Protein intake: effects on bone mineral density and the rate of bone loss in elderly women

Prema B Rapuri, J Christopher Gallagher, and Vera Haynatzka

ABSTRACT

Background: The role of dietary protein in bone metabolism is controversial.

Objective: We investigated the associations of dietary protein intake with baseline bone mineral density (BMD) and the rate of bone loss over 3 y in postmenopausal elderly women.

Design: Women aged 65–77 y (n = 489) were enrolled in an osteoporosis intervention trial. We studied the associations of protein intake as a percentage of energy with baseline BMD and the rate of bone loss in 96 women in the placebo group (n = 96). We also examined the effect of the interaction of dietary calcium intake with protein intake on BMD.

Results: In the cross-sectional study, a higher intake of protein was associated with higher BMD. BMD was significantly higher (P < 0.05) in the spine (7%), midradius (6%), and total body (5%) in subjects in the highest quartile of protein intake than in those in the lower 2 quartiles. This positive association was seen in women with calcium intakes > 408 mg/d. There was no significant effect of protein intake on hip BMD. In the longitudinal study of the placebo group, there was no association between protein intake and the rate of bone loss.

Conclusions: The highest quartile of protein intake (≥ 72 g/d) was associated with higher BMD in elderly women at baseline only when the calcium intake exceeded 408 mg/d. In the longitudinal study, no association was seen between protein intake and the rate of bone loss, perhaps because the sample size was too small or the follow-up period of 3 y was not long enough to detect changes.

KEY WORDS Protein, bone mineral density, bone loss, calcium intake, bone markers, calcitropic hormones, elderly, parathyroid hormone

INTRODUCTION

The relation between dietary protein intake and bone metabolism is controversial, and questions about this relation are unresolved. Excess dietary protein was shown to cause urinary calcium loss, negative calcium balance, and bone loss in young and elderly men and women, and these effects are mainly attributable to high acid load from metabolism of animal protein (1–11). It has been proposed that bone buffers the excess acid load, which results in urinary calcium loss that leads to reduced bone mineral content and bone mass (12). The type of dietary protein has also been suggested to play a role, but the results of studies on this aspect remain unclear (13–19). At the other end of the spectrum, protein undernutrition is suspected to be a risk factor for bone loss and osteoporosis. There is convincing evidence from the literature that indicates that low protein intake is associated with low bone mineral density (BMD) (20–23) and greater fracture risk (24, 25). Studies of protein supplementation after hip fracture in the elderly further substantiate the importance of adequate protein intake in bone biology (26–28).

The relations of dietary protein intake with BMD and bone loss in postmenopausal women and the elderly, who have the highest risk of developing osteoporosis, are not very clear. Among cross-sectional studies, some showed a positive association between protein intake and BMD (20–22, 29, 30), whereas others did not find any such association (23, 31–34). There are few longitudinal studies that examined the relation between dietary protein intake and bone loss in postmenopausal women and the elderly, and the results of these studies are conflicting. Freudenheim et al (32) and Hannan et al (18) reported that higher protein intake is associated with lower rates of bone loss, whereas Nordin and Polley (35) and, more recently, Sellmeyer et al (15) reported contrasting results. Studies examining the association between dietary protein intake and fracture risk also reported conflicting observations (14–16, 19, 26, 27).

Dietary calcium was shown to influence the association between dietary protein and BMD by some researchers (17, 19, 36–39) but not by others (21). Promislow et al (17) reported that under conditions of low calcium intake, increasing protein intake increased BMD. On the other hand, Dawson-Hughes and Harris (38) reported that in elderly subjects supplemented with calcium and vitamin D, dietary protein was inversely associated with the rate of bone loss. Feskanich et al (19) and Meyer et al (39) observed a positive association between protein intake and fracture risk when calcium intake was < 540 mg/d. In contrast, Kerstetter et al (21) reported that the association between dietary protein intake and BMD is not related to calcium intake.
In the present study, we examined the association at baseline between different dietary protein intakes and bone metabolism in postmenopausal elderly women. Furthermore, in women receiving the placebo treatment, we prospectively studied whether different baseline protein intakes influence the rate of bone loss and changes in biochemical markers. In addition, we examined whether dietary calcium intake influences the associations of protein intake with bone density, biochemical variables, and the rate of bone loss in both the population studied cross-sectionally and the population studied longitudinally.

SUBJECTS AND METHODS

Subjects

The study population comprised 489 women aged 65–77 y who participated in a double-blind, randomized osteoporosis intervention trial, Sites Testing Osteoporosis Prevention/Intervention (STOP IT). The main objective of the study was to compare the effect of placebo on bone density with the effects of the following 3 therapies: an estrogen-medroxyprogesterone combination, a vitamin D analogue [1,25-dihydroxyvitamin D₃ (Rocaltril; Hoffman-La Roche Inc, Nutley, NJ)], and a combination of both the estrogen-medroxyprogesterone and 1,25-dihydroxyvitamin D₃. The participants were healthy, ambulatory women who were recruited into the 3-y study by mass mailing of letters or through advertisements in local newspapers. Of the 489 women, 470 were white, 13 were black, 4 were Hispanic, 1 was Asian, and 1 was of mixed race. Women were excluded if they had severe chronic illness, primary hyperparathyroidism, or active renal stone disease or were taking medications, such as bisphosphonates, anticonvulsants, estrogen, or fluoride. The Institutional Review Board at Creighton University approved the study, and written informed consent was obtained from each subject before enrollment.

The cross-sectional data presented in this report were derived from the information collected from these 489 women at baseline. One subject with suspected Paget’s disease was excluded from the analysis. In addition, data for protein intake from the 7-d food diary were not available for 15 women. Thus, the analyses were performed on the remaining 473 women. BMD measurements and biochemical indexes were compared on the basis of quartiles of protein intake. Data on BMD and biochemical indexes at baseline and at 36 mo from 96 women who received the placebo treatment and completed the 3-y study were used to study the longitudinal effect of protein. Food diary data were not available for 4 of those women, and the analyses were therefore performed on 92 women. Both at baseline and prospectively, we also examined the influence of calcium intake on the associations of protein intake as a percentage of energy with BMD, rate of bone loss, and biochemical variables.

Dietary intakes and histories of alcohol use and smoking

Dietary intake data at baseline and at the end of the study were collected with the use of 7-d food diaries. Participants were carefully instructed by a dietitian on how to complete a 7-d food diary and nutrient supplement record. Plastic food models (NASCO, Fort Atkinson, WI) were used to help participants better estimate the quantities consumed. Average daily intakes of energy, fiber, protein, calcium, vitamin D, and caffeine were calculated by using the FOOD PROCESSOR II PLUS nutrition and diet analysis system (version 5.1; Esha Research, Salem, OR). The subjects’ histories of alcohol use and smoking were assessed via a questionnaire. Current smokers were classified as smokers, whereas past smokers and subjects who never smoked were classified as nonsmokers. With regard to alcohol intake, the subjects were stratified into drinkers and nondrinkers.

Calcium absorption test

While the subjects were in a fasting state, calcium absorption was measured at the beginning and the end of the study by oral administration of 18.5 × 10⁶ Bq (5 μCi) ⁴⁵Ca (Amersham, Arlington Heights, IL) in 100 mg CaCl₂ carrier given in a total volume of 250 mL distilled water (40). A blood sample was collected 2 h after the oral dose. ⁴⁵Ca activity was counted in 2 mL serum with a 1900 CA Tricarb Liquid scintillation analyzer (Packard Instrument, Meriden, CT). A parallel standard taken from the patient’s dose before ingestion was counted at the same time as the sample. Calcium absorption was expressed as a percentage of the actual dose per liter of blood and was corrected for body weight.

Biochemical analysis

Fasting blood and 24-h urine samples were obtained from the subjects at baseline and at the end of the study. Blood specimens were allowed to clot and were then centrifuged at 4°C for 15 min at 2056 × g to separate the serum. All samples were stored frozen at −70°C until analyzed.

Serum and urine chemistry measurements

All serum and urine measurements were made with fresh samples. Serum ionized calcium and total calcium and creatinine in serum and urine samples were measured by automated procedures (Nova Nucleus Chemistry Analyzer; Nova Biochemical, Waltham, MA). Serum albumin and alkaline phosphatase were measured by using automated procedures (Technicon SMAC Analyzer; Technicon Corp, Tarrytown, NY).

Serum calcitropic hormones

Serum 25-hydroxyvitamin D (calcidiol) was measured by using a competitive protein binding assay (41) after extraction and purification of serum on Sep-Pak cartridges (Waters Associates, Milford, MA) (42). The limit of detection was 12.5 nmol/L (5 μg/L), and the interassay variation was 5%. Serum 1,25-dihydroxyvitamin D (calcitriol) was measured by using a nonequilibrium radioreceptor assay (Instar Corp, Stillwater, MN) with calf thymus receptor. The samples were extracted and purified before assay on nonporlar C₁₈ silanol silica cartridges (43, 44). The limit of detection was 12 pmol/L (5 ng/L), and the interassay variation was 10%. Allelochrome immunoradiometric assay (Nichols Institute, San Juan Capistrano, CA) was used to measure serum intact parathyroid hormone (45). The limit of detection was 1 ng/L (1 pg/mL), and the interassay variation was 5%.

Bone markers

Serum osteocalcin was measured by radioimmunoassay (Instar Corp). The limit of detection was 0.78 μg/L (0.78 ng/mL), and the interassay variation was 5%. Urine collagen crosslinks were measured by enzyme-linked immunosorbent assay (Osteomark International, Seattle) as N-telopeptides, a specific marker for bone type I collagen (expressed as nmol bone collagen equivalents/mmol creatinine).
Bone mineral density

BMD measurements of the spine (L1–L4), proximal femur (femoral neck, trochanter, and total hip), total body, and radial midshaft were performed by using dual-energy X-ray absorptiometry on a DPXL scanner (Lunar Radiation, Madison, WI) with standardized protocols for uniform subject positioning, scan mode, and scan analysis. The spine scans were performed on the L1–L4 vertebrae. Hip and spine scans were performed in duplicate, and the average value computed was used for the analysis. The percentage of change in BMD was calculated as the difference between the values at baseline and 36 mo divided by the baseline BMD and multiplied by 100.

Statistical analysis

Data were analyzed with the SAS statistical package (version 8.8.2; SAS Institute Inc, Cary, NC). The populations considered in both the cross-sectional and the longitudinal studies were divided into quartiles of protein intake as a percentage of total energy. The baseline characteristics of the 4 quartile groups in the cross-sectional and longitudinal studies were compared by using one-way analysis of variance for the continuous variables and the chi-square test for the categorical variables. The biochemical indexes and BMD measurements at baseline of the subjects in the 4 groups were compared by using analysis of covariance with adjustments for various relevant confounders (age; body mass index; intakes of calcium, caffeine, fiber, and vitamin D; smoking status, and alcohol use) selected with the use of a stepwise selection method. For the longitudinal analyses, we used similar analysis of covariance models in which the outcome variables were the percentage change in BMD and the biochemical variables were added to the list of independent variables. In both the baseline and the longitudinal models, we adjusted for total energy intake. The residuals of the final models were tested for deviation from normality by using graphic methods. The effect of protein intake on BMD and biochemical variables in both the cross-sectional and the longitudinal analyses is summarized by the unadjusted least-squares means and their respective SEs. Tukey’s post hoc multiple comparison test was used to determine the significance of differences in adjusted data between the 4 quartile groups. To establish the combined effect of the outcome of total calcium intake and protein intake as a percentage of energy in both the baseline and the longitudinal analyses, both a categorical calcium intake variable (defined from quartiles of calcium intake) and its interaction with protein intake as a percentage of energy were added. These interactions were examined for significance. In addition, the slopes of the fitted regression lines were examined for associations between the BMD variables and protein intake as a percentage of energy for the 4 calcium-intake groups, with adjustment for the relevant covariates mentioned above.

RESULTS

Characteristics

Age and height were not significantly different across the quartiles of protein intake as a percentage of energy (Table 1). The weight of the women in the highest quartile of protein intake was significantly (P < 0.05) lower than that of the women in the lowest quartile. Dietary energy intake was significantly (P < 0.05) lower in the women in the highest quartile than in the women in the lower 2 quartiles. The women in the lowest quartile consumed an average of 54 g protein/d, which was 0.84 g protein/kg body wt, and 13% of the total energy that they consumed was contributed by protein. The women in the highest quartile consumed 71 g protein/d, which was 1.02 g protein/kg body wt, and 19.8% of the total energy that they consumed was contributed by protein. The women in the highest quartile consumed significantly higher vitamin D intake than did those in quartiles 2 and 3. Dietary calcium intake, dietary fiber intake, and dietary caffeine intake were not significantly different between the 4 quartiles. The percentage of smokers was also not significantly different between the 4 quartiles. The percentage of alcohol drinkers was significantly (P < 0.05) lower in the highest quartile than in the lowest quartile.

The baseline characteristics of the 96 women who were followed prospectively are given in Table 2. There were no significant differences in age; height; dietary calcium, fiber, and caffeine intakes; and percentages of smokers and alcohol drinkers between the quartiles of protein intake as a percentage of energy. The

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TABLE 1

Characteristics of the population at baseline by quartile (Q) of protein intake as a percentage of energy

| Variable | Q1 (13.1 ± 0.12%) | Q2 (15.1 ± 0.11%) | Q3 (16.7 ± 0.12%) | Q4 (19.8 ± 0.12%) | P
<table>
<thead>
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<tbody>
<tr>
<td>Age (y)</td>
<td>71.4 ± 0.33</td>
<td>72.0 ± 0.33</td>
<td>71.2 ± 0.33</td>
<td>71.3 ± 0.33</td>
<td>0.3781</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>159.3 ± 0.60</td>
<td>159.2 ± 0.60</td>
<td>159.2 ± 0.60</td>
<td>158.8 ± 0.60</td>
<td>0.9425</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65.0 ± 1.13</td>
<td>68.2 ± 1.12</td>
<td>68.5 ± 1.13</td>
<td>72.0 ± 1.13</td>
<td>0.0003</td>
</tr>
<tr>
<td>Dietary energy intake (kcal/d)</td>
<td>1643 ± 29</td>
<td>1585 ± 28</td>
<td>1557 ± 29</td>
<td>1453 ± 29</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dietary protein intake (g/d)</td>
<td>53.7 ± 1.18</td>
<td>59.9 ± 1.17</td>
<td>65.2 ± 1.18</td>
<td>71.2 ± 1.18</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Protein (g/kg body wt)</td>
<td>0.84 ± 0.02</td>
<td>0.91 ± 0.02</td>
<td>0.99 ± 0.02</td>
<td>1.02 ± 0.03</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dietary vitamin D intake (µg/d)</td>
<td>2.62 ± 0.18</td>
<td>3.35 ± 0.18</td>
<td>3.53 ± 0.18</td>
<td>4.43 ± 0.18</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dietary calcium intake (mg/d)</td>
<td>687 ± 29</td>
<td>736 ± 28</td>
<td>759 ± 29</td>
<td>788 ± 29</td>
<td>0.0774</td>
</tr>
<tr>
<td>Dietary fiber intake (g/d)</td>
<td>14.5 ± 0.45</td>
<td>14.8 ± 0.45</td>
<td>14.69 ± 0.45</td>
<td>14.87 ± 0.45</td>
<td>0.9570</td>
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<tr>
<td>Dietary caffeine intake (mg/d)</td>
<td>284 ± 21</td>
<td>283 ± 21</td>
<td>268 ± 21</td>
<td>261 ± 21</td>
<td>0.8390</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>17.0 ± 2.9</td>
<td>8.4 ± 2.9</td>
<td>8.5 ± 2.9</td>
<td>11.9 ± 2.9</td>
<td>0.1291</td>
</tr>
<tr>
<td>Alcohol drinkers (%)</td>
<td>42.4 ± 4.3</td>
<td>29.4 ± 4.3</td>
<td>33.1 ± 4.3</td>
<td>25.4 ± 4.3</td>
<td>0.0372</td>
</tr>
</tbody>
</table>

\( ^1 \) ± SEM. Values in the same row with different superscript letters are significantly different, \( P < 0.05 \) (Tukey’s post hoc multiple comparison test).

\( ^2 \) ± SEM.

\( ^3 \) ANOVA for continuous variables, and chi-square test for categorical variables.
TABLE 2
Baseline characteristics of the population studied longitudinally by quartile (Q) of protein intake as a percentage of energy

| Variable                        | Q1 (13.3 ± 0.24%) | Q2 (15.2 ± 0.24%) | Q3 (16.7 ± 0.24%) | Q4 (19.5 ± 0.24%) | P
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<tbody>
<tr>
<td>Age (y)</td>
<td>71.3 ± 0.76</td>
<td>72.2 ± 0.76</td>
<td>70.1 ± 0.76</td>
<td>69.9 ± 0.76</td>
<td>0.1268</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160.6 ± 1.35</td>
<td>160.6 ± 1.34</td>
<td>160.5 ± 1.35</td>
<td>158.6 ± 1.34</td>
<td>0.6525</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>62.8 ± 2.78</td>
<td>69.4 ± 2.78</td>
<td>69.9 ± 2.78</td>
<td>75.5 ± 2.78</td>
<td>0.0192</td>
</tr>
<tr>
<td>Dietary energy intake (kcal/d)</td>
<td>1757 ± 68</td>
<td>1657 ± 68</td>
<td>1573 ± 68</td>
<td>1486 ± 68</td>
<td>0.0390</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>58.3 ± 2.73</td>
<td>63.3 ± 2.73</td>
<td>65.7 ± 2.73</td>
<td>71.8 ± 2.73</td>
<td>0.0078</td>
</tr>
<tr>
<td>Dietary vitamin D intake (µg/d)</td>
<td>3.0 ± 0.45</td>
<td>3.5 ± 0.45</td>
<td>3.5 ± 0.45</td>
<td>4.7 ± 0.45</td>
<td>0.0612</td>
</tr>
<tr>
<td>Dietary calcium intake (mg/d)</td>
<td>759 ± 63</td>
<td>830 ± 63</td>
<td>755 ± 63</td>
<td>750 ± 63</td>
<td>0.7832</td>
</tr>
<tr>
<td>Dietary fiber intake (g/d)</td>
<td>15.5 ± 0.98</td>
<td>15.6 ± 0.98</td>
<td>14.9 ± 0.98</td>
<td>13.3 ± 0.98</td>
<td>0.3409</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>17.4 ± 6.75</td>
<td>0.0 ± 6.75</td>
<td>13.0 ± 6.75</td>
<td>17.4 ± 6.75</td>
<td>0.2235</td>
</tr>
<tr>
<td>Alcohol drinkers (%)</td>
<td>52.2 ± 9.61</td>
<td>34.8 ± 9.61</td>
<td>17.4 ± 9.61</td>
<td>26.1 ± 9.61</td>
<td>0.0743</td>
</tr>
</tbody>
</table>

1 x ± SEM. Values in the same row with different superscript letters are significantly different, P < 0.05 (Tukey’s post hoc multiple comparison test).
2 x ± SEM.
3 ANOVA for continuous variables, and chi-square test for categorical variables.

weight and vitamin D intake of the women in the highest quartile were significantly (P < 0.05) higher than those of the women in the lowest quartile, and the women in the highest quartile had significantly (P < 0.05) lower dietary energy intake than did those in the lowest quartile. The women in the lowest quartile consumed 58 g protein/d, which was 0.95 g protein/kg body wt, and 13% of the total energy that they consumed was contributed by protein. The women in the highest quartile consumed 71.8 g protein/d, which was 0.99 g protein/kg body wt, and 19.5% of the total energy that they consumed was from protein.

Bone mineral density
At baseline the women in the highest quartile of protein intake as a percentage of energy had higher BMD in the spine (7.5–8%), midradius (5.5–7%), and total body (5%) than did those in the lower 3 quartiles, although for the midradius and total body, the only significant difference was that between the highest quartile and quartile 2 (Figure 1). In the spine, the difference in BMD between quartiles 3 and 4 was also significant. The difference in total body BMD between the lowest and the highest quartiles was also marginally significant (P = 0.07).

FIGURE 1. Unadjusted mean (±SEM) bone mineral density (BMD) of the women at baseline by quartile (Q) of protein intake as a percentage of energy (n = 118, 118, 118, and 119 in quartiles 1–4, respectively). Means were compared by analysis of covariance with adjustment for BMI; total energy, calcium, fiber, and vitamin D intakes; smoking status, and alcohol use. Values with different superscript letters are significantly different, P < 0.05 (Tukey’s post hoc multiple comparison test). After adjustment for the confounders listed above, the total body and total femur BMD values for the women in quartile 1 were marginally different (P = 0.07 and 0.08, respectively) from those for the women in quartile 4.
Similar trends were observed in the femoral sites measured (femoral neck, 3%; trochanter, 5–7%; and total femur, 5.5%) although these differences were not significant (Figure 1). Total femur BMD was marginally higher ($P = 0.08$) in quartile 4 than in quartile 2.

The interaction of dietary calcium intake and protein intake as a percentage of energy was marginally significant for the spine ($P = 0.08$) at baseline. As calcium intake increased, the association between protein intake and BMD became positive (Figure 2). Among the women in the lowest quartile of calcium intake (<408 mg/d), there was no significant association between protein intake and spinal BMD. However, in the upper 2 quartiles of calcium intake, a significant positive association was seen between protein intake and spinal BMD (quartile 3, $P = 0.04$; quartile 4, $P = 0.03$). Although the interaction between calcium intake and protein intake was not significant for total body BMD ($P = 0.10$), a positive association between protein intake and total body BMD was observed with increasing calcium intake, as seen for the spine (Figure 2, bottom). In the upper 2 quartiles of calcium intake there was a significant positive association between protein intake and total body BMD (quartile 3, $P < 0.01$; quartile 4, $P = 0.01$). There was no significant interaction between calcium intake and protein intake for BMD in the other skeletal sites.

When these women were followed longitudinally (only 96 women who were assigned to the placebo group in the original study), there were no significant differences in the rate of bone loss at various skeletal sites between the quartiles of protein intake as a percentage of energy (Figure 3). Furthermore, no significant influence of calcium intake was noted on the association between protein intake and the rate of bone loss at any of the skeletal sites measured (data not shown).

Biochemical variables

Serum chemistry measures, calcitropic hormone measures, and calcium absorption values were not significantly different between the quartiles of protein intake as a percentage of energy either at baseline or in the longitudinal analyses (Tables 3 and 4). Both the 24-h and 2-h urinary ratios of calcium to creatinine were also not significantly different between the quartiles of protein intake because dietary protein intake was not assessed on the day of urine collection. At baseline, the bone remodeling markers, serum osteocalcin and urinary N-telopeptides, were marginally higher in the lowest quartile of protein intake than in the highest quartile, but the difference was not significant (Table 3). In the longitudinal study, serum osteocalcin and urinary N-telopeptides were not significantly different between the quartiles of protein intake (Table 4). There was no significant influence of calcium intake on the association between protein intake and any of the biochemical variables measured either at baseline or in the longitudinal study (data not shown).

DISCUSSION

In the present study of elderly women aged 65–77 y, long-term dietary protein intake was positively associated with BMD when the data were examined cross-sectionally. The women in the highest quartile of protein intake as a percentage of energy (>18%) had significantly higher BMD in the spine (7.5–8%), midradius (5.5–7%), and total body (5%) than did those in the lower 2 quartiles. The higher BMD in the women with higher protein intake could be partly due to less bone remodeling. When the effect of dietary protein intake was examined in relation to dietary calcium intake, positive associations of protein intake with baseline spinal and total body BMD were observed when calcium intake was >408 mg/d. The association between dairy protein intake and BMD at multiple skeletal sites was not different from the association between nondairy protein intake and BMD at various skeletal sites. In the longitudinal study, baseline dietary protein intake was not significantly associated with the rate of bone loss, probably because either the sample size was too small or the follow-up period of 3 y was not long enough to detect the changes. In addition, protein intake during the follow-up period remained unchanged. Dietary calcium had no significant influence on this association.
FIGURE 3. Unadjusted mean (± SEM) percentages of change from baseline in bone mineral density (BMD) by quartile (Q) of protein intake as a percentage of energy in the placebo group (n = 23 in each quartile). Means were compared by analysis of covariance with adjustment for BMI; total energy, calcium, fiber, and vitamin D intakes; smoking status; and alcohol use. No significant differences were found between the groups.

Our baseline results are in agreement with the results of some earlier cross-sectional studies. Chiu et al (30) reported that energy intake from protein is a significant correlate of spinal BMD in women aged 61 y. Similar observations were made by Michaels-son et al (46) and Lacey et al (22). Geinoz et al (20) reported that in elderly hospitalized patients aged 80 y, women with a protein intake of > 1 g/kg ideal body wt had higher BMD in the spine (7%), femoral neck (18%), and femoral shaft (15%) than did those

<table>
<thead>
<tr>
<th>Variable</th>
<th>Q1 (13.1 ± 0.12%)</th>
<th>Q2 (15.1 ± 0.11%)</th>
<th>Q3 (16.7 ± 0.12%)</th>
<th>Q4 (19.8 ± 0.12%)</th>
<th>P$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
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</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.33 ± 0.01</td>
<td>2.34 ± 0.01</td>
<td>2.32 ± 0.01</td>
<td>2.33 ± 0.01</td>
<td>0.5978</td>
</tr>
<tr>
<td>Ionized calcium (mmol/L)</td>
<td>1.24 ± 0.004</td>
<td>1.24 ± 0.004</td>
<td>1.23 ± 0.004</td>
<td>1.24 ± 0.004</td>
<td>0.1681</td>
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<tr>
<td>Phosphorus (mmol/L)</td>
<td>1.14 ± 0.015</td>
<td>1.17 ± 0.015</td>
<td>1.14 ± 0.015</td>
<td>1.15 ± 0.015</td>
<td>0.1961</td>
</tr>
<tr>
<td>Alkaline phosphatase (μkat/L)</td>
<td>1.62 ± 0.03</td>
<td>1.51 ± 0.03</td>
<td>1.51 ± 0.03</td>
<td>1.51 ± 0.03</td>
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<tr>
<td>Albumin (g/L)</td>
<td>40.4 ± 0.25</td>
<td>40.3 ± 0.25</td>
<td>40.1 ± 0.25</td>
<td>40.4 ± 0.25</td>
<td>0.7833</td>
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<td>iPTH (ng/L)</td>
<td>36.03 ± 1.34</td>
<td>38.19 ± 1.34</td>
<td>36.98 ± 1.34</td>
<td>36.90 ± 1.34</td>
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<tr>
<td>25(OH)D (nmol/L)</td>
<td>76.4 ± 2.4</td>
<td>78.1 ± 2.4</td>
<td>78.6 ± 2.4</td>
<td>80.95 ± 2.4</td>
<td>0.9045</td>
</tr>
<tr>
<td>1,25(OH)$_2$D (pmol/L)</td>
<td>84.6 ± 1.8</td>
<td>81.9 ± 1.8</td>
<td>82.7 ± 1.8</td>
<td>81.8 ± 1.8</td>
<td>0.8526</td>
</tr>
<tr>
<td>Osteocalcin (g/L)</td>
<td>4.07 ± 0.012</td>
<td>3.74 ± 0.012</td>
<td>3.81 ± 0.012</td>
<td>3.57 ± 0.012</td>
<td>0.4967</td>
</tr>
<tr>
<td>Calcium absorption (% of AD/L, wt corrected)</td>
<td>26.24 ± 0.69</td>
<td>26.27 ± 0.69</td>
<td>25.44 ± 0.69</td>
<td>26.22 ± 0.69</td>
<td>0.4012</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium:creatinine (24 h)</td>
<td>0.174 ± 0.01</td>
<td>0.164 ± 0.01</td>
<td>0.167 ± 0.01</td>
<td>0.158 ± 0.01</td>
<td>0.9586</td>
</tr>
<tr>
<td>Calcium:creatinine (2 h)</td>
<td>0.148 ± 0.01</td>
<td>0.138 ± 0.01</td>
<td>0.149 ± 0.01</td>
<td>0.143 ± 0.01</td>
<td>0.8440</td>
</tr>
<tr>
<td>NTx:creatinine</td>
<td>56.20 ± 2.45</td>
<td>51.82 ± 2.45</td>
<td>50.36 ± 2.47</td>
<td>44.35 ± 2.46</td>
<td>0.4898</td>
</tr>
</tbody>
</table>

$^1$Unadjusted ± SEM. iPTH, intact parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; AD/L, actual dose/L blood; NTx, N-telopeptides.

$^2$Analysis of covariance with adjustment for BMI; calcium, total energy, fiber, and vitamin D intakes; smoking status; alcohol intake; and physical activity score. When Tukey’s post hoc multiple comparison test was used to determine post hoc significance, no significant differences were found between the groups.

$^3$Value is also ± SEM.
with a protein intake of < 1 g/kg ideal body wt. In the National Health and Nutrition Examination Survey, Kerstetter et al (21) observed 3–4% higher total hip BMD in women in the highest quartile of protein intake than in those in the lower 2 quartiles.

There are very few longitudinal studies that examined the relation between dietary protein intake and bone loss. In the Framingham Osteoporosis Study, Hannan et al (18) reported that over a 4-y period, the greatest bone loss in the femoral neck and the spine occurred in subjects in the lowest quartile of dietary protein (mainly from animal sources) intake as a percentage of energy. Freudenheim et al (32) also reported that higher protein intake correlates with slower bone loss in postmenopausal women. In contrast, Dawson-Hughes and Harris (38) recently reported that in subjects supplemented with calcium and vitamin D (calcium intakes of 1200 mg/d), high protein intake was associated with bone gain.

In the present study, the women who had a protein intake as a percentage of energy that was high but within the normal range tended to have marginally lower bone remodeling (lower serum osteocalcin and urinary N-telopeptides) at baseline, which probably explains the high BMD seen in these women. There was no influence of dietary calcium intake on the association between dietary protein intake and bone markers. The mechanisms explaining the associations of dietary protein intake with changes in BMD and in the rate of bone loss are not clearly established. In agreement with our results, Dawson-Hughes and Harris (38) recently reported that women with a high protein intake had nonsignificantly lower serum osteocalcin concentrations than did those with a lower protein intake. However, they did not find any difference in urinary N-telopeptides across tertiles of protein intake as a percentage of energy. In contrast, Kerstetter et al (7) reported that under controlled conditions, a high-protein diet increases bone resorption (urinary N-telopeptides) in young women. They also reported a nonsignificant decrease in serum osteocalcin concentrations in the high-protein group, which is consistent with our results.

In the present study at baseline, the beneficial effect of higher protein intake on BMD was seen at calcium intakes >408 mg/d. Contrasting results on the effect of the interaction between protein and calcium intakes on BMD have been published. Promislow et al (17) observed that increasing protein intake is beneficial for women with a low calcium intake; however, the interaction was not very strong. Kerstetter et al (21) reported a positive association between protein intake and total femur BMD in women receiving either < 500 or 800 mg Ca/d. Feskanich et al (19) reported that the risk of forearm fractures with a high intake of protein (> 90 g/d) is exacerbated by a low calcium intake (< 541 mg/d). Similar observations were made by Meyer et al (39), who reported an elevated risk of fracture in elderly men and women with a high intake of protein from nondairy sources and calcium intakes < 400 mg/d. However, both Feskanich et al (19) and Meyer et al (39) did not find a positive effect of high calcium and high protein intakes on fracture risk. In the present study, we found no influence of calcium intake on the association between protein intake and the rate of bone loss. In contrast, Dawson-Hughes and Harris (38) recently reported that in subjects supplemented with calcium and vitamin D (calcium intakes of 1200 mg/d), high protein intake was associated with bone gain.

### Table 4

<table>
<thead>
<tr>
<th>Variable</th>
<th>Q1 (13.3 ± 0.24%)</th>
<th>Q2 (15.2 ± 0.24%)</th>
<th>Q3 (16.7 ± 0.24%)</th>
<th>Q4 (19.5 ± 0.24%)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Calcium</td>
<td>0.29 ± 0.62</td>
<td>0.90 ± 0.62</td>
<td>0.61 ± 0.62</td>
<td>0.32 ± 0.62</td>
<td>0.4822</td>
</tr>
<tr>
<td>Ionized calcium</td>
<td>0.81 ± 0.62</td>
<td>1.36 ± 0.59</td>
<td>0.61 ± 0.59</td>
<td>0.71 ± 0.59</td>
<td>0.0634</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.26 ± 0.78</td>
<td>4.0 ± 0.78</td>
<td>3.7 ± 0.78</td>
<td>5.6 ± 0.78</td>
<td>0.1064</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>0.04 ± 0.57</td>
<td>0.52 ± 0.57</td>
<td>0.69 ± 0.57</td>
<td>0.71 ± 0.57</td>
<td>0.0274</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.14 ± 0.18</td>
<td>1.50 ± 0.18</td>
<td>1.91 ± 0.18</td>
<td>0.13 ± 0.18</td>
<td>0.9421</td>
</tr>
<tr>
<td>iPTH</td>
<td>29.00 ± 7.49</td>
<td>23.03 ± 7.49</td>
<td>23.03 ± 7.49</td>
<td>40.61 ± 7.49</td>
<td>0.4822</td>
</tr>
<tr>
<td>25(OH)D</td>
<td>35.15 ± 5.72</td>
<td>10.56 ± 5.72</td>
<td>19.42 ± 5.72</td>
<td>21.19 ± 5.72</td>
<td>0.0634</td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>6.57 ± 7.55</td>
<td>15.75 ± 7.55</td>
<td>15.75 ± 7.55</td>
<td>6.51 ± 7.55</td>
<td>0.0634</td>
</tr>
<tr>
<td>Calcium absorption</td>
<td>0.60 ± 4.38</td>
<td>5.26 ± 4.38</td>
<td>3.56 ± 4.28</td>
<td>4.08 ± 4.28</td>
<td>0.0634</td>
</tr>
<tr>
<td>Calcium:creatinine (2 h)</td>
<td>15.12 ± 15.74</td>
<td>67.28 ± 15.74</td>
<td>34.62 ± 15.74</td>
<td>36.92 ± 15.74</td>
<td>0.1064</td>
</tr>
<tr>
<td>Calcium:creatinine (2 h)</td>
<td>14.06 ± 22.60</td>
<td>66.45 ± 21.54</td>
<td>8.02 ± 21.07</td>
<td>40.34 ± 21.07</td>
<td>0.1766</td>
</tr>
<tr>
<td>NTX:creatinine</td>
<td>10.40 ± 10.97</td>
<td>16.00 ± 10.97</td>
<td>30.55 ± 10.97</td>
<td>12.08 ± 11.22</td>
<td>0.2262</td>
</tr>
</tbody>
</table>

1 Unadjusted t ± SEM. iPTH, intact parathyroid hormone; 25(OH)D, 25-hydroxyvitamin D. NTx, N-telopeptides.
2 t ± SEM.
3 Analysis of covariance with adjustment for BMI; calcium, total energy, fiber, and vitamin D intakes; smoking status; alcohol intake; and physical activity score. When Tukey’s post hoc multiple comparison test was used to determine post hoc significance, no significant differences between the groups were found.
of dietary protein on intestinal calcium absorption (6, 9, 49–51). On the other hand, Dawson-Hughes and Harris (38) observed that high protein intakes are associated with high calcium absorption under conditions of low calcium intake but not when calcium intakes are well above normal. A high-protein diet was also reported not to influence circulating concentrations of parathyroid hormone and calcitriol (6, 51–55). On the other hand, Licata (56) reported a decrease in parathyroid hormone concentrations with consumption of a high-protein diet. In contrast, Kerstetter et al (57) reported that the ingestion of a low-protein diet for 4 d by young healthy women led to a 1.5–3% increase in serum concentrations of parathyroid hormone and 1,25-dihydroxyvitamin D₃, and to a decrease in urinary calcium excretion. The hyperparathyroidism induced by the low-protein diet was attributed to depressed calcium absorption (58).

Thus, the results of the present study in postmenopausal elderly women suggest that a higher protein intake (≥71 g/d) as a percentage of energy is associated with higher BMD in the presence of an adequate calcium intake. This translates to a recommended dietary allowance of 1.0 g/kg body wt (the current recommended dietary allowance is 0.8 g/kg body wt). Our results suggest that in the elderly, who are at the highest risk of osteoporosis, a higher protein intake is important for the maintenance of good bone health.

We thank Karen A Rafferty for her help in the collection and analysis of food diary data. We also thank Kurt E Balhorn for the laboratory analyses. JCG and PBR were involved in designing the study, interpreting the data, and preparing the manuscript. PBR was also involved in laboratory analyses. VH carried out the statistical analysis of the data. All the authors reviewed the manuscript, provided suggestions for revision, and approved the final version submitted for publication. None of the authors had any conflicts of interest.

REFERENCES


