Dietary protein and calcium interact to influence calcium retention: a controlled feeding study

Janet R Hunt, LuAnn K Johnson, and ZK Fariba Roughhead

ABSTRACT
Background: The effect of meat protein on calcium retention at different calcium intakes is unresolved.
Objective: The objective was to test the effect of dietary protein on calcium retention at low and high intakes of calcium.
Design: In a randomized controlled feeding study with a 2 x 2 factorial crossover design, healthy postmenopausal women (n = 27) consumed either ≈675 or ≈1510 mg Ca/d, with both low and high protein (providing 10% and 20% energy) for 7 wk each, separated by a 3-wk washout period. After 3 wk, the entire diet was extrinsically labeled with 47Ca, and isotope retention was monitored by whole-body scintillation counting. Clinical markers of calcium and bone metabolism were measured.
Results: High compared with low dietary protein significantly increased calcium retention from the low-calcium (29.5% compared with 26.0% absorbed) but not the high-calcium diet (18% absorbed). For the low-calcium diet, this effect nearly balanced a protein-related 0.5-mmol/d greater urinary calcium excretion. Protein-related calciuretic effects were independent of dietary calcium. Testing at 1, 2, 3, 5, and 7 wk showed no long-term adaptation in urinary acidity or urinary calcium excretion. High compared with low dietary protein decreased urinary deoxypyridinoline and increased serum insulin-like growth factor 1 without affecting parathyroid hormone, osteocalcin, bone-specific alkaline phosphatase, or tartrate-resistant acid phosphatase.
Conclusions: In healthy postmenopausal women, a moderate increase in dietary protein, from 10% to 20% of energy, slightly improved calcium absorption from a low-calcium diet, nearly compensating for a slight increase in urinary calcium excretion. Under practical dietary conditions, increased dietary protein from animal sources was not detrimental to calcium balance or short-term indicators of bone health. Am J Clin Nutr 2009:89:1357-65.

INTRODUCTION

Whether dietary protein has an anabolic or catabolic effect on bone remains a controversial issue. Although dietary protein from purified protein sources generally increases urinary calcium excretion (1-4), this effect is reduced if not eliminated when meat is the protein source (5-7). Furthermore, the calciuretic effect of protein may be counterbalanced by an increase in dietary calcium absorption (8, 9). Protein is a primary bone constituent, and the anabolic effect of dietary protein may be beneficial for bone mass, especially if dietary calcium is adequate (10). However, concerns persist about the effect of protein on acid-base balance, its associated effect on calcium excretion, and possible negative consequences for bone health (11, 12), especially if calcium intakes are low.

In this controlled feeding study, the objectives were to determine the nature of interaction between dietary protein and calcium on 1) calcium retention (by using 47Ca radiotracer and whole-body scintillation counting) and 2) blood and urinary biomarkers of calcium and bone metabolism. Multiple urinary measurements during a 7-wk dietary treatment period also enabled examination of possible adaptations in urinary acidification and calciurea.

SUBJECTS AND METHODS

General protocol and treatment assignment

This controlled feeding trial was conducted as a randomized crossover design (2 x 2 factorial); each subject consumed one amount of dietary calcium but alternately consumed 2 amounts of dietary protein. After statistically blocking to attain an even distribution of body mass index (BMI; in kg/m²), subjects were randomly assigned to either high-calcium (HC) or low-calcium (LC) treatment groups. Subjects were further randomly assigned to a sequence for consuming both low-protein (LP) and high-protein (HP) diets. Thus, every subject consumed 2 experimental diets for 7 wk each. The 2 diet periods were separated by a 3-wk washout period when diets were not controlled.

Subjects

Healthy postmenopausal women were recruited through public advertising and direct mailings. The women were selected after an interview and blood analysis if they met the following criteria:

1. From the US Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND (JRH and ZKFR), and the University of North Dakota, Grand Forks, ND (LKJ).
2. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the US Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.
3. Supported by the USDA Agricultural Research Service, the National Cattlemen's Beef Association, and the North Dakota Beef Commission.
4. Reprints not available. Address correspondence to JR Hunt, US Department of Agriculture, ARS, GPHNRC, 2420 2nd Avenue N, STOP 9034, Grand Forks, ND 58202-9034. E-mail: janetrhunt@gmail.com.

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age 50–80 y; ≥3 y since last menses; follicle-stimulating hormone >40 IU/L; no apparent underlying disease; normal bone mineral density (femoral neck T score ≥−2.5) as determined by dual-energy X-ray absorptiometry (Hologic Delphi QDR, Bedford, MA); normal thyroid, liver, and kidney functions; BMI ≤35; and no regular use of medications. Hormone replacement therapy was allowed if it had been regularly used for ≥1 y. Tobacco smokers were also allowed. Subjects were willing to discontinue any nutritional supplements.

The study was approved by the University of North Dakota's Radioactive Drug Research Committee and Institutional Review Board, and by the US 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 34 women enrolled, 7 withdrew: 2 with dietary intolerance, 2 with illness, and 3 with personal scheduling conflicts. Subject characteristics such as age, BMI, bone mineral density, previous calcium and protein intakes, serum and urinary calcium, urine pH, and use of hormone replacement therapy or tobacco did not differ between the 2 randomly assigned treatment groups (Table 1). As estimated from 3-d food records, the women's diets before the study contained 749 ± 220 mg Ca and 66 ± 16 g protein daily.

**Diets**

Four controlled experimental diets with 2 amounts of calcium and 2 of protein (LCLP, LCHP, HCLP, HCHP) were planned with US Department of Agriculture food composition data (13) supplemented with published phytate data (14), using ordinary foods in a 2-d menu cycle. On the basis of an average daily energy intake of 9.5 ± 0.8 MJ (2270 ± 190 kcal), the LC and HC diets contained ≈675 and 1510 mg Ca, respectively (Table 2). The primary source of calcium for the LC diets was skim milk, divided into equal portions at each meal. Differences in calcium content between the diets were attained by increasing portions of meat (mainly beef, plus chicken and pork) and milk (Table 2), with isocaloric reductions in fruit and fruit juices, and smaller changes in grains, sweets, and fat portions. Vegetable contents were the same for all diets. Calculation of potential renal acid load (15) predicted 25 mEq less acid load with the LP compared with the HP diets, and 9–10 mEq less acid load with the HC compared with the LC diets (Table 2). All diets were supplemented with a daily chewable multivitamin tablet containing 10 µg cholecalciferol (Table 2 footnote).

To maintain body weights, energy intakes were adjusted by proportionally changing the amounts of all foods. Coffee, tea, and artificially sweetened, noncola carbonated beverages (containing citric acid rather than phosphoric acid) were individualized, limited to 2 total servings daily and kept constant. Similarly, salt consumption was individualized, and kept constant. City water and chewing gum were consumed as desired, after analyses indicating minimal mineral content. The subjects were given a list of approved over-the-counter medications, toothpaste, and dental adhesives that contained minimal amounts of calcium or other minerals. All diet ingredients except water were weighted to 1% accuracy and as possible were purchased from single production lots. Subjects consumed the food quantitatively, with the aid of spatulas and rinse bottles, consuming one meal at the Research Center on weekdays and the remaining foods elsewhere.

**Calcium retention measurements with 47Ca**

Dietary calcium retention was measured with a 47Ca radiotracer and whole-body scintillation counting (16), with adjustments to disregard 47Sc activity. The 47Ca isotope (with a radioactive half-life of 4.5 d) was obtained by neutron activation (University of Missouri, Columbia, MO) of stable 46Ca (as calcium carbonate, 30.89% enriched; Oak Ridge National Research Laboratory, Oak Ridge, TN). The custom-made scintillation counter detects γ radiation and allows for energy discrimination.

**TABLE 1**

Baseline subject characteristics for the 2 calcium treatment groups

<table>
<thead>
<tr>
<th></th>
<th>Low calcium (n = 13)</th>
<th>High calcium (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low calcium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>58 ± 6 (50–69)</td>
<td>57 ± 5 (51–67)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28 ± 4 (22–34)</td>
<td>28 ± 4 (20–32)</td>
</tr>
<tr>
<td>Total hip BMD T score</td>
<td>−0.4 ± 0.9 (−1.8 to 1.5)</td>
<td>−0.6 ± 0.9 (−1.8 to 0.7)</td>
</tr>
<tr>
<td>Lumbar spine (L1 to L4) BMD T score</td>
<td>−0.4 ± 1.2 (−2.0 to 1.6)</td>
<td>−0.7 ± 1.1 (−2.4 to 1.3)</td>
</tr>
<tr>
<td>Prestudy dietary calcium intake (mg/d)</td>
<td>729 ± 216 (519–1174)</td>
<td>768 ± 231 (304–1201)</td>
</tr>
<tr>
<td>Prestudy dietary protein intake (g/d)</td>
<td>63 ± 15 (36–92)</td>
<td>69 ± 17 (44–96)</td>
</tr>
<tr>
<td>Serum calcium (mmol/L)</td>
<td>2.67 ± 0.26 (2.27–3.16)</td>
<td>2.72 ± 0.31 (2.33–3.52)</td>
</tr>
<tr>
<td>Urine calcium (mmol/d)</td>
<td>3.3 ± 1.4 (1.1–5.7)</td>
<td>4.5 ± 1.8 (1.3–6.8)</td>
</tr>
<tr>
<td>Urine pH</td>
<td>6.2 ± 0.5 (5.1–6.9)</td>
<td>6.4 ± 0.4 (5.8–7.1)</td>
</tr>
<tr>
<td>Hormonal replacement therapy (n)</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Smokers of tobacco (n)</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

1 BMD, bone mineral density. All subjects were white women, except for one woman of Asian descent who was in the high-calcium group. Baseline subject characteristics did not differ significantly between calcium treatment groups (Student's t test).

2 Mean ± SD; range in parentheses (all such values).

3 To convert serum calcium from mmol/L to mg/dL, multiply by 0.0357. To convert urine calcium from mmol/d to mg/d, multiply by the molecular weight of 40 for calcium.
The precision of the whole-body counting measurements was the measurement of a 47Ca standard distributed in water (19). Activity was corrected to the midpoint of the 2 d of labeled first meal. Whole-body calcium retention was monitored for body count 1-3 research center. Constant for the radiolabeled meals between the 2 diets for each meals and adjusted for background and minor fluctuations in was excreted), divided by the fraction of the total activity in the subject. All labeled meals were consumed under supervision at the night. The specific activity (ratio of 47Ca to elemental calcium) the tracer was mixed with milk and allowed to equilibrate over-night. The concern that ingested calcium from some dietary sources may not form a common absorptive pool (18), all diets were de-signed with milk as a primary source of calcium. For each meal, the tracer was mixed with milk and allowed to equilibrate overnight. The specific activity (ratio of 47Ca to elemental calcium) was constant for all meals for each subject. The dietary energy was constant for all meals for each subject. All labeled meals were consumed under supervision at the research center.

The initial total body activity was determined from the whole-body emissions with 32 crystal NaI(Tl) detectors (10 × 10 × 41 cm each) arranged in 2 planes above and below a bed on which the subjects lie (17).

After 3 wk of equilibration to each diet, all the meals of the 2-d menu were extrinsically labeled with a total of 148 kBq (4 μCi) 47Ca radiotracer (the activity as of midnight between the 2 d). Because of the concern that ingested calcium from some dietary sources may not form a common absorptive pool (18), all diets were designed with milk as a primary source of calcium. For each meal, the tracer was mixed with milk and allowed to equilibrate overnight. The specific activity (ratio of 47Ca to elemental calcium) was constant for all meals for each subject. The dietary energy was constant for the radiolabeled meals between the 2 diets for each subject. All labeled meals were consumed under supervision 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 in the first meal. Whole-body calcium retention was monitored for 25 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 47Ca standard distributed in water (19). The precision of the whole-body counting measurements was 1.4%.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>LCLP</th>
<th>LCHP</th>
<th>HCLP</th>
<th>HCHP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Meat (g)</strong></td>
<td>60</td>
<td>323</td>
<td>44</td>
<td>297</td>
</tr>
<tr>
<td><strong>Milk (g)</strong></td>
<td>371</td>
<td>371</td>
<td>516</td>
<td>578</td>
</tr>
<tr>
<td><strong>Protein (g)</strong></td>
<td>58</td>
<td>112</td>
<td>59</td>
<td>115</td>
</tr>
<tr>
<td><strong>Fat (% of energy)</strong></td>
<td>20</td>
<td>20</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td><strong>Carbohydrate (% of energy)</strong></td>
<td>61</td>
<td>51</td>
<td>61</td>
<td>51</td>
</tr>
<tr>
<td><strong>Dietary fiber (g)</strong></td>
<td>20</td>
<td>18</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td><strong>Dietary phytate (mg)</strong></td>
<td>511</td>
<td>479</td>
<td>481</td>
<td>478</td>
</tr>
<tr>
<td><strong>Calcium (mg)</strong></td>
<td>688 ± 19</td>
<td>667 ± 15</td>
<td>1525 ± 216</td>
<td>1498 ± 93</td>
</tr>
<tr>
<td><strong>Phosphorus (mg)</strong></td>
<td>1307 ± 29</td>
<td>1784 ± 3</td>
<td>1514 ± 130</td>
<td>1957 ± 81</td>
</tr>
<tr>
<td><strong>Magnesium (mg)</strong></td>
<td>271 ± 39</td>
<td>310 ± 42</td>
<td>291 ± 36</td>
<td>328 ± 39</td>
</tr>
<tr>
<td><strong>Potassium (mg)</strong></td>
<td>3342 ± 326</td>
<td>4220 ± 136</td>
<td>3627 ± 161</td>
<td>4520 ± 33</td>
</tr>
<tr>
<td><strong>Sodium (mg)</strong></td>
<td>4288 ± 813</td>
<td>4961 ± 1129</td>
<td>4348 ± 749</td>
<td>5022 ± 1129</td>
</tr>
<tr>
<td><strong>Chloride (mg)</strong></td>
<td>4703</td>
<td>5474</td>
<td>4875</td>
<td>5646</td>
</tr>
<tr>
<td><strong>Sulfate (mg)</strong></td>
<td>607 ± 27</td>
<td>1066 ± 35</td>
<td>637 ± 11</td>
<td>1079 ± 35</td>
</tr>
<tr>
<td><strong>PRAL (mEq)</strong></td>
<td>-10</td>
<td>16</td>
<td>-19</td>
<td>6</td>
</tr>
</tbody>
</table>

1. Based on energy intake of 9.5 ± 0.8 MJ (2270 ± 190 kcal). Macronutrients were calculated from US Department of Agriculture food composition data (13). Phytate was calculated from published data (14). All diets were supplemented with a daily chewable multivitamin tablet containing retinol (500 μg), ascorbic acid (60 mg), cholecalciferol (10 μg), α-tocopherol (10 mg), pyridoxine (2 mg), cyanocobalamin (6 μg), thiamin (1.5 mg), riboflavin (1.7 mg), folic acid (400 μg), and pantothenic acid (10 mg). Minerals and electrolytes (mean ± SD for analysis at a single energy level) were analyzed as described in the text. LCLP, low-calcium, low-protein diet; LCHP, low-calcium, high-protein diet; HCLP, high-calcium, low-protein diet; HCHP, high-calcium, high-protein diet; PRAL, potential renal acid load. Statistical comparisons were not conducted between the diets because of the limited df associated with the measures or calculations of the dietary constituents.

2. Mean ± SD (all such values), as analyzed at a single energy level; the mineral content varied further with the variation in energy intake (see footnote 1).

3. Data shown do not include an allowance of ≤2 g/d optional salt, which was weighed and added to both the subject’s high- and low-protein diets. Including 14 subjects who did not add salt, 0.53 ± 0.7 g added salt/d was consumed per subject, providing an additional ≈210 mg Na/d and ≈320 mg Cl/d.

4. Data shown do not include an allowance of ≤2 g/d optional salt, which was weighed and added to both the subject’s high- and low-protein diets. Including 14 subjects who did not add salt, 0.53 ± 0.7 g added salt/d was consumed per subject, providing an additional ≈210 mg Na/d and ≈320 mg Cl/d.

### Analyses

The subjects provided total 48-h urine collections during weeks 0, 1, 2, 3, 5, and 7 of each dietary period, and these were used for all urine analyses with the exception of deoxypyridinoline (DPD). Because it is affected by diurnal variation (20), DPD was measured in first morning urine samples from weeks 1 and 7 of each diet period, using a solid-phase chemiluminescent enzyme-labeled immunoassay (Innolite 1000 Pylinks-D; Diagnostic Products Corporation, Los Angeles, CA).

Fasting blood samples were drawn at the beginning (week 1) and end (week 7) of each dietary period. Calcium and other mineral elements in the serum, urine, and acid-digested diet aliquots were determined by inductively coupled argon plasma emission spectrophotometry. Analytic quality was monitored by analyzing standard reference materials (Seronorm Trace Elements Urine, Lot 2525; SERO AS, Billingstad, Norway; Typical Diet, 1548b; US National Institute of Standards and Technology, Gaithersburg, MD). Despite care in the mixing and preparing of dietary aliquots, greater variation was observed in the calcium and phosphorous contents of the HC diets (Table 2), probably because of an incomplete solubility of the fortificants in the commercially fortified drinks.

Urinary ammonium was determined colorimetrically (Raichem; Hemagen Diagnostics, San Diego, CA). Titratable acidity was determined in undiluted urine by titrating to pH 7.40 with 0.1
mold NaOH/L. Urinary free organic acids were measured by the method of Van Slyke and Palmer (21) as modified by Lemann et al. (22). Urinary sulfates were determined turbidimetrically (23). Urine oxalate was measured colorimetrically (Oxalate Kit no. 591-D; Sigma Diagnostics, St Louis, MO). Enzyme-linked immunosassays were used to determine serum bone-specific alkaline phosphatase (Metra Biosystems, Mountain View, CA) and serum estradiol (Abbott Laboratories, Abbott Park, IL). Serum tartrate-resistant acid phosphatase (TRAP) activity was determined with a-naphthylphosphate and diazotized-2-amino-5-chlorotoluene as substrates (24). Creatinine clearance was calculated from serum and urinary creatinine, which were measured using alkaline picric acid (25) (Cobas Mira; Roche Diagnostic Systems Inc, Sommerville, NJ). Serum parathyroid hormone, calcium, osteocalcin, and 25-OH-cholecalciferol were determined by radioimmunassay (Diasorin, Stillwater, MN). Serum insulin-like growth factor 1 (IGF-I; Diagnostic Systems Laboratory, Webster, TX) was determined by enzyme-linked immunosorbent assays. Plasma ionized calcium was measured with an electrode (Nova 8+ Electrolyte Analyzer; Nova Biomedical, Waltham, MA) (26).

### Statistics

Individual $^{47}$Ca retention data were modeled with a 2-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 since dose administration in hours, and the coefficients $\beta_1$ and $\beta_2$ represent the fractional biological turnover of the radiotracer, expressed as a percentage of dose, at rates $k_1$ and $k_2$, respectively. The biological half-life of each component, $T_i$, is calculated (in h) as $\ln(2)/k_i$. Some calcium retention data ≤5 days after isotope administration were excluded from the model because they primarily represented a delay in elimination of the unabsorbed isotope. In addition to calcium retention, the percentage of $^{47}$Ca initially absorbed was separately estimated from the $y$-intercept of the linear portion (days 8–25 for most subjects; days 12–25 for 2 subjects) of a semi-logarithmic retention plot of the percentage of $^{47}$Ca retained compared with time.

Subject baseline characteristics were compared by using Student’s $t$ test and chi-square analyses (SAS, version 9.1.3; SAS Institute Inc, Cary, NC). Dietary protein, dietary calcium, protein × calcium interaction, and sequence effects were evaluated by repeated-measures analysis of variance followed by Tukey’s contrasts. For examining correlations between calcium absorbed and urinary calcium excreted, $R^2$ values were calculated with a linear mixed model that allowed random intercepts for each volunteer (27). With the use of 2-tailed probabilities, $P < 0.05$ was considered significant. As an indication of possible nonsignificant trends, $P$ values between 0.05 and 0.1 are also shown.

### RESULTS

Dietary calcium retention, based on whole-body retention of the calcium isotope, was significantly affected by dietary calcium, dietary protein, and the interaction between these 2 components (Table 3, Figure 1). As expected, high compared with low dietary calcium significantly decreased the fraction of ingested calcium retained while increasing the absolute amount retained. The increase in dietary calcium of 835 mg/d (from 675 to 1510 mg/d) increased the amount of absorbed calcium by 73 mg/d, or 1.8 mmol/d (see treatment means, Table 3).

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>LCLP</th>
<th>LCHP</th>
<th>HCLP</th>
<th>HCHP</th>
<th>Pooled SD</th>
<th>$P$ for calcium</th>
<th>$P$ for protein</th>
<th>$P$ for calcium × protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_1$ (% of dose)</td>
<td>75.5</td>
<td>71.7</td>
<td>84.5</td>
<td>84.3</td>
<td>2.3</td>
<td>0.0001</td>
<td>0.005</td>
<td>0.01</td>
</tr>
<tr>
<td>$\beta_2$ (% of dose)</td>
<td>24.6</td>
<td>28.3</td>
<td>15.8</td>
<td>15.9</td>
<td>2.2</td>
<td>0.0001</td>
<td>0.005</td>
<td>0.01</td>
</tr>
<tr>
<td>$T_1$ (d)</td>
<td>ln</td>
<td>0.18</td>
<td>0.22</td>
<td>0.26</td>
<td>0.29</td>
<td>0.17</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>1.2</td>
<td>1.3</td>
<td>1.3</td>
<td>1.3</td>
<td>0.5</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>$T_2$ (d)</td>
<td>ln</td>
<td>3.7</td>
<td>3.6</td>
<td>4.0</td>
<td>3.9</td>
<td>0.5</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>40</td>
<td>37</td>
<td>54</td>
<td>47</td>
<td>22</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

1. $n = 13$ low-calcium diets; $n = 14$ high-calcium diets. All times are relative to the time of administration of the isotope. LCLP, low-calcium, low-protein diet; LCHP, low-calcium, high-protein diet; HCLP, high-calcium, low-protein diet; HCHP, high-calcium, high-protein diet. Main effects were evaluated by ANOVA. When the interaction term was significant, means were compared by Tukey’s contrasts, means with different superscript letters differ significantly. A 2-component exponential model $y = \beta_1 e^{-k_1 t} + \beta_2 e^{-k_2 t}$ fit the data ($R^2$ of 0.97-0.99) for models of subjects on each diet, after exclusion of 1 subject from the LC group who apparently had a longer gastrointestinal transit time. In this model, $\beta_1$ and $\beta_2$ represent the percentage of the isotope having a biological half-life of $T_1$ and $T_2$, respectively, where $T_i$ is calculated (in h) as $\ln(2)/k_i$. Retention data at days 7, 14, 21, and 28 were derived from these models. See Figure 1 for measurement data.

2. Intestinal calcium absorption, as percentage of dose, was estimated by using the linear portion (days 8–25 after $^{47}$Ca administration for most subjects; days 12–25 for 2 subjects who apparently excreted unabsorbed isotope more gradually) of a semilogarithmic retention plot $\ln(\%$ of retention compared with time) and extrapolating back to the time of tracer administration.

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**TABLE 3**

Whole-body retention of the calcium radiotracer ($^{47}$Ca) as affected by the calcium and protein content of controlled diets consumed by healthy postmenopausal women for 7 wk.

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See Figure 1 for...
Dietary calcium and protein, but not their interaction, also affected urinary calcium excretion. Compared with low dietary calcium, high calcium increased urinary calcium excretion by 1.9 mmol/d (see treatment means, Table 4), approximately matching the 1.8 mmol/d increase in absorption (Table 3) and suggesting no net increase in body calcium retention with the higher calcium intake. Dietary protein significantly increased urinary calcium by 0.5 mmol/d, whether dietary calcium was low or high (Table 4). Urinary calcium excretion was significantly correlated with calcium absorption (both expressed in mmol/d; $R^2 = 0.14$, $P < 0.01$; $n = 54$). High dietary calcium, especially when dietary protein was low, also resulted in serum calcium that was slightly greater, although within the expected range of normal concentrations (Table 5).

Dietary calcium and protein independently affected urinary acidity. Dietary calcium directly influenced urinary acidity and calcium excretion without significantly affecting other urinary minerals and electrolytes. The HC diets resulted in a nearly significant ($P < 0.06$) decrease in urinary phosphate (Table 4), despite the higher phosphorus content of these diets (Table 2). HC compared with LC diets decreased titratable acidity and increased urinary pH by 0.1–0.2 units (Table 4).

Urinary acidity was more substantially affected by the differences in protein than the differences in calcium in these experimental diets. The HP compared with the LP diets significantly increased urinary calcium, phosphorus, magnesium, sodium, potassium, chloride, sulfate, and ammonium ions, as well as free organic acid, without changing oxalic acid (Table 4). These changes increased titratable acidity and reduced urinary pH by 0.3–0.4 units with the higher protein diets. Urine analyses at 1, 2, 3, 5, and 7 wk did not significantly change with sampling time (data not shown), suggesting that full adaptation of these urinary variables occurred within the first week of the study. Although high dietary protein increased urinary creatinine excretion (Table 4), presumably because of greater exogenous creatinine from increased meat consumption, neither serum creatinine nor renal creatinine clearance were significantly affected by diet.

Dietary protein significantly influenced 2 possible markers of bone metabolism. High compared with low dietary protein increased by 27% the circulating concentrations of serum IGF-I, a polypeptide with anabolic influence on bone and other tissues (Table 5). High dietary protein also decreased urinary DPD excretion (Table 4), which is suggestive of decreased bone collagen catabolism. This occurred whether DPD was expressed relative to urinary creatinine (which was also affected by dietary protein) or relative to urine volume of the first morning void. Neither serum IGF-I nor DPD excretion was affected by calcium intake. The dietary treatments did not significantly affect several other potential biomarkers of calcium and bone metabolism, including ionized calcium, 25-hydroxycholecalciferol, parathyroid hormone, osteocalcin, bone-specific alkaline phosphatase, and TRAP (Table 5).

**DISCUSSION**

These results indicate that dietary protein interacted with dietary calcium to increase fractional calcium absorption from 26.0% to 29.5% when calcium intake was low (=675 mg/d) but not when calcium intake was high (=1510 mg/d). The results with the lower calcium intake are generally consistent with a similar study in
which we observed a slight but nonsignificant increase in fractional calcium absorption, from 28.4% to 29.9% of 600 mg dietary Ca/d, with low compared with high intake of meat protein, respectively (28). Kerstetter et al (9) reported a greater increase in calcium absorption, from 28.4% to 29.9% of 600 mg dietary Ca/d with LP compared with HP diets, formulated to also keep calcium, high-protein diet. By ANOVA, time did not significantly influence the results; thus, each mean represents all samples from each diet period. Main effects were evaluated by ANOVA. When the interaction term was significant, means were compared by Tukey’s contrasts; means with different superscript letters differ significantly.

To convert from mmol/d to mg/d, multiply by the molecular weights of 40 for calcium, 31 for phosphorus, 23 for sodium, 39 for potassium, 35 for chloride, and 32 for the sulfur in the sulfate anion.

The protein-calcium interaction that affected the percentage of calcium absorption in the present study did not affect urinary calcium excretion, which was increased to a similar degree by high dietary protein whether dietary calcium was low or high. This increase in urinary calcium excretion with an HP diet cannot be attributed directly to an increase in calcium absorption, because calcium absorption did not change when dietary calcium was high. Interpretation of these somewhat divergent findings between the whole-body calcium isotope retention and the urinary elemental calcium excretion must consider the strengths and limitations of the isotopic retention measurements. Although the isotopic measurements sensitively indicate initial calcium absorption or retention from the diet, these measurements are less sensitive for detecting endogenous calcium excretion. This is because once absorbed, the isotopic label mixes with a larger pool of readily exchangeable endogenous calcium. As a result, the ratio of isotope to elemental calcium may be considerably greater in the labeled diet than in the subsequently excreted endogenous calcium, which provides greater analytic power to sensitively detect a small difference in calcium absorption than an equivalent difference in endogenous calcium excretion. Together, the isotope retention and urinary analysis data indicate that high dietary protein increased the percentage of calcium absorption from only a (nonsignificant) difference of 0.4 mmol/d. Although with the higher calcium intake there was no such compensatory increase in calcium absorption, the protein-related increase of 0.5 mmol/d in urinary calcium excretion was modest compared with the greater quantities of calcium absorbed (1.8 mmol) and excreted (1.9 mmol), which likely provided substantial homeostatic control to counter any nutritional calcium imbalance. The similar dietary calcium-associated increases in calcium absorption and urinary excretion also suggest minimal nutritional benefit of the higher calcium intake.
Concerns about the calciuretic effect of dietary protein on calcium balance and bone health have largely been based on evidence with purified proteins or with animal proteins with dietary adjustments to control phosphorous intake. At least partly because of the phosphorous content of meat, protein from meat is less calciuretic than that from purified protein sources (5–7). Of other reports testing increased meat intake without adjustments to control dietary phosphorous, 4 reported little or no calciuretic effect (28–31). Two others reported increased urinary calcium (8, 32), but in one of these studies the calciuretic effect of meat was balanced by a decrease in fecal calcium excretion (8), which is consistent with an increase in calcium absorption.

As predicted from the dietary composition (15) (Table 2), urinary acidity was increased (pH was decreased) with greater protein or less calcium intake (Table 4). In a similar earlier investigation of postmenopausal women consuming low- and high-meat diets, differences in pH and titratable acidity at 3 wk were reduced as the urinary measurements were extended to 5 and 8 wk, suggesting a possible long-term adaptation in renal acid excretion (8), which is consistent with an increase in calcium absorption.

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Protein-associated hypercalcemia has been attributed to an increased renal acid load (4). Higher estimates of renal acid load, resulting from higher protein as well as lower potassium intake, have been cross-sectionally associated with lower bone mineral density and markers of poorer bone health (33, 34). An increased acid load may partly explain the increase in urinary calcium in the present study, in which both urinary calcium and urinary acidity were similarly influenced by dietary protein whether dietary calcium was low or high. However, meat-induced changes in urinary calcium are not entirely attributable to changes in the renal acid load. For example, dietary phosphorous increases urinary acidity (5, 15) but decreases urinary calcium excretion (5–7). The calciuric effect of the high-meat–HP diets in the present study cannot be attributed to differences in creatinine clearance or urinary oxalate, which were unaffected by diet.

The present study measured several potential biomarkers of altered bone metabolism, but most of these (ionized calcium, 25-hydroxycholecalciferol, parathyroid hormone, osteocalcin, bone-specific alkaline phosphatase, and TRAP) were not significantly affected by dietary treatment. The HP diets resulted in a 27% increase in serum IGF-I (Table 5), an anabolic peptide hormone proposed to be a biomarker of increasing bone mass (35–37). Although not all studies have found a significant effect of dietary protein on serum IGF-I (28, 38), the present data are consistent with several other experiments showing that increased dietary protein under a variety of conditions increases serum IGF-I (36, 39–43). Consistent with other experiments comparing high and low intakes of calcium (44, 45), calcium intake in the present study did not influence serum IGF-I. Although IGF-I effects are not specific to bone tissue, a protein-related increase in serum IGF-I may be conducive to bone anabolism.

Urinary DPD was another bone biomarker influenced by dietary treatment in the present study. A marker of the breakdown of bone collagen cross-links, urinary DPD decreased with high dietary protein, suggesting reduced bone catabolism. Consistent with this finding, Dawson-Hughes et al (43) reported significantly lower urinary N-telopeptide, another marker of cross-links from the breakdown of bone collagen, with protein-supplemented diets. However, experimental results have been inconsistent about the effect of protein on the excretion of bone collagen cross-links, including no effect of meat protein treatment on such markers (28, 46), and of increased, rather than decreased, urinary N-telopeptide with protein supplementation (42). Reports of calcium intake influencing collagen cross-link markers are also inconsistent: 2 reports of lower DPD with increased dietary calcium are in contrast to the unresponsiveness of urinary DPD to calcium in the present study (44, 46) (Table 4), and of urinary C-telopeptide cross-links to 600 mg additional dietary...
calcium either as supplements or fortified dairy products (45). Although the present urinary DPD results are compatible with a reduction in bone catabolism with high protein, but not high calcium intake, the inconsistency of such findings in the literature suggest a cautious interpretation. Healthy bone maintenance involves continuous tissue turnover, so catabolism of collagen, accompanied by anabolic replacement and remodeling, is not necessarily detrimental.

In conclusion, in healthy postmenopausal women, a moderate increase in protein intake, from 10% to 20% of energy, mostly as meat, increased urinary acidity and increased calcium excretion by 0.5 mmol/d; however, at a low intake of calcium of ≈675 mg/d, this increase was nearly compensated for by an increase in fractional calcium absorption (although the difference in absolute calcium absorbed was not statistically significant). With a high intake of calcium of ≈1510 mg/d, there was no dietary protein-related increase in fractional calcium absorption, but, compared with the dietary calcium-related increases in calcium absorption and excretion, the protein-related increase in urinary calcium seemed insubstantial. Overall, the caleuretic effect of increased meat protein in this study was not detrimental to calcium retention, and the increase in plasma IGF-I may indicate a beneficial effect on bone.

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