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Legumes and meat analogues consumption are associated with hip fracture risk independently of meat intake among Caucasian men and women: the Adventist Health Study-2

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Abstract

Objective: In contrast to non-vegetarians, vegetarians consume more legumes and meat analogues as sources of protein to substitute for meat intake. The present study aimed to assess the association between foods with high protein content (legumes, meat, meat analogues) by dietary pattern (vegetarians, non-vegetarians) and hip fracture incidence, adjusted for selected lifestyle factors.
Design: A prospective cohort of Adventist Health Study-2 (AHS-2) enrollees who completed a comprehensive lifestyle and dietary questionnaire between 2002 and 2007.
Setting: Every two years after enrolment, a short questionnaire on hospitalizations and selected disease outcomes including hip fractures was sent to these members.
Subjects: Respondents (*n* 33 208) to a baseline and a follow-up questionnaire.
Results: In a multivariable model, legumes intake of once daily or more reduced the risk of hip fracture by 64 % (hazard ratio = 0·36, 95 % CI 0·21, 0·61) compared with those with legumes intake of less than once weekly. Similarly, meat intake of four or more times weekly was associated with a 40 % reduced risk of hip fracture (hazard ratio = 0·60, 95 % CI 0·41, 0·87) compared with those whose meat intake was less than once weekly. Furthermore, consumption of meat analogues once daily or more was associated with a 49 % reduced risk of hip fracture (hazard ratio = 0·51, 95 % CI 0·27, 0·98) compared with an intake of less than once weekly.
Conclusions: Hip fracture incidence was inversely associated with legumes intake and, to a lesser extent, meat intake, after accounting for other food groups and important covariates. Similarly, a high intake of meat analogues was associated with a significantly reduced risk of hip fracture.

Keywords
Legumes
Meat analogues
Vegetable protein
Hip fracture
Dietary protein

In 2009, the rate of hip fracture per 100 000 person-years among US Caucasian men and women 65+ years of age was 524·9 and 1088·7, respectively[1]. However, while hip fracture accounts for about 14 % of all fractures, it accounts for nearly 75 % of total fracture costs[1]. By 2025, the burden of incident osteoporosis-related fractures and costs are projected to increase by almost 50 %[2].

Findings from a meta-analysis suggest that the proportion of bone mineral density attributable to dietary protein intake is 1–2 %[3]. A slight positive association between protein supplement and lumbar bone mineral density was observed from this pooled analysis. However, there was no significant association between dietary protein and hip fracture[3]. Up until now, the association between dietary protein and the risk of hip fracture remains inconclusive[3–6].

According to the food guide pyramid, two or three daily servings of legumes or meat intake are recommended as sources of protein[7]. The source of dietary protein differs significantly between vegetarians and non-vegetarians. Vegetarians acquire protein from foods such as meat analogues (i.e. gluten, soya products), legumes and dairy. Among omnivores, a high percentage of protein comes from meat, poultry and fish. The fact that one-half of our study population in the Adventist Health Study-2 (AHS-2) are vegetarians (meat/fish less than once weekly) provides a unique opportunity to examine the influence of two food patterns (vegetarian or

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omnivore) with different sources of protein on the occurrence of hip fracture among a large cohort of males and females adjusting for demographic factors, medical history and lifestyle factors (smoking, physical activity, dairy intake, etc.). In the present study, due to a small number of hip fractures among other races in our cohort, we limited our analytical population to Caucasians.

Participants and methods

Study population

Participants were enrollees in the AHS-2, a large prospective cohort study investigating the relationship between lifestyle factors and several disease outcomes. The study has been described in detail elsewhere[8] and consists of Adventists throughout the USA and Canada who completed a comprehensive lifestyle and dietary questionnaire between 2002 and 2007. The study was approved by the Loma Linda University Institutional Review Board.

A total of 58 137 Caucasian men and women, aged 30 years and above, were enrolled into the study from 2002 to 2007; of these, 47 154 responded to the biennial hospital history surveys (Fig. 1). With the exclusion of persons with baseline self-reported osteoporosis, baseline minor trauma fracture and extreme values of daily energy intake, a total of 33 208 particiants were available for the present analysis.

FFQ

Dietary information was collected as part of enrolment into the AHS-2 study using a comprehensive self-administered and validated FFQ[9] reporting on the participant's dietary intake during the last 12 months. Participants were asked to report how frequently they consumed a food: 'never', '1–3 times per month', '1 time per week', '2–4 times per week', '5–6 times per week', '1 time per day' and '2 or more times per day'. Frequency per month was calculated for each food group (e.g. legumes, meat analogues, meat, dairy, soya milk, fruits). Soyabeans, tofu and soya cheese were not included in the legumes category as they were consumed very infrequently and were evaluated separately. Similarly, soya-containing meat analogues were evaluated separately. Thus, legumes in our study consist of refried beans, navy beans, garbanzo beans, pinto beans and lentils. Meat consumption was assessed using the combined monthly intake of beef, poultry, lamb, pork and fish. Vegetarian status was defined by the intake of meat/fish less than four times per month. By this definition, non-vegetarians consumed meat/fish at least once weekly. According to the definition, a little over 50 % of the participants were classified as vegetarians (n 17 300), 15 831 were non-vegetarians and seventy-seven could not be classified due to missing values. Monthly consumption was further categorized into times per week or times per day for statistical analysis.

Lifestyle questionnaire

At enrolment, in addition to the FFQ, participants completed a comprehensive questionnaire on exercise, medical history, smoking, anthropometrics, education, personal and household incomes as well as other demographic variables. Two surveys questions identified participants likely to have osteoporosis at baseline. The first asked 'Have you ever been told by a doctor that you had any of these conditions?' The second asked if participants had experienced any fracture due to minor accident in the year prior to enrolment. A total of 3719 men and women responded affirmatively to only the first question, 7741 men and women responded affirmatively only to the second question, and 2133 reported affirmatively to both. All were excluded from analysis (Fig. 1).

Outcome measurement

Approximately every two years after enrolment into the parent study, the Biennial Hospitalization History questionnaire (HHQ) was sent to participants. Eighty-one per cent (n 47 154) of Caucasian participants responded to either the first Biennial Hospital History Survey (HHQ1) or the third Biennial Hospital History Survey (HHQ3). These HHQ included questions on incident disease including the following on hip fracture in the HHQ1: 'During the last two years, have you developed fracture of the hip (broken hip bone) for the first time?', and the following in HHQ3: 'Have you had any fractures (broken bones) of the hip after 2001?' A total of 286 men and women who answered yes to one of these questions were identified as incident hip fracture cases for our study population. Thirty-nine per cent of the hip fractures (n 111) came from HHQ1 and the other 61 % (n 175) came from HHQ3. Participants were followed until the last response date of the HHQ or fracture date. The average follow-up time was 5·1 years. We also linked our database with the National Death Index database and used ICD10-S 72·0–72·2 (International Classification of Diseases, 10th edition, Clinical Modification) codes to identify additional hip fracture cases among those who died after enrolment and therefore were unable to return the HHQ. Nineteen additional hip fractures were identified for a total of 305 hip fractures. These additional hip fracture cases were followed until the average of HHQ returned date.

Statistical analysis

The χ^2 test was used to determine the statistical significance of the association between hip fractures and selected predictor variables (age, gender, physical activity (based on walking/running/jogging: low, 0–119 min/week or <3 miles/week; medium, 120–179 min/week or 3–8·9 miles/week; high, ≥180 min/week or ≥9 miles/week), total Ca intake, self-reported health status, smoking

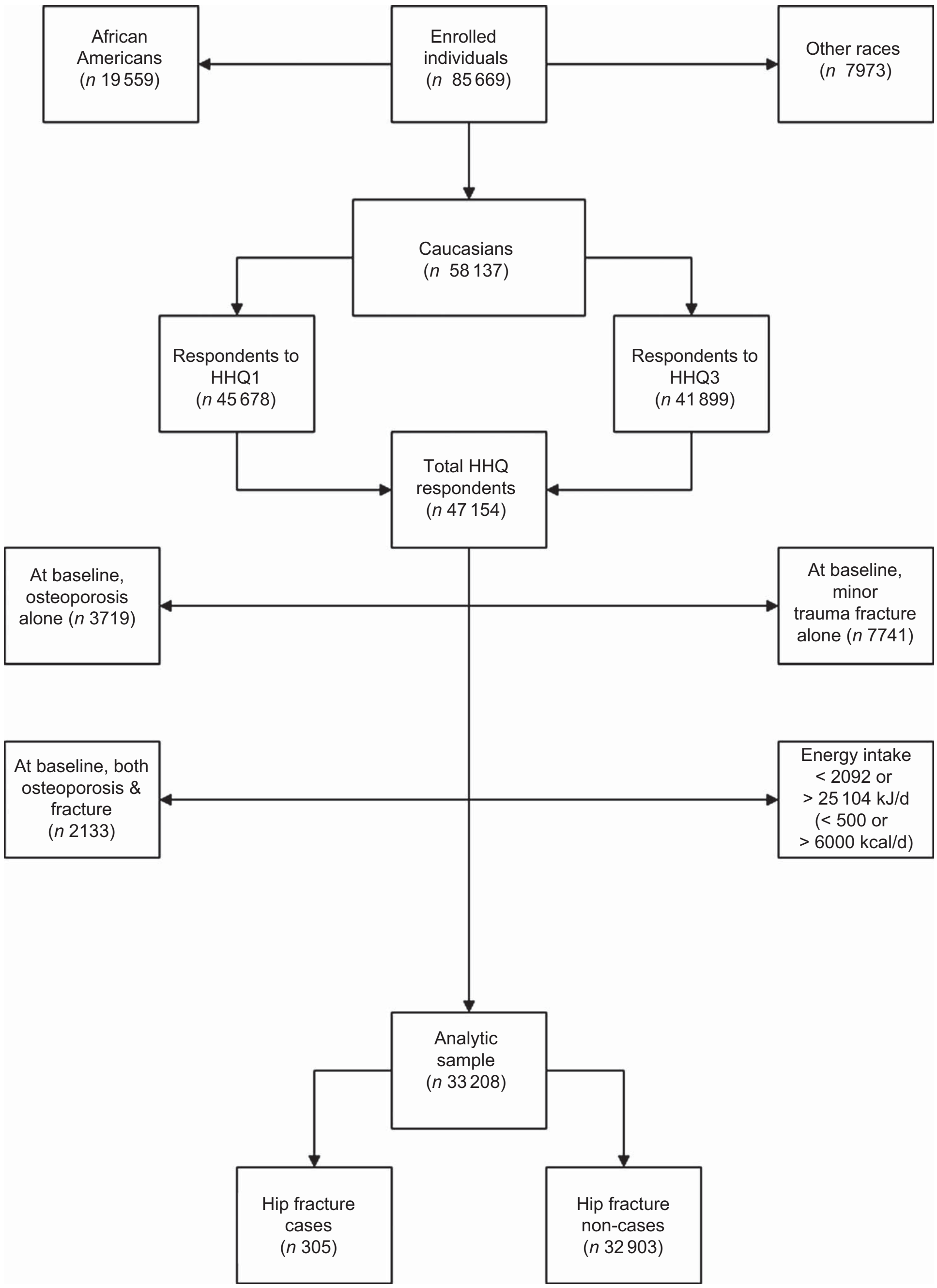

Fig. 1 Study population (HHQ, Biennial Hospitalization History questionnaire; HHQ1, first Biennial Hospital History Survey; HHQ3, third Biennial Hospital History Survey)

(ever smoker *v.* never smoker) and foods (meat, fruits, salads, vegetables, legumes, nuts, meat analogues, grains, soya milk, soya foods)). The *t* test was used to compare the continuous variables (height, weight, total energy intake) between cases and non-cases.

Cox proportional hazard regression analyses were used to determine the associations between high-protein foods (legumes, meat, meat analogues) and occurrence of hip fracture. Hazard ratios (HR) and 95 % confidence intervals were calculated with attained age as time variable adjusted for all the above covariates. Left truncation of failure time and time at risk was used to select only ages after participants joined AHS-2. Log likelihood ratio tests were used to determine the *P* value of χ^2 difference of the likelihood ratio test between the full model (with all foods) and the reduced model (without one particular food). Any independent variable which changed the estimated hazard ratio of the primary exposure variables (legumes, meat, meat analogues) by at least 10 % remained in the final model.

The final model estimated the hazard ratio of high-protein foods (i.e. frequency of legumes, meat analogues, meat) in the total population as well as by dietary pattern (vegetarian or omnivore). All statistical analyses were performed using the statistical software package SAS version 9·2.

Results

Our annual incidence rate of women and men who experienced a hip fracture during the follow-up period was 1·94/1000 person-years and 1·91/1000 person-years, respectively (Table 1). Vegans experienced the highest rate of hip fractures (Table 1). Compared with non-cases, the cases were older (74 *v.* 59 years), had lower weight (72·5 *v.* 77·6 kg), were more likely to report their health status as fair/poor (20 % *v.* 11 %) and had somewhat lower levels of physical activity (Table 2).

The percentage of people who consumed legumes, meat and meat analogues less than once weekly was higher among cases compared with non-cases (legumes: 14 % *v.* 8 %; meat: 61 % *v.* 52 %; meat analogues: 42 % *v.* 37 %). The daily median intakes of various foods and their daily ranges by vegetarian status are shown in Table 3. Overall, vegetarians consumed more legumes, vegetables, salad, nuts, soya foods, soya milk, fresh fruits and grains than their non-vegetarians counterparts. Non-vegetarians consumed more meat and dairy than vegetarians (Table 3).

Multivariable analyses

Independent and significant reductions in the risk of hip fracture by 37 % were found with increasing meat intake (more than three times weekly *v.* less than once weekly) and by more than 60 % with increasing consumption of legumes (once daily or more *v.* less than once weekly). There was a non-significant negative association between hip fracture risk and intake of meat analogues, dairy, nuts and grains. Soya foods and soya milk consumption were not associated with the risk of hip fracture (Table 4).

Final model

From our findings, none of the other investigated independent food variables (e.g. dairy, nuts, soya milk, grains) changed the effect of the statistically significant high-protein foods (legumes, meat, meat analogues) on the hazard ratio of hip fracture by more than 10 %. Thus, in the final model (Table 5), we included the three protein foods (legumes, meat/fish and meat analogues) adjusted for age, height, weight, gender, energy intake, total Ca intake, self-reported health status, smoking and physical activity level and stratified by vegetarian status. In the total study population, consumption of legumes, meat and meat analogues showed independent and significant protective effects on risk of hip fracture by 64 %, 40 % and 49 %, respectively, for those who consumed these three foods at the highest level of intake compared with the lowest level.

When stratifying on vegetarian status, the associations with legumes and meat were strengthened among non-vegetarians with a reduction of 82 % and 46 %, respectively. Among vegetarians, the association with meat analogues was strengthened (HR = 0·34, 95 % CI 0·12, 0·95) and the association with legumes was somewhat weakened (HR = 0·48, 95 % CI 0·24, 0·97), but remained statistically significant. Among vegetarians with infrequent intake of meat less than once weekly, meat intake showed some protection (HR = 0·83), although non-significant, compared with those who never ate meat.

Discussion

Our hip fracture incidence rate is comparable to the rate reported in 2006 by a large health-care organization in California, which was 2·24/1000 person-years and 1·29/1000 person-years among men and women, respectively[10]. As expected, the women experienced a higher rate of hip fracture than the men (58 % females *v.* 42 % males)[1,10].

In our study population, both legumes intake and meat intake independently reduced the risk of hip fracture by 40–64 %. Both food groups are high in protein, but from

Table 1 Hip fracture rates among 33 208 Caucasian males and females by gender and vegetarian status*, Adventist Health Study-2, 2002–2007

	No. of participants	Hip fracture cases	Mean age (years) at fracture*	Age-adjusted rate per 1000 person-years
Overall	33 208	305	76·8	1·93
Men	14 044	127	76·3	1·91
Women	19 164	178	77·2	1·94
Non-vegetarians†	15 831	120	75·2	1·60
Vegetarians (including vegans)†	17 300	184	77·8	2·21
Vegetarians (excluding vegans)†	13 524	130	78·0	1·99
Vegans†	3776	54	77·2	2·99

*Mean age calculation excluded ninteen hip fracture cases identified from the National Death Index database.
†Seventy-seven individuals were not classified as vegetarians or non-vegetarians due to missing values.

Table 2 Food intakes and other lifestyle characteristics of hip fracture cases and non-cases among 33 208 Caucasian males and females by vegetarian status*, Adventist Health Study-2, 2002–2007

	All participants		Non-vegetarians*		Vegetarians*	
	Non-cases (*n* 32 903)	Cases (*n* 305)	Non-cases (*n* 15 711)	Cases (*n* 120)	Non-cases (*n* 17 116)	Cases (*n* 184)
Age (years, %)						
<55 years	42	6	42	8	42	5
55–64 years	24	14	26	17	22	13
65–74 years	20	23	20	26	19	21
75–84 years	12	42	11	38	14	45
85+ years	3	15	2	12	3	17
P value†	<0·0001		<0·0001		<0·0001	
Height (cm)						
Mean	169·9	169·3	169·8	169·6	170·0	168·8
SD	10·2	10·7	10·3	10·8	10·0	10·6
P value†	0·3		0·96		0·14	
Weight (kg)						
Mean	77·6	72·5	81·5	78·7	74·1	68·4
SD	18·2	16·7	19·1	18·3	16·4	14·2
P value†	<0·0001		0·01		<0·0001	
Energy intake (kJ)						
Mean	7505	7647	7658	7812	7372	7552
SD	3000	3155	3151	2912	2845	3310
P value†	0·4		0·6		0·4	
Energy intake (kcal)						
Mean	1793·7	1827·6	1830·2	1867·1	1761·9	1804·9
SD	717	754	753	696	680	791
P value†	0·4		0·6		0·4	
Gender (%)						
Female	58	58	58	58	58	59
Male	42	42	42	43	42	41
P value†	>0·82		0·95		0·67	
Oestrogen use (females, %)						
Past/never user	84	85	82	78	87	89
Current user	16	15	18	22	13	11
P value†	0·9		0·4		0·5	
Smoking status (%)						
Never smoker	80	85	74	72	87	93
Ever smoker	20	15	26	28	13	7
P value†	0·07		0·64		0·01	
Health status (%)						
Excellent/good	89	80	87	73	92	85
Fair/poor	11	20	13	27	8	15
P value†	<0·0001		<0·0001		0·0008	
Physical activity‡ (%)						
Walking/running/jogging						
0–119 min/week or <3 miles/week	47	53	52	61	42	47
120–179 min/week or 3–8·9 miles/week	33	29	31	23	36	33
≥180 min/week or ≥9 miles/week	20	18	17	16	22	20
P value†	0·14		0·13		0·44	
Total daily Ca intake (%)						
<400 mg/d	11	10	9	5	13	13
400–1200 mg/d	57	59	55	53	60	63
1200+ mg/d	31	31	36	42	27	24
P value†	0·59		0·20		0·62	
Meat consumption (%)						
No meat intake	52	61	n/a	n/a	89	91
Less than once weekly			n/a	n/a	11	9
One to three times weekly	22	26	45	65	n/a	n/a
More than three times weekly	26	14	55	35	n/a	n/a
P value†	<0·0001		<0·0001		0·27	
Legumes consumption (%)						
Less than once weekly	8	14	12	22	5	9
Once weekly to less than once daily	80	76	80	73	80	78
Once daily or more	12	10	8	5	15	13
P value†	0·001		0·004		0·04	
Meat analogues consumption (%)						
Less than once weekly	37	42	45	46	29	39
Once weekly to less than once daily	55	54	47	48	62	59
Once daily or more	8	4	8	6	8	3
P value†	0·01		0·72		0·001	

Table 2 *Continued*

	All participants		Non-vegetarians*		Vegetarians*	
	Non-cases (n 32 903)	Cases (n 305)	Non-cases (n 15 711)	Cases (n 120)	Non-cases (n 17 116)	Cases (n 184)
Dairy consumption (%)						
Less than once weekly	16	23	3	6	28	34
Once weekly to less than once daily	35	30	32	24	38	34
Once daily or more	48	47	65	70	33	32
P value†	0·007		0·07		0·22	
Vegetables consumption (%)						
Less than once daily	37	38	42	43	32	34
Once daily to less than twice daily	38	36	36	38	40	35
Twice daily or more	25	26	22	20	28	30
P value†	0·79		0·86		0·46	
Salad consumption (%)						
Less than once daily	28	24	32	28	24	21
Once daily to less than twice daily	38	36	37	35	39	37
Twice daily or more	34	40	31	37	37	42
P value†	0·11		0·34		0·45	
Nuts consumption (%)						
Less than once weekly	4	4	6	7	2	2
Once weekly to less than once daily	43	32	52	37	36	29
Once daily or more	53	64	42	57	62	69
P value†	0·0002		0·004		0·13	
Fruits consumption (%)						
Less than once daily	10	6	14	9	6	3
Once daily to less than three times daily	35	31	40	38	31	28
Three times daily or more	55	63	46	53	63	69
P value†	0·004		0·15		0·13	
Grains consumption (%)						
Less than once daily	19	12	26	16	13	10
Once daily to less than twice daily	34	36	37	42	32	33
Twice daily or more	46	51	37	43	54	57
P value†	0·009		0·04		0·50	
Soya milk intake (%)						
Less than once weekly	52	49	68	68	37	36
Once weekly to less than once daily	25	20	18	18	32	22
Once daily or more	23	31	14	14	31	42
P value†	0·002		0·98		0·003	
Tofu, soya cheese intake§ (%)						
Never	35	34	52	54	21	21
Less than once weekly	20	17	20	14	19	18
Once weekly or more	45	50	28	32	60	61
P value†	0·19		0·24		0·93	

n/a, not appliccable.
*Seventy-seven individuals were not classified as vegetarians or non-vegetarians due to missing values
†*P* value of χ^2 test or *t* test.
‡945 missing values for physical activity levels.
§450 missing values for tofu, soya cheese.

different sources. With the elimination of meat from the diet among vegetarians, legumes are one of the major sources of protein and are thus placed at the bottom of the vegetarian food guide pyramid[11]. In our study, we excluded soyabeans and soya-containing meat analogues from the larger legume category because we wished to evaluate them separately given their isoflavone content. With the exception of soyabeans, the nutritional benefit of legumes has not been previously studied extensively for potential effects on bone metabolism.

None of the studies examining the effect of legumes intake on fracture risk found any association, possibly because of low intake levels of legumes in these study populations[12–14]. In the Iowa Women's Health Study, women who experienced hip fracture and those who did not each consumed about 0·05 serving of legumes daily and showed no significant difference in the level of intake[15]. As suggested by the authors, it may be difficult to detect the beneficial effect of vegetable protein intake due to a homogeneous and relatively low level of vegetable protein intake in this population.

In a cohort of elderly volunteers in the European Prospective Investigation into Cancer and Nutrition (EPIC) study, legumes intake at the highest quintile level showed a non-significant protective trend for hip fracture (HR = 0·95; 95 % CI 0·87, 1·04)[13]. However, the mean daily legumes intake across five participating countries ranged from 2·9 g for women in Sweden to 20·9 g for men in Germany, compared with the median daily intake of legumes without soyabeans of 36 g (range 0–189 g) in

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Table 3 Median and range* of food intakes among 33 208 Caucasian males and females by vegetarian status†, Adventist Health Study-2, 2002–2007

		All participants (*n* 33 208)		Non-vegetarians† (*n* 15 831)		Vegetarians† (*n* 17 300)	
Food	Average serving size	Median (serving/d)	Range* (serving/d)	Median (serving/d)	Range* (serving/d)	Median (serving/d)	Range* (serving/d)
Legumes	½ cup	0·4	0–2·1	0·3	0–2·0	0·5	0–2·3
Meat	3–4 oz	0·1	0–2·1	0·5	0·1–2·5	0·0	0–0·07
Meat analogues	2 slices	0·4	0·1–2·6	0·4	0·1–2·7	0·4	0·1–2·6
Dairy	2 slices or 6–8 oz cup	0·9	0–5·4	1·3	0–5·9	0·5	0–4·8
Vegetables	½ cup	1·3	0·1–4·9	1·1	0·1–4·7	1·3	0·1–5·2
Salad	1 cup	1·5	0·1–6·0	1·4	0·1–5·9	1·6	0·1–6·0
Nuts	14 nuts	1·0	0–6·5	0·8	0–6·0	1·3	0·1–6·8
Tofu/soya cheese	½ cup	0·1	0–1·7	0·0	0–1·1	0·1	0–2·5
Soya milk	1 cup	0·8	0·1–4·4	0·7	0·1–3·5	0·8	0·1–4·5
Fresh fruits	1 medium or ⅓ cup	3·3	0·2–20·6	2·7	0·1–18·8	3·8	0·3–21·9
Grains	1 cup	1·9	0·1–6·6	1·5	0·1–6·2	2·1	0·1–6·8

*1st percentile–99th percentile.
†Seventy-seven individuals were not classified as vegetarians or non-vegetarians due to missing values.

our population. The high consumption of legumes in our population may be an important reason for our findings. Our findings are in line with our earlier findings from the Adventist Health Study-I Cohort (AHS-1), where a non-significant negative association was found between vegetable protein intake (beans, nuts, vegetarian meat analogues) at one or more time(s) daily and the risk of wrist fracture (HR = 0·79; 95 % CI 0·43, 1·46)[15]. Compared with other sources of plant protein, legumes consist of protein that is high in essential amino acids such as lysine, which is common in animal sources but much less common in plant sources[16]. Without any dairy or meat intake or supplements, it is unlikely that anyone is able to meet the daily dietary recommended levels of lysine[17]. For an adult weight of 60 kg, about 2280 mg of lysine is recommended per day. Two cups of cooked navy beans would yield approximately 2160 mg of lysine. Therefore, an individual who adheres to a vegan diet, which excludes meat and dairy products, will need at least two cups of cooked beans to meet the recommended lysine intake requirement.

Lysine and hydroxylysine are the main amino acids in the cross-linking process of bone collagen[18]. Among osteoporotic individuals, bone collagen taken from cancellous bone showed a substantial reduction in the concentration of collagen cross-links compared with their matched controls[18]. A deficiency in lysine is evident among lysinuric protein intolerance patients, who showed a significant reduction in collagen synthesis in fibroblast cultures compared with their age-matched controls at 5, 14 and 30 years of age[19]. Additionally, lysine has been shown to enhance intestinal Ca absorption and renal Ca preservation compared with the amino acids valine or tryptophan among forty-five osteoporotic patients[20]. Lysine can also influence bone health through its end product carnitine. Carnitine supplements have been shown to improve bone density in some animal and human studies[21]. Furthermore, legumes are a good source of Ca and thus important for bone mineralization[22].

Soyabeans and soya-containing meat analogues were not part of the legumes intake category used in the present study. Our study population has a much higher soya intake than other Western populations, mostly from meat analogue sources and less so from tofu. We found no effect on hip fracture risk of soya milk, tofu or soyabeans in our study. Soyabeans, soya products and derivatives have been the subject of intense focus in the past decade. Both animal and human studies have indicated that soya isoflavones may prevent bone loss[23–26]. The inverse association between soya intake and fracture risk is believed by some to be caused by an increase in bone mineral density by a metabolite of the soya isoflavone known as equol[27]. Intervention trials have consistently found that soya isoflavones increase bone formation markers and reduce bone resorption markers[24]. In a meta-analysis reviewing nine randomized controlled trials of isoflavone supplements with follow-up periods of 3 to 12 months, there was a significant decrease in urinary deoxypyridinoline, a bone resorption marker, and a significant increase in serum bone-specific alkaline phosphatase, a bone formation marker, among individuals who consumed soya isoflavone compared with those who did not[24]. However, the effect of soya on bone mineral density has been inconsistent in several studies[25]. A meta-analysis reviewing ten studies with follow-up periods of at least 1 year found that daily intake of >80 mg of isoflavone showed a weak increase in bone mineral density only at the lumbar spine[25]. The Shanghai Women's Health Study, a prospective cohort study of 24 403 postmenopausal women, found a significant negative association between fracture incidence and quintiles of soya protein intake (HR = 0·63; 95 % CI 0·53, 0·76)[26]. It is, of course, possible that the effect of tofu intake seen in these women could just as well be a protein effect *per se* rather than an isoflavone effect.

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Table 4 Associations between high-protein foods and hip fracture incidence among 33 208* Caucasian males and females in multivariable Cox regression model†, Adventist Health Study-2, 2002–2007

	All participants (non-cases, *n* 32 903; cases, *n* 305)		Non-vegetarians‡ (non-cases, *n* 15 711; cases, *n* 120)		Vegetarians‡ (non-cases, *n* 17 116; cases, *n* 184)	
	HR	95 % CI	HR	95 % CI	HR	95 % CI
Legumes intake						
Less than once weekly	1·00	Reference	1·00	Reference	1·00	Reference
Once weekly to less than once daily	0·49	0·34, 0·70	0·44	0·27, 0·72	0·55	0·31, 0·99
Once daily or more	0·34	0·20, 0·59	0·18	0·06, 0·54	0·45	0·22, 0·94
P value§	0·0003		0·0008		0·11	
Meat intake					1·00	Reference (none)
Less than once weekly	1·00	Reference			0·84	0·50, 1·43
One to three times weekly	1·11	0·82, 1·51	1·00	Reference		
More than three times weekly	0·63	0·42, 0·94	0·55	0·36, 0·83		
P value§	0·01		0·004		0·52	
Meat analogues intake						
Less than once weekly	1·00	Reference	1·00	Reference	1·00	Reference
Once weekly to less than once daily	0·96	0·74, 1·24	1·08	0·71, 1·61	0·90	0·65, 1·26
Once daily or more	0·52	0·27, 1·00	0·80	0·34, 1·92	0·34	0·12, 0·95
P value§	0·09		0·77		0·06	
Dairy consumption						
Less than once weekly	1·00	Reference	1·00	Reference	1·00	Reference
Once weekly to less than once daily	0·74	0·52, 1·04	0·42	0·18, 0·97	0·85	0·57, 1·26
Once daily or more	0·80	0·55, 1·18	0·51	0·22, 1·18	0·89	0·56, 1·42
P value§	0·23		0·17		0·72	
Nuts consumption						
Less than once weekly	1·00	Reference	1·00	Reference	1·00	Reference
Once weekly to less than once daily	0·73	0·39, 1·34	0·64	0·30, 1·39	0·88	0·31, 2·49
Once daily or more	0·77	0·41, 1·44	0·89	0·40, 1·95	0·74	0·26, 2·10
P value§	0·6		0·25		0·63	
Grains consumption						
Less than once daily	1·00	Reference	1·00	Reference	1·00	Reference
Once daily to less than twice daily	1·31	0·88, 1·95	1·46	0·83, 2·54	1·19	0·67, 2·12
Twice daily or more	0·79	0·52, 1·21	0·88	0·48, 1·61	0·71	0·39, 1·28
P value§	0·002		0·07		0·02	
Soya milk intake						
Less than once weekly	1·00	Reference	1·00	Reference	1·00	Reference
Once weekly to less than once daily	0·93	0·66, 1·31	1·00	0·58, 1·75	0·90	0·58, 1·41
Once daily or more	1·04	0·76, 1·44	0·84	0·47, 1·53	1·13	0·76, 1·69
P value§	0·81		0·84		0·54	
Tofu, soya cheese intake						
Never	1·00	Reference	1·00	Reference	1·00	Reference
Less than once weekly	1·02	0·70, 1·48	0·72	0·40, 1·31	1·30	0·78, 2·16
Once weekly or more	1·22	0·87, 1·69	1·27	0·77, 2·09	1·21	0·77, 1·91
P value§	0·45		0·19		0·58	

HR, hazard ratio.
*1736 individuals were excluded from the model due to censored or missing values.
†All foods in the same model adjusted for fruits and vegetables intake, age, height, weight, gender, energy intake, physical activity, smoking, health status and total Ca intake.
‡Seventy-seven individuals were not classified as vegetarians or non-vegetarians due to missing values.
§*P* value of χ^2 difference of the likelihood ratio test between the full model (with all three foods) and the reduced model (without one of the three foods).

Among our participants, intake of meat analogues of at least one serving daily reduced the risk of hip fracture by up to 49 %. Although the benefit of soya products has been investigated for the past decade, little is known about the effect of meat analogues on bone metabolism. The main protein ingredients in meat analogues are soya, wheat, gluten, eggs and milk. A typical serving of meat analogues (1 serving ≈ 73 g in AHS-2) contains at least 10 g of protein, but can vary from 9 to 18 g. In our study population, the median daily intake of meat analogues is 29 g (range 7–190 g), much higher than that found among health-conscious Europeans[28]. The mean daily meat analogues intake among Greek men and Danish men is 0·46 g and 0·13 g, respectively[28]. It is likely that the wide range of meat analogues intake in our population may have allowed us to capture its beneficial effect on hip fracture risk.

A number of studies have examined the effects of animal protein consumption on fracture risk in various ways[12,29–40], but few have reported on the effect of meat consumption specifically. Our finding of an inverse association between meat consumption and hip fracture risk is in agreement with others[12,15,36,37]. In the Iowa Women's Health Study, postmenopausal women without hip fracture had a significantly higher intake of meat than women with hip fracture[12]. In a case–control study of

Table 5 Associations between legumes, meat and meat analogues consumption and hip fracture incidence among 33 208* Caucasian males and females in final multivariable Cox regression model†, Adventist Health Study-2, 2002–2007

	All participants (non-cases, *n* 32 903; cases, *n* 305)		Non-vegetarians‡ (non-cases, *n* 15 711; cases, *n* 120)		Vegetarians‡ (non-cases, *n* 17 116; cases, *n* 184)	
	HR	95% CI	HR	95% CI	HR	95% CI
Legumes intake						
Less than once weekly	1·00	Reference	1·00	Reference	1·00	Reference
Once weekly to less than once daily	0·50	0·35, 0·71	0·44	0·28, 0·70	0·58	0·33, 1·02
Once daily or more	0·36	0·21, 0·61	0·18	0·06, 0·53	0·48	0·24, 0·97
P value§	0·0003		0·0004		0·13	
Meat intake					1·00	Reference (none)
Less than once weekly	1·00	Reference			0·83	0·50-1·41
One to three times weekly	1·05	0·79, 1·40	1·00	Reference		
More than three times weekly	0·60	0·41, 0·87	0·54	0·36, 0·82		
P value§	0·009		0·003		0·49	
Meat analogues intake						
Less than once weekly	1·00	Reference	1·00	Reference	1·00	Reference
Once weekly to less than once daily	0·96	0·75, 1·24	1·10	0·74, 1·65	0·91	0·66, 1·26
Once daily or more	0·51	0·27, 0·98	0·85	0·36, 2·00	0·34	0·12, 0·95
P value§	0·08		0·77		0·05	

HR, hazard ratio.
*1736 individuals were excluded from the model due to censored or missing values.
†All three foods in the same model adjusted for age, height, weight, gender, energy intake, physical activity, smoking, total Ca intake and health status.
‡Seventy-seven individuals were not classified as vegetarians or non-vegetarians due to missing values.
§*P* value of χ^2 difference of the likelihood ratio test between the full model (with all three foods) and the reduced model (without one of the three foods).

Chinese women, a greater proportion of those without a forearm fracture recalled eating meat (OR = 0·13; 95 % CI 0·02, 0·74) and pork ribs (OR = 0·37; 95 % CI 0·17, 0·83) more frequently between the ages of 16 and 29 years than their matched controls[36]. However, neither of these two studies reported the effect of meat consumption in a final model. In the AHS-I cohort, meat intake of four or more times weekly significantly reduced the risk of wrist fracture by 56 % (HR = 0·44; 95 % CI 0·23, 0·84) compared with no meat intake[15]. In a prospective population-based cohort of elderly people in Japan, three dietary patterns (meat, vegetable, traditional Japanese) were identified through factor analysis[37]. Results showed that participants who ranked moderately high on the meat pattern were significantly less likely to experience any fall-related fracture (HR = 0·36; 95 % CI 0·13, 0·94). A similar reduction in risk was observed in the group that ranked highest on the meat pattern. These findings should be interpreted in the context of the Japanese diet which is typically low in meat consumption relative to Western countries. According to the 2002 national nutritional survey in Japan, average meat consumption per capita was approximately one-third that in the USA (77 g/d *v.* 245 g/d)[37].

In contrast, the Nurses' Health Study found no association between intake of beef, pork or lamb during the teenage years and forearm or hip fracture[30]. Similarly, the EPIC cohort study of men and women 60 years of age and older followed for 8 years found no association between meat intake and hip fracture risk (HR = 1·01; 95 % CI 0·91, 1·11)[13]. However, a non-significant protective effect of fish/shellfish intake on hip fracture risk was observed (HR = 0·93; 95 % CI 0·85, 1·02)[13]. A case–control study conducted in Spain also reported no association between fracture risk and meat intake[14]. The contrasting findings between our study and the European studies could be due to the striking difference in the level of meat intake. The mean daily meat intake in the EPIC cohort ranged from 52·4 g in women to 120·4 g in men and was 136·3 g and 125·5 g for cases and controls, respectively, in Spain. In our study, the average meat intake is approximately 0·5 serving/d or 32·5 g/d (1 serving ≈ 65 g) among non-vegetarians. It may be that the beneficial effect of meat on fracture risk may be most readily observable in populations with generally low meat consumption. In other words, it is possible that meat intake is beneficial to bone up to a certain threshold level of intake.

Although there are multiple factors that contribute to the risk of hip fracture such as physical activity, risk of falls, muscle strength and other nutrients such as vitamin D, the independent and beneficial effect of dietary protein on bone health is well supported by a number of studies[38]. Protein is recognized for its ability to improve Ca balance, suppress parathyroid hormone, increase lean body mass and increase production of the bone growth regulator insulin-like growth factor-1[39]. From our findings, foods with high content of either animal or vegetable protein can significantly reduce the risk of hip fracture by at least 40 %. It appears that vegans may be especially vulnerable to fractures; as reported in one cohort study of Taiwanese postmenopausal women, long-term vegans suffered a 4-fold risk of osteopenia at the femoral neck and a 2·5-fold risk of lumbar fracture due to low protein intake[40]. In our study, the highest rate of hip fracture was observed among vegans as well.

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Our findings indicate that consumption of legumes may be beneficial to both vegetarians and non-vegetarians in addition to their staple protein food source such as meat or meat analogues.

The present study has several strengths and some limitations. First, it is the first study to examine the association between legumes intake and hip fracture risk excluding soyabeans intake. Second, our study population consists predominantly of vegetarians or lower meat consumers compared with the general US population. This unique population provides us with non-homogeneous food consumption levels which enhances statistical power to detect associations. Third, our FFQ was validated and has shown high correlations for estimates of forty-seven foods and food groups and 24 h dietary recalls[9]. Fourth, our study has a prospective design with a follow-up period of at least 2 years. Fifth, we adjusted for important confounders which may influence the risk of hip fracture. A limitation is that there might have been a change in the dietary pattern during the period between the baseline questionnaire and the hip fracture incidence. However, we have found that individuals in this population rarely change their overall dietary pattern over short periods of time such as in the present study[41]. Our self-reported hip fractures were not verified by hospital records. However, others have shown that the validity of self-reported hip fracture when compared with hospital records is very high, ranging from 81 % to 93 %[42,43]. In one study, self-reports of hip fracture were verified by radiological reports and had a true-positive rate of 83 %[44]. In addition, higher education increases the accuracy of self-reported fractures[44]. Since 93 % of our study population were high-school graduates and 53 % had at least an associate degree, this likely contributes to a high true-positive rate of self-reported hip fractures in our study population.

Furthermore, non-response bias may exist because some individuals might fail to report their hip fracture status. With our non-response rate of 19 %, we assume that there is no difference in the level of dietary intake between those who responded to the follow-up survey and those who did not.

In summary, our study indicates that higher intakes of legumes, meat and meat analogues can reduce the risk of hip fracture. Among vegetarians, meat analogues and legumes are recommended as food sources to maintain an adequate protein intake. Among non-vegetarians, legumes and meat intake are food sources which can independently reduce the risk of hip fracture.

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