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ORIGINAL ARTICLE

Protein intake and risk of hip fractures in postmenopausal women and men age 50 and older

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Abstract
Summary In this study, we followed postmenopausal women and men aged 50 and above for up to 32 years and found no evidence that higher protein intake increased the risk of hip fracture. Protein intake from specific sources was inversely associated with risk, but these associations appeared to differ by gender.
Introduction We examined the association between intakes of total and specific sources of protein and hip fracture risk in postmenopausal women and men over 50 years of age. Our hypothesis was that a higher protein intake would not be associated with a higher risk of hip fractures.
Methods In this analysis, we followed 74,443 women in the Nurses' Health Study between 1980 and 2012 and 35,439 men from the Health Professionals Follow-up Study between 1986 and 2012. Health and lifestyle information and hip fractures were self-reported on biennial questionnaires. Protein was assessed approximately every 4 years with a food frequency questionnaire. Relative risks (RR) were computed for hip fracture by quintiles of total, animal, dairy, and plant protein intakes using Cox proportional hazard models, adjusting for potential confounders.
Results During follow-up, we ascertained 2156 incident hip fractures in women and 595 fractures in men. Among men, we observed significant inverse associations for each 10 g increase of total protein (RR = 0.92, 95% CI = 0.85–0.99) and animal protein (RR = 0.91, 95% CI = 0.85–0.98) intakes. Total and animal proteins were not significantly associated with hip fractures in women. Both plant (RR = 0.88, 95% CI 0.79–0.99 per 10 g) and dairy protein (RR = 0.92, 95% CI 0.86–0.97) were associated with significantly lower risks of hip fracture when results for men and women were combined. None of these associations were modified by BMI, smoking, physical activity, age, or calcium intake.
Conclusion We found no evidence that higher protein intake increases risk of hip fracture in these Caucasian men and women. Protein intake from specific sources was inversely associated with risk, but these associations appeared to differ by gender.

Keywords Diet · Fractures · Hip · Nutrition · Protein

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Introduction

Hip fractures are a major cause of morbidity in the USA. In 2010, the rate of hospitalization for individuals aged 65 and older was 778/100,000 in women and 443/100,000 in men [1]. Mortality risk after hip fractures is also high, with a 5-fold increase in women and 8-fold increase in men within 3 months of fractures [2] and 22% mortality within 1 year [3]. In the elderly, osteoporosis is a major risk factor for fractures [4]. Dietary protein has been suggested to be both deleterious and beneficial for bone health. On one hand, dietary protein, especially animal protein, may contribute to net acid load, and mild, persistent metabolic acidosis may lead to demineralization [5]. However, higher protein intake can also increase intestinal calcium absorption [6], provide amino acids for collagen synthesis, and increase secretion of insulin-like growth factor-1 (IGF-1) which in turn increases osteoblast activity [7]. In addition, adequate protein intake is essential in preserving muscle mass and in turn may help maintain balance and prevent falls [8].

Studies examining the association of protein intake and bone mineral density (BMD) were conducted predominantly in women. Results generally showed no association [9] or a small positive association with BMD in both men and women [10, 11]. Studies that examined fractures risk similarly did not suggest higher protein intake as a risk factor for fractures. An earlier meta-analysis did not find a significant association between total, animal or vegetable protein and hip fracture risks [11]. Several recent cohort studies generally showed no association between total protein intake and hip fracture risk [10, 12, 13]. On the other hand, a small study from the Framingham Offspring cohort among participants with mean age 55 found a higher risk for hip fractures among individuals with high animal protein but low calcium intake [14]. In a Norwegian cohort study among middle aged women and men, an increased risk of hip fracture was found in those with higher intake of non-dairy animal proteins combined with a lower intake of calcium [15]. However, in a study that included adults age 30 and older, an inverse association was observed between legumes and meat intake and risk of hip fractures [16].

In previous studies, data on protein intake and fracture risk were primarily from women. In the few studies that included men, there was no separate analysis to examine risk in men. Many studies had follow-up duration of less than 10 years and diet was assessed only once at baseline. Moreover, few studies examined specific sources of protein.

To address these limitations, we examined total as well as specific sources of protein and the risk of hip fractures in two large cohorts of Caucasian US men and women, the Health Professionals Follow-up Study (HPFS, men) and Nurses' Health Study (NHS, women), with up to 32 years of follow-up. Diet was assessed multiple times to account for change in intake. In addition, we examined if age would influence the association as higher protein intake might be more important in older people due to declining levels of anabolic hormones [17, 18]. We hypothesized that a higher protein intake would not be associated with increased risk of hip fractures.

Methods

Participants

The NHS is an ongoing, prospective cohort study of women that began in 1976 with 121,700 nurses aged 30–55 years who were living in 11 US states [19]. A questionnaire assessing lifestyle and disease information were sent to participants every 2 years and a food-frequency questionnaire (FFQ) was sent in 1980, 1984, 1986, and every 4 years thereafter to update dietary information. Follow-up rate was approximately 90% at each follow-up cycle. The HPFS was a similar cohort of men that began in 1986 with 51,529 health professions aged 40–75 [20]. A self-administered lifestyle questionnaire was sent to participants every 2 years and a FFQ was sent every 4 years. Follow-up has been consistently over 90%.

In this analysis, follow-up began in 1980 for women who were postmenopausal at the time they completed the 1980 FFQ. Otherwise, follow-up began in the year of the follow-up questionnaire on which they reported having reached menopause, including surgical menopause. Follow-up began for men in 1986 if they were at least 50 years old or as they reached age 50 at each follow-up questionnaire cycle. We only included white men and women because of the small number of non-white participants. Both men and women were excluded from the current analysis if they had not completed the most-recent dietary assessment at entry, which had previously reported a hip fracture or diagnosis of cancer or osteoporosis. Therefore, 74,443 Caucasian women and 35,439 men were included in the analysis. This study was approved by the Institutional Review Boards of the Brigham and Women's Hospital and the Harvard TH Chan School of Public Health, Boston MA.

Dietary assessment

A validated FFQ was administered in 1980, 1984, and 1986 and every 4 years thereafter for a total of nine times in NHS until 2010 and seven times between 1986 and 2010 in HPFS [21, 22]. Each FFQ contained over 130 food items with nine frequency choices ranging from <1 time/month to ≥6 times/day [23, 24]. A standard portion size was also specified for each food item. Intakes of macro- and micro-nutrients and energy, including calcium, vitamin D, and vitamin K, were computed using data from the United States Department of Agriculture, other published data, and food manufacturers. Total protein and specific protein intake from animal (including dairy), plant, and dairy sources were calculated by summing the contribution from each food item. We also examined major sources of protein. Depending on the year of the FFQ, there were approximately five foods listed for non-processed red meats, four for processed meats, five for fish and seafood, two for chicken or poultry, seven for dairy (milk, yogurt, cheese), and three each for nuts and legumes. The correlation coefficient for protein intake between FFQ and multiple weeks of food record was 0.50 in women and similar in men [20, 22].

Assessment of fractures

Hip fractures were self-reported by participants on biennial questionnaires, including information on the fracture site and month and year of the fracture. Participants also reported the circumstances under which the fracture occurred to categorize the fracture by the level of trauma. Fractures sustained from high impact trauma such as motor vehicle accidents, horse-back riding, skiing, and other high-trauma events were excluded because these events could have resulted in a fracture even in the absence of low bone mineral density. Because all participants were health professionals, the self-reporting of fractures was likely highly accurate. In a validation study in the NHS, a medical record review confirmed all reported fractures in all 30 sampled cases [19].

Assessment of covariates

Height was reported at baseline. Weight and the calculated BMI, smoking, thiazide diuretic use (yes or no), and diagnosis of osteoporosis and diabetes (yes or no) were assessed on all biennial questionnaires. Recreational physical activity was assessed with ten activities that were assigned a metabolic equivalent task score for energy expenditure in relation to sitting, and reported hours per week were multiplied by these scores and summed over all activities to create a value in metabolic equivalent task hours per week. In women, post-menopausal hormone use was assessed in each biennial questionnaire.

Statistical analysis

We computed cumulative averages for food and nutrient intakes from available FFQs to reduce the within-person variation and represent long-term intake [25]. For example, if follow-up began for a participant in 1990, dietary intake in 1994 was calculated as the mean of 1990 and 1994. We used Cox proportional hazard models to examine the association between proteins and protein sources and risk of hip fractures. Participants were censored on the date of fracture, last questionnaire response, or end of follow-up in 2012 (June 1 for women and January 31 for men), which ever occurred first. Energy-adjusted intakes of total protein, specific types of protein, and major protein sources were classified into cohort-specific quintiles. We also modeled the risk of fractures for each 10 g increase in protein intake. Risk estimates for each 10 g increase in protein intake in men and women were then pooled using a fixed effects model by Der Simonian and Laird [26].

In multivariable analysis, we adjusted for age (in months), leisure-time physical activity (five categories), thiazide use (yes or no), smoking (ten categories to represent smoking history and number of cigarettes per day), alcohol (five categories), caffeine (quintiles), and the following nutrients in energy-adjusted quintiles: calcium, retinol, vitamin D, and vitamin K [22]. However, in analysis of dairy protein, we adjusted for supplemental intake of vitamin D (yes/no) and calcium (yes/no) instead of total calcium and vitamin D intake to avoid collinearity. All models were also adjusted for sugar-sweetened beverages (five categories) as they were associated with hip fractures in a previous analysis in NHS [27]. The aforementioned variables were updated at each questionnaire follow-up period. For categorical variables, missing data were assigned to a separate category. We did not adjust for self-reported diagnoses of osteoporosis because it may have been in the causal pathway. In analysis of major protein food groups, regression models were adjusted for total energy intake.

We conducted additional analyses in which we stratified by smoking status (current compared with non current smokers), BMI (<25 compared with ≥25), age (<65 compared with ≥65 y), physical activity (above or below the median), and calcium intake (<600 mg/d compared with ≥600 mg/d) to explore whether the influence of protein intake on fracture risk varied by the level of these variables and formally tested for interaction. We also examined whether the association with fractures was different among different proteins. First, we fitted a fully adjusted model with total protein intake. Then, we fitted another one with total protein and plant protein in the same model and use the likelihood ratio test to examine if the model including plant protein had better fit than the model with total protein only. A significant likelihood ratio test indicates a better fit and reflects that the risk estimate for plant protein is different from that of animal protein. We then repeat the same procedure for dairy protein.

Results

During up to 32 years of follow-up, we identified 2156 incident hip fractures in women and 595 in men. Protein intake in our cohorts was generally higher in men than women (Table 1). In both cohorts, participants with higher protein intake tended to be non-smokers and consumed less alcohol but more vegetables, retinol, calcium, and vitamin D. Men with lower protein intake tended to consume more sugar-sweetened beverages. On the other hand, women with higher protein intake also had higher BMI ($\rho = 0.16$, $p < 0.001$) and higher level of physical activity but this was not observed in men.

Table 1 Demographical and lifestyle characteristics (SD) of men and women at entry to the follow-up by quintiles of energy-adjusted total protein intake

	Women			Men		
	Q1	Q3	Q5	Q1	Q3	Q5
Weight (kg)	64.8 ± 16.5	66.5 ± 17.1	68.6 ± 17.2	80.9 ± 11.2	81.7 ± 11.4	82.7 ± 12.0
Height (cm)	164 ± 6	164 ± 6	164 ± 6	179 ± 7	178 ± 7	178 ± 7
BMI (kg/m^2)	24.7 ± 4.5	25.3 ± 4.7	26.1 ± 4.9	25.3 ± 3.0	25.6 ± 3.1	26.0 ± 3.2
Current smoker (%)	27	22	21	9	7	6
Physical activity (METs/week)	14.6 ± 15.2	15.5 ± 15.2	16.2 ± 15.9	23.9 ± 27.8	23.9 ± 26.1	23.0 ± 25.3
Menopausal hormone use (%)	29	33	29	N/A	N/A	N/A
Multivitamin use (%)	35	39	42	42	43	45
Alcohol (g/day)	9 ± 15	6 ± 10	4 ± 7	17 ± 21	11 ± 14	6 ± 10
Caffeine (mg/day)	350 ± 254	334 ± 248	338 ± 259	243 ± 227	234 ± 224	209 ± 218
Mean intake (±SD)						
Energy intake (kcal/day)	1648 ± 556	1690 ± 506	1602 ± 515	1962 ± 621	2016 ± 591	1935 ± 624
Total protein (g/day)	56.9 ± 5.9	74.4 ± 1.8	96.9 ± 11.0	70.6 ± 6.7	91.3 ± 2.3	115.9 ± 11.2
Total protein (%energy)	14.3 ± 1.5	18.6 ± 0.8	24.4 ± 3.0	14.2 ± 1.4	18.3 ± 0.8	23.4 ± 2.4
Animal protein (g/day)	39.8 ± 8.5	56.4 ± 6.9	79.2 ± 14.5	45.3 ± 9.5	65.8 ± 6.2	91.3 ± 13.4
Animal protein (%energy)	9.8 ± 2.0	14.1 ± 1.6	20.3 ± 3.4	9.1 ± 1.9	13.2 ± 1.4	18.5 ± 2.8
Vegetable protein (g/day)	17.6 ± 5.5	17.6 ± 5.3	16.2 ± 5.3	25.3 ± 6.8	25.5 ± 5.8	24.6 ± 7.0
Vegetable protein (%energy)	4.5 ± 1.4	4.4 ± 1.3	4.1 ± 1.3	5.1 ± 1.4	5.1 ± 1.2	4.9 ± 1.4
Dairy protein (g/day)	11.1 ± 6.1	15.1 ± 7.8	20.3 ± 11.7	12.4 ± 6.6	16.1 ± 8.6	18.3 ± 11.4
Dairy protein (%energy)	2.8 ± 1.5	3.8 ± 1.9	5.1 ± 2.9	2.5 ± 1.3	3.2 ± 1.7	3.6 ± 2.3
Calcium (mg/day)	749 ± 407	889 ± 428	1015 ± 479	784 ± 355	922 ± 402	1029 ± 532
Vitamin D (IU/day)	268 ± 247	329 ± 241	442 ± 320	332 ± 277	412 ± 290	429 ± 368
Retinol (mcg/day)	3925 ± 5251	4433 ± 4878	5812 ± 6604	4662 ± 5891	5298 ± 6119	6335 ± 7143
Vitamin K (mcg/day)	84 ± 90	97 ± 102	91 ± 131	159 ± 105	184 ± 103	222 ± 137
Red meat (servings/day)	0.5 ± 0.3	0.7 ± 0.4	0.7 ± 0.6	0.5 ± 0.3	0.6 ± 0.5	0.7 ± 0.6
Processed meat (servings/day)	0.3 ± 0.3	0.3 ± 0.3	0.2 ± 0.3	0.3 ± 0.4	0.4 ± 0.4	0.3 ± 0.4
Poultry (servings/day)	0.4 ± 0.1	0.3 ± 0.2	0.5 ± 0.4	0.2 ± 0.1	0.3 ± 0.2	0.6 ± 0.4
Fish (servings/day)	0.1 ± 0.1	0.2 ± 0.2	0.4 ± 0.4	0.2 ± 0.2	0.4 ± 0.2	0.6 ± 0.5
Nuts (servings/day)	0.3 ± 0.5	0.4 ± 0.5	0.3 ± 0.5	0.5 ± 0.7	0.5 ± 0.7	0.4 ± 0.6
Legumes (servings/day)	0.4 ± 0.3	0.4 ± 0.3	0.5 ± 0.4	0.4 ± 0.3	0.4 ± 0.3	0.5 ± 0.4
Fruit (servings/day)	2.4 ± 1.9	2.3 ± 1.4	2.2 ± 1.3	2.1 ± 1.8	2.1 ± 1.4	2.0 ± 1.4
Vegetables (servings/day)	2.0 ± 1.5	2.4 ± 1.7	2.7 ± 1.9	2.4 ± 1.6	2.8 ± 1.6	3.3 ± 2.0
Sugar-sweetened beverages (servings/day)	0.8 ± 1.1	0.7 ± 1.0	0.8 ± 1.1	0.6 ± 0.9	0.3 ± 0.4	0.1 ± 0.3

Entry was age 50 for men (HPFS cohort) and menopause for women (NHS cohort)

After adjusting for age, each 10 g increase in total protein intake was associated with a significant 10% lower risk of hip fractures in women (RR = 0.90, 95% CI = 0.87–0.94) (Table 2). However, this association was greatly attenuated after adjustment for BMI and after further adjustment for other lifestyle factors, it was no longer statistically significant (RR = 0.97, 95% CI = 0.93–1.02). A similar pattern was seen for animal protein. However, for plant protein, there was a suggestion of an inverse association with hip fractures (RR for each 10 g increase = 0.87, 95% CI = 0.76–1.00) in women. On the other hand, dairy protein intake was significantly associated with fracture risk, even after adjusting for use of calcium and vitamin D supplements (RR for each 10 g increase = 0.92, 95% CI = 0.86–0.99).

In men, higher total and animal protein were associated with lower risk of hip fractures. The RR for each 10 g increase was 0.92 (95% CI = 0.85–0.99) for total protein intake and 0.91 (95% CI = 0.85–0.98) for animal protein intake in the multivariable models. All results were not changed after

Table 2 Relative risks (95% CI) for hip fractures by quintiles of energy-adjusted protein intake

	Q1	Q2	Q3	Q4	Q5	Per 10 g increase
Total protein						
Women						
Median intake (g/day)	60.2	68.0	73.5	79.3	88.6	
No. of cases	466	476	435	436	343	
Age adjusted	1	0.90 (0.79, 1.02)	0.81 (0.71, 0.93)	0.82 (0.72, 0.94)	0.74 (0.65, 0.85)	0.90 (0.87, 0.94)
Age and BMI adjusted	1	0.95 (0.83, 1.08)	0.88 (0.77, 1.00)	0.93 (0.81, 1.06)	0.89 (0.77, 1.03)	0.95 (0.92, 1.00)
Multivariable adjusted[a]	1	1.00 (0.88, 1.14)	0.94 (0.82, 1.08)	1.01 (0.88, 1.16)	0.94 (0.81, 1.10)	0.97 (0.93, 1.02)
Men						
Median intake (g/day)	73.6	83.1	89.9	97.1	108.3	
No. of cases	136	132	118	130	79	
Age adjusted	1	0.96 (0.75, 1.22)	0.86 (0.67, 1.09)	0.97 (0.76, 1.25)	0.77 (0.58, 1.02)	0.93 (0.87, 0.99)
Age and BMI adjusted	1	0.97 (0.76, 1.24)	0.87 (0.68, 1.12)	0.98 (0.77, 1.26)	0.78 (0.58, 1.03)	0.93 (0.87, 1.00)
Multivariable adjusted[a]	1	0.96 (0.75, 1.24)	0.88 (0.67, 1.14)	0.96 (0.73, 1.25)	0.74 (0.54, 1.02)	0.92 (0.85, 0.99)
					Pooled	0.96 (0.92, 1.00)
Animal protein						
Women						
Median intake (g/day)	39.0	47.0	52.8	59.0	69.7	
No. of cases	433	487	414	452	370	
Age adjusted	1	0.95 (0.83, 1.08)	0.78 (0.68, 0.89)	0.86 (0.75, 0.98)	0.81 (0.70, 0.93)	0.93 (0.90, 0.97)
Age and BMI adjusted	1	0.99 (0.87, 1.13)	0.85 (0.74, 0.97)	0.97 (0.85, 1.11)	0.98 (0.85, 1.13)	0.99 (0.95, 1.03)
Multivariable adjusted[a]	1	1.01 (0.88, 1.15)	0.86 (0.75, 0.99)	0.98 (0.85, 1.13)	0.94 (0.83, 1.10)	0.97 (0.93, 1.01)
Men						
Median intake (g/day)	46.2	56.3	63.5	71.3	83.6	
No. of cases	137	130	118	120	90	
Age adjusted	1	0.93 (0.73, 1.19)	0.84 (0.66 1.09)	0.90 (0.70, 1.16)	0.89 (0.68, 1.17)	0.95 (0.89, 1.00)
Age and BMI adjusted	1	0.94 (0.73, 1.20)	0.87 (0.68, 1.12)	0.93 (0.72, 1.20)	0.93 (0.70, 1.23)	0.96 (0.90, 1.02)
Multivariable adjusted[a]	1	0.93 (0.72, 1.20)	0.83 (0.64, 1.08)	0.87 (0.66, 1.15)	0.81 (0.59, 1.11)	0.91 (0.85, 0.98)
					Pooled	0.95 (0.92, 0.99)
Plant protein						
Women						
Median intake (g/day)	14.7	17.9	19.9	21.8	25.1	
No. of cases	476	505	429	407	339	
Age adjusted	1	0.88 (0.77, 0.99)	0.77 (0.67, 0.87)	0.80 (0.70, 0.91)	0.83 (0.72, 0.95)	0.85 (0.75, 0.95)
Age and BMI adjusted	1	0.88 (0.78, 1.00)	0.77 (0.68, 0.88)	0.79 (0.69, 0.91)	0.78 (0.67, 0.89)	0.80 (0.72, 0.90)
Multivariable adjusted[a]	1	0.94 (0.83, 1.08)	0.85 (0.74, 0.98)	0.87 (0.75, 1.01)	0.85 (0.72, 1.00)	0.87 (0.76, 1.00)
Men						
Median intake (g/day)	19.6	23.2	25.8	28.6	33.4	
No. of cases	113	114	129	131	108	
Age adjusted	1	0.87 (0.66, 1.14)	0.87 (0.67, 1.13)	0.94 (0.73, 1.22)	0.82 (0.63, 1.08)	0.95 (0.81, 1.11)
Age and BMI adjusted	1	0.88 (0.67, 1.15)	0.87 (0.67, 1.12)	0.92 (0.71, 1.20)	0.77 (0.59, 1.02)	0.91 (0.77, 1.06)
Multivariable adjusted[a]	1	0.86 (0.65, 1.14)	0.83 (0.62, 1.11)	0.90 (0.67, 1.22)	0.75 (0.53, 1.06)	0.89 (0.73, 1.10)
					Pooled	0.88 (0.79, 0.99)
Dairy protein						
Women						
Median intake (g/day)	6.8	10.6	13.8	17.8	24.6	
No. of cases	395	468	439	437	417	
Age adjusted	1	0.99 (0.86, 1.13)	0.86 (0.75, 0.98)	0.83 (0.73, 0.95)	0.82 (0.71, 0.94)	0.91 (0.85, 0.96)
Age and BMI adjusted	1	1.03 (0.90, 1.18)	0.90 (0.78, 1.03)	0.88 (0.77, 1.01)	0.87 (0.76, 1.01)	0.93 (0.87, 0.99)
Multivariable adjusted[a]	1	1.07 (0.93, 1.22)	0.94 (0.82, 1.08)	0.92 (0.80, 1.06)	0.87 (0.75, 1.01)	0.92 (0.86, 0.99)

Table 2 (continued)

	Q1	Q2	Q3	Q4	Q5	Per 10 g increase
Men						
Median intake (g/day)	6.8	10.6	14.0	18.2	26.5	
No. of cases	122	116	119	110	128	
Age adjusted	1	0.79 (0.60, 1.02)	0.81 (0.62, 1.05)	0.70 (0.54, 0.92)	0.83 (0.65, 1.07)	0.96 (0.86, 1.07)
Age and BMI adjusted	1	0.80 (0.62, 1.05)	0.82 (0.64, 1.07)	0.72 (0.55, 0.94)	0.85 (0.65, 1.09)	0.96 (0.87, 1.07)
Multivariable adjusted[a]	1	0.79 (0.60, 1.03)	0.75 (0.57, 0.98)	0.63 (0.47, 0.83)	0.70 (0.53, 0.93)	0.89 (0.79, 1.00)
					Pooled	0.91 (0.86, 0.97)

Animal protein models adjusted or plant protein and vice versa. Dairy protein models adjusted for non-dairy protein and adjusted for calcium and vitamin supplement use instead of total calcium and vitamin D intake

[a] Adjusted for age, height, alcohol intake, sugar-sweetened beverages, total vitamin D, total calcium, retinol, caffeine, vitamin K, physical activity, BMI, smoking, menopausal hormone use, and thiazide use

additional adjustment for a history of osteoporosis. When we pooled the results for men and women together, the RRs for each 10 g increase in protein intake were 0.96 (95% CI = 0.92–1.00) for total protein, 0.95 (95% CI = 0.92–0.99) for animal protein, 0.88 (95% CI = 0.79–0.99) for plant protein, and 0.91 (95% CI = 0.86–0.97) for dairy protein. No heterogeneity of RR between the cohorts was detected for any protein type.

We also explored major food sources of protein and hip fractures risk while simultaneously adjusting for all sources of protein and potential confounders. In women, we observed an inverse association for total high protein dairy food intake (RR comparing fifth vs first quintile = 0.84, 95% CI = 0.70–1.01, p trend = 0.05) (Table 3). This is consistent with the inverse association that was observed for dairy protein. A somewhat similar pattern was also seen in men, but the confidence intervals were wider. In men, poultry foods had the strongest inverse association with hip fracture (RR comparing fifth vs first quintile = 0.71, 95% CI = 0.51–0.98, p trend =0.01). This is consistent with the inverse association we observed for total and animal protein. We did not find any difference in the association between specific protein type or the specific protein food sources and fracture risk.

When we stratified the analysis by major risk factors of osteoporosis, total protein intake was more strongly associated with a lower risk of hip fracture among men with a physical activity level below the median (RR for each 10 g increase = 0.91, 95% CI = 0.83–0.99). In women, a suggestion of an inverse association was observed in the group younger than 65 years (RR for each 10 g protein = 0.92, 95% CI = 0.84–1.00) but not in the older age groups (Table 4). However, the tests for interaction were not statistically significant. The association between protein intake and hip fractures did not appear to differ by BMI or calcium intake in either cohort.

Discussion

In this analysis, we found that each additional 10 g of total protein and animal protein intakes in men were associated with significant 9 and 8% lower risks of hip fractures, respectively. Total and animal proteins were not associated with hip fractures in women. A 9% lower risk of hip fractures was observed for dairy protein in pooled analysis although data in men were not significant on its own despite similar RR to women. Plant protein was not significantly associated with hip fracture in each cohort separately, likely due to both a lower intake from plant sources and a narrow range of distribution, limiting the ability to observe a significant association. However, a significant 12% lower risk was observed after pooling data from both cohorts.

A recent meta-analysis showed total protein was associated with lower risk of hip fractures [28]. However, when protein animal and plant sources were separately analyzed, neither reached statistical significance. Our results are consistent with a recent US cohort in older men that found an inverse association with higher protein intake [29]. A small case-control study in Spain, however, did not detect any association [30].

While current literature appears to be somewhat consistent in suggesting a lower hip fracture risk with higher total protein intake, data on plant and animal proteins have been inconsistent. In a US prospective study, intakes of both meat and legumes were inversely associated with hip fracture risks [16]. However, another US prospective study showed no significant association, although results trended toward a positive association with animal protein and an inverse association with plant protein [14]. In a case-control study from Spain, neither animal nor plant protein was significantly associated with fracture risk; however, individuals with higher animal protein intake tended to have lower risk [30]. In all of these studies, as in ours, the intake of

Table 3 Multivariable RR (95% CI) for hip fractures by quintiles of major protein groups

	Q1	Q2	Q3	Q4	Q5	P trend
Red meat						
Women						
Median intake (servings/day)	0.2	0.4	0.5	0.7	1.0	
No. of cases	391	429	457	466	413	
Multivariable RR[a]	1	1.00 (0.87, 1.16)	1.03 (0.89, 1.19)	1.03 (0.88, 1.20)	1.06 (0.89, 1.25)	0.50
Men						
Median intake (servings/day)	0.1	0.3	0.5	0.7	1.1	
No. of cases	129	112	131	116	106	
Multivariable RR	1	0.81 (0.61, 1.08)	0.90 (0.68, 1.21)	0.85 (0.62, 1.16)	0.83 (0.59, 1.17)	0.50
Processed meats						
Women						
Median intake (servings/day)	0.0	0.1	0.2	0.4	0.7	
No. of cases	391	446	461	436	422	
Multivariable RR	1	1.10 (0.95, 1.26)	1.11 (0.96, 1.28)	1.07 (0.92, 1.24)	1.15 (0.97, 1.35)	0.57
Men						
Median intake (servings/day)	0	0.1	0.2	0.4	0.7	
No. of cases	116	118	119	130	111	
Multivariable RR	1	1.02 (0.77, 1.36)	1.02 (0.77, 1.36)	1.03 (0.76, 1.41)	0.94 (0.67, 1.31)	0.34
Poultry						
Women						
Median intake (servings/day)	0.1	0.2	0.3	0.4	0.6	
No. of cases	458	530	492	358	318	
Multivariable RR	1	0.95 (0.84, 1.09)	0.95 (0.83, 1.09)	0.88 (0.75, 1.02)	0.96 (0.81, 1.12)	0.51
Men						
Median (servings/day)	0.1	0.2	0.4	0.5	0.7	
No. of cases	131	136	136	115	76	
Multivariable RR	1	1.02 (0.79, 1.32)	0.99 (0.76, 1.29)	0.89 (0.67, 1.17)	0.71 (0.51, 0.98)	0.01
Fish and seafood						
Women						
Median intake (servings/day)	0.1	0.16	0.24	0.4	0.6	
No. of cases	434	470	444	453	355	
Multivariable RR	1	1.07 (0.93, 1.22)	1.05 (0.91, 1.21)	1.21 (1.05, 1.40)	1.15 (0.98, 1.36)	0.17
Men						
Median (servings/day)	0.1	0.2	0.3	0.4	0.7	
No. of cases	125	130	119	109	111	
Multivariable RR	1	0.96 (0.74, 1.25)	0.89 (0.68, 1.17)	0.83 (0.62, 1.10)	0.83 (0.60, 1.13)	0.41
High protein dairy[b]						
Women						
Median intake (servings/day)	0.5	1.0	1.4	2.0	3.0	
No. of cases	392	451	468	447	398	
Multivariable RR	1	1.03 (0.89, 1.18)	0.99 (0.85, 1.15)	0.88 (0.75, 1.03)	0.84 (0.70, 1.01)	0.05
Men						
Median (servings/day)	0.3	0.8	1.1	1.6	2.9	
No. of cases	111	136	107	115	125	
Multivariable RR	1	1.04 (0.79, 1.36)	0.78 (0.57, 1.07)	0.72 (0.51, 1.02)	0.77 (0.52, 1.14)	0.44
Legumes						
Women						
Median intake (servings/day)	0.2	0.3	0.4	0.5	0.8	
No. of cases	372	422	501	500	361	

Table 3 (continued)

	Q1	Q2	Q3	Q4	Q5	P trend
Multivariable RR	1	0.94 (0.82, 1.09)	1.06 (0.92, 1.22)	1.10 (0.95, 1.27)	0.89 (0.76, 1.05)	0.20
Men						
Median (servings/day)	0.1	0.3	0.4	0.5	0.9	
No. of cases	101	112	123	133	125	
Multivariable RR	1	1.00 (0.75, 1.33)	1.05 (0.79, 1.40)	1.14 (0.85, 1.53)	1.17 (0.85, 1.61)	0.34
Nuts						
Women						
Median intake (servings/day)	0.0	0.1	0.2	0.4	0.8	
No. of cases	380	452	437	440	447	
Multivariable RR	1	1.05 (0.91, 1.20)	0.94 (0.81, 1.08)	0.92 (0.79, 1.06)	0.96 (0.82, 1.11)	0.63
Men						
Median (servings/day)	0.05	0.1	0.3	0.5	1.1	
No. of cases	100	126	126	128	114	
Multivariable RR	1	1.25 (0.95, 1.65)	1.11 (0.84, 1.46)	1.01 (0.76, 1.34)	0.86 (0.64, 1.16)	0.25

[a] Adjusted for age, height, energy intake, alcohol intake, sugar-sweetened beverages, vitamin D, calcium, retinol, caffeine, vitamin K, physical activity, BMI, smoking, menopausal hormone use, thiazide use, and all other protein groups

[b] Includes milk, yogurt, hard cheeses, and cottage cheese

animal protein was much higher than plant protein. Our study is somewhat consistent with the other US studies suggesting a possible inverse association with plant protein.

Dietary protein has been proposed to act negatively on bone remodeling based on the hypothesis that an increase in bone resorption is needed to maintain acid-base balance when metabolic acid load increases due to higher amino-acid intake [31]. However, it is now recognized that although higher protein increases urinary calcium excretion, it also increases intestinal calcium absorption [6]. In addition, in experimental studies, a higher calcium excretion in response to increased net acid load due to higher protein intake failed to detrimentally impact net calcium balance or N-telopeptide levels, which reflect bone turnover [32]. Low protein intake, on the other hand, can induce secondary hyperparathyroidism [6] and high levels of parathyroid hormone is associated with low bone mineral density [33]. Dietary protein stimulates the production of insulin-like growth factor-1 (IGF-1) [34] which in turn stimulates formation of osteoblasts and reduces apoptosis [35]. Cross-sectional studies have shown a direct association between IGF-1 and bone mineral density [35] and fractures [36]. In a randomized trial in postmenopausal women, supplementation of whey protein increased IGF-1 levels but hip bone mineral density was not different from the placebo group [37]. On the other hand, higher protein intake has been associated with higher bone mineral density in both men and women [10, 12, 38]. In a cross-sectional analysis of the Framingham Offspring Study, a dietary protein pattern characterized by high intake of low fat dairy was associated with higher femoral neck bone mineral density [39]. Falls are a major cause of fractures in the elderly and adequate protein intake to maintain muscle mass might reduce the risk of falls. However, the literature in this area has been sparse and with mixed results [40, 41].

This is one of the few studies to separately examine the association between protein intake and hip fractures in men. We had detailed and updated dietary information to examine different sources of protein, as well comprehensive lifestyle data to control for confounding. The range of intake for plant protein was much lower than animal protein. This might have limited our ability to detect a significant inverse association in the separate cohorts even though the magnitude of the RRs suggested this possibility. The finding of an inverse association between poultry intake and hip fracture risk in men was surprising and therefore need to be confirmed in other populations. Although all information was self-reported and some degree of error is unavoidable, we used a validated dietary questionnaire, and our cohort participants have shown to report health and medical information to a high degree of accuracy [19].

In conclusion, we found no evidence that higher protein intake increases risk of hip fracture in these Caucasian men and women. Rather, protein intakes from animal, plant, and dairy food sources were inversely associated with risk, but the strength of these associations may differ by gender.

Table 4 Multivariable RR (9% CI) of energy-adjusted total protein intake for hip fractures stratified by major risk factors

	Q1	Q2	Q3	Q4	Q5	Per 10 g increment	P interaction
Age							
Women							0.28
<65 (case = 455)	1	1.04 (0.79, 1.37)	0.87 (0.65, 1.17)	0.88 (0.65, 1.18)	0.76 (0.54, 1.06)	0.92 (0.84, 1.00)	
65 to <75 (case = 782)	1	0.86 (0.69, 1.08)	0.99 (0.79, 1.24)	0.97 (0.77, 1.23)	0.92 (0.71, 1.18)	0.98 (0.91, 1.06)	
75+ (case = 919)	1	1.12 (0.92, 1.37)	0.96 (0.77, 1.19)	1.15 (0.93, 1.43)	0.91 (0.69, 1.20)	1.02 (0.95, 1.11)	
Men							0.34
<65 (case = 73)	1	1.11 (0.54, 2.30)	0.80 (0.35, 1.82)	1.53 (0.73, 3.18)	1.17 (0.51, 2.65)	1.02 (0.84, 1.23)	
65 to <75 (case = 157)	1	0.90 (0.56, 1.46)	0.70 (0.42, 1.17)	0.79 (0.47, 1.32)	0.59 (0.33, 1.07)	0.85 (0.74, 0.98)	
75+ (case = 365)	1	0.95 (0.69, 1.32)	0.95 (0.68, 1.32)	0.96 (0.68, 1.35)	0.77 (0.51, 1.15)	0.94 (0.85, 1.03)	
BMI							
Women							0.32
BMI <25 (case = 1308)	1	1.00 (0.85, 1.17)	0.91 (0.77, 1.09)	1.03 (0.86 1.23)	1.07 (0.86, 1.31)	1.00 (0.94, 1.06)	
BMI >= 25 (case = 848)	1	0.98 (0.78, 1.24)	0.97 (0.77, 1.22)	0.95 (0.75, 1.20)	0.82 (0.63, 1.05)	0.95 (0.88, 1.02)	
Men							0.93
BMI <25 (case = 321)	1	0.93 (0.71, 1.40)	0.76 (0.53, 1.10)	0.94 (0.65, 1.35)	0.81 (0.52, 1.25)	0.94 (0.85, 1.04)	
BMI >= 25 (case = 274)	1	1.13 (0.74, 1.71)	1.16 (0.76, 1.75)	1.17 (0.77, 1.80)	0.83 (0.51, 1.34)	0.94 (0.84, 1.06)	
Physical activity							
Women							0.49
< median (case = 1179)	1	0.96 (0.81, 1.15)	0.90 (0.74, 1.08)	1.00 (0.83, 1.21)	0.95 (0.77, 1.17)	0.98 (0.92, 1.04)	
>= median (case = 977)	1	1.03 (0.83, 1.26)	0.99 (0.80, 1.22)	1.01 (0.82, 1.26)	0.92 (0.72, 1.16)	0.96 (0.90, 1.03)	
Men							0.15
< median (case = 393)	1	1.01 (0.74, 1.38)	0.96 (0.69, 1.33)	0.91 (0.65, 1.27)	0.68 (0.46, 1.01)	0.91 (0.83, 0.99)	
>= median (case = 202)	1	0.95 (0.60, 1.50)	0.71 (0.44, 1.15)	1.03 (0.64, 1.65)	0.98 (0.58, 1.67)	0.97 (0.85, 1.11)	
Smoking							
Women							0.72
Non-smoker (case = 1857)	1	1.01 (0.88, 1.17)	0.93 (0.80, 1.08)	1.04 (0.89, 1.21)	0.94 (0.79, 1.11)	0.98 (0.93, 1.02)	
Current (case = 299)	1	0.89 (0.62, 1.27)	0.98 (0.68, 1.42)	0.78 (0.51, 1.19)	1.00 (0.63, 1.58)	0.97 (0.86, 1.10)	
Men							0.12
Non-smoker (case = 300)	1	1.07 (0.75, 1.54)	0.79 (0.54, 1.17)	1.02 (0.70, 1.50)	0.76 (0.49, 1.19)	0.94 (0.85, 1.04)	
Current (case = 295)	1	0.86 (0.59, 1.25)	1.01 (0.69, 1.47)	0.80 (0.54, 1.20)	0.75 (0.48, 1.18)	0.90 (0.81, 1.00)	
Calcium intake							
Women							0.18
<600 mg/day (case = 218)	1	1.21 (0.83, 1.77)	1.10 (0.71, 1.70)	0.78 (0.45, 1.33)	0.93 (0.51, 1.67)	0.98 (0.86, 1.12)	
600+ mg/day (case = 1938)	1	0.97 (0.84, 1.12)	0.93 (0.80, 1.07)	1.00 (0.86, 1.16)	0.91 (0.77, 1.07)	0.96 (0.92, 1.01)	
Men							0.34
<600 mg/day (case = 71)	1	1.42 (0.51, 4.00)	0.80 (0.26, 2.43)	1.08 (0.33, 3.53)	0.66 (0.14, 3.02)	0.89 (0.66, 1.22)	
600+ mg/day (case = 524)	1	0.93 (0.71, 1.23)	0.86 (0.65, 1.15)	0.92 (0.69, 1.23)	0.75 (0.54, 1.05)	0.92 (0.85, 1.00)	

Adjusted for age, height, alcohol intake, sugar-sweetened beverages, total vitamin D, total calcium, retinol, caffeine, vitamin K, physical activity, BMI, smoking, menopausal hormone use, and thiazide use

Data from the 2013–2014 National Health and Nutrition Examination Survey showed mean protein intake for age 60–69 was 86.9 g in men and 67.6 g in women. This amount decreased to 80.8 g in men and 60.0 g in women for those age 70 or older [42]. While these amounts might still meet the Recommended Dietary Allowances of 0.8 g/kg [43], results from this and other studies suggest higher levels might have additional benefits for fracture prevention. However, recommendations for dietary change should take into account effects on all major health outcomes. Notably, within these cohorts, intake of animal protein has been associated with higher risk of cardiovascular mortality and intake of plant protein with lower risk of this outcome [44]. In addition, further research is needed to confirm whether associations between protein intake and risk of hip fractures differ by source and gender.

Compliance with ethical standards

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Conflicts of interest None.

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