



Mini Review

High Versus low Dietary Protein Intake and Bone Health in Older Adults: a Systematic Review and Meta-Analysis

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ABSTRACT

Protein may play a beneficial role in the prevention of bone loss and in slowing down osteoporosis. The effect of dietary protein may be different in older adults compared to younger adults, since this population has a greater need for protein. The aim of this systematic review and meta-analysis was to investigate the impact of a dietary protein intake above the Recommended Dietary Allowance (RDA) of 0.8 g/kg body weight/day from any source on Bone Mineral Density (BMD)/Bone Mineral Content (BMC), bone turnover markers, and fracture risk in older adults compared to a lower dietary protein intake. A systematic search was conducted through October 2018 in 3 databases: CENTRAL, MEDLINE, and EMBASE. We included all prospective cohort studies and Randomized Controlled Trials (RCTs) among adults aged ≥65 years that examined the relation between protein intake on bone health outcomes. Two investigators independently conducted abstract and full-text screenings, data extractions, and risk of bias assessments. Authors were contacted for missing data. After screening of 523 records, twelve cohort studies and one RCT were included. Qualitative evaluation showed a positive trend between higher protein intakes and higher femoral neck and total hip BMD. Meta-analysis of four cohort studies showed that higher protein intakes resulted in a significant decrease in hip fractures (pooled hazard ratio: 0.89; 95% confidence interval: 0.84, 0.94). This systematic review supports that a protein intake above the current RDA may reduce hip fracture risk and may play a beneficial role in BMD maintenance and loss in older adults.

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Contents

1.	Introduction	1102
2.	Methods	1102
2.1.	Data Sources and Searches	1102
2.2.	Study Eligibility Criteria.	1102
2.3.	Study Selection Process.	1102
2.4.	Data Extraction	1103
2.5.	Risk of Bias in Individual Studies.	1103
2.6.	Data Synthesis	1103
2.6.1.	Qualitative Synthesis	1103
2.6.2.	Quantitative Synthesis	1103
3.	Results	1103
3.1.	Quality of Evidence.	1103
3.2.	Prospective Cohort Studies	1104
3.2.1.	BMC–Total Body.	1104
3.2.2.	BMD–Total Body.	1104
3.2.3.	BMD–Total Hip	1104

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3.2.4.	BMD–Femoral Neck	1106
3.2.5.	BMD–(Lumbar) Spine.	1106
3.2.6.	Bone Turnover Markers	1106
3.2.7.	Fractures	1108
3.3.	RCTs	1108
3.4.	Meta-analysis	1108
4.	Discussion	1109
5.	Conclusions.	1110
	Acknowledgements	1110
Appendix A.	Supplementary data.	1110
	References.	1111

1. Introduction

Osteoporosis is an increasing public health problem worldwide [1]. The prevalence in nine industrialized countries is estimated at 9–38% for women and 1–8% for men, affecting up to 49 million people [2]. The rising prevalence of osteoporosis leads to an increase in the number of falls and fractures, which in turn affects mortality and morbidity, and increases the economic burden [1]. Protein may play a role in the prevention of bone loss and in slowing down osteoporosis [3].

An adequate intake of dietary protein is important for bone acquisition and maintenance. Older adults may become protein malnourished due to an inadequate intake of protein and a reduced ability to use available protein, because of age-related changes in metabolism, immunity, and hormone levels and sensitivity [4,5]. At the same time there is a greater need for protein [5]. The current recommended dietary allowance (RDA) for protein is 0.8 g/kg body weight/day [6]. For the preservation of muscle function, evidence supports a protein intake of 1.0–1.2 for healthy older adults and 1.2–1.5 g/kg body weight/day for older adults suffering from acute or chronic illnesses [7].

Over the past years, the relation between dietary protein intake and bone health has received much attention. Safety concerns of a high dietary protein intake have been raised, but beneficial effects on bone health have also been found. As yet, several systematic reviews and meta-analyses [8–11] have been published investigating the effect of dietary protein intake on bone health. All of these publications pooled cohort studies and trials over a wide age range with specific exclusion criteria to find beneficial effects of protein intake on Bone Mineral Density (BMD), Bone Mineral Content (BMC), bone turnover markers, and/or fracture risk. Some reviews include trials that have an intervention duration of <6 months [8,10]. However, it is questionable if dietary interventions can already lead to a measurable change in BMD within such a short time span. To get a reliable estimate of the impact on changes in BMD, a minimum intervention duration of 6 months seems appropriate [12].

An expert consensus paper from 2018 summarized the systematic reviews and meta-analyses looking at the effects of dietary protein on bone health in adults [3]. It states that protein intakes above the RDA, in combination with an adequate calcium intake, is associated with higher BMD, a lower rate of bone loss, and a modestly reduced fracture risk. Furthermore, it was not proven that the acid load caused by a high protein intake is harmful for bone health.

None of the above reviews focussed on older adults (age of 65 years and older) specifically. The effect of dietary protein may be different in older adults compared to adults, since this population has a greater need for protein. The aim of this systematic review and meta-analysis was to investigate the impact of a dietary protein intake above the RDA of 0.8 g/kg body weight/day from any source on BMD/BMC, bone turnover markers, and fracture risk in older adults compared to a lower dietary protein intake.

2. Methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement is followed in reporting this systematic review [13].

2.1. Data Sources and Searches

A systematic search was conducted through October 29, 2018, in 3 databases: CENTRAL (<http://www.thecochranelibrary.com>), MEDLINE (<https://www.ncbi.nlm.nih.gov/pubmed/>), and EMBASE (<https://www.ovid.com/>). The searches were limited to the English language, and prospective cohort and human intervention studies that examined the relations of protein intake (food or supplemental sources) on bone health outcomes of interest. The search strategy per database is presented in Supplementary Table A1.

2.2. Study Eligibility Criteria

We included all prospective cohort studies and randomized controlled trials (RCTs) among older adults aged ≥ 65 years that examined the relation between protein intake from any source on several bone health outcomes (Table 1). Studies including both young and older individuals were still included if stratification was performed. Studies had to have an intervention duration of at least 6 months. Studies enrolling exclusively subjects with a diagnosed disease or where >20% of the baseline population was diagnosed with a disease were excluded. Furthermore, studies were excluded if they were designed to examine outcomes in response to protein type but not protein quantity and if protein was supplemented in the form of soy isoflavone. The reason for the latter is that these plant oestrogens present in soy can independently have an effect on bone loss [14]. Also studies designed for weight loss were excluded.

2.3. Study Selection Process

First, citation duplicates across the 3 literature searches were removed. Second, titles were screened by a single investigator to exclude

Table 1
Bone health outcomes of interest.

Outcome	Sites or markers
BMC	Total body
BMD	Total body, total hip, femoral neck, lumbar spine
Bone turnover markers	Alkaline phosphatase, bone alkaline phosphatase, bone-specific alkaline phosphatase, collagen type I cross-linked C-terminal telopeptide, collagen type I cross-linked N-terminal telopeptide, C-terminal type 1 procollagen, N-terminal type 1 procollagen, deoxypyridinoline, hydroxyproline, pyridinoline, osteocalcin
Fracture	All sites

Note. BMC = Bone Mineral Content; BMD = Bone Mineral Density.

cross-sectional, animal, in vitro studies, and review articles. All abstracts were then independently screened by 2 investigators. When an abstract was regarded as potentially relevant, full-text articles were retrieved and independently screened by 2 investigators based on study eligibility criteria. All abstract and full-text articles screening conflicts were resolved through discussion between the 2 investigators and a final decision was made by the consensus of the entire research team.

2.4. Data Extraction

To capture data of interest from the eligible studies, a data extraction sheet was created in Excel. One investigator extracted the data from all studies, which was reviewed and confirmed by another investigator. The following items were extracted: study characteristics, baseline population characteristics, intervention details, relevant outcomes and their assessment methods, data details (including dropouts), confounders and effect modifiers used in statistical analysis, and results.

2.5. Risk of Bias in Individual Studies

The Newcastle–Ottawa Scale (NOS) was used to assess risk of bias of included prospective cohort studies [15]. For intervention studies, the Cochrane Collaboration's tool for assessing risk of bias was used to assess internal validity [16]. This tool addresses risk of selection bias, performance bias, detection bias, attrition bias, reporting bias, and other potential biases. Two investigators independently assessed the risk of bias in included studies. Disagreements were discussed among the research team and resolved via group consensus.

2.6. Data Synthesis

All included studies were summarized in narrative form and in tables. Items of the summary tables include study characteristics (first author, publication year, cohort name), participant characteristics, baseline mean age or age range, exposure assessment, follow-up period, and outcomes. Summary tables were organized by study type (cohort and RCT). Results were qualitatively and, if possible, quantitatively summarized by study type and outcome of interest. Meta-analysis was performed using R (v3.5.3; package meta) [17].

2.6.1. Qualitative Synthesis

Quality of the evidence was judged with the use of a grading system developed by the GRADE collaboration [18]. According to the GRADE approach, evidence was graded as 'High', 'Moderate', 'Low', or 'Very Low' depending on several criteria. Quality of evidence was downgraded based on five GRADE categories: risk of bias, imprecision, inconsistency, indirectness, and publication bias [19]. Quality was graded upwards when effects were sufficiently large, when all plausible biases would underestimate the effect, or when there was a dose-response gradient [20].

2.6.2. Quantitative Synthesis

If sufficient data were available and homogeneity in terms of participants, interventions and outcomes between studies were reasonable, a meta-analysis was conducted. Authors of relevant articles were contacted if required data were not reported. The methods described in the Cochrane Handbook for conducting meta-analyses was followed [16]. Results were pooled using a random-effects meta-analysis, with standardized mean differences for continuous outcomes and risk ratios (RR) or hazard ratios (HR) for binary outcomes. The extent of statistical heterogeneity was quantified using both the chi-squared test and the I-squared statistic. An I-squared value >50% was used as a threshold for indicating substantial statistical heterogeneity [16]. Random-effects model was used when

studies were drawn from populations that differ from each other in such a way that it could influence the effect estimate. When heterogeneity was not present, a fixed-effect model was used. Sensitivity analyses were performed to explore the impact of excluding studies that were judged to be at high risk of bias and to detect the influence of a single study on the overall estimate.

3. Results

The search yielded 659 citations and two additional articles [21,22] were identified by searching in the reference lists of the other articles included in the analyses. After removal of 138 duplicates and exclusion of 53 articles because the title made clear that it was not a prospective cohort study or RCT, 470 articles were identified for dual abstract screening. A total number of 84 articles were identified for full-text screening, of which 13 were included for data extraction (12 prospective cohort studies and 1 RCT; Fig. 1). The characteristics of the included studies are shown in Table 2 and Table 3.

3.1. Quality of Evidence

Risk of bias assessment using NOS in selected prospective cohort studies is presented in Table 4. Risk of bias was classified as high (score 1–3), potential limitations (score 4–6), or low (score 7–9). However, one study with a score of 4 was classified as having a high risk of bias due to a substantial large loss of follow-up (42.5%) [31]. Six cohort studies were classified as having a low risk of bias, five had potential limitations, and one study had a high risk of bias. With respect to adjustment of important confounders, 1 point was given if they controlled for age, gender, weight or BMI, energy intake, physical activity, smoking, alcohol, vitamin D, and calcium. Another point was given if family history of osteoporosis, fractures, (certain) illnesses, and (certain) drugs were included. Only one study took all those confounders into account [24]. Nine studies adjusted for a part of the relevant confounders [21–23,25,26,29,30,32,33] and in three studies adjustment was inadequate [27,28,31]. The dropout rate was not clear in one cohort study [27] and another study stated that the rate was around 10% per 4 years with a total follow-up of 32 years [21]. Dropout rates were high ($\geq 20\%$) in five studies varying from 24.7% to 81.2%, which (potentially) leads to attrition bias [25,28,29,31,32]. In the other five cohort studies, rates varied from 2.0% to 12.1%. Publication bias could not be assessed, because the numbers of studies in the meta-analysis were too small.

Using the Cochrane Collaboration's tool, overall risk of bias in the selected intervention study [33] was classified as 'low to some concerns' (Table 5). Those concerns were raised with respect to attrition and reporting bias. The dropout rate was 10.5% after 1 year and 18.3% after 2 years. An expected dropout rate of 30% was taken into account in the sample size calculation. Furthermore, there were no significant differences between the protein and control group in the number of people lost to follow-up.

According to the GRADE approach, evidence from observational studies should be rated as low quality (Table 6). Five cohort studies reported the protein intake per a defined unit increase in energy-adjusted protein intake (dose-response gradient). In absence of serious limitations in other categories, three of those cohort studies were rated up one level to moderate quality with respect to their BMD and fracture outcomes [24,29,30]. Quality of evidence for the outcomes total body BMC and bone turnover markers was rated down to 'very low' due to risk of bias, imprecision, and limited number of studies. In addition, quality of evidence for total fractures and spine fractures was rated 'very low', because both were assessed in one study only.

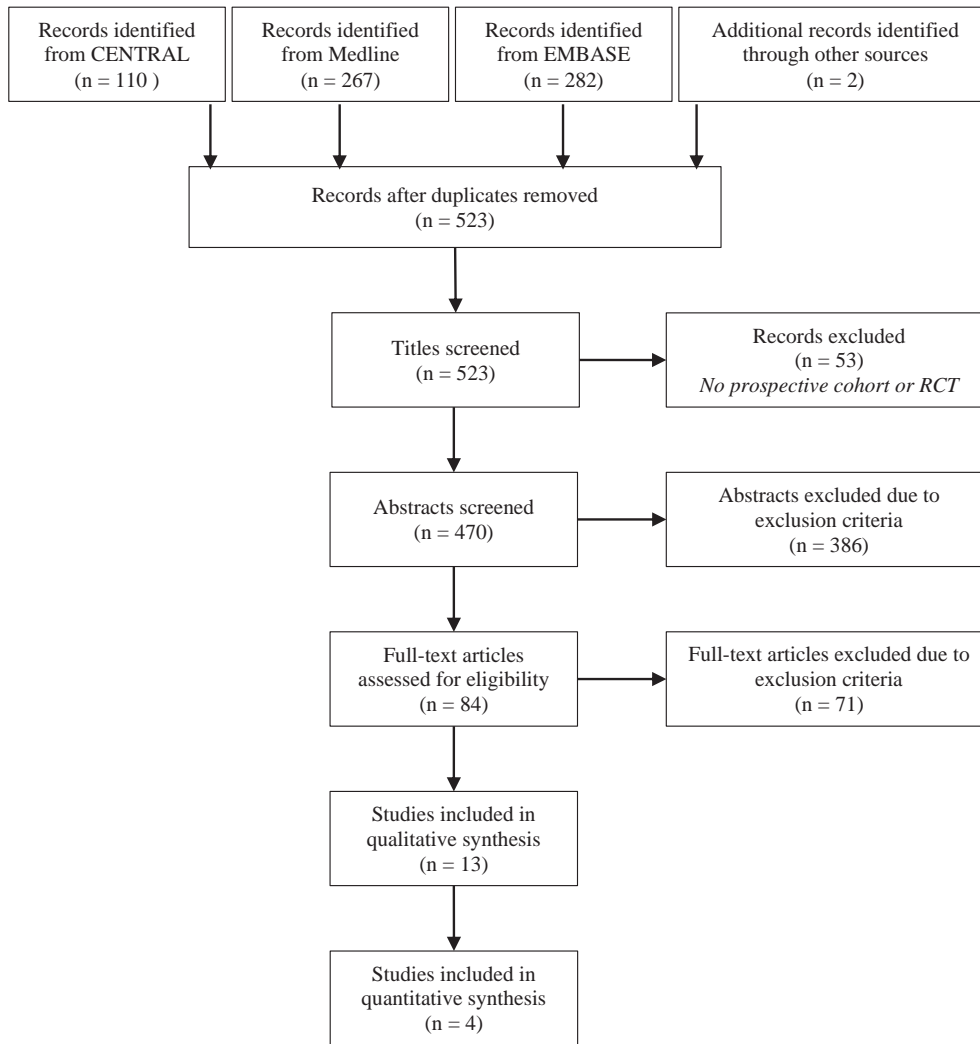


Fig. 1. Flow diagram of study selection.

3.2. Prospective Cohort Studies

3.2.1. BMC–Total Body

Two studies assessed the effect of protein intake on total body BMC and showed different results [29,31]. Meng et al. [31] found that postmenopausal women with a protein intake above the RDA (>1.6 g/kg bw/d) had a significant 5.3% higher whole body BMC compared to women with a low protein intake (<0.8 g/kg bw/d) after 5 years of follow-up. Isanejad et al. [29] found that protein intake was not significantly associated with total body BMC over 3 years of follow-up.

3.2.2. BMD–Total Body

Four studies assessed the effect of protein intake on total body BMD, of which one saw a beneficial effect [26] and three found no significant effects [23,29,32]. Dawson-Hughes et al. [26] found that the highest tertile of protein intake (18.2–29.1% of total energy; estimated mean 1.17 g/kg bw/d) was associated with significantly higher total body BMD after 3 years of follow-up compared to the lowest tertile of protein intake (9.6–15.5% of total energy; estimated mean 0.96 g/kg bw/d; figure-derived: mean percent change 0.6% vs -0.2%, respectively). This effect was seen in a group who took calcium and vitamin D supplements for 3 years, but no association was found in the placebo group. Beasley et al. [23] found that each 20% increase in calibrated protein intake was associated with a non-significant increase in total body BMD in

women aged 65 and 75 years at baseline after 3 years of follow-up. Isanejad et al. [29] found that protein intake was not significantly associated with total body BMD over 3 years of follow-up and Rapuri et al. [32] found no differences in total body BMD between four quartiles of protein intake above the RDA (0.95, 0.94, 0.98, 0.99 g/kg bw/d; 13.3, 15.2, 16.7, 19.5% of energy intake) after 3 years of follow-up.

3.2.3. BMD–Total Hip

Five studies assessed the effect of protein intake on total hip BMD, of which two found beneficial effects [27,30] and three found no significant effects [23,25,32]. Devine et al. [27] found that women with a protein intake above the RDA (>1.6 g/kg bw/d) had a significantly higher hip BMD compared to women with a low protein intake (<0.8 g/kg bw/d) after 1 year of follow-up (figure-derived: mean 824 vs 798 mg/cm², respectively). Langsetmo et al. [30] showed that a higher protein intake (each standard deviation (SD) increase in total energy from protein intake) was associated with higher hip BMD in men after 10.5–11.2 years of follow-up (beta = 0.06, standard error = 0.01). On the other hand, Beasley et al. [23] found that each 20% increase in calibrated protein intake was associated with a non-significant increase in hip BMD in women aged 65 and 75 years at baseline after 3 years of follow-up. Chan et al. [25] found that protein intake was not associated with % change in hip BMD in men and women after 4 years of follow-up. Rapuri et al. [32] found no differences in hip BMD between

Table 2

Summary table of cohort studies included in the analysis.

First author, year [ref]	Cohort name (country)	Participants	N baseline/ analysed	Baseline mean age(SD) or age range (y)	Exposure assessment	Mean protein intake ^a	Follow-up (y)	Relevant outcomes	Effect sizes ^b
Beasley, 2014 [23]	Women's Health Initiative (US)	Post- menopausal women	161,808/144,580 (whole sample)	55–79; subgroups: 65 & 75	FFQ + calibrated with biomarkers	0.52, 0.75, 0.92, 1.11, 1.50 (quantiles, 6 whole sample)		TB BMD Hip BMD Any fracture Hip fracture	Per 20% increase in protein intake: 65 y: mean 0.003 (0.001, 0.005) ns 75 y: mean 0.003 (0.001, 0.007) ns 65 y: mean 0.003 (0.001, 0.005) ns 75 y: mean 0.004 (0.001, 0.007) ns 65 y: HR 0.99 (0.96, 1.01) ns 75 y: HR 0.96 (0.91, 1.02) ns 65 y: HR 0.91 (0.82, 0.99) ns 75 y: HR 0.92 (0.83, 1.02) ns
Cauley, 2016 [24]	Osteoporotic Fractures in Men Study (US)	Men >65 y	5994/5876	No fracture: 73.5(5.8); fracture: 77.8(6.1)	Block semi-quantitative FFQ	No fracture: 16.1%; fracture: 15.3% of EI		Hip fracture	Per SD increase in protein intake (2.9% of EI): HR 0.82 (0.69, 0.97) p<0.05
Chan, 2011 [25]	- (China)	Men and women ≥65 y	2944/2217 (1225 men, 992 women)	Men: 71.6(4.6); women: 72.0(5.1)	FFQ	Men 88.8, women 65.7 g/d. Men 1.42; women 1.19 g/kg bw/d (estimated values)	4	Hip BMD FN BMD	Per unit increase in energy-adjusted protein intake: Men: B -0.007 SE 0.005 p 0.147 Women: B 0.003 SE 0.009 p 0.744 Men: B -0.013 SE 0.008 p 0.088 Women: B 0.010 SE 0.013 p 0.416
Dawson-Hughes, 2002 [26]	- (US)	Men and women ≥65 y	389/342	Supplemented group: 70(5), 71(4), 70(4); placebo group: 71(5), 71(5), 71(5) (tertiles)	Willett semi-quantitative FFQ	9.6–15.5, 15.5–18.2, 18.2–29.1% of EI (tertiles). Supplemented: 0.96, 1.07, 1.17; placebo: 0.90, 1.08, 1.20 g/kg bw/d (estimated values)	3	TB BMD FN BMD Spine BMD Osteocalcin N-telopeptide Hip BMD	Supplemented group protein T3 vs T1: NR, less loss/gain p 0.042 NR, less loss/gain p 0.011 NR, no difference ns NR, no difference ns NR, no difference ns Protein T3 vs T1: NR, higher p<0.05
Devine, 2005 [27]	- (Australia)	Women >70 y	1077	75(3)	ACCV semi-quantitative FFQ	<0.84, 0.84–1.6, >1.6 (tertiles)	1	FN BMD Hip fracture	NR, higher p<0.05 Protein Q5 vs Q1: Women 65–75 y: RR 0.92 (0.71, 1.18) Women 75+ y: RR 0.91 (0.69, 1.20) Men 65–75 y: RR 0.59 (0.33, 1.07) Men 75+ y: RR 0.77 (0.51, 1.15)
Fung, 2017 [21]	Nurses' Health Study & Health Professionals Follow-Up Study (US)	Men ≥50 y and post-menopausal women	74,443 women; 35,439 men	Whole sample ≥50 y; stratification: <65, 65–75, ≥75	Semi-quantitative FFQ	Women 14.3, 18.6, 24.4% of EI; men 14.2, 18.3, 23.4% of EI (whole sample). Women 0.88, 1.12, 1.41; men 0.87, 1.12, 1.40 g/kg bw/d (estimated values)	32	FN BMD Hip fracture	Protein Q5 vs Q1: Women 65–75 y: RR 0.92 (0.71, 1.18) Women 75+ y: RR 0.91 (0.69, 1.20) Men 65–75 y: RR 0.59 (0.33, 1.07) Men 75+ y: RR 0.77 (0.51, 1.15)
Hannan, 2000 [28]	Framingham Osteoporosis Study (US)	Men and women	855/615	75(4.4), 68–91	Willett semi-quantitative FFQ	0.21–0.71; 0.72–0.96; 0.97–1.23; 1.24–2.78 (quartiles)	4	FN BMD LS BMD	Protein Q4 vs Q1: Mean - 2.32(0.74)% vs -4.61(0.70)% p<0.001 Mean - 1.11(1.10)% vs -3.72(0.97)% p<0.05
Isanejad, 2017 [29]	Osteoporosis Risk Factor and Fracture Prevention Study (Finland)	Women ≥65 y	750/544	68.1(1.9), 65–72	3 d food records	0.79, 0.90, 0.96, 1.18 (quartiles)	3	TB BMC TB BMD FN BMD LS BMD	Per unit increase in energy-adjusted protein intake: B -0.16 SE 30.04 p 0.159 B 0.04 SE 0.01 p 0.507 B -0.01 SE 0.01 p 0.918 B -0.31 SE 0.01 p 0.001
Langsetmo, 2017 [30]	Osteoporotic Fractures in Men Study (US)	Men ≥65 y	5994/5875	73.6(5.9)	Modified Block FFQ	0.67, 0.75, 0.83, 0.93 (quartiles)	10.5–11.2	Hip BMD Spine fracture Hip fracture	Per SD increase in protein intake (2.9% of EI): B 0.06 SE 0.01 p<0.001 HR 1.06 (0.92, 1.22) p 0.45 HR 0.84 (0.73, 0.95) p 0.01
Meng, 2009 [31]	- (Australia)	Women 70–85 y	1500/862	74.9(2.6)	ACCV quantitative FFQ	<0.84, 0.84–1.6, >1.6 (tertiles)	5	TB BMC	Protein T3 vs T1: 5.3% higher p<0.05

(continued on next page)

Table 2 (continued)

First author, year [ref]	Cohort name (country)	Participants	N baseline/ analysed	Baseline/ or age range (y)	Exposure assessment	Mean protein intake ^a	Follow-up (y)	Relevant outcomes	Effect sizes ^b
Misra, 2011 [22]	Framingham Osteoporosis Study (US)	Men and women ≥68 y	976/946	No fracture: 75(5.0); fracture: 76(5.2)	Williett semi-quantitative FFQ	46.5, 59.6, 67.7, 82.7 g/d (quartiles); 0.69, 0.88, 1.00, 1.22 g/kg bw/d (estimated values)	11.6 (median)	Hip fracture	Protein Q2–4 vs Q1: HR 0.63 (0.37, 1.09) p 0.04
Rapuri, 2003 [32]	Sites Testing Osteoporosis Prevention/ Intervention (US)	Women 65–77 y	489/92	71.3(0.8), 72.2(0.8), 70.1(0.8), 69.9(0.8) (quartiles)	7 d food diaries	0.95, 0.94, 0.98, 0.99 g/kg bw/d; 13.3, 15.2, 16.7, 19.5% of EI (quartiles)	3	TB BMD Hip BMD FN BMD Spine BMD Osteocalcin N-telopeptide	Protein Q4 vs Q1: NR, no difference ns NR, no difference ns NR, no difference ns NR, no difference ns Mean – 6.5(7.6)% vs –5.8(7.6)% p 0.042 Mean 12.1(11.2)% vs 10.4(11.0)% p 0.226

Note. BMC = bone mineral content; BMD = bone mineral density; EI = energy intake; FFQ = Food Frequency Questionnaire; FN = femoral neck; HR = hazard ratio; TB = total body; LS = lumbar spine; NR = not reported; ns = not significant; RR = risk ratio; SD = standard deviation; SE = standard error; US = United States.

^a Unit is g/kg bw/d, unless stated otherwise. Values presented as mean or range.

^b Values presented as mean(SE), mean (95% CI) or RR/HR (95% CI). BMD in g/cm².

four quartiles of protein intake above the RDA (0.95, 0.94, 0.98, 0.99 g/kg bw/d; 13.3, 15.2, 16.7, 19.5% of energy intake) after 3 years of follow-up.

3.2.4. BMD–Femoral Neck

Six studies assessed the effect of protein intake on femoral neck BMD, half of them showed beneficial effects [26–28] and the other half showed no significant effects [25,29,32]. Dawson-Hughes et al. [26] found that highest tertile of protein intake (18.2–29.1% of total energy; estimated mean 1.17 g/kg bw/d) was associated with significantly higher femoral neck BMD after 3 years of follow-up compared to the lowest tertile of protein intake (9.6–15.5% of total energy; estimated mean 0.96 g/kg bw/d; figure-derived: mean percent change 2.5% vs –0.4%, respectively). This effect was seen in a group who took calcium and vitamin D supplements for 3 years, but no association was found in the placebo group. Devine et al. [27] found that women with a protein intake above the RDA (>1.6 g/kg bw/d) had a significantly higher femoral neck BMD compared to women with a low protein intake (<0.8 g/kg bw/d) after 1 year of follow-up (figure-derived: mean 702 vs 679 mg/cm², respectively). Hannan et al. [28] showed that men and women with a protein intake above the RDA (1.24–2.78 g/kg bw/d) had significantly less femoral neck BMD loss than those with a protein intake below the RDA (0.21–0.71 g/kg bw/d) after 4 years of follow-up (mean percent change –2.32% vs –4.61%). With regard to the studies showing no effect, Chan et al. [25] found that protein intake was not associated with % change in femoral neck BMD in men and women after 4 years of follow-up. Isanejad et al. [29] found that protein intake was not significantly associated with femoral neck BMD over 3 years of follow-up and Rapuri et al. [32] found no differences in femoral neck BMD between four quartiles of protein intake above the RDA (0.95, 0.94, 0.98, 0.99 g/kg bw/d; 13.3, 15.2, 16.7, 19.5% of energy intake) after 3 years of follow-up.

3.2.5. BMD–(Lumbar) Spine

Four studies assessed the effect of protein intake on (lumbar) spine BMD, of which one found a beneficial effect [28], one found a negative effect [29], and two found no significant effects [26,32]. Hannan et al. [28] found a beneficial effect, they showed that men and women with a protein intake above the RDA (1.24–2.78 g/kg bw/d) had significantly less lumbar spine BMD loss than those with a protein intake below the RDA (0.21–0.71 g/kg bw/d) after 4 years of follow-up (mean percent change –1.11% vs –3.72%). On the contrary, Isanejad et al. [29] found that in women with protein intakes ranging from 0.79 to 1.18 g/kg bw/d, protein was significantly negatively associated with total body BMD over 3 years of follow-up (beta = –0.31, standard error = 0.01). Dawson-Hughes et al. [26] found no association between the protein tertile of 18.2–29.1% of total energy (estimated mean 1.17 g/kg bw/d) vs 9.6–15.5% of total energy (estimated mean 0.96 g/kg bw/d) for spine BMD after 3 years of follow-up. In addition, Rapuri et al. [32] found no differences in spine BMD between four quartiles of protein intake above the RDA (0.95, 0.94, 0.98, 0.99 g/kg bw/d; 13.3, 15.2, 16.7, 19.5% of energy intake) after 3 years of follow-up.

3.2.6. Bone Turnover Markers

Two studies assessed the effect of protein intake on the bone turnover markers osteocalcin and N-telopeptide [26,32]. Dawson-Hughes et al. [26] found no association between the highest and lowest protein tertile (18.2–29.1% vs 9.6–15.5% of total energy; estimated means 1.17 vs 0.96 g/kg bw/d) for both serum osteocalcin and urinary N-telopeptide cross-links after 3 years of follow-up. Rapuri et al. [32] also found no differences in serum osteocalcin and urinary N-telopeptides between four quartiles of protein intake above the RDA (0.95, 0.94, 0.98, 0.99 g/kg bw/d; 13.3, 15.2, 16.7, 19.5% of energy intake) after 3 years of follow-up.

Table 3

Summary table of the intervention study included in the analysis.

First author, year [ref]	RCT name (country)	Participants	N baseline/ analysed	Baseline mean age(SD)	Protein source	Control diet intake	High protein intake	Study length (y)	Relevant outcomes	Effect sizes
Zhu, 2011 [33]	- (Australia)	Healthy ambulant post-menopausal women 70–80 y	219/196 (after 1y) /179 (after 2y)	Protein: 74.2(2.8); placebo: 74.3(2.6)	Skim milk + whey protein isolate	2.1 g (skim milk); after 2y: 1.1 g/kg bw/d	30 g (skim milk + whey protein isolate); after 2y: 1.4 g/kg bw/d	2	Hip BMD FN BMD	Mean change(SD) protein vs placebo (mg/cm ²): After 1y: –8.3(21) vs –5.1(22) p 0.31 After 2y: –10.8(25) vs –8.2(22) p 0.46 After 1y: –5.7(22) vs –2.6(24) p 0.34 After 2y: –8.7(26) vs –7.9(27) p 0.84

Note. BMD = Bone Mineral Density; NR = Not Reported; ns = not significant; RCT = Randomized Controlled Trial; SD = Standard Deviation.

Table 4

Newcastle - Ottawa quality assessment scale for selected cohort studies.

First author, year [ref]	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of the exposure	Outcome of interest absent at baseline	Control for important confounders	Outcome assessment	Adequate follow-up duration	Completeness of cohort follow-up	Total points out of 9	Risk of bias
Beasley, 2014 [23]	1	1	1	0	1	1	1	1	7	Low
Cauley, 2016 [24]	1	1	0	1	2	1	1	1	8	Low
Chan, 2011 [25]	0	1	0	0	1	1	1	0	4	Some concerns
Dawson-Hughes, 2002 [26]	0	1	0	1	1	1	1	1	6	Low
Devine, 2005 [27]	1	1	0	0	0	1	1	0	4	Some concerns
Fung, 2017 [21]	0	1	0	1	1	1	1	1	6	Some concerns
Hannan, 2000 [28]	1	1	0	1	0	1	1	0	5	Some concerns
Isanejad, 2017 [29]	1	1	1	1	1	1	1	0	7	Low
Langsetmo, 2017 [30]	1	1	0	0	1	1	1	1	6	Low
Meng, 2009 [31]	1	1	0	0	0	1	1	0	4	High
Misra, 2011 [22]	1	1	0	1	1	1	1	1	7	Low
Rapuri, 2003 [32]	1	1	1	0	1	1	1	0	6	Some concerns

Note. A study can be awarded a maximum of one point for each numbered item within the selection and outcome categories. A maximum of two points can be given for comparability.

Table 5
Risk of bias in the selected intervention study using Cochrane Collaboration's tool.

Author	Risk of selection bias		Risk of performance bias		Risk of attrition bias		Risk of detection bias			Risk of reporting bias		Overall risk of bias						
	Random sequence allocation	Allocation concealment	Baseline differences	Total Blinding of participants	Blinding of personnel	Appropriate analysis	Total outcome data	Complete outcome data	Missing outcome data	Total outcome data	Inappropriate outcome assessment		Outcome assessment group differences	Blinding Total	Analysis as pre-specified	Selection on multiple outcome measurements	Selection on multiple analyses	Total
Zhu	L	L	L	Low	L	Low	PH	PH	PL	Some concerns	L	L	L	Low	NI	NI	Some concerns	Low to some concerns

Note. H = high; L = low; NI = no information; PH = probably high; PL = probably low.

3.2.7. Fractures

Only Beasley et al. [23] looked at total fractures and found that each 20% increase in calibrated protein intake was associated with a non-significant decrease in risk of total fractures in women aged 65 and 75 years at baseline after 3 years of follow-up.

With regard to risk of spine fracture specifically, only Langsetmo et al. [30] studied this outcome and found no association between a higher protein intake (each SD increase in total energy from protein intake) and risk of spine fracture in men after 10.5–11.2 years of follow-up.

More often investigated was the risk of getting a hip fracture. Five studies assessed the effect of protein intake on hip fracture risk, of which four showed a beneficial effect [21,22,24,30] and one found no effect [23]. Cauley et al. [24] showed that each SD increase in total energy from protein was associated with an 18% decrease in risk of hip fractures in men after 8.6 years of follow-up. In the same cohort population but after 10.5–11.2 years of follow-up, Langsetmo et al. [30] found a significant association between each SD increase in total energy from protein intake and a decreased hip fracture risk of 16%. Fung et al. [21] showed an 8% lower risk of hip fractures in women aged 65–75 years consuming 1.4 g protein/kg bw/d (estimated value, 24.4% of energy intake) compared to a protein intake of 0.9 g/kg bw/d (estimated value, 14.3% of energy intake) and a 9% lower risk in women aged ≥75 years after 32 years of follow-up. In men aged 65–75 years, a 41% lower hip fracture risk was found between the highest vs lowest protein tertile (23.4% vs 14.2% of energy intake, estimated mean 1.4 vs 0.9 g/kg bw/d) and this reduction in risk was 23% in men aged ≥75 years. Misra et al. [22] found that a mean protein intake above the RDA (three quartiles: 59.6, 67.7, 82.7 g/d; estimated 0.88, 1.00, 1.22 g/kg bw/d) was significantly positively associated with a decreased hip fracture risk of 37% after 11.6 years of follow-up compared to a mean protein intake below the RDA (46.5 g/d; estimated 0.69 g/kg bw/d). However, Beasley et al. [23] found that each 20% increase in calibrated protein intake was associated with a non-significant decrease in risk of hip fractures in women aged 65 and 75 years at baseline after 3 years of follow-up (9% and 8% respectively).

3.3. RCTs

No eligible RCTs were found investigating the effect of dietary protein intake on BMC, total body and lumbar spine BMD, and bone turnover markers. Only one RCT with total hip and femoral neck BMD as outcome was suitable for inclusion [33]. Zhu et al. [33] investigated the effect of a high-protein drink containing 30 g of protein compared to a placebo drink with 2.1 g of protein. Protein intake after 2 years was above the RDA for both groups (1.4 and 1.1 g/kg bw/d for protein and placebo group, respectively). No significant differences in hip and femoral neck BMD between women in the protein or placebo group after 1 and 2 years of protein supplementation were found. Hip and femoral neck BMD in both groups fell significantly from baseline.

3.4. Meta-analysis

A meta-analysis could be conducted for the outcome hip fracture among four cohort studies including eight different groups (Fig. 2). The study of Cauley et al. [24] was excluded from the meta-analysis, since the same cohort population was used as in the study of Langsetmo et al. [30]. The latter was preferred because the follow-up duration was longer. The association between protein intake and risk of hip fracture was expressed as weighted HR (RR reported in one study was considered as HR). HR was transformed to its natural logarithm (ln) and the corresponding 95% CIs were used to calculate the standard errors. Inverse variance weighting was used to obtain the pooled HR for higher compared to lower protein intakes. Heterogeneity was not significantly present for hip fracture ($I^2 = 0.0\%$, heterogeneity chi-squared $p = 0.614$) and a fixed-effect model was used. The meta-analysis of the

Table 6
Quality of evidence per outcome of interest from selected studies.

Outcome	Number of cohort studies	Number of RCTs	Risk of bias	Imprecision	Consistency	Number of moderate quality studies	Overall quality rating
BMC - total body	2	0	Some concerns	Some concerns	1 p, 1 ns	0	Very low
BMD							
Total body	4	0	Low	Low	1 p, 3 ns	1	Low
Total hip	5	1	Some concerns	Low	2 p, 3 ns	1	Low
Femoral neck	6	1	Some concerns	Low	3 p, 3 ns	1	Low
Lumbar spine	4	0	Some concerns	Low	1 p, 1 n, 2 ns	1	Low
Bone turnover markers							
Osteocalcin	2	0	Some concerns	Some concerns	2 ns	0	Very low
N-telopeptide	2	0	Some concerns	Some concerns	2 ns	0	Very low
Fracture							
Total	1	0	Low	Low	1 ns	0	Very low
Spine	1	0	Low	Low	1 ns	1	Very low
Hip	5	0	Low	Low	4 p, 1 ns	2	Low

Note. According to the GRADE approach, evidence was graded as 'High', 'Moderate', 'Low', or 'Very Low' depending on several criteria. Risk of bias is a combined judgement from risk of bias in the individual studies. Indirectness was rated low for all outcomes. BMC = Bone Mineral Content; BMD = Bone Mineral Density; n = negative; ns = not significant; p = positive; RCT = Randomized Controlled Trial.

cohort studies showed that high vs low protein intake resulted in a statistically significant decrease in hip fractures (pooled HR: 0.89; 95% CI: 0.84, 0.94; $p < 0.001$).

Sensitivity analyses showed that there was no single study affecting the overall estimate considerably. A subgroup analysis was performed for gender. One study had to be excluded because no separate data was available for men and women [22]. A pooled HR of 0.82 (95% CI: 0.73, 0.93; $p = 0.002$) was found for men and 0.91 (95% CI: 0.86, 0.98; $p = 0.007$) for women. This difference was not statistically different ($p = 0.126$).

Meta-analyses with other outcomes of interest could not be performed, because missing quantitative data could not be provided for each relevant article after contact with authors.

4. Discussion

The aim of this systematic review and meta-analysis was to investigate the impact of a dietary protein intake above the RDA of 0.8 g/kg body weight/day from any source on BMD/BMC, bone turnover markers, and fracture risk in older adults compared to a lower dietary protein intake. Our findings showed a positive trend between higher protein intakes and higher femoral neck and total hip BMD. Conflicting results were reported for lumbar spine BMD and no association was found for total body BMD. No conclusions could be drawn regarding BMC and bone turnover markers. Meta-analysis of four prospective cohort studies showed a statistically significant decrease of 11% in hip fracture risk.

Total body BMC was measured in only two cohort studies, of which one had a high risk of bias [31] and the other one had imprecise results [29]. Therefore, quality of evidence for total body BMC was rated as very low. Quality of evidence for the bone turnover markers was also rated as very low due to eligibility of only two cohort studies, of which one was rated down due to imprecision [32]. Risk of bias of the other cohort studies did not affect the quality of evidence.

The selected cohort studies compared varying levels of protein intakes, which makes comparisons between studies difficult and it can influence the magnitude of the measured effect. In addition, some studies looked at very high levels above the RDA, while others reported protein intakes closer to the RDA. For instance, two studies divided the protein intake in tertiles with the highest protein category being >1.6 g/kg bw/d [27,31] and the highest category in the study of Fung et al. was 1.4 g/kg bw/d. While in three other cohort studies, the highest protein category was around 1.2 g/kg bw/d [22,26,29]. Rapuri et al. divided protein intake in four quartiles, which were very close to each other when transferred from percentage of energy intake to g/kg bw/d as unit. This minimal difference in protein intake may be one of the reasons why in this study no significant differences in bone health outcomes were found.

No sufficient evidence was available from intervention studies, only one RCT was eligible for inclusion in this review. This RCT showed no significant difference in hip and femoral neck BMD between protein and placebo group after 2 years. However, these outcomes fell significantly from baseline in both groups. Since protein intake after 2 years was above the RDA for both groups, this may indicate that a protein intake above the RDA is beneficial for hip and femoral neck BMD. This is in

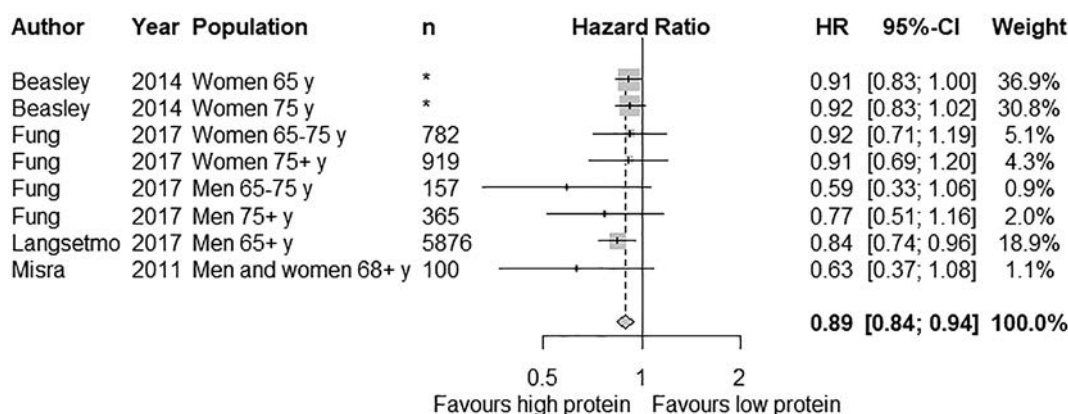


Fig. 2. Effect of protein intake on hip fractures. Fixed-effect pooled hazard ratio (HR) analysis was used. Grey boxes represent the point estimates with the size of the box representing the weight of the study. Horizontal lines depict the length of the 95% CI. The diamond represents the pooled effect estimate. * No exact sample size can be stated; the hazard ratio is the estimate of the effect at specific age levels (65 and 75 y) selected from a continuous distribution. Total sample size was 144,580 persons.

line with some of the cohort studies investigating hip BMD (3 studies showed no differences and 2 showed beneficial differences) and femoral neck BMD (3 studies showed no differences and 3 showed beneficial differences). The overall risk of bias in this RCT was classified as low to some concerns. Since this study was only done in women, representativeness is limited.

Over the years, several systematic reviews and meta-analyses assessing the impact of protein intake on bone health have been published. Darling et al. (2009) investigated the effect of varying protein intakes in healthy adults of 18 years and older and included 31 observational studies and 19 RCTs [8]. They concluded that there might be a small beneficial effect of protein supplementation on lumbar spine BMD (weighted mean difference = 0.02 g/cm²; 95% CI: 0.00, 0.04), but that it is unknown if this also results in a reduced fracture risk. However, they also included studies with a duration of <6 months, leading to a less a reliable estimate of the impact on changes in BMD [12]. In comparison with the current review, we did not have enough data to assess lumbar spine BMD. We did find an effect on hip fracture risk, which can partly be explained by the differences between study populations (adults ≥18 years vs adults ≥65 years).

A more recent systematic review of Shams-white et al. [9] investigated the effect of varying protein intakes in healthy adults of 18 years and older and excluded studies with a duration of <6 months [9]. A total of 20 observational studies and 16 RCTs were included, which showed that there were positive trends of high compared to low protein intakes on BMD at different bone sites, especially the lumbar spine (net percentage change = 0.52%; 95% CI: 0.06, 0.97). Meta-analyses of RCTs assessing lumbar spine, total hip and femoral neck BMD were performed; no meta-analysis was done with hip fracture risk. Since insufficient evidence is available from RCTs in the current review, meta-analysis results cannot be compared.

The systematic review of Wallace & Frankenfeld [10] included healthy adults of 18 years and older and specifically focused on protein intakes above the current RDA [10]. They also excluded weight loss studies and studies in which women used hormone replacement therapy (HRT), resulting in the inclusion of 15 observational studies and 16 RCTs. They conclude that a protein intake above 0.8 g/kg body weight/day can potentially have a beneficial effect on hip fracture risk (pooled RR = 0.84; 95% CI: 0.73, 0.95) and BMD loss (qualitative evaluation). Although this meta-analysis included a younger population, the reduction in hip fracture risk is similar to what we have found. They also included studies with a duration of <6 months, leading to a less a reliable estimate of the impact on changes in BMD [12]. Therefore, results for BMD outcomes cannot be compared.

Lastly, results of the meta-analysis performed by Wu et al. [34] were the same as the current meta-analysis: a significant decrease in hip fracture risk of 11% (RR 0.89; 95% CI 0.82, 0.97) [34]. Two of the six included prospective cohort studies were also added to the current meta-analysis, the other four were performed with adults aged <65 years. Despite addition of recent publications investigating older adults in our analysis, no differences in hip fracture risk reduction could be observed.

The prevalence of osteoporosis and fracture risk are higher in women than in men [35,36]. Therefore, the effect of a high protein intake on hip fracture risk may be gender-specific. However, subgroup analysis for gender showed no statistically significant difference in hip fracture risk reduction and therefore it was assumed that pooling the effect of men and women together was fair. Currently, much research is focused on postmenopausal women, but since men tend to have a higher mortality risk after a fracture, men should not be overlooked [36,37].

Previously it was believed that a high dietary protein intake could have a negative effect on bone health by inducing chronic metabolic acidosis, which eventually leads to osteoporosis [38]. However, an increase in urinary calcium excretion observed after a high protein diet likely originates from an increased intestinal absorption instead of

from bone calcium loss [39]. In addition, an expert consensus paper from 2018, assessing the risks and benefits of dietary protein for bone health, concluded that a protein intake above the RDA is beneficial for older adults [3]. A long-term protein intake of 2 g/kg body weight/day is reported safe for healthy adults, higher values may lead to digestive, renal, and vascular problems [40]. No conclusive evidence is available to set a new RDA for older adults in the light of bone health.

Two comments must be made with respect to the study eligibility criteria of the current review. In the pre-specified protocol, it was mentioned that studies including women using oral contraceptives or HRT would be excluded, except when stratification was done or when it was controlled for in the analyses. In principle, women aged ≥65 years do not take oral contraceptives anymore. Regarding HRT, a majority of the included studies did not report if women used this. Since we have older adults as study population, exclusion of this group of people might harm the generalizability. Therefore, we decided to remove this exclusion criterion. Secondly, it was stated that vegan individuals would be excluded, but this was not reported in the included studies. We assumed that the prevalence of vegan individuals in this generation is negligible [41] and therefore the expected impact on the results is minimal.

For the meta-analysis, standard errors were estimated from the HR or RR and its 95% CI, reported by the individual studies. When standard errors were back transformed to CIs, this indirect variance estimation resulted into a close but not similar value of the actually reported CIs. However, the differences were small (maximum interval difference of 0.02) and assumed not to influence the results.

A strength of this study was that only studies investigating older adults (aged ≥65 years) were included, making the recommendation specific for this more vulnerable group that has a greater protein need than younger adults. As a consequence, the review is limited by inclusion of only one intervention study. This makes clear that large and long-term RCTs in older adults are needed to judge if a protein intake above the current RDA can improve bone health and/or prevent osteoporosis. Another strength was the exclusion of studies with a duration of <6 months, studies that supplemented protein in the form of soy isoflavone, and studies designed for weight loss. These exclusion criteria eliminate a proportion of confounding issues.

5. Conclusions

This systematic review supports that there is an association between a dietary protein intake above the current RDA of 0.8 g/kg body weight/day and a reduced hip fracture risk in older adults. In addition, positive trends for total hip and femoral neck BMD were found. In comparison with younger adults, the body of evidence from the included studies is not strong enough to increase the protein recommendation for older adults with respect to bone health.

Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.csbj.2019.07.005>.

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