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# Dietary protein and bone health: a systematic review and meta-analysis[1–3]

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## ABSTRACT

**Background:** There has been a resurgence of interest in the controversial relation between dietary protein and bone health.

**Objective:** This article reports on the first systematic review and meta-analysis of the relation between protein and bone health in healthy human adults.

**Design:** The MEDLINE (January 1966 to September 2007) and EMBASE (1974 to July 2008) databases were electronically searched for all relevant studies of healthy adults; studies of calcium excretion or calcium balance were excluded.

**Results:** In cross-sectional surveys, all pooled *r* values for the relation between protein intake and bone mineral density (BMD) or bone mineral content at the main clinically relevant sites were significant and positive; protein intake explained 1–2% of BMD. A meta-analysis of randomized placebo-controlled trials indicated a significant positive influence of all protein supplementation on lumbar spine BMD but showed no association with relative risk of hip fractures. No significant effects were identified for soy protein or milk basic protein on lumbar spine BMD.

**Conclusions**: A small positive effect of protein supplementation on lumbar spine BMD in randomized placebo-controlled trials supports the positive association between protein intake and bone health found in cross-sectional surveys. However, these results were not supported by cohort study findings for hip fracture risk. Any effects found were small and had 95% CIs that were close to zero. Therefore, there is a small benefit of protein on bone health, but the benefit may not necessarily translate into reduced fracture risk in the long term. *Am J Clin Nutr* 2009;90:1674–92.


## INTRODUCTION

The bone disease osteoporosis is becoming epidemic, with 1 in 4 women >70 y of age having at least one fracture in their lifetime (1). The incidence of osteoporosis is likely to worsen, and an increase in hip fracture rates to 6.62 million per year is predicted by 2030 (2). The increasing burden of osteoporosis globally means modifiable factors such as nutrition have become of larger importance.

There is a requirement for amino acid precursors from dietary protein to maintain bone structure. In addition, the anabolic drive of amino acids on the organism includes an influence on bone mediated in part through the stimulation of growth factors such as insulin-like growth factor I (IGF-I) (3). IGF-1 has been suggested to increase bone mass by increasing osteoblast activity and may also increase the mineralization of bone matrix (4) in part by increasing calcium absorption (5). Therefore, an inadequate anabolic drive due to insufficient dietary protein (6) may decrease bone strength through adverse changes in bone microarchitecture (7). This indicates a need for adequate protein intakes for both the elderly and the general population to help optimize bone health. However, the balance between beneficial and detrimental influences of dietary protein on bone health is a long-standing debate (8–11). Dietary protein is a major contributor to acid production (12) as a result of the oxidation of the sulfur amino acids, and declining pH values influence the balance between osteoblastic and osteoclastic activity (13) and increases urinary calcium excretion (14).

Because protein is a modifiable factor in osteoporosis prevention, dietary protein clearly has a role in bone health. However, to our knowledge, the evidence to date has not been systematically reviewed. Therefore, we conducted a systematic review and meta-analysis of the effect of protein intake on indexes of bone mineral density (BMD), bone turnover, and fracture risk and present the results herein.

## METHODS

### Search strategy

We conducted a systematic search of the MEDLINE (http://www.ncbi.nlm.nih.gov/pubmed/) and EMBASE (http://www.embase.com/home) databases. The MEDLINE database (January 1966 to September 2007) was searched via PubMed. The EMBASE database (1974 to July 2008) was also searched to ensure broad coverage. The search phrase used for both searches was ''(protein intake OR dietary protein OR protein supplement OR protein consumption) AND (bone OR fracture OR BMD OR bone turnover)'' limited to human studies written in the English language and from 1975 to the present day. Articles that

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were >30 y old were excluded if the details provided in the publications did not provide sufficient details of the results for this meta-analysis. Two of the authors (AD and SLN) screened titles and abstracts to identify potentially eligible studies. Any disagreements were resolved by consensus or deferred to a third party, if necessary. Full papers for potentially eligible studies were obtained and assessed for inclusion independently by 2 of the authors (ALD and SAL-N).

### Study eligibility criteria

All studies of any design reporting influences of any protein type on BMD or bone mineral content (BMC), bone turnover, and fracture risk in healthy human adults were considered for inclusion in the review. Studies investigating subjects with a preexisting medical condition (including obesity), children, and pregnant or lactating women were excluded, as were studies involving only indirect measures of bone health such as calcium balance or metabolism. Supplementation trials were not excluded on the basis of the type of control used, the duration of the trial or the dose of protein in the experimental group. The exception to this was that studies using milk basic protein (MBP) as a control treatment were excluded because in these trials the dosage of protein was the same in the experimental and control groups. Crossover supplementation trials were included in the qualitative review but were excluded from the meta-analysis.

### Data extraction

For the tables of characteristics, relevant information (ethnicity, age, sex, and protein intake) about the populations studied was extracted. Protein intake was expressed in $g \cdot kg^{-1} \cdot d^{-1}$ by dividing mean total daily protein intake by mean weight (kg) of the participants. If no weight was available, mean intakes (g/d) or the ranges of intake (g/d) were extracted. For quality-assessment purposes, data on potential confounders as well as drop-out rates and methods of dietary assessment were extracted.

When data on study outcomes were extracted, multivariate-adjusted analyses were used wherever possible in preference over crude or age-adjusted measures. For the cross-sectional surveys, the correlation coefficients ($r$), $n$ (number of participants) values, and $P$ value were extracted for each outcome. In the cohort studies, any relevant data were extracted, such as percentage change in bone mass over time, means and SDs, or $r$ coefficients for the slope of bone loss in different protein intake groups. Also, odds ratios (ORs) or the relative risk of fracture estimates (with 95% CIs) for the highest and lowest quartiles or quintiles of intakes for cases were also extracted, with $n$ and $P$ if available.

For each of the supplementation trials, the mean, SD, and $n$ for follow-up measurements were extracted for each relevant outcome in each arm of the study. If SEMs were presented, they were converted to SDs by using the standard formula ($SEM = SD/\sqrt{n}$). One soy protein supplementation trial (15) had 2 experimental groups, so the low-isoflavone group was chosen for comparison with the control group. Only follow-up data were extracted because it was assumed that participants were randomized at baseline.

All 29 authors of relevant articles with missing data were contacted. Replies were received from 16 authors, with 8 authors being able to provide the requested data. The other 8 authors were not able to provide data because the data were not available. The articles not providing complete data (ie, not able to calculate the SD or the SEM) were not able to be included in the meta-analysis. These articles with incomplete data were therefore included in the analysis in a qualitative form only. Therefore, no articles were excluded from the whole review simply for having incomplete data.

### Data synthesis

All studies were analyzed qualitatively, and the studies with suitable data were also analyzed quantitatively. Microsoft Excel (16) was used for pooling $r$ coefficients, and RevMan version 4.2 (17) was used for the meta-analyses.

#### *Pooling of correlation coefficients*

From the cross-sectional surveys, the $r$ values were pooled by bone site (BMD and BMC) or by bone marker and were then repooled by population subgroup (men, premenopausal women, and postmenopausal women).

To calculate pooled values, all $r$ values were transformed by using Fisher's $z$ transformation and then weighted by using the standard formula (18). They were then inverse Fisher transformed to give the pooled $r$ ($r_p$) values; 95% CIs were calculated by using a standard formula (mean ± 1.96 SD). This gave a pooled $r$ value with a 95% CI for each BMD and BMC site, bone marker, or population subgroup. Levels of heterogeneity of pooled $r$ values were calculated by using the chi-square statistic.

#### *Meta-analysis*

For the meta-analysis, results could only be pooled when there were ≥2 studies looking at the same outcome within protein type. For example, there were not enough studies to assess forearm fracture risk. This is because there was only one study assessing forearm as an outcome, unlike hip fracture, for which there were >2 studies. Also, for the fracture meta-analysis, separate analyses were made for each protein type (total, animal, and vegetable), but not all protein types were analyzed together.

The meta-analysis of the supplementation trials examined the main effects for protein supplementation on lumbar spine BMD. Only lumbar spine BMD was used in the meta-analysis of the supplementation trials because there were not enough compatible studies to assess other bone sites. The analysis for protein intake and lumbar spine BMD assessed all protein types, including MBP. Soy studies were not included here because protein dose was not varied between experimental and control groups. A separate soy analysis was therefore run to compare soy protein (experimental group) with nonsoy protein (control group).

In addition, a separate comparison was also made for the effect of MBP and lumbar spine BMD. Not enough studies examining animal or vegetable protein separately that satisfied the search strategy were identified to justify a separate analysis. It was not possible to run a meta-analysis for BMC and for bone markers and all protein because there were not enough studies.

#### *Heterogeneity, sensitivity, and publication bias—meta-analysis*

The $I^2$ statistic was used to assess heterogeneity between studies because this is more effective than the chi-square

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statistic when small numbers of studies are included in meta-analyses (19). As suggested by Higgins et al (19), $I^2$ values of 25%, 50%, and 75% were considered low, moderate, and high, respectively. Random-effects (heterogeneous comparisons) and fixed-effects (homogenous comparisons) models were used accordingly. Weighted mean differences were used throughout (BMD and fracture risk). Unfortunately, the numbers of studies in the meta-analysis were too small to assess publication bias.

### Quality analysis

Because there is no clearly defined method for assessing the quality of cross-sectional surveys and cohort, case-control, and ecologic, and nonrandomized comparative trials, this was done subjectively by one author (ALD). The randomized placebo-controlled trials were assessed for quality by using the CONSORT statement checklist (20) by one author (ALD). Scales relating to randomization and to concealment and blinding (CONSORT items 8–11) were used to assess study quality because these have been found to be linked to estimates of effect size and risk of bias (21).

## RESULTS

The QUORUM (quality of reporting of meta-analyses) flow diagram (22), showing the flow of articles through the selection process, is shown in **Figure 1**. Sixty-one studies were included in the systematic review, including 31 cross-sectional surveys, ecologic and cohort studies, and 19 supplementation trials examining BMD, BMC, or bone markers. Also included were 11 cohort and case-control studies examining fracture risk.

### BMD, BMC, and bone markers

Thirty-one cross-sectional surveys examining BMD, BMC, and bone markers were included in the systematic review (**Tables 1** and **2**): 22 studies from Western countries (United States, Australia, Europe, and Canada), 8 from Asian countries (Japan, Taiwan, and China), and 1 from Brazil. Of these, 23 were studies of women alone (with 6 pre-, 9 post-, and 8 pre- and postmenopausal), 4 of men alone, and 4 of men and women. Most studies examined total protein ($n = 30$), but one study examined soy and total protein (31).

#### *Study quality*

In terms of dietary assessment methods, 11 studies (25, 27, 30, 34, 37, 45–47, 49, 50, 52) used food-frequency questionnaires (FFQ), with most FFQs being previously validated. Thirteen studies used dietary records (24, 26, 28, 29, 31–33, 35, 38, 41, 43, 44, 53). Of these, 3 (28, 31, 41) clearly stated this was a weighted method, although most studies using nonweighted measures made efforts to use food models or photos to enable more accurate estimation by the participants. The final 7 studies used either recall methods (36, 40), other questionnaires (42, 48), or a mixture of recall and FFQs (23, 51) or a duplicate-portion method (39).

For the 12 studies reporting *r* values that were pooled, it was not clear whether confounders had been adjusted for (23, 25, 29, 31, 36, 39, 40, 43, 46, 48). Only 5 studies (24, 33, 35, 38, 50) clearly reported adjustment for at least one relevant confounder such as age, body weight or BMI, physical activity, or energy intake.

Some studies in the qualitative analysis only reported regression analyses and these were adjusted for at least one confounder (27, 30, 32, 37, 42, 45, 47, 49). For those that reported correlations, adjustment for at least one confounder was present in one study (41) but not another that mentioned crude correlation only (34). Last, 3 (44, 52, 53) of the 4 (44, 51–53) studies looking at percentage change in BMD adjusted for at least one confounder in the analysis.

Potential bias could arise from the use of volunteers in 7 of the studies (28, 29, 35, 37, 45, 46, 48). Potential confounders may include other aspects of the diet or lifestyle that may influence bone health (eg, phosphorus, calcium, sodium, potassium, latitude, sun exposure, smoking, alcohol, educational attainment, socioeconomic status, and physical activity) in addition to physiologic variables such as BMI, age, menopausal status, and effects of chronic conditions or medications.

#### *BMD*

Overall, there was very little evidence of a deleterious influence of protein intake on BMD, with most cross-sectional surveys and cohort studies reporting either no influence or a positive influence. Thus, 15 cross-sectional surveys found a statistically significant positive relation between protein intake and at least one BMD site (24, 25, 27–33, 37, 38, 42, 44, 46, 50). However, 18 studies found no significant correlation between protein intake and at least one BMD site (23, 24, 26–28, 30, 33–38, 42–44, 46–48).

The cohort studies also identified little evidence of any deleterious influence of protein intake on bone. Thus, of those studies reporting *r* values or percentage BMD loss as a function of protein intake, no studies showed a significant increase in BMD loss with increased protein intake, and only one study showed a significant decrease in BMD loss with increased animal and total protein intakes (52). However, 3 studies found no correlation between protein intake and bone loss at one or more sites (26, 44) or that protein intake was not a significant predictor of BMD (51). In the only study examining mean BMD (53), subjects with a higher mean protein intake had a significantly higher mean femoral neck BMD than did those with a lower mean intake.

#### *BMC*

For BMC, 4 cross-sectional surveys found a positive correlation between protein intake and BMC for at least one site (30, 37, 43, 47), whereas 2 found no significant correlation (26, 34). Only one survey found a significant negative correlation between protein intake and BMC (37).

#### *Ultrasonic measures*

Most cross-sectional surveys examining ultrasonic measures of bone density have found a positive (25, 27, 45, 49) correlation or no correlation (41) between total protein intake and BMD at the phalanges or at the calcaneus. Only one survey (49) reported a negative correlation for total protein and BMD, which was at the calcaneus.

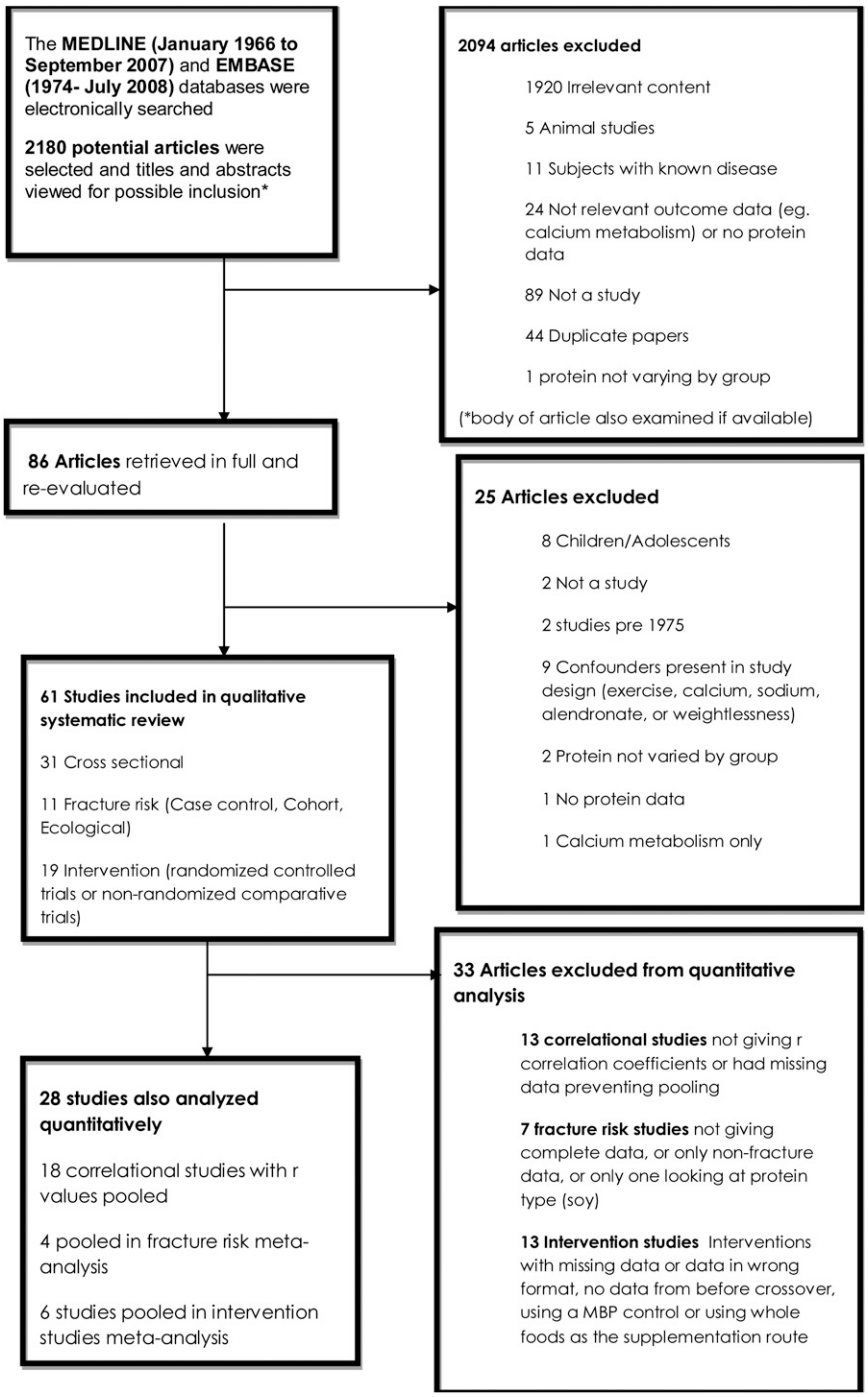


**FIGURE 1.** Quality of reporting of meta-analyses (QUOROM) statement flow diagram. MBP, milk basic protein.

*Bone markers*

The cross-sectional surveys provided little evidence of any influence of protein intake on bone markers. Thus, the 4 studies of markers of bone formation (31, 38, 39, 44) and 3 studies of markers of bone resorption (31, 39, 44) reported no significant correlation with protein intake, and only one study reported a significant (positive) correlation with markers of bone resorption (31).

*Pooled correlation coefficients*

Eighteen cross-sectional surveys gave *r* correlation coefficients suitable for pooling (40, 23–26, 28, 29, 31, 33, 35, 36, 38, 39, 43, 44, 46, 48, 50). These *r* correlation coefficients were pooled by population subgroup (**Table 3**) and then by outcome type (**Table 4**) as described in Tables 1 and 2. All pooled values for population subgroups and for outcome type were positive, except for ulna BMC, deoxypyridinoline, and hydroxyproline.

**TABLE 1**
Characteristics and outcomes of cross-sectional surveys and cohort studies of protein intake and bone health[1]

| Study and country | Mean protein intake | Method | Population | *n* | Outcome | Coefficient[2] | *P* |
|---|---|---|---|---|---|---|---|
| Chiu et al, 1997 (23) Taiwan | 1.09 g · kg$^{-1}$· d$^{-1}$ | DPA (BMD) | Older post F | 258 | LS BMD | 0.107 | 0.09 |
| | | | | | FN BMD | 0.085 | 0.18 |
| Cooper et al, 1996 (24) USA | 72 g/d | DPA/SPA (BMD) | Pre ($n$ = 72) and post ($n$ = 218) F | 290 | LS BMD (pre F) | 0.20 | NS |
| | | | | | Troch BMD (pre F) | 0.36 | <0.01 |
| | | | | | FN BMD (pre F) | 0.26 | <0.05 |
| | | | | | DR BMD (pre F) | 0.35 | <0.01 |
| | | | | | MR BMD (pre F) | 0.27 | <0.05 |
| | | | | | FS BMD (pre F) | 0.22 | NS |
| | | | | | LS BMD (post F) | 0.13 | NS |
| | | | | | Troch BMD (post F) | 0.20 | <0.01 |
| | | | | | FN BMD (post F) | 0.25 | <0.001 |
| | | | | | DR BMD (post F) | 0.19 | <0.01 |
| | | | | | MR BMD (post F) | 0.21 | <0.01 |
| | | | | | FS BMD (post F) | 0.24 | <0.001 |
| Devine et al, 2005 (25) Australia | 1.2 g · kg$^{-1}$ · d$^{-1}$ | DXA, QUS | Elderly F, mean age 75 ± 3 y, white | 1077 | Hip BMD | 0.138 | — |
| | | | | | BUA calc | 0.136 | — |
| Freudenheim et al, 1986 (26) USA, cross-sectional data | 1.02 g · kg$^{-1}$ · d$^{-1}$ | SPA | Pre and post F, 35–65 y, white | 99 (17 pre F, 67 post F) | R BMC (post F) | 0.087 | 0.484 |
| | | | | | R BMC (pre F) | 0.08 | 0.76 |
| | | | | | R BMD (post F) | −0.017 | 0.889 |
| | | | | | R BMD (pre F) | 0.384 | 0.128 |
| | | | | | U BMC (post F) | −0.041 | 0.743 |
| | | | | | U BMC (pre F) | 0.063 | 0.81 |
| | | | | | U BMD (post F) | 0.044 | 0.725 |
| | | | | | U BMD (pre F) | 0.282 | 0.272 |
| Gregg et al, 1999 (27) USA | 0.9 g · kg$^{-1}$ · d$^{-1}$ | QUS | Middle-aged F, mean age 45.5 y | 393 | BUA calc | 3.15 (univ assoc) | 0.0008 |
| | | | | | SOS calc | 0.96 (univ assoc) | 0.02 |
| | | | | | LS BMD | 0.015 (univ assoc) | 0.02 |
| | | | | | FN BMD | 0.010 (univ assoc) | 0.09 |
| Henderson et al, 1995 (28) Australia | 1 g · kg$^{-1}$ · d$^{-1}$ | DXA | Pre F, mean age 18 y | 115 | FN BMD | 0.22 | <0.05 |
| | | | | | Intertroch BMD | 0.19 | <0.05 |
| | | | | | Troch BMD | 0.27 | <0.005 |
| | | | | | DTB BMD | 0.05 | >0.05 |
| | | | | | TF BMD | 0.21 | <0.05 |
| | | | | | FS BMD | 0.09 | >0.05 |
| | | | | | LS BMD | 0.05 | >0.05 |
| Hirota et al, 1992 (29) Japan | 1.13 g · kg$^{-1}$ · d$^{-1}$ | SPA (BMD) | Young pre F, 19–25 y | 161 | FB MD | 0.2307 | 0.002 |
| Ho et al, 2003 (30) China, soy protein | 1.01 g · kg$^{-1}$ · d$^{-1}$ | DXA | <12 y post F, 48–62 y, Asian | 454 (269 <4 y post F, 185 >4 y post F) | Spine BMD (<4 y meno) | $r^2$ = 0.1 (−) | 0.705 |
| | | | | | FN BMD (<4 y meno) | $r^2$ = 0.03 (+) | 0.764 |
| | | | | | Troch BMD (<4 y meno) | $r^2$ = 0.002 (+) | 0.945 |
| | | | | | Intertroch BMD | $r^2$ = 0.1 (−) | 0.616 |
| | | | | | TH BMD (<4 y meno) | $r^2$ = 0.0002 (−) | 0.981 |
| | | | | | TB BMD (<4 y meno) | $r^2$ = 0.7 (−) | 0.160 |
| | | | | | TB BMC (<4 y meno) | $r^2$ = 0.4 (−) | 0.304 |

*(Continued)*

**TABLE 1** (*Continued*)

| Study and country | Mean protein intake | Method | Population | *n* | Outcome | Coefficient[2] | *P* |
|---|---|---|---|---|---|---|---|
| | | | | | Spine BMD (>4 y meno) | $r^2 = 1.0$ (+) | 0.172 |
| | | | | | FN BMD (>4 y meno) | $r^2 = 1.3$ (+) | 0.123 |
| | | | | | Troch BMD (>4 y meno) | $r^2 = 2.7$ (+) | 0.025 |
| | | | | | Intertroch BMD (>4 y meno) | $r^2 = 3.4$ (+) | 0.012 |
| | | | | | TH BMD (>4 y meno) | $r^2 = 3.3$ (+) | 0.013 |
| | | | | | TB BMD (>4 y meno) | $r^2 = 2.0$ (+) | 0.058 |
| | | | | | TB BMC (>4 y meno) | $r^2 = 2.8$ (+) | 0.024 |
| Horiuchi et al, 2000 (31) Japan | TP, 62.5 g/d; SP, 12.6 g/d | DXA | Post F, 52–83 y | 85 | LS BMD (soy) | 0.251 | 0.05 |
| | | | | | Osteocalcin (soy) | −0.097 | NS |
| | | | | | ALP (soy) | −0.017 | NS |
| | | | | | Pyridinoline (soy) | −0.132 | NS |
| | | | | | Deoxypyd (soy) | −0.229 | <0.05 |
| | | | | | Total protein (soy) | | |
| | | | | | LS BMD (soy) | 0.223 | <0.05 |
| | | | | | Pyridinoline | −0.229 | <0.05 |
| | | | | | Deoxypyd (soy) | −0.218 | NS |
| | | | | | Osteocalcin (soy) | −0.131 | NS |
| | | | | | ALP (soy) | −0.09 | NS |
| Ilich et al, 2003 (32) USA | 1.04 g · kg$^{-1}$ · d$^{-1}$ | DXA | Older F, >5 post, white, mean age 68.7 ± 7.1 y | 136 | TB BMD | $r^2 = 51.8$ (+) | — |
| | | | | | TB BMC | $r^2 = 73.4$ (+) | — |
| | | | | | W BMD | $r^2 = 47.0$ (+) | — |
| | | | | | H BMD | $r^2 = 51.6$ (+) | — |
| Jaime et al, 2006 (33) Brazil | 1.2 g · kg$^{-1}$ · d$^{-1}$ | DXA | Men >50 y | 277 | FN BMD (black) | 0.359 | 0.040 |
| | | | | | FN BMD (white) | 0.055 | 0.505 |
| Kyriazopoulos et al, 2006 (34) Greece | 0.35 g · kg$^{-1}$ · d$^{-1}$ | SPA | Men 18–30 y | 300 | DR BMC | 0.025 (correlation with number of protein meals in childhood) | 0.835 |
| | | | | | DR BMD | 0.065 (correlation with number of protein meals in childhood) | 0.583 |
| Lacey et al, 1991 (35) Japan | 1.35 g · kg$^{-1}$ · d$^{-1}$ | SPA | Asian pre F, 35–40 y; post F, 55–60 y | 178 (89 pre F, 89 post F) | MR BMC (pre F) | 0.22 | 0.08 |
| | | | | | MR BMC (post F) | 0.21 | 0.07 |
| Lau et al, 1998 (36) China | 0.65 g · kg$^{-1}$ · d$^{-1}$ | DXA | Pre and post F, 70–89 y | 76 | LS BMD | 0.09 | — |
| | | | | | FN BMD | 0.13 | — |
| | | | | | Intertroch BMD | 0.084 | — |
| | | | | | W BMD | 0.042 | — |
| Metz et al, 1993 (37) USA | 1.24 g · kg$^{-1}$ · d$^{-1}$ | SPA | Pre F white, 24–28 y | 38 | DR BMC | $r^2 = 0.123$ (−) | 0.019 |
| | | | | | DR BMD | $r^2 = 0.114$ (−) | 0.032 |
| | | | | | MR BMC | $r^2 = 0.153$ (−) | 0.009 |
| | | | | | MR BMD | $r^2 = 0.038$ (−) | 0.248 |

*(Continued)*

**TABLE 1** (*Continued*)

| Study and country | Mean protein intake | Method | Population | *n* | Outcome | Coefficient[2] | *P* |
|---|---|---|---|---|---|---|---|
| Michaelsson et al, 1995 (38) Sweden | 59 g/d | DXA (dietary records data used, not FFQ) | F, 28–74 y, white | 175 | TB BMD | 0.189 | 0.018 |
| | | | | | LS BMD | 0.058 | 0.474 |
| | | | | | FN BMD | 0.117 | 0.151 |
| | | | | | Osteocalcin | −0.036 | 0.669 |
| Nakamura et al, 2004 (39) Japan | 1.29 g · kg$^{-1}$ · d$^{-1}$ | Bone markers | Elderly post F, mean age 68.3 y | 43 | Osteocalcin | −0.197 | >0.05 |
| | | | | | Bone ALP | −0.039 | >0.05 |
| | | | | | Deoxypyd | −0.241 | >0.05 |
| | | | | | *N*-telopeptide | −0.205 | >0.05 |
| Orwoll et al, 1987 (40) USA | — | CT (vertebrae), SPA (radius) | Study 1: M | 62 | PR BMC (study 1) | 0.2 | NS |
| | | | | | DR BMC (study 1) | 0.03 | NS |
| | | | Study 2: M 30–90 y | 92 | Vertebral BMC (study 1) | 0.27 | <0.05 |
| | | | | | DR BMC (study 2) | 0.22 | NS |
| | | | | | PR BMC (study 2) | 0.15 | NS |
| | | | | | Vertebral BMC (study 2) | 0.30 | <0.01 |
| Pedrera et al, 2001 (41) Spain | 1.4–1.5 g · kg$^{-1}$ · d$^{-1}$ | Phalangeal Ad-SOS | 228 pre, peri, and post F (mean age 48.9 y) | 228 | Phalangeal Ad-SOS | — | NS |
| Promislow et al, 2002 (42) USA | 72.5 g/d | DXA | M/F 55–92 y; 572 F 388 M | 960 | TH BMD (F) | $\beta = 0.0143$ | 0.02 |
| | | | | | FN BMD (F) | $\beta = 0.0092$ | 0.07 |
| | | | | | TS BMD (F) | $\beta = 0.0150$ | 0.08 |
| | | | | | TB BMD (F) | $\beta = 0.0158$ | 0.002 |
| | | | | | TH BMD (M) | $\beta = 0.0057$ | 0.48 |
| | | | | | FN BMD (M) | $\beta = 0.0032$ | 0.66 |
| | | | | | TS BMD (M) | $\beta = 0.0057$ | 0.62 |
| | | | | | TB BMD (M) | $\beta = -0.0005$ | 0.94 |
| Quintas et al, 2003 (43) Spain | 1.4–1.7 g | DPA | Pre F | 74 | F BMC | 0.236 | <0.05 |
| | | | | | F BMD | 0.06968 | >0.05 |
| | | | | | LS BMC | 0.434 | <0.05 |
| | | | | | Hip BMC | 0.411 | <0.05 |
| | | | | | LS BMD | 0.317 | <0.05 |
| | | | | | Hip BMD | 0.301 | <0.05 |
| Rapuri et al, 2003 (44) USA | 53.7–71.2 g · kg$^{-1}$ · d$^{-1}$ | DXA | Post F, 65–77 y | 489 | MR BMD | 0.097 | 0.036 |
| | | | | | FN BMD | 0.092 | 0.047 |
| | | | | | Troch BMD | 0.155 | 0.001 |
| | | | | | TF BMD | 0.136 | 0.003 |
| | | | | | LS BMD | 0.065 | 0.163 |
| | | | | | TB BMD | 0.129 | 0.005 |
| | | | | | *N*-telopeptide | −0.022 | 0.641 |
| | | | | | Osteocalcin | 0.01 | 0.832 |
| Tanaka et al, 2001 (45) Japan | 1.3 g · kg$^{-1}$ · d$^{-1}$ | Ultrasonic bone absorptiometry | Pre F 18–22 y | 965 | OSI calcaneus | 0.234 (regression coefficient) | 0.009 |

(*Continued*)

**TABLE 1** (*Continued*)

| Study and country | Mean protein intake | Method | Population | *n* | Outcome | Coefficient[2] | *P* |
|---|---|---|---|---|---|---|---|
| Teegarden et al, 1998 (46) USA | 1.21 g · kg$^{-1}$ · d$^{-1}$ | DXA | Young pre F | 215 | TB BMD | 0.11 | NS |
| | | | | | R BMD | 0.16 | <0.05 |
| | | | | | LS BMD | 0.19 | <0.05 |
| | | | | | FN BMD | 0.08 | NS |
| | | | | | Troch BMD | 0.10 | NS |
| | | | | | W BMD | 0.08 | NS |
| | | | | | TB BMC | 0.12 | NS |
| | | | | | R BMC | 0.08 | NS |
| | | | | | Spine BMC | 0.23 | <0.05 |
| Tylavsky and Anderson, 1988 (47) USA | 1.01 g · kg$^{-1}$ · d$^{-1}$ | SPA | Elderly F, 60–98 y | 375 | DR BMC | $\beta = 2.72$ | 0.03 |
| | | | | | DR BMD | $\beta = 0.63$ | 0.25 |
| | | | | | MR BMC | $\beta = 2.96$ | 0.003 |
| | | | | | MR BMD | $\beta = 1.36$ | 0.06 |
| Wang et al, 1997 (48) USA | 0.97 g · kg$^{-1}$ · d$^{-1}$ | DXA | Older post F | 125 | LS BMD | 0.04 | NS |
| | | | | | FN BMD | −0.01 | NS |
| Weikert et al, 2005 (49) Germany | 67.9 g/d | QUS/BUA | F, 35–67 y | 8178 | TP (os calcis) | $\beta = -0.03$ | 0.017 |
| | | | | | AP (os calcis) | $\beta = -0.03$ | 0.010 |
| | | | | | VP (os calcis) | $\beta = 0.11$ | 0.007 |
| Whiting et al, 2002 (50) Canada | 1.15 g · kg$^{-1}$ · d$^{-1}$ | DXA (BMD) | M, 39–42 y | 57 | TB BMD | 0.383 | <0.01 |
| | | | | | LS BMD | 0.419 | <0.01 |
| | | | | | TH BMD | 0.322 | <0.05 |

[1] LS, lumbar spine; FN, femoral neck; Troch, trochanter; DR, distal radius; MR, midradius; FS, femoral shaft; calc, calcaneus; R, radius; U, ulna; intertroch, intertrochanter; TP, total protein; SP, soy protein; DTB, distal tibia/fibular; TF, total femur; F, forearm spine; TH, total hip; TB, total-body; ALP, alkaline phosphatase; deoxypyd, deoxypyridinoline; W, Wards area; peri F, perimenopausal women; H, hand; pre F, premenopausal women; post F, postmenopausal women; TS, total spine; PR, proximal radius; AP, animal protein; DXA, dual-energy X-ray absorptiometry; BMD, bone mineral density; BMC, bone mineral content; CT, computed tomography; meno, menopausal; SPA, single-photon absorptiometry; DPA, dual-photon absorptiometry; QUS, qualitative ultrasound; BUA, broadband ultrasound attenuation; Ad-SOS, amplitude-dependent speed of sound; OSI, Osteo-Sono Assessment Index; univ assoc, univariate association; FFQ, food-frequency questionnaire.

[2] Simple *r* coefficients unless otherwise stated; for $r^2$, the + or − in parentheses indicates whether the corresponding regression coefficient is positive or negative.

**TABLE 2**
Studies that provide *r* values or percentages of bone loss[1]

| Study and country | Mean protein intake | Population | Duration | Total *n* | Fracture/BMD site | Protein type | Percentage change, *r*, and/or *P* |
|---|---|---|---|---|---|---|---|
| Lukert et al, 1987 (51) USA, 4–5-y cohort study | 29–158 g/d | Peri F, elderly M and F | 4–5y | 114 | Bone density | — | — |
| Hannan et al, 2000 (52) USA | 1 g · kg$^{-1}$ · d$^{-1}$ | Elderly M and F, 68–91 y | 4 y | 615 | LS BMD | TP | 3.72% (Q1) |
| | | | | | LS BMD | AP | −3.79% (Q1) |
| | | | | | FN BMD | TP | −4.61% (Q1) |
| | | | | | FN BMD | AP | −3.95% (Q1) |
| | | | | | Troch BM | TP | −8% (Q1) |
| | | | | | Troch BM | AP | −2.57% (Q1) |
| | | | | | W BMD | TP | −7.05% (Q1) |
| | | | | | W BMD | AP | −4.02% (Q1) |
| | | | | | R BMD | TP | −4.21% (Q1) |
| | | | | | R BMD | AP | −4.6% (Q1) |
| Rapuri et al, 2003 (44) USA, longitudinal data | 53.7–71.2 g/d | Elderly women | 3 y | 489 | ALP | Total | −5.04% (Q1) |
| | | | | | *N*-telopeptide | Total | 10.4% (Q1) |
| | | | | | Osteocalcin | Total | −5.76% (Q1) |
| | | | | | Spine BMD | Total | −1.95% (Q1) |
| | | | | | TB BMD | Total | −2.63% (Q1) |
| | | | | | TF BMD | Total | −1.25% (Q1) |
| | | | | | Troch BM | Total | −1.92% (Q1) |
| | | | | | FN BMD | Total | 0.32% (Q1) |
| | | | | | MR BMD | Total | −3.32% (Q1) |
| Freudenheim et al, 1986 (26) USA, longitudinal data | 1.02 g · kg$^{-1}$ · d$^{-1}$ | Pre and post F, 35–65 y, white | 4 y | 99 | R BMD (pre F) | TP | $r = 0.384$ ($P > 0.05$) |
| | | | | | Hu BMD (pre F) | TP | $r = 0.157$ ($P > 0.05$) |
| | | | | | Ulna (pre F) | TP | $r = 0.282$ ($P > 0.05$) |
| | | | | | R BMD (post F) | TP | $r = -0.017$ ($P > 0.05$) |
| | | | | | Hu BMD (post F) | TP | $r = 0.138$ ($P > 0.05$) |
| | | | | | Ulna (post F) | TP | $r = 0.044$ ($P > 0.05$) |
| Geinoz et al, 1993 (53) Switzerland | 37.8–59.4 g/d | DXA | Elderly M and F, mean age 82 y (F) and 80 y (M) | 74 | F: FN BMD (>1 g protein · kg$^{-1}$ · d$^{-1}$) | 0.679 ± 0.09[2] | NS |
| | | | | | F: FS BMD (>1 g protein · kg$^{-1}$ · d$^{-1}$) | 1.288 ± 0.35 | NS |
| | | | | | F: Spine BMD (>1 g protein · kg$^{-1}$ · d$^{-1}$) | 0.935 ± 0.24 | NS |
| | | | | | F: FB BMD (≤1 g protein · kg$^{-1}$ · d$^{-1}$) | 0.574 ± 0.13 | $P < 0.05$ |
| | | | | | F: FS BMD (≤1 g protein · kg$^{-1}$ · d$^{-1}$) | 1.120 ± 0.33 | NS |
| | | | | | F: Spine BMD (≤1 g protein · kg$^{-1}$ · d$^{-1}$) | 0.877 ± 0.36 | NS |
| | | | | | M: FN BMD (>1 g protein · kg$^{-1}$ · d$^{-1}$) | 0.761 ± 0.12 | NS |
| | | | | | M: FS BMD (>1 g protein · kg$^{-1}$ · d$^{-1}$) | 1.516 ± 0.19 | NS |
| | | | | | M: Spine BMD (>1 g protein · kg$^{-1}$ · d$^{-1}$) | 1.094 ± 0.26 | NS |
| | | | | | M: FN BMD (≤1 g protein · kg$^{-1}$ · d$^{-1}$) | 0.643 ± 0.14 | $P < 0.05$ |
| | | | | | M: FS BMD (≤1 g protein · kg$^{-1}$ · d$^{-1}$) | 1.318 ± 0.34 | NS |
| | | | | | M: Spine BMD (≤1 g protein · kg$^{-1}$ · d$^{-1}$) | 0.847 ± 0.18 | $P < 0.05$ |

[1] LS, lumbar spine; FN, femoral neck; Troch, trochanter; MR, midradius; R, radius; TP, total protein; TF, total femur; TB, total-body; peri F, perimenopausal women; post F, postmenopausal women; pre F, premenopausal women; AP, animal protein; Hu, humerus; DXA, dual-energy X-ray absorptiometry; ALP, alkaline phosphatase; BMD, bone mineral density; Q, quartile; W, Wards area.
[2] Mean ± SD (all such values).

**TABLE 3**
Pooled *r* values for protein intake and bone health by sex and age subgroup[1]

| Sub group | Total *n* | Pooled *r* value (±95% CI) | $r^2$ | Percentage | Heterogeneity test $\chi^2$ | Heterogeneity test *P* | Studies |
|---|---|---|---|---|---|---|---|
| Men, BMD | 448 | 0.2 (0.11, 0.29) | 0.04 | 4 | 12.45, df = 4 | 0.0143 | Jaime (33), Whiting (50) |
| Men, BMC | 462 | 0.212 (0.122, 0.302) | 0.04 | 4 | 3.07, df = 5 | 0.6892 | Orwoll (40) |
| Post F, BMD | 6494 | 0.09 (0.07, 0.11) | 0.008 | 0.8 | 29.51, df = 23 | 0.1640 | Rapuri (44), Freudenheim (26), Cooper (24), Chiu (23), Lau (36), Wang (48), Devine (25), Horiuchi (31) |
| Post F, BMC | 357 | 0.11 (0.00, 0.22) | 0.01 | 1 | 2.72, df = 4 | 0.6057 | Freudenheim (26), Lacey (35) |
| Pre F, BMD | 3015 | 0.249 (0.209, 0.289) | 0.06 | 6 | 55.38, df = 26 | 0.0007 | Freudenheim (26), Cooper (24), Chiu (23), Lau (36), Wang (48), Devine (25), Horiuchi (31) |
| Pre F, BMC | 933 | 0.14 (0.11, 0.17) | 0.02 | 2 | 4.10, df = 8 | 0.8480 | Freudenheim (26), Teegarden (46), Cooper (24), Hirota (29), Quintas (43), Henderson (28) |

[1] Pre F, premenopausal women; post F, postmenopausal women; BMC, bone mineral content; BMD, bone mineral density.

They were also all significant, except for BMC and BMD at the ulna and humerus and for osteocalcin and hydroxyproline. Overall, heterogeneity was low, with only 3 of 20 pooled estimates showing significant heterogeneity, which were the men (BMD), premenopausal women (BMD), and radius (BMD) estimates.

## Fracture risk

As can be seen in **Tables 5**, **6**, and **7**, the 11 studies examining fracture risk were all conducted in the United States, China, or Europe. All studies assessed hip fractures, except for one that also assessed forearm fractures (54) and one that examined all fractures (60). Seven were cohort studies (54–60) 2 were case-control (63, 64) studies, and 2 were ecologic studies (61, 62). The studies were mainly of women (5 postmenopausal and 3 pre- and postmenopausal); 4 studies were conducted in both men and women and 1 was in men alone. The 2 ecologic studies reported the relation between protein intake and fracture risk in 16 of 33 countries worldwide. Overall, these fracture studies examined total protein in 6 studies (54, 56, 57, 62–64), animal protein in 8 studies (54–56, 58, 59, 61, 62, 64), vegetable protein in 6 studies (54, 56, 58, 59, 62, 64), and soy protein in 1 study (60).

### *Study quality*

Nearly all studies clearly stated that they had adjusted for relevant confounders such as age, sex, weight, BMI, physical activity, menopausal status, smoking, use of hormones or medications, alcohol, and calcium intake. One of the ecologic studies reported adjustment for age (61). For 2 studies it was not clear whether adjustments had been made (51, 62). Overall, the cohort studies may have been less affected by confounders than the studies looking at BMD, BMC, and bone markers.

In 3 studies, fracture incidence was examined by self report from the participants followed up by confirmation from medical records or medical practitioner (55, 56, 59). Three studies relied on self report alone (54, 60, 64), and one study looked at medical records alone (57). The 2 case-control studies (63, 64), as would be expected, chose cases from medical records and lists of hospital patients who had had a confirmed fracture. The 2 ecologic studies (61, 62) used published survey data of reported incident fractures.

In terms of dropout rates, this was not clear for 4 studies, but was reported in 5 studies and varied from 1.3% to 28%. The final 2 studies were of case-control design, so this was less applicable. In terms of dietary-assessment methods, 8 studies (54–56, 58–60, 63, 64) used FFQ and 3 studies used national survey data (57, 61, 62).

### *Hip fracture*

Overall, the cohort studies indicated either a benefit or no effect of protein intake on hip fracture relative risk, with only one study reporting a significant increase in risk with increasing animal protein intake and increasing animal to vegetable protein ratio (59). Three studies found a decreased relative risk of hip fracture with increasing animal (56), total (56), and vegetable (59) protein intakes. Two studies found no significant association of animal protein with fracture risk (54, 55), whereas 2 studies found no association of total protein with fracture risk (54, 57). Last, 2 studies found no relation between fracture risk and vegetable protein (54, 56).

In contrast, the 2 ecologic studies (61, 62) found a positive correlation between animal protein intake and hip fracture risk and a negative association of increasing vegetable protein (62). Of the 2 case control studies, 1 reported no significant relation between protein intake and risk of hip fracture (63), but the other reported a beneficial association with a significant substantial reduction in hip fracture in 50–69-y-olds (OR = 0.35, highest compared with lowest quartile of total protein intake; 64). However, in this study, the beneficial influence of animal or vegetable protein alone (OR = 0.43 and 0.52) did not achieve significance for 50–69-y-olds, and there was no identifiable influence of any type of protein in the older 70–89-y-old groups (64).

### *Forearm fracture*

The more limited literature on forearm fracture indicates mixed results, with one study indicating a significant increase in

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**TABLE 4**
Pooled *r* values for protein intake and bone health by outcome[1]

| Bone site | Total *n* | Pooled *r* value (±95% CI) | $r^2$ | Percentage | Heterogeneity $\chi^2$ | Heterogeneity *P* | Studies |
|---|---|---|---|---|---|---|---|
| Radius BMC (all areas) | 859 | 0.150 (0.08, 0.22) | 0.02 | 2 | 4.24, df = 9 | 0.8949 | Freudenheim (26), Orwoll (40), Lacey (35), Quintas (43) |
| Radius BMD (all areas) | 1584 | 0.124 (0.07, 0.17) | 0.02 | 2 | 19.29, df = 9 | 0.0228 | Rapuri (44), Freudenheim (26), Teegarden (46), Cooper (24), Hirota (29), Quintas (43), Cooper (24) |
| Ulna BMC | 84 | −0.022 (−0.24, 0.20) | −0.0005 | <0.05 | 0.12, df = 1 | 0.7290 | Freudenheim (26) |
| Ulna BMD | 84 | 0.088 (−0.13, 0.31) | 0.007 | 0.7 | 0.69, df = 1 | 0.4062 | Freudenheim (26) |
| Hip BMD | 4771 | 0.117 (0.09, 0.15) | 0.01 | 1 | 32.41, df = 22 | 0.0707 | Rapuri (44), Jaime (33), Chiu (23), Cooper (24), Lau (36), Wang (48), Henderson (28), Teegarden (46), Michaelsson (38), Whiting (50), Devine (25), Quintas (43) |
| Femur, tibia, and fibula BMD | 1085 | 0.108 (0.05, 0.17) | 0.01 | 1 | 4.23, df = 5 | 0.5168 | Rapuri (44), Henderson (28), Cooper (24) |
| Humerus BMC | 84 | 0.159 (−0.1, 0.38) | 0.03 | 3 | 0.24, df = 1 | 0.6242 | Freudenheim (26) |
| Humerus BMD | 84 | 0.141 (−0.08, 0.36) | 0.02 | 2 | 0.004, df = 1 | 0.9496 | Freudenheim (26) |
| Lumbar spine BMD | 1933 | 0.143 (0.10, 0.20) | 0.02 | 2 | 17.73, df = 11 | 0.0597 | Rapuri (44), Horiuchi (31), Henderson (28), Quintas (43), Michaelsson (38) Whiting (50), Chiu (23), Cooper (24), Lau (36), Wang (48), Teegarden (46) |
| Lumbar spine BMC | 443 | 0.285 (0.20, 0.38) | 0.08 | 8 | 2.868, df = 3 | 0.4124 | Quintas (43), Teegarden (46), Orwoll (40) |
| Total-body BMD | 911 | 0.152 (0.08, 0.22) | 0.02 | 2 | 4.271, df = 3 | 0.2336 | Rapuri (44), Whiting (50), Teegarden (46), Michaelsson (38) |
| Osteocalcin | 1043 | 0.005 (−0.06, 0.07) | 0.00003 | <0.003 | 6.61, df = 5 | 0.2513 | Rapuri (44), Nakamura (39), Michaelsson (38), Cooper (24), Horiuchi (31) |
| Deoxypyridinoline | 128 | −0.226 (−0.41, −0.05) | 0.05 | 5 | 0.02, df = 1 | 0.8875 | Nakamura (39), Horiuchi (31) |
| Hydroxyproline | 290 | −0.07 (−0.19, 0.05) | 0.005 | 0.5 | 3.12, df = 1 | 0.0773 | Cooper (24) |

[1] BMC, bone mineral content; BMD, bone mineral density.

risk with increasing total and animal protein intakes, but no effect of vegetable protein (54) and one study (58) indicated a reduced risk of wrist fracture with increasing consumption of meat products. Finally for all fractures, one study (60) reported a significant reduction in risk with increased soy protein intake.

*Fracture risk meta-analysis*

Studies excluded from the quantitative meta-analysis of fracture risk ($n = 6$) included the ecologic studies (results in the wrong format), 2 case control studies (ORs not relative risks), 1 study with missing data, 1 study based on food-frequency measurements, and 1 study in which risk estimates were for combined fractures. This left 4 suitable studies (54–57) which were pooled in a meta-analysis. Heterogeneity was moderate to low for total ($I^2 = 22.0\%$) animal ($I^2 = 48.3\%$), and vegetable ($I^2 = 2.0\%$) proteins, respectively. Therefore, all relative risk (RR) estimates were pooled by using random-effects models and are denoted by the notation as $RR_{(random)}$.

As shown in **Figure 2**, no significant effect was found for all protein on the RR of fractures in the highest compared with the lowest quintile/quartile of protein intake for total protein, $RR_{(random)} = 0.75$ (0.47–1.21, $P = 0.24$) animal protein, $RR_{(random)} = 0.83$ (0.54–1.30, $P = 0.42$) or vegetable protein: $RR_{(random)} = 1.21$ (0.82–1.79, $P = 0.34$).

## Supplementation trials

*Study characteristics*

The 19 supplementation trials (**Table 8**) were randomized controlled trials or nonrandomized comparative trials. They included 2 studies of perimenopausal women, 5 studies of premenopausal women, and 6 of postmenopausal women. Four studies examined men and women, and 2 examined men alone. Fourteen studies were from Western countries, (United States, the Netherlands, and Switzerland), and 5 studies were from Japan. Seven studies used soy protein for the intervention, 5 used MBP (all Japanese studies), 1 study compared high- with

**TABLE 5**
Characteristics of the cohort studies that assessed fracture risk[1]

| Study and country | Mean protein intake | Population and age | Length | Total *n* | Fracture/BMD site | Protein type | RR[2] | 95% CI | *P* |
|---|---|---|---|---|---|---|---|---|---|
| Feskanich et al, 1996 (54) USA | 79.6 g/d (median) | White F, 35–59 y | 12 y | 85,900 | FF | AP | 1.25 | 1.07, 1.46 | 0.004 |
| | | | | | | TP | 1.22 | 1.04, 1.43 | 0.01 |
| | | | | | | VP | 0.9 | 0.77, 1.06 | 0.17 |
| | | | | | HF | AP | 0.98 | 0.65, 1.47 | 0.7 |
| | | | | | | TP | 0.96 | 0.64, 1.45 | 0.7 |
| | | | | | | VP | 1.11 | 0.75, 1.66 | 0.58 |
| Meyer et al, 1997 (55) Norway | 0.8 g/d | M and F, mean age 47.1 y | 11.4 y | 19,752 F | HF (F) | AP | 0.96 | 0.62, 1.49 | 0.37 |
| | | | | 20,035 M | HF (M) | AP | 1.3 | 0.63, 2.68 | 0.48 |
| Munger et al, 1999 (56) USA | 1.2 g/d | Post F, 55–69 y | 1–3 y | 32,050 | HF | AP | 0.31 | 0.10, 0.93 | 0.037 |
| | | | | | | TP | 0.44 | 0.16, 1.22 | 0.049 |
| | | | | | | VP | 1.92 | 0.72, 5.11 | 0.11 |
| Mussolino et al, 1998 (57) USA | <56 to >98 g/d | White M, 45–74 y | 22 y | 2879 | HF | TP | 0.55 | 0.20, 1.55 | — |
| Thorpe et al, 2007 (58) USA | — | Peri and post F | 25 y | 1865 | Hazard ratio wrist fracture | Meat >4 times/wk | 0.44 | 0.23, 0.84 | 0.02 |
| | | | | | | VP > 1/d | 0.79 | 0.43, 1.46 | 0.31 |
| Sellmeyer et al, 2001 (59) USA | 49.8 g/d | White F, >65 y | 7 y | 1035 | Hip fracture | VP | 0.3 | — | 0.03 |
| | | | | | | Ratio AP:VP | 3.7 | — | 0.04 |
| | | | | | | AP | 2.7 | — | 0.04 |
| Zhang et al, 2005 (60) | 3.3–18.5 g/d | Post F, 40–70 y | — | 24,403 | All fractures | SP | 0.63 | 0.53, 0.76 | <0.001 |

[1] RR, relative risk; TP, total protein; AP, animal protein; VP, vegetable protein; SP, soy protein; BMD, bone mineral density; FF, forearm fracture; HF, hip fracture; peri F, perimenopausal women; post F, postmenopausal women.
[2] Highest quartile/quintile of intake; lowest quartile = 1.

low-vegetable protein, and 1 compared high- with low-animal (meat) protein. The other 5 studies examined total protein (3 compared high- with low-protein diets, and 2 compared supplement with placebo). Outcome indicators were either bone turnover markers or BMD and BMC.

### *Study quality*

There were 12 randomized placebo-controlled trials (15, 65–68, 70, 75, 76, 78, 80–82). The quality of these trials was assessed by using CONSORT. All of these studies, except 3 (67, 70, 78), were clearly stated as double blinded. In only 5 studies (67, 68, 70, 76, 82) were the background protein intakes clearly stated, and these varied from an average of 45 to 112 g/d. Only 4 of the 12 studies (67, 76, 80, 82) stated they had used a random number generation method, with only 1 giving details of stratification of subjects during this process (80). Only one study assessed participants' beliefs about their allocation (65), but most did attempt to mask the flavor of the supplements or attempted to ensure that they were as identical as possible. However, it was unclear in many studies how successful this masking was. Only one study (76) stated that the allocation to treatment was undertaken by persons not involved in the investigation.

Trials of other design were quality assessed separately. Two trials were crossovers (73, 77), whereas one study switched all participants from higher to lower intakes (72), and one was a before and after supplementation comparison in the same participants (79). Last, in 3 studies, participants were allocated to high-, medium-, or low-protein diets (74); to high- or low-protein diets (71); or to soyfoods or control foods (69). Only

**TABLE 6**
Characteristics of the ecologic studies that assessed fracture risk[1]

| Study and country | Mean protein intake | Method | Population and age | *n* | Outcome | Coefficient[2] | *P* |
|---|---|---|---|---|---|---|---|
| Abelow et al, 1992 (61) USA | 10.4–77.8 g/d (AP) | Fracture | F, >50 y | 34 studies, 16 countries | Hip fracture and AP | $r^2 = 0.66$ (+), by study | <0.001 |
| | | | | | | $r^2 = 0.67$ (+), by country | <0.001 |
| Frassetto et al, 2000 (62) USA, cross-cultural | 48–110.9 g/d | Fracture | F, >50 y | 33 countries | TP | $r = 0.67$ | <0.001 |
| | | | | | AP | $r = 0.82$ | <0.001 |
| | | | | | VP | $r = -0.370$ | <0.04 |

[1] TP, total protein; AP, animal protein; VP, vegetable protein.
[2] For $r^2$, the + or − in parentheses indicates whether the corresponding regression coefficient is positive or negative.

**TABLE 7**
Characteristics of the case-control studies that assessed fracture risk[1]

| Study and country | Protein intake | Population and age | *n* | | Group/outcome | OR[2] | *P* |
|---|---|---|---|---|---|---|---|
| Nieves et al, 1992 (63) USA | <24 to >55 g/d | F, 50–103 y | 329 (161 cases, 168 controls) | Hip (OR) | Hip fracture | 1.04 | — |
| Wengreen et al, 2004 (64) USA | 1.2 g · kg$^{-1}$ · d$^{-1}$ | M and F, 50–89 y | 2501 (1157 cases, 1334 controls) | Hip (OR) | 50–69 y (TP) | 0.35 | <0.001 |
| | | | | | 70–89 y (TP) | 1.28 | 0.06 |
| | | | | | 50–69 y (AP) | 0.43 | 0.21 |
| | | | | | 70–89 y (AP) | 1.54 | 0.95 |
| | | | | | 50–69 y (VP) | 0.52 | 0.19 |
| | | | | | 70–89 y (VP) | 0.79 | 0.46 |

[1] TP, total protein; AP, animal protein; VP, vegetable protein; OR, odds ratio.
[2] Highest quartile/quintile of intake; lowest quartile = 1.

one study (69) stated that allocation to treatment was undertaken by persons not involved in the investigation.

### *Bone markers*

Overall, the bone-markers intervention studies indicated mixed results. Of the bone-resorption markers, hydroxyproline excretion was significantly reduced with a low-meat diet in one study (77), but *N*-telopeptide excretion was significantly increased (with increased vegetable protein intake) in another study (73), and there was no significant effect of protein supplementation for at least one marker in 2 studies: *N*-telopeptide (77) and deoxypyridinoline (75).

For the markers of bone formation, there was no effect of increased animal protein intake (77) or increased total protein intake (74) on osteocalcin. Also, for bone alkaline phosphatase there was no significant reduction when overall protein intake was increased (74), although no significant effect was found on this bone marker with increased vegetable protein (73) or soy protein (75) in other studies.

### *BMD and BMC*

In terms of BMD, protein supplementation reduced bone loss in 2 studies of older people (78, 82). However, there was no effect of soy on BMD in 3 studies (15, 69, 76), although soy reduced BMD loss in one study compared with control (65). A benefit of MBP on BMD was found in 4 studies (66, 67, 80, 81). Three of the soy protein supplementation studies also measured BMC, and no effect was found (15, 65, 69).

### *Meta-analysis of supplementation trials*

Thirteen of the intervention trials (66, 68–75, 77, 79, 81, 82) were not suitable for meta-analysis because of incomplete data, the data were in the wrong format, the study was not randomized, the study was not placebo-controlled, or the study was not a dietary intervention or had MBP as a control. There

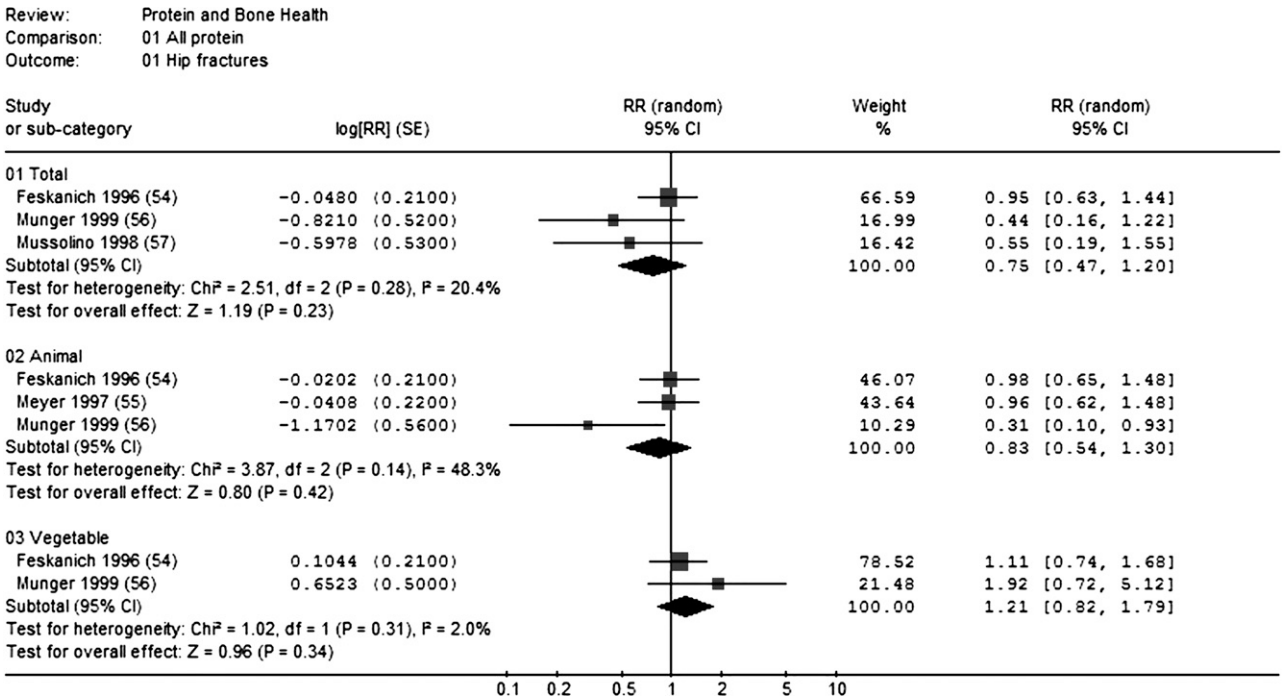


**FIGURE 2.** The effect of protein intake on hip fractures. Random-effects pooled relative risk (RR) analysis was used to compare highest with lowest quintile/quartile of protein intake.

**TABLE 8**
Characteristics of the supplementation trials[1]

| Study and country | Duration | Supplementation vs control | Total *n* and/or age | Outcomes measured | Intervention | | Placebo | | *P* |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | *n* | Mean ± SD | *n* | Mean ± SD | |
| Alekel et al, 2000 (65) USA | 24 wk | Soy (80.4 mg/d) vs whey | 2002 Peri F | LS BMC | 24 | 52.96 ± 8.72 | 21 | 56.57 ± 9.74 | NS |
| | | | F | LS BMD | 24 | 0.933 ± 0.12 | 21 | 0.989 ± 0.132 | NS |
| | | | | BSAP | 24 | 15.05 ± 5.11 | 21 | 12.51 ± 4.3 | — |
| Aoe et al, 2001 (66) Japan | 6 mo | MBP (40 mg/d) vs placebo | Pre F | % Change in calc BMD | 17 | 3.42 ± 2.05 | 16 | 2.01 ± 1.75 | 0.04 |
| Aoe et al, 2005 (67) Japan | 6 mo | 40 mg/d MBP vs inactive placebo | 27 Peri F | *N*-telopeptide | 14 | 47.3 ± 8.3 | 13 | 58.7 ± 8.3 | NS |
| | | | | Osteoalcin | 14 | 5.73 ± 0.59 | 13 | 5.82 ± 0.59 | NS |
| | | | | LS BMD | 14 | 1.11 ± 0.03 | 13 | 1.09 ± 0.03 | <0.05 |
| Arjmandi et al, 2003 (68) USA | 3 mo | Soy protein (40 g/d) vs MBP | 42 Post F | BSAP | 20 | 0.41 ± 0.14 | 22 | 0.35 ± 0.15 | — |
| | | | | Deoxypyd | 20 | 7.19 ± 3.31 | 22 | 6.79 ± 3.24 | — |
| Arjmandi et al, 2005 (69) USA | 1 y | Soy protein (25 g/d) in soyfoods vs nonsoyfoods | 62 Post F, <60 y | Osteocalcin | 35 | 18.1 ± 11.83 | 27 | 16.2 ± 11.43 | 0.365 |
| | | | | BSAP | 35 | 24.9 ± 6.51 | 27 | 25.3 ± 6.24 | 0.796 |
| | | | | Deoxypyd | 35 | 5.1 ± 1.78 | 27 | 4.9 ± 2.08 | 0.888 |
| | | | | LS BMD | 35 | 0.93 ± 0.13 | 27 | 0.93 ± 0.14 | 0.958 |
| | | | | W BMC | 35 | 2003 ± 289.9 | 27 | 1994 ± 301.3 | 0.944 |
| | | | | TH BMD | 35 | 0.852 ± 0.11 | 27 | 0.87 ± 7 | 0.512 |
| | | | | TH BMC | 35 | 57.18 ± 10.31 | 27 | 57.14 ± 0.109 | 0.967 |
| | | | | LS BMC | 35 | 54.15 ± 10.74 | 27 | 52.806 ± 10.7 | 0.662 |
| | | | | W BMD | 35 | 1.036 ± 0.11 | 27 | 1.036 ± 11.1 | 0.986 |
| Dalais et al, 2003 (70) Australia | 3 mo | Soy protein (40 g/d) vs casein placebo | 106 Post F, 50–75 y | Pyridinoline | 38 | 70 ± 24.97 | 40 | 72.72 ± 21.31 | NS |
| | | | | Deoxypyd | 38 | 14.48 ± 8.15 | 40 | 14.19 ± 6.58 | NS |
| Dawson-Hughes et al, 2004 (71) USA | 63 d | High (0.75 g · kg$^{-1}$ · d$^{-1}$) vs low (0.04 g · kg$^{-1}$ · d$^{-1}$) protein | 32 Elderly M and F | *N*-telopeptide | 16 | 102.3 ± 34.5 (high protein) | 16 | 170 ± 118.4 (low protein) | 0.038 |
| | | | | Osteocalcin | 16 | 3.4 ± 0.9 (high protein) | 16 | 3.2 ± 1.5 (low protein) | 0.795 |
| Ince et al, 2004 (72) USA | 2wk | High (1.1 g · kg$^{-1}$ · d$^{-1}$) vs low (0.8 g · kg$^{-1}$ · d$^{-1}$) protein | 39 Pre F, 22–39 y | *N*-telopeptide | 39 | 442 ± 124.9 (high protein) | 39 | 360 ± 99.9 (low protein) | <0.001 |
| | | | | Osteocalcin | 39 | 15.8 ± 8.74 (high protein) | 39 | 13.4 ± 8.1 (low protein) | 0.166 |
| Jenkins et al, 2003 (73) USA | 2 mo | Vegetable (27% protein) vs control (16% protein) | 20 Middle-aged M and F | *N*-telopeptide | 20 | 584 ± 340 | 20 | 461 ± 259 | — |
| | | | | BSAP | 20 | 20 ± 4.5 | 20 | 19 ± 4.5 | — |
| Kerstetter et al, 1999 (74) USA | 4 d | High (2.1 g · kg$^{-1}$ · d$^{-1}$) vs low (0.7 g · kg$^{-1}$ · d$^{-1}$) protein | 16 Pre F, 20–40 y | Osteocalcin | — | 5.7 ± 0.8[2] | — | 7.6 ± 1.4 | NS |
| | | | | BSAP | — | 57.2 ± 7.8[2] | — | 69.4 ± 8.8 | NS |
| | | | | *N*-telopeptide | — | 48.2 ± 7.2[2] | — | 32.7 ± 5.3 | <0.05 |
| Khalil et al, 2002 (75) USA | 3 mo | Soy (40 g/d) vs milk protein | 64 M, 59.2 ± 17.6 y | BSAP | 24 | — | 22 | — | NS |
| | | | | Deoxypyd | 24 | — | 22 | — | NS |

*(Continued)*

**TABLE 8** (*Continued*)

| Study and country | Duration | Supplementation vs control | Total *n* and/or age | Outcomes measured | Intervention | | Placebo | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | *n* | Mean ± SD | *n* | Mean ± SD | *P* |
| Kreijkamp-Kaspers et al, 2004 (76) Netherlands | 1 y | Soy protein (25.6 g/d) vs total milk protein | 202 Elderly F, 60–75 y | FN BMD | 88 | 0.718 ± 0.01 | 87 | 0.691 ± 0.108 | 0.89 |
| | | | | Hip BMD | 88 | 0.86 ± 0.11 | 87 | 0.83 ± 0.12 | 0.27 |
| | | | | Intertroch BMD | 88 | 1.01 ± 0.14 | 87 | 0.969 ± 0.149 | 0.02 |
| | | | | Troch BMD | 88 | 0.666 ± 0.10 | 87 | 0.638 ± 0.097 | 0.28 |
| | | | | W BMD | 88 | 0.549 ± 0.13 | 87 | 0.527 ± 0.126 | 0.33 |
| | | | | LS BMD | 88 | 0.92 ± 0.160 | 87 | 0.89 ± 0.17 | 0.79 |
| | | | | BSAP | 88 | 12.1 ± 4.4 | 87 | 12.4 ± 3.7 | 0.55 |
| Potter et al, 1998 (15) USA | 6 mo | Soy protein (40 g/d) vs milk protein | 66 Post F | LS BMD | 22 | 0.969 ± 0.143 | 22 | 0.934 ± 0.153 | NS |
| | | | | LS BMC | 22 | 55.1 ± 11.7 | 22 | 55.4 ± 10.3 | NS |
| Roughead et al, 2003 (77) USA | 8 wk | High-meat (20% of energy) vs low-meat (12% of energy) diet | 15 Post F | Hydroxy | 15 | 71.5 (high meat) | 15 | 64.5 (low meat) | 0.001 |
| | | | | Osteocalcin | 15 | 4.01 (high meat) | 15 | 3.94 (low meat) | NS |
| | | | | *N*-telopeptide | 15 | 3.79 (high meat) | 15 | 3.83 (low meat) | NS |
| | | | | BSAP | 15 | 18.1 (high meat) | 15 | 18.3 (low meat) | NS |
| Tkatch et al, 1992 (78) Switzerland | 38 d | Protein (20.4 g/d) vs no protein supplement[3] | 62 Elderly M and F, mean age 82 y | Change in FN BMD | 25 | 0.569 ± 0.105 | 23 | 0.579 ± 0.12 | — |
| | | | | Change in FS BMD | 24 | 0.24 ± 0.049 | 22 | 1.257 ± 0.3 | |
| | | | | Change in LS BMD | 25 | 0.88 ± 0.18 | 23 | 0.81 ± 0.17 | |
| | | | | Change in osteocalcin | 24 | 6.94 ± 2.45 | 18 | 4.96 ± 2.93 | |
| Toba et al, 2001 (79) Japan | 16 d | MBP (30 mg/d) vs inactive placebo | 30 M, 36.2 ± 8.5 y | *N*-telopeptide | 30 | 26.8 ± 9.6 | 30 | 31.5 ± 10.2 | <0.001 |
| | | | | Osteocalcin | 30 | 5.4 ± 1.8 | 30 | 3.7 ± 1.8 | <0.001 |
| Uenishi et al, 2007 (80) Japan | 6 mo | MBP (40 mg/d) vs inactive placebo | 35 Pre F | LS BMD | 17 | 1.16 ± 0.14 | 18 | 1.13 ± 0.16 | — |
| Yamamura et al, 2002 (81) Japan | — | MBP (40 mg/d) vs inactive placebo | 33 Pre F | R BMD | 17 | — | 16 | — | — |

*(Continued)*

**TABLE 8** (*Continued*)

| Study and country | Duration | Supplementation vs control | Total *n* and/or age | Outcomes measured | Intervention | | Placebo | | *P* |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | *n* | Mean ± SD | *n* | Mean ± SD | |
| Schurch et al, 1998 (82) Switzerland, 6 mo | | Total protein (20 g/d) vs placebo | 82 Elderly M and F, 80.7 ± 7.4 y | % Change in deoxypyd | — | −9.2[4] | — | 1.4[4] | >0.2 |
| | | | | % Change in FS BMD | — | −1.61[4] | — | −1.23[4] | >0.2 |
| | | | | % Change in LS BMD | — | −3.05[4] | — | −6.11[4] | >0.2 |
| | | | | % Change in osteocalcin | — | 7.9[4] | — | 6.9[4] | >0.2 |
| | | | | % Change in PF BMD | — | −2.95[4] | — | −3.37[4] | >0.2 |
| | | | | % Change in pyridinoline | — | 6.6[4] | — | 17[4] | >0.2 |
| | | | | % Change in Troch BMD | — | −3.02[4] | — | −3.65[4] | >0.2 |
| | | | | % Change in W BMD | — | −3.77[4] | — | −3.1[4] | >0.2 |
| | | | | % Change in AntLS BM | — | −0.48[4] | — | −0.82[4] | >0.2 |

[1] LS, lumbar spine; AntLS, anterior LS; FN, femoral neck; Troch, trochanter; FS, femoral shaft; calc, calcaneus; intertroch, intertrochanter; TH, total hip; BSAP, bone-specific alkaline phosphatase; deoxypyd, deoxypyridinoline; peri F, perimenopausal women; pre F, premenopausal women; post F, postmenopausal women; R, radius; PF, proximal femur; BMD, bone mineral density; BMC, bone mineral content; MBP, milk basic protein; W, Wards area.

[2] Mean ± SEM.

[3] Experimental supplement = 20.4 g protein/d with calcium (0.525 g), 750 IU vitamin A, and 25 IU vitamin D3. Control = same supplement without protein.

[4] Values are means.

were only enough studies for all protein types to pool lumbar spine BMD as an outcome; therefore, one study was excluded because it did not give lumbar spine as an outcome measure (70). The remaining 6 studies (15, 65, 67, 76, 78, 80) were pooled in the meta-analyses. Here, random- and fixed-effects models are denoted by the subscript (random) or (fixed) as appropriate.

For all protein (total protein and MBP), a statistically significant effect of protein supplementation on lumbar spine BMD was observed [weighted mean difference $(WMD)_{(fixed)} = 0.02$; 95% CI: 0.00, 0.04; $P = 0.04$; **Figure 3**]. For soy protein studies (**Figure 4**), no statistically significant effect was found for lumbar spine BMD ($WMD_{(random)} = 0.01$ (95% CI: −0.05, 0.06; $P = 0.85$). For MBP studies alone (**Figure 5**), no statistically significant effect was found for all lumbar spine BMD ($WMD_{(fixed)} = 0.02$; 95% CI: 0.00, 0.04; $P = 0.07$). Overall heterogeneity was low for the influence of protein on BMD at $I^2 = 0\%$. The effects of soy protein and MBP were of high and low heterogeneity: $I^2 = 54.1\%$ and $I^2 = 0.0\%$, respectively.

## DISCUSSION

The relation between dietary protein intakes and bone health has a contentious history, with much research examining a wide range of indirect and direct measures of bone health. Our analysis was limited to direct measures in terms of BMD and BMC and fracture rates as well as markers of bone turnover. This literature is restricted because it does not examine calcium metabolism. However, it shows that, in contrast with the adverse relation between protein intake and fracture risk shown between populations (in ecologic studies), there is little support for an apparently adverse relation within populations. Indeed, only a small minority of studies reviewed here reported an adverse influence of dietary protein. Also, no cross-sectional survey or cohort studies showed any adverse association of protein with BMD, and only one showed an adverse association with BMC. Several cross-sectional surveys and cohort studies indicated a beneficial association between protein intakes and BMD and BMC.

Indeed, there were positive pooled correlations for the relation between total protein intake and BMD and BMC for nearly all bone sites, and nearly all correlations were significant. This relation was consistent among all population subgroups studied. The proportion of BMD attributable to protein intake indicated in the cross-sectional surveys of this review was 1–2%. This shows a beneficial association of protein with bone health, albeit the effect size was very small. Most importantly, the small but significant positive effect of protein supplementation on lumbar spine BMD suggested by the meta-analysis results gives support to a causal beneficial influence of protein.

However, within the studies identified here, the translation of this potential benefit into a reduced risk of hip fractures in cohort studies in the meta-analysis was not found. This was the case even though multivariate-adjusted analyses were used, which minimized the likely influence of confounding. Thus, there was no clear relation between fracture risk and dietary protein in the qualitative review or in the meta-analysis. This included the analyses looking at vegetable or animal protein separately. Unfortunately there were no intervention studies available here with complete data to further investigate the effect of animal and vegetable protein supplementation on fracture risk or BMD.

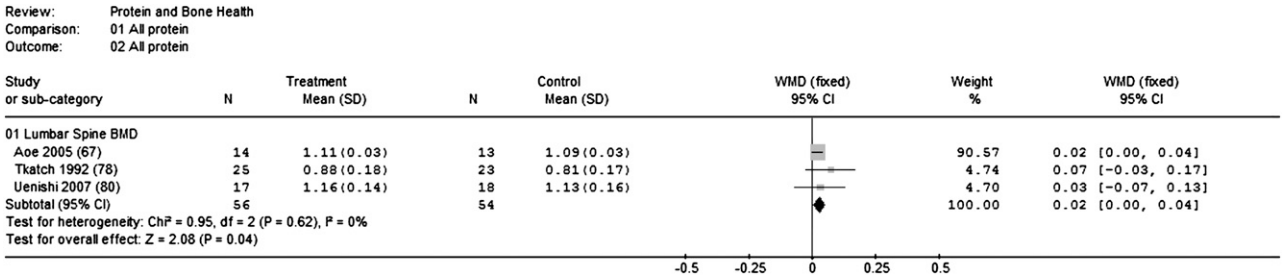


**FIGURE 3.** The effect of protein supplementation on lumbar spine bone mineral density (BMD). WMD, weighted mean difference.

In the absence of long-term intervention studies, the issue of whether protein intake does influence fracture risk must remain an open question. Fracture risk is indeed the most important clinical outcome, and it is disappointing that a significant association of protein and fracture risk was not found here. Indeed, the lack of an association with fracture risk may weaken the theory that protein intake influences bone health.

Still, taken together, our analysis suggests that the strongly debated cross-cultural direct relation between animal protein intake and fracture risk is highly unlikely to represent a causal influence. Indeed, as pointed out many years ago (83), the relation is the same as the positive relation between cross-cultural intakes of calcium and fracture risk, which few would argue to be a causal influence. As others have remarked (9), there are many difficulties in interpreting cross-cultural comparisons of national dietary data with specific multifactorial outcomes, such as fracture risk.

In terms of soy protein, no evidence was found in this meta-analyses to support any significant effect compared with other protein on lumbar spine BMD. This could be due to no differential effect of soy protein compared with other protein types on bone. It could also be due to methodologic issues, including the very small number of studies available and the small numbers of participants.

MBP is a specific dietary protein source that appears to have an influence on bone and has been examined as one potential mediator of the beneficial influence of milk on bone health. However, in our analysis, no effect was found for MBP compared with inactive control on lumbar spine BMD. It must be noted that the overall literature is small and limited to studies conducted in Japanese populations. Clearly, further studies especially within the context of white populations and diets are required.

## Limitations of this review

The quality of the intervention studies was reasonable, but most studies showed omissions in the reporting of randomization and investigator blinding, although participant blinding was reported in nearly all studies. Therefore, risk of bias was moderate, but it may have affected the reliability of the meta-analysis.

Although publication bias was not specifically measured, it is reasonable to assume that it existed to a certain degree in our analysis and may have led to a larger selection of positive findings over no effect. Reference lists in articles were not searched, and this may be a limitation of this review. Also, some authors did not report findings that were not significant, and these could not always be obtained from authors, which again may exaggerate a positive effect.

The situation is also confused by the clear heterogeneity present in the pooled r values and the main meta-analyses. The studies had a wide variety of supplement types and dosages, durations, clinical outcomes, and designs. This made it very difficult to draw firm conclusions from the data about the existence of effects and their clinical importance. Study durations were often not long enough to see effects on outcomes such as BMD or fracture, which may need months to years.

Last, many of the intervention studies were potentially underpowered to detect any effect of supplementation, should one exist. However, it should be considered that the studies summarized in this review are the best evidence available at present. In the future, more long-term, homogeneous, adequately powered intervention studies looking at total protein and BMD and fracture in a set of clinically relevant outcomes (lumbar spine, femoral neck, and radius) are required.

## Conclusions

A positive association between protein intake and BMD, BMC, and a reduction in bone resorption markers was indicated in the studies reviewed here. However, no separate effects of soy supplementation or MBP on lumbar spine BMD were found. However, the studies were highly heterogeneous from one another, and confounding may partly explain any positive effects of protein found in the cross-sectional surveys and cohort studies. Importantly, there was no relation between dietary protein and fracture risk in the qualitative review or meta-analysis.

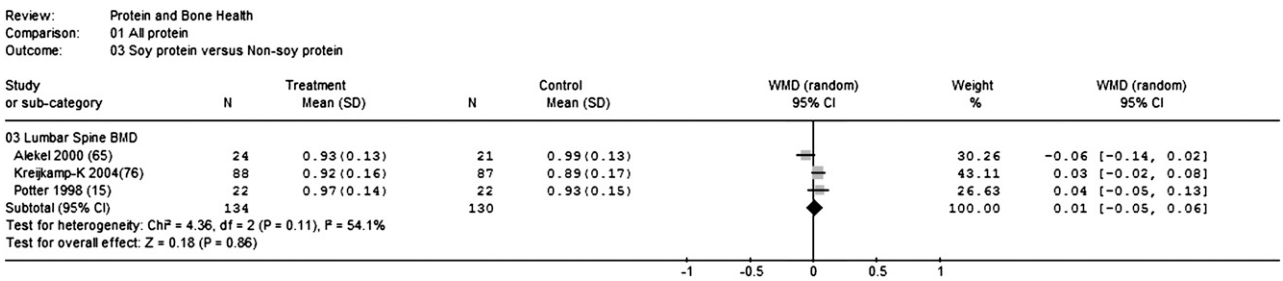


**FIGURE 4.** The effect of soy protein supplementation on lumbar spine bone mineral density (BMD). WMD, weighted mean difference.

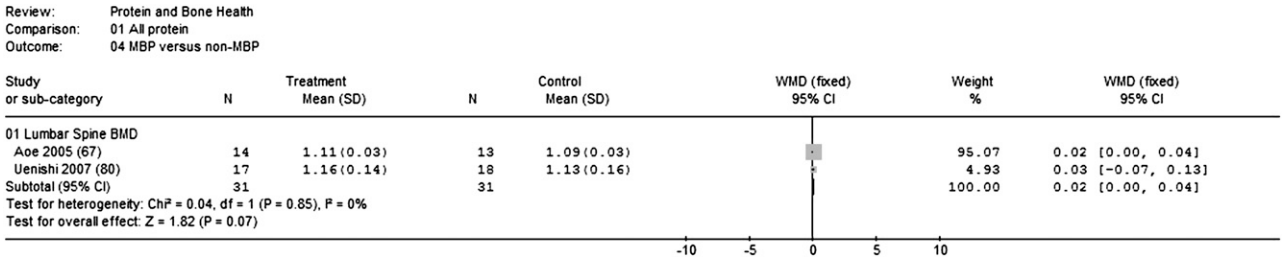


**FIGURE 5.** The effect of milk basic protein (MBP) supplementation on indexes of lumbar spine bone mineral density (BMD). WMD, weighted mean difference.

Overall, the weight of the evidence shows that the effect of dietary protein on the skeleton appears to be favorable to a small extent or, at least, is not detrimental. However, the long-term clinical importance of the effect is unclear, and a reduction in fracture risk was not seen. More research is required to resolve the protein debate. In the meantime the protein intakes and balance of different protein sources as indicated in the current healthy eating guidelines (eg, Balance of Good Health) represent appropriate dietary advice.

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