Interaction of Dietary Calcium and Protein in Bone Health in Humans

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ABSTRACT Protein has both positive and negative effects on calcium balance, and the net effect of dietary protein on bone mass and fracture risk may be dependent on the dietary calcium intake. In addition to providing substrate for bone matrix, dietary protein stimulates the production of insulin-like growth factor-1 (IGF-1), a factor that promotes osteoblast-mediated bone formation. Protein also increases urinary calcium losses, by several proposed mechanisms. Increasing calcium intake may offset the negative impact of dietary protein on urinary calcium losses, allowing the favorable effect of protein on the IGF-1 axis to dominate. Several, although not all, studies are either compatible with or support this hypothesis. Protein supplements significantly reduced bone loss in elderly hip-fracture patients in a study in which both the protein and control groups received supplemental calcium. In an observational study, total protein intake was positively associated with favorable 3-y changes in femoral neck and total body bone mineral density in volunteers who received supplemental calcium citrate malate and vitamin D, but not in volunteers taking placebos. In conclusion, an adequate calcium intake may help promote a favorable effect of dietary protein on the skeleton in older individuals. J. Nutr. 133: 852S–854S, 2003.

KEY WORDS: ● protein ● calcium ● interaction ● bone mineral density ● fractures

Protein and calcium are major components of bone tissue. By weight, bone tissue is 70% mineral, 8% water and 22% protein. Bone undergoes continuous remodeling, and an adequate supply of mineral and amino acid substrate is needed to support the formation phase of bone remodeling. In addition to their passive roles as substrate for bone formation, dietary calcium and protein play active roles in bone metabolism. An inadequate intake of calcium results in a sequential reduction in the circulating ionized calcium concentration and an increase in parathyroid hormone (PTH) secretion. PTH normalizes the circulating ionized calcium concentration by promoting bone resorption, by reducing renal calcium excretion and, indirectly, by stimulating intestinal calcium absorption. Small increases in PTH over time that result from an inadequate dietary calcium (or vitamin D) intake cause a chronic increase in bone turnover and a steady loss of bone mass, both of which increase risk of fracture.

Dietary protein has long been known to increase renal calcium excretion. Dietary protein of both animal and plant origin leads to endogenous acid production, and diet-induced low grade metabolic acidosis causes hypercalcuria by several mechanisms. These include decreasing renal tubular reabsorption of calcium (1), increasing cell-mediated bone resorption (2) and direct physicochemical dissolution of bone (3).

It has been recognized more recently that dietary protein increases circulating levels of insulin-like growth factor-1 (IGF-1), a growth factor thought to play an important role in bone formation. In a randomized, controlled study, supplementation with 20 g/d of protein for 6 mo caused an 80% increase in serum IGF-1 levels in relatively malnourished elderly patients with recent hip fractures (4). Serum IGF-1 binding protein levels did not change significantly with protein supplementation in this study. Other supporting evidence comes from two randomized, controlled milk intervention studies in healthy subjects with normal usual diets. Milk contains 300 mg of calcium and 9 g of protein per 8 oz serving, in addition to many other components. Cadogan et al. (5) reported about a 20% increase in serum IGF-1 levels in girls (mean age 12 y) who consumed an extra pint of milk/d, although this change was not statistically significant (P = 0.23). In the other study, adult men and women who consumed three extra servings of milk/d had 14% higher serum IGF-1 levels after 12 wk than did unsupplemented controls, a difference that was statistically significant (P < 0.001) (6).

Dietary protein, bone mass and fractures: observational studies

Among observational studies, evidence for associations between protein intake and bone mass or fracture rates is mixed and includes positive, negative and no associations. Dietary protein and total hip bone mineral density (BMD) were pos-

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itively associated in postmenopausal women in the NHANES III Survey (7). The association persisted in the subset of women with calcium intakes >800 mg/d. No information was given on any association among the women with lower calcium intakes. Hannon et al. (8) found that higher protein intake (both total and animal protein) was associated with favorable 4-y changes in BMD of the femoral neck and spine in elderly, community-dwelling men and women. These subjects consumed an average of 68 g/d of protein and 800 mg/d of calcium. Munger et al. (9) identified a significant 69% lower risk of hip fracture as animal protein intake increased in a large cohort of postmenopausal women with an average total calcium intake of 1100 mg/d. In contrast, in the Nurses Health Study, Feskanich et al. (10) found no association between protein intake and risk of hip fracture but identified a significant 25% higher in risk of forearm fracture in women consuming >80 g/d compared with those consuming <51 g/d. The nurses' mean calcium intake was 720 mg/d. These reports do not allow an in-depth examination of a potential influence of calcium on the association between dietary protein and BMD.

Protein intervention studies

Two studies have examined the impact of dietary protein supplementation in elderly patients with recent hip fractures and, in both, supplemental calcium was given along with the protein. In a randomized study of 59 patients (11) a dietary supplement containing 20 g of protein and 500 mg of calcium taken daily for only 4 wk improved the clinical course of these patients over the following 6 mo. Notably, the patients in the control group had a low mean calcium intake of 400 mg/d and a low mean protein intake of about 32 g/d. In a subsequent study in acute hip fracture patients, supplementation with 20 g/d of protein for 6 mo increased serum IGF-1 levels (as indicated earlier) and reduced the rate of bone loss in the contralateral hip during the year after the fracture (4). In this study, both the protein and control groups were given a large oral dose of vitamin D at the outset and 550 mg/d of calcium throughout the study. From this study, we can conclude that, in elderly subjects with low usual intakes of dietary protein and adequate intakes of calcium and vitamin D, supplementation with 20 g/d of protein has skeletal benefits. We cannot know, of course, what role calcium played in the patients taking supplemental protein, in either of these intervention studies.

Potential interaction of calcium and protein with bone

There are several reasons to believe that the calcium intake may influence the net effect of protein on the skeleton. Higher calcium intake results in more absorbed calcium, which may help offset the urine losses induced by dietary protein. Calcium, by lowering the turnover rate, may also reduce the adverse effect of mild acidosis on bone resorption. An observational study by Meyer et al. (12) suggests that the calcium intake may in fact influence the impact of dietary protein on the skeleton. In that study, neither calcium intake nor protein intake from nondairy animal sources was associated with the incidence of hip fracture. However, subjects with the combination of low calcium intake (<435 mg/d, lowest quartile bound) and high protein intake (>20.6 g/d, highest quartile bound) were at approximately double the risk of hip fracture (RR 1.96, CI 95% 1.09, 3.56) compared with other subjects in the study. In contrast, Promislow (13), who reported a positive association between animal protein intake and BMD in older men and women, found that increasing protein intake was more beneficial to those with lower calcium intakes, although evidence for this interaction was inconsistent.

We recently examined the association between protein intake and changes in BMD in healthy men and women age 65 and older who had participated in a 3-y randomized controlled trial. In the trial, subjects took either 500 mg/d of calcium as calcium citrate malate plus 700 IU of vitamin D or double placebo. The mean total calcium intakes of the two groups were 1346 ± 378 (SD) and 871 ± 413 mg/d and mean vitamin D intakes were 22.5 μg (900 IU) and 5 μg (200 IU), respectively. The supplemented group also consumed an additional 25 meq of alkali potential, from the citrate malate. The main results of the trial were that supplementation lowered the bone turnover rate by 10 to 15%; reduced bone loss from the spine, hip and total body; and lowered clinical fracture rates. It is likely that the calcium, vitamin D and the citrate malate contributed to the supplement effect (14).

For the protein and bone analyses, 342 subjects were divided into tertiles of total protein as percentage of energy. Mean total protein intakes of the three tertiles were 69, 80 and 88 g/d. In ANCOVAs there was a significant interaction of treatment group by protein, indicating that supplement status would influence any association of protein with change in bone density at the total body. We therefore examined associations of dietary protein with change in BMD separately in the supplemented and unsupplemented subjects. We did not find any indication that potassium intake (or urinary excretion), an index of potential alkali, influenced the association of protein with change in BMD at any skeletal site. Selected dietary and biochemical values for the protein tertiles are shown in Table 1. Calcium and potassium intakes and serum PTH levels differed only slightly across the tertiles in either treatment group. Similarly, 24-h urinary calcium, sodium and potassium, each corrected for creatinine excretion, did not differ across the tertiles. As reported earlier (14), the supplemented group had lower levels of PTH and higher levels of urinary calcium. Changes in BMD by tertile of total protein intake are shown for the supplemented (upper panels) and

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Dietary and laboratory characteristics by tertile of protein intake (% of energy)</th>
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<tbody>
<tr>
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<td>Tertile 1</td>
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<tr>
<td>Total protein intake, g/d</td>
<td>67.4</td>
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<tr>
<td>Supplement</td>
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<td>Calcium, mg/d</td>
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<td>Placebo</td>
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<tr>
<td>Supplement</td>
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<tr>
<td>Potassium, mg/d</td>
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<td>Supplement</td>
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<td>Serum PTH,1 pmol/L</td>
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<tr>
<td>Placebo</td>
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<td>Placebo</td>
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<tr>
<td>24-h Urinary Na/Cr, mg/g</td>
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<td>Placebo</td>
<td>3815</td>
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1 PTH, parathyroid hormone.
FIGURE 1  The association of protein intake with rates of bone loss in elderly men and women treated for 3 y with 500 mg of calcium as citrate malate plus 17.5 µg (700 IU) of vitamin D (solid bars) and with placebo (open bars). For the total body there was a significant interaction of treatment group X protein tertile (*P* = 0.044). (Adapted with permission by the American Journal of Clinical Nutrition. © Am J Clin Nutr American Society for Clinical Nutrition.)

placebo groups (lower panels) in Figure 1 (15). Higher protein intake was associated with a favorable change in total body BMD in the supplemented but not in the placebo group. Femoral neck BMD also increased with increasing protein intake in the supplemented group but not in the placebo group, although the interaction at this site was not significant. There was no association of protein intake with change in BMD of the spine in this study. Serum IGF-1 levels did not differ across the protein tertiles in either the supplemented or the placebo subjects. Serum osteocalcin and 24-h urinary N-telopeptide levels were lower in the supplemented than the placebo group, but did not differ across protein intake tertiles in either treatment group. Collectively, these findings suggest that a higher calcium intake may reduce or offset the negative effect of protein on calcium retention and/or amplify the positive effect of IGF-1 or other factors on bone mass.

Theoretically, to optimize the impact of dietary protein on the skeleton, one would want to minimize the impact of protein on renal calcium losses but not impair its impact on promoting the production of IGF-1. One approach to reducing the negative impact of protein on calcium balance, that examined here, is to increase calcium intake (from 800 to 1300 mg/d in this study). The alkali potential of the supplements used in our study (25 meq/d) may also have limited the renal calcium losses, although the supplements probably represented a relatively small contribution to total intake of potential alkali in this study population. By our estimates, the subjects consumed an average of 85 meq/d of potential alkali from dietary potassium sources alone. A practical alternative strategy to enhance a net positive effect of dietary protein on the skeleton may be to substantially increase the intake of alkali. Although we found no indication in our study that fruit and vegetable intake (as estimated by intake and urinary excretion of potassium) influenced the association of dietary protein with change in BMD, this possibility should be examined prospectively. In addition, protein intervention studies are needed to examine more rigorously the impact of dietary protein on serum IGF-1, biochemical markers of bone turnover and other skeletal measures at different calcium intake levels.

In conclusion, the impact of dietary protein on the skeleton appears to be favorable in older subjects who are meeting their dietary calcium requirements but not in those with lower calcium intakes. The optimal protein intake for bone health in the elderly needs to be determined, and this determination should be made in subjects who are meeting the dietary calcium requirement.

**LITERATURE CITED**