

# Peripheral skeleton bone strength is positively correlated with total and dairy protein intakes in healthy postmenopausal women<sup>1,2</sup>

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## ABSTRACT

**Background:** Bone mineral content (BMC) and bone mineral density (BMD) are positively correlated with dietary protein intakes, which account for 1–8% of BMC and BMD variances. However, the relation between bone strength and microstructure, which are variables that are not captured by areal bone mineral density (aBMD), and dietary protein intakes, particularly from specific dietary sources, has not been clearly established.

**Objective:** We investigated the association between the peripheral skeleton–predicted failure load and stiffness, bone microstructure, and dietary protein intakes from various origins (animal, divided into dairy and nondairy, and vegetable origins) in healthy postmenopausal women.

**Design:** In a cross-sectional study in 746 Caucasian women aged  $65.0 \pm 1.4$  y, we measured the aBMD with the use of dual-energy X-ray absorptiometry, the distal radius and tibia bone microstructures with the use of high-resolution peripheral quantitative computerized tomography, and bone strength with the use of a finite element analysis, and we evaluated dietary protein and calcium with the use of a validated food-frequency questionnaire.

**Results:** Mean dietary calcium and protein intakes were greater than recommended amounts for this class of age. The predicted failure load and stiffness at the distal radius and tibia were positively associated with total, animal, and dairy protein intakes but not with vegetable protein intake. Failure load differences were accompanied by modifications of the aBMD and of cortical and trabecular bone microstructures. The associations remained statistically significant after adjustment for weight, height, physical activity, menopause duration, calcium intake, and the interaction between calcium and protein intake. A principal component analysis of the volumetric BMD and bone microstructure indicated that trabecular bone mainly contributed to the positive association between protein intakes and bone strength.

**Conclusions:** These results, which were recorded in a very homogeneous population of healthy postmenopausal women, indicate that there is a beneficial effect of animal and dairy protein intakes on bone strength and microstructure. Specifically, there is a positive association between the bone failure load and stiffness of the peripheral skeleton and dietary protein intake, which is mainly related to changes in the trabecular microstructure. This trial was registered at [www.controlled-trials.com](http://www.controlled-trials.com) as ISRCTN11865958. *Am J Clin Nutr* 2017;105:513–25.

**Keywords:** bone fragility, bone microstructure, dairy products, finite element analysis, fracture risk, HR-pQCT, nutrition, osteoporosis, protein intake

## INTRODUCTION

Adequate supplies of dietary protein are required for the maintenance of healthy bone (1). Dietary protein intakes account for 1–8% of the bone mineral density (BMD)<sup>7</sup> variance in adults (2). In older people with osteoporosis, higher protein intakes ( $\geq 0.8$  g · kg body weight<sup>-1</sup> · d<sup>-1</sup> or 24% of total energy intake) are associated with higher BMD (2–10), a slower rate of bone loss (11, 12), and reduced risk of hip (13–16) or forearm (6, 17) fractures. An intervention with dietary protein supplements has been shown to attenuate a postfracture-related or weight loss–associated BMD decrease (18–20) and reduced bone turnover markers (21, 22), together with an increase in insulin-like growth factor I (18, 23–26) and a decrease in parathyroid hormone (21, 27).

However, the role of the protein source, such as animal protein (including dairy and nondairy products) or vegetable protein, is still debated. For instance, some positive associations between bone health and vegetable protein have been reported (5, 28, 29), but inconsistently (8, 30). The association between bone health and dairy-product intakes has been investigated in both cross-sectional and longitudinal observational studies and in intervention trials

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<sup>2</sup> Supplemental Figures 1 and 2 and Supplemental Tables 1–3 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at <http://ajcn.nutrition.org>.

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<sup>7</sup> Abbreviations used: aBMD, areal bone mineral density; BMC, bone mineral content; BMD, bone mineral density; DXA, dual-energy X-ray absorptiometry; FEA, finite element analysis; HR-pQCT, high-resolution peripheral quantitative computerized tomography; PC, principal component; PCA, principal component analysis; RDA, Recommended Dietary Allowance; vBMD, volumetric bone mineral density.

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with some positive effects [see Rizzoli (31) for a review]. The study of the original Framingham cohort indicated that there is some inverse association between hip-fracture risk and milk intakes (32). However, a meta-analysis of 6 studies that included 195,102 women showed no overall association between hip-fracture risk and total milk intake (33). In addition, the positive association between BMD and dietary protein intakes appears to depend on a sufficient dietary calcium supply (11, 15, 34–36).

Bone strength is a major contributor to fracture risk and can be estimated noninvasively *in vivo* through a finite element analysis (FEA) that is based on images obtained with the use of high-resolution peripheral quantitative computerized tomography (HR-pQCT). Bone strength and, hence, resistance to fracture depend on various quantitative traits such as the bone mineral content (BMC) and BMD, bone turnover, bone geometry and microstructure, and bony tissue material-level properties (37). To our knowledge, the association between bone strength and various sources of protein intakes in humans has never been previously reported, nor has the interaction of protein with calcium intakes on bone strength. We hypothesized that proteins—particularly those from dairy, which provide calcium as well—would be positively associated with peripheral skeleton strength in relation to a better bone microstructure.

In this cross-sectional study, we evaluated the association between distal radius- and tibia-estimated failure load and stiffness, together with areal bone mineral density (aBMD), cortical and trabecular volumetric BMDs and microstructures, and dietary protein intakes (total and from animal, dairy, nondairy, and vegetable origins) in 746 healthy Caucasian postmenopausal women. A principal component analysis (PCA) of bone-microstructure variables was applied to assess the relative contribution of the bone microstructure to the association between failure load and protein intakes. We also tested the interactions on predicted failure load between protein intakes and weight, height, physical activity, and calcium intakes because all these variables are associated with protein intakes and bone strength.

## METHODS

### Subjects

The Geneva Retirees Cohort comprised 759 healthy Caucasian women, who were recruited in Geneva at the time of retirement (i.e., at 65 y of age) through an advertisement in the local newspapers, among the Geneva University Hospitals staff, or in large, local companies between 2008 and 2012. Exclusion criteria were mainly an active disease that was capable of influencing bone turnover or muscle performance. Dietary intakes were available in 746 women (**Supplemental Figure 1**). All subjects signed a written informed consent before undergoing a series of interviews and determinations to investigate the genetic, environmental, and nutritional determinants of bone microstructure. The study protocol received approval from the Geneva University Hospitals' Ethics Committee. Participants in the current cross-sectional study were women who were investigated at baseline.

### Anthropometric and lifestyle variables and fracture history

Body weight (with a scale at the nearest 0.1 kg) and standing height [determined with the use of a Holtzmann stadiometer

(Holtain Ltd.)] were measured, and BMI (in kg/m<sup>2</sup>) was calculated. Physical activity was assessed with the use of a face-to-face questionnaire that assessed the usual amount of time spent walking and cycling, using stairs, and participating in organized sports and recreational activities over the preceding year, which was classified on a list of 45 activities (38). The collected data were converted and expressed as the physical activity energy expenditure (kilocalories per day) with the use of established conversion formulae (39). Tobacco (current use compared with never or past use) and alcohol consumption ( $\geq 7$  compared with  $< 7$  U/wk) were assessed as were the age of menopause and current or past drug use with a specific focus on bone-targeted treatments. Fracture history was recorded including the fracture site, age at the time of fracture, and type and intensity of trauma associated with the fracture. Only low-energy trauma fractures (defined as a fracture that resulted from a fall from standing height) in adult age were taken into account. Vitamin D (25-hydroxyvitamin D) was measured batchwise on a Cobas-6000 instrument with Elecsys reagents (Roche Diagnostics).

### Dietary intakes

Dietary calcium and protein intakes were estimated with the use of a validated food-frequency questionnaire that was specifically developed for the evaluation of calcium and protein intakes (40, 41). The questionnaire, which comprised 23 items, was administered face-to-face by a certified dietitian. The frequency was either daily or weekly according to the food item. The period assessed was the preceding year. The size of portions was estimated with the help of pictures that were used in the large multicenter survey [Supplementation en Vitamines et Minéraux Antioxydants (SU.VI.MAX)] (42, 43). The amounts recorded were translated into quantities of protein with the use of Prodi 5.3 Expert Nutrition Software Program (Nutri-Science GmbH) and the table provided by the French Information Center on Food Quality (<https://pro.anses.fr/TableCIQUAL/index.htm>). Total protein intake was quantified in addition to protein intake, according to dietary sources of proteins by specifically calculating animal (divided into dairy products and nondairy products) and vegetable protein intakes.

### Bone-related outcomes

#### Dual-energy X-ray absorptiometry

The lumbar spine, proximal femur, and distal radius aBMDs, whole-body BMC, and lean and fat masses were determined with the use of dual-energy X-ray absorptiometry (DXA) with a Hologic QDR Discovery instrument (Hologic Inc.) that was located in a mobile truck. The CV of repeated measurements varied between 1.0% and 1.6% for BMD (44, 45).

#### HR-pQCT

Volumetric BMD and microstructure variables were determined at the distal radius and distal tibia with the use of HR-pQCT on an XtremCT instrument (Scanco Medical). A stack of 110 tomography slices were acquired over a 9-mm length with an isotropic voxel size of 82  $\mu$ m that started proximally at 9.5 and 22.5 mm from a joint-margin reference line for the distal radius and distal tibia, respectively. The effective dose was 3  $\mu$ Sv, and the measurement time 2.8 min. Short-term reproducibility that was assessed with repositioning was 0.6–1.0% and 2.8–4.9% for density variables

and the trabecular microstructure, respectively (44, 45). Determinations were performed on the nondominant limb unless a fracture was reported in the region of interest. Recorded variables were as follows: total, cortical, and trabecular volumetric bone mineral densities (vBMDs), which were expressed as milligrams of hydroxyapatite per cubic centimeter; the trabecular number (per millimeter) and thickness and spacing (micrometer); the mean cortical thickness (millimeter); and the cross-sectional area (square millimeters). Relative bone volume (percentage) was derived from the trabecular density divided by  $1200 \text{ mg/cm}^3$ , thereby assuming fully mineralized bone tissue. The trabecular number was defined as the inverse of the mean spacing between trabecular ridges. Trabecular thickness and spacing were calculated from the relative bone volume with the use of conventional histomorphometry formulae. The trabecular spacing SD was an estimate of the heterogeneity of the trabecular structure and was calculated as the SD of the individual distribution of the trabecular spacing. Cortical thickness was defined as the mean cortical area divided by the outer bone surface. Cortical porosity was calculated as the number of void voxels in each binary cortex image divided by the total number of voxels (46).

#### FEA

Bone strength was estimated with the use of an FEA. Finite element models of the radius and tibia were created directly from the segmented HR-pQCT images. In summary, a voxel-conversion procedure was used to convert each voxel of bone tissue into an equally sized brick element, thereby creating microfinite element models that could represent the actual microarchitecture in detail. The models contained  $\sim 2$  million elements for the distal radius and 5 million elements for the distal tibia and could be solved in  $\sim 3$  and 5 h, respectively. Material properties were chosen as isotropic and elastic. Both cortical and trabecular bone elements were assigned a Young's modulus of 10 GPa and a Poisson's ratio of 0.3. A compression test was simulated to represent loading conditions during a fall from standing height. The bone predicted failure load was calculated as the force for which 2% of the bone tissue would be loaded beyond a 0.7% strain (47). In addition to the failure load (Newtons), the microfinite element analysis-derived variables also included stiffness (kiloNewtons per millimeter) and the apparent modulus (Newtons per millimeters squared), which was calculated as the stiffness multiplied by the height of the model (9 mm in all cases) and divided by the projected cross-sectional area. Thus, the latter measure provided information about stiffness that was corrected for differences in height and the cross-sectional area. All microfinite element analyses were done with the use of the FE solver that is integrated into IPL software (version 1.15; Scanco Medical AG).

#### Statistical analysis

The various anthropometric, BMD, and microstructure variables are presented as means  $\pm$  SDs or SEMs and percentages. The Shapiro-Francia *W* test and skewness and kurtosis tests were used to verify the normality of the distributions, and non-Gaussian variables were normalized with the use of simple mathematical transformations (the square root for all sources of protein intakes and tibia failure load and log for the radius failure load). The primary outcomes were distal radius and tibia predicted failure loads as assessed with the use of an FEA from the HR-pQCT results.

Secondary outcomes were as follows: predicted stiffness and apparent modulus, which were assessed with the use of an FEA, the aBMD, and whole-body BMC; lean and fat masses, which were assessed with the use of DXA; and the peripheral skeleton vBMD and microstructure, which were as assessed with the use of HR-pQCT. Associations between outcome variables and protein intakes are presented according to tertile cutoffs of total protein intake and of various sources of protein and with protein intakes as continuous variables. The associations between outcome and tertiles of protein intakes were assessed with the use of a 1-factor ANOVA. Comparisons between the 3 tertiles groups were made with the use of post hoc multiple comparisons with Tukey's honestly significant difference test. A chi-square test was used when appropriate. Because this trial was an explanatory study, and because many variables that were obtained from the HR-pQCT data were interdependent, no adjustment for multiple comparisons was applied.

Associations of normalized bone variables (dependent variables) and protein intakes (independent variables) were evaluated with the use of a linear regressions analysis in 3 separate models of independent variables as follows: model 1 included total protein intake, model 2 included animal protein and vegetable protein intakes, and model 3 included dairy protein, nondairy animal protein, and vegetable protein intakes. These models enabled us to adjust animal and vegetable protein intakes for each other in model 2 and dairy protein and nondairy protein intakes for each other in model 3.

Multivariable regressions were also applied with weight, height, physical activity, menopause duration, calcium intake, and the interaction of calcium-protein intakes as independent variables in addition to variables relative to protein intakes in the 3 models. These additional variables were used as confounding variables because of their significant associations with the failure load in simple regression models. In addition, we tested a priori interactions between protein intakes and weight, height, and physical activity or calcium intakes on the predicted failure load in separate models. Only the interaction between protein and calcium intakes on failure loads was significant and, therefore, was included in the multiple regressions models.

The relative contribution of bone microstructure in the association between the failure load and protein intakes was assessed in additional models. Because there is multicollinearity between many microstructure variables that are measured with the use of HR-pQCT, a PCA was applied for the radius and tibia separately. A PCA is a statistical method that transforms a series of correlated variables into a smaller series of uncorrelated variables, which are defined as principal components (PCs). All normalized variables that were obtained from the HR-pQCT analysis were introduced in the PCA except for trabecular thickness and volumetric cortical density, which are strongly affected by partial volume effects (48). After optimization with the use of a varimax rotation step, the PCA identified 3 uncorrelated PCs at the radius and tibia (i.e., PCs with eigenvalues  $> 1.0$ ). Associations of each PC (dependent variable) and protein intake (independent variable) were evaluated with the use of a linear regression analysis in the 3 separate models (i.e., models 1–3). To assess the specific contribution of each PC to the association between biomechanical properties and protein intakes, we built additional models by adding each PC in the 3 models that tested the association between the failure load and protein intakes. The data were analyzed with the use of STATA software (version 14.0; StataCorp LP).



## RESULTS

## Subjects' characteristics

The 746 postmenopausal women with dietary intake data were very homogenous (mean age  $65.0 \pm 1.4$  y) (Table 1). Mean dietary calcium intake ( $1156 \pm 423$  mg/d) and protein intake ( $1.11 \pm 0.37$  g · kg body weight<sup>-1</sup> · d<sup>-1</sup>) were within or slightly greater than the recommended amounts for this class of age (700–1300 mg/d and 0.8–1.2 g · kg body weight<sup>-1</sup> · d<sup>-1</sup>, respectively) (1, 31). Protein from animal origins represented approximately

two-thirds of total protein intake, and specifically, dairy products represented more than a one-quarter of total protein intake. Menopause hormone therapy and calcium and vitamin D supplements were recorded with a similar prevalence in all 3 tertiles of total protein intake.

With the use of aBMD values at the spine, femoral neck, and total hip, 19.7% of subjects were osteoporotic, and 57.6% of subjects were osteopenic. Approximately 20% of the subjects had a prevalent low-trauma fracture. The prevalence of sarcopenia, as defined by the appendicular lean mass per squared

**TABLE 1**  
Characteristics of postmenopausal women according to tertiles of total protein intake<sup>1</sup>

	Tertile 1 (<60.9 g/d; n = 249)	Tertile 2 (60.9–79.1 g/d; n = 249)	Tertile 3 (>79.1 g/d; n = 248)	P
Total protein intake				
Subject characteristic				
Age, y	65.0 ± 1.4 <sup>2</sup>	65.0 ± 1.5	64.9 ± 1.4	0.458
Height, cm	161.6 ± 6.4	162.3 ± 6.8	162.7 ± 6.1	0.135
Weight, kg	64.5 ± 11.9 <sup>a</sup>	65.9 ± 11.2 <sup>a,b</sup>	68.4 ± 12.5 <sup>b</sup>	<0.001
BMI, kg/m <sup>2</sup>	24.7 ± 4.5 <sup>a</sup>	25.1 ± 4.3 <sup>a,b</sup>	25.8 ± 4.7 <sup>b</sup>	0.008
Time since menopause, y	8.9 ± 7.2	7.8 ± 6.2	8.1 ± 6.6	0.202
Tobacco consumption, current, %	8.8	8.0	9.3	0.884
Alcohol consumption, ≥7 U/wk, %	14.1	13.6	15.3	0.858
Previous low-trauma fracture, <sup>3</sup> %	17.7	19.3	21.8	0.510
Vitamin D, nmol/L	68.1 ± 28.1	66.6 ± 27.3	66.4 ± 27.4	0.771
Nutrition and physical activity				
Dietary calcium intake, mg/d	852 ± 254 <sup>a</sup>	1115 ± 301 <sup>b</sup>	1502 ± 409 <sup>c</sup>	<0.001
Dietary total protein intake, g/d	48.8 ± 8.9 <sup>a</sup>	69.4 ± 5.1 <sup>b</sup>	96.9 ± 15.1 <sup>c</sup>	NA
Dietary total protein intake, g · kg <sup>-1</sup> · d <sup>-1</sup>	0.78 ± 0.18 <sup>a</sup>	1.08 ± 0.19 <sup>b</sup>	1.46 ± 0.32 <sup>c</sup>	<0.001
Dietary animal protein intake, g/d	30.7 ± 8.6 <sup>a</sup>	46.6 ± 7.3 <sup>b</sup>	69.7 ± 15.9 <sup>c</sup>	<0.001
Dietary vegetable protein intake, g/d	18.1 ± 6.0 <sup>a</sup>	22.8 ± 6.0 <sup>b</sup>	27.5 ± 8.2 <sup>c</sup>	<0.001
Dietary dairy protein intake, g/d	13.0 ± 6.3 <sup>a</sup>	18.7 ± 8.3 <sup>b</sup>	28.8 ± 12.4 <sup>c</sup>	<0.001
Dietary nondairy animal protein intake, g/d	17.7 ± 8.1 <sup>a</sup>	27.9 ± 9.2 <sup>b</sup>	40.9 ± 14.9 <sup>c</sup>	<0.001
Physical activity, kcal/d	521.7 ± 278.4	520.2 ± 285.5	566.4 ± 330.0	0.266
Bone-targeting treatment, %				
Calcium supplements	37.7	34.5	39.1	0.557
Vitamin D supplements	35.3	39.0	38.7	0.650
Menopausal hormone therapy	22.5	24.5	21.0	0.642
Current or past use of anti-osteoporotic drug <sup>4</sup>	8.4	7.6	6.8	0.804
Body composition (DXA)				
Whole-body BMC, g	1888 ± 291 <sup>a</sup>	1961 ± 288 <sup>b</sup>	1974 ± 307 <sup>b</sup>	0.002
Whole-body fat mass, kg	22.9 ± 8.0 <sup>a</sup>	23.7 ± 7.6 <sup>a,b</sup>	25.0 ± 8.4 <sup>b</sup>	0.009
Whole-body lean mass, kg	39.5 ± 5.0 <sup>a</sup>	40.1 ± 4.8 <sup>a</sup>	41.2 ± 5.0 <sup>b</sup>	<0.001
Appendicular lean mass, kg/m <sup>2</sup>	6.3 ± 0.8 <sup>a</sup>	6.4 ± 0.8 <sup>a</sup>	6.6 ± 0.8 <sup>b</sup>	0.002
Sarcopenia, <sup>5</sup> %	14.1 <sup>a</sup>	11.6 <sup>a</sup>	6.0 <sup>b</sup>	<0.001
Areal BMD (DXA)				
Lumbar spine BMD, g/cm <sup>2</sup>	0.901 ± 0.145	0.928 ± 0.165	0.917 ± 0.135	0.157
Femoral neck BMD, g/cm <sup>2</sup>	0.689 ± 0.105 <sup>a</sup>	0.707 ± 0.102 <sup>a,b</sup>	0.722 ± 0.11 <sup>b</sup>	0.002
Total hip BMD, g/cm <sup>2</sup>	0.827 ± 0.105 <sup>a</sup>	0.845 ± 0.111 <sup>a,b</sup>	0.856 ± 0.116 <sup>b</sup>	0.017
Distal 1/3 radius BMD, g/cm <sup>2</sup>	0.622 ± 0.063 <sup>a</sup>	0.637 ± 0.066 <sup>b</sup>	0.642 ± 0.064 <sup>b</sup>	0.002
Osteoporotic status, <sup>6</sup> %				0.056
Osteoporosis	21.3	20.1	18.2	—
Osteopenia	62.6	53.8	55.9	—
Normal BMD	16.1	26.1	25.9	—

<sup>1</sup> P values were determined with the use of an ANOVA with tertiles of total protein intake. All sources of protein intakes were normalized as square root. Means or percentages that do not share a common superscript letter were significantly different at  $P < 0.05$  on the basis of an ANOVA with the use of Tukey's post hoc test or chi-square test. BMC, bone mineral content; BMD, bone mineral density; DXA, dual-energy X-ray absorptiometry; NA, not applicable.

<sup>2</sup> Mean ± SD (all such values).

<sup>3</sup> In adult age (>20 y).

<sup>4</sup> Bisphosphonates, raloxifene, and strontium ranelate.

<sup>5</sup> Defined as an appendicular lean mass  $\leq 5.45$  kg/m<sup>2</sup> according to the criteria proposed by Baumgartner et al. (49).

<sup>6</sup> Osteoporosis was defined as  $\geq 1$  T score  $\leq -2.5$  SDs, and osteopenia was defined as  $\geq 1$  T score between  $-1$  and  $-2.5$  SDs, with no score  $\leq -2.5$  SDs at the lumbar spine, total hip, or femoral neck.

height of 2 SDs below the values of young healthy women (49), was lower in subjects in the highest tertile of total protein intake (6%) than in subjects in the lowest and medium tertiles of total protein intake (14.1% and 11.6%, respectively). Subjects in the highest tertile of total protein intake were heavier, had higher BMI, and consumed more calcium, animal, dairy, and vegetable proteins (Table 1). They also had higher aBMDs at the total hip, femoral neck, and distal 1/3 radius as well as higher whole-body BMCs and fat and lean masses.

### Association of bone strength with dietary protein intakes

Unadjusted values of distal radius and tibia predicted failure loads, which were estimated from microstructure values with the use of an FEA, increased with tertiles of total and animal protein intakes (Figure 1, Table 2). The difference was mainly observed between the first and third tertiles ( $P = 0.058$  and  $P = 0.003$  at the radius and tibia, respectively) and between the first and second tertiles of total protein intake ( $P = 0.076$  and  $P = 0.051$  at the radius and tibia, respectively), but there was no difference between the second and third tertiles of total protein intake, which suggested that there was some plateau effect. Radius and tibia failure loads were positively associated with total and animal protein intakes (as continuous variables), and the tibia failure load was positively associated with dairy protein intakes (Table 3). The relation with vegetable proteins was NS. A quite-similar pattern was shown for predicted bone stiffness (Table 2, Supplemental Figure 2).

### Interaction between calcium intakes with dietary protein intakes on predicted failure load

We tested the interactions between protein intakes and weight, height, and physical activity or calcium intakes on the predicted failure load (Supplemental Table 1). A significant interaction was detected only between protein and calcium intakes with  $P$  values of 0.001 and 0.014 for the interaction of total protein intake  $\times$  calcium intakes (continuous variable) on radius and tibia failure loads, respectively. There were significant interactions between calcium intakes and animal, dairy, and nondairy animal proteins but not with vegetable proteins. The positive association between bone strength and protein intakes was observed only in women with low calcium intakes ( $<1081$  mg/d according to the median of dietary calcium intakes) (Supplemental Table 2).

### Adjusted association of bone strength and dietary protein intakes

Distal radius and tibia failure loads were significantly associated with height, body weight, physical activity, and calcium intakes in univariate models (data not shown). Thus, we tested the association of bone strength with dietary protein intakes in multiple regression models with adjustment for height, body weight, physical activity, calcium intakes, and interactions between calcium and total, animal, dairy, and nondairy animal protein intakes as reported previously. The corresponding adjusted  $P$  values for each category of protein intakes are presented in Table 3. After adjustment, a positive association was shown between distal radius and tibia predicted failure load or stiffness and dairy protein intakes ( $P = 0.025$  and  $P = 0.048$  for failure load, and  $P = 0.032$  and  $P = 0.045$  for stiffness, at the

radius and tibia, respectively). The association between the failure load and stiffness and animal proteins remained significant at the radius only ( $P = 0.005$  for both). At the distal radius, positive associations were also shown for the modulus in relation to animal and dairy protein intakes in nonadjusted and adjusted models. Irrespective of the variables included in the models, the predicted failure load, stiffness, and modulus were not correlated to vegetable and animal nondairy protein intakes.

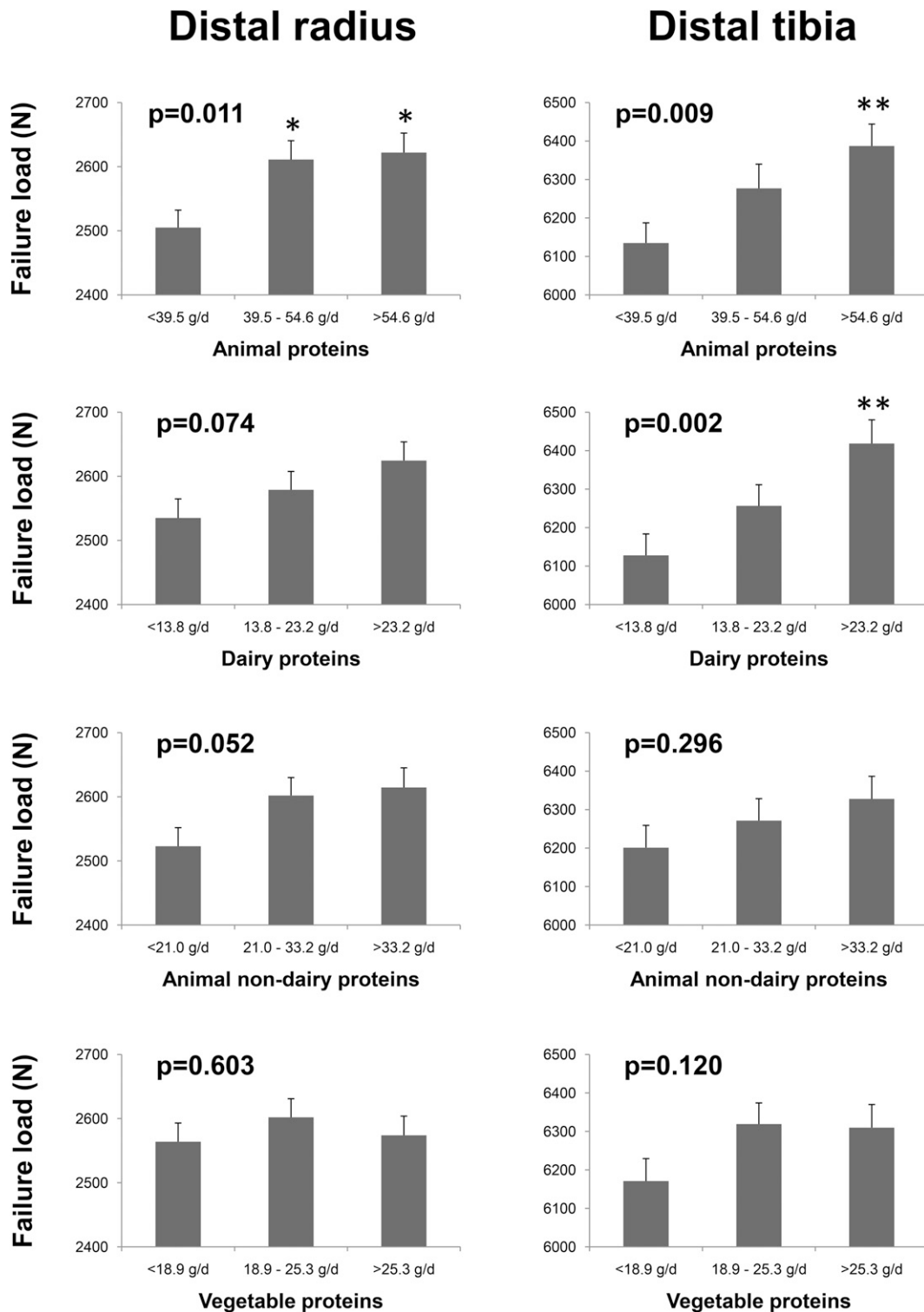
### Bone microstructure in relation to dietary protein intakes

All microstructure variables were in agreement with those that were expected for this age classification (50, 51) (Table 2). In the highest compared with lowest tertiles of protein intake, trabecular spacing and spacing SD were lower both at the distal radius and tibia, whereas the cortical area and trabecular number were higher at the distal tibia only (unadjusted  $P$  values).

To evaluate the contribution of microstructure components to the estimated bone strength, a PCA was applied for the radius and tibia separately. The PCA identified 3 uncorrelated PCs at the distal radius and tibia (Supplemental Table 3). The first component (PC1) could be considered a trabecular bone microstructure, the second component (PC2) could be considered a bone morphology, and the third component (PC3) could be considered a cortical microstructure. PC1 included the trabecular vBMD, number, spacing, and spacing SD. PC2 was composed of total and trabecular areas and the cortical perimeter. PC3 comprised the cortical area, thickness, and porosity. Together, these PCs accounted for 89% of the variance of the original data set for the 2 sites. In simple and multiple regression analyses that were adjusted for height, body weight, physical activity, calcium intakes, and interactions between calcium and protein intakes, the trabecular bone microstructure (PC1) was significantly associated with animal and dairy protein intakes but not with vegetable protein intake for both skeletal sites (Table 4). Bone morphology (PC2) at the radius was positively associated with vegetable protein intake only. The cortical microstructure (PC3) was associated with animal and dairy protein intakes but not with vegetable protein intake for the distal radius only.

To evaluate the relative contribution of each component in the association between the failure load and total, animal, and dairy protein intakes, we introduced the various PCs into the models that are presented in Table 3 (Table 5). The association between the failure load and total protein intake was no longer significant at the distal radius after adjustment for PC1 (trabecular bone microstructure), whereas it persisted after adjustment for PCs 2 and 3. These data indicate the importance of the trabecular bone component in the relation between the predicted failure load and total protein intake. For animal protein intakes, the associations of radius and tibia failure loads and protein intakes disappeared after adjustment for PC1 (trabecular bone microstructure) or PC3 (cortical bone microstructure) but not for PC2 (bone morphology). The same result occurred for the associations between the tibia failure load and dairy protein intakes. These data indicate that the positive associations between the failure load and animal and dairy protein intakes are mediated via the positive associations between trabecular and cortical bone microstructures and animal and dairy protein intakes. Similar results were obtained for the analyses of the relative contribution of each component in the association between bone stiffness and protein intakes (data not shown).





**FIGURE 1** Finite element analysis of mean  $\pm$  SEM distal radius and distal tibia predicted failure loads according to tertiles of dietary animal, dairy, nondairy animal, and vegetable protein intakes ( $n = 703$  at the distal radius;  $n = 725$  at the distal tibia). \*\*\*Compared with the lowest protein intake tertile (ANOVA with Tukey's post hoc test): \* $P < 0.05$ , \*\* $P < 0.01$ .

## DISCUSSION

In this cohort of seven hundred forty-six 65-y-old healthy women, higher protein intakes, particularly from animal and dairy products, were associated with higher values of peripheral skeleton predicted failure load and stiffness, which were likely

related to changes in both cortical and trabecular compartments. All of these associations at the distal radius, and the association with dairy protein intakes at the distal tibia, were maintained after adjustment for weight, height, physical activity, menopause duration, calcium intake, and interactions with calcium intakes.



**TABLE 2**  
Bone strength and microstructure variables at the distal radius and tibia according to tertiles of total protein intake<sup>1</sup>

	Distal radius				Distal tibia				
	Tertile 1 (<60.9 g/d; n = 231)	Tertile 2 (60.9–79.1 g/d; n = 239)	Tertile 3 (>79.1 g/d; n = 233)	P <sup>2</sup>	Tertile 1 (<60.9 g/d; n = 243)	Tertile 2 (60.9–79.1 g/d; n = 239)	Tertile 3 (>79.1 g/d; n = 243)	P <sup>2</sup>	P <sup>3</sup>
<b>Bone strength</b>									
Estimated failure load, N	2514 ± 407 <sup>a</sup>	2610 ± 465 <sup>a</sup>	2614 ± 465 <sup>a</sup>	0.037	6112 ± 816 <sup>a</sup>	6306 ± 913 <sup>a,b</sup>	6383 ± 947 <sup>b</sup>	0.003	0.174
Stiffness, N/mm	67,129 ± 12,212 <sup>a</sup>	70,097 ± 14,070 <sup>a</sup>	70,058 ± 14,001 <sup>a</sup>	0.042	180,326 ± 27,185 <sup>a</sup>	186,955 ± 30,604 <sup>a,b</sup>	189,540 ± 32,065 <sup>b</sup>	0.004	0.142
Apparent modulus, N/mm <sup>2</sup>	1679 ± 360	1748 ± 409	1729 ± 396	0.247	2003 ± 347	2061 ± 425	2068 ± 406	0.197	0.148
<b>Total bone</b>									
Total area, mm <sup>2</sup>	258 ± 46	259 ± 42	262 ± 43	0.592	692 ± 111	698 ± 109	703 ± 106	0.450	0.292
Cortical perimeter, mm	68.1 ± 6.3	68.2 ± 5.7	68.7 ± 5.8	0.421	103.1 ± 8.3	104 ± 9.6	104.1 ± 7.8	0.333	0.190
Total vBMD, mg HA/cm <sup>3</sup>	289 ± 63	298 ± 65	296 ± 66	0.238	248 ± 46 <sup>a</sup>	257 ± 53 <sup>a</sup>	259 ± 53 <sup>a</sup>	0.045	0.080
<b>Cortical bone</b>									
Cortical vBMD, mg HA/cm <sup>3</sup>	856 ± 66	863 ± 67	860 ± 63	0.451	813 ± 62	817 ± 61	821 ± 64	0.405	0.877
Cortical area, mm <sup>2</sup>	46.8 ± 10.2	48.6 ± 10.7	48.7 ± 11.3	0.104	93.6 ± 19.4 <sup>a</sup>	96.8 ± 21.6 <sup>a,b</sup>	98.6 ± 22.6 <sup>b</sup>	0.029	0.860
Cortical thickness, mm	0.69 ± 0.17	0.72 ± 0.17	0.71 ± 0.18	0.270	0.92 ± 0.22	0.94 ± 0.24	0.96 ± 0.25	0.212	0.899
Cortical porosity, %	2.6 ± 1.4	2.7 ± 1.5	2.7 ± 1.4	0.872	8.5 ± 3.2	8.7 ± 3.4	8.2 ± 3.2	0.278	0.330
<b>Trabecular bone</b>									
Trabecular vBMD, mg HA/cm <sup>3</sup>	134 ± 34	140 ± 41	140 ± 38	0.077	144 ± 32 <sup>a</sup>	151 ± 37 <sup>a,b</sup>	152 ± 36 <sup>b</sup>	0.023	0.005
Trabecular area, mm <sup>2</sup>	205 ± 46	204 ± 43	207 ± 43	0.824	589 ± 114	591 ± 117	595 ± 114	0.812	0.339
Trabecular number, mm <sup>-1</sup>	1.76 ± 0.3	1.79 ± 0.32	1.82 ± 0.36	0.075	1.64 ± 0.31 <sup>a</sup>	1.69 ± 0.3 <sup>a,b</sup>	1.73 ± 0.34 <sup>b</sup>	0.011	0.055
Trabecular thickness, μm	63 ± 10	64 ± 12	64 ± 11	0.309	74 ± 13	75 ± 14	74 ± 13	0.685	0.059
Trabecular spacing, μm	526 ± 135 <sup>a</sup>	514 ± 130 <sup>a,b</sup>	521 ± 215 <sup>b</sup>	0.049	559 ± 135 <sup>a</sup>	537 ± 129 <sup>a,b</sup>	530 ± 138 <sup>b</sup>	0.007	0.040
Trabecular spacing SD, μm	263 ± 158 <sup>a</sup>	250 ± 132 <sup>a,b</sup>	265 ± 259 <sup>b</sup>	0.039	281 ± 161 <sup>a</sup>	267 ± 146 <sup>a,b</sup>	264 ± 174 <sup>b</sup>	0.006	0.107

<sup>1</sup> All values are means ± SDs. Strength variables, total area, cortical perimeter and porosity, and trabecular thickness at the radius, and stiffness, total area, and trabecular thickness at the tibia were normalized as log. The radius total vBMD and trabecular area and tibia failure load, modulus, total vBMD, cortical thickness and porosity, and trabecular vBMD and area were normalized as square root. The radius trabecular spacing SD and tibia cortical perimeter, trabecular spacing, and trabecular spacing SD were normalized as inverse. Radius and tibia cortical vBMDs were normalized as cubic. The radius trabecular number was normalized as square, and the radius trabecular spacing was normalized as inverse square. Means that do not share a common superscript letter were significantly different at  $P < 0.05$  on the basis of an ANOVA with the use of Tukey's post hoc test. HA, hydroxyapatite; vBMD, volumetric bone mineral density.

<sup>2</sup> Determined with the use of an ANOVA with tertiles of total protein intake.

<sup>3</sup> Adjusted for weight, height, physical activity, menopause duration, calcium intake, and the interaction between calcium intake and tertiles of total protein intake.

**TABLE 3**

Associations (linear regressions) between bone strength at the distal radius and tibia (dependent variables) and various sources of protein intakes (independent variables) in separate models<sup>1</sup>

	Distal radius ( <i>n</i> = 703)			Distal tibia ( <i>n</i> = 725)		
	$\beta$ (95% CI)	<i>P</i>	<i>P</i> <sup>2</sup>	$\beta$ (95% CI)	<i>P</i>	<i>P</i> <sup>2</sup>
<b>Failure load</b>						
Model 1						
Total protein	0.012 (0.002, 0.021)	0.019	0.008	0.575 (0.263, 0.886)	<0.001	0.071
Model 2						
Animal protein	0.013 (0.003, 0.022)	0.008	0.005	0.484 (0.181, 0.787)	0.002	0.102
Vegetable protein	-0.001 (-0.018, 0.015)	0.881	0.845	0.323 (-0.200, 0.846)	0.226	0.448
Model 3						
Dairy protein	0.009 (-0.001, 0.020)	0.076	0.025	0.461 (0.128, 0.793)	0.007	0.048
Nondairy animal protein	0.009 (0.000, 0.019)	0.052	0.070	0.278 (-0.021, 0.577)	0.068	0.697
Vegetable protein	-0.002 (-0.018, 0.015)	0.854	0.793	0.307 (-0.216, 0.830)	0.250	0.237
<b>Stiffness</b>						
Model 1						
Total protein	0.013 (0.002, 0.024)	0.023	0.010	0.016 (0.007, 0.025)	<0.001	0.065
Model 2						
Animal protein	0.014 (0.004, 0.025)	0.008	0.005	0.014 (0.005, 0.022)	0.002	0.094
Vegetable protein	-0.003 (-0.021, 0.016)	0.780	0.755	0.010 (-0.006, 0.025)	0.214	0.420
Model 3						
Dairy protein	0.011 (-0.001, 0.023)	0.063	0.032	0.013 (0.003, 0.022)	0.010	0.045
Nondairy animal protein	0.010 (-0.001, 0.021)	0.065	0.058	0.008 (-0.001, 0.017)	0.067	0.711
Vegetable protein	-0.003 (-0.021, 0.015)	0.754	0.843	0.009 (-0.006, 0.024)	0.237	0.232
<b>Modulus</b>						
Model 1						
Total protein	0.007 (-0.006, 0.020)	0.288	0.050	0.177 (-0.064, 0.419)	0.149	0.109
Model 2						
Animal protein	0.014 (0.002, 0.027)	0.027	0.014	0.224 (-0.011, 0.458)	0.062	0.199
Vegetable protein	-0.019 (-0.041, 0.002)	0.074	0.184	-0.123 (-0.528, 0.282)	0.551	0.855
Model 3						
Dairy protein	0.014 (0.000, 0.028)	0.045	0.019	0.220 (-0.038, 0.478)	0.095	0.094
Nondairy animal protein	0.008 (-0.005, 0.020)	0.222	0.150	0.113 (-0.119, 0.345)	0.338	0.685
Vegetable protein	-0.020 (-0.041, 0.001)	0.068	0.501	-0.128 (-0.534, 0.278)	0.536	0.427

<sup>1</sup> All radius strength variables and tibia stiffness were normalized as log, tibia failure load and modulus and all sources of protein intakes as square root. Model 1 included total protein intake as an independent variable; model 2 included animal protein and vegetable protein intakes as independent variables; and model 3 included dairy protein, nondairy animal protein, and vegetable protein intakes as independent variables.

<sup>2</sup> Adjusted for weight, height, physical activity, menopause duration, calcium intake, and the interaction between calcium and protein intakes (for total, animal, dairy, and nondairy animal protein intakes).

To our knowledge, this is the first study to show a relation between the estimated bone strength as assessed with the use of an FEA and dietary protein intakes.

An FEA is used to determine bone mechanical behavior. When applied to HR-pQCT data, this method allows for the estimation of the failure load, stiffness, and apparent modulus of skeletal specimens and to specifically distinguish the contribution of cortical and trabecular components on the resistance to load bearing. Because bone strength, which is a major component in the resistance to fracture, is determined by bone geometry, mass, microstructure, and material-level properties, we decided to assess the influence of dietary proteins from various origins on the predicted failure load and stiffness because these variables integrate all components of bone strength except material-level properties. We showed that the distal radius- and tibia-predicted failure load and stiffness were positively associated with total, animal, and dairy protein intakes. These changes were likely related to significant modifications of aBMD and cortical and trabecular microstructures that accompany various dietary protein

intakes. To further decipher the relative importance of the various bone-strength components in the association between the failure load and dietary protein intakes, we applied a PCA, which identified a component that included the trabecular microstructure, a second component that corresponded to bone morphology, and a third component that corresponded to the cortical microstructure. These PCs accounted for a large proportion of the variance of the original data set. In simple and multiple regression analyses, the first 2 components were significantly associated with total, animal, and dairy protein intakes for both skeletal sites, and the third component was associated with animal protein intakes at the radius. After adjustment for the trabecular microstructure component, the associations between the failure load and total, animal, or dairy protein intakes were no longer significant, thereby indicating a major effect of the trabecular bone microstructure in the association between bone strength and dietary protein intakes.

Our subjects were 65-y-old women who were investigated at the usual age of retirement from professional activity. The women





TABLE 4

Associations (linear regressions) between bone-microstructure components at the distal radius and tibia (dependent variable) and various sources of protein intakes (independent variables) in separate models<sup>1</sup>

	Distal radius (n = 703)			Distal tibia (n = 725)		
	$\beta$ (95% CI)	P	P <sup>2</sup>	$\beta$ (95% CI)	P	P <sup>2</sup>
Trabecular microstructure (PC1)						
Model 1						
Total protein	0.115 (0.003, 0.227)	0.045	0.007	0.181 (0.071, 0.291)	0.001	0.008
Model 2						
Animal protein	0.121 (0.012, 0.231)	0.030	0.002	0.169 (0.062, 0.276)	0.002	0.013
Vegetable protein	0.005 (-0.182, 0.192)	0.958	0.874	0.050 (-0.134, 0.235)	0.590	0.988
Model 3						
Dairy protein	0.144 (0.024, 0.263)	0.019	0.001	0.171 (0.054, 0.289)	0.004	0.012
Nondairy animal protein	0.062 (-0.047, 0.171)	0.263	0.151	0.094 (-0.011, 0.199)	0.080	0.375
Vegetable protein	-0.004 (-0.191, 0.184)	0.968	0.563	0.043 (-0.141, 0.227)	0.643	0.698
Morphology (PC2)						
Model 1						
Total protein	0.070 (-0.031, 0.171)	0.177	0.721	-0.065 (-0.170, 0.039)	0.220	0.392
Model 2						
Animal protein	-0.012 (-0.110, 0.086)	0.815	0.365	-0.018 (-0.120, 0.084)	0.729	0.600
Vegetable protein	0.238 (0.070, 0.406)	0.005	0.035	-0.145 (-0.321, 0.030)	0.104	0.790
Model 3						
Dairy protein	-0.034 (-0.141, 0.073)	0.536	0.064	-0.011 (-0.122, 0.101)	0.853	0.385
Nondairy animal protein	0.005 (-0.092, 0.103)	0.917	0.873	-0.020 (-0.120, 0.081)	0.702	0.760
Vegetable protein	0.241 (0.073, 0.409)	0.005	0.127	-0.144 (-0.319, 0.032)	0.108	0.738
Cortical microstructure (PC3)						
Model 1						
Total protein	0.055 (-0.036, 0.146)	0.238	0.064	0.089 (0.006, 0.172)	0.035	0.479
Model 2						
Animal protein	0.128 (0.040, 0.216)	0.005	0.021	0.128 (0.047, 0.208)	0.002	0.291
Vegetable protein	-0.200 (-0.351, -0.049)	0.009	0.064	-0.099 (-0.238, 0.039)	0.160	0.439
Model 3						
Dairy protein	0.063 (-0.033, 0.159)	0.200	0.025	0.115 (0.027, 0.204)	0.011	0.148
Nondairy animal protein	0.112 (0.024, 0.199)	0.013	0.354	0.073 (-0.006, 0.152)	0.072	0.888
Vegetable protein	-0.200 (-0.351, -0.049)	0.009	0.135	-0.102 (-0.24, 0.037)	0.151	0.807

<sup>1</sup> All sources of protein intakes were normalized as square root. Model 1 included total protein intake as an independent variable; model 2 included animal protein and vegetable protein intakes as independent variables; and model 3 included dairy protein, nondairy animal protein, and vegetable protein intakes as independent variables. PC, principal component.

<sup>2</sup> Adjusted for weight, height, physical activity, menopause duration, calcium intakes, interaction calcium-protein intakes (for total, animal, dairy and nondairy animal protein intakes).

had very healthy lifestyles with regular physical activity and consumed well-balanced diets with mean intakes of 1156 mg Ca/d and 71.7 g protein/d. Except for women in the lowest protein-intake tertile, with a mean intake at the Recommended Dietary Allowance (RDA), all of the other women in the other tertiles had higher consumption of dietary proteins. When translated into the number of servings per day, the tertile intakes were <3,  $\geq 3$  to 4, and  $\geq 4$  servings/d, respectively. There was no evidence of any harm even at higher protein intakes, as shown by the positive association between bone-strength mineral mass and density, bone microstructure, and dietary protein intakes. Because our finding showed a higher axial skeleton BMD and higher peripheral bone strength that were associated with higher protein intakes, particularly from animal and dairy origins, our results provide some support for the recommendation of intakes that are higher than the RDA in the oldest old to attenuate the age-dependent decrease in bone and muscle in this population (1, 52, 53).

These results are in agreement with and extend those of Radavelli-Bagatini et al. (9) who used a pQCT instrument with a

lower resolution in an oldest-old population. The meta-analysis of Darling et al. (2) concluded that there was a positive association between DXA-determined BMC and BMD at various skeletal sites and dietary protein intakes, the latter association of which explained between 1% and 8% of the variance of BMC and BMD. In agreement with epidemiologic studies that have shown a correlation between both axial and peripheral BMDs and dietary protein intakes (3, 4), observational studies with a fracture as the outcome have shown an inverse relation between fracture risk, particularly of the hip, and dietary proteins (13–17). In one of the studies, the association was detected with animal but not vegetable proteins (13). Correction for the low protein intakes of patients with a recent hip fracture reduced the BMD decrease that occurred in the contra-lateral intact hip by 50% over 1 y (18).

However, the majority of randomized controlled trials on bone in response to a protein intervention have been performed with dairy products. Dairy products are sources of both protein (32 g/L milk, which represents approximately  $\leq 50\%$  of the RDA for

**TABLE 5**

Associations (linear regressions) between failure load at the distal radius and tibia (dependent variable) and various sources of protein intakes in separate models including each principal microstructure component (independent variables) for the testing of the relative contribution of each component in the association between bone strength and protein intakes<sup>1</sup>

	Distal radius ( <i>n</i> = 703)		Distal tibia ( <i>n</i> = 725)	
	$\beta$ (95% CI)	<i>P</i>	$\beta$ (95% CI)	<i>P</i>
<b>Model 1</b>				
Total protein intakes	0.012 (0.002, 0.021)	0.019	0.575 (0.263, 0.886)	<0.001
<b>Model 1 + trabecular microstructure (PC1)</b>				
Total protein intakes	0.005 (−0.002, 0.013)	0.167	0.244 (0.006, 0.481)	0.045
PC1	0.056 (0.052, 0.061)	<0.001	1.860 (1.700, 2.020)	<0.001
<b>Model 1 + bone morphology (PC2)</b>				
Total protein intakes	0.012 (0.002, 0.021)	0.021	0.584 (0.271, 0.897)	<0.001
PC2	0.003 (−0.005, 0.010)	0.480	0.082 (−0.136, 0.300)	0.461
<b>Model 1 + cortical microstructure (PC3)</b>				
Total protein intakes	0.008 (0.001, 0.015)	0.033	0.429 (0.149, 0.709)	0.003
PC3	0.075 (0.069, 0.080)	<0.001	1.693 (1.447, 1.939)	<0.001
<b>Model 2</b>				
Animal protein	0.013 (0.003, 0.022)	0.008	0.484 (0.181, 0.787)	0.002
Vegetable protein	−0.001 (−0.018, 0.015)	0.881	0.323 (−0.200, 0.846)	0.226
<b>Model 2 + trabecular microstructure (PC1)</b>				
Animal protein	0.006 (−0.001, 0.013)	0.107	0.172 (−0.059, 0.403)	0.144
Vegetable protein	−0.002 (−0.014, 0.011)	0.809	0.239 (−0.157, 0.635)	0.237
PC1	0.056 (0.0514, 0.061)	<0.001	1.860 (1.703, 2.017)	<0.001
<b>Model 2 + bone morphology (PC2)</b>				
Animal protein	0.013 (0.003, 0.022)	0.008	0.488 (0.185, 0.792)	0.002
Vegetable protein	−0.002 (−0.018, 0.014)	0.815	0.339 (−0.186, 0.864)	0.205
PC2	0.003 (−0.004, 0.010)	0.419	0.082 (−0.136, 0.301)	0.459
<b>Model 2 + cortical microstructure (PC3)</b>				
Animal protein	0.003 (−0.004, 0.010)	0.357	0.270 (−0.003, 0.542)	0.053
Vegetable protein	0.014 (0.002, 0.026)	0.021	0.501 (0.033, 0.970)	0.036
PC3	0.075 (0.069, 0.081)	<0.001	1.703 (1.457, 1.950)	<0.001
<b>Model 3</b>				
Dairy protein	0.009 (−0.001, 0.02)	0.076	0.461 (0.128, 0.793)	0.007
Non-dairy animal protein	0.009 (0.000, 0.019)	0.052	0.278 (−0.021, 0.577)	0.068
Vegetable protein	−0.002 (−0.018, 0.015)	0.854	0.307 (−0.216, 0.830)	0.250
<b>Model 3 + trabecular microstructure (PC1)</b>				
Dairy protein	0.001 (−0.007, 0.009)	0.745	0.143 (−0.111, 0.397)	0.270
Non-dairy animal protein	0.006 (−0.001, 0.013)	0.110	0.108 (−0.120, 0.335)	0.353
Vegetable protein	−0.001 (−0.014, 0.011)	0.836	0.236 (−0.160, 0.633)	0.243
PC1	0.056 (0.051, 0.061)	<0.001	1.858 (1.700, 2.016)	<0.001
<b>Model 3 + bone morphology (PC2)</b>				
Dairy protein	0.010 (−0.001, 0.020)	0.073	0.465 (0.131, 0.799)	0.006
Non-dairy animal protein	0.009 (−0.000, 0.019)	0.052	0.281 (−0.019, 0.580)	0.066
Vegetable protein	−0.002 (−0.019, 0.014)	0.786	0.323 (−0.202, 0.848)	0.228
PC2	0.003 (−0.004, 0.010)	0.411	0.083 (−0.135, 0.301)	0.455
<b>Model 3 + cortical microstructure (PC3)</b>				
Dairy protein	0.005 (−0.003, 0.012)	0.218	0.266 (−0.033, 0.566)	0.081
Non-dairy animal protein	0.001 (−0.006, 0.008)	0.778	0.158 (−0.033, 0.566)	0.248
Vegetable protein	0.014 (0.002, 0.025)	0.024	0.489 (0.196, 0.958)	0.041
PC3	0.075 (0.069, 0.081)	<0.001	1.699 (1.452, 1.946)	<0.001

<sup>1</sup> Radius failure load was normalized as log, and tibia failure load and all sources of protein intakes were normalized as square root. Model 1 included total protein intake as an independent variable; model 2 included animal protein and vegetable protein intakes as independent variables; and model 3 included dairy protein, nondairy animal protein, and vegetable protein intakes as independent variables. PC1 represented trabecular microstructure, PC2 bone morphology, and PC3 cortical microstructure. PC, principal component.

nonobese adults) and calcium (1200 mg/L milk, which is equivalent to recommended daily intakes). In agreement with observational studies that have reported some benefits of dairy intake on bone mineral mass [(54–56); reviewed in Rizzoli (31)], various short-term controlled intervention studies with the use of milk, calcium-fortified milk, or cheese have shown

reductions in parathyroid hormone and biochemical markers of bone turnover together with an increase in circulating insulin-like growth factor I (21, 57–65). In studies of longer duration and with BMD as the outcome, fortified milk or dairy attenuated proximal femur bone loss or even increased the spine or hip BMD (20, 66–68). In contrast, an intervention-controlled study

with whey protein failed to find a significant difference on the axial bone of elderly women (69). A meta-analysis of cohort studies did not show any association between hip-fracture risk and milk intake in women but did show a trend toward a negative association in men (33).

The strengths of this study are the homogenous age of a large cohort of healthy postmenopausal women and, to our knowledge, the hitherto unreported analysis of bone strength and bone microstructure in relation to dietary protein intakes of various origins. The limitation of the study is mostly due to the cross-sectional design of the study, which precluded a causal relation to be firmly established. Dietary intakes were estimated with the use of a food-frequency questionnaire with risk of limited accuracy for the measurement of absolute intakes although the questionnaire was administered face to face, not autoadministered, by a certified dietitian with the help of food pictures. In addition, the questionnaire did not allow for the estimation of total energy and nutrient intakes. However, our results were adjusted for weight, height, and physical activity, which are associated with energy intakes (70). In addition, although we adjusted for possible confounders that are strongly associated with bone health, there was still the possibility of residual or unmeasured confounding from additional unmeasured factors. However, the tertiles of protein-intake groups did not differ for tobacco or alcohol consumption, use of menopausal hormone therapy, of vitamin D status. There were no malnourished subjects in this population of healthy retirees with dietary calcium and protein intakes within or slightly above the recommended amounts for this class of age, optimal vitamin D status, and a high level of socioeconomic status and living conditions. The generalization of these findings to more-heterogeneous populations may be limited because of many other factors that interfere with the bone microstructure and strength in addition to protein intakes. Last, another limitation is the multiple comparisons because of the high number of variables obtained from the HR-pQCT analysis. Therefore, the significant findings of this exploratory study need to be confirmed by studies in other populations.

In conclusion, within this cohort of 65-y-old healthy women, there is a positive association between peripheral skeleton predicted failure load and stiffness as well as bone microstructure and dietary total, animal, and dairy product protein intakes. These findings should be taken into account in the design of regimens for the nutritional prevention of bone fragility.

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