

## Protein Consumption and Bone Mineral Density in the Elderly

### The Rancho Bernardo Study

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The role of dietary protein in osteoporosis is unclear, with previous studies having suggested both protection and harm. The associations of total, animal, and vegetable protein with bone mineral density (BMD) and the variations in these associations with calcium intake were studied in a community-dwelling cohort of 572 women and 388 men aged 55–92 years (Rancho Bernardo, California). Multiple linear regression analyses adjusted for standard osteoporosis covariates showed a positive association between animal protein consumption, assessed by food frequency questionnaires in 1988–1992, and BMD, measured 4 years later. This association was statistically significant in women. For every 15-g/day increase in animal protein intake, BMD increased by 0.016 g/cm<sup>2</sup> at the hip ( $p = 0.005$ ), 0.012 g/cm<sup>2</sup> at the femoral neck ( $p = 0.02$ ), 0.015 g/cm<sup>2</sup> at the spine ( $p = 0.08$ ), and 0.010 g/cm<sup>2</sup> for the total body ( $p = 0.04$ ). Conversely, a negative association between vegetable protein and BMD was observed in both sexes. Some suggestion of effect modification by calcium was seen in women, with increasing protein consumption appearing to be more beneficial for women with lower calcium intakes, but evidence for this interaction was not consistently strong. This study supports a protective role for dietary animal protein in the skeletal health of elderly women. *Am J Epidemiol* 2002;155:636–44.

aged; bone density; dietary proteins; osteoporosis

Evidence suggests that dietary protein may have an important influence on skeletal health, but the nature of its role has remained controversial. Central to this controversy has been the endogenous acid hypothesis. Proposed by Wachman and Bernstein in 1968 (1), this hypothesis speculates that diets rich in protein will increase bone loss due to the acid produced by protein catabolism and the consequent need for calcium to leave the skeleton in order to buffer the elevated acidity of the endogenous medium. While some studies have in fact found that urinary calcium level increases with greater protein intake (2–5), another found that commonly consumed proteins do not cause calcium loss (6), possibly because of the naturally high phosphorus content of most protein-rich foods, and still another found that increased protein intake leads to increases in both net calcium absorption and urinary calcium concentration (7).

Epidemiologists have weighed in with evidence on both sides of the debate surrounding dietary protein and skeletal integrity. Cross-cultural surveys have been frequently cited as supporting the endogenous acid hypothesis and implicating excess protein consumption in the high incidence of hip fracture observed in industrialized nations (8, 9). Conversely, clinical trials have suggested a beneficial effect of protein supplementation in hip fracture patients (10–12). Results from population-based cohort studies have been inconsistent (13–16).

In this study, we prospectively examined the associations of total, animal, and vegetable protein consumption with bone mineral density (BMD) and bone loss in community-dwelling elderly women and men. By evaluating modification of each of these associations by calcium intake, we also obtained new information on a potentially important interaction.

## MATERIALS AND METHODS

### Participants and protocol

Participants in this study were members of the Rancho Bernardo Heart and Chronic Disease Study (17). This population-based cohort comprises elderly, upper-middle-class Caucasian residents of Rancho Bernardo, a southern California community. Between 1988 and 1992, surviving community-dwelling members of the Rancho Bernardo cohort aged 55 years or older were invited to participate in a study of osteoporosis. All participants gave written informed consent. The institutional review board of the University of California, San Diego, approved the investigation.

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Abbreviations: BMD, bone mineral density; CI, confidence interval; SD, standard deviation.

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At the 1988–1992 visit, information on dietary intake was obtained for 1,526 participants aged 55 years or more (882 postmenopausal women, 644 men) using the Harvard-Willet diet assessment questionnaire (18). The questionnaire was self-administered and contained questions regarding portion size and consumption frequency of 128 common food items. Information on smoking habits, alcohol intake, exercise frequency, reproductive history, and use of vitamins, thiazides, thyroid hormones, steroids, and estrogen (women only) was also obtained via questionnaire. All pills and prescriptions were brought to the study center for confirmation of current use of dietary supplements and medications. Height and weight were measured with participants wearing light clothing and no shoes.

During the 1988–1992 visit, baseline BMD ( $\text{g}/\text{cm}^2$ ) was measured at the hip, femoral neck, and lumbar spine using dual-energy x-ray absorptiometry (Hologic QDR, model 1000; Hologic, Inc., Waltham, Massachusetts). Total hip BMD was obtained by summing bone mineral content at the femoral neck, intertrochanter, and greater trochanter and dividing this value by the composite area of the three sites. Spine BMD was defined as the average BMD of lumbar vertebrae L1–L4. Instruments were calibrated daily and had measurement precisions of  $\leq 1$  percent for the spine and  $\leq 1.5$  percent for the hip.

In 1992–1996, 572 (65 percent) of the postmenopausal women and 388 (60 percent) of the men returned for a follow-up visit and a second BMD measurement. BMD was again measured at the hip, femoral neck, and spine, and a separate scan was also performed to determine total body BMD. Participants were seen as close as possible to 4 years after their initial visit. The majority of participants who did not return had died. Diabetes status was determined at this visit; diabetes was defined as a fasting plasma glucose level of  $>125$  mg/dl, a 2-hour postchallenge plasma glucose level of  $\geq 200$  mg/dl, or previously established diabetes mellitus.

### Statistical analyses

All protein measures ( $\text{g}/\text{day}$ ) were adjusted for total energy intake ( $\text{kcal}/\text{day}$ ) by regression analysis for evaluation of protein consumption independent of energy intake (19). Additional analyses were also conducted in which the protein measures were not energy-adjusted and instead 1) total energy intake was included as a covariate in the regression models or 2) energy intake was not adjusted for in any way. All of these methods yielded very similar results; thus, only the results from the analyses using energy-adjusted protein measures are presented. Protein and calcium ( $\text{mg}/\text{day}$ ) intakes were retained in continuous form in all analyses to prevent loss of information and to maximize our ability to evaluate an interaction between the two.

Two sets of outcome measures were evaluated: 1) absolute BMD measured at the 1992–1996 visit at the hip, femoral neck, spine, and total body and 2) rate of bone loss between the two visits at the hip, femoral neck, and spine. Rate of bone loss was calculated as the percentage change in BMD per year. Since relative change in BMD, though commonly evaluated, has been suggested to be a less useful

measure than absolute change, annualized absolute change in BMD was also evaluated (20). These results were not meaningfully different, however, and only the results for percentage change in BMD are presented. An additional cross-sectional analysis was also performed using data from the 1988–1992 visit for both protein and BMD. Both the evidence for effect modification by calcium and the associations of total, animal, and vegetable protein intake with BMD were similar to those observed using the 1992–1996 BMD data; thus, only the results from the prospective analyses are presented.

Participants who completed the study were compared with those who did not using  $\chi^2$  tests and  $t$  tests. Linear regression was used to investigate the associations of total, animal, and vegetable protein consumption with BMD and rate of bone loss. For absolute BMD, all models included age and body mass index ( $\text{weight (kg)}/\text{height (m)}^2$ ); in the final models, data were also adjusted for calcium intake (including calcium from supplements), diabetes status, number of years postmenopausal (women only), current exercise ( $\geq 3$  times per week), and current use of cigarettes, alcohol, thiazides, thyroid hormones, steroids, and estrogen (women only). Analyses of the rate of bone loss additionally included percentage change in body weight.

The linearity of the relation between each protein variable and BMD was tested by including a quadratic protein term in the regression. Evidence of a nonlinear relation was found for vegetable protein and BMD in the multivariable models for men, and that association was consequently evaluated with models that retained the quadratic vegetable protein term. In all other models, the quadratic term was not significant ( $p > 0.10$ ), and a linear relation was thus assumed. An interaction between dietary protein and calcium was also evaluated. Since this interaction was significant ( $p < 0.05$ ) at just fewer than half of the BMD sites for women, results are presented both for models that included this interaction term and for models that did not.

Each of the BMD and protein variables followed a normal distribution. Tolerances were checked for all variables and, when necessary, variables were centered to avoid collinearity. For all regression models, plots of the residuals versus the predicted values were examined to ascertain that basic model assumptions were being met. SAS software, version 8.1 (SAS Institute, Cary, North Carolina), was used to perform all analyses.

### RESULTS

Baseline characteristics of the final study sample and participants who did not return for follow-up are compared in table 1. Men who did not complete the study consumed significantly less total protein and animal protein, while women who did not return consumed somewhat more vegetable protein. Men who did not complete the study were also older, less likely to exercise, and more likely to use thiazides and steroids and to have a lower body mass index and calcium intake. Women who did not complete the study were also older, had been menopausal for a longer time, and were less likely to exercise and to use estrogen. In the final sample,

**TABLE 1. Baseline characteristics of participants in the 1988–1992 visit of the Rancho Bernardo Study, according to follow-up status at the 1992–1996 visit**

Variable	Women					Men				
	Final sample ( <i>n</i> = 572)		Lost participants ( <i>n</i> = 310)		<i>p</i> value*	Final sample ( <i>n</i> = 388)		Lost participants ( <i>n</i> = 256)		<i>p</i> value*
	Mean or %	SD†	Mean or %	SD		Mean or %	SD	Mean or %	SD	
Protein intake (g/day)										
Total protein	71.2	24.8	72.3	27.9	0.55	73.8	23.4	68.9	23.1	0.01
Animal protein	48.5	19.4	48.3	22.1	0.89	50.7	18.8	45.9	17.5	0.001
Vegetable protein	22.7	9.3	24.0	10.4	0.06	23.1	8.0	23.0	10.0	0.91
Age (years)	71.2	8.7	76.0	8.4	<0.0001	70.0	8.5	77.7	8.2	<0.0001
Body mass index‡	24.6	3.7	24.7	4.4	0.85	26.4	3.4	25.6	3.3	0.004
Total calcium intake (mg/day)	985.0	577.9	972.6	527.9	0.75	797.2	458.2	689.6	361.2	0.001
Energy intake (kcal/day)	1,666.1	568.9	1,718.7	643.0	0.23	1,800.7	524.7	1,755.1	626.6	0.34
No. of years menopausal	24.3	10.8	28.7	10.8	<0.0001					
Current exercise§ (%)	70.9		58.4		0.001	78.9		68.8		0.004
Current use (%) of:										
Cigarettes	9.2		10.0		0.69	8.8		8.2		0.80
Alcohol	86.1		83.9		0.36	90.2		90.2		1.00
Thiazides	24.7		29.9		0.10	15.0		26.8		0.001
Thyroid hormones	21.0		20.4		0.84	4.4		5.5		0.53
Steroids	3.9		5.3		0.34	2.1		5.2		0.03
Estrogen	39.9		30.3		0.005					

\* Calculated using  $\chi^2$  tests or *t* tests, as appropriate.

† SD, standard deviation.

‡ Weight (kg)/height (m)<sup>2</sup>.§ Defined as exercising  $\geq 3$  times per week.

both women and men ranged in age from 55 years to 92 years at baseline, with an average age of 71 for women and 70 for men.

Table 2 shows the associations of total, animal, and vegetable protein consumption with absolute BMD after adjustment for age and body mass index. In women, both total and animal protein intake were positively associated with BMD at each site. For total protein, the association reached statistical significance at the hip and total body and was borderline-significant at the femoral neck and spine. Protein from animal sources, which accounted for 67 percent of total protein intake in women (68 percent in men), was responsible for this positive association with BMD. At each measurement site, the positive association between consumption of animal protein and BMD was highly significant for women. Vegetable protein, conversely, was negatively correlated with BMD in women, with the association reaching significance at the hip, femoral neck, and spine.

Table 2 shows a similar pattern for men, with total and animal protein consumption generally being positively correlated with BMD and vegetable protein being negatively correlated with BMD after adjustment for age and body mass index. However, for the men, the positive associations of total and animal protein with BMD were weaker and not significant. The negative association between vegetable protein consumption and BMD was also not significant for men, although in this case the magnitude of each estimated coefficient was comparable to that for the women.

Effect modification by calcium was investigated for each protein term in the multivariable analyses. There was no suggestion of a protein  $\times$  calcium interaction in men ( $p >$

0.2), but there was in women. The effect of calcium intake on the association between dietary protein and BMD in women was qualitatively similar for total, animal, and vegetable protein; as calcium intake decreased, the association between protein intake and BMD became more positive. This pattern is illustrated for total protein in figure 1. The figure shows that while BMD increased quite markedly with increasing protein consumption for women with median (835 mg/day) and especially low (350 mg/day) calcium intakes, the association was negligible (and at the spine actually negative) in women with high (1,800 mg/day) calcium intakes. For total protein, effect modification by calcium was statistically significant for women at the femoral neck ( $p = 0.01$ ) and spine ( $p = 0.01$ ) and borderline-significant for the total body ( $p = 0.08$ ). A similar pattern can be seen at the other sites at which this interaction was significant: the femoral neck ( $p = 0.05$ ) and spine ( $p = 0.05$ ) for animal protein (figure 2) and the total body ( $p < 0.001$ ) for vegetable protein (figure 3). The  $p$  value for the interaction term was greater than 0.2 at all other sites. Figures 2 and 3 also illustrate the overall positive association between animal protein consumption and BMD and the overall negative association between vegetable protein consumption and BMD in women, as well as the consistently higher BMD values associated with greater calcium intakes.

Since evidence for effect modification by calcium was not consistently strong, models without the interaction term were also evaluated. As table 3 shows, after adjustment for age, body mass index, dietary calcium, years menopausal, diabetes status, current exercise, and current use of estrogen, steroids, cigarettes, alcohol, thiazides, and thyroid hormones,

**TABLE 2. Results from linear regression analysis\* of bone mineral density (g/cm<sup>2</sup>) measured in 1992–1996 versus dietary protein variables measured in 1988–1992 for participants in the Rancho Bernardo Study**

Variable	Total hip (n = 562 women, n = 382 men)†			Femoral neck (n = 562 women, n = 382 men)			Total spine (n = 568 women, n = 387 men)			Total body (n = 536 women, n = 369 men)		
	Estimated β coefficient	95% CI‡	p value	Estimated β coefficient	95% CI	p value	Estimated β coefficient	95% CI	p value	Estimated β coefficient	95% CI	p value
<b>Women</b>												
Protein§ (15 g)	0.0143	0.0026, 0.0261	0.02	0.0092	-0.0007, 0.0192	0.07	0.0150	-0.0020, 0.0320	0.08	0.0158	0.0058, 0.0257	0.002
Animal protein (15 g)	0.0182	0.0070, 0.0293	0.001	0.0125	0.0032, 0.0219	0.01	0.0215	0.0054, 0.0375	0.01	0.0161	0.0067, 0.0254	0.001
Vegetable protein (5 g)	-0.0097	-0.0184, -0.0009	0.03	-0.0078	-0.0151, -0.0005	0.04	-0.0147	-0.0273, -0.0021	0.02	-0.0039	-0.0114, 0.0037	0.31
<b>Men</b>												
Protein (15 g)	0.0057	-0.0102, 0.0217	0.48	0.0032	-0.0110, 0.0174	0.66	0.0057	-0.0172, 0.0286	0.62	-0.0005	-0.0127, 0.0117	0.94
Animal protein (15 g)	0.0102	-0.0054, 0.0259	0.20	0.0059	-0.0081, 0.0199	0.41	0.0120	-0.0104, 0.0345	0.29	0.0016	-0.0104, 0.0136	0.80
Vegetable protein (5 g)	-0.0108	-0.0244, 0.0029	0.12	-0.0065	-0.0187, 0.0057	0.30	-0.0152	-0.0349, 0.0046	0.13	-0.0048	-0.0154, 0.0058	0.38

\* Data were adjusted for age and body mass index.

† Minor site variations in participant numbers relate to problems of positioning, hip replacement, and participant fatigue.

‡ CI, confidence interval.

§ All protein terms were energy-adjusted with units that approximated one standard deviation.

the association between total protein consumption and BMD in women was positive but not significant at each site. The adjusted association between animal protein consumption and BMD in women was also positive at all sites, significantly so at three of the four sites. For every 15-g/day increase in animal protein intake, BMD was 0.016 g/cm<sup>2</sup> greater at the hip ( $p = 0.005$ ), 0.012 g/cm<sup>2</sup> greater at the femoral neck ( $p = 0.02$ ), 0.015 g/cm<sup>2</sup> greater at the spine ( $p = 0.08$ ), and 0.010 g/cm<sup>2</sup> greater for the total body ( $p = 0.04$ ). The only site at which statistical significance was not attained, the spine, was also a site with significant evidence for effect modification by calcium. At the spine, as animal protein consumption increased, BMD increased rapidly in women with low dietary calcium intakes but remained unchanged for those with high calcium intakes.

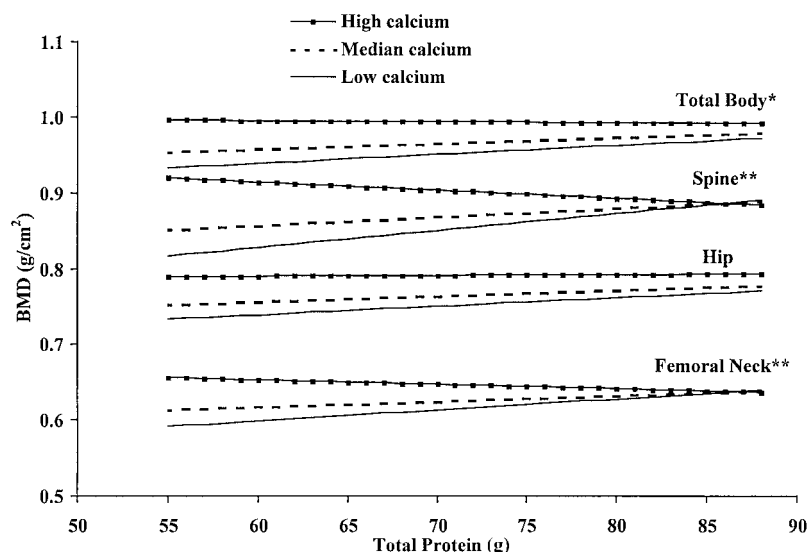
In contrast, the adjusted association between vegetable protein consumption and BMD for women was negative at each site and significantly so at three of the four. As was the case for animal protein, the only BMD measurement site at which statistical significance was not attained for vegetable protein, the total body, was also the one site with a significant vegetable protein  $\times$  calcium interaction. For the total body, as vegetable protein consumption increased, BMD decreased for women with high calcium intakes, remained relatively unchanged for women with the median calcium intake, and increased for women with low calcium intakes.

No significant association with BMD was detected at any site for total or animal protein consumption in the adjusted analyses for men (table 3). There was, however, evidence of a nonlinear relation between vegetable protein consumption and BMD in men; the quadratic vegetable protein term was statistically significant at the hip, spine, and total body. As figure 4 illustrates, the negative association between BMD and vegetable protein consumption appeared to be stronger in men with lower vegetable protein intakes.

Repeat BMD measurements were obtained in 567 women and 387 men. The mean time between measurements was 4.1 years (standard deviation (SD) 0.5) for both women and men. The mean annual percentage change in BMD for women was -0.54 percent (SD 1.6) at the hip, -0.21 percent (SD 2.0) at the femoral neck, and 0.02 percent (SD 1.3) at the spine. For men, the annual percentage change in BMD was -0.45 percent (SD 1.2) at the hip, -0.36 percent (SD 1.4) at the femoral neck, and 0.23 percent (SD 1.2) at the spine. No association ( $p > 0.1$ ) between total, animal, or vegetable protein intake and rate of bone loss was found for either women or men, nor was there any indication of effect modification by calcium.

## DISCUSSION

In this prospective study of elderly women and men, consumption of animal protein was positively associated with BMD in women but not in men. Conversely, in both women and men, protein from nonanimal sources was negatively correlated with BMD. Some evidence of effect modification by calcium was seen in women, with the association between protein consumption and BMD being more positive as calcium intake decreased. This interaction, which was not

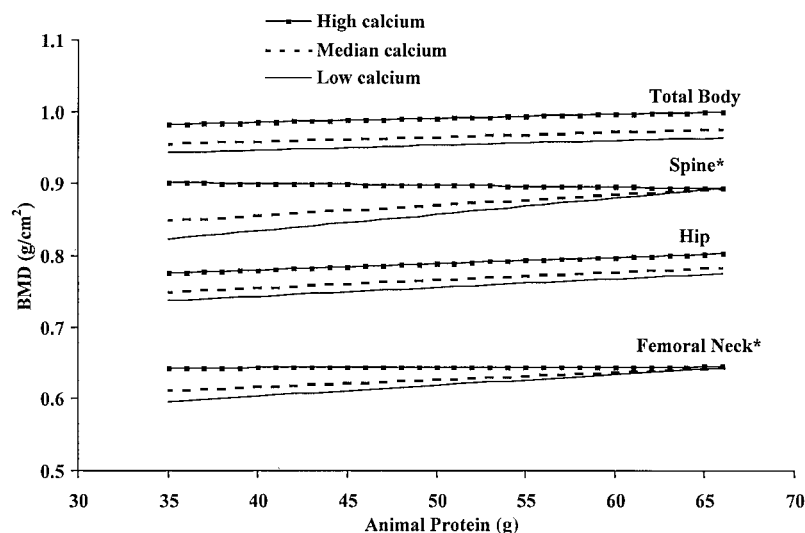


**FIGURE 1.** Bone mineral density (BMD) ( $\text{g}/\text{cm}^2$ ) in 1992–1996 versus total protein intake ( $\text{g}/\text{day}$ ) in 1988–1992 for women in the Rancho Bernardo Study with three different calcium intakes: low (350  $\text{mg}/\text{day}$ ), median (835  $\text{mg}/\text{day}$ ), and high (1,800  $\text{mg}/\text{day}$ ). The plots were generated for the typical female profile, as described in table 1, using the fitted multiple linear regression model with the protein calcium interaction term. The energy-adjusted protein consumption values represent the range between the 10th and 90th percentiles for the female participants. Similarly, the low and high calcium levels represent the 10th and 90th percentiles of calcium intake for the female participants. (\* $p$  for interaction = 0.08; \*\* $p$  for interaction = 0.01).

seen in men, was most pronounced for total protein and suggests that any benefits of increased protein consumption would be greater for women with low calcium intakes.

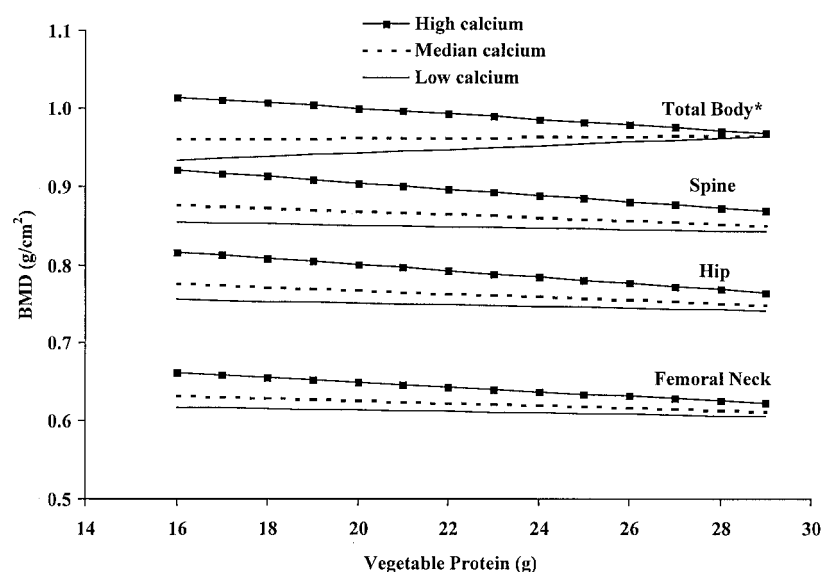
No evidence of an association between protein consumption and rate of bone loss was observed in this study.

Assessing bone loss has the advantage that it may better represent the effect of current exposures, but it also has serious limitations. Change in BMD cannot be measured as precisely as BMD itself, and it may also be more susceptible to information bias due to changes in covariates between the



**FIGURE 2.** Bone mineral density (BMD) ( $\text{g}/\text{cm}^2$ ) in 1992–1996 versus animal protein intake ( $\text{g}/\text{day}$ ) in 1988–1992 for women in the Rancho Bernardo Study with three different calcium intakes: low (350  $\text{mg}/\text{day}$ ), median (835  $\text{mg}/\text{day}$ ), and high (1,800  $\text{mg}/\text{day}$ ). The plots were generated for the typical female profile, as described in table 1, using the fitted multiple linear regression model with the animal protein  $\times$  calcium interaction term. The energy-adjusted animal protein consumption values represent the range between the 10th and 90th percentiles for the female participants. Similarly, the low and high calcium levels represent the 10th and 90th percentiles of calcium intake for the female participants. (\* $p$  for interaction = 0.05).





**FIGURE 3.** Bone mineral density (BMD) (g/cm<sup>2</sup>) in 1992–1996 versus vegetable protein intake (g/day) in 1988–1992 for women in the Rancho Bernardo Study with three different calcium intakes: low (350 mg/day), median (835 mg/day), and high (1,800 mg/day). The plots were generated for the typical female profile, as described in table 1, using the fitted multiple linear regression model with the animal protein  $\times$  calcium interaction term. The energy-adjusted vegetable protein consumption values represent the range between the 10th and 90th percentiles for the female participants. Similarly, the low and high calcium levels represent the 10th and 90th percentiles of calcium intake for the female participants. (\* $p$  for interaction = 0.0005).

baseline and final measurements. In the elderly, changes in bone density are also obscured by osteoarthritic processes. The increase in spine BMD observed in this cohort, for example, is typical for this age group but stems from osteophyte formations rather than a meaningful gain in bone mineral (21). Finally, it has been observed in postmenopausal women that women with higher initial bone mass have slightly faster rates of bone loss, which further suggests that bone mass, rather than rate of bone loss, may be the more relevant indicator of bone health in the elderly (22).

Although indirect evidence has provided some support for the endogenous acid hypothesis (2–5, 8, 9), which argues for a deleterious effect of protein-rich diets, epidemiologic studies have pointed more strongly to a beneficial role for dietary protein in bone health (23). Protein intake has been positively associated with BMD cross-sectionally in both premenopausal women (24, 25) and postmenopausal women (24, 26) and has been positively associated prospectively in elderly men and postmenopausal women (15). Clinical trials in hip fracture patients have consistently observed that patients who receive protein supplements experience significantly improved recoveries (10–12) and reduced bone loss (27, 28).

Studies involving a dietary protein-fracture association have yielded inconsistent results. While one population-based cohort study in postmenopausal women observed an inverse association of total or animal protein intake with hip fracture risk (14), another reported that women in the Nurses' Health Study who consumed the most total and animal protein suffered an increased risk of forearm fracture (though not hip fracture) relative to those who consumed the

least (13). Finally, in a population-based cohort of elderly women, those in the highest quintile of ratio of animal protein intake to vegetable protein intake had the highest baseline BMD but a significantly increased risk of hip fracture and bone loss relative to those in the lowest quintile (16). The conflicting reports regarding animal protein and bone health could derive in part from differences in participant ages, protein intake distributions, protein measures evaluated, and anatomic sites assessed. Contradictory findings for fracture may also arise because the association between diet and fracture is diluted by other variables, such as risk factors for falls, that do not impact on the relation between diet and BMD.

Dietary protein has historically been investigated largely in regard to its effect on calcium balance. However, protein itself is an important structural component of bone, accounting for approximately half of bone volume and one fourth of bone mass, including the skeletal matrix (29). Therefore, by influencing the functionality of bone-related proteins, dietary protein may have considerable ramifications for bone health beyond its effect on calcium. Dietary protein could also affect skeletal integrity through its influence on the production of insulin-like growth factor I, which exerts several positive effects on the skeleton (30). This mechanism is supported by the observation of a positive association between insulin-like growth factor I levels and BMD among female participants in this study (31).

Substantial evidence also exists for an association between very low protein intake, a marker of malnutrition, and frailty and fracture (27, 32, 33). Undernutrition has been reported to be relatively common in the elderly (34), but the

**TABLE 3. Results from multiple linear regression analysis\* of bone mineral density (g/cm<sup>3</sup>) measured in 1992–1996 versus dietary protein variables measured in 1988–1992 for participants in the Rancho Bernardo Study**

Variable	Total hip (n = 537 women, n = 367 men) <sup>†</sup>			Femoral neck (n = 537 women, n = 367 men)			Total spine (n = 541 women, n = 372 men)			Total body (n = 512 women, n = 356 men)		
	Estimated β coefficient	95% CI <sup>‡</sup>	p value	Estimated β coefficient	95% CI	p value	Estimated β coefficient	95% CI	p value	Estimated β coefficient	95% CI	p value
<b>Women</b>												
Total protein§ (15 g)	0.0094	-0.0025, 0.0214	0.12	0.0063	-0.0039, 0.0165	0.22	0.0084	-0.0090, 0.0258	0.34	0.0081	-0.0017, 0.0179	0.11
Animal protein (15 g)	0.0162	0.0049, 0.0275	0.005	0.0115	0.0019, 0.0211	0.02	0.0149	-0.0016, 0.0314	0.08	0.0098	0.0005, 0.0191	0.04
Vegetable protein (5 g)	-0.0133	-0.0219, -0.0047	0.002	-0.0102	-0.0175, -0.0028	0.01	-0.0129	-0.0255, -0.0003	0.04	-0.0047	-0.0121, 0.0026	0.20
<b>Men</b>												
Total protein (15 g)	-0.0003	-0.0180, 0.0174	0.97	-0.0045	-0.0202, 0.0112	0.57	-0.0095	-0.0345, 0.0155	0.45	-0.0078	-0.0212, 0.0057	0.26
Animal protein (15 g)	0.0059	-0.0112, 0.0230	0.50	0.0007	-0.0145, 0.0159	0.93	-0.0007	-0.0249, 0.0235	0.96	-0.0036	-0.0167, 0.0095	0.59
Vegetable protein (5 g) <sup>¶</sup>	0.0100	0.0032, 0.0169	0.004	0.0034	-0.0028, 0.0031	0.28	0.0200	0.0103, 0.0298	<0.0001	0.0054	0.0000, 0.0107	0.05
Vegetable protein (5 g)	-0.0206	-0.0357, -0.0054	0.01	-0.0131	-0.0267, 0.0006	0.06	-0.0327	-0.0542, -0.0112	0.003	0.0124	-0.0243, -0.0004	0.04

\* Covariates included in the model were age, body mass index, calcium intake, years menopausal (women only), diabetes status, current exercise, and current use of estrogen (women only), steroids, cigarettes, alcohol, thiazides, and thyroid hormones.

<sup>†</sup> Minor site variations in participant numbers relate to problems of positioning, hip replacement, and participant fatigue.

<sup>‡</sup> CI, confidence interval.

<sup>§</sup> All protein terms were energy-adjusted with units that approximated one standard deviation.

<sup>¶</sup> The regression of bone mineral density on vegetable protein for men included both linear and quadratic centered vegetable protein terms.

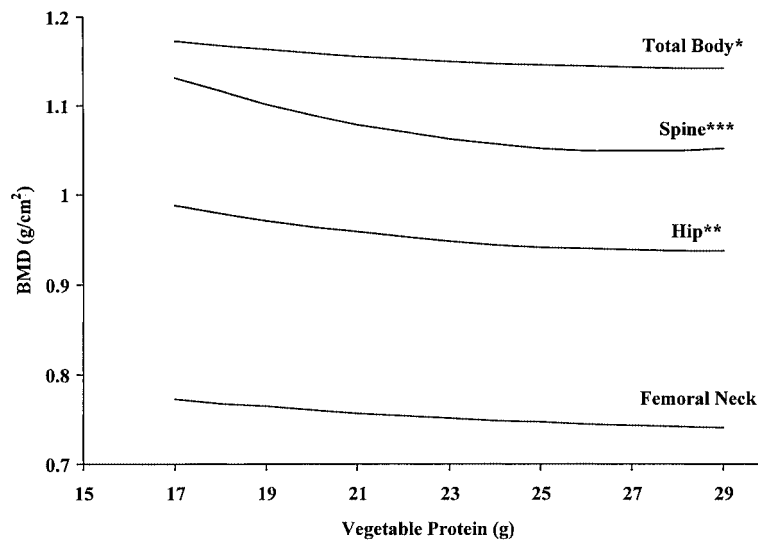
mean protein intake in this cohort of community-dwelling elderly persons was similar to recommended values, as well as national values (35). Although fully 20 percent of the female participants in this study had protein intakes below the Recommended Dietary Allowance for protein, 95 percent reported consuming at least two thirds of the Recommended Dietary Allowance, a common estimate of dietary adequacy (15).

A significant negative association between vegetable protein intake and BMD was observed. While not expected, this result is also not inconsistent with previous work; Munger et al. (14) reported an increase in age-adjusted hip fracture risk with increasing quartile of vegetable protein consumption. In Rancho Bernardo participants, the negative association between vegetable protein and BMD existed despite a positive correlation between vegetable protein and both animal ( $r = 0.42$ ) and total ( $r = 0.70$ ) protein consumption.

Differences in the effects of animal and vegetable sources of protein on bone are not simple to predict from the endogenous acid theory; the greater alkaline ash content of vegetable foods should theoretically provide a protective buffering effect, but sulfur-containing amino acid content, which increases the acid load, varies markedly among vegetable foods relative to animal foods (36). Since the associations observed in this and other populations do not generally support a dominant role for the endogenous acid mechanism in bone health, other explanations for the differing effects of animal and vegetable protein should also be considered.

To our knowledge, this study was the first with the power to effectively evaluate an interaction between dietary protein and calcium. It has been proposed that increased calcium intake may attenuate the hypothesized negative effect of protein consumption on bone (37, 38). The evidence found in this study for effect modification by calcium in women does not support this view. In direct contradiction to the implications of the endogenous acid hypothesis, the positive association between total and animal protein consumption and BMD was greatest for women with the lowest calcium intakes. This pattern would be consistent with a concentration-dependent influence of one nutrient on the absorption of the other. Effect modification by calcium could partially explain the inconsistent findings of previous studies of dietary protein and osteoporosis.

It is noteworthy that only the negative association observed between vegetable protein and BMD in women was reproduced in men. Although only two thirds as many male participants as female participants were studied, the magnitudes of the estimated coefficients indicate that the absence of statistically significant associations for men cannot be attributed to this reduced power. The protein consumption distributions were similar for women and men and thus also cannot explain the discrepant findings. Perhaps selection bias could have played a role in obscuring the association between dietary protein and BMD in men. The male participants who did not return for a BMD measurement had significantly lower total and animal protein intakes than those who did return, and it has been observed in osteoporosis studies that the participants who do not



**FIGURE 4.** Bone mineral density (BMD) ( $\text{g}/\text{cm}^2$ ) in 1992–1996 versus vegetable protein intake ( $\text{g}/\text{day}$ ) in 1988–1992 for men in the Rancho Bernardo Study. The plots were generated for the typical male profile, as described in table 1, using the fitted multiple linear regression model with the quadratic vegetable protein term. The energy-adjusted vegetable protein consumption values represent the range between the 10th and 90th percentiles for the male participants. (\* $p$  for quadratic term = 0.05; \*\* $p$  for quadratic term = 0.004; \*\*\* $p$  for quadratic term < 0.0001).

return for follow-up typically have lower BMD values (39). The observed gender differences could also reflect a true difference in the association of dietary protein with BMD for women and men, stemming from their different osteoporosis etiologies. In support of this theory, the gender differences observed in this study were consistent with the finding of a positive association between insulin-like growth factor I and BMD in female members of this cohort but not in male members (31).

This study had several strengths. It was, to our knowledge, the first to specifically investigate the associations of each protein component with an absolute measure of bone mass in the elderly. The elderly participants in this population-based study were representative of the population most at risk for osteoporotic fractures. This was also one of the first studies of this topic to include a male cohort. Finally, to our knowledge, the interaction between dietary calcium and protein with respect to bone health was effectively evaluated for the first time.

This study also had a number of limitations. The food frequency questionnaire used is well suited for ranking individuals by their habitual intakes, but it is semiquantitative and subject to recall bias. Although we adjusted for many factors that influence bone density, participants with higher intakes of protein may have differed from those with lower intakes in ways that are not known. The high loss to follow-up due to death could also have introduced bias. As participants in an osteoporosis study, cohort members may have had a heightened awareness of their bone health, which may have led them to alter modifiable osteoporosis risk factors between the baseline and follow-up visits. Such an effect is unlikely to have influenced protein consumption, since protein is not commonly perceived to be an osteoporosis risk factor, but the potential misclassification with respect to covariates could have biased

the observed associations toward the null value. Finally, study participants were upper-middle-class Caucasians, which limits the generalizability of these results to other populations.

This prospective study supports the possibility of a positive role for dietary animal protein in the skeletal health of elderly women. It also provides some indication of an interaction between dietary protein and calcium in women, with increased protein consumption appearing to be most beneficial for those with low calcium intakes. This study also suggests that dietary protein may play less of a role in the skeletal health of elderly men as compared with women. These findings, along with the intriguing observation of a negative association between vegetable protein consumption and BMD, have significant implications for osteoporosis prevention strategies and warrant further investigation in elderly cohorts.

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