolled, two dropped out on the second day of the study (one because of transportation problems and the other without providing a reason). The remaining 13 (all white, six using hormone replacement therapy) were age 59.9 ± 5.0 yr (mean ± SD; range, 52–69), with a body mass index of 26.0 ± 5.0 kg/m^2; range, 19.7–38.5; median, 24.8; and femoral neck bone mineral density, 0.742 ± 0.093 (T-score range, −2.33 to −0.56). As estimated from 3-d food records, their calcium and protein intakes before the study were 857 ± 336 mg/d and 76 ± 23 g/d, respectively.

Diets

Registered dietitians planned two experimental diets using ordinary foods in a 2-d menu cycle (Table 1). The subjects consumed both diets for 7 wk, in a randomized crossover design, with a 2-wk break during which they consumed their self-selected diets but continued to take the multivitamin provided (Table 1). The weighed control diet (CONTROL) and 25-g high-isoflavone soy protein (SOY) diets provided (mean ± SD) similar amounts of protein (15% of energy; 1.32 ± 0.19 and 1.33 ± 0.17 g/kg body weight) but contained 170 and 55 g/d meat, respectively (Table 1). The amount of calcium in the diet was chosen to be similar to typical intakes by postmenopausal women in the United States (28). Although the diets were planned with similar amounts of calcium, the

TABLE 1. Ingredient difference and nutrient composition of controlled high- and low-meat diets consumed by healthy postmenopausal women for 7 wk each in a crossover design[a,b]

	CONTROL	SOY
Diet ingredient difference (g/d)		
d 1:		
Soy protein isolate (added to banana bread)[c]		+10
Mandarin oranges		+10
Beef[d]	+55	
Soy protein isolate (added to beef casserole)[c]		+10
Chicken[d]	+55	
White dinner roll		+5
Soy protein isolate (added to brownie)[c]		+10
d 2:		
Soy protein isolate (added to peanut butter)[c]		+5
Ham[d]	+60	
Soy protein isolate (added to gingerbread)[c]		+15
Chicken[d]	+60	
Soy protein isolate (added to cornbread)[c]		+10
Tub margarine	+1	
Nutrient composition		
Protein (% of energy)	15	15
Sulfur amino acids (g)	3.0	2.7
Fat (% of energy)	30	30
Saturated fat	7	7
Monounsaturated fat	13	13
Polyunsaturated fat	7	7
Cholesterol (mg)	220	157
Carbohydrate (% of energy)	55	55
Dietary fiber (g)	19	19
Calcium (mg)	690 (670 ± 7)	746 (754 ± 17)
Potassium (mg)	2617 (2584 ± 290)	2303 (2587 ± 339)
Sodium (mg)	3657 (3450 ± 152)	3504 (3323 ± 268)
Phosphorus (mg)	1505 (1596 ± 87)	1533 (1638 ± 104)
Magnesium (mg)	306	321
Phytate (mg)	1673	2246

[a] All values are based on energy intake of 9.2 MJ (2200 kcal). Values for protein, carbohydrate, fat, cholesterol, and minerals were calculated from U.S. Department of Agriculture food composition data. Data in *parentheses* represent analyzed values for minerals (mean ± SD).

[b] A chewable multivitamin tablet containing 100% daily value (DV) for vitamins A, C, D, E, B_6, B_{12}, thiamin, riboflavin, niacin, folic acid, and pantothenic acid was taken daily by the participants.

[c] The soy protein isolate contained 3.72 mg isoflavones/g protein (2.28 mg aglycone/mg protein; 1.22, 0.87, 0.19 mg/g protein of genistein, daidzein, and glycitein, respectively).

[d] The protein content of beef, chicken, and ham was calculated from U.S. Department of Agriculture, Nutrient Database for Standard Reference, Release 14 (Nutrient Data Laboratory home page, http//www.nal.usda.gov/fnic/foodcomp) and was 22.03, 19.35, and 23.09 (g/100 g), respectively.

analyzed values for calcium were slightly higher in the SOY diet than the CONTROL (754 ± 17 *vs.* 670 ± 7 mg calcium/2200 kcal, respectively), reflecting the calcium content of the soy protein isolate. The isoflavone content of the isolate was 2.28 mg aglycone/G protein (product: IB1.2UN30CA, lot no. 038A-02; Solae Company, St. Louis, MO) (Table 1). Phytate content of the diets was calculated from food composition tables (29) (Table 1). To maintain body weights, energy intakes were adjusted by proportionally changing the amounts of all foods. The energy intakes were similar during the two dietary periods (2216 ± 271 and 2189 ± 300 kcal for CONTROL and SOY, respectively). Amounts of coffee, tea, and artificially sweetened, noncola, carbonated beverages (limited to two total servings daily), salt, and pepper were individualized and kept constant. City water and chewing gum were consumed as desired. Participants were given a list of approved over-the-counter medications and mouth products. The food items were prepared in oven-/microwave-oven-safe containers. Participants consumed the food quantitatively, using spatulas and rinse bottles, eating one meal at the research center on weekdays and the remaining foods elsewhere.

Calcium retention measurements with ^{47}Ca

Dietary calcium retention was measured with a ^{47}Ca radiotracer and whole-body scintillation counting (30) with adjustments to account for ^{47}Sc activity. The ^{47}Ca isotope was obtained by neutron activation (University of Missouri, Columbia, MO) of stable ^{46}Ca (as calcium carbonate, 30.89% enriched; Oak Ridge National Research Laboratory, Oakridge, TN). The custom-made scintillation counter (31) detects γ-emissions with 32 crystal NaI(T1) detectors (10 × 10 × 41 cm each), arranged in two planes above and below a bed on which the subjects lie.

After 3 wk of dietary equilibration, all the meals in the 2-d menu were labeled with a total of 148 kBq (4 μCi) ^{47}Ca (<4 μg elemental calcium). Because of the concern that ingested calcium from some dietary sources may not form a common absorptive pool (32), both diets were designed with milk as the primary source of calcium. For each meal, the tracer was mixed with milk and allowed to equilibrate overnight. The specific activity (ratio of ^{47}Ca to elemental calcium) was constant for all meals for each individual during both dietary periods. The energy provided by the radiolabeled meals was constant during each 2-d administration. All labeled meals were consumed at the research center.

The initial total body activity was determined from the whole-body count, 1–3 h after the first labeled meal (before any isotope was excreted), divided by the fraction of the total activity that was in the first meal. Whole-body calcium retention was monitored for 28 d. Activity was corrected to the midpoint of the 2 d of labeled meals and adjusted for background and minor fluctuations in the measurement of a ^{47}Ca standard distributed in water (33). The precision of the whole-body counting measurements was 1.4%.

Analyses

The subjects provided total 48-h urine collections during wk 0, 3, 5, and 7 of each dietary period. Fasting blood samples were drawn at the beginning (wk 1) and end (wk 7) of each dietary period. For homocysteine and serum lipids, fasting blood samples were collected twice at the beginning and end of the study, separated by a few days, and the values were averaged. Calcium in the urine and acid-digested diet aliquots (34) was determined by inductively coupled argon plasma emission spectrophotometry. Mean (±SD) measurements were 98 ± 4% of certified values for calcium in a standard reference material (Typical Diet, 1548b, United States National Institute of Standards and Technology).

Urinary ammonium was determined colorimetrically (35) (Raichem, Hemagen Diagnostics, San Diego, CA). Titratable acidity was determined in undiluted urine by titrating to pH 7.40 with 0.1 N NaOH. Free organic acids were measured by the Van Slyke and Palmer (36) method as modified by Lemann *et al.* (37). Urinary sulfates were determined turbidometrically (38). ELISAs were used to determine bone-specific alkaline phosphatase (Metra Biosystems, Mountain View, CA) and estradiol (Abbott Laboratories, Abbott Park, IL). The inter- and intraassay variabilities were 5.2 and 5.0%, respectively, for bone-specific alkaline phosphatase and 6.2 and 6.7%, respectively, for estradiol. Serum tartrate-resistant acid phosphatase activity was determined using α-naphthylphosphate and diazotized-2-amino-5-chlorotoluene as substrates (39). Creatinine clearance was calculated from serum and urinary creatinine, which were measured using alkaline picric acid (40). RIAs were used to determine serum TSH (TSH, T_3, T_4) (Abbott Laboratories). The intra- and interassay variabilities were 4.2 and 5.4%, respectively, for TSH and 3.64 and 4.23%, respectively, for T_4. RIAs were also used for intact PTH (iPTH), osteocalcin, and 25-hydroxyvitamin D (Diasorin, Stillwater, MN). The intra- and interassay variabilities were 3.6 and 3.4%, respectively, for iPTH; 4.3 and 11.9%, respectively, for osteocalcin; and 8.2 and 8.6%, respectively, for 25-hydroxyvitamin D. Serum IGF-I and IGF-I binding protein-3 (IGF-IBP3) (Diagnostic Systems Laboratory, Webster, TX) and urinary N-telopeptides (Ostex, Seattle, WA) were determined by ELISAs. The intra- and interassay variabilities were 7.1 and 5.4%, respectively, for IGF-I; 9.6 and 11.4% for IGF-IBP3; and 8.0 and 5.1% for N-telopeptides. Plasma ionized calcium was measured with an electrode (41) (8^+ Electrolyte Analyzer, Nova, Waltham, MA). The inter- and intraassay variabilities for this assay were 2.0 and 3.0%, respectively. Serum cholesterol fractions and triglycerides were determined using an automated procedure (Cobas Mira, Roche Diagnostic Systems, Inc., Sommerville, NJ). The inter- and intraassay variabilities were 1.0 and 1.2%, respectively, for total cholesterol; 2.1 and 2.6%, respectively, for high-density lipoprotein (HDL) fraction; and 1.0 and 3.0%, respectively, for triglycerides. Serum homocysteine was analyzed by fluorescence polarization immunoassay using an automated procedure (Abbott Laboratories). The inter- and intraassay variabilities were 2.2 and 5.2%, respectively.

Urine samples from wk 3 were analyzed for the unconjugated isoflavones (aglycones) and for total isoflavone content as previously described (42, 43). Briefly, for excreted aglycones, samples were extracted twice with 5 ml diethyl ether, and the organic layers were evaporated to dryness at 55 C under nitrogen. For conjugated isoflavones, samples were enzymatically hydrolyzed with *Helix pomatia* (sulfatase/glucuronidase, 100/1000 U) at 37 C for 3 h. All samples were then extracted twice with 5 ml diethyl ether, and the organic layers were evaporated to dryness at 55 C under nitrogen. Dried extracts were reconstituted in 0.5 ml of a solvent containing a known amount of biochanin A and injected into the LC-MS system to determine the aglycone concentrations using conditions reported previously (1, 2). All samples were analyzed in triplicate, and the results were expressed as nanograms/milligrams creatinine after normalization with biochanin A. Genistein (5,7,4′-trihydroxyisoflavone) and daidzein (7,4′-dihydroxyisoflavone) were purchased from Indofine Chemical Company, Inc. (Belle Mead, NJ). Sulfatase type V (aryl-sulfate sulfohydrolase) from *Helix pomatia* with reported sulfatase activity of 15–40 U/mg and glucuronidase activity of 400–600 U/mg was purchased from Sigma Chemical Company (St. Louis, MO).

Statistics

Individual ^{47}Ca retention data were modeled with a two-component exponential equation, $y = \beta_1 e^{-k_1 t} + \beta_2 e^{-k_2 t}$, where y represents ^{47}Ca retention as a percentage of the administered dose, t represents the time in hours, and coefficients β_1 and β_2 represent the fractional biological turnover of the radiotracer, expressed as percent of dose, at rates k_1 and k_2, respectively. The calcium retention data for d 2–5 were not included in the model because they primarily represent the delay in elimination of the unabsorbed isotope. The percentage of ^{47}Ca initially absorbed was separately estimated from the y-intercept of the linear portion (d 9–25) of a semilogarithmic retention plot of percent ^{47}Ca retained *vs.* time.

Diet and sequence effects were evaluated for the parameter estimates in the calcium retention models, calcium retention on d 7, 14, 21, and 28, and initial calcium absorption. Urinary and blood measurements (wk 3, 5, and 7) were analyzed by using repeated-measures ANOVA followed by Tukey's contrasts (44). Variances in the data were expressed as pooled SD from the ANOVA. Sequence effects were evaluated before testing for diet and time effects and were found to be significant ($P < 0.1$) only for serum creatinine, homocysteine, low-density lipoprotein (LDL), total HDL, and HDL-3. For these variables, the mixed model was modified to use the diet × time × sequence interaction as the error term for the primary effects of diet and time. When data were highly skewed, they were logarithmically transformed so that the distribution would more closely approximate a normal distribution. For these, geometric means are reported in addition to the mean and pooled SD of the transformed data. The urinary isoflavone data were highly variable and were therefore ranked before ANOVA, and median and range of values are re-

ported. Using two-tailed probabilities, $P \leq 0.05$ was considered significant.

Results

Whole-body retention and intestinal absorption of calcium

Substitution of 25 g soy protein for meat protein did not affect the efficiency of calcium retention at any of the weekly time points tested (Table 2 and Fig. 1). By d 28, 14.0 and 14.1 (percent dose, ±1.6) of the calcium tracer was retained from the CONTROL and SOY diets, respectively (Table 2, Fig. 1). As indicated by the two-component exponential model, with both diets, more than 75% of the tracer was eliminated rapidly (biological half-life, 1.5 d), indicating the excretion of the unabsorbed isotope and early endogenous losses. The remaining tracer (~20%) was eliminated less rapidly, with a biological half-life of 45–49 d (Table 2). Despite the higher phytate content of the SOY diet (by 573 mg/d), the initial absorption of calcium, expressed as percent dose, was also similar between the two diets [mean ± pooled SD, 26.1 *vs.* 27.0% ± 7.0; not significant (NS); Table 2]. Calcium retention was not different between the two diets, at any time points tested, in women using hormone replacement therapy (n = 6).

Urine composition

Urinary pH was higher with the SOY, compared with CONTROL, by an average of about 0.15 pH units across all time points tested (overall mean, 6.33 *vs.* 6.19, respectively; $P \leq 0.0001$; Table 3) and decreased with time on both diets ($P = 0.002$). Titratable acidity was also lower by wk 3 of the SOY diet, and this difference was sustained throughout the diet period (overall mean, 41.5 *vs.* 53.4 mEq/d for SOY and CONTROL, respectively; $P = 0.03$; Table 3). Diet did not affect ammonium excretion at any of the time points tested (overall mean, 37.6 *vs.* 43.9 mEq/d for SOY and CONTROL, respectively, NS; Table 3), at least partially reflecting the similar nitrogen content of the two menus. However, renal acid excretion, defined as the sum of ammonium and titratable acidity, was consistently lower during the SOY dietary period (overall mean, 97.2 *vs.* 79.1 mEq/d; $P = 0.0001$ for CONTROL and SOY diets, respectively; Table 3). Urinary free organic acid (representing compounds such as citric, acetic, and lactic acids) was similar during the two dietary periods. Urinary sulfate excretion was initially lower during the SOY than the CONTROL diet at wk 3, but this difference abated by wk 7 because of a gradual decrease during the CONTROL and an increase during the SOY dietary period (diet × time interaction, $P = 0.01$, Table 3). Diet did not affect creatinine clearance measured at the end of each dietary period (1.33 *vs.*1.27 ml/sec for CONTROL and SOY diets, respectively, NS; Table 3).

Despite the lower urine pH, and approximately15–20% lower renal acid excretion during the SOY diet, urinary calcium excretion was similar between the two diets at all time points tested (overall mean, 3.53 and 3.48 mmol/d, NS, for the CONTROL and SOY diets, respectively; Table 3). No correlation between renal acid and urinary calcium excretion was detected. Urinary calcium, phosphorus, and oxalate excretion slightly increased, and urinary pH decreased, over time during both dietary periods ($P < 0.002$; Table 3).

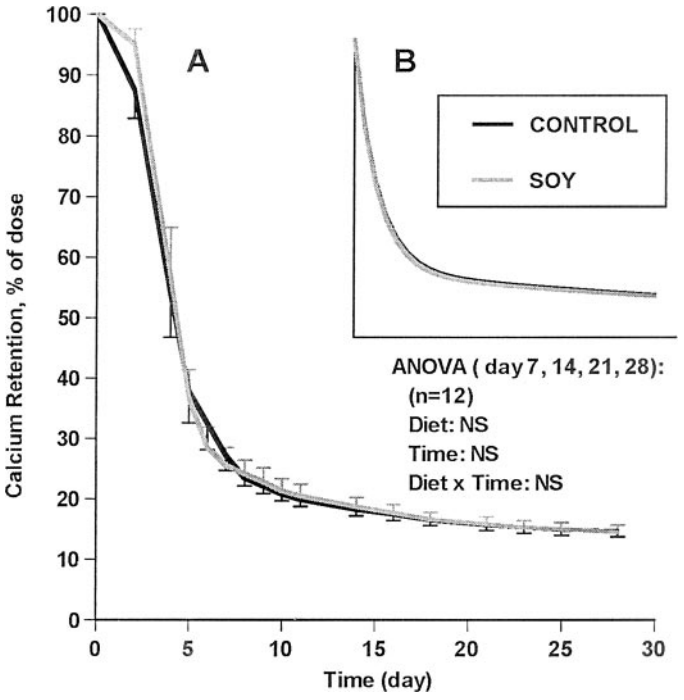

FIG. 1. A, Whole-body calcium retention, as percent of dose (mean ± SEM, n = 13) over time. Calcium retention was similar between the CONTROL and SOY diets at all weekly time points tested (see Table 2). B, The two component exponential models that fit the data for 12 of the 13 subjects. These group models ($y = 77.4\ e^{-0.0196t} + 22.7\ e^{-0.0007t}$ for high-meat and $y = 78.5\ e^{-0.0191t} + 21.6\ e^{-0.0007t}$ for CONTROL, where t is expressed in hours) use the mean coefficients from the modeled retention curves for each individual. Because of the variability in the early elimination of the isotope, calcium retention data for the first 2–5 d were omitted before the modeling of the data. The retention curves, modeled separately for individual on each diet, had coefficients of determination (R^2) of 0.97–0.99.

TABLE 2. Whole-body retention of a calcium radiotracer (^{47}Ca), measured by whole-body scintillation counting, as affected by daily substitution of SOY for an equivalent amount of CONTROL for 7 wk in healthy postmenopausal women[a,b]

	CONTROL	SOY	Pooled SD	*P*
β_1 (% dose)	77.4	78.5	3.0	NS
β_2 (% dose)	22.7	21.6	2.9	NS
ln (T_1)	0.43	0.43	0.09	NS
[Days][c]	[1.5]	[1.5]		
ln (T_2)	3.8	3.9	0.13	NS
[Days][c]	[44.7]	[49.4]		
Retention @ d 7 (%)	24.4	23.5	4.8	NS
Retention @ d 14 (%)	18.2	17.7	2.3	NS
Retention @ d 21 (%)	15.9	15.6	1.7	NS
Retention @ d 28 (%)	14.1	14.0	1.6	NS
Absorption (% of dose)[d]	26.1	27.0	7.0	NS

[a] Mean ± SD, n = 12.

[b] A two-component exponential model fit the data (R^2 of 0.97 to 0.99 for models of individuals on each diet). β_1 and β_2 represent the percentage of the isotope having a biological half-life of T_1 and T_2, respectively. One subject's data could not be modeled using the two-component exponential equation.

[c] Geometric means.

[d] Intestinal calcium absorption, as percentage of dose, was estimated by using the linear portion (d 9–25 after ^{47}Ca administration) of a semilogarithmic retention plot [ln(% retention *vs.* time)] and extrapolating back to the time of tracer administration.

TABLE 3. Urine composition of total 48-h composites as affected by a daily substitution of SOY for an equivalent amount of CONTROL for 7 wk in healthy postmenopausal women

	CONTROL			SOY			Pooled SD	Diet *P* value	Time *P* value
	Wk 3	Wk 5	Wk 7	Wk 3	Wk 5	Wk 7			
pH	6.25	6.17	6.13	6.41	6.29	6.30	0.12	0.0001	0.002
Titratable acidity (mEq/d)	28.2	25.9	25.1	19.9	20.4	20.2	8.1	0.03	NS
Ammonium (mmol/d)	43.5	41.9	46.1	35.0	35.5	42.2	5.8	NS	NS
Renal acid excretion (mEq/d)[a]	70.9	66.3	69.4	55.0	56.4	62.3	10.5	0.0001	NS
ln (free organic acid) (mEq/d)	1.82 [6.17]	1.94 [6.96]	1.57 [4.81]	1.22 [3.39]	1.63 [5.10]	1.75 [5.75]	0.56	NS	NS
Sulfate (mmol/d)[b]	17.0[x]	15.3[x,y]	16.8[x]	14.3[x]	15.4[x,y]	16.4[x]	1.7	0.01	0.03
ln (calcium) [mg/d (mmol/d)]	4.86 (3.25) [130.0]	4.81 (3.09) [123.6]	5.06 (3.97) [158.8]	4.86 (3.22) [128.7]	4.88 (3.32) [132.6]	5.05 (3.93) [157.2]	0.22	NS	0.001
Phosphorus [mg/d (mmol/d)]	964.9 (31.2)	866.8 (28.0)	1167.5 (37.7)	874.4 (28.2)	896.8 (29.0)	1097.5 (35.4)	173.2	NS	0.0001
Oxalate [mg/d (μmol/d)]	26.2 (291)	24.5 (272)	28.3 (314)	21.4 (238)	24.2 (269)	27.5 (305)	3.6	NS	0.0004
Creatinine [g/d (mmol/d)]	1.10 (9.7)	1.11 (9.8)	1.14 (10.1)	0.98 (8.6)	1.01 (8.9)	1.07 (9.5)	0.12	0.003	NS
ln (N-telopeptide), nM BCE/mM creatinine			3.08 [21.8]			3.20 [25.0]	0.24	NS	
Hydroxyproline [mg/d (μmol/d)]			0.108 (107.9)			0.105 (104.5)	17	NS	
Creatinine clearance (ml/sec)			1.38			1.37	0.16	NS	

Data are means with pooled SD from ANOVA (n = 13). *P* values are from ANOVA. Data in *brackets* are geometric means.
[a] Defined as sum of titratable acidity and ammonium excretion. BCE, Bone collagen equivalents.
[b] Significantly affected by diet × time interaction. Means without a *common superscript* (*x*, *y*) are significantly different as determined by Tukey's contrasts ($P < 0.05$).

Biomarkers of soy intake

Urinary isoflavone concentrations were used as a confirmatory marker of soy protein intake. The mean (±SEM) total urinary isoflavone concentrations were 100.41 ± 11.39 μmol/mg creatinine in the soy-consuming women and 7.5 ± 1.30 μmol/mg creatinine when no added soy protein was consumed. Based on our previous experience, the total urinary isoflavone excretion was consistent with the amount of soy consumed (42, 43). Furthermore, none of the women in this study were considered equol producers, because the mean equol excretion rate during the soy intake periods was 13.69 ± 4.46 μmol/d (range, 1.22–9.49).

Biomarkers of bone metabolism

Substitution of soy protein for meat did not affect bone metabolism as indicated by specific blood biomarkers of bone formation (serum bone-specific alkaline phosphatase, osteocalcin, and IGF-I) or of bone resorption (serum tartrate-resistant acid phosphatase) (Table 4). Several other blood and urinary indices of bone and mineral metabolism, iPTH, 25-hydroxyvitamin D (Table 4), cortisol, plasma zinc, magnesium, calcium, phosphorus, ionized calcium, ionized magnesium (data not shown), and urinary N-telopeptide and hydroxyproline excretion, were also unaffected by dietary treatments (Table 3). Diet did not change any of the measured indicators of thyroid function (Table 4). The subjects were replete with vitamin D as indicated by serum 25-hydroxyvitamin D at wk 1 of both dietary periods (71.8 and 68.2 nmol/liter for CONTROL and SOY, respectively). Although this study was conducted during October–March in Grand Forks, ND (latitude, 47.5° N), the vitamin D status of the subjects was successfully maintained and even slightly increased with a daily vitamin D_3 supplement of 20 μg/d (Table 4).

Biomarkers of cardiovascular health

Daily substitution of soy protein for meat protein did not affect serum homocysteine concentration or the lipid profile (LDL, HDL, total cholesterol, and triacylglycerol) (Table 5). Diet also did not change any of the measured hemostatic indicators, such as fibrinogen, protime, and partial thromboplastin time (data not shown).

Discussion

Effects of meat vs. soy protein on calcium homeostasis

The results of this carefully controlled feeding study indicate that a daily substitution of 25 g intact, high isoflavone soy protein for an equivalent amount of meat protein for several weeks, in a mixed diet with typical calcium content, does not improve or impair calcium homeostasis in healthy postmenopausal women. This conclusion is supported by the whole-body calcium retention data (Table 2 and Fig. 1) and by a host of biomarkers of bone metabolism (Tables 3 and 4).

The design of this study was optimized for comparison of calcium retention by assuring sufficient statistical power (12 subjects were needed to detect a difference of 2.5 percentage points in calcium retention in a crossover design; 90% power; $\alpha = 0.05$; residual SD = 1.5). The measurements were made

TABLE 4. Blood biomarkers of bone and mineral status as affected by daily substitution of SOY for an equivalent amount of CONTROL for 7 wk in healthy postmenopausal women

	CONTROL	SOY	Pooled SD	Diet *P* value	Time *P* value
Bone-specific alkaline phosphatase [U/liter (μkat/liter)]					
Wk 1	17.9 (0.30)	18.4 (0.31)	1.9	NS	0.01
Wk 7	19.7 (0.33)	19.6 (0.33)			
ln (Osteocalcin) [ng/ml (nmol/liter)]	1.16 (−0.58) [3.20]	1.15 (−0.59) [3.16]	0.22	NS	NS
Serum IGF-I [ng/ml (nmol/liter)]	112.5 (14.8)	125.1 (16.8)	23.6	NS	NS
Serum IGF-IBP3 [ng/ml (nmol/liter)]	4711.3 (162.5)	4670.8 (161.1)	10.2	NS	NS
Tartrate-resistant acid phosphatase [U/liter (μkat/liter)]	2.66 (44.3)	2.75 (45.8)	0.36	NS	NS
25-OH vitamin D [ng/ml (nmol/liter)]					
Wk 1	28.7 (71.8)	27.8 (68.2)	4.4	NS	0.004
Wk 7	30.5 (76.3)	33.8 (83.4)			
ln (iPTH) [pg/ml (pmol/liter)]	3.48 (1.25) [32]	3.49 (1.26) [33]	0.13	NS	NS
Total T_3 [ng/ml (nmol/liter)]	71.0 (1.09)	72.9 (1.12)	6.5	NS	NS
Total T_2 [μg/dl (nmol/liter)]	8.8 (113.0)	9.1 (116.9)	0.6	NS	NS
TSH [μU/ml (mU/liter)]	2.51 (2.51)	2.62 (2.62)	0.60	NS	NS

Data are mean with pooled SD (n = 13). *P* values are from ANOVA. The data in *brackets* are geometric means. Blood was drawn twice at the beginning and the end of each dietary period. When no time or diet × time interaction was detected, the data from the four blood draws were pooled. μkat, Microkatal.

after allowing for both equilibration and a break between the diets (total of 5 wk) and using sensitive radiotracer and whole-body counting methodology, in a crossover design. The subjects continued to consume the controlled diets during the whole-body counting measurements (4 additional wk); thus, the final retention data reflect not only the initial bioavailability of calcium but also the net effect of the subsequent loss through multiple excretory pathways. For this reason, the diets were designed to be similar except for the protein components, and the intakes of other factors known to influence calcium excretion (*e.g.* salt, potassium, caffeine) (28) were kept constant for each individual. The phosphorus content of the diets was similar without manipulation of the menus.

The hypercalciuric effect of protein is thought to be related to increased glomerular filtration rate (GFR) and reduced renal reabsorption of calcium in response to the acid-ash produced from sulfur amino acid catabolism (45–49). In the current study, despite lower urinary acidity and lower sulfate and renal acid excretions during the SOY diet, no change in creatinine clearance, a surrogate measure of GFR, was observed (Table 3). Therefore, it is not surprising that urinary calcium and whole-body calcium retention were not affected by the diets. This lack of responsiveness of urinary calcium excretion to both sulfate and renal acid excretions is consistent with our earlier findings (20). In a study of similar design, although renal acid excretion was about 45% higher during wk 3 of the high-meat period (20), urinary calcium excretion was not different between controlled high- and low-meat diets (20% *vs.* 12% of energy as protein). This difference abated to only 18% by wk 8 of the study, indicating adaptation in renal acid excretion over time (20). These collective findings indicate that changes in urinary acidity within the range that may result from common, practical dietary practices do not reach a threshold that triggers an increase in GFR and/or use of body calcium as a buffering agent. Remer *et al.* (50) have suggested that a net acid excretion of more than 120 mEq/d is required for the depletion of plasma bicarbonate and use of alkali from the skeleton. In this study, the acid excretion (uncorrected for bicarbonate excretion) during the CONTROL diet (97 mEq/d) was below this threshold.

It is possible that diet-induced metabolic acidosis induces bone resorption through a cell-mediated mechanism (51), by inhibition of osteoblasts and stimulation of osteoclasts (52), without the direct involvement of the renal system. However, lack of changes in both bone resorption and formation biomarkers in this study do not support this hypothesis.

The current study provides strong evidence that a daily incorporation of soy protein (with high isoflavone content) in place of meat protein, in a mixed diet with typical calcium intakes of approximately 700 mg/d, does not improve calcium retention. However, several supplementation studies have suggested a bone sparing effect for high-isoflavone soy

TABLE 5. Serum homocysteine and lipid concentrations as affected by daily substitution of SOY for an equivalent amount of CONTROL for 7 wk in healthy postmenopausal women

	CONTROL	SOY	Pooled SD	Diet *P* value
Homocysteine [μg/L (μmol/liter)]	1162.7 (8.6)	1149.2 (8.5)	94.6	NS
Cholesterol [mg/dl (mmol/liter)]	244.1 (6.31)	240.9 (6.23)	12.8	NS
LDL [mg/dL (mmol/liter)]	141.9 (3.67)	138.4 (3.58)	0.09	NS
Total HDL [mg/dl (mmol/liter)]	82.0 (2.12)	82.4 (2.13)	6.6	NS
HDL-3 [mg/dl (mmol/liter)]	68.4 (1.77)	68.8 (1.78)	3.5	NS
ln (Triacylglycerols) [mg/dl (mmol/liter)]	4.54 (0.06) [93.7]	4.52 (0.04) [91.8]	0.14	NS

Data are mean with pooled SD (n = 13) and represent average of two blood draws at the beginning (wk 0, 1) and end of each dietary period (wk 6 and 7).

Data in *brackets* are geometric means. None of the variables were significantly affected by diet, time, or diet × time interaction.

protein compared with milk proteins in peri-and postmenopausal women (22, 23, 53). Studies in ovarioectomized rats have also indicated protection from estrogen deficiency bone loss in animals fed intact soy protein *vs.* casein (54–56). Although the results of the human studies cited above (22, 23, 53) must be interpreted with full recognition of their short duration and small sample size, the favorable effects of soy protein supplementation on bone are intriguing and imply improved calcium retention compared with isolated milk proteins. This difference may be related to the higher calciuric effect of milk-based proteins *vs.* soy protein. In a recent study, urinary calcium excretion increased by 33% from baseline in those receiving 40 g/d isolated milk-based proteins compared with no change in those receiving soy protein (23).

Alternatively, the putative beneficial effect of soy protein supplementation in the previous studies may be related to the higher combined doses of both soy protein (40 g/d) and supplemental calcium (650–1400 mg/d) used (22, 23, 53). The results of two recent studies (57, 58) suggest that calcium intake may modulate the effect of protein on the skeleton and that, at high intakes, dietary calcium and protein may synergistically interact to favorably affect bone mass. The mechanism for this interaction is not known but is thought to be at least partially related to an increase in the concentration of serum IGF-I, an osteotrophic growth factor. This stimulatory effect on serum IGF-I has been observed with protein supplements alone (59) and with milk supplementation providing both protein and calcium (60, 61). A recent study has indicated that supplemental soy protein may be more potent in increasing serum IGF-I than milk-based proteins in postmenopausal women (23), suggesting that the source of protein may modify the protein effect on serum IGF-I. This notion is not supported by the findings of the current study, given that both soy and meat protein consumption failed to affect serum IGF-I. However, the study design was not optimal to test this idea, because the subjects were generally well-nourished, and their protein intake was not supplemented, closely mimicking their usual diets.

Effects of meat vs. soy protein on indicators of cardiovascular health

In this study, daily substitution of 25 g soy protein for meat for 7 wk did not improve serum lipids (Table 5) in mildly hypercholesterolemic women. Although the dose of soy protein conformed to the FDA-approved health claim (27), the lack of an effect was somewhat expected, because the fat composition of the diets was similar, and also the beneficial effects of soy protein are recently shown to be less than initially reported. The landmark meta-analysis of 38 studies (62), the basis of the health claim, predicted a decrease in total and LDL cholesterol concentrations of 9% and 13%, respectively, with an average intake of 47 g/d soy protein compared with casein. However, other studies have shown smaller effects, of 2–7% or no effect, of soy protein or soy products (63, 64) and a slower response in postmenopausal women (21). The hypocholesterolemic effect of soy protein is thought to be related to increased T_4 levels (65). In this study, thyroid function was unaffected by soy protein intake (Table 4). The small increase in free T_3 during the SOY dietary period is consistent with a previous report (66) but may not be of clinical consequence.

Plasma total homocysteine concentration has been shown to be an independent indicator of atherosclerotic disease (67). The results of a recent study indicated that, compared with casein, 30–50 g/d soy, added to self-selected diets, improves plasma total homocysteine concentrations in hyperlipidemic individuals (68). In this controlled diet study, soy protein did not affect plasma total homocysteine in mildly hypercholesterolemic women with normal homocysteine levels.

In summary, preliminary evidence from previous supplementation studies indicates improvements in bone and cardiovascular health from consuming soy protein compared with isolated milk proteins. In this carefully controlled feeding study, the calcium retention data, with supportive clinical chemistry, provide strong evidence that incorporation of soy protein in place of meat protein, in amounts currently recommended and combined with typical calcium intakes, provides no improvements or deterioration in calcium homeostasis or cardiovascular health. The potential synergistic effects between dietary protein and calcium, and the mechanisms of how protein source may modulate this interaction to affect bone health, should be rigorously investigated. Defining the relationship between dietary protein (source and quantity) and calcium metabolism will have important implications for nutrition policy and evidence-based advice for prevention of the growing problem of osteoporosis.

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Erratum

In the article "Preoperative Evaluation of Infants with Focal or Diffuse Congenital Hyperinsulinism by Intravenous Acute Insulin Response Tests and Selective Pancreatic Arterial Calcium Stimulation" by Charles A. Stanley, Paul S. Thornton, Arupa Ganguly, Courtney MacMullen, Patricia Underwood, Pooja Bhatia, Linda Steinkrauss, Laura Wanner, Robin Kaye, Eduardo Ruchelli, Mariko Suchi, and N. Scott Adzick (*The Journal of Clinical Endocrinology & Metabolism* **89:**288–296, 2004), there is an error in Table 5. The K_{ATP} channel mutation $K_{ir}6.2$ region should be listed as A101N instead of A102N. *The authors regret the error.*

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