

1 **Association of protein intake with bone mineral density and bone mineral content among**  
2 **elderly women: the OSTPRE Fracture Prevention Study.**

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## 25 Abstract

26 It has been hypothesized that high protein intakes is associated with lower bone mineral content  
27 (BMC). Previous studies yield conflicting results and thus far no studies has undertaken the  
28 interaction of body mass index (BMI) and physical activity with protein intakes in relation to BMC  
29 and bone mineral density (BMD). **Objective:** To evaluate the associations of dietary total protein  
30 (TP), animal protein (AP) and plant protein (PP) intakes with BMC and BMD and their changes.  
31 We tested also the interactions of protein intake with, obesity (BMI  $\leq 30$  vs.  $>30$  kg/m<sup>2</sup>) and  
32 physical activity level (passive vs. active). **Design/ Setting:** Prospective cohort study  
33 (Osteoporosis Risk-Factor and Fracture-Prevention Study). **Participants/measures:** At the  
34 baseline, 554 women aged 65-72 years filled out a 3-day food record and a questionnaire covering  
35 data on lifestyle, physical activity, diseases, and medications. Intervention group received calcium  
36 1000 mg/d and cholecalciferol 800 IU for 3 years. Control group received neither supplementation  
37 nor placebo. Bone density was measured at baseline and year 3, using dual energy x-ray  
38 absorptiometry. Multivariable regression analyses conducted to examine the associations between  
39 protein intake and BMD and BMC. **Results:** In cross-sectional analyses energy-adjusted TP  
40 ( $P \leq 0.029$ ) and AP ( $P \leq 0.045$ ) but not PP (g/d) were negatively associated with femoral neck (FN)  
41 BMD and BMC; women with  $TP \geq 1.2$  g/kg/body weight (BW) ( $P_{\text{trend}} \leq 0.009$ ) had lower FN, lumbar  
42 spine (LS) and total BMD and BMC. In follow-up analysis, TP (g/kg/BW) was inversely  
43 associated with LS BMD and LS BMC. The detrimental associations were stronger in women with  
44 BMI  $< 30$  kg/m<sup>2</sup>. In active women, TP (g/kg/BW) was positively associated with LS BMD and FN  
45 BMC changes. **Conclusions:** This study suggests detrimental associations between protein intake  
46 and bone health. However, these negative associations were counteracted by BMI  $> 30$  kg/m<sup>2</sup> and  
47 physical activity.

48 **Keywords:** Dietary protein intake. Source of protein intake. Bone mineral density. Physical  
49 activity. Body mass index

## 50 **Introduction**

51 Osteoporosis is major public health problem, particularly in women (1). Bone mineral density  
52 (BMD) and bone mineral content (BMC) measured by dual energy x-ray absorptiometry (DXA),  
53 have been considered as important determinants of osteoporotic fractures (2). It is crucial to  
54 identify risk factors associated with low BMD due to its importance to fracture, functional quality  
55 of ageing as well as significant health costs (3). The role of dietary protein in bone health is unclear  
56 and also might be dependent on the presence of other factors (3-6). In meta-analysis by Darling et  
57 al. (6) for cross-sectional studies of protein intakes and BMD no association or a small positive  
58 association have been suggested. The source of protein consumed may be differentially associated  
59 with bone health in adults (7). It has been suggested that consumption of animal protein sources  
60 (AP) containing high acidifying amino acids might increase the risk of bone loss (8), while plant  
61 protein (PP) based diets contain isoflavones that may have protective effects on bone health (9).  
62 Further studies examining the sources of protein and their potential differentiating associations  
63 with bone health are warranted. Further understanding of the mechanisms behind how protein  
64 modifies bone metabolism, will provide future therapeutic targets in forestalling bone loss with  
65 aging (10, 11).

66 Protein might increase the protein-sensitive anabolic mediator of calcium such as insulin like  
67 growth factor (IGF-1) and increase intestinal calcium absorption (12, 13), whereas short term  
68 intervention study using purified protein supplements have shown that 1 mg calcium is on average  
69 lost in the urine for every 1 g increase in protein intake (14). However, whether bone is the source  
70 of this calcium loss has not been shown. Furthermore, body weight (BW) is an important  
71 determinant of BMD, individuals with higher BW have higher BMD and reduced fracture risk  
72 (15). Between-individuals variation in BW accounts for about 30% of variation in BMD, making  
73 it one of the strong determinants of BMD (16). Besides, it is evident from previous studies that  
74 physical activity has strong beneficial effect on bone health (17). It was shown also that physical  
75 activity and protein-containing supplement have positive effect on femoral neck (FN) BMD (18).  
76 However, whether greater physical activity combined with dietary protein are associated with  
77 increased BMD has not been investigated in cohort studies (19).

78 In this study, we evaluated the associations of total protein (TP), and protein intake by food source  
79 (AP and PP intakes) with BMD and BMC at lumbar spine (LS), FN and total body among elderly

80 women at the baseline and over 3 year of follow-up. We further tested the interaction of TP (g/kg  
81 BW) with BMI and physical activity in relation to BMD and BMC.

## 82 **Materials and methods**

### 83 *Study design and participants*

84 Data of the present study were collected from the Osteoporosis Risk Factor and Fracture  
85 Prevention Study (OSTPRE-FPS), which was a 3-year intervention to investigate the effect of  
86 calcium and vitamin D supplementation on incidence of falls and fractures among elderly women.  
87 Inclusion criteria were being older than 65 years of age by the end of November 2002, residing in  
88 Kuopio region and no previous participation in OSTPRE bone densitometry sample. The  
89 intervention (supplementation) group (n=287) received daily cholecalciferol 800 IU (20 µg) and  
90 calcium 1000 mg for 3 years while the control group (n=306) received neither supplementation  
91 nor placebo (20). In total 750 women were randomly taken into this subsample for participating in  
92 detailed examinations including measurement of bone density and body composition and food  
93 records. Out of those, 554 returned valid food record and had valid body composition  
94 measurements for both at the baseline and after 3 year (21). All clinical measurements were  
95 performed in Kuopio Musculoskeletal research unit of the Clinical research center of the  
96 University of Kuopio, Kuopio, Finland. All participants provided written permission for  
97 participation. The study was approved in October 2001 by the ethical committee of Kuopio  
98 University Hospital. The study was registered in Clinical trials.gov by the identification  
99 NCT00592917.

### 100 *Bone density measurements*

101 BMC (g) was measured at the baseline and year 3, using DXA (Lunar Prodigy, Wisconsin, USA)  
102 for LS (L2-L4), FN and total body by trained nurses. BMD ( $\text{g}/\text{cm}^2$ ) was calculated as BMC  
103 (g)/bone area ( $\text{cm}^2$ ). DXA is a standard and the most widely used technique to determine BMD  
104 since the late 1980s (22). Technical quality of measurements was double checked and those with  
105 any measurement errors were excluded from the statistical analysis. The long-term reproducibility  
106 (CV) of the DXA instrument for BMD during the study period, as determined by regular phantom  
107 measurements, was 0.4% (20). Absolute changes in BMD and BMC were further calculated with  
108 the use of baseline and year 3 values. Height and weight of participants were measured in light  
109 indoor clothing without shoes, and body mass index (BMI) was calculated ( $\text{kg}/\text{m}^2$ ).

### 110 *Dietary intakes*

111 Dietary intake was collected by using 3-day food record at the baseline. A questionnaire and  
112 instructions were sent to participants beforehand, and they were returned on the visiting day.  
113 Participants were advised to fill the questionnaire for 3 consecutive days, including 2 days during  
114 the week and one day in the weekend (Saturday or Sunday). In case of uncertainties in the food  
115 record, a nutritionist called the participant for additional information (23). To assess the  
116 underreporting the ratio of energy intake to estimated basal metabolic rate was calculated based  
117 on BW according to equations given by Department of Health in the UK (24). The ratio of energy  
118 intake to basal metabolic rate cutoff value for under-reporting was chosen to be 1.49, as derived  
119 from Goldberg et al.(25) and Black (26) and none of the participants was excluded from the  
120 analyses (27). Collected data provided calculations of AP (including egg, dairy, poultry and meat)  
121 and PP sources (including cereals, grains, vegetables and fruits) of protein in addition to TP intake.  
122 Nutritional intake from food was calculated using Nutrica program (version 2.5, Finnish social  
123 insurance institute, Turku, Finland).

### 124 *Questionnaire*

125 All lifestyle related information was gathered by the self-administered questionnaire. The  
126 questionnaire included questions on age, hormone therapy use (never used, used), time since  
127 menopause (years), smoking status (present status), self-reported calcium and vitamin D  
128 supplementation (yes, no) and alcohol consumption (portions/ week). Total exercise time/week  
129 was based on self-reported amounts and types of exercise/week. Participants were questioned also  
130 for their mobility status and categorized as no restriction, restricted and no mobility at the baseline.  
131 Diseases possibly affecting BMD included hyperthyroidism, disease of parathyroid gland, chronic  
132 liver disease, chronic intestinal disease, celiac disease, ventricle operation, chronic nephropathy  
133 arthritis, osteoporosis, and lactose intolerance. Medications that may influence BMD included  
134 loop-diuretics, insulin, antiepileptics, glucocorticoids and cancer chemotherapy (20).

### 135 *Statistical analysis*

136 All statistical analysis were executed using SPSS software version 21 for Windows (IBM Corp.,  
137 Armonk, NY). Result was significant if a *P* value was < 0.05. The protein intakes (TP, AP and  
138 PP) were adjusted for energy intake utilizing the residual method (28). An advantage of this

139 method is that it provides a measure of protein intake which is independent of total energy  
140 intake. Protein intake g/kg BW was calculated using crude protein intake divided per BW.  
141 Further, the selection of TP (g/kg BW) cut-offs were based on three different nutrition  
142 recommendations, RDA (29) ( $\leq 0.8$  g/kg BW), PROT-AGE Study Group recommendation (30)  
143 ( $0.81-1.19$  g/kg/BW), and Nordic Nutrition recommendation ( $\geq 1.2$  g/kg BW) (31).

144 One way ANOVA was used to test differences in means of baseline characteristics of participants  
145 among quartiles of energy-adjusted protein intake. Each of the BMD and BMC measures at the  
146 baseline and changes in them over 3 year of follow-up were set as dependent variable in multiple  
147 linear regression or logistic regression models. Tests for a linear trend across categories of protein  
148 intake (g/kg BW) were conducted by using the median value in each category of protein intake as  
149 a continuous variable in the linear and logistic regression models.

150 Model 1 was adjusted for age, energy intake, height, weight, and study group (intervention calcium  
151 and vitamin D). Model 2 was further adjusted for variables in model 1 plus dietary calcium and  
152 vitamin D intake, self-reported vitamin D and calcium supplementation, smoking status, physical  
153 activity level, hormone therapy use, time since menopause (years), diseases and use of medications  
154 which affect BMD. BMD and BMC variables at the baseline were entered in longitudinal models  
155 as an independent variable to account for differential subsequent changes of BMD and BMC  
156 depending on initial measures. AP and PP intakes were included in the same regression model to  
157 adjust for each other. To manage the strong collinearity of the protein intake as expressed per BW  
158 (dependent variable) and BW as covariate, in analysis using TP (g/kg BW), BW was dropped from  
159 the adjusted covariates (32, 33).

#### 160 *Subgroup analysis*

161 We tested the interaction of TP (g/kg BW) with obesity and physical activity level. Obesity was  
162 defined using WHO criteria where women with BMI  $>30$  g/kg m<sup>2</sup> were categorized as obese (34).  
163 The physical activity level was compiled from frequency of exercise times per week and mobility  
164 status. Women were classified as passive if they had restricted or no mobility and exercise  $\leq 2$   
165 times/week and those with no mobility restriction and exercise  $> 2$  times/week were classed as  
166 active. Interactions between TP intake g/kg BW with obesity status (BMI  $\leq 30$  and  $> 30$  kg/m<sup>2</sup>)  
167 and physical activity level (passive/active) were tested by introducing an interaction term in model  
168 2. In this data total intake of calcium at the baseline did not predict annual BMD changes (20).We

169 also checked for the interaction of dietary calcium intake, self-reported calcium supplement and  
170 total calcium intake (dietary + self-reported calcium supplement) with protein intake in relation to  
171 BMD and BMC, and associations were not significant.

## 172 **Results**

173 The mean age was 68.1 (SD 1.9) years, and mean energy intake was 6560 (SD 1556) kJ/d (Table  
174 1). Total protein intake was 68.2 g/d which constituted 17% of total energy intake and  
175 corresponded to 0.96 g/kg BW. Women in the second and fourth quartiles of energy-adjusted TP  
176 intakes had significantly higher BW. Women in the first and third quartiles of TP intake reported  
177 more use of HT (46%) as compared to women in the second and fourth quartiles. Those in the third  
178 quartile had higher percentage of participation in calcium and vitamin D interventional  
179 supplementation and also had higher self-reported vitamin D supplementation.

180 Total energy intake (kJ/d), dietary calcium and total calcium intake (mg/d) were significantly  
181 higher in higher quartiles of protein intake and total fat intake (g/d) was highest in the fourth  
182 quartile. TP and AP intakes were significantly higher in higher quartiles of protein intake, while  
183 no significant association was observed for PP intake. Dietary carbohydrate (g/d) and phosphorus  
184 (mg/d) intakes were highest in the first quartile and dietary magnesium intake (mg/d) increased by  
185 higher protein intake. Mean BMD at the baseline was 1.096 g/cm<sup>2</sup> (T-score: -0.78), 0.869 g/cm<sup>2</sup>  
186 (T-score: -0.924) and 1.077 g/cm<sup>2</sup> (T-score: -0.603) for LS, FN and total body, respectively. In 3  
187 years of follow up FN BMD decreased by -1.89%, while LS and total body BMD increased by  
188 +0.93% and +0.56%, respectively.

### 189 *Cross-sectional BMD and BMC*

190 At the baseline in model 2 energy adjusted TP ( $\beta \geq -0.19$  and  $P \leq 0.029$ ) and AP ( $\beta \geq -0.02$  and  $P$   
191  $\leq 0.029$ ) were negatively associated with FN BMD and FN BMC, while no such association was  
192 observed for PP intake (**Table 2**). Further, TP (g/kg BW) ( $\beta \geq -0.28$  and  $P \leq 0.009$ ) was in negative  
193 associations with FN, LS and total BMD and BMC. Similar results were observed using categories  
194 of protein intake (g/kg BW) where women with higher protein intake  $\geq 1.2$ g/kg BW had the lowest  
195 LS, FN and total BMD and BMC at the baseline (data not shown).



### 196 *Longitudinal changes in BMD and BMC*

197 Results for the prospective analysis are presented in total population in **Table 3**. The interactions  
198 between energy-adjusted TP, AP and PP intakes (g/d) as well as TP (g/kg BW) and interventional  
199 vitamin D and calcium supplementation were not significant ( $P \geq 0.660$ ) so groups are kept  
200 together. In the prospective analysis in model 2, TP intake (g/kg BW) was negatively associated  
201 with changes of LS BMD and LS BMC ( $\beta \geq -0.30$  and  $P \leq 0.002$ ).

### 202 *Protein and BMI interaction*

203 The interaction between protein intake and BMI was significant only for association with FN and  
204 LS BMC ( $P_{\text{interaction}} \leq 0.007$ ). At the baseline, in women with  $\text{BMI} \leq 30 \text{ kg/m}^2$ , TP (g/kg BW) was  
205 negatively associated with LS and FN and total BMD ( $\beta \geq -0.25$  and  $P \leq 0.050$ ) as well as FN and  
206 total BMC ( $\beta \geq -0.31$  and  $P \leq 0.007$ ) (**Table 4**). In prospective analysis, among women with  $\text{BMI}$   
207  $\leq 30 \text{ kg/m}^2$ , TP intake (g/kg BW) was negatively associated with change of LS BMD ( $\beta = -0.31$   
208 and  $P = 0.016$ ).

### 209 *Protein and physical activity interaction*

210 Association of TP (g/kg BW) at the baseline and over 3 year of follow-up was further explored  
211 according to physical activity level of the participants (**Table 5**). Interaction between TP and  
212 physical activity level was significant only in association with total BMC and BMD ( $P_{\text{interaction}} \leq$   
213  $0.050$ ). At the baseline TP (g/kg BW) was negatively associated with FN BMD ( $\beta \geq -0.26$  and  $P$   
214  $\leq 0.041$ ) and FN BMC ( $\beta \geq -0.22$  and  $P \leq 0.036$ ) in both physically passive and active women. In  
215 prospective analysis, among passive women TP (g/kg BW) was negatively associated with LS  
216 BMD and LS BMC loss ( $\beta \geq -0.43$  and  $P \leq 0.003$ ), while among active women TP (g/kg BW) was  
217 in positive relationships with changes of LS BMD ( $\beta = 0.23$  and  $P = 0.047$ ) and FN BMC ( $\beta = 0.21$   
218 and  $P = 0.049$ ) over 3 years of follow-up.

## 219 **Discussion**

220 In our data at the baseline energy-adjusted TP (g/d) and AP (g/d) but not PP (g/d) were negatively  
221 associated with FN BMD and BMC. Women with higher protein intake (g/kg BW) also had lower  
222 FN, LS and total BMD and BMC. In follow-up analysis TP (g/kg BW) was associated with loss  
223 of LS BMD and LS BMC. To the best of our knowledge this is the first cohort study which focused  
224 on different modifiers in association of protein intake with BMD and BMC. We evaluated and

225 suggested that association of dietary protein intake with bone density may differ according to  
226 participants' lifestyle characteristics. TP (g/kg BW) negatively associated with BMD and BMC  
227 only in women with  $BMI \leq 30\text{kg/m}^2$ , and it was in positive relationship with changes of LS BMD  
228 and FN BMC in active women. These findings were observed independent of relevant covariates  
229 and confounders.

230 Most of the previous cross-sectional observational studies reported positive association between  
231 protein intake and higher BMD (6, 7, 35) or did not detect detrimental associations (36, 37).  
232 Findings by Sahni et al.(35) showed that protein intake was positively associated with FN,  
233 trochanter and LS BMD in women, while no significant associations were seen in men at any bone  
234 site. In contrast, in study by Darling et al.(38) in 176 postmenopausal women (aged 58 years and  
235 older) protein intake was negatively associated with LS and FN BMD as well as FN BMC.

236 Protein intake from different dietary sources may influence bone health by different mechanisms,  
237 including increasing calcium absorption or regulating plasma IGF-1 that increases bone formation  
238 (38, 39). PP based diets contain isoflavones that may have protective effects on bone health,  
239 however, their protective effects were not observed when used as dietary supplementation (9). AP  
240 sources contain more sulphur-containing amino acids such as methionine and cysteine as compared  
241 to PP sources that can release protons which may decrease the pH and therefore increase the bone  
242 dissolution and bone loss (38, 40, 41). Previous epidemiological studies regarding association of  
243 PP and AP intakes and BMD have reported inconsistent results (3, 4, 8, 42, 43). Among white  
244 women (aged 80 years or older), higher PP intake was associated with higher BMD, while there  
245 were no consistent significant associations for TP and PP intakes among white women or other  
246 sex and racial/ethnic groups (42). In this data AP but not PP was negatively associated with FN  
247 BMD and BMC. Further investigations are warranted to evaluate whether AP and PP intakes have  
248 different associations with bone health.

249 Different study designs and population, including the length of follow-up, predominant protein  
250 sources of the diet, calcium content, lifestyle factors as well as discrepancies in data reporting, can  
251 all lead to inconsistency of the results of previous studies regarding the relationship of protein  
252 intake with bone health (4, 44). Given that we observed negative associations for protein intakes  
253 and BMC and BMD, stratified analysis was conducted to evaluate whether BMI and physical  
254 activity level mediate these associations. In postmenopausal elderly women BW and BMI are

255 strongly associated with bone health through weight bearing (15, 45, 46). Several data indicated  
256 that women with high BMI (25.0-29.9 kg/m<sup>2</sup>) are protected from osteoporosis (47). Recent  
257 findings by Yang et al. in 5287 men and women aged between 8-69 years showed that greater BMI  
258 was associated with increased LS and FN BMD (48). However, it has been suggested that BMI >  
259 30 kg/m<sup>2</sup> may be harmful to bone health (46). In this study negative associations of protein intake  
260 and BMD and BMC were more pronounced in those with BMI ≤ 30 kg/m<sup>2</sup> as compared to their  
261 counterparts with BMI > 30 kg/m<sup>2</sup>. Mean protein intake did not differ between women with BMI  
262 ≤ 30 and BMI >30 kg/m<sup>2</sup> (17.4 % and 17.8 % of energy, respectively). Findings by Rikkonen et  
263 al.(49) in this population also showed that women with osteoporosis (FN BMD T score ≤ 2.5 SD)  
264 had a lower BMI, lower lean mass, but not fat mass proportion as compared to their normal  
265 counterparts. However, for the interaction between protein intakes with obesity, muscle mass and  
266 bone health more investigations are required.

267 It is evident from previous studies that physical activity has strong beneficial effect on bone health  
268 (17). In a 6-month, RCT in 19 healthy early postmenopausal women allocated to either  
269 postexercise consumption of a protein-containing nutrient supplement (with additional calcium  
270 and vitamin D) or a placebo supplement (with minimal energy); results revealed that there was a  
271 positive effect of the protein-containing supplement on FN BMD (18). However, trials are limited  
272 by short durations and small sample sizes. Results of the present study demonstrated that at the  
273 baseline protein intake (g/kg BW) was inversely associated with FN BMD and BMC in both  
274 passive and active women. While, follow-up results showed that in passive women protein intake  
275 (g/kg BW) was negatively associated with changes of LS BMD and BMC while in active women  
276 protein intake (g/kg BW) was in positive relationships with changes of FN BMD and BMC.  
277 Therefore, this data suggests that the interaction of physical activity and dietary protein might have  
278 positive relationship with bone density in elderly women. To our knowledge this was the first  
279 cohort study in elderly women exploring the exercise combined with dietary protein association  
280 and bone health and further studies are warranted.

281 Current study contains also some limitations. The 3-day dietary records method has been described  
282 as a suitable instrument for assessing energy and protein intake in elderly people (50, 51) , which  
283 has been also used and applied to measure AP and PP intake (52) . However, a single 3 day dietary  
284 record at the baseline might not be appropriate method to capture long term protein intake. Albeit

285 we covered a wide selection for several known confounders that might influence BMD and BMC,  
286 other factors might have affected the observed results. Participants who took part in an  
287 osteoporosis study may have had a heightened awareness of their bone health. This may have led  
288 them to alter some of their modifiable osteoporosis risk factors between the baseline and follow-  
289 up visits. However, such an effect is unlikely to have influenced protein consumption; since protein  
290 is not commonly perceived to be an osteoporosis risk factor. We cannot exclude also the possible  
291 effect of body composition on BMD background (53). Likewise to other studies observed effects  
292 in longitudinal analyses were weaker than what would be predicted by cross-sectional assessments.  
293 Lastly, based on the observational nature of our study we cannot establish a causal association.

294 Observed results could be confounded by mechanical errors. Fat mass loss during weight loss can  
295 affect tissue thickness and bone area measurements; therefore, present study reported both BMD  
296 and BMC (54). The availability of each BMD and BMC measures at the baseline as well as over  
297 a 3 year period added significant strength to our study. The analyses were adjusted for total energy  
298 intake and protein was reported as energy-adjusted and expressed as per BW, therefore, results  
299 showed separated effect of protein intake on BMD and BMC independent of the intake of energy  
300 from other sources.

## 301 **Conclusion**

302 Findings of the present study suggest that protein intake g/d and g/kg BW were negatively  
303 associated with BMD and BMC. This study highlights the importance of higher BMI and physical  
304 activity in counteracting the adverse association of protein intake and bone health. However, due  
305 to several unestablished aspects of these interactions, further cohort and intervention studies are  
306 warranted.

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**Table 1.** Baseline characteristics of participants across quartiles of energy-adjusted total protein intake (g/d).

Characteristics	Q 1 (<54.73 g/d) n=138		Q 2 (54.73-66.0 g/d) n=139		Q 3 (66-80.3 g/d) n=139		Q 4 (>80.3 g/d) n=138		P
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Age (years)	68.1	1.9	67.9	1.8	67.6	1.7	67.8	1.9	0.078
Weight (kg)	71.2	12.2	73.7	11.9	71.5	11.3	73.4	12.7	0.014
Height (cm)	157.9	5.6	158.4	5.5	159.4	4.8	158.7	5.3	0.139
BMI (kg/m <sup>2</sup> )	27.2	4.6	26.8	3.6	27.8	4.1	28.0	4.2	0.085
Current smoker (%)	7.5		4.4		4.3		2.9		0.194
Portions of alcohol/week (n)	3.0	0.7	2.9	0.6	3.0	0.6	4.4	0.7	0.081
Physical activity level (%) <sup>b</sup>									0.660
Passive	39.1		33.8		40.3		39.9		
Active	60.9		66.2		59.7		60.1		
Hormone therapy use (%)	46.0		41.3		46.0		41.3		0.008
Interventional calcium and vitamin D supplement (%)	14.5		26.8		30.2		21.2		0.010
Disease or medication affecting bone (%)	38.4		33.1		37.0		37.0		0.816
<b>Bone measurements</b>									
Baseline total BMD	1.06	0.93	1.07	0.92	1.07	0.86	1.08	0.99	0.988
Baseline FN BMD	0.85	0.11	0.87	0.11	0.85	0.11	0.84	0.11	0.383
Baseline lumbar BMD	1.08	0.17	1.09	0.19	1.06	0.14	1.08	0.19	0.797
Baseline total BMC	2.12	0.34	2.23	0.57	2.21	0.30	2.24	0.32	0.832
Baseline FN BMC	4.11	0.57	4.22	0.31	4.14	0.59	4.15	0.60	0.320
Baseline lumbar BMC	4.30	0.11	4.41	0.12	4.22	0.91	4.45	0.11	0.723
<b>Dietary intakes</b>									
Total energy (kJ/d)	5091	1108	6150	1071	6907	1037	8083	1238	0.036
Fat (g/d)	55.6	9.9	54.1	10.1	51.3	8.9	66.8	17.6	0.005
Carbohydrate (g/d)	204.0	51.5	190.5	45.5	187.6	48.0	193.3	47.8	0.028
Protein (g/d)	47.0	7.7	60.6	3.2	72.7	4.3	92.0	10.5	<0.001
Animal protein (g/d)	24.7	5.9	35.2	2.0	42.5	2.4	54.3	6.7	<0.001
Plant protein (g/d)	23.5	4.4	24.0	4.5	24.4	4.0	24.1	4.2	0.451
Protein g/ kg body weight	0.79	0.24	0.90	0.23	0.96	0.27	1.18	0.29	<0.001
Magnesium (mg/d)	311.4	74.5	323.8	66.6	339.9	67.6	371.4	69.0	<0.001
Phosphorus (mg/d)	357.9	48.7	296.1	43.2	329.8	44.0	315.3	42.57	<0.001
Dietary calcium intake (mg/d)	799.4	317.6	908.2	285.3	1077.8	308.9	1257.7	385.9	0.001
Total calcium (mg/d) <sup>c</sup>	879.6	318.1	981.1	344.3	1187.1	358.9	1341.4	392.0	0.001
SR Calcium supplement (%)	20.3		24.6		31.7		27.7		0.170
SR vitamin D supplement (%)	14.5		26.8		30.2		21.2		0.010

Abbreviations: BMD, bone mineral density. FN, femoral neck. SD, standard deviation. SR, self-reported.

<sup>a</sup> ANOVA or chi-square tests were used to evaluate the distribution. <sup>b</sup> Passive: those women with restricted or no mobility and exercise  $\leq 2$  times/week. Active: those women with no mobility restriction and exercise  $> 2$  times/week were classed as active. <sup>c</sup> Total calcium consists of dietary calcium and SR calcium supplement.

**Table 2.** Cross-sectional association between protein intake and BMD (g/cm<sup>2</sup>) and BMC (g).

	FN BMD			LS BMD			Total BMD			FN BMC			LS BMC			Total BMC		
	$\beta$	SE	<i>P</i>	$\beta$	SE	<i>P</i>	$\beta$	SE	<i>P</i>	$\beta$	SE	<i>P</i>	$\beta$	SE	<i>P</i>	$\beta$	SE	<i>P</i>
<b>Total protein (g/d)</b>																		
Model 1 <sup>a</sup>	-0.09	0.01	0.094	-0.05	0.01	0.366	-0.01	0.01	0.794	-0.06	0.01	0.186	-0.01	0.04	0.875	-0.01	1.23	0.979
Model 2 <sup>b</sup>	-0.19	0.01	0.029	-0.08	0.01	0.307	-0.11	0.01	0.185	-0.19	0.01	0.018	-0.06	0.07	0.943	-0.05	2.07	0.480
<b>Animal protein (g/d) <sup>c</sup></b>																		
Model 1	-0.09	0.01	0.093	-0.04	0.01	0.364	-0.01	0.01	0.790	-0.06	0.01	0.185	-0.01	0.04	0.867	-0.01	1.23	0.978
Model 2	-0.20	0.01	0.029	-0.09	0.01	0.307	-0.01	0.01	0.185	-0.02	0.01	0.018	-0.01	0.07	0.943	-0.05	2.07	0.480
<b>Plant protein (g/d) <sup>c</sup></b>																		
Model 1	-0.07	0.01	0.194	-0.03	0.01	0.599	-0.02	0.01	0.668	-0.04	0.01	0.367	-0.02	0.11	0.700	-0.02	3.39	0.608
Model 2	-0.06	0.01	0.325	-0.01	0.01	0.821	-0.01	0.01	0.790	-0.05	0.01	0.411	-0.01	0.14	0.989	-0.03	4.02	0.487
<b>Total protein (g/kg body weight) <sup>d</sup></b>																		
Model 1	-0.23	0.03	0.001	-0.23	0.04	0.002	-0.25	0.02	0.001	-0.23	0.03	0.001	-0.18	2.47	0.009	-0.26	72.9	<0.001
Model 2	-0.39	0.04	0.001	-0.36	0.06	0.001	-0.51	0.03	<0.001	-0.38	0.21	<0.001	-0.28	3.80	0.009	-0.47	10.61	<0.001

Abbreviations: BMD, bone mineral density. FN, femoral neck. LS, lumbar spine. TP, total protein. AP, animal protein. PP, plant protein. SE, standard error.

<sup>a</sup> Model 1 was adjusted for age, total energy intake, height (cm), weight (kg) and study group.

<sup>b</sup> Model 2 was adjusted for variables in model 1 plus dietary vitamin D, dietary calcium intake, self-reported vitamin D and calcium supplementation, smoking status (current, former and nonsmokers), physical activity level (passive and active), hormone therapy use (never used, used), time since menopause (years); diseases and use of medications which affect BMD.

<sup>c</sup> Models for animal protein were also adjusted for plant protein intake. Models for plant protein were also adjusted for animal protein intake.

<sup>d</sup> Body weight was excluded from adjusted variables in analysis using protein as expressed per body weight due to high collinearity. However, result remained significant even after controlling for body weight.

**Table 3.** Prospective association of protein intake and changes in BMD (g/cm<sup>2</sup>) and BMC (g).

	FN BMD			LS BMD			Total BMD			FN BMC			LS BMC			Total BMC		
	$\beta$	SE	<i>P</i>	$\beta$	SE	<i>P</i>	$\beta$	SE	<i>P</i>	$\beta$	SE	<i>P</i>	$\beta$	SE	<i>P</i>	$\beta$	SE	<i>P</i>
<b>TP (g/d)</b>																		
Model 1 <sup>a</sup>	0.07	0.01	0.077	0.05	0.01	0.273	0.11	0.01	0.044	0.08	0.01	0.050	0.07	0.01	0.138	0.03	0.36	0.505
Model 2 <sup>b</sup>	0.08	0.01	0.239	-0.03	0.01	0.617	0.12	0.01	0.174	0.10	0.01	0.164	-0.06	0.02	0.420	-0.08	0.58	0.064
<b>AP (g/d)<sup>c</sup></b>																		
Model 1	0.08	0.01	0.056	0.08	0.01	0.075 <sup>†</sup>	0.11	0.01	0.035	0.09	0.01	0.038	0.07	0.01	0.110	0.04	0.35	0.442
Model 2	0.10	0.01	0.160	0.03	0.01	0.712	0.17	0.01	0.077	0.12	0.01	0.123	-0.04	0.02	0.569	-0.05	0.59	0.531
<b>PP (g/d)<sup>c</sup></b>																		
Model 1	-0.07	0.01	0.095	-0.10	0.01	0.075	-0.09	0.01	0.070	-0.07	0.01	0.091	-0.05	0.03	0.247	-0.10	0.95	0.053
Model 2	-0.05	0.01	0.301	-0.11	0.01	0.066	-0.14	0.01	0.054	-0.04	0.01	0.409	-0.04	0.04	0.492	-0.08	1.10	0.208
<b>TP (g/kg body weight)<sup>d</sup></b>																		
Model 1	0.02	0.01	0.692	-0.14	0.01	0.038	0.05	0.01	0.471	0.09	0.05	0.141	-0.09	0.70	0.168	-0.01	21.12	0.928
Model 2	-0.01	0.01	0.918	-0.31	0.01	0.001	0.04	0.01	0.507	0.16	0.07	0.083	-0.30	1.02	0.002	-0.16	30.04	0.159

Abbreviations: BMD, bone mineral density. FN, femoral neck. LS, lumbar spine. TP, total protein. AP, animal protein. PP, plant protein. SE, standard error.

<sup>a</sup> Model 1 was adjusted for age, total energy intake, height (cm), weight (kg), study group and baseline BMD and BMC values .

<sup>b</sup> Model 2 was adjusted for variables in model 1 plus dietary vitamin D, dietary calcium intake, self-reported vitamin D and calcium supplementation, smoking status (current, former and nonsmokers), physical activity level (passive and active), hormone therapy use (never used, used), time since menopause (years); diseases and use of medications which affect BMD.

<sup>c</sup> Models for animal protein were also adjusted for plant protein intake. Models for plant protein were also adjusted for animal protein intake.

<sup>d</sup> Body weight was excluded from adjusted variables in analysis using protein as expressed per body weight due to high collinearity. However, result remained significant even after controlling for body weight.

**Table 4.** Cross-sectional and prospective association of protein intake (g/kg body weight) and BMD (g/cm<sup>2</sup>) and BMC (g) according to BMI category.

	BMI ≤ 30 kg/m <sup>2</sup> (n=401)			BMI > 30 kg/m <sup>2</sup> (n=151)		
	β	SE	P <sup>a</sup>	β	SE	P
<b>Lumbar spine BMD (g/cm<sup>2</sup>)</b>						
Baseline	-0.25	0.08	0.050	0.31	0.27	0.472
Change	-0.31	0.02	0.016	-0.05	0.05	0.778
<b>Femoral neck BMD (g/cm<sup>2</sup>)</b>						
Baseline	-0.34	0.05	0.006	-0.12	0.27	0.776
Change	0.03	0.01	0.802	-0.01	0.04	0.940
<b>Total BMD (g/cm<sup>2</sup>)</b>						
Baseline	-0.38	0.04	0.002	0.28	0.17	0.518
Change	0.02	0.01	0.869	-0.19	0.05	0.694
<b>Lumbar spine BMC (g)</b>						
Baseline	-0.16	4.42	0.191	0.22	16.183	0.525
Change	-0.21	1.38	0.104	-0.19	2.88	0.314
<b>Femoral neck BMC (g)</b>						
Baseline	-0.31	0.24	0.007	-0.23	1.41	0.551
Change	0.12	0.08	0.299	0.09	0.30	0.601
<b>Total BMC (g)</b>						
Baseline	-0.41	120.99	<0.001	-0.06	686.71	0.877
Change	-0.21	32.24	0.100	0.39	207.94	0.425

Abbreviations: BMD· bone mineral density, BMD, bone mineral density. BMC, bone mineral content.

<sup>a</sup> Model was adjusted for age, total energy intake, height, study group, dietary vitamin D and calcium intakes, self-reported vitamin D and calcium supplementation, smoking status (current, former and nonsmokers), physical activity level (passive and active), hormone therapy use (never used, used), time since menopause (years); diseases and use of medications which affect BMD and baseline BMD and BMC values for longitudinal analysis.

**Table 5.** Cross-sectional and prospective association of protein intake (g/kg body weight) and BMD (g/cm<sup>2</sup>) and BMC (g) according to physical activity level.

	Passive (n=211)			Active (n=341)		
	$\beta$	SE	P <sup>a</sup>	$\beta$	SE	P
<b>Lumbar spine BMD (g/cm<sup>2</sup>)</b>						
Baseline	0.01	0.16	0.963	-0.20	0.10	0.268
Change	-0.43	0.02	0.003	0.23	0.02	0.047
<b>Femoral neck BMD (g/cm<sup>2</sup>)</b>						
Baseline	-0.26	0.06	0.041	-0.30	0.04	0.006
Change	-0.16	0.02	0.264	0.13	0.01	0.467
<b>Total BMD (g/cm<sup>2</sup>)</b>						
Baseline	-0.11	0.07	0.590	-0.26	0.05	0.134
Change	-0.07	0.01	0.678	0.024	0.01	0.882
<b>Lumbar spine BMC (g)</b>						
Baseline	0.07	9.61	0.732	-0.10	5.90	0.578
Change	-0.46	1.50	0.002	0.20	1.40	0.125
<b>Femoral neck BMC (g)</b>						
Baseline	-0.22	0.30	0.036	-0.31	0.21	0.004
Change	-0.02	0.14	0.840	0.21	0.08	0.049
<b>Total BMC (g)</b>						
Baseline	-0.05	2.47	0.788	-0.12	1.62	0.435
Change	-0.11	55.40	0.545	0.24	38.72	0.146

Abbreviations: BMD, bone mineral density. BMC, bone mineral content.

<sup>a</sup> Model was adjusted for age, total energy intake, height, weight, study group, dietary vitamin D and calcium intakes, self-reported vitamin D and calcium supplementation, smoking status (current, former and nonsmokers), hormone therapy use (never used, used), time since menopause (years); diseases and use of medications which affect BMD and baseline BMD and BMC values for longitudinal analysis.