HHS Public Access

Author manuscript

J Acad Nutr Diet. Author manuscript; available in PMC 2016 October 01.

Published in final edited form as:

J Acad Nutr Diet. 2015 October ; 115(10): 1605–1613.e1. doi:10.1016/j.jand.2015.04.001.

Bone mineral density and protein derived food clusters from the Framingham Offspring Study

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Abstract

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Conflicts of interest: KMM, SS, KLT, ABD and MTH state they have no conflicts of interest to disclose. DPK reports being on the scientific advisory board of Merck Sharp and Dohme, Amgen, Eli Lilly, Ammonett Pharma and Novartis, consulting for Guidepoint Clinical Advisors, has institutional grants from Merck Sharp and Dohme, Amgen, and reports receiving royalties from Springer for editorial work and author royalties from Kluwer Wolter for UpToDate®.

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Background—Dietary protein is beneficial to bone health; however, dietary patterns of protein intake and their relation with bone mineral density (BMD) have not been evaluated.

Objective—To examine the relation of dietary protein food clusters with BMD at the femoral neck, trochanter, total femur and lumbar spine among middle-aged and older men and women.

Design—Cross-sectional study.

Participants and setting—2,758 community-dwelling individuals from the Framingham Offspring Study.

Methods—BMD was measured by Lunar DPX-L in 1996–2001. Dietary intakes were estimated using the Willett food frequency questionnaire in either 1995–1998 or 1998–2001, and the exam closest to a participant's BMD measurement was used. Cluster analysis (fastclus procedure, k-means method) was used to classify participants into groups, determined by major sources of protein. Generalized linear regression was used to compare adjusted least-squares mean BMD across protein food clusters for all pairwise comparisons.

Results—From 2,758 participants (44% men; mean age 61 ± 9y, range 29–86y), five protein food clusters were identified (chicken, fish, processed foods, red meat, low-fat milk). Three of these food clusters showed associations with BMD. The red meat protein food cluster presented with significantly lower femoral neck BMD compared to the low-fat milk cluster (red meat: 0.898 ± 0.005 versus low-fat milk: 0.919 ± 0.007, p=0.04). Further, the processed foods protein cluster presented with significantly lower femoral neck BMD compared to the low fat milk cluster (processed foods: 0.897 ± 0.004 versus low-fat milk: 0.919 ± 0.007, p=0.02). A similar, yet non-significant trend was observed for other BMD sites examined.

Conclusions—Diets with the greatest proportion of protein intake from red meat and processed foods may not be as beneficial to the skeleton compared to dietary patterns where the highest proportion of protein is derived from low-fat milk.

Keywords

Bone mineral density; dietary protein; cohort; dietary patterns; aging

INTRODUCTION

Osteoporosis and low bone mass currently affect approximately 44 million US adults over the age of 50 years.[1] Worldwide, one in three women in this age group will experience osteoporotic related fractures, as will one in five men.[2–4] The debilitating health consequences of osteoporotic fracture include chronic pain, reduced mobility, disability, and increasing degree of dependence. Perhaps most strikingly, mortality rates increase 20–24% within the first year after experiencing a hip fracture.[5] Therefore, it is of utmost public health importance to prevent this widespread disease.

Modifiable lifestyle interventions, such as altering dietary intake, have the potential to prevent or forestall bone loss associated with aging. Studies suggest that dietary protein is protective of bone loss over time[6] and may benefit the skeleton by increasing insulin-like growth factor-1 (IGF-1)[7], augmenting intestinal calcium absorption[8, 9], and improving

muscle strength and mass.[10, 11] However, in most epidemiologic studies, protein intake is examined as a single macro nutrient (g/d) with little consideration of its food source and consumption with other foods in the diet. Protein-rich foods differ not only in their protein content, but also in their amino acid composition, digestibility, and synergy with other nutrients.[12] Dietary protein may interact with nutrients found in non-protein rich foods consumed simultaneously in a meal.[13] Previous research by our group has shown different dietary patterns (derived from energy intake) to be associated with bone health.[14] Therefore, it is crucial to expand this dietary pattern methodology to examine patterns of protein intake to understand the complex relation of dietary protein (usual intake, as consumed with other foods and nutrients) with bone health in independent living adults.

The purpose of this cross-sectional study was to examine the association of dietary protein food clusters (derived from novel dietary pattern techniques) with bone mineral density (BMD) at the hip and spine among middle-aged and older Framingham Offspring Study participants. In contrast to previous studies with *a-priori* hypotheses that specific protein rich foods may be more beneficial to bone health, we chose to examine the diets of community dwelling middle-aged and older adults, utilizing novel protein-centric food patterning techniques. Although our use of protein as the primary nutrient in cluster analysis is novel (typically total energy intake is used), the dietary patterning methodology in this study has been previously validated in the Framingham Cohorts.[15] We hypothesized that multiple protein food clusters could be created using this systematic method of grouping, and that not all protein food clusters would be equally beneficial to bone health.

METHODS

Subjects

The Framingham Offspring Study is a longitudinal cohort study which began in 1971 by enrolling 5,124 adult children of the Original Framingham Study and their spouses.[16] The purpose of the Framingham Study was to identify risk factors for coronary artery disease, including familial factors. Visits occur every 4 to 8 years, where participants take part in physical examinations, blood chemistries, assessment of risk factors and questionnaires. Of the 5,124 Offspring participants, 2,764 men and women completed a validated food frequency questionnaire (FFQ) either in 1995–1998 or 1998–2001. We excluded participants with missing/incomplete FFQ, based on the criteria of more than 12 food items left blank or with energy intakes <600kcal or >4000kcal/day. Of the 2,764 men and women, 6 participants were removed following outlier analyses (as explained in statistical analysis section). Two thousand seven hundred and fifty eight participants were included in the cluster analysis to create protein dietary patterns (described thoroughly in the statistics section based on previously used[17, 18] and validated techniques[15]). Participants with missing covariate information on age, height, body mass index, smoking status, calcium and vitamin D supplement use or estrogen status were excluded after performing cluster analysis (n=17). Hence, 2,741 participants were used to describe the sample. In this cohort, BMD measures were performed between the years 1996–1998 or 1998–2001. Dietary information collected closest to participants' BMD measurement date was used in subsequent analyses (mean time difference between FFQ and BMD measurements: 255 ± 235 days). The final analytic

sample included 2,721 Framingham Offspring Cohort study participants with protein cluster and BMD data. All participants provided informed consent for their participation. This study was approved by the Institutional Review Board at Hebrew SeniorLife.

Bone mineral density

BMD was measured at the hip (regions of interest: femoral neck, trochanter, total femur) and lumbar spine (average BMD of L2–L4) in g/cm^2 using dual energy X-ray absorptiometry (Lunar DPX-L; Lunar Radiation Corporation, Madison, WI, USA). The right hip was scanned unless there was a history of previous fracture or hip joint replacement, in which case the left hip was scanned. The precision (CV) was 1.7% at the femoral neck, 2.5% at the trochanter, and 0.9% at the spine, which is similar to the range of 1.8–1.9% reported by others.[19, 20]

Dietary assessment

Usual dietary intakes of foods and nutrients were assessed with a semi-quantitative and validated 126-item food-frequency questionnaire (FFQ).[21, 22] Questionnaires were mailed to participants before each examination, and the participants were asked to complete them and bring them to the exam. This FFQ has been validated for many foods and nutrients and against multiple diet records or blood measures in several populations.[21, 23–25] Total daily protein contribution in g/day from each food consumed was calculated from the food list section of the FFQ. Percent protein contribution from individual foods to total protein intake was calculated for all participants: [(protein from specific food, in g/total protein intake, in g) × 100] for use in cluster analysis.

Covariates

Covariates known to affect bone health were included in all statistical analyses. Covariates were captured at the exam when diet was measured (either 1995–1998 or 1998–2001). These covariates included age (y), sex, menopause status and use of estrogen (women only), height (m), body mass index (BMI, kg/m^2), physical activity (continuous score), total energy intake (kcal/d), smoking status (never, former, current), alcohol intake (g/d), calcium supplement use and vitamin D supplement use. Height was measured without shoes to the nearest 0.25 inch (0.64 cm) with the use of a stadiometer. Weight was measured in pounds with the use of a standard balance-beam scale (Detecto, Worcester Scal Co., Inc.). These measures were converted to meters and kilograms, respectively, and BMI was then calculated as weight/height^2 (kg/m^2). Physical activity level was assessed using the Physical Activity Scale for the Elderly (PASE), a validated questionnaire of self-reported activity over the past seven days.[26]

Usual intakes of total energy and alcohol were assessed with the FFQ. Smoking status was defined as never, former or current smoker. Women were classified as “estrogenic” (premenopausal or currently taking post-menopausal estrogen) or “non-estrogenic” (post-menopausal and non-estrogen user) based on the following self-reported variables: current estrogen use (yes/no) and menopausal status (menstrual periods stopped for one year - yes/no).

Supplement use was captured in the supplement section of the FFQ. Calcium supplement use was then categorized as: non-supplement user (0 mg/d); supplement use from a multivitamin (supplemental calcium intake >0 and <200mg/d); or additional supplement use (supplemental calcium ≥200 mg/d). Vitamin D supplement use was categorized similarly: non-supplement user; supplement use from a multivitamin (vitamin D >0 and ≤400 IU/d); or additional supplement use (vitamin D >400 IU/d). These supplement categories were selected to identify and separate individuals receiving calcium and/or vitamin D intake from a multivitamin (a marker of a healthy lifestyle) from those who were using calcium and vitamin D supplements, possibly in an effort to improve their bone health.

Statistical analysis

Among 2,764 men and women with complete dietary information, first, the percent total daily protein contributed from each food was calculated for each individual. Foods (as a percentage of total protein intakes) were then grouped into 20 pre-defined food groups, based on similar nutrient composition, protein type or source (Supplement 1). Food groups contributing <0.5% total daily protein were removed from subsequent analyses. Dietary patterns were generated using the FASTCLUS procedure in SAS, which applies statistical methods to generate protein food clusters *a-posteriori*. This procedure applies the K-means method of cluster analysis to classify subjects into mutually exclusive groups by comparing Euclidean distances between each subject and each cluster center in an interactive process. As cluster analysis is sensitive to outliers, we verified that there were no individuals with protein contributions from food groups that were >5 standard deviations away from the mean protein contribution for that group. To further identify potential outliers, we also ran the FASTCLUS procedure with a predefined number of 20 clusters and removed individuals who fell into clusters with <8 subjects (n participants removed=6). Therefore, with the final sample of 2,758 individuals (all participants with a valid FFQ, 6 outliers removed) we re-ran the FASTCLUS procedure 7 times, requesting the procedure to produce 2 through 8 clusters to determine which number of clusters provided the most meaningful interpretation of dietary protein intake. The 5-cluster set was selected because it presented the most meaningfully separated clusters, a high F-ratio and well distributed participants between all food groups (each cluster contained >100 participants). Discussion on the methods used in the current study to interpret which cluster set was the most meaningful have been described in detail elsewhere.[27] These procedures were repeated for men and women separately; however, the cluster groups were not meaningfully different between the sexes. Therefore, men and women were combined for all subsequent analyses.

Means and standard deviations for continuous variables and proportion of participants for relevant categorical variables were calculated. Means and standard deviations were also calculated for percent of protein intake from individual food groups, and for participant characteristics across dietary protein clusters. Nutrient intakes were adjusted for energy intake using the residual method[28] prior to assessment across dietary clusters. General linear modeling was used to compare the percent protein intake from foods across protein food cluster and p-values were adjusted for multiple comparisons using Tukey-Kramer test.

Separate analyses were conducted for each BMD measure (femoral neck, trochanter, total femur and lumbar spine). Generalized linear regression was used to compare adjusted least-squares mean BMD across protein food clusters. Initial models were adjusted for age, sex, estrogen status, height, BMI, total energy intake, smoking status, energy-adjusted alcohol intake, calcium supplement use and vitamin D supplement use. Final models were further adjusted for daily physical activity. Differences in association with BMD by sex were tested in final models with an interaction term. The interaction term was not significant at any BMD site (p-range: 0.37–0.93); therefore, men and women are combined for all analyses. Resulting least squares means for each BMD site were compared across all pairwise combinations of protein food cluster groups. The Tukey-Kramer test was used to adjust for multiple comparisons. All analyses were performed using SAS software, version 9.3 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Anthropometric, demographic and vitamin supplement use characteristics of the 2,741 Offspring participants are presented in Table 1. In addition, Table 1 shows mean BMD for our bone areas of study. The ranges of BMD experienced in our cohort were as follows: femoral neck BMD ranged from 0.478 to 1.482 g/cm^2; trochanter BMD range was 0.365 to 1.445 g/cm^2; total hip range was 0.534 to 1.595 g/cm^2 and lumbar spine BMD range was 0.608 to 2.168 g/cm^2. Average BMD values for the current cohort are similar to the US average of adults 20 years and older.[29]

In Table 2, the protein food clusters are described by showing the percentage protein contribution from each pre-determined food group. Clusters were named based on the highest percentage of intake from one or more food groups (bolded in Table 2). Food groups contributing significantly greater percentages of protein intake within a cluster compared to 3 or more protein food clusters are identified by asterisks. Relative to all other groups, the members of protein food cluster 1 (labeled as the “chicken cluster”, n=564) derived most of their total protein intake from chicken. Members of the second protein food cluster (labeled as the “fish cluster”, n=322) derived most of their total protein intake from fish. Protein food cluster 3 (labeled as the “processed food cluster”, n=833) showed the greatest variation in protein intake from all sources. Members of this protein pattern received most of their protein intake from pizza and French fries, snacks, white grains and cheese products. Members of the fourth protein food cluster (labeled the “red meat cluster”, n=666) derived the majority of their protein intake from red meat. Lastly, the fifth protein food cluster (labeled the “low-fat milk cluster”, n=356) derived most of their protein intake from low fat milk.

Descriptive characteristics of participants across protein food clusters are shown in Table 3. Age, BMI and percentage of calcium and vitamin D supplement users were similar across protein food clusters. More women consumed protein within the chicken cluster than men. Processed foods and red meat clusters included the greatest percentage of smokers. The lowest total protein intake in g/d was present among individuals in the processed foods cluster. The highest protein intake was present among individuals in the chicken cluster.

Individuals in the low-fat milk cluster presented with the least amount of alcohol intake in g/d (the highest intake in the red meat cluster).

Results on the comparisons of BMD across protein food clusters are presented in Table 4. After adjustment for relevant confounders and covariates, femoral neck BMD was significantly lower among participants in the processed foods cluster (p=0.02) and red meat cluster (p=0.049) compared to the low-fat milk cluster. BMD at other hip sites, but not the lumbar spine, showed similar trends, where BMD was lowest among participants in the processed foods and red meat clusters compared to the low-fat milk cluster; however, these associations did not reach statistical significance. Adjustment for physical activity did not change the least squares mean estimates at the femoral neck, although the p-value attenuated slightly for the difference between red meat and low-fat milk protein food clusters from p=0.049 to p=0.056 (see Table 4 for unchanged least squares mean estimates). Similarly, upon adjustment for physical activity, the least squares mean estimates for femoral neck did not change for the test of difference between processed foods protein food cluster and the low-fat milk protein food cluster (Table 4; p-value unchanged at 0.02).

DISCUSSION

Five protein food clusters were indentified in this cohort of largely middle-aged men and women. Overall, our results show that individuals in the processed foods and red meat protein food clusters had significantly lower femoral neck BMD compared to individuals in the low-fat milk protein food cluster. Similar associations were observed for the total femur, trochanter; however the results did not reach statistical significance. Patterns in differences of lumbar spine BMD across protein food clusters remain less clear and did not reach statistical significance.

Cohort studies examining the relation between dietary protein (absolute intake, g/d) and bone health in older adults support an overall positive relation.[6, 30] Previous work from our group using data from the Framingham Osteoporosis Study showed that greater dietary protein is associated with decreased odds of falling[31] and is protective against the risk of hip fracture.[32] However, long term intervention trials supplementing protein to older individuals show less conclusive results.[33, 34] The difference in results may be due to the type of protein intervention used in each study. The first study provided additional protein via dietary intervention and observed less bone loss over 12 months[33]; where the second study provided a whey protein supplement and observed no change in BMD over 2 years.[34] Thus, the differing results may be due in part to how additional protein is supplemented (dietary source versus protein powder). Results from the current study highlight that the association of dietary protein with bone varies dependent upon protein food source and the synergy of these protein rich foods with nutrients consumed concurrently. These results are the first to suggest that protein food source and other components of the diet consumed with protein-rich foods should be considered when evaluating the relation between dietary protein and bone health.

Protein-rich foods from various sources may differentially affect bone health because they differ in their protein content, amino acid composition, digestibility, and synergy with other

nutrients.[12] For example, protein quality, defined by essential amino acid composition, varies between foods. In many studies, animal protein (meat and dairy, which provide all essential amino acids) has been associated with higher BMD[35, 36] and improved calcium metabolism[37] in comparison to plant protein sources, which do not provide all essential amino acids from one source alone. Studies have reported high animal protein intake to be associated with greater BMD[38] and decreased risk of fracture.[35, 39, 40] However, a few studies have reported that a high ratio of animal:plant protein in diet was associated with greater fracture risk.[41, 42] Although these studies provide insight into protein food sources and their differing associations with bone, they do not capture the interaction of dietary protein with other nutrients consumed in the diet. In the current study, when all components of the diet were taken into account (see supplemental table), the different animal sources of protein were not uniform in their association with BMD. In fact, the red meat protein food cluster presented with the lowest BMD compared to other protein food clusters. This may be explained by the higher saturated fat content found in red meat compared to other animal protein sources. Saturated fat has been shown to be detrimental to bone in adults[43], possibly by reducing calcium absorption from the intestine[44], reducing bone formation[45], and enhancing bone resorption.[46]

Individuals in the processed protein foods cluster also presented with lower bone density measurements compared to other clusters. The processed food cluster consisted of a high percentage of protein intakes from cheese, processed meat, sweet baked products, pizza and French fries, snacks and white grains in comparison to the other protein food clusters. Processed meats and processed cheeses are high in sodium. High sodium diets have been shown to alter calcium metabolism[47] and to increase bone resorption in postmenopausal women.[48] Conversely, low sodium diets have been shown to be protective of bone health[49] by reducing bone turnover and improving calcium balance.[50] Sodium phosphate salts, also found in processed foods and cheeses, have been shown to increase serum parathyroid hormone levels, which is unfavorable to bone metabolism.[51] The presence of these nutrients in processed foods and red meat may explain in part why the relation of protein with bone health was attenuated among individuals in these food clusters. It is also likely that consumption of high protein processed foods accompany other unhealthy food choices linked with reduced bone loss. Greater consumption of less nutrient-dense foods may explain in part, why the processed foods group had significantly less total protein intake (g/d) compared to all other clusters.

Not surprisingly, the low-fat milk protein food cluster was beneficially related to BMD among older adults. Milk proteins have been uniquely linked with altered bone metabolism and improved BMD among adults.[52] Furthermore, low-fat milk is rich in calcium, a nutrient that has been reported to modify the effect of protein on bone[53] likely due to protein's ability to increase intestinal calcium absorption.[7, 8] A randomized controlled trial found that higher protein intake was associated with a favorable 3-year change in BMD, but only under conditions of calcium plus vitamin D supplementation (500mg + 700IU daily).[53] Similar findings were reported for fracture outcomes in a longitudinal cohort study of Framingham Offspring participants (average age 55years), which reported that among individuals with calcium intakes less than 800mg/d, the highest tertile of animal protein intake had 2.8 times the risk of hip fracture versus the lowest tertile of intake.[40] However, in the 800mg/d or

more calcium intake group, the highest tertile of animal protein intake had an 85% reduced hip fracture risk versus the lowest tertile. The current study builds on our previous protein studies by using novel patterning techniques to create protein clusters, which account for synergistic effects of individuals nutrients. Thus results from the previous studies in combination with the latest findings from our protein food cluster analysis suggest that the synergy between protein and calcium within low-fat milk may play a role in the bone health of older adults.

Although all bone sites showed a similar trend in being lower among individuals in the processed foods and red meat clusters, the only site which reached statistical significance was the femoral neck. Among clinical risk factors, measurement of BMD particularly at the femoral neck has the most robust predictive value for risk of various fractures[54, 55] and is the most used site for osteoporosis assessment.[56] The magnitude of difference in femoral neck BMD among individuals in the processed foods group compared to the low-fat milk group is similar to the difference observed in BMD between current smokers and never smokers. Therefore, the results from the current study provide clinically meaningful results which could impact the bone health of middle-aged and older adults. Further study of longitudinal design is needed to determine whether long term dietary protein pattern has the potential to forestall bone loss and prevent hip fracture. The BMD values for the current cohort, in terms of mean values and ranges, are similar to the US average of adults 20 years and older.[29] The similarities between our study participants and the national experience of typical BMD values speaks to possible generalization on the distributions in each population. It is important to note that we are evaluating BMD as it is measured, and not the clinical cut points of osteoporosis as estimated by T-scores in clinical settings.

While we adjusted for several potential confounders, residual confounding may be a concern. Another limitation to this study is its cross-sectional design. Due to the study design, it is difficult to distinguish whether chronic protein intake in these whole diet groupings would alter bone density over time. It will be important to look at longitudinal bone changes and their relation with protein food groups in future research. Further, it is difficult to determine whether the differences in BMD between food clusters is due solely to the differing proteins among these foods, or additionally due to other nutrients consumed and their interaction with protein. Answers to these questions require further mechanistic and randomized study. This study has several important strengths. These include the large sample size from a community-based cohort of middle aged and older adults. Further, this study used comprehensive dietary assessments that have been shown to estimate usual nutrient intake. The examination of percent contribution of protein intake to the total diet, and assessing intake by cluster analysis are unique aspects of this study. It is important to note that previous single nutrients studies were often unable to isolate individual nutrient effects of calcium and protein upon bone due to high correlation of these nutrients in some foods. Assessment of intake by dietary pattern analysis permitted us to examine dietary protein's association with bone health, taking into consideration its synergy with other nutrients in the diet and food source. Cluster analysis offers advantages over the alternative quantitative approaches as it aims to identify distinct, relatively homogeneous groups based upon selected attributes: in this case, percentage contribution of protein. Disadvantages to cluster analysis include its sensitivity to outliers and the need for subjective interpretation of

the clusters after the statistical modeling is complete. The current study employed methods to remove outliers and outlined decisions regarding naming and interpretation of the 5-cluster set used in detail.

In conclusion, cluster analysis by percentage contribution of foods to total protein intake produced five distinct food clusters. The processed foods and red meat protein food clusters were related to lower bone mineral density compared to other protein derived food clusters in this study of largely middle-aged and older adults. The low-fat milk protein food cluster presented with the greatest hip bone mineral density compared to all other clusters. It is important to examine the association of protein intake with bone health in the context of the whole diet. Future intervention trials to alter bone health with protein dense foods should take this into consideration and can be used for potential therapeutic targets in benefiting the bone health of adults.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

KMM, SS, MTH, KLT designed research; KMM, SS, DPK, KLT, MTH conducted research; KMM and ABD analyzed data; KMM, SS, DPK, KLT, ABD, MTH wrote the paper; KMM and MTH had primary responsibility for final content. All authors read and approved the final manuscript

Sources of support: NIH's National Institute of Aging (T32-AG023480), NHLBI Framingham Heart Study (N01-HC-25195), Framingham Osteoporosis Study (NIH R01 AR041398), Academy of Nutrition and Dietetics Healthy Aging Dietetics Practice Group Research Award.

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Table 1

Characteristics of participants from the Framingham Offspring Study with valid dietary data and hip or spine bone mineral density between the years of 1995–2001 (n=2,741)

Characteristics	Mean ± SD or %
Men (%)	43.9
Age (years)	60.8 ± 9.3 (range 29, 86)
Height (cm)	167 ± 9
Body mass index (kg/m^2)	28.0 ± 5.1
Smoking status (current, %)	12.1
Total energy intake (Kcal/d)	1833 ± 592
Dietary protein (g/d)	78 ± 27
Alcohol intake (g/d)	9.9 ± 14.9
Physical Activity Scale for the Elderly	144.7 ± 79.2
Bone Mineral Density (BMD, g/cm^2)	
Femoral Neck BMD	0.913 ± 0.149
Trochanter BMD	0.788 ± 0.162
Total Femur BMD	0.968 ± 0.161
Lumbar Spine BMD	1.226 ± 0.219
Estrogen status (among women, %)	
Estrogenic	45.8
Non-estrogenic	54.2
Calcium supplement use (%)	
None	57
Calcium from multivitamins (0<intake<200 mg/d)	9
Calcium from other supplements (≥200 mg/d)	34
Vitamin D supplement use (%)	
None	52
Vitamin D from multivitamins (0<intake≤400 IU/d)	36
Vitamin D from other supplements (>400 IU/d)	12

Estrogenic status was defined as: estrogenic if pre-menopausal, or post-menopausal taking hormone replacement therapy; non-estrogenic if post-menopausal, not taking hormone replacement therapy.

Table 2

Average percentage of total protein intake from individual food group across protein food clusters among 2,741 men and women from the Framingham Offspring Cohort[1]

Food Group[2]	Cluster 1 Chicken (n=564)	Cluster 2 Fish (n=322)	Cluster 3 Processed foods (n=833)	Cluster 4 Red meat (n=666)	Cluster 5 Low fat milk (n=356)
Beans and peas	1.7 ± 2.1	2.4 ± 2.3*	2.3 ± 2.4	1.6 ± 1.3	1.9 ± 2.3
Low fat milk	5.0 ± 5.5	4.5 ± 4.6	5.2 ± 4.4	3.8 ± 4.5	**24.0 ± 7.7***
Whole milk	0.4 ± 1.7	0.4 ± 1.6	1.0 ± 3.3*	1.2 ± 3.7*	0.3 ± 1.9
Cream	1.3 ± 1.5	1.4 ± 1.5	1.9 ± 1.7*	1.5 ± 1.3	1.4 ± 1.4
Yogurt	1.9 ± 3.4	2.2 ± 3.5	2.2 ± 4.0	1.0 ± 2.4	2.2 ± 3.8
Cheese products	3.3 ± 3.4	3.6 ± 3.4	**5.8 ± 5.7***	3.9 ± 3.2	3.1 ± 3.1
Red meat	10.4 ± 6.2	9.7 ± 6.0	13.8 ± 5.0	**29.5 ± 6.7***	11.8 ± 7.1
Processed meat	1.0 ± 1.5	1.0 ± 1.4	1.9 ± 2.0*	1.9 ± 1.9*	1.1 ± 1.3
Chicken	**34.6 ± 7.8***	16.9 ± 7.6*	15.2 ± 6.4	15.7 ± 7.3	12.9 ± 6.5
Fish	9.0 ± 5.4	**23.3 ± 6.6***	9.2 ± 4.2	7.6 ± 4.7	8.7 ± 5.3
Eggs	1.5 ± 1.8	1.6 ± 1.7	2.1 ± 2.4*	2.1 ± 2.1*	1.4 ± 1.4
Fruit and vegetables	8.3 ± 3.4	9.8 ± 3.7*	9.7 ± 4.0*	7.7 ± 2.9	8.6 ± 3.6
Nuts	1.7 ± 2.8	2.4 ± 3.5	2.9 ± 3.8*	1.9 ± 2.6	1.6 ± 1.9
Cereal	2.5 ± 2.9	3.2 ± 3.2	3.2 ± 3.4	1.8 ± 2.2	4.0 ± 3.5*
Sweet baked products	2.4 ± 2.2	2.6 ± 2.1	**3.6 ± 2.7***	3.0 ± 2.4	2.9 ± 2.7
Pizza and French fries	3.9 ± 3.4	3.6 ± 3.1	**5.7 ± 5.2***	4.4 ± 3.4	3.3 ± 2.4
Snacks	1.4 ± 2.1	1.2 ± 1.3	**2.0 ± 2.5***	1.6 ± 1.8	1.2 ± 1.5
White grains	6.0 ± 4.1	6.0 ± 3.7	**7.8 ± 5.1***	6.3 ± 3.7	5.9 ± 3.6
Whole grains	2.0 ± 2.4	2.6 ± 2.6	2.5 ± 2.9	1.7 ± 2.0	2.4 ± 2.7
Other protein sources	0.5 ± 2.0	0.4 ± 1.7	0.5 ± 2.2	0.3 ± 1.4	0.5 ± 2.1

[1] Mean ± SE

[2] Percentage of protein intake from individual food groups cumulatively adds up to approximately 100%, and may be <100% due to the omission of food groups providing <0.5% total protein intake to the diet

* Significantly greater percent protein intake within a food group compared to 3 or more protein food clusters, on the basis of comparisons in general linear models, adjusted for multiple comparisons using Tukeys test P<0.05

Table 3

Characteristics of 2,741 study participants from the Framingham Offspring Cohort across protein food clusters[1]

Characteristic	Chicken (n=564)	Fish (n=322)	Processed foods (n=833)	Red meat (n=666)	Low fat milk (n=356)
Age (years)	59.1 ± 8.7	62.1 ± 8.8	61.2 ± 9.6	60.3 ± 9.3	61.8 ± 9.7
Male (%)	37	44	45	49	42
Smoking status (current, %)	11	7	13	16	8
Body mass index (kg/m^2)	28.3 ± 5.5	27.4 ± 4.8	27.5 ± 4.7	28.7 ± 5.4	27.9 ± 5.2
Height (in)	65.4 ± 3.7	65.9 ± 3.8	65.9 ± 3.8	66.0 ± 3.7	65.7 ± 3.6
Physical Activity Scale for the Elderly	151.3 ± 77.5	145.6 ± 85.4	143.1 ± 77.1	139.0 ± 80.3	147.7 ± 78.3
Non-estrogenic women (%)	32	31	31	26	32
Calcium supplement user (%)					
None	55	51	58	63	54
Multivitamin (<200mg/d)	8	10	10	8	10
Additional (≥200mg/d)	36	39	32	29	36
Vitamin D supplement user (%)					
None	49	50	52	57	49
MVI (≤400IU/d)	39	33	37	34	37
Additional (>400IU/d)	12	17	11	9	14
Nutrient intakes[2]					
Total energy (kcal/d)	1668 ± 559	1716 ± 529	1892 ± 594	1954 ± 613	1830 ± 576
Total protein (g/d)	88 ± 16	86 ± 15	76 ± 13	82 ± 14	84 ± 14
Dietary calcium (mg/d)	774 ± 211	777 ± 202	822 ± 202	697 ± 212	1270 ± 299
Dietary vitamin D (IU/d)	215 ± 89	291 ± 114	206 ± 84	186 ± 85	390 ± 106
Alcohol (g/d)	12.7 ± 14.2	12.5 ± 11.7	12.7 ± 14.5	14.9 ± 18.1	8.9 ± 9.8

[1] Mean ± SD

[2] Energy-adjusted nutrient intakes, mean ± SD

Table 4

Association of dietary protein food clusters with bone mineral density (g/cm^2) in 2,721 men and women from the Framingham Offspring Cohort

Variable	*n*	Least squares means ± SE[1] for bone mineral density by protein food group				
		Cluster 1 Chicken	Cluster 2 Fish	Cluster 3 Processed foods	Cluster 4 Red meat	Cluster 5 Low fat milk
Bone Mineral Density						
Model 1 [2]						
Femoral neck	2720	0.909 ± 0.005	0.910 ± 0.007	0.897 ± 0.004[a]	0.898 ± 0.005[a]	0.919 ± 0.007[b]
Trochanter	2720	0.772 ± 0.005	0.773 ± 0.007	0.765 ± 0.004	0.765 ± 0.005	0.778 ± 0.007
Total Femur	2720	0.956 ± 0.006	0.956 ± 0.007	0.948 ± 0.005	0.948 ± 0.005	0.964 ± 0.007
Lumbar Spine	2721	1.224 ± 0.009	1.208 ± 0.011	1.208 ± 0.008	1.212 ± 0.009	1.226 ± 0.011
Model 2 [3]						
Femoral neck	2689	0.908 ± 0.006	0.911 ± 0.007	0.897 ± 0.005[a]	0.899 ± 0.006	0.920 ± 0.007[b]
Trochanter	2689	0.771 ± 0.005	0.774 ± 0.007	0.766 ± 0.004	0.765 ± 0.005	0.777 ± 0.007
Total Femur	2689	0.956 ± 0.006	0.957 ± 0.007	0.949 ± 0.005	0.949 ± 0.005	0.965 ± 0.007
Lumbar Spine	2690	1.223 ± 0.009	1.209 ± 0.011	1.208 ± 0.008	1.212 ± 0.009	1.223 ± 0.011

[1] The analyses were adjusted for multiple comparisons using Tukeys test (different superscripts represent statistically significant differences at P<0.05).

[2] Adjusted for age, sex, estrogen status, BMI, height, total energy intake, current smoking status, energy adjusted alcohol intake, calcium supplement use, vitamin D supplement use

[3] Further adjusted for physical activity (measured by Physical Activity Scale for the Elderly)