Dietary protein and bone health: a systematic review and meta-analysis from the National Osteoporosis Foundation\textsuperscript{1,2}

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\section*{ABSTRACT}

\textbf{Background:} Considerable attention has recently focused on dietary protein’s role in the mature skeleton, prompted partly by an interest in nonpharmacologic approaches to maintain skeletal health in adult life.

\textbf{Objective:} The aim was to conduct a systematic review and meta-analysis evaluating the effects of dietary protein intake alone and with calcium with or without vitamin D (Ca\textsubscript{\textordmasculine}±D) on bone health measures in adults.

\textbf{Design:} Searches across 5 databases were conducted through October 2016 including randomized controlled trials (RCTs) and prospective cohort studies examining \textit{I}) the effects of “high versus low” protein intake or \textit{2}) dietary protein’s synergistic effect with Ca\textsubscript{\textordmasculine}±D intake on bone health outcomes. Two investigators independently conducted abstract and full-text screenings, data extractions, and risk of bias (ROB) assessments. Strength of evidence was rated by group consensus. Random-effects meta-analyses for outcomes with \( \geq 4 \) RCTs were performed.

\textbf{Results:} Sixteen RCTs and 20 prospective cohort studies were included in the systematic review. Overall ROB was medium. Moderate evidence suggested that higher protein intake may have a protective effect on lumbar spine (LS) bone mineral density (BMD) compared with lower protein intake (net percentage change: 0.87\%; 95\% CI: 0.18\%, 1.56\%; \( I^2 \): 0\%; \( n = 4 \)) but no effect on total hip (TH), femoral neck (FN), or total body BMD or bone biomarkers. Limited evidence did not support an effect of protein with Ca\textsubscript{\textordmasculine}±D on LS BMD, TH BMD, or forearm fractures; there was insufficient evidence for FN BMD and overall fractures.

\textbf{Conclusions:} Current evidence shows no adverse effects of higher protein intakes. Although there were positive trends on BMD at most bone sites, only the LS showed moderate evidence to support benefits of higher protein intake. Studies were heterogeneous, and confounding could not be excluded. High-quality, long-term studies are needed to clarify dietary protein’s role in bone health. This trial was registered at www.crd.york.ac.uk as CRD42015017751.

\textbf{Keywords:} protein, bone health, osteoporosis, bone density, diet

\section*{INTRODUCTION}

Protein makes up ~50\% of bone volume and approximately one-third of its mass (1). It provides the structural matrix of bone, whereas calcium is the dominant mineral within that matrix. Collagen and a variety of noncollagenous proteins form the organic matrix of bone, so an adequate dietary protein intake would seem to be essential for optimal acquisition and maintenance of adult bone mass. Consistent with this idea, a recent systematic review of available data found a positive effect of dietary protein on skeletal acquisition in children and adolescents (2). Considerable attention has recently also focused on dietary protein’s role in the mature skeleton, prompted in part by an increasing interest in nonpharmacologic approaches to maintaining skeletal health in adult life and later adult years.

In 1920, Sherman (3) reported that increasing dietary protein led to greater urinary calcium excretion. Subsequent studies have repeatedly confirmed this association, such that for every 40-g increase in dietary protein, urinary calcium excretion increases by ~50 mg (4). In the 1970s and early 1980s a series of metabolic balance studies in which dietary calcium was carefully controlled found no change in intestinal calcium absorption with higher dietary protein intakes, leading to the conclusion that the source of the additional urinary calcium must be from the breakdown of the skeleton (5–12). It was proposed that increasing dietary protein with a commensurate increase in sulfur-containing amino acids resulted in the imposition of a systemic metabolic acid load, which could not—particularly in older adults
individuals—be adequately buffered by the respiratory apparatus. Instead, this required buffering from alkali stores in bone resulting in skeletal calcium loss. However, current short-term dietary intervention studies that used dual-stable isotope techniques to assess calcium kinetics found that increasing dietary protein is associated with a significant increase in intestinal calcium absorption, such that nearly the entire increase in urinary calcium can be accounted for by improved calcium absorption efficiency and that, in the short term, there is no increase in skeletal catabolism (13–15).

Studies that examined the association between protein intake and bone health outcomes are limited. A meta-analysis published in 2009 found null effects of protein intake on fracture risk (16); however, a more recent 2015 meta-analysis indicated a slight reduction in hip fractures (17). Moreover, the effects of simultaneous consumption of protein and calcium intake on bone have not been widely studied, although a higher protein intake does increase intestinal calcium absorption in dual-stable isotope studies (12–14). Because the body only absorbs limited amounts of these 2 essential nutrients at a time, one could speculate that concurrent consumption of moderate amounts of protein and calcium at each meal might offer an advantage. In light of several new studies and a plethora of available data on bone mineral density (BMD),12 bone biomarkers, and fracture outcomes, we undertook a comprehensive systematic review in an effort to clarify the impact of 1) dietary protein and 2) dietary protein and calcium with or without vitamin D (Ca±D) on these bone health outcomes in healthy adults.

**METHODS**

The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement is followed in reporting this systematic review (18). A prospectively developed protocol for this systematic review was registered on PROSPERO (http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42015017751; CRD42015017751) (19).

**Data sources and searches**

A search strategy was developed in consultation with 2 librarians, first by using nomenclatures for Ovid Medline and then adjusted for other electronic databases. The searches were implemented in 5 databases: Ovid Medline (gateway.ovid.com; 1946 to 4 October 2016), Cochrane Central Register of Controlled Trials (gateway.ovid.com; 1991 to 31 October 2016), Scopus (+ EMBASE; www.scopus.com; 1974 to 31 October 2016), Web-of-Science (webofknowledge.com; 1864 to 31 October 2016), and Global Health (1910 to 31 October 2016; www.ebscohost.com/academic/global-health). The searches were limited to the English language and human studies that examined the relations of protein intake (foods or supplement sources) on bone health outcomes of interest. The complete search strategy is presented in Supplemental Table 1.

**Study eligibility criteria**

We included all intervention trials and prospective cohort studies in healthy adults aged ≥18 y that examined the relations between varying doses of protein intake from any source and fracture and bone health outcomes. We excluded studies that compared equal amounts of protein from different protein sources, studies in children and adolescents (i.e., <18 y of age), and studies in pregnant or lactating women. The complete list of bone health outcomes is described in Table 1, and further details describing the included study populations and outcome-specific inclusion and exclusion criteria are described in Supplemental Methods 1.

**Study selection process**

For citations identified from the non-Medline databases, titles were first screened by a single investigator to exclude in vitro, cell, and stem cell studies; animal studies; cross-sectional studies; retrospective case-control studies; interrupted time series studies; and review articles. All of the abstracts identified in the literature searches were then independently screened by ≥2 investigators by using the open-source online software Abstrackr (http://abstrackr.cebm.brown.edu/) (20). For all abstracts that were deemed potentially relevant by 2 screeners, full-text articles were retrieved and independently screened by 2 investigators on the basis of the final study eligibility criteria. For studies conducted in the same cohort population and time period, the first published study was retained. All abstract and full-text screening conflicts were resolved through discussion, and final decisions were reached by the consensus of the entire research team. Additional study eligibility criteria were added after full-text screening was completed to make the scope of this systematic review manageable. Specifically for this systematic review, any study with an intervention duration of <6 mo was excluded, and the bone health biomarkers of interest were limited to osteocalcin and collagen type 1 cross-linked C-terminal telopeptide (CTX) as measures of bone formation and bone turnover, respectively. These decisions were made on the basis of their biological and clinical importance in relation to bone health (e.g., bone density changes cannot reliably be measured in shorter intervals) (2).

**Data extraction**

Data extraction forms were tailored to our topic and outcomes of interest by modifying the data extraction forms used in the systematic review “Vitamin D and Calcium: A Systematic Review of Health Outcomes” (21). The items extracted included the following: study characteristics, baseline population characteristics, background diet data, dietary assessment methods, interventions (for intervention studies only), confounders and effect modifiers used in statistical analysis, relevant outcomes assessed, and results (complete data extraction forms are available on request). Each study was extracted by one investigator and reviewed and confirmed by another investigator. Any disagreements were discussed among the research team and resolved via group consensus.

Intervention and cohort study results were extracted quantitatively unless such data were not provided; in the latter case, qualitative results were extracted only. For all studies, multivariate-adjusted analyses were extracted in preference over crude or age-adjusted measures. In order of preference, risk ratios, HRs, incidence ratios, and ORs were extracted.

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12 Abbreviations used: BMC, bone mineral content; BMD, bone mineral density; Ca±D, calcium with or without vitamin D; CTX, collagen type 1 cross-linked C-terminal telopeptide; FFQ, food-frequency questionnaire; FN, femoral neck; LS, lumbar spine; RCT, randomized controlled trial; ROB, risk of bias; SOE, strength of evidence; TB, total body; TH, total hip.
TABLE 1
Included bone outcomes of interest

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Sites or markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMC</td>
<td>TB only</td>
</tr>
<tr>
<td>BMD</td>
<td>TB, TH, FN, LS</td>
</tr>
<tr>
<td>Fractures</td>
<td>All sites</td>
</tr>
<tr>
<td>Falls</td>
<td>N/A</td>
</tr>
<tr>
<td>Bone quality</td>
<td>For example, via Ad-SOS</td>
</tr>
<tr>
<td>Bone metabolism biomarkers</td>
<td>ALP, BAP, BSAP, CTX, NTX, C1NP or</td>
</tr>
<tr>
<td></td>
<td>P1NP, DPD, hydroxyproline, OC, PYD</td>
</tr>
</tbody>
</table>

1 Ad-SOS, amplitude-dependent speed of sound; ALP, alkaline phosphatase; BAP, bone alkaline phosphatase; BMC, bone mineral content; BMD, bone mineral density; BSAP, bone-specific alkaline phosphatase; C1NP, C-terminal type 1 procollagen; CTX, collagen type 1 cross-linked C-terminal telopeptide; DPD, deoxypyridinoline; FN, femoral neck; LS, lumbar spine; NTX, collagen type 1 cross-linked N-telopeptide; N/A, not applicable; OC, osteocalcin; P1NP, N-terminal type 1 procollagen; PYD, pyridinoline; TB, total body; TH, total hip.

2 Ratios of the biomarkers (e.g., with creatinine) were included as outcomes of interest.

Risk of bias in individual studies

We assessed the methodologic quality of each study on the basis of predefined criteria. For intervention and prospective cohort studies, we used a modified version of the Cochrane risk of bias (ROB) tool (http://www.cochrane.org/handbook) and the Newcastle Ottawa Scale, respectively. Two independent investigators conducted ROB assessments, with discrepancies discussed among the research team and resolved via group consensus. Further details can be found in Supplemental Methods 2.

Data synthesis

All of the included studies were summarized in narrative form and in summary tables that tabulated key features of the study populations, design, intervention, outcomes, and results. Summary tables were organized by study type [i.e., randomized controlled trial (RCT) compared with cohort study]. Results were quantitatively and qualitatively summarized by study type and outcome of interest.

Qualitative synthesis

The strength of evidence (SOE) for major comparisons and outcomes was assessed through a consensus process of the entire research team, with the use of a modified version of a grading system used by the American Diabetes Association and other prominent groups (22, 23). Briefly, SOE levels could be categorized as A (strong), B (moderate), C (limited), D (inadequate), E (expert consensus or clinical experience), or NA (not applicable). Details of the grading system can be found in Supplemental Table 2.

Meta-analysis (quantitative synthesis)

The methods outlined in the Cochrane Handbook for conducting meta-analysis of RCTs were followed (24). Observational studies were not pooled due to insufficient data for dose-response meta-analysis.

For BMD outcomes in RCTs, we used the reported or calculated net percentage change between the higher- and lower-protein groups as the effect size measure in the meta-analysis because most RCTs reported within-group percentage changes in BMD outcomes. The mean within-group percentage change, if not reported, was calculated by using the postintervention mean minus the baseline mean, then divided by the baseline mean and multiplied by 100%. Its SD was estimated by using the SD of the mean change divided by the baseline mean. The net percentage change was the difference in the 2 within-group percentage changes by using the lower-protein group as the reference group; thus, a positive net percentage change indicates an effect (i.e., less bone loss) favoring higher protein intake. The SD of the net percentage change was the pooled SD of the 2 SDs of the mean change: SD_pooled = √[(n1−1) × SD1 + (n2−1) × SD2]/(n1 + n2 − 2), where n1 and n2 are the sample sizes and SD1 and SD2 are the SDs of the mean within-group percentage changes of the higher- and lower-protein groups, respectively. For studies that did not report final sample sizes, the baseline sample size was used in our calculations (25, 26).

For other outcomes [i.e., total body (TB) bone mineral content (BMC), osteocalcin, and CTX], we used the reported or calculated net change (difference of the 2 within-group changes from baseline) between the higher- and lower-protein groups as the effect size measure in the meta-analysis. If the SDs of the within-group changes were not reported, the SD of the mean within-group change was estimated by using the following formula: SD_diff = √[(SD_b1^2 + SD_b2^2 − 2 × Corr × SD_b1 × SD_b2), where SD_b1 is the SD at baseline and SD_b2 is the SD at the end of the study. We assumed a correlation coefficient (Corr) value of 0.50 to impute the missing SD of the mean within-group change. Sensitivity analyses that used Corr values of 0.20 and 0.80 were conducted to evaluate the impacts of the correlation assumptions on the meta-analysis results, and none showed appreciable impacts (Supplemental Table 3).

Studies were excluded from meta-analyses if required information for the aforementioned calculations was not reported for any given outcome. The original authors were contacted to obtain missing quantitative data that were needed for meta-analysis (26–31); 3 authors responded with the requested information (26, 28, 31).

In light of clinical heterogeneity (different doses and types of protein interventions), we performed random-effects meta-analyses for outcomes with ≥4 unique RCTs (32). We used both the Q statistic (considered significant when P < 0.10) and the I² index to quantify the extent of statistical heterogeneity (24). We defined low, moderate, and high heterogeneity as I² values of 25%, 50%, and 75%, respectively. These cutoffs are arbitrary and were used for descriptive purposes only (33).

All of the calculations and meta-analyses were conducted in Stata SE 13 (StataCorp). The analytic data sets can be found in the Supplemental Analytic Data Sets. Two-tailed P values <0.05 were considered significant.
RESULTS

Our search yielded 1999 citations: 1280 articles were identified for dual abstract screening, of which 207 were identified for full-text screening and 36 were included for data extraction (16 RCTs and 20 prospective cohort studies). The details are summarized in Figure 1. Results shown below are organized by question, namely 1) the effect of higher compared with lower protein intake on bone health outcomes and 2) the synergistic effects of Ca\(\text{D}\) with protein on bone health outcomes. ROB assessments can be found by study in Supplemental Tables 4 and 5.

Higher compared with lower protein intake

Lumbar spine BMD RCTs

Seven RCTs examined lumbar spine (LS) BMD (Supplemental Table 6) (26, 29, 34–38). Three studies prescribed supplements (35–37). The doses of milk-based protein in each of the higher-protein groups were 13.2 and 45 g/d for 7 d/wk and 30 g/d for 5 d/wk, and the dose in the lower-protein groups was 0 g/d. The other 4 studies prescribed dietary composition changes (26, 29, 34, 38). The 4 higher-protein group interventions consisted of 90 g protein/d, 25% and 30% of total energy from protein/d, and 1.4 g protein \(\text{kg}^{-1}\text{d}^{-1}\) with 3 daily servings of dairy, whereas the lower-protein group’s interventions consisted of <80 g protein/d, 15% and 18% of total energy from protein/d, and 0.8 g protein \(\text{kg}^{-1}\text{d}^{-1}\) with 2 daily servings of dairy, respectively. Studies were conducted in different populations (Supplemental Table 6). Overall, ROB was medium, primarily due to low compliance (<80%) and incomplete outcome data (i.e., dropout rate >20%) (Supplemental Figure 1, Supplemental Table 4).

Findings from the 7 RCTs were inconsistent: 3 RCTs (29, 36, 38) reported that the lower-protein groups lost significantly more or gained significantly less LS BMD than the higher-protein groups after 1 y, whereas 4 RCTs (26, 34, 35, 37) showed no significant difference in LS BMD (Supplemental Table 7).

Our random-effects meta-analysis of 5 RCTs showed that higher protein intake, on average, had a protective effect on LS BMD compared with lower protein intake, increasing BMD by an average of 0.52% with no statistical heterogeneity (pooled net percentage change: 0.52%; 95% CI: 0.06%, 0.97%; \(I^2: 0.0\%\)) (Figure 2). The meta-analysis was rerun without the study by Kukuljan et al. (36), which had more than half the weight in the meta-analysis, to see if the discrepancy was due to this one study alone, and the pooled result was no longer significant (\(n = 4\); pooled net percentage change: 0.35%; 95% CI: −0.20, 0.89; \(I^2: 0.0\%\); data not shown). Findings from the 2 studies that could not be included in the meta-analysis were also inconsistent: Thorpe et al. (29) found that those in the high-protein group showed significantly higher LS BMD gain than did the low-protein group after 1 y (data in figure only; \(P < 0.01\), whereas no differences between groups were found by Schürch et al. (37) after 1 y (net difference; BMD \(\text{T}\) score: 0.010; 95% CI: −0.01, 0.03; \(P > 0.2\)) (Supplemental Table 7).

Cohort studies

Seven cohort studies examined LS BMD for 2.5–6 y (Supplemental Table 8) (39–45). One cohort study comprised young,
nulliparous women (44), 1 included perimenopausal women (42), 1 comprised postmenopausal women (39), and the other 4 (40, 41, 43, 45) comprised both men and women [of which 2 (40, 41) were in older adults only]. Protein intake was assessed by using 7-d diary in 1 study (44) and food-frequency questionnaires (FFQs) in the remaining studies. Overall, ROB was low, except most studies did not address if there was an adequate sample size or power for any significant findings (Supplemental Table 5).

There was no significant association between protein intake and LS BMD change in 6 of the 7 studies (Supplemental Table 7) (39, 40, 42–45). Only 1 cohort study (41) conducted in older adults found that those in the lowest protein category (7.3–13.5% and 9.6–15.49% of total energy) showed the most LS BMD loss, whereas those in the highest category (17.9–27.4% of total energy) showed the least LS BMD loss.

Total hip BMD

**RCTs**

Eight RCTs examined total hip (TH) BMD (Supplemental Table 6) (26, 29, 31, 34–36, 38, 46). Four studies prescribed supplements (31, 35, 36, 46). The doses of milk-based protein in the higher protein groups ranged from 13.2 to 45 g/d, and in the lower protein groups from 0 to 2.1 g/d. Four studies prescribed dietary composition changes (26, 29, 34, 38). In these 2 studies, the higher-protein groups consumed 90 g protein/d, 25% and 30% of total energy from protein/d, and 1.4 g protein/kg^2^/d with 3 daily servings of dairy, whereas the lower-protein groups consumed <80 g protein/d, 15% and 18% of total energy from protein/d, and 0.8 g protein/kg^2^/d with 2 daily servings of dairy, respectively. Studies were conducted in different populations (Supplemental Table 6). Overall, ROB was medium, primarily due to low compliance (<80%) and incomplete outcome data (i.e., dropout rate >20%) (Supplemental Figure 1, Supplemental Table 4).

Six of the 8 RCTs (26, 31, 34–36, 46) found no significant difference in the net changes in TH BMD over 1–2 y (Supplemental Table 7). Two RCTs (29, 38) that administered dietary composition changes found that the lower-protein groups lost significantly more or gained significantly less TH BMD than the higher-protein groups after 1 y (Supplemental Table 7).

Our random-effects meta-analysis of 7 RCTs showed similar but smaller effects of higher compared with lower protein intakes on TH BMD (pooled net percentage change: 0.30%; 95% CI: −0.02%, 0.62%; I^2^: 0.0%) (Figure 3). One study could not be meta-analyzed: Thorpe et al. (29) reported that those in the lower-protein group had significantly greater TH BMD loss than those in the higher-protein group after 1 y (data in figure only; P = 0.01) (Supplemental Table 7).

**Cohort studies**

Two cohort studies examined TH BMD for 4 and 6 y (Supplemental Table 8) (39, 43). One cohort study comprised postmenopausal women (39) and the second included adults aged ≥55 y (43). Protein intake was assessed by using FFQs. ROB was medium, mainly due to unclear power to detect a difference in one study and the lack of completeness of the cohort follow-up in both studies (e.g., >20% lost to follow-up or differential loss of follow-up) (Supplemental Table 5). No significant association between protein intake and BMD change was found in either study (Supplemental Table 9) (39, 43).

Femoral neck BMD

**RCTs**

Eight RCTs examined femoral neck (FN) BMD (25, 26, 31, 34–38) (Supplemental Table 6). Five studies (25, 31, 35–37) prescribed supplements. The doses of milk-based protein in the higher-protein groups ranged from 13.2 to 45 g/d, and in the lower protein groups from 0 to 2.1 g/d. Three studies (26, 34, 38) prescribed dietary composition changes. In these 2 studies, the higher-protein groups consumed >90 g protein/d, 25% or
30% of total energy from protein, whereas the lower-protein groups consumed <80 g protein/d, 15% or 18% of total energy from protein, respectively. Studies were conducted in different populations (Supplemental Table 6). Overall, ROB was medium, primarily due to low compliance (<80%) and incomplete outcome data (i.e., dropout rate >20%) (Supplemental Figure 1, Supplemental Table 4).

Seven of the 8 RCTs (26, 31, 34–38) found no significant difference in the net changes in FN BMD (Supplemental Table 7). Only one study (25) in postmenopausal women with recent hip fracture found that the lower-protein group lost significantly more FN BMD in 1 y than the higher-protein group.

Our meta-analysis of 6 RCTs showed no difference in the effects of higher compared with lower protein intake on FN BMD, with no statistical heterogeneity (pooled mean percentage change: −0.14%; 95% CI: −0.60%, 0.32%; $I^2$: 0.0%) (Figure 4). Two studies (25, 37) could not be meta-analyzed: no significant net difference in FN BMD was found between groups.
after 1 y by Schürch et al. (37) (net difference; \( T \) score: 0.010; 95% CI: 0.000, 0.030; \( P = 0.111 \)) or Tengstrand et al. (25) (net difference; \( z \) score: 0.260; 95% CI: \(-0.046, 0.566\); \( P > 0.05 \)) (Supplemental Table 7).

**Cohort studies**

Five cohort studies examined FN BMD for 2.5–4.6 y (Supplemental Table 8) (40–43, 45). One cohort study (42) comprised perimenopausal women and the other 4 comprised both men and women [of which 2 (40, 41) were conducted in older adults only]. Protein intake was assessed by using FFQs. Overall, ROB was low, except that no study addressed if there was adequate sample size or power for any significant findings (Supplemental Table 5).

There was no significant association between protein intake amounts and FN BMD change in 3 studies (Supplemental Table 9) (42, 43, 45). The 2 cohort studies (40, 41) conducted in older adults found that those in the lowest protein category (7.3–13.5% and 9.64–15.49% of total energy) showed the least FN BMD loss, whereas those in the highest category (17.9–27.4% and 18.16–29.14% of total energy, respectively) showed the most FN BMD loss (Supplemental Table 9).

**TB BMD**

**RCTs**

Five RCTs examined TB BMD (Supplemental Table 6) (25, 29, 38, 46, 47). Two studies (25, 46) prescribed supplements. The doses of milk-based protein in the higher-protein groups were 20 and 40 g/d and those in the lower-protein groups were 0 g/d. The other 3 studies (29, 38, 47) prescribed dietary composition changes. The higher-protein groups consumed 2.2 g protein \( \cdot \) kg lean body mass\(^{-1} \) \( \cdot \) d\(^{-1} \), 30% of total energy from protein/d, and 1.4 g protein \( \cdot \) kg\(^{-1} \) \( \cdot \) d\(^{-1} \) with 3 daily servings of dairy, whereas the lower-protein groups consumed 1.1 g \( \cdot \) kg lean body mass\(^{-1} \) \( \cdot \) d\(^{-1} \), 18% of total energy from protein/d, and 0.8 g protein \( \cdot \) kg\(^{-1} \) \( \cdot \) d\(^{-1} \) with 2 daily servings of dairy, respectively. Studies were conducted in different populations (Supplemental Table 6). Overall, ROB was medium, primarily due to low compliance (<80%) and incomplete outcome data (dropout rate >20%) (Supplemental Table 4).

Findings from the 5 RCTs were inconsistent: 2 RCTs (25, 29) found that the lower-protein groups lost significantly more TB BMD than did the higher-protein groups after 1 y; 3 RCTs (38, 46, 47) found no significant difference in the net changes in TB BMD after 1 y (Supplemental Table 7). Data were not sufficient to conduct a meta-analysis.

**Cohort studies**

Two cohort studies examined TB BMD for 3 and 6 y (Supplemental Table 8) (39, 40). One cohort study (40) comprised older adults and 1 study (39) comprised postmenopausal women, respectively. Protein intake was assessed by using FFQs. ROB was medium, primarily due to the lack of completeness of the cohort follow-up in 1 study (i.e., >20% lost to follow-up) (Supplemental Table 5). One study (39) found higher protein intake to be associated with a significant increase in TB BMD over time. The second study (40) found higher protein intake to be associated with significantly less TB BMD loss over time compared with lower protein intake, but only among those supplemented with calcium and vitamin D; no such association was found among those given a placebo (Supplemental Table 9).

**TB BMC**

**RCTs**

Three RCTs examined TB BMC (Supplemental Table 6) (29, 37, 38). One study (37) prescribed supplements: 20 g milk protein/d for 5 d/wk to the higher-protein group and 0 g protein to the lower-protein group. The other 2 studies (29, 38) prescribed dietary composition changes. The higher-protein groups consumed 30% of total energy from protein/d and 1.4 g protein \( \cdot \) kg\(^{-1} \) \( \cdot \) d\(^{-1} \) with 3 daily servings of dairy, whereas the lower-protein groups consumed 18% of total energy from protein/d and 0.8 g protein \( \cdot \) kg\(^{-1} \) \( \cdot \) d\(^{-1} \) with 2 daily servings of dairy, respectively. All of the studies were conducted in different populations (Supplemental Table 6). Overall, ROB was high, primarily due to low compliance in 2 of the studies (<80%) and incomplete outcome data in all 3 studies (i.e., dropout rate >20%) (Supplemental Table 4).

Findings from the 3 RCTs were inconsistent: 1 study (29) found that the lower-protein group lost significantly more TB BMC than did the higher-protein group after 1 y; 2 RCTs (37, 38) found no significant difference in the net changes in TB BMC after 1 y (Supplemental Table 7). Data were not sufficient to conduct a meta-analysis.

**Cohort studies**

One cohort study, which was composed of perimenopausal women, examined TB BMC for 2.5 y (Supplemental Table 8) (42). Protein intake from soy was assessed by using an FFQ. Overall ROB for this study was medium, primarily due to >20% of participants being lost to follow-up over the course of the study (Supplemental Table 5). Higher protein intake was associated with a significant increase in TB BMC over time compared with lower protein intake (Supplemental Table 9) (42).

**Falls**

**RCTs**

No RCTs examining the risk of falls met the inclusion criteria.

**Cohort studies**

Two cohort studies in older adults examined the risk of falls over a 1- to 2-y period (Supplemental Table 8) (48, 49). Protein intake was assessed by using an FFQ. Overall ROB for these studies was low, although it is important to note that it is unclear if there was enough statistical power to detect an association with continuous protein intake data in either study (Supplemental Table 5). One study (49) found higher protein intake to be associated with a lower risk of falls compared with lower protein intake, whereas the other study (48) found no significant association (Supplemental Table 10).

**Spine fracture**

**RCTs**

No RCTs examining spine fracture risk met the inclusion criteria.
Cohort studies

One cohort study in postmenopausal women examined spine fracture risk over a 6-y period (Supplemental Table 8) (39). Protein intake was assessed by using an FFQ. The ROB for this study was medium, primarily due to the completeness of the cohort follow-up (i.e., >20% lost to follow-up) and selective outcome reporting (Supplemental Table 5). There was a non-significant association found between protein intake and spine fracture risk (Supplemental Table 10) (39).

Hip fractures

RCTs

No RCTs examining hip fracture risk met the inclusion criteria.

Cohort studies

Nine cohort studies examined hip fracture risk for 3–17 y (Supplemental Table 8) (39, 50–57). The studies were conducted in different populations: 3 studies (39, 50, 54) were conducted in women [2 (39, 54) of which were conducted in postmenopausal women], 2 (55, 57) were conducted in men, and 4 studies (51–53, 56) were conducted in adults of both sexes. Protein intake was assessed by using FFQs. Overall, ROB was low, although 3 studies adjusted for neither calcium nor vitamin D and sample size adequacy was either unclear or not met in 5 studies (Supplemental Table 5).

Six studies (39, 50, 52, 54–56) did not find a significant association between protein intake and hip fracture risk (Supplemental Table 10). One study in men (57) found that increased protein intake was associated with a decreased risk of hip fracture. Another study (51) in adults stratified men and women and only found a significant inverse association between higher protein quartiles and the risk of hip fracture when compared with the lowest quartile in women; there was no significant association in men. Last, one study (53) found a significantly lower risk of hip fracture only among participants in the third quartile compared with those in the lowest quartile of protein intake; no association was found in the second or highest quartile (Supplemental Table 10).

Forearm fractures

RCTs

No RCTs examining forearm fracture risk met the inclusion criteria.

Cohort studies

Two cohort studies examined forearm fracture risk for 6 and 12 y (Supplemental Table 8) (39, 50). One cohort study comprised postmenopausal women (39) and the other study (50) comprised middle-aged women. Protein intake was assessed by using FFQs. Overall, ROB was medium, because one study reported inadequate sample size and power and self-reported fracture (50) and the other study (39) had loss to follow-up (<80%) and incomplete outcome reporting (Supplemental Table 5).

The 2 studies had opposite findings. In one study (50), which compared the higher quintiles with the lowest quintile (<68 g/d), the highest quintile (>95 g/d) showed a significant positive association between total protein and forearm fracture. Conversely, in the second study (39), when protein was examined in quintiles by percentage of total energy, women who had 20% higher protein intakes were 7% less likely to have a fracture (Supplemental Table 10).

Overall fractures

RCTs

No RCTs examining overall fracture risk met the inclusion criteria.

Cohort studies

Four cohort studies examined overall fracture risk for between 5 and 13 y (Supplemental Table 8) (39, 58–60). Three cohort studies comprised postmenopausal women (39, 58, 59) and 1 comprised adults aged ≥55 y (60). Protein intake was assessed by using FFQs. Overall, ROB was low, although 3 studies captured FFQ data only at baseline for ≥6 y of follow-up, and 2 studies used self-reported data for the outcome assessment (Supplemental Table 5).

There was no significant association between protein intake and overall fracture risk in 3 of the studies (Supplemental Table 10) (39, 58, 60). The fourth cohort study (59) found an inverse association of overall fracture risk with higher soy protein intake (>4.98 g/d) with the use of the lowest quintile of soy protein intake (<4.98 g/d) as the reference group.

Osteocalcin

RCTs

Ten RCTs examined osteocalcin concentrations (Supplemental Table 6) (25, 27, 28, 30, 34, 35, 37, 38, 61, 62). Eight studies (25, 27, 28, 30, 35, 37, 38, 62) prescribed milk protein–based supplements: the doses of milk-based protein in the higher-protein groups in 7 studies ranged from 10 to 45 g/d and 1 study (62) supplemented 250-mg capsules of milk ribonuclease–enriched lactoferrin/d, whereas doses in the lower-protein groups ranged from 0 to 2.1 g milk-based protein/d. In the higher-protein groups, 3 received 10–45 g milk-based protein/d, 3 received 40 mg milk-based protein/d, and 1 received 250 mg milk lactoferrin capsules that were RNase-enriched. The lower-protein groups received 0 g protein/d. The 2 remaining studies (34, 38) prescribed dietary composition changes. The higher-protein groups consumed >90 g protein or 30% of total energy from protein, whereas the lower-protein groups consumed <80 g protein or 18% of total energy from protein, respectively. Eight studies were conducted in different populations of women, 1 study was conducted in healthy adults, and 1 study was conducted in adults with recent hip fracture (Supplemental Table 6). Overall, ROB was low, although it is important to note that 4 studies had low compliance (<80%) and 4 studies had incomplete outcome data (i.e., dropout rate >20%) (Supplemental Figure 1, Supplemental Table 4).

Findings were inconsistent across studies: 6 of the 10 RCTs (25, 27, 35, 37, 38, 61) found no significant difference in the net changes in osteocalcin concentrations (Supplemental Table 11). Two studies (30, 62) found that osteocalcin concentrations increased significantly more in the higher-protein group than in
the lower-protein groups, whereas another study found that osteocalcin concentrations decreased significantly more in the higher-protein group than in the lower-protein group (34). Findings in the remaining study (28) were unclear, because the higher-protein group showed significantly different osteocalcin concentrations at both baseline and follow-up compared with the lower-protein group, and final and change quantitative values were not provided (i.e., data in figure only) (Supplemental Table 11).

Our meta-analysis of 8 RCTs showed that higher protein intakes had no significant effects on osteocalcin compared with lower protein intakes, with wide uncertainty (i.e., wide CIs) and low statistical heterogeneity (pooled net change: 0.06 ng/mL; 95% CI: −0.49, 0.60 ng/mL; $I^2$: 27.2%) (Figure 5). Two 6-mo studies could not be included in the meta-analysis: Aoe et al. (27) found no difference between protein groups (data in figure only; $P > 0.05$), whereas Uenishi et al. (30) found the higher-protein group to have higher osteocalcin concentrations over time compared with the lower-protein group (data in figure only; $P = 0.033$) (Supplemental Table 11).

Cohort studies

The association between protein intake and osteocalcin concentrations was not examined for this outcome in any of the included cohort studies.

CTX

RCTs

Five RCTs examined CTX concentrations (25, 28, 34, 35, 46) (Supplemental Table 6). Four studies (25, 28, 35, 46) prescribed supplements. The doses of milk-based protein in the higher-protein groups ranged from 10 to 45 g/d, whereas the lower-protein groups received 0 g protein/d. One study (34) prescribed dietary composition changes. The higher-protein group consumed >90 g protein/d, whereas the lower-protein group consumed <80 g protein/d. Studies were conducted in different populations (Supplemental Table 6). Overall, ROB was medium, primarily due to low compliance (<80%) in 3 studies and incomplete outcome data (dropout rate >20%) in 2 studies (Supplemental Figure 1, Supplemental Table 4).

Three RCTs (25, 28, 46) found no significant difference in the net changes in CTX concentrations (Supplemental Table 11). One study (34) found that the lower-protein group showed significantly greater increases in CTX concentrations compared with the higher-protein group after 2 y, whereas another study (35) found that the higher-protein group showed significantly higher concentrations than the lower-protein group after 1.5 y (Supplemental Table 11).

Our meta-analysis of 5 RCTs showed that higher protein intake, on average, increased CTX compared with lower protein intake, although the pooled result was not significant, with wide uncertainty (i.e., wide CIs) and substantial heterogeneity (pooled net change: 47.72 ng/L; 95% CI: −27.34, 122.78 ng/L; $I^2$: 61.3%) (Figure 6).

Cohort studies

The association between protein intake and CTX concentrations was not examined for this outcome in any of the included cohort studies.

Synergistic effects of protein and Ca$\pm$D

Most RCTs supplemented all of the participants with the same or similar amounts of Ca$\pm$D, but none examined their synergistic effects for our included outcomes of interest. The following results are thus from cohort studies only (Supplemental Table 8). No studies examined the interaction between protein intake and Ca$\pm$D.
and Ca±D on TB BMC, falls, osteocalcin, or CTX and thus we conclude there is D level of evidence for these outcomes.

**BMD**

Four (39, 40, 43, 45), 2 (39, 43), and 3 (40, 43, 45) cohort studies examined the interaction between protein and Ca±D on LS BMD, TH BMD, and FN BMD outcomes, respectively (Supplemental Table 8). Overall, ROB was medium for all 3 BMD outcomes, primarily due to loss to follow-up (>20%) and not addressing if there was adequate sample size or power to detect significant findings in 3 studies (Supplemental Table 5). All except for one of the studies (40) did not find a significant interaction (Supplemental Table 9). This study (40) was a cohort study that used data from a 3-y, randomized, placebo-controlled trial with or without Ca±D supplementation and found that higher dietary protein intake was associated with less TB BMD loss than was lower protein intake only in the group supplemented with Ca±D (Supplemental Table 9).

**Spine fractures**

One cohort study examined the interaction between protein and calcium (Supplemental Table 8) (39), but its findings were not significant (Supplemental Table 10). Overall ROB for this study was medium, primarily due to loss to follow-up (>20%) and incomplete reporting for this outcome (Supplemental Table 5).

**Hip fractures**

Four cohort studies also examined an interaction between protein and calcium intake (39, 52, 55, 56) (Supplemental Table 8). Overall, ROB was medium, primarily due to 2 studies that lost >20% of their subjects during follow-up, 4 studies only ascertaining nutrient exposure at baseline for ≥6 y of follow-up, and 3 studies either not addressing or not having an adequate sample size or power to detect significant findings (Supplemental Table 5). Of these 4 studies, 2 (39, 55) did not find a significant interaction. One study (56) did find a significant interaction between protein and calcium, but once stratified by calcium, findings were not significant for either of the calcium categories. The fourth study (52) reported on men and women separately and found an increased risk of hip fracture only among women with both the highest quartile of protein intake and the lowest quartile of calcium intake (Supplemental Table 10).

**Forearm fractures**

Two cohort studies examined an interaction between protein and calcium intake (39, 50) (Supplemental Table 8), but neither of these studies found a significant interaction (Supplemental Table 10). Overall, ROB was medium, because one study (50) reported an inadequate sample size and power and self-reported fracture and the other study (39) had loss to follow-up (>20%) and incomplete reporting for this outcome (Supplemental Table 5).

**Overall fractures**

Two cohort studies examined an interaction between protein and dietary calcium intake, with inconsistent findings (Supplemental Table 8) (39, 58). Overall, ROB was low, although both studies only captured FFQ data at baseline for ≥6 y, and 1 study used self-reported data for the outcome assessment (Supplemental Table 5). One study (39) did not find a significant interaction between protein and calcium. The second study (58) found that, when the lowest protein quartile plus the lowest calcium quartile was used as the reference group, a higher protein intake (g/1000 kcal) was positively associated with overall fracture risk in the lowest calcium quartile. It was not significantly associated with overall fracture risk in the higher calcium quartiles (Supplemental Table 10).
<table>
<thead>
<tr>
<th>Outcome</th>
<th>Studies, n (ref)</th>
<th>Cohort studies</th>
<th>SOE grade</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD LS</td>
<td>7 (26, 29, 34–38)</td>
<td>7 (39–45)</td>
<td>B</td>
<td>We conclude a B level of evidence that a higher protein intake may cause less LS BMD loss than lower protein intake in older adults. Three RCTs (2 in older men and 1 in postmenopausal women) found this effect, whereas 4 (2 in healthy adults, 1 in postmenopausal women, and 1 in older adults with current hip fracture) found that protein intake had no significant effect on LS BMD. Studies in various populations are currently limited, varying doses and dietary compositions were used or prescribed, respectively, and there was medium ROB among the RCTs. Cohort studies overall did not support this association between higher protein intake and LS BMD loss, although there may be ROB because of loss to follow-up or because it is unclear if they had adequate sample size and power to detect an association.</td>
</tr>
<tr>
<td>TH</td>
<td>8 (26, 29, 31, 34–36, 38, 46)</td>
<td>2 (39, 43)</td>
<td>B</td>
<td>We conclude a B level of evidence that protein intake amount does not affect TH BMD loss. Most of the RCTs did not find protein intake to significantly affect TH BMD in various populations, and only 2 studies found a difference. Studies in each population are also limited, varying doses were used, and there was medium ROB. Two cohort studies also found no association between protein intake and TH BMD loss, although it is important to note that the number of cohort studies included were limited with medium ROB.</td>
</tr>
<tr>
<td>FN</td>
<td>8 (25, 26, 31, 34–38)</td>
<td>5 (40–43, 45)</td>
<td>B</td>
<td>We conclude a B level of evidence that protein intake amount does not affect FN BMD loss. Although RCTs overall did not find protein intake to significantly affect FN BMD in various populations, studies in each population are limited, varying doses were used, and there was medium ROB. Some cohort studies found no association between higher protein intake and BMD loss, although 2 in older adults reported high protein intake to be associated with less BMD loss.</td>
</tr>
<tr>
<td>TB</td>
<td>5 (25, 29, 38, 46, 47)</td>
<td>2 (39, 40)</td>
<td>B</td>
<td>We conclude a B level of evidence that higher protein intake may cause less TB BMD loss than lower protein intake. Two RCTs (1 in postmenopausal women with FN fractures and 1 in men) found this effect, whereas 3 (1 in postmenopausal women, 1 in adults with recent hip fracture, and 1 in adults) found that protein intake had no significant effect on TB BMD. Multiple studies in various populations are currently limited, varying doses and dietary compositions were used or prescribed, respectively, and there was medium ROB among the RCTs. Cohort studies overall supported this association between higher protein intake and less TB BMD loss.</td>
</tr>
<tr>
<td>TB BMC</td>
<td>3 (29, 37, 38)</td>
<td>1 (42)</td>
<td>C</td>
<td>No firm conclusions can currently be drawn. One RCT and 1 cohort study support the hypothesis that higher protein intake causes less TB BMC loss than lower protein intake, whereas 2 RCTs support the hypothesis that protein intake has no significant effect on TB BMC. Furthermore, varying protein doses were used in each RCT intervention group, studies in various populations are limited, and there was medium ROB among both the RCTs and cohort study.</td>
</tr>
</tbody>
</table>

(Continued)
SOE grading

An evidence level of B was assigned for the association between higher compared with lower protein intakes and BMD of the LS, TH, FN, and TB, with no effect for the TH and FN; possibly less BMD loss for the LS; and inconclusive effects for the TB. An evidence level of C was assigned for the association of higher compared with lower protein intakes and total body BMC, with no firm conclusions possible. With regard to fracture outcomes, an evidence level of B was assigned for no relation between higher compared with lower protein intakes and hip fractures, but a level of D was assigned for fractures of other sites (i.e., spine, forearm, and overall fracture risk) due to insufficient or inconsistent data. An evidence level of D was assigned for falls due to insufficient data.

With regard to biochemical markers of bone turnover, an evidence level of B was assigned for no relation between higher compared with lower protein intakes and osteocalcin or CTX (Table 2).

An evidence level of C was assigned for no synergistic effect between protein and Ca\(^{2+}\) on LS BMD, TH BMD, and forearm fractures, as well for the inconclusive effect on FN BMD, TB BMD, and overall fractures. An evidence level of D was assigned for all remaining outcomes due to insufficient data to support a hypothesis (Table 3).

DISCUSSION

The relation between dietary protein intake and bone health has been a topic of great debate over the past several decades.
There is an apparent dietary requirement for protein to build and maintain bone; however, reports of detrimental effects of protein made this more in-depth analysis necessary to better inform the clinical community and stimulate additional research. Our analysis was limited to direct measures in terms of fracture outcomes, BMD, BMC, and select bone turnover markers. A reductionist approach is often used (particularly in meta-analyses) to assess the impact of a particular food or nutrient on a health endpoint or disease outcome. Although this approach is useful in guiding us in establishing reference intakes, it is often confounded by the many synergies that exist with other essential nutrients that promote healthy cell function.

<table>
<thead>
<tr>
<th>TABLE 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SOE grading: synergistic effect of protein with calcium with or without vitamin D by outcome</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcome</th>
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</tr>
</thead>
<tbody>
<tr>
<td>BMD LS</td>
<td>0 4 (39, 40, 43, 45)</td>
<td>C</td>
<td>We conclude a C level of evidence that there is no synergistic effect of protein with calcium with or without vitamin D on FN BMD loss. Most of the cohort studies included addressed this association with no significant findings, although there may be ROB because of loss to follow-up or because it is unclear if they had adequate sample size and power to detect an association.</td>
</tr>
<tr>
<td>TH</td>
<td>0 2 (39, 43)</td>
<td>C</td>
<td>We conclude a C level of evidence that protein intake does not have a synergistic effect with calcium on TH BMD loss. Although 2 cohort studies did not find this interaction to be significant, the power to detect an association was unclear in 1 study and we have concerns with &gt;20% of participants being lost to follow-up in both studies.</td>
</tr>
<tr>
<td>FN</td>
<td>0 3 (40, 43, 45)</td>
<td>C</td>
<td>No firm conclusions can currently be drawn. Only a few cohort studies addressed this association with inconsistent findings, and it is unclear if they had adequate sample size and power to detect an association.</td>
</tr>
<tr>
<td>TB</td>
<td>0 2</td>
<td>C</td>
<td>No firm conclusions can currently be drawn. Only 2 cohort studies addressed this association with inconsistent findings.</td>
</tr>
<tr>
<td>TB BMC</td>
<td>0 0</td>
<td>D</td>
<td>There are insufficient data to support a hypothesis: no study examined this association.</td>
</tr>
<tr>
<td>Falls</td>
<td>0 0</td>
<td>D</td>
<td>There are insufficient data to support a hypothesis: no study examined this association.</td>
</tr>
<tr>
<td>Fractures Spine</td>
<td>0 1 (39)</td>
<td>D</td>
<td>There are insufficient data to support a hypothesis: only one cohort study examined this association.</td>
</tr>
<tr>
<td>Hip</td>
<td>0 4 (39, 52, 55, 56)</td>
<td>D</td>
<td>There are inconsistent results among the limited number of studies that addressed this possible interaction between protein and calcium.</td>
</tr>
<tr>
<td>Forearm</td>
<td>0 2 (39, 50)</td>
<td>C</td>
<td>We conclude a C level of evidence that there is no synergistic effect of protein with calcium with or without vitamin D on forearm fractures. Two cohort studies addressed this association with no significant findings. However, there may be ROB because it is unclear if they had adequate sample size and power to detect an effect in one study, or because of loss to follow-up in the other study.</td>
</tr>
<tr>
<td>Overall</td>
<td>0 2 (39, 58)</td>
<td>C</td>
<td>No firm conclusions can currently be drawn. Only 2 cohort studies addressed this association with inconsistent findings, and it is unclear if the existing studies had adequate sample size and power to detect synergistic effects, or adequate follow-up time to accurately capture fracture risk.</td>
</tr>
<tr>
<td>Osteocalcin</td>
<td>0 0</td>
<td>D</td>
<td>There are insufficient data to support a hypothesis: no study examined this association.</td>
</tr>
<tr>
<td>CTX</td>
<td>0 0</td>
<td>D</td>
<td>There are insufficient data to support a hypothesis: no study examined this association.</td>
</tr>
</tbody>
</table>

1 BMC, bone mineral content; BMD, bone mineral density; CTX, collagen type 1 cross-linked C-terminal telopeptide; FN, femoral neck; LS, lumbar spine; RCT, randomized controlled trial; ref, reference; SOE, strength of evidence; TB, total body; TH, total hip.
Although the scientific literature is somewhat limited with regard to the beneficial effects of protein intake, our analysis does not indicate the presence of any adverse relations. Only a small number of studies indicate an adverse association of protein intakes and bone health. Positive trends for the relation of high compared with low protein intakes and BMD and/or BMC were identified for nearly all bone sites, although many findings were inconsistent across studies. An evidence level of B was assigned for the association between higher compared with lower protein intakes and BMD of the LS, TH, FN, and TB, with no effect for TH and FN and inconclusive effects for LS and TB. An evidence level of C was assigned for the association of higher compared with lower protein intakes and total body BMC, with no firm conclusions possible. An evidence level of D was assigned for falls due to insufficient data. With regard to biochemical markers of bone turnover, an evidence level of B was assigned for no relation between higher compared with lower protein intakes and osteocalcin or CTX (Table 2).

With regard to fracture outcomes, an evidence level of B was assigned for no relation between higher compared with lower protein intakes and hip fractures; however, a level of D was assigned for fractures of other sites (i.e., spine, forearm, and overall fracture risk) due to insufficient or inconsistent data. Thus, our study of limited data found no significant beneficial or detrimental effects on high compared with low protein intakes and fracture outcomes. In the absence of long-term intervention studies, however (most study durations were ≤2 y), it is difficult to assess at this time whether high compared with low protein intakes affect fracture risk. Because fractures indeed represent the most important outcome from a clinical standpoint, further study is warranted.

Furthermore, significant interactions between dietary protein with Ca±D intake were not shown in the included prospective cohort studies (Table 3). However, it is important to reiterate the limited number of cohort studies included and the level C (i.e., “limited”) and level D (i.e., “inadequate”) SOE gradings across all the outcomes. Because Ca±D supplementation has recently been shown to significantly decrease the risk of both hip and total fractures (63), further studies are merited.

Our systematic review and meta-analysis has several caveats in addition to the inherent limitations related to the ROB from the included studies. First, RCTs reported diverse BMD outcome metrics, which make meta-analysis very challenging. Some studies did not report sufficient data for meta-analysis, and several assumptions were made to impute missing SDs of the effect sizes, further limiting our confidence in the meta-analysis results. Second, there is unaccountable clinical heterogeneity (e.g., different protein intervention doses and comparators) in our meta-analyses, and the small number of RCTs for each outcome did not allow us to perform subgroup meta-analyses or meta-regression to quantitatively examine the influences of clinical heterogeneity on our pooled results. We were also unable to identify apparent reasons to explain the inconsistent findings across RCTs through qualitative evaluations, due to the diversity of population characteristics. However, we considered all of these limitations, including clinical heterogeneity, in our qualitative SOE synthesis. Third, many RCTs had low adherence, whereas some cohort studies experienced loss to follow-up and many did not report if they had adequate sample sizes and power. The presence of these biases across studies may have influenced the results. Finally, intake estimates from the observational studies relied on 24-h dietary recalls and/or FFQs, which have known limitations due to measurement errors. The measurement errors may have had an impact on the results, but the directions of these impacts are unpredictable.

Conclusions are further limited by the clear heterogeneity present across studies. Studies included in this systematic review used a variety of designs, doses, durations, and outcomes. Many studies also may not have been long enough to see clear effects on BMD, BMC, and fractures. Thus, future large RCTs that span many years in duration are needed to accurately assess the effect of protein intakes on fracture risk, along with surrogate endpoints (e.g., BMD) on bone health. Additional, well-designed cohort studies may also facilitate better insight, because large RCTs are expensive and often not possible.

In conclusion, although positive trends for the relation of high compared with low protein intakes and BMD and/or BMC were identified for nearly all bone sites, only the LS had a moderate SOE, showing that a higher protein intake may cause less BMD loss than a lower protein intake in older adults. However, studies were highly heterogeneous and confounding by variables that were not accounted for could not be excluded when hypothesizing why many outcomes were not significant. We found no significant relation between dietary protein and fracture risk in this systematic review. Larger, long-term RCTs are greatly needed to clarify the role of dietary protein and fracture risk and BMD changes. Overall, the body of evidence shows that the effect of dietary protein on the skeleton appears to be favorable to a small extent and is not detrimental. However, the existing data are too heterogeneous and the SOE is not strong enough to warrant a clinical guideline to recommend an increase in protein intake as a standard care protocol.

We thank Amy Lapidow and Amy LaVertu for aiding MMS-W in the development of all search strategies. We also thank Lars Holm, Kun Zhu, and Russell de Souza for providing original data from their research studies upon request for inclusion in the meta-analyses. The authors’ responsibilities were as follows—MMS-W, MC, KLI, MSL, SAS, TCW, and CMW: designed the research, developed the overall research plan, and interpreted the results; MC and TCW: conceived of the project conception and obtained funding; MMS-W: was responsible for study oversight; MMS-W, MD, ZF, MC, MCK, and JS: conducted the research; MMS-W, MC, KLI, MCK, MSL, SAS, JS, and CMW: conducted qualitative synthesis; MMS-W, MC, and ZF: conducted quantitative analyses; MMS-W, KLI, and TCW: wrote the manuscript; MMS-W, MC, KLI, MCK, MSL, SAS, TCW, and CMW: had primary responsibility for the final content and all authors: read and approved the final manuscript. TCW is a consultant for the National Osteoporosis Foundation. None of the other authors report a conflict of interest. MC, MD, KLI, MSL, SAS, and CMW contributed their efforts without receiving funding or salary support. The Egg Nutrition Center and Dairy Management Inc. had no role in the design, analysis, interpretation, or presentation of the data or the results.

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