

# Is protein intake associated with bone mineral density in young women?<sup>1–3</sup>

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## ABSTRACT

**Background:** The range of protein intakes for optimizing bone health among premenopausal women is unclear. Protein is a major constituent of bone, but acidic amino acids may promote bone resorption.

**Objective:** The objective was to examine cross-sectional and longitudinal associations between baseline dietary protein and bone mineral density (BMD) among 560 females aged 14–40 y at baseline enrolled in a Pacific Northwest managed-care organization. The role of protein source (animal or vegetable) and participant characteristics were considered.

**Design:** Dietary protein intake was assessed by using a semiquantitative food-frequency questionnaire in participants enrolled in a study investigating associations between hormonal contraceptive use and bone health. Annual changes in hip, spine, and whole-body BMD were measured by using dual-energy X-ray absorptiometry. Cross-sectional and longitudinal associations between baseline protein intake (% of energy) and BMD were examined by using linear regression analysis and generalized estimating equations adjusted for confounders.

**Results:** The mean ( $\pm$ SD) protein intake at baseline was  $15.5 \pm 3.2\%$ . After multivariable adjustment, the mean BMD was similar across each tertile of protein intake. In cross-sectional analyses, low vegetable protein intake was associated with a lower BMD ( $P = 0.03$  for hip,  $P = 0.10$  for spine, and  $P = 0.04$  for whole body). For every percentage increase in the percentage of energy from protein, no significant longitudinal changes in BMD were observed at any anatomic site over the follow-up period.

**Conclusions:** Data from this longitudinal study suggest that a higher protein intake does not have an adverse effect on bone in premenopausal women. Cross-sectional analyses suggest that low vegetable protein intake is associated with lower BMD. *Am J Clin Nutr* 2010;91:1311–6.

## INTRODUCTION

The effects of dietary protein intake on bone health are controversial. Protein is a major constituent of bone (1), so adequate protein intake is critical to maintaining bone health. Whereas a detrimental effect of insufficient protein intake on bone health has been documented (2), there also has been considerable concern over reports that higher-protein diets increase urinary calcium (3–6). Furthermore, acidifying amino acids such as cysteine and methionine, released after protein digestion, can stimulate osteoclastic bone resorption, thereby reducing bone mineral density (BMD) (7). Other data suggest the

increased urinary calcium excretion due to higher protein intakes is compensated for by increased intestinal absorption of calcium (4), or that the adverse effects of high protein intake on bone are observed only among individuals with insufficient intakes of calcium (5).

The appropriate range of protein intakes for optimizing bone health among premenopausal women is unclear, as reflected by the width of the Acceptable Macronutrient Distribution Range for protein (10–35% of energy for adults aged >18 y) (8). To help inform public health recommendations for protein intake, we analyzed data from 2 longitudinal studies of bone health among females aged 14–40 y at baseline. We investigated the association of baseline total protein intake and baseline BMD and changes in BMD over time. We also evaluated whether the observed associations between protein and BMD varied by type of protein intake (animal compared with vegetable), age, BMI, physical activity, recent depot medroxyprogesterone acetate (DMPA) injectable contraceptive use, smoking, or calcium intake.

## SUBJECTS AND METHODS

### Subjects

This analysis used data from 2 cohort studies that enrolled a total of 631 participants to examine the effects of DMPA contraception on BMD, conducted at Group Health Cooperative, a Pacific Northwest nonprofit managed health care organization. The first study (1994–1999) enrolled 457 women aged 18–40 y and followed participants for 3 y. The second cohort (1999–2004) consisted of 174 females aged 14–18 y who were followed for 2 to 3 y. Women with conditions or using other medications known

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to affect bone mass were excluded from participation, and all adolescents had attained menarche before enrollment. For these analyses, additional exclusion criteria were invalid data on dual-energy X-ray absorptiometry (DXA) measurements ( $n = 6$ ), misclassification of primary exposure (DMPA use;  $n = 21$ ), or food-frequency questionnaires (FFQ) with implausible energy intakes ( $<500$  or  $>5000$  calories;  $n = 44$ ). The Group Health Human Subjects Committee reviewed and approved all study procedures. All participants provided written informed consent or assent with parental approval for adolescents.

### Data collection

At baseline, participants completed questionnaires querying for demographic information (age, race-ethnicity, and education), behaviors (smoking and physical activity), and medical history (age at menarche, family history of fracture, pregnancies, and hormonal contraceptive use). Height and weight were measured in the clinic, and BMI was calculated [weight (kg)/height<sup>2</sup> (m)]. Weight-bearing physical activity was assessed by adapting 2 validated measures (9, 10) for use in young women. To derive the weight-bearing physical activity score, the frequency (6-point scale) of 17 specific activities, up to 2 write-in sports activities, and up to 2 other regular activities was collected. Each activity was assigned a weight-bearing value of 0 (none), 1 (low/medium), or 2 (medium/high). The physical activity score was obtained by multiplying the weight-bearing value, and the frequency of each activity and summing up over all activities. Recent DMPA exposure was defined as current use, as reported by questionnaire and in-person interview by the clinic staff.

### Outcome assessment

BMD and bone mineral content (BMC) of the total hip, the lumbar spine, and the whole body were measured semiannually by DXA. The Hologic 2000 and Hologic 4500 (Hologic Inc, Bedford, MA) were used for the cohort study conducted in 1994–1999, and the Hologic 4500 and Hologic Delphi (MNAP, Philadelphia, PA) were used for the study conducted in 1999–2004. A cross-calibration was performed in each study, in which measurements were taken on both machines for an independent measure and an adjustment was calculated to calibrate the BMD measures, as previously described (11). CVs ranged from 0.72% to 1.5%. Standard techniques were used by densitometrists who were trained by the manufacturer.

### Exposure assessment

Protein intake was assessed at baseline and at annual follow-up visits by using an FFQ developed and evaluated in the Women's Health Initiative at the Fred Hutchinson Cancer Research Center (Seattle, WA) (12). Consistent with current dietary guidelines, protein was evaluated as a percentage of total energy. All nutrient measures were evaluated by using the FFQ.

### Statistical analysis

Participant characteristics of women included and those excluded because of extreme caloric intakes on the FFQ ( $n = 44$ ) were compared. Baseline characteristics and other dietary factors that can affect bone health were compared by tertile of baseline protein intake by analysis of variance for continuous

variables and chi-square tests for categorical variables. Adjusted mean BMD at baseline was calculated by tertile of baseline protein intake by using linear regression models. Potential confounders of the association between protein intake and bone health were evaluated by testing bivariate associations based on the previous literature and the original study designs (race-ethnicity, age of menarche, time since menarche, family history of fracture, age, BMI, physical activity score, calories, dietary calcium, phosphorous, dietary vitamin D, magnesium, fluoride, alcohol, smoking, DMPA use, prior pregnancy, and education). The association of baseline protein intake with baseline BMD was examined by using models adjusted for variables associated with protein intake and BMD (age, BMI, physical activity, smoking, and recent DMPA use) as well as nutritional factors significantly associated with both the exposure and outcome in bivariate analyses (energy intake, phosphorous, and magnesium). Additional models estimated associations by type of protein intake by including separate terms for animal and vegetable protein in regression models.

To estimate longitudinal associations, we used linear regression to model absolute and percentage changes in BMD from baseline to each follow-up visit (12, 24, and 36 mo) as a function of baseline protein intake. Percentage energy from protein was modeled both continuously and categorically (tertiles) to allow for the possibility of nonlinear associations between protein intake and BMD. Likelihood ratio tests estimated  $P$  values for the main effect of categorized protein on BMD at each site (hip, spine, and whole body).

We also evaluated models that included baseline and annual data with protein exposure updated annually, accounting for the dependence of repeated observations within a study participant by using generalized estimating equations with an independent correlation structure. Cross-sectional analyses were repeated characterizing protein in absolute amounts (g/d) and expressed as a function of body weight (g/kg body wt). Cross-sectional and longitudinal analyses were repeated by using BMC as the outcome measure.

Statistical tests for interaction with baseline age category, BMI, physical activity, recent DMPA use, smoking, and calcium intake were conducted by using the likelihood ratio test statistic for cross-sectional analyses and the Wald test statistic for analyses of generalized estimating equations. Data were analyzed by using SAS (version 9.2; SAS Institute, Cary, NC), and statistical significance was defined as  $P < 0.05$  by using 2-sided tests.

## RESULTS

### Baseline characteristics

Participants excluded because of invalid FFQs were more likely to be overweight ( $P = 0.005$ ), nonwhite ( $P < 0.0001$ ), and a recent DMPA user ( $P = 0.02$ ). Overall, mean ( $\pm$ SD) protein intake as a proportion of energy was  $15.5\% \pm 3.2\%$ . Women consuming a higher proportion of energy from protein had a higher BMI and were less likely to smoke than were women consuming less protein (Table 1). Although the amount of vegetable protein was consistent across tertiles, the amount of animal protein intake was higher among women with a high total protein intake (Table 1). Higher protein intake was associated

**TABLE 1**  
Baseline characteristics by tertile of protein intake<sup>1</sup>

Characteristic	Tertile of protein intake (% of energy)			<i>P</i> <sup>2</sup>
	Low (5.7–14.3)	Medium (14.4–17.1)	High (17.2–27.6)	
No. of subjects	186	187	187	
Age (y)	24.2 ± 6.6 <sup>3</sup>	24.3 ± 6.9	25.4 ± 7.4	0.2
Ethnicity [ <i>n</i> (%)]				
White	143 (77)	154 (82)	142 (76)	
Black	21 (11)	18 (10)	28 (15)	
Other	22 (12)	15 (8)	17 (9)	0.35
BMI (kg/m <sup>2</sup> )	24.9 ± 5.2	24.5 ± 5.9	25.9 ± 5.3	0.04
Current smoker [ <i>n</i> (%)]	53 (28)	46 (25)	31 (17)	0.02
Physical activity <sup>4</sup>	78.2 ± 50.0	74.5 ± 47.9	81.6 ± 53.6	0.40
Time since menarche (y)	11.6 ± 0.48	12.1 ± 0.50	12.7 ± 0.6	0.33
Family history of fracture [ <i>n</i> (%)]	37 (26)	46 (31)	49 (35)	0.47
Ever pregnant [ <i>n</i> (%)]	85 (46)	82 (44)	87 (47)	0.87
DMPA user [ <i>n</i> (%)]	81 (44)	69 (37)	83 (44)	0.27
Baseline diet				
Energy intake (kcal)	1725 ± 733 (520–4183) <sup>5</sup>	1654 ± 633 (541–3975)	1646 ± 681 (547–4817)	0.47
Animal protein (g)	33.2 ± 18.1 (8.0–99.3)	43.3 ± 18.8 (11.4–117.4)	58.7 ± 25.8 (17.6–152.8)	<0.0001
Vegetable protein (g)	18.6 ± 10.2 (3.4–65.1)	19.9 ± 9.2 (4.3–62)	18.6 ± 8.2 (5.8–49.2)	0.28
Carbohydrate (% of energy)	55 ± 9 (36–81)	52 ± 9 (31–79)	49 ± 9 (29–70)	<0.0001
Fat (% of energy)	33 ± 8 (8–52)	33 ± 8 (10–55)	32 ± 8 (11–51)	0.58
Alcohol (g)	4.5 ± 10.4 (0–87)	2.7 ± 5.6 (0–39)	2.9 ± 6.8 (0–55)	0.06
Calcium (mg)	673 ± 376 (132–2827)	832 ± 416 (124–2362)	1035 ± 636 (156–3666)	<0.0001
Phosphorous (mg)	953 ± 446 (215–3139)	1136 ± 467 (321–2791)	1348 ± 602 (362–3483)	<0.0001
Vitamin D (μg)	3.50 ± 2.41 (0.34–15.91)	4.56 ± 2.91 (0.61–16.40)	6.45 ± 4.43 (1.27–27.00)	<0.0001
Vitamin K (mg)	66.68 ± 39.39 (15.55–223.58)	66.20 ± 41.49 (15.75–232.16)	60.71 ± 31.10 (22.82–169.96)	0.68
Magnesium (mg)	216 ± 101 (49.0–651.4)	239 ± 97 (68.7–619.9)	262 ± 102 (66.7–582.4)	<0.0001
Caffeine (mg)	118 ± 131 (0–766)	87 ± 107 (0–686)	85 ± 108 (0–686)	0.01
Fruit (servings/d)	1.8 ± 1.6 (0.1–8.0)	1.7 ± 1.4 (0.1–8.0)	1.7 ± 1.4 (0.1–7.0)	0.89
Vegetable (servings/d)	1.4 ± 1.1 (0.1–5.7)	1.5 ± 1.0 (0.1–5.1)	1.8 ± 1.5 (0.1–11.5)	0.001
Bone mineral density (g/cm <sup>2</sup> )				
Hip	0.93 ± 0.12	0.93 ± 0.12	0.96 ± 0.13	0.06
Spine	1.00 ± 0.13	1.01 ± 0.12	1.03 ± 0.12	0.25
Whole body	1.08 ± 0.09	1.08 ± 0.09	1.10 ± 0.10	0.10

<sup>1</sup> DMPA, depot medroxyprogesterone acetate.<sup>2</sup> *P* values from ANOVA for continuous variables or chi-square tests for categorical variables.<sup>3</sup> Mean ± SD (all such values).<sup>4</sup> Weight-bearing physical activity was assessed by querying the frequency (6-point scale) of specific activities. Each activity was assigned a weight-bearing value of 0 (none), 1 (low/medium), or 2 (medium/high).<sup>5</sup> Range in parentheses (all such values).

with lower carbohydrate ( $P < 0.0001$ ) and caffeine ( $P = 0.01$ ) intakes but with higher calcium ( $P < 0.0001$ ), phosphorous ( $P < 0.0001$ ), vitamin D ( $P < 0.0001$ ), magnesium ( $P < 0.0001$ ), and vegetable ( $P = 0.001$ ) intakes (Table 1).

### Associations between protein intake and BMD

In unadjusted analyses, those in the highest tertile of protein had slightly higher baseline BMD at the hip and whole body compared with those in the lowest tertile of protein ( $P \leq 0.10$ ) (Table 1). After adjustment for age, BMI, physical activity, smoking, recent DMPA use, energy intake, phosphorous, and magnesium, mean baseline BMD was similar across each tertile of protein intake (Table 2). Women in the lowest tertile of vegetable protein intake had a lower BMD at each site than did women consuming more vegetable protein ( $P = 0.03$  for hip,  $P = 0.10$  for spine, and  $P = 0.04$  for whole body).

Results modeling protein intake continuously were similar. For every percentage increase in the percentage of energy from

protein, there were no significant longitudinal changes in BMD at any anatomic site at any time point (Table 3). Type of protein intake (animal/vegetable) was not associated with change in BMD at any site or time point (Table 3). No significant overall associations were observed when BMC was used as the outcome measure, modeling protein intake as g/d or as g/kg body wt, absolute change in protein as the exposure, or analyses by using generalized estimating equations (data not shown).

### Evaluation of effect modification

Evaluation of the relation between protein intake and BMD showed no statistically significant interactions for calcium, BMI, physical activity, smoking, recent DMPA exposure, or age category at baseline (all  $P > 0.05$ ). In the longitudinal analyses, there was a suggestion of effect modification by age category (Figure 1;  $P < 0.10$  at each site): among women younger than 30 y, a higher protein intake was associated with a higher BMD at each of the 3 anatomic sites, whereas for women aged  $\geq 30$  y,

**TABLE 2**Adjusted mean (95% CI) bone mineral density (BMD; in g/cm<sup>2</sup>), by protein intake at baseline (*n* = 560)<sup>1</sup>

BMD site	Tertile of protein intake (% of energy)			<i>P</i> value <sup>2</sup>
	Low (5.7–14.3)	Medium (14.4–17.1)	High (17.2–27.6)	
Total protein (% of energy)				
Hip	0.93 (0.91, 0.95)	0.93 (0.92, 0.95)	0.93 (0.91, 0.96)	0.94
Spine	1.00 (0.98, 1.02)	1.02 (1.00, 1.03)	1.02 (1.00, 1.04)	0.37
Whole body	1.08 (1.07, 1.10)	1.08 (1.07, 1.09)	1.08 (1.06, 1.10)	0.98
Animal protein (% of energy)				
Hip	0.93 (0.91, 0.95)	0.94 (0.92, 0.95)	0.94 (0.92, 0.96)	0.99
Spine	1.00 (0.98, 1.02)	1.02 (1.00, 1.04)	1.02 (1.00, 1.04)	0.40
Whole body	1.08 (1.07, 1.10)	1.08 (1.07, 1.09)	1.08 (1.07, 1.10)	0.80
Vegetable protein (% of energy)				
Hip	0.92 (0.90, 0.94)	0.95 (0.94, 0.97)	0.94 (0.92, 0.96)	0.03
Spine	1.00 (0.98, 1.01)	1.02 (1.01, 1.04)	1.01 (0.99, 1.04)	0.10
Whole body	1.07 (1.06, 1.09)	1.09 (1.08, 1.11)	1.08 (1.06, 1.09)	0.04

<sup>1</sup> Values were adjusted for age, BMI, physical activity, smoking, recent depot medroxyprogesterone acetate exposure, energy intake, phosphorous, and magnesium. Models estimating associations by type of protein intake (animal/vegetable) contain terms for each type of protein in multivariable linear regression models.

<sup>2</sup> *P* values derived from a likelihood ratio test of significance of the categorical exposure.

the association between protein intake and BMD was inverse. However, the only statistically significant stratum-specific estimate was among 14–19-y-olds at the spine.

## DISCUSSION

Data from this large population-based study of premenopausal women suggest that women consuming more protein do not have a lower BMD than do women consuming less protein, irrespective of the site measured. In cross-sectional analyses, women consuming less vegetable protein had a lower BMD. The annual change in BMD was also similar across tertiles of protein intake, and there was no evidence that type of protein intake influenced associations. Although there were no statistically significant interactions across all 3 sites (hip, spine, and whole body), there was evidence of effect modification by age category, with higher

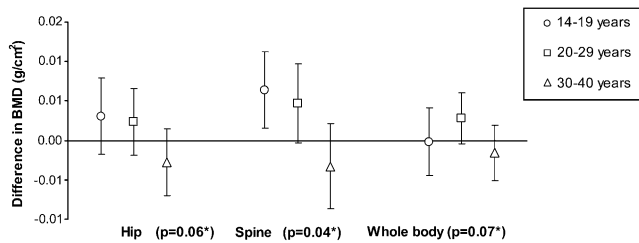
protein intake being more beneficial for women aged <30 y than for women who have already achieved peak bone mass.

Studies to date of the association between protein intake and BMD report inconsistent results, with some finding beneficial associations (2, 13–16), others reporting no association (17, 18), and others finding adverse associations (19). A recent systematic review including 61 studies reported a small beneficial association between total protein intake and BMD and BMC, estimating the proportion of BMD attributable to protein was 1–2% (20). A longitudinal study reported that, with adequate calcium intake (>1000 mg/d), protein intake predicted BMC, BMD, and net gain in BMC in females followed from adolescence to young adulthood (21). A weight-loss feeding study in middle-aged adults found that a high-protein diet (1.4 g · kg<sup>-1</sup> · d<sup>-1</sup>) with 3 dairy servings attenuated bone loss relative to a diet consistent with the current Recommended Dietary Allowance for protein

**TABLE 3**Adjusted mean absolute change (in g/cm<sup>2</sup>) from baseline in bone mineral density (BMD) by baseline protein intake<sup>1</sup>

BMD site	Year 1 ( <i>n</i> = 423)		Year 2 ( <i>n</i> = 378)		Year 3 ( <i>n</i> = 224)	
	Per 1% of energy	<i>P</i> value	Per 1% of energy	<i>P</i> value	Per 1% of energy	<i>P</i> value
Total protein intake (% of energy)						
Hip	-0.0002	0.64	0	0.99	-0.0002	0.88
Spine	-0.0004	0.51	-0.0008	0.28	0.0004	0.71
Whole body	0.0005	0.29	-0.0004	0.43	-0.0012	0.19
Animal protein (% of energy)						
Hip	-0.0002	0.67	0.0002	0.83	-0.0002	0.87
Spine	-0.0004	0.54	-0.0008	0.31	0.0005	0.69
Whole body	0.0004	0.36	-0.0005	0.4	-0.0011	0.21
Vegetable protein (% of energy)						
Hip	-0.0017	0.15	-0.003	0.11	-0.0023	0.4
Spine	-0.0015	0.29	-0.0016	0.37	-0.0019	0.5
Whole body	0.0008	0.43	-0.0011	0.4	0.0009	0.69

<sup>1</sup> Values were adjusted for age, BMI, physical activity, smoking, recent depot medroxyprogesterone acetate exposure, energy intake, phosphorous, and magnesium. Models estimating associations by type of protein intake (animal/vegetable) contain terms for each type of protein in multivariable linear regression models.



**FIGURE 1.** Adjusted mean difference in bone mineral density (BMD) per 1% total energy from protein, by anatomic site and age group. \**P* for interaction estimated from generalized estimating equation models adjusted for age, BMI, physical activity, smoking, recent depot medroxyprogesterone acetate injectable contraceptive exposure, energy intake, phosphorus, and magnesium.

( $0.8 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ ) during both weight loss (4 mo) and maintenance of weight loss (8 mo) (22).

Studies with fracture as the outcome among women aged  $>50$  y also report inconsistent results. Some studies of higher protein intake among women older than our study population found an increased risk of fracture (23, 24), whereas others show a decreased risk (25, 26).

Protein source (ie, animal or vegetable) may influence protein's effect on bone health. Because protein from animal sources is rich in acidifying amino acids, such as cysteine and methionine, and animal sources have fewer base precursors than vegetable sources, researchers have suggested that diets rich in protein from animal sources increase the risk of osteoporosis and sarcopenia (7). Studies to date investigating the role of protein source on bone health have been conducted primarily among postmenopausal women and report disparate findings. Among a cohort of adults aged  $\geq 55$  y, a higher animal protein intake was associated with a higher BMD, whereas vegetable protein intake was inversely correlated with BMD (27). A recent study found no overall association between protein intake and fracture risk, but did see a trend toward increased fracture risk with an increased intake of animal protein (28). A 2008 study in older women found increased odds of osteoporosis for total protein, but a decrease in odds with increased vegetable protein intake (29). An investigation of postmenopausal women in a large cohort study [the European Prospective Investigation into Cancer and Nutrition, Potsdam (EPIC)] found an adverse association between increased animal protein and bone structure assessed by ultrasound, but a beneficial association with a higher vegetable protein intake (30). Some recent feeding studies, one in postmenopausal females and 2 in young women, found no detrimental effect of animal-based protein on biochemical indicators of bone health (31–33).

In the current study, participants consumed similar amounts of vegetable protein in each tertile of protein intake; thus, much of the difference between protein intake groups was due to higher consumption of animal protein. Therefore, these data do not support a detrimental effect of animal protein on bone health in the consumption range that typified this study population.

Findings in young women could be different from those in older women because bone mass is still accruing in the young women. Peak bone density at the hip occurs at about age 19 y, and bone mass at age 30 y is used to define the “T-score,” which is the most frequently used measurement in reporting bone density results. We found suggestive, but not definitive, evidence of a qualitative interaction by age. Among women younger than 30

y, a higher protein intake was associated with a higher BMD at each of the 3 anatomic sites, whereas for women aged  $\geq 30$  y, the association between protein intake and BMD was inverse. Several mechanisms could explain age-related differences in the association between protein intake and BMD. Kerstetter et al (34) studied protein-induced effects on net bone balance in young women and showed increased gastrointestinal calcium absorption as well as a nonsignificant trend toward decreased bone resorption with a high-protein diet. Proteins likely increase the production of insulin-like growth factor I (35, 36), an important hormone that increases bone formation and that has positive effects on the growth plates. Renal function often decreases in older women, so the kidneys are less able to excrete the fixed acids from dietary protein and more of the fixed acids will be buffered by the bone, which causes bone loss.

Limitations should be considered in interpreting our findings. The FFQ has considerable measurement error and thus may have substantially attenuated diet-disease associations (37). However, calibration studies using urinary nitrogen as a biomarker of protein intake suggest that much of the measurement error lies in misreporting energy, rather than protein, intake, at least among postmenopausal women (38). Another limitation is that protein intake did not vary across the entire recommended range of 10% to 35% of protein intake, because the interquartile range of protein intake at baseline was 13.4–17.6% of energy. So, whereas these inferences apply to typical protein intake in the population, data are not available to evaluate lower and upper bounds of recommended ranges of intake. The study population was predominantly non-Hispanic white, so our findings may not be generalizable to other racial-ethnic groups with differences in bone metabolism. The sample consisted of a higher proportion of DMPA users compared with the general population, but recent DMPA use was controlled for in all models.

Strengths of the current study include the large sample size of population-based premenopausal women, allowing us to examine associations between dietary intake and bone health among a group of women who are still developing bone or have recently achieved peak bone mass. With 560 women, we had  $>80\%$  power to detect a 2% difference in BMD with a 2-sided test at a level of significance of  $P < 0.05$ . Having longitudinal measures of BMD as measured by DXA provided us the opportunity to accurately and precisely detect changes in bone health over time. Data were collected on multiple exposures related to bone health in addition to dietary intake, such as physical activity and smoking, and these factors were accounted for in the analysis.

Data from this large population-based cohort provide evidence that protein intake in the upper range of typical consumption in the United States does not negatively affect bone mass in premenopausal women. These data suggest that the relation between protein intake and bone health among women who consume low amounts of vegetable protein or are still developing bone warrants further investigation. Additional studies in populations consuming protein in the upper end of the recommended range (25–35% of energy from protein) may be informative.

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