

# Calcium intake influences the association of protein intake with rates of bone loss in elderly men and women<sup>1-4</sup>

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## ABSTRACT

**Background:** There is currently no consensus on the effect of dietary protein intake on the skeleton, but there is some indication that low calcium intakes adversely influence the effect of dietary protein on fracture risk.

**Objective:** The objective of the present study was to determine whether supplemental calcium citrate malate and vitamin D influence any associations between protein intake and change in bone mineral density (BMD).

**Design:** Associations between protein intake and change in BMD were examined in 342 healthy men and women (aged  $\geq 65$  y) who had completed a 3-y, randomized, placebo-controlled trial of calcium and vitamin D supplementation. Protein intake was assessed at the midpoint of the study with the use of a food-frequency questionnaire and BMD was assessed every 6 mo by dual-energy X-ray absorptiometry.

**Results:** The mean ( $\pm$ SD) protein intake of all subjects was  $79.1 \pm 25.6$  g/d and the mean total calcium intakes of the supplemented and placebo groups were  $1346 \pm 358$  and  $871 \pm 413$  mg/d, respectively. Higher protein intake was significantly associated with a favorable 3-y change in total-body BMD in the supplemented group (in a model containing terms for age, sex, weight, total energy intake, and dietary calcium intake) but not in the placebo group. The pattern of change in femoral neck BMD with increasing protein intake in the supplemented group was similar to that for the total body.

**Conclusion:** Increasing protein intake may have a favorable effect on change in BMD in elderly subjects supplemented with calcium citrate malate and vitamin D. *Am J Clin Nutr* 2002; 75:773-9.

**KEY WORDS** Calcium intake, protein intake, bone loss, bone mineral density, potential alkali, calcium absorption, vitamin D, elderly

## INTRODUCTION

Several studies have identified associations between dietary protein intake and bone mineral density (BMD) (1), rates of bone loss (2, 3), and fracture incidence (3-5). In the original Framingham cohort, subjects with lower total and animal protein intakes had greater rates of bone loss from the femoral neck and spine than did subjects consuming more protein (2). Munger et al (6) reported that higher total (and animal) protein intake was associated with a

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reduced incidence of hip fracture in postmenopausal women. In contrast, a high intake ratio of animal to plant protein was associated with greater bone loss from the femoral neck and a greater risk of hip fracture in women aged  $\geq 65$  y (3). Higher total and animal protein intakes were also associated with an increased risk of forearm fracture in younger postmenopausal women (4). Meyer et al (5) noted no association between protein intake and risk of hip fracture in most women, but among those with very low calcium intakes (400 mg/d), a higher protein intake was associated with an increased risk of hip fracture. In a controlled, 1-y intervention study, 20 g supplemental dietary protein/d improved hip BMD in elderly patients with a recent hip fracture (7). All the patients in that study received supplemental calcium and vitamin D.

Dietary protein has several opposing effects on calcium balance. First, it influences urinary calcium excretion over at least the ensuing several months (8-10). Women placed on high-protein diets have increased urinary calcium excretion and rises in *N*-telopeptide, suggesting that some of the increase in urinary calcium results from increased bone resorption (11). In contrast, Shapses et al (12) found that increased protein intake has no effect on bone resorption markers in subjects with calcium intakes in the high-normal range. Dietary protein may affect intestinal calcium absorption, but the evidence for this is mixed. Rats consuming high-protein diets appear to compensate for increased urinary calcium losses by increasing net calcium absorption (13). Balance studies in humans found little to no effect of dietary protein on calcium absorption (8, 14, 15).

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Heaney (16) recently noted, however, that at low calcium intakes, absorption can increase sufficiently to offset urinary losses. In women with a mean calcium intake of 800 mg/d, lowering protein intake from 158 to 52 g/d lowered calcium absorption within 4 d (17). Finally, dietary protein also promotes the production of insulin-like growth factor 1 (IGF-1), a factor that stimulates bone growth (18), and low protein intakes have been associated with low serum concentrations of IGF-1 in humans (19).

Thus, although no consensus currently exists on the effect of dietary protein intake on bone health, the results of several studies suggest that calcium intake may influence the effect of dietary protein on the skeleton. In the present study, we examined and compared associations of protein intake with changes in BMD in healthy older men and women who were treated with either placebo or calcium citrate malate and vitamin D for 3 y.

## SUBJECTS AND METHODS

### Subjects

The study included 342 healthy subjects (161 men and 181 women) aged  $\geq 65$  y who participated in a 3-y, randomized, placebo-controlled trial designed to assess the effect of calcium and vitamin D supplementation on rates of change in BMD (20). Of the 389 subjects who completed the trial (20), 37 did not attend the 18-mo visit at which the food-frequency questionnaire was completed, and an additional 9 attended the visit but did not complete the questionnaire. Of the remaining 343 subjects, one man in the calcium- and vitamin D-treated (supplemented) group was excluded from the analysis because of an unreasonably high reported energy intake (28.6 J/d compared with a range of 3.3–18.7 J/d in all other subjects).

All subjects had baseline femoral neck BMD within 2 SDs of the age-matched reference mean given in the densitometer software, and subjects with medical conditions or who used medications known to alter rates of bone loss were excluded from the trial. Subjects were randomly assigned to treatment with either 500 mg Ca as calcium citrate malate and 17.5  $\mu$ g (700 IU) vitamin D daily or with double placebos. Subjects came to the Nutrition Center every 6 mo for BMD and other measurements. The protocol was approved by the Human Investigation Review Committee at Tufts University and written, informed consent was obtained from each subject.

### Dietary assessments

Dietary intakes of protein, calcium, phosphorus, magnesium, fiber, vitamin D, and total energy intake over the preceding year were assessed in all subjects at the 18-mo visit with the use of the 126-item Willett food-frequency questionnaire (1988 version). The self-administered questionnaire was filled out at the Nutrition Center and was reviewed for completeness by staff during the visit. The 126 food items included 14 meat and fish items and 13 dairy foods. The validity of the food and nutrient assessments from the original questionnaire has been studied (21–25), and estimates with this questionnaire correlated reasonably well with protein (21) and meat (22) intakes derived from four 1-wk dietary records in 173 subjects (21, 22). This semi-quantitative questionnaire is reproducible and is an effective means of ranking protein intake in men and women (21, 23).

Because absolute protein intake is strongly influenced by total energy intake, protein intake is expressed throughout the present

article as energy from protein as a percentage of total energy intake (% of energy). Similarly, the 2 components of total protein intake, animal and plant protein, are also expressed as energy from animal (or plant) protein as a percentage of total energy intake. Intakes of other nutrients are expressed in the usual manner of weight consumed per day.

### Bone mineral density measurements

BMD of the femoral neck, spine, and total body were measured every 6 mo with a model DPX-L dual-energy X-ray absorptiometer (Lunar Radiation Corp, Madison, WI). Software version 1.2 was used for data acquisition and version 1.3y for analysis. The CVs of the measurements were 2.0% (femoral neck), 1.0% (spine), and 0.6% (total body) (26). Femur scans were performed in duplicate and the mean value was used in the analyses. Baseline BMD and percentage change after 3 y are reported.

### Calcium absorption measurements

Fractional calcium absorption was measured at 18 mo with a single isotope method ( $^{45}\text{Ca}$ ; Amersham Corporation, Chicago). As described previously (27), test solutions for the absorption measurements contained 3  $\mu\text{Ci}$   $^{45}\text{Ca}$  and 100 mg elemental Ca as calcium chloride in deionized water for a total volume of 100 mL. Duplicate aliquots of 50  $\mu\text{L}$  each were taken from the test dose to calculate the total dose given. After he or she had fasted overnight, each subject consumed the test solution and 3 serial 50-mL rinses of the test solution container. After 2 h, during which the subjects continued to fast, 15 mL blood was drawn. Two 2-mL aliquots of serum were counted in a beta-scintillation counter (Beckman Instruments, Fullerton, CA). Weight-corrected fractional calcium absorption was calculated as the percentage of total  $^{45}\text{Ca}$  counts administered that were recovered per liter serum after 2 h multiplied by  $0.15 \times$  body weight. The CV of this measure is 15%. Absorbed calcium was calculated by multiplying weight-corrected fractional calcium absorption by total calcium intake (from diet and study supplements, assessed at 18 mo).

### Biochemical and other measurements

Blood was drawn between 0700 and 0930 after the subjects had fasted overnight. Serum parathyroid hormone was measured by immunometric assay (Nichols Institute, San Juan Capistrano, CA), serum osteocalcin by immunoradiometric assay (Nichols Institute), and urinary *N*-telopeptide cross-links by enzyme-linked immunosorbent assay (Ostex International, Seattle) with CVs of 5.6–7.7%. Serum IGF-1 was measured in the laboratory of Cliff Rosen by use of a double-antibody radioimmunoassay after extraction from serum IGF binding proteins, as previously described (28). Urinary calcium, sodium, and creatinine were measured with a Nova Nucleus Chemistry Analyzer (Nova Biochemical, Waltham, MA) with CVs of  $<3\%$ .

Leisure, household, and occupational activity were estimated with use of the Physical Activity Scale for the Elderly questionnaire (29). Cigarette smoking was determined by questionnaire.

### Statistical analysis

Linear associations were described with partial correlation coefficients. Protein intake was computed as tertiles (in the entire sample of 342 subjects) as % of energy. Characteristics of the subjects across protein tertiles were compared by analysis of variance (ANOVA) for continuous variables and by chi-square test for categorical variables. Adjusted means for the text and



**TABLE 1**  
Dietary and clinical characteristics by tertile of energy intake from protein among subjects treated with placebo or calcium plus vitamin D for 3 y<sup>1</sup>

| Variable and group                               | Tertile of protein intake (% of total energy) |                |                |
|--|---|----------------|----------------|
|  | 1: 9.64–15.49                                 | 2: 15.53–18.15 | 3: 18.16–29.14 |
| No. of subjects                                  |   |                |                |
| Placebo group                                    | 62  | 57             | 65             |
| Supplemented group                               | 56  | 53             | 49             |
| Total protein intake (% of energy) <sup>2</sup>  |   |                |                |
| Placebo group                                    | 13.9 ± 1.1 <sup>3</sup>                       | 16.9 ± 0.8     | 20.3 ± 2.2     |
| Supplemented group                               | 13.8 ± 1.5                                    | 16.7 ± 0.8     | 20.1 ± 1.6     |
| Animal protein intake (% of energy) <sup>2</sup> |   |                |                |
| Placebo group                                    | 8.7 ± 1.5                                     | 11.3 ± 1.4     | 15.0 ± 2.6     |
| Supplemented group                               | 8.8 ± 1.7                                     | 11.3 ± 1.5     | 14.9 ± 2.1     |
| Plant protein intake (% of energy)               |   |                |                |
| Placebo group                                    | 5.1 ± 1.2                                     | 5.6 ± 1.1      | 5.3 ± 1.3      |
| Supplemented group                               | 5.0 ± 1.1                                     | 5.5 ± 1.2      | 5.2 ± 1.0      |
| Total protein intake (g) <sup>2</sup>            |   |                |                |
| Placebo group                                    | 67.4 ± 20.9                                   | 81.8 ± 27.9    | 86.7 ± 22.8    |
| Supplemented group                               | 71.7 ± 25.7                                   | 79.3 ± 21.0    | 88.6 ± 29.3    |
| Total energy intake (J) <sup>2</sup>             |   |                |                |
| Placebo group                                    | 8.1 ± 2.3                                     | 8.1 ± 2.7      | 7.2 ± 1.9      |
| Supplemented group                               | 8.7 ± 3.0                                     | 7.9 ± 2.1      | 7.4 ± 2.2      |
| Calcium intake (mg)                              |   |                |                |
| Placebo group                                    | 755 ± 315                                     | 918 ± 471      | 940 ± 425      |
| Supplemented group                               | 809 ± 382                                     | 885 ± 334      | 847 ± 358      |
| Phosphorus intake (mg)                           |   |                |                |
| Placebo group                                    | 1174 ± 368                                    | 1382 ± 521     | 1375 ± 415     |
| Supplemented group                               | 1251 ± 468                                    | 1342 ± 398     | 1332 ± 427     |
| Magnesium intake (mg)                            |   |                |                |
| Placebo group                                    | 304 ± 94                                      | 334 ± 115      | 328 ± 109      |
| Supplemented group                               | 324 ± 110                                     | 337 ± 103      | 315 ± 97       |
| Fiber intake (g)                                 |   |                |                |
| Placebo group                                    | 20.9 ± 8.4                                    | 23.3 ± 10.2    | 20.6 ± 9.1     |
| Supplemented group                               | 21.1 ± 9.2                                    | 22.7 ± 8.0     | 20.1 ± 7.9     |
| Vitamin D intake (μg/d)                          |   |                |                |
| Placebo group                                    | 6.1 ± 2.7                                     | 6.9 ± 4.0      | 7.8 ± 4.2      |
| Supplemented group                               | 6.3 ± 3.4                                     | 7.1 ± 3.2      | 6.8 ± 3.2      |
| Age (y)  |   |                |                |
| Placebo group                                    | 71 ± 5  | 71 ± 5         | 71 ± 5         |
| Supplemented group                               | 70 ± 5  | 71 ± 4         | 70 ± 4         |
| Men (%) <sup>2</sup>                             |   |                |                |
| Placebo group                                    | 60  | 42             | 35             |
| Supplemented group                               | 68  | 40             | 37             |
| Weight (kg)                                      |   |                |                |
| Placebo group                                    | 75 ± 14                                       | 76 ± 15        | 72 ± 14        |
| Supplemented group                               | 75 ± 14                                       | 74 ± 14        | 76 ± 13        |
| Current smokers (%)                              |   |                |                |
| Placebo group                                    | 5   | 2              | 6              |
| Supplemented group                               | 9   | 6              | 4              |
| PASE activity score                              |   |                |                |
| Placebo group                                    | 122 ± 62                                      | 119 ± 50       | 113 ± 55       |
| Supplemented group                               | 124 ± 53                                      | 114 ± 57       | 111 ± 57       |
| Total-body BMD (g/cm <sup>2</sup> )              |   |                |                |
| Placebo group                                    | 1.12 ± 0.13                                   | 1.10 ± 0.11    | 1.07 ± 0.14    |
| Supplemented group                               | 1.15 ± 0.13                                   | 1.10 ± 0.14    | 1.09 ± 0.13    |
| Femoral neck BMD (g/cm <sup>2</sup> )            |   |                |                |
| Placebo group                                    | 0.89 ± 0.14                                   | 0.86 ± 0.12    | 0.86 ± 0.14    |
| Supplemented group                               | 0.92 ± 0.16                                   | 0.89 ± 0.16    | 0.87 ± 0.15    |
| Spine BMD (g/cm <sup>2</sup> )                   |   |                |                |
| Placebo group                                    | 1.17 ± 0.23                                   | 1.17 ± 0.20    | 1.11 ± 0.25    |
| Supplemented group                               | 1.22 ± 0.23                                   | 1.14 ± 0.24    | 1.14 ± 0.22    |

<sup>1</sup>Clinical characteristics were determined at baseline and dietary values at 18 mo. PASE, Physical Activity Scale for the Elderly (27); BMD, bone mineral density. There were no significant interactions between protein tertile and treatment.

<sup>2</sup>Significant effect of protein tertile,  $P < 0.05$ .

<sup>3</sup> $\bar{x} \pm SD$ .

figures were computed and compared across protein tertiles by analysis of covariance (ANCOVA). Covariates considered were sex, age, weight, total energy, dietary calcium, physical activity score, smoking, and interaction terms for treatment group  $\times$  protein intake as % of energy. The statistical significance of potential interactions was examined by including interaction terms in the ANCOVA models. Pairwise comparisons from these models were examined by  $t$  test only when the  $P$  values for the protein tertile terms in the models were  $<0.05$ , and a Bonferroni correction for multiple comparisons was applied.  $P$  values from these terms were considered statistically significant if  $<0.05$ . Statistical analyses were conducted with SPSS version 10.1 (SPSS Inc, Chicago).

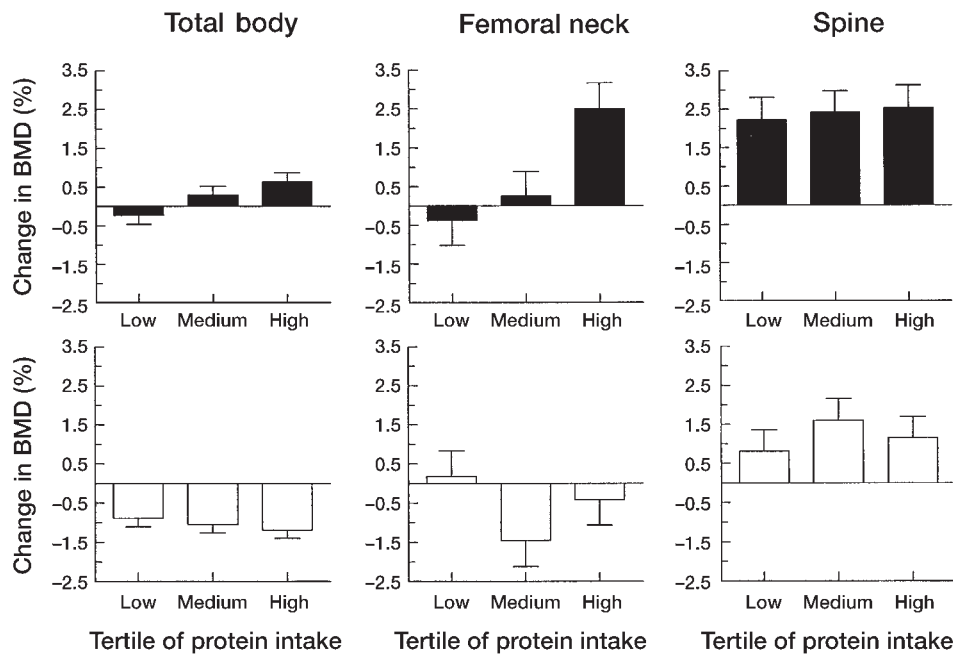
## RESULTS

The mean protein intake of the study participants was  $79.1 \pm 25.6$  g/d. Mean total calcium intake was  $871 \pm 413$  mg/d in the placebo group and  $1346 \pm 358$  mg/d in the supplemented group. In the group as a whole, protein intake as a percentage of energy was not significantly correlated with total-body, femoral neck, or spine BMD (partial  $r$  values:  $-0.03$  to  $-0.07$  after adjustment for age, sex, and weight).

The clinical characteristics of the subjects, by tertile of protein intake as a percentage of energy and by treatment group, are shown in **Table 1**. By definition, total protein intake as a percentage of energy increased across the tertiles in each treatment group. Intake of plant protein as a percentage of energy did not differ significantly across the tertiles, whereas intake of animal protein as a percentage of energy increased significantly. Total energy intake declined across the tertiles of protein intake. The higher proportion of men in the lowest tertile of protein intake indicated that the men generally consumed a lower percentage of energy as protein than did the women. There were no significant interactions of treatment with protein intake as a percentage of energy in analyses of any of the variables in **Table 1**.

To determine whether treatment with placebo or supplemental calcium plus vitamin D influenced any associations between protein intake as a percentage of energy and rates of bone loss, we performed ANCOVAs that included interaction terms for treatment group  $\times$  protein intake as a percentage of energy along with terms for sex, age, weight, total energy, and dietary calcium intake in the models (inclusion of adjustments for physical activity score and smoking did not alter the results and so these terms were omitted). The interaction of treatment group  $\times$  protein intake as a percentage of energy was significant for the total body ( $P = 0.044$ ).

For each treatment group, mean changes in BMD of the total body, femoral neck, and spine, by tertile of total protein intake, are shown in **Figure 1**. At each skeletal site, mean changes in BMD were determined in ANCOVA models that contained terms for age, sex, weight, total energy intake, and dietary calcium intake (inclusion of adjustments for vitamin D and phosphorus intakes and for baseline BMD did not alter the results and so these terms were omitted). For the total body, a higher protein intake as a percentage of energy was associated with significantly less BMD loss (or greater gain) in subjects in the supplemented group (ANCOVA  $P = 0.046$ ), whereas protein intake as a percentage of energy was not significantly associated with change in BMD in the placebo group. Although there was no significant interaction of treatment group  $\times$  protein intake as a



**FIGURE 1.** Mean ( $\pm$ SEM) percentage change in bone mineral density (BMD) by tertile of protein intake as a percentage of total energy intake (adjusted for sex, age, weight, total energy intake, and dietary calcium intake) in 342 men and women treated with calcium citrate malate and vitamin D (■) or placebo (□) for 3 y. For the total body, there was a significant interaction of treatment group  $\times$  protein tertile ( $P = 0.044$ ); in the supplemented group, the high tertile differed from the low tertile ( $P = 0.042$ ; adjusted for multiple comparisons). For the femoral neck, the interaction term was not significant, but the pattern of change in BMD in the supplemented group was similar to that for the total body: the high tertile differed from the low ( $P = 0.011$ ) and middle ( $P = 0.042$ ) tertiles.

percentage of energy at the femoral neck ( $P = 0.215$ ), the pattern of change in femoral neck BMD across the tertiles of protein intake in the supplemented group was similar to that for the total body (ANCOVA  $P = 0.008$ ). At the lumbar spine, there was no significant interaction of treatment group  $\times$  protein intake as a percentage of energy and change in BMD was not significantly associated with protein intake in either treatment group.

To evaluate whether protein source, animal or plant, was important in the association between protein intake as a percentage of energy and change in total-body BMD in the supplemented group, a term for animal protein as a percentage of total protein was added to the ANCOVA model containing terms for tertile of protein intake, age, sex, weight, total energy intake, and dietary calcium intake. In this model, the term for animal protein as a percentage of total protein was not significant, indicating that it was the total amount rather than the source of protein that was related to change in total-body BMD in subjects supplemented with calcium and vitamin D. A similar result was seen for the femoral neck.

Absorbed calcium was examined in relation to protein intake as a percentage of energy in the 282 subjects (128 men and 154 women) who had absorption measurements taken at the 18-mo visit. There was a significant interaction of treatment group  $\times$  protein intake as a percentage of energy ( $P = 0.046$ ) in analyses of absorbed calcium that included adjustments for age, sex, weight, total energy intake, and dietary calcium intake. Within the supplemented group, absorbed calcium did not differ significantly among the tertiles of protein intake after adjustment for age, sex, weight, total energy intake, and dietary calcium intake (Figure 2). In the placebo group, however, absorbed calcium declined with increasing protein intake as a

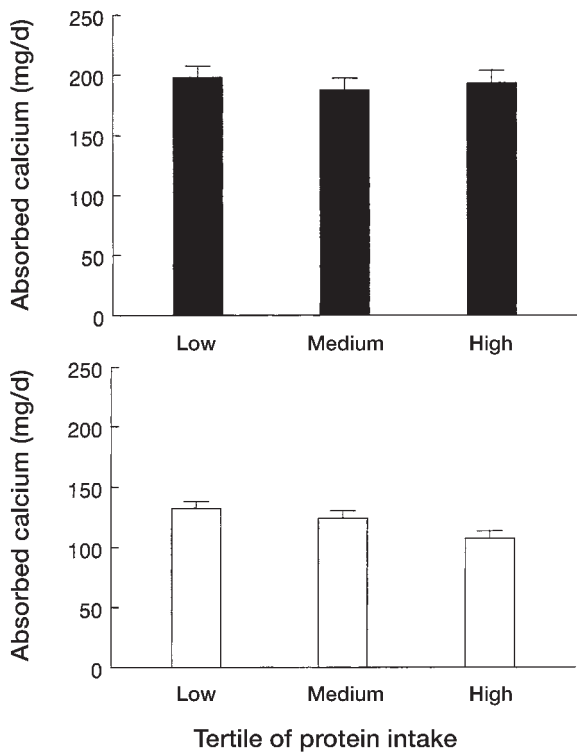
percentage of energy (ANCOVA  $P = 0.017$ , after the same adjustments; additional adjustment for dietary fiber and phosphorus did not alter the results). As shown in Figure 2, absorbed calcium in the placebo group was significantly lower in tertile 3 than in tertile 1. As expected, absorbed calcium was higher in the supplemented group as a whole than in the placebo group ( $P < 0.001$ ).

Biochemical measurements, by tertile of protein intake as a percentage of energy and by treatment group, are shown in Table 2. There were no significant interactions of treatment assignment  $\times$  protein intake as a percentage of energy in analyses of any of the variables in Table 2, after adjustments for age, sex, weight, total energy, and dietary calcium intake. As previously reported (20), serum osteocalcin and parathyroid hormone were lower and 24-h urinary calcium:creatinine was higher in the supplemented group than in the placebo group ( $P < 0.001$ ).

## DISCUSSION

Among subjects supplemented with calcium citrate malate and vitamin D, a 20% higher mean protein intake was associated with favorable changes in total-body BMD. In unsupplemented subjects, there was no association between protein intake and change in BMD. We also observed a favorable association between protein intake and change in BMD at the femoral neck in the supplemented group. The lack of a significant interaction term at this site may reflect the greater variability in femoral neck BMD than in total-body BMD measurements (26).

Of the available longitudinal observational studies (2–5), 2 examined the effect of protein intake on the skeleton as a function of calcium intake (4, 5). Feskanich et al (4) noted a trend



**FIGURE 2.** Mean ( $\pm$ SEM) absorbed calcium by tertile of protein intake as a percentage of total energy intake (adjusted for sex, age, weight, total energy intake, and dietary calcium intake) in 282 men and women after 18 mo of treatment with calcium citrate malate and vitamin D (■) or placebo (□). The interaction term was significant ( $P = 0.046$ ), and in the placebo group the high tertile differed from the low tertile ( $P = 0.015$ ).

toward more forearm fractures with higher protein intake at calcium intakes  $<540$  mg/d. Meyer et al (5) identified a significant positive association of protein intake with hip fracture risk at calcium intakes  $<400$  mg/d in older men and women. In contrast with our findings [in subjects who had an average total calcium intake that was slightly higher than the current recommendation of 1200 mg/d (30)], neither Feskanich et al nor Meyer et al observed any effect of calcium intake on the association of protein intake and fracture rates among subjects with calcium intakes  $\geq 540$  mg/d.

The design of the present study does not allow us to identify the active component or components of the supplements, and both the calcium and the citrate malate may have been important. The citrate malate in our supplement provided 25 mEq potential alkali. Ingestion of alkali decreases net acid excretion and urinary calcium excretion (31–34). For example, Lutz (31) reported that ingestion of 70 mEq alkali/d as sodium bicarbonate reversed the hypercalciuria induced by 58 g dietary protein/d. Sebastian et al (32) found that potassium bicarbonate administration also has a favorable net effect on bone turnover (raised serum osteocalcin concentrations and lowered urinary hydroxyapatite excretion). The effect of supplemental alkali on BMD and fracture rates is unknown. Of note, the amount of protein consumed by the subjects in the lowest intake tertile in our study,  $\approx 1.0$  g/kg, is higher than the current recommended dietary allowance of 0.80 g/kg (35).

It was not the source of protein but the total protein intake that appeared to influence change in BMD in our supplemented subjects. Hannan et al (2) found both total and animal protein intake to predict favorable changes in BMD in elderly men and women, whereas other investigators found that animal protein has an adverse association with bone loss (3) and fracture risk (3, 4). The reasons for the discordant associations of both total and animal protein intake with bone loss and fracture rates are not clear. They do not appear to be related to differences in mean animal protein intakes or in mean calcium intakes or to the use of different instruments for the assessment of protein intake.

The decline in serum osteocalcin with increasing protein intake in the supplemented group, although not significant, may be suggestive of a reduction in bone turnover that would be consistent with the observed changes in BMD. This finding is also consistent with the results of a short-term study that found serum osteocalcin to be 25% lower (also not significant) in healthy persons consuming 2.1 compared with 0.7 g protein  $\cdot$  kg $^{-1}$   $\cdot$  d $^{-1}$  (11). Higher protein intake was also associated with higher urinary cross-link concentrations in that study (11). We saw no change in urinary *N*-telopeptide cross-links across protein tertiles, in agreement with the results of another short-term study (12).

The calcium intake also appeared to influence the association of protein intake with absorbed calcium. At the lower mean calcium intake of  $\approx 800$  mg/d, a 20% higher protein intake was associated with a 23% (or an  $\approx 25$  mg/d) lower absorbed amount of

**TABLE 2**

Laboratory values by tertile of energy intake from protein among subjects treated with placebo or calcium plus vitamin D for 3 y<sup>1</sup>


| Variable and group                        | Tertile of protein intake (% of total energy) |                  |                  |
|---|---|------------------|------------------|
|   | 1: 9.64–15.49                                 | 2: 15.53–18.15   | 3: 18.16–29.14   |
| <b>Serum parathyroid hormone (pmol/L)</b> |   |                  |                  |
| Placebo group                             | 4.4 $\pm$ 1.8                                 | 4.9 $\pm$ 2.3    | 4.9 $\pm$ 3.0    |
| Supplemented group                        | 3.2 $\pm$ 1.5                                 | 3.2 $\pm$ 1.3    | 3.5 $\pm$ 1.3    |
| <b>Serum osteocalcin (nmol/L)</b>         |   |                  |                  |
| Placebo group                             | 1.1 $\pm$ 0.3                                 | 1.1 $\pm$ 0.4    | 1.1 $\pm$ 0.4    |
| Supplemented group                        | 0.9 $\pm$ 0.3                                 | 0.9 $\pm$ 0.2    | 0.8 $\pm$ 0.3    |
| <b>Serum IGF-1 (<math>\mu</math>g/L)</b>  |   |                  |                  |
| Placebo group                             | 125 $\pm$ 42                                  | 130 $\pm$ 39     | 126 $\pm$ 46     |
| Supplemented group                        | 137 $\pm$ 44                                  | 142 $\pm$ 37     | 132 $\pm$ 42     |
| <b>Creatinine clearance (mL/s)</b>        |   |                  |                  |
| Placebo group                             | 1.3 $\pm$ 0.3                                 | 1.3 $\pm$ 0.4    | 1.3 $\pm$ 0.3    |
| Supplemented group                        | 1.3 $\pm$ 0.3                                 | 1.2 $\pm$ 0.3    | 1.3 $\pm$ 0.3    |
| <b>24-h Urinary calcium:creatinine</b>    |   |                  |                  |
| Placebo group                             | 282 $\pm$ 125                                 | 354 $\pm$ 180    | 305 $\pm$ 171    |
| Supplemented group                        | 421 $\pm$ 224                                 | 421 $\pm$ 184    | 474 $\pm$ 210    |
| <b>24-h Urinary sodium:creatinine</b>     |   |                  |                  |
| Placebo group                             | 13380 $\pm$ 3377                              | 13781 $\pm$ 4448 | 13682 $\pm$ 3318 |
| Supplemented group                        | 13169 $\pm$ 3371                              | 14887 $\pm$ 4839 | 15251 $\pm$ 5238 |
| <b>24-h Urinary N-telopeptide (nmol)</b>  |   |                  |                  |
| Placebo group                             | 231 $\pm$ 172                                 | 218 $\pm$ 115    | 232 $\pm$ 218    |
| Supplemented group                        | 197 $\pm$ 101                                 | 198 $\pm$ 121    | 173 $\pm$ 86     |

<sup>1</sup> $\bar{x} \pm$  SD. All variables were measured at 18 mo with the exception of serum insulin-like growth factor 1 (IGF-1), 24-h urinary sodium:creatinine, and 24-h urinary *N*-telopeptide, values for which represent the mean of measurements made at baseline and 3 y. There were no significant interactions between protein tertile and treatment.

calcium, whereas at the higher calcium intake, absorbed calcium was greater overall and did not change significantly with increasing protein intake. It is somewhat surprising that calcium absorption decreased across the tertiles in the placebo group because short-term calcium balance studies have not documented a primary effect of dietary protein intake on calcium absorption. If anything, one might expect an increase in fractional and total calcium absorption to offset protein-induced urinary calcium losses (13). We could not attribute the decline in absorbed calcium across the protein tertiles in the placebo group to concurrent increases in dietary fiber, phosphorus, or magnesium intake. However, we cannot rule out possible effects of other components of the diet. The greater absorbed calcium in the supplemented group as a whole appears to have been sufficient to offset any expected (but not actually identified) protein-related deficits in calcium balance (such as increasing endogenous fecal calcium excretion and urinary calcium excretion) and in fact may have facilitated a favorable effect of dietary protein on the skeleton.

Urinary calcium excretion has been positively linked with protein intake in studies lasting  $\leq 2$  mo (8–10). The fact that we could identify no significant association between protein intake and urinary calcium excretion may be related to the high degree of variability in this urine measure and to the fact that calcium intake over the past year was estimated by use of a food-frequency questionnaire, whereas urinary calcium reflected dietary calcium intake on the day of the collection.

Serum IGF-1 is known to stimulate osteoblast proliferation and differentiation and bone matrix formation (18). Serum IGF-1 concentrations are influenced by protein intake (36). Increasing milk consumption was shown to increase serum IGF-1 in older men and women (37). Our failure to see an association between serum IGF-1 concentrations and protein intake may have been related to the fact that we did not measure IGF binding protein 3 or to the fact that the difference in protein intake across the tertiles was only 20%.

In conclusion, we identified a positive association between dietary protein intake and change in total-body and femoral neck BMD in healthy older men and women supplemented with calcium citrate malate and vitamin D. We saw no evidence that the type of protein (animal compared with plant) mattered. The present study suggests that BMD may be improved by increasing protein intake in many older men and women, as long as they meet the currently recommended intakes of calcium and vitamin D. Further research is needed to determine whether a similar association would be observed in older adults consuming these nutrients from other sources. 

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