Dietary protein and bone health across the life-course: an updated systematic review and meta-analysis over 40 years

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Word Count: 4131 (excluding title page, abstract, tables, references), 4 tables and 2 figures

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Acknowledgements: This work received no funding. The authors are grateful to Dr Helen Lambert for her help with translation of papers.

Conflict of Interest Statement: SS has received institutional grants from Dairy Management Inc. and she serves as a member of the National Dairy Council's Nutrition Research Scientific Advisory Committee. SLN has received consultancy fees from the following companies/organisations: National Dairy Council UK, Yoplait, Kelloggs, Danone, Nestle. All other authors have nothing to declare.

Authors' contribution: ALD, RM and S-LN designed the research: ALD, RM and S-LN conducted the research. ALD analysed data. ALD wrote the paper. ALD and S-LN have primary responsibility for final content. ALD, CEH, DJM, KZ, RM, RP, SS, S-LN interpreted data and aided with statistical analyses. ALD, CEH, DJM, KZ, RM, RP, SS and S-LN revised manuscript content for intellectual content. ALD, CEH, DJM, KZ, RM, RP, SS and S-LN approved the final version of manuscript.

Awards: ALD received a Young Investigator Travel Award from the 10th International Symposium on the Nutritional Aspects of Osteoporosis Conference in Hong Kong, 28th November to 1st December 2017 for this work.

Please cite this article in press as: Darling AL et al. (2019) Dietary protein and bone health across the life-course: an updated systematic review and meta-analysis over 40 years. Osteoporosis International (in press).

Link to publisher version: http://link.springer.com/article/10.1007/s00198-019-04933-8

DOI : 10.1007/s00198-019-04933-8

Abstract

Purpose: This systematic review and meta-analysis analysed the relationship between dietary protein and bone health across the life-course.

Methods: The PubMed database was searched for all relevant human studies from the 1st January 1976 to 22nd January 2016, including all bone outcomes except calcium metabolism.

Results: The searches identified 127 papers for inclusion, including 74 correlational studies, 23 fracture or osteoporosis risk studies and 30 supplementation trials. Protein intake accounted for 0 - 4% of areal BMC and areal BMD variance in adults and 0-14% of areal BMC variance in children and adolescents. However, when confounder adjusted (5 studies) adult lumbar spine and femoral neck BMD associations were not statistically significant. There was no association between protein intake and relative risk (RR) of osteoporotic fractures for total ($RR_{(random)}$ = 0.94; 0.72 to 1.23, I^2=32%), animal ($RR_{(random)}$ = 0.98; 0.76 to 1.27, I^2 = 46%) or vegetable protein ($RR_{(fixed)}$= 0.97 (0.89 to 1.09, I^2 = 15%). In total protein supplementation studies, pooled effect sizes were not statistically significant for LSBMD (total n=255, $MD_{(fixed)}$=0.04 g/cm^2 (0.00 to 0.08, P=0.07), I^2=0%) or FNBMD (total n=435, $MD_{(random)}$=0.01 g/cm^2 (-0.03 to 0.05, P=0.59), I^2=68%).

Conclusions: There appears to be little benefit of increasing protein intake for bone health in healthy adults but there is also clearly no indication of any detrimental effect, at least within the protein intakes of the populations studied (around 0.8-1.3 g/Kg/day). More studies are urgently required on the association between protein intake and bone health in children and adolescents.

Key Words: Aging, Epidemiology, IGF-1, Nutrition, Osteoporosis, Diet

Mini Abstract: We undertook a systematic review and meta-analysis of published papers assessing dietary protein and bone health. We found little benefit of increasing protein intake for bone health in healthy adults but no indication of any detrimental effect, at least within the protein intakes of the populations studied.

Introduction

The association between dietary protein intake and bone health has been debated worldwide for decades. It is unclear as to whether dietary protein exerts a positive or detrimental influence on bone and there are competing theories as to the effects of dietary protein on bone health. Proponents of a positive link between protein intake and bone health cite the known anabolic effects of dietary protein on bone, via dietary protein's known ability to increase secretion of insulin-like growth factor 1 (IGF-1). Dietary protein may also increase calcium absorption from the gut [1], which is likely to be beneficial for bone mineralisation.

On the other hand, proponents of a detrimental association between protein intake and bone argue that high protein intakes may be bad for bone health. This is on the basis of known in-vitro increased osteoclast activity with increasing body acidity[2] and predicted subsequent bone demineralisation. However, it is important to consider dietary protein type as only proteins that are rich in sulphur amino acids are likely to increase net physiological acid production. Also, protein is consumed in the diet with other components which may modify the overall net acid concentration (e.g. fruits and vegetables which have an alkalizing effect, soy isoflavones which have estrogenic effects, and dairy products which contain calcium). These nutrients may modify the association between protein intake and bone health [3, 4].

A previous systematic review and meta-analysis published in 2009, covering published papers from January 1966 to July 2008 [5], showed a small beneficial effect of dietary protein supplementation on areal bone mineral density (aBMD) in human adults, as well as showing that nearly all published cross-sectional studies demonstrate a positive association between dietary protein intake and bone health. However, there was no association between dietary protein intake and fracture risk in cohort studies, and much of the findings of the cross-sectional studies may be due to inadequate controlling for confounding factors. Also, the

previous systematic review and meta-analysis did not assess dietary protein and bone health in children and adolescents, which is an important area of research due to the known importance of obtaining peak bone mass at this time of life.

Since the publication of the dietary protein and bone health systematic review and meta-analysis in 2009 [5] the number of relevant articles on the topic of dietary protein and bone health has doubled. This highlights the need for an updated systematic review of this topic, including consideration of peak bone mass development in children and adolescents, as well as bone health in adults. Further systematic reviews have been conducted on the topic since 2009 [6-10], but none to date have included all study designs (cross-sectional, longitudinal and randomized control trials) and included both adults and children. Our objective was to perform a systematic review and meta-analysis of the evidence published over the last 40 years assessing dietary protein intake and all indices of bone health across the human life-course, including all relevant observational and intervention studies examining intakes of all types of protein.

Materials and Methods

Search Strategy

The PUBMED databases, as well as reference lists of relevant journal papers were searched to ensure broad coverage. The search phrase used for the electronic search 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, in any language, from 1st January 1976 to 22nd January 2016. Two of the authors (ALD, SLN) identified potential studies for inclusion by first screening titles and abstracts, and then looking at the full version of papers if necessary. Any disagreements were resolved by consensus and deferred to a third party if required.

Study Design and Criteria

Papers assessing relevant bone outcomes (specifically BMD or BMC, bone turnover markers and/or fracture risk) in healthy human adults, adolescents or children were considered for inclusion. Studies investigating subjects with a pre-existing medical condition affecting bone or calcium metabolism, or studying animals, infants, and pregnant or lactating women were excluded as were studies involving only calcium balance or calcium metabolism.

All cross-sectional, longitudinal and intervention studies were eligible for inclusion, including intervention studies of any design. However, due to problems of statistically combining crossover and parallel intervention designs, crossover studies were not included in the meta-analysis and were discussed in the systematic review only. Studies were excluded if they were published before 1975, were weight loss studies, did not have usable protein or bone data, were not on the relevant topic, were review articles or correspondence, or only had data on dietary patterns. They were also excluded if they had confounders present in the central study design (e.g. differing exercise levels in the intervention and control groups as part of the intervention, for example protein and exercise as intervention vs. no protein and exercise). All bone sites in the body were eligible for inclusion in the review, as were all markers of bone metabolism.

Soy protein studies were excluded if they did not have data on isoflavone devoid soy protein isolate. Studies on whole dietary measures (e.g. meat intake, frequency of high protein meals) were also excluded due to the confounding potential of other dietary constituents (such as isoflavones in soy protein; fat or iron in meat). However, whole diet intervention studies were included as long as the nutritional contents of the diets were shown in the paper and the diets had been designed to minimise the impact of confounders (e.g. ensuring high and low protein

diets were isocaloric, similar in dietary calcium etc.). No ethical approval was required as the analysis was conducted only on already published data.

Data synthesis

See Online Resource for full details of the data extraction process. In the meta-analyses, two sided tests with an alpha value of 5% were used, and Bonferroni adjustment for multiple testing was applied when necessary.

The systematic review included all eligible studies, but only studies with data suitable for meta-analysis were analysed quantitatively. The R software (version 3.1.2) [11] add-on packages 'metacor'[12], 'meta'[13], 'metafor'[14] and 'metagen'[15] were used to pool correlation coefficients, intervention and fracture risk data for meta-analysis, as well as creating all plots. For cross-sectional and longitudinal data, multivariate adjusted analyses were used wherever possible, rather than unadjusted or age adjusted measures. Linear and non-linear modelling was undertaken to assess whether there was an association between correlation coefficients or actual BMD values with the following: calcium: protein ratio (mg/g/d); protein dose (g/kg/d) and calcium dose (mg/kg/d).

It was planned *a priori* to run a combined analysis of hip and non-hip fracture data as all fracture outcome first, then a subsequent analysis of hip fracture data only. Unfortunately the calcium: protein ratios (calcium mg/protein g) in the fracture risk studies were too constant (10-12 mg/g/d) to be able to perform an assessment of calcium: protein ratio vs. fracture risk. Also, there were too few studies within the same protein type to be able to plot protein or calcium dose against fracture risk.

The intervention trials meta-analysis examined the main effects of protein supplementation (all protein types but excluding studies specifically looking at soy protein isolate and milk basic protein (MBP)) on BMD, BMC and bone turnover markers, based on mean differences.

The decision was made *a priori* to also perform a separate analysis for MBP trials as this type of protein is highly concentrated and is supplemented in very high doses. Similarly, experience from the previous meta-analysis suggested that soy protein studies need to be analysed separately than non-soy studies, as soy studies usually assess the effect of soy protein vs. another protein type (hence the actual protein intake is the same, just different protein type). Conversely, studies of mixed protein supplementation usually compare higher with lower doses of protein (or have another type of control, e.g. maltodextrin), hence the protein intake varies in each arm of the study.

Finally, due to the differences in skeletal biology between early life and in older age, as well as differences in nutritional requirements, children and adults were analysed separately in all meta-analyses. Also, all adult groups were broken down into gender and life stage specific analyses wherever there was enough data to do so. Only for the cross-sectional studies were there enough data to perform a meta-analysis in children.

<u>Heterogeneity, Sensitivity and Publication Bias- meta-analysis</u>

It was pre-specified that the I^2 statistic was to be used to assess heterogeneity between studies as this is recommended for analyses with smaller numbers of studies[16]. I^2 values of 25%, 50% and 75% were considered low, moderate and high, respectively[16]. Random-effects (heterogeneous comparisons) and fixed effects (homogenous comparisons) models were used accordingly, with the decision based on size of I^2 (%), rather than the $P_{(heterogenity)}$ value from the Q test, as number of studies in the meta-analyses were predicted to be small. It was also pre-specified that funnel plots were used to assess possible biases in meta-analyses containing 10 or more studies[17]. Sensitivity analyses were also planned to assess the impact of removal of each study in turn on the pooled effect size.

155 Quality Analysis

156 Intervention study quality analysis was undertaken at the study level. This was conducted
157 independently by 2 authors (AD, SLN) using the Jadad Scale[18]. Due to the extensive
158 number of observational studies, only one author (AD) assessed quality for these papers,
159 using the Newcastle-Ottawa adapted scales [19] for cohort and case-control study designs as
160 appropriate. Cross-sectional studies were not assessed for study quality due to the very large
161 number of papers included in the review. For all study types, due to the small predicted
162 numbers of potential studies for the fracture and intervention study meta-analyses, we decided
163 a *priori* that the quality analysis information was to be used to guide result interpretation
164 rather than to exclude studies *per se*.

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Results

Figure 1 shows the Quality of Reporting of Meta-analyses (QUOROM) flow diagram[20] illustrating the search and selection process. The systematic review included 127 studies, including 74 studies reporting correlation or regression coefficients **(Table 1)**. These included either just cross-sectional data (55 studies), just longitudinal change data (4 studies) or both (9 studies). These also included 2 studies reporting both longitudinal change in BMD data and fracture data and 4 studies reporting cross-sectional data and odds of low BMD or osteoporosis diagnosis (**Table 2**). In addition there were 23 studies reporting only fracture or osteoporosis risk (**Table 3**), and 30 intervention studies (**Table 4**). Due to the extensive number of studies to be discussed here, see the Online Resource for the systematic review and the study quality assessments.

Pooling of studies reporting correlation or regression coefficients

By population subgroup

See Table 1 (and **Online Resource Table S1**) for details of the studies reporting correlation or regression coefficients. Of these 74 studies, 30 studies in adults [21-50] and 5 studies in children [51-55] gave cross-sectional R correlation coefficients suitable for pooling. Of note, only 5 of these studies [22, 24, 25, 36, 56] reported adjusted data, so the majority (n=30) were non-adjusted for confounders. When pooling by population subgroup the R^2 values suggested that <1-8% of adult bone BMD or BMC (depending on age and biological sex) as well as 10% of child BMC was explained by protein intake (**Online Resource Tables S2-S3**, Online Resource Material Text). Heterogeneity was highly variable with values ranging from 0-74% depending on population subgroup.

By bone parameter

For adults, **Online Resource Figures 1-2** illustrate the pooled effect size for FNBMD and LSBMD. FNBMD (total n=4786) showed $r_{(fixed)}$=0.07 (0.04 to 0.09) R^2=0.005 (0.5%) P<0.0001, I^2=26% $P_{(heterogenity)}$=0.15. LSBMD (total n=4257) showed $r_{(random)}$=0.09 (0.04 to 0.14) R^2=0.008 (0.8%) P<0.001, I^2=58% $P_{(heterogenity)}$=0.001. Of note, when only studies reporting multivariate adjusted data were shown, the pooled effect sizes for FNBMD (total n=1169, $r_{(random)}$=0.04 (-0.05 to 0.12) R^2=0.0016 (0.2%) P=0.37, I^2=45% $P_{(heterogenity)}$=0.14), and for LSBMD (total n=728, $r_{(fixed)}$=0.0 (-0.07 to 0.07), R^2=0 (0%) P=0.97, I^2=0% $P_{(heterogenity)}$=0.61) were no longer statistically significant. Other bone outcomes ranged in R^2 value from 0-10% (including unadjusted data). See Online Resource Material for full results of pooling for other bone sites in adults. Heterogeneity was highly variable with values ranging from 0-94% depending on adult bone site.

In children and adolescents, the R^2 values suggested that 0-14% of child/adolescent BMC and 21% child/adolescent total body bone area (TBBA) were explained by protein intake (all unadjusted data). Heterogeneity was 0% (All BMC and total body bone mineral content (TBBMC)), 79% (TBBA) and 87% total body bone mineral density (TBBMD)). See Online Resource Material for further details of all the above correlational analyses including sensitivity analyses and funnel plots, as well as associations with protein and calcium dose, and calcium: protein ratio (**Online Resource Table S4**).

<u>Fracture risk meta-analysis</u>

See Table 3 and **Online Resource Table S5** for details of all studies presenting fracture or osteoporosis risk data. Of 29 studies (6 of which have already appeared in correlational section of review), 5 studies were pooled in the meta-analysis for cohort studies assessing relative risk of fracture [57-61] (**Figure 2**), all of which provided multivariate adjusted risk

estimates. No statistically significant association was found between total protein intake and relative risk (RR) of all fractures ($RR_{(random)}$=0.94 (0.72 to 1.23, P=0.55 n=4 studies, I^2=32% $P_{(heterogenity)}$=0.30). Similar results were seen for animal protein intake, $RR_{(random)}$=0.98 (0.76 to 1.27, P=0.87, n=4 studies, I^2=46% $P_{(heterogenity)}$=0.13) and vegetable protein intake: $RR_{(fixed)}$=0.97 (0.89 to 1.09, P=0.61, n=3 studies, I^2=15% $P_{(heterogenity)}$=0.31). For three cohort studies reporting hazard ratios (HR) (**Online Resource Figure S3**)[62-64], no significant association was found between total protein intake and HR for all fractures ($HR_{(random)}$=0.82 (0.59 to 1.14, P=0.24), n=4 studies (5 data points as 2 studies had independent subgroups which can both be entered), I^2=35% $P_{(heterogenity)}$=0.19). Of note, removal of the Sahni (Low calcium) data[64] led to a reduced risk of fracture with increased protein intake (HR (fixed)=0.79 (0.64 to 0.97, P=0.02), n=4 studies, I^2=0% P(heterogenity)=0.66), which would be of borderline statistical significance after adjustment for multiple testing (using a cut-off of 0.02 for 3 fracture meta-analyses). For three case-control studies reporting odds ratio (OR) for fracture[65-67] (**Online Resource Figure S4**) there was no significant association between total protein intake and odds of fracture: ($OR_{(random)}$=0.69 (0.30 to 1.58, P=0.38), n=4 studies (4 data points as 1 study had independent subgroups which can both be entered) I^2=65% $P_{(heterogenity)}$=0.03). See Online Resource Material for details of relevant sensitivity analyses.

Intervention Studies Meta-analysis

Thirty papers were intervention studies (Table 4**, Online Resource Table S6**). See Online Resource Material for details of study quality assessment for these studies as well as systematic review of intervention studies.

Meta-analysis- summary of included studies

Nine of the 30 intervention studies [68-76] were suitable for meta-analysis. The other 20 studies were excluded due to missing data [77], cross over design study with no report of data prior to crossover [1, 78-85], and not having data that is compatible with other studies to allow meta-analysis [86-93], being a food-based intervention [94] or presenting data for change in bone indices only[95, 96].

Total protein intake

The pooled effect size for protein supplementation on LSBMD[68, 72] showed no statistically significant effect (P=0.07) (total n=255, $MD_{(fixed)}$=0.04 (0.00 to 0.08, P=0.07), I^2=0% $P_{(heterogenity)}$=0.47; **Online Resource Figure S5**) Equivalent data for FNBMD (3 studies)[68, 72, 76], were also not statistically significant (total n=435, $MD_{(random)}$=0.01 (-0.03 to 0.05, P=0.59) I^2=68% $P_{(heterogenity)}$=0.04)(**Online Resource Figure S6**).

MBP and Soy Protein

For MBP studies, comparing MBP supplementation with a nutrient matched drink [69, 70] or no control[71], the pooled effects sizes were as follows: LSBMD Mean Difference $(MD)_{(fixed)}$= 0.02 (0.00-0.04, P=0.08), I^2=0% $P_{(heterogenity)}$=0.87, 3 studies) (**Online Resource Figure S7**). For soy protein studies no statistically significant effect was found for LSBMD ($MD_{(random)}$ = -0.01 (-0.07 to 0.06, P=0.82), I^2=51% $P_{(heterogenity)}$=0.13) , FNBMD ($MD_{(random)}$ =0.01 (-0.06 to 0.07, P=0.87), I^2=74% $P_{(heterogenity)}$=0.05) or BAP ($MD_{(random)}$ = -1.75 (-10.50 to 7.01, P=0.70), I^2=91% $P_{(heterogenity)}$=0.0009). There were not enough studies with compatible data to assess any other bone sites or bone markers for soy protein or MBP supplementation.

Overall heterogeneity was low for the influence of MBP on LSBMD at I^2=0% but high for soy protein LSBMD, FNBMD and BAP (I^2=51%, 71% and 91% respectively). For both MBP and soy protein sensitivity analyses showed that removal of each study in turn had no effect on the pooled effect sizes (see Online Resource Material). The results for the soy and MBP subgroup analyses are unlikely to remain statistically significant after Bonferroni adjustment for multiple testing.

Discussion

This systematic review and meta-analysis reviewed evidence, published over the last 40 years, assessing the association between dietary protein and bone health across the life-course. Dietary protein intake explained 0 to 4% of adult BMD, BMC and bone markers, as well as 0-14% of child/adolescent BMC and 21% child/adolescent TBBA. However, when only studies adjusted for confounders (5 studies) were included in the adult analyses for FNBMD and LSBMD these effects did not remain statistically significant, suggesting the effect sizes seen could be caused by potential confounding. The larger pooled effect sizes for the children and adolescents suggest a stronger relationship between protein and bone health at this life stage than in later life. Indeed, nutritional requirements for skeletal growth in childhood and adolescence are different to those for reducing senescence related bone loss, as the cellular mechanisms involved differ at these two life stages. Protein intake may have a strong relationship with bone health in childhood due to the involvement of amino acids in endochondral ossification during bone growth. However, this finding of a stronger association between protein intake and bone health in children may also be an artefact of the smaller number of participants included in the pooled effect sizes in the child and adolescent analyses compared with adults.

In adults, neither calcium: protein ratio [calcium (mg)/protein (g /d)] nor protein (g/kg/d) or calcium (mg/kg/d) dose were associated with strength of effect for the association between protein intake and bone health. As seen in the previous 2009 systematic review and meta-analysis, there was no association between dietary protein intake and fracture risk in cohort and case-control studies, for total protein, animal or vegetable protein. In intervention studies, no statistically significant effects of total protein supplementation (excluding MBP and soy protein) were seen for FNBMD and LSBMD, or for higher vs. lower soy protein supplementation.

The lack of an effect of dietary protein on bone seen in the above fracture risk and supplementation trials may be for a variety of reasons. It could be that the opposing anabolic and catabolic actions of dietary protein cancel each other out to some extent. Alternatively dietary protein, at the protein intakes seen in the populations in this review, may not affect bone health and it may be that there is a small window whereby very low protein intakes are detrimental to bone, due to low IGF-1 production and dietary calcium absorption from the gut. Few studies in the review contained populations with low population intakes, with most studies reporting intakes of over 0.75g/Kg/d-0.84g/Kg/d which is considered adequate by western countries such as Australia [97], Europe [98] and the US [99]. Some studies that contained older people or Buddhist Nuns had intakes nearer to, or slightly lower than, 0.75g/Kg/d [66, 95, 100-102]. Twelve of the trials had no baseline protein intake data which makes it difficult to fully assess the degree of sufficiency. However, observational studies included this information with only one observational study not reporting overall protein intake [42]. In only 2 of the childhood studies was there a deficient protein intake [53, 92], and these were studies of malnourished children so this is as would be expected. However, none of the other child or adolescent studies showed deficient protein intakes.

Equally, other aspects of the diet that the authors of the original studies did not control for in the analysis (e.g. calcium intake) may be affecting the results. Of note, the study by Sahni et al. (2010) [64] showed a higher risk of hip fracture with higher protein intake when calcium intake was low (<800 mg/d) but conversely a lower risk of fracture with higher protein intake when calcium was higher (≥800 mg/d). Failure to control for calcium intake in the fracture analyses could therefore obscure any relationship between protein intake and fracture risk. Of importance, the meta-analysis for cohort studies reporting hazard ratios was borderline statistically significant for a reduced risk of fracture with increasing protein intake, when the low calcium arm of the study by Sahni et al. 2010 [64] was removed, suggesting this study

arm may be masking an association between protein intake and fracture which was evident when only the high calcium arm was included. Moreover, the meta-analysis result for the case-control studies (hazard ratios, see Online Resource Figure 4) would have been statistically significant if using fixed models, which were not used due to heterogeneity present in our analysis. This may explain some of the differing results of our study from other meta-analyses, in that other analyses have combined data from cohort and case-control studies, which we did not, and the results of other meta-analyses may only be significant because of the inclusion of case-control data.

Since the publication of our original meta-analysis in 2009 [5] there have been a series of meta-analyses published on the subject of dietary protein and bone [6-9]. However, these differ from our meta-analysis in significant ways. Wu et al. (2015) only assessed fracture risk, and Wallace and Frankenfeld (2017) did not assess cross-sectional studies. Santesso et al. (2012) only included bone data from weight loss trials and Shams-White et al. (2017) [6] included a mixture of weight loss and non-weight loss trials, which may partially explain their finding of a protective effect of higher protein intake against LSBMD loss. Moreover, only one study in the latter review's change in LSBMD meta-analysis showed a positive effect, so the analysis was reliant on this one study for the significant result. The most recent meta-analysis, Shams-White (2018)[10] included only studies on animal protein vs. soy protein with isoflavones, so differs substantially from our current review. Finally, none of these meta-analyses assessed data from children and adolescents.

There have also been 13 relevant protein and bone health papers [103-115] published since our electronic searches were completed. All of these studies have found either a positive association, or no association of protein take with bone health, except for one study which found poorer bone health with increased protein intake [107]. All these studies were of observational study design except for two randomised control trials; one of soy protein in

children which found no effect on bone marker concentration [110] and one of collagen supplementation in postmenopausal women which found a positive effect of supplementation on aBMD [108]. Overall, our current meta-analysis reported here is the most comprehensive to date on the subject and the inclusion of studies published since 2016 are unlikely to have significantly changed our results. As stated above, the separation of cohort and case-control studies may explain differences from our study to that of other meta-analyses.

The strengths of this analysis include the fact that we included papers published in both English and non-English languages, and from both observational and intervention studies. Moreover, inclusive coverage both in terms of timescale (i.e. 1975 to the present day; 40 years), topic area (all types of protein, BMD, BMC, BA and bone turnover markers) and population age subgroups (children, adolescents, adults, older adults) was achieved. This makes this analysis a comprehensive overview of dietary protein and bone health across the life-course, using data from the last 40 years. This makes the work novel and of importance to scientists, public health providers and clinicians alike.

However, the review was limited by the following factors. First, studies did not vary enough in calcium intake, as well as too few studies, to be able to assess whether the association between dietary protein and fracture risk varies by calcium intake. Second, there were concerns due to the low methodological quality of some intervention and cross-sectional studies. Many cross-sectional studies did not report any multivariate adjusted correlation coefficients in the paper (only unadjusted r values). Third, there is a clear lack of intervention studies, particularly parallel trials and cross-over studies reporting data from before the cross-over point, so effect sizes in the intervention meta-analysis are likely to have been underpowered due to a small number of studies with a small number of participants.

Fourth, the diets of the populations represented in this analysis generally showed protein intakes that meet (and usually slightly exceed) protein intake recommendations. Results of

the analysis could differ if persons with a low protein intake (or very high protein intake) were included. Indeed, as discussed above, few studies had populations with inadequate intake and more research is required into bone health in these populations. Moreover, it is also difficult to answer the question as to whether there is a threshold whereby protein intake becomes so high that is detrimental to bone due to the lack of data in persons with high intakes. Fifth, the Newcastle Ottawa tool was not always a good fit to the studies, especially for nested case-control studies where it is not clear whether they should be scored on the case-control or cohort scales. Therefore, the results of the quality analysis should be seen as a general guide to study quality, rather than a definitive and accurate score. Finally, the funnel plots for the correlation studies showed some evidence of publication bias in terms of a lack of small to medium studies showing negative associations between protein intake and bone health.

Future research needs to include larger-scale intervention studies, particularly in understudied population sub-groups such as children and adolescents and persons with very low protein and calcium intakes (e.g. the frail elderly) or very high protein and calcium intakes. Fracture risk studies need to be undertaken in the elderly, followed up to increased age (e.g. 80s) to truly see the effect of increased protein intake on long term fracture risk.

Overall, this systematic review and meta-analysis of the association between dietary protein and bone health across the life-course assessed evidence from the last 40 years. A positive cross-sectional association was shown between protein intake and bone health in most pooled analyses of r values in adults. However, these associations disappeared when only covariate adjusted data were used and there was no association between fracture risk and intake of any protein type in the fracture risk meta-analysis. There was no effect of any form of protein supplementation on any indices of bone health. The public health and clinical implications of this work are that there may be little benefit of increasing protein intake for bone health in

399 healthy adults with an adequate protein intake but there is clearly no indication of any
400 detrimental effect.
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Table 1: Characteristics of 74 cross-sectional and/or longitudinal correlational studies

Study	Design	Mean Protein **	Method	Population	n	Outcomes measured
Alexy et al, 2005, Germany[116]	CS	1.4-2.0 +/- 0.3 g/Kg/d	pQCT	Prepubescent and pubescent boys and girls	229	Periosteal Circumference, Cortical Area, BMC, Polar SSI
Alissa et al, 2011, Saudi Arabia[117]	CS	1.03 g/Kg/d	DXA	Postmenopausal women aged 46-70 years old	122	BMD
Alissa et al, 2014, Saudi Arabia[21]	CS	71.4+/-1.55 g/d	DXA	Postmenopausal women, aged 46-88 years	300	BMD
Beasley et al. 2010, USA[118]	CS	TP: 5.7 - 27.6% energy AP:45g/d, VP:19g/d	DXA	Premenopausal women	560	BMD
Beasley et al. 2014, USA[119]	LS	15% total energy	DXA	Postmenopausal women 50-79 years	144,580	BMD
Bounds et al, 2005, USA[51]	CS	55g/d (1.9g/Kg/d)	DXA	6-8 year old children, 25 Boys and 27 Girls	52	BMD/BMC
Budek et al. 2007a, Denmark [120]	CS	2.67 g/Kg/d	BTM	Pubertal boys	81	OC, BAP, CTX
Budek et al. 2007b, Denmark [121]	CS	TP: 1.2 (Girls), 1.3 (Boys), AP: 0.4 (Girls), 0.5 (Boys), DP: 0.4 (Both Girls and Boys) g/Kg/d	DXA	17-year-olds: 63 girls and 46 boys	109	BMC
Chan et al, 2009, Hong Kong/Beijing[22]	CS	65.4-77.5g/d	DXA	Premenopausal women	441	BMD
Chan et al. 2011, Hong Kong[122]	LS	1.3 g/Kg/d	DXA	Older men and women	2217	BMD
Chevalley et al. 2008, Switzerland[52]	CS	47.3 g/d, 1.78 g/Kg/d	DXA	Pre-pubertal boys	232	BMC
Chevalley et al. 2014, Switzerland[123]	LS	Age 7: 1.8; Age 15: 1.1 (g/Kg/d)	DXA; High resolution pQCT	Adolescent boys	176	BMC/BMD/Area at 7.4 and 15.2 years; HR-pQCT distal tibia microstructure and strength

Study	Design	Mean Protein **	Method	Population	n	Outcomes measured
Chiu et al, 1997, Taiwan [23]	CS	1.09 g/Kg/d	DPA (BMD)	Older post F	258	BMD
Coin et al, Italy, 2008[25]	CS	1.02 g/Kg/d	DXA	Males, mean age 73.9+/-5.6 years	136	BMD
Cooper et al, 1996, USA[24]	CS	72 g/d	DPA SPA	Pre (72) and post (218) F	290	BMD, HPO. OC
Dawson-Hughes et al, 2002, USA[124]	CS	79 g/d	DXA	184 men and women(>=65 years old) in placebo (inactive) arm of calcium supplementation trial	184	BMD, OC, NTX
Devine et al, 2005, AUS[26]	CS	1.2 g/Kg/d	DXA , QUS	Elderly F mean age 75y+/-3y Caucasian	1077	BMD, BUA calcaneus
Ekbote et al, 2011, India[53]	CS	18.6g/d-normal and malnourished children combined	DXA	2-3 year old children	71	BMC,BA
Fairweather-Tait et al, 2011, UK[125]	CS	81.3g/d	DXA	Postmenopausal female twin pairs (Monozygotic or dizygotic twins)	2464 pairs	BMD
Freudenheim et al, 1986, USA[27]	CS	1.02 g/Kg/d	SPA	Pre and post F, 35-65y, Caucasian	84	BMC
Geinoz et al, 1993, Switzerland[100]	CS	Mean Intake in g/d by group: 37.8-59.4 g/d	DXA	Elderly M and F Mean age 82y(F); 80y(M)	74	BMD
Genaro et al, 2015, Brazil[126]	CS	66g/d	DXA	Women over 65 years old	200	BMC, BMD
Gregg et al, 1999, USA[29]	CS	0.9 g/Kg/d	QUS	Middle aged (premenopausal) F- mean age= 45.5y	393	BUA Calcaneus, BMD
Gunn et al, 2014, New Zealand[28]	CS	79g/d	BTM, DXA	Postmenopausal women, 60 years of age	142	BMD, CTX,P1NP
Hannan et al. 2000, USA[127]	LS	68g/d (16% of total energy) 0.97 g/kg/d	DXA	224 older men and 391 older women	615	BMD
Henderson et al, 1995 , AUS[30]	CS	1.0 g/Kg/d	DXA	Pre F- mean age=18y	115	BMD
Hernandezavila et al, 1993, USA[128]	CS	76g/d	SPA	Women (50-60 years old)	281	BMD

Study	Design	Mean Protein **	Method	Population	n	Outcomes measured
Hirota et al, 1992, Japan[31]	CS	1.13 g/Kg/d	SPA (BMD)	Young PRE women: 19-25y	161	BMD
Ho et al, 2003, China [56]	CS	1.01 g/Kg/d SP	DXA	<12y POST women (48-62y), Asian	454	BMC, BMD
Ho et al, 2008, China[49]	CS	5.2g/d SP 48.6 g/d TP	DXA	Pre and perimenopausal women 45-55 years old	438	BMC, BMD
Ho-Pham et al, 2009, Vietnam[101]	CS	TP: 35.4-62.6 g/d	DXA	105 Post F Buddhist vegan Nuns and 105 omnivorous women 62+/-10 years old	210	BMD
Ho-Pham et al. 2012, Vietnam[129]	CS/LS	49 g/d; 0.92g/Kg/d	DXA, BTM	Postmenopausal Buddhist Nuns	210	BMD, CTX, PINP
Hoppe et al. 2000, Denmark[54]	CS	73-82g/d	DXA	10 year old children	105	BMC, BA
Horiuchi et al, 2000, Japan[32]	CS	TP: 62.5g/d SP: 12.6g/d	DXA	Post F, 52-83y	85	BMD,OC,BAP,PYD, DPYD
Hu et al, 2014, USA[130]	CS	TP: 11.6-20.4% energy	DXA, QCT	801 women and 857 men (age 62+/-10 years)	1658	BMD
Ilich et al, 2003, USA[131]	CS	1.04 g/kg/d	DXA	Older F, >5 post, 68.7+/-7.1y	136	BMD, BMC
Iuliano-Burns et al, 2005, AUS[132]	CS	76g/d	DXA	7-20 year old Male twins (Monozygotic n=30) and Dizygotic (n=26)	56	BMC, CT, PW, EW
Jaime et al, 2006, Brazil[33]	CS	1.2 g/kg/d	DXA	Men- Over 50y	277	BMD
Jones et al. 2001, Tasmania[55]	CS	83g/d	DXA	Boys and Girls Aged 8 years old	330	BMD
Knurick et al. 2015, USA[34]	CS	69-97g/d	DXA	Adult men and women, 18-50 y	81	BMD
Kumar et al, 2010, India[35]	CS	45.7g/d	DXA	Women aged 20-69 years	225	BMD
Lacey et al, 1991, Japan[36]	CS	1.35 g/kg/d	SPA	Asian pre F(35-40y) and post F (55-60y)	178	BMC
Langsetmo et al., 2015 Canada[62]	LS	0.79g/Kg/d	DXA	Men and women aged over 25 years old	6510	BMD

Study	Design	Mean Protein **	Method	Population	n	Outcomes measured
Lau et al, 1998, China[37]	CS	0.65 g/kg/d	DXA	Vegetarian Post F, 70-89y	76	BMD
Libuda et al. 2008, Germany[133]	CS	1.3 g/Kg/d	pQCT	228 Children and adolescents 8-14 years old		
Libuda et al. 2011, Germany[134]	CS	42.7-46.1 g/d	pQCT	Pre-pubertal children	107	BMC, Cortical Area
Loenekke et al. 2010, USA[50]	CS	72-91g/d	DXA	Males and Females, 22+/-3 years	27	BMD, BMC
MacDonald et al, 2005, UK[135]	CS	79.4g/d	BTM, DXA	45-54y women	5119	PYD, DPYD, BMD
Meng et al. 2009 AUS[136]	LS	80.6 g/d, 1.2 g/kg/d	DXA	Community-dwelling premenopausal women aged 75 +/- 3 years old	862	BMC
Metz et al, 1993, USA[137]	CS	1.24 g/kd/d	SPA	Pre F Caucasian (24-28y)	38	BMD, BMC
Michaelsson et al, 1995, Sweden[38]	CS	59 g/d	DXA	F 28-74y, Caucasian	175	BMD, OC
Nakamura et al, 2004, Japan[39]	CS	1.29 g/kg/d	BTM	Elderly post F, mean age=68.3y , range 43-79	43	OC,BAP,DPYD,NTX
Neville et al, 2002, UK[138]	CS	66-98 g/d	DXA	238 M and 205 F, at both 15 and 20-25 y	443	BMD
New et al, 1997, UK[40]	CS	81+/-22 g/d	DXA	Women aged 44-50 years (Premenopausal)	994	BMD
Oh et al, 2013, Korea[102]	CS	TP 45.0-52.3 g/d	QUS	Men and POM F aged 50-70 years	3330	Calcaneal Bone Stiffness Index
Orozco et al, 1998, Spain[41]	CS	TP: 73.4(17.9) g/d	DXA	Premenopausal women aged 42years old	76	BMD
Orwoll et al, 1987, USA[42]	CS	-	CT,SPA	Men	154	BMC
Pearce et al. 2010, UK[139]	CS	Median: 87.7g/d	Bone Markers	Men aged 49-52 years	412	CTX
Promislow et al, 2002 USA[140]	CS	72.5 g/d	DXA	M/F 55-92y; 572F 388M	960	BMD
Quintas et al, 2003, Spain[43]	CS	1.4-1.7g/d	DPA	Pre F	164	BMD

Study	Design	Mean Protein **	Method	Population	n	Outcomes measured
Rapuri et al, 2003, USA[44]	CS	53.7-71.2 g/d	DXA	Post F- 65-77y	473	BMD,NTX, OC
Rubinacci et al, 1992, Italy[45]	CS	68-83g/d	SPA	Post F	120	BMC
Sahni et al. 2013, USA[141]	CS/LS	81g/d (Men) 77g/d (Women)	DXA	1,280 men and 1,639 women	2919	BMD
Tanaka et al, 2001, Japan[142]	CS	1.3 g/Kg/d	QUS	Pre F- 18-22y	965	Stiffness Index Calcaneus
Teegarden et al, 1998, USA[46]	CS	1.21 g/Kg/d	DXA	Young pre F	215	BMC, BMD
Thorpe et al, 2008, USA[47]	CS	74.7g/d	DXA	Postmenopausal women mean age 68+/-6 years	161	BMD
Tylavsky and Anderson, 1988, USA[143]	CS	1.01 g/Kg/d	SPA	60-98y elderly F	375	BMC, BMD
Vatanparast et al, 2007, Canada[144]	CS	20-25 years: 68-119g/d	DXA	Young adults (59 males, 74 females)	133	BMC, BMD
Wang et al, 1997, USA[48]	CS	0.97 g/Kg/d	DXA	Older post F	125	BMD, BMC
Wang et al. 1999, USA[145]	CS	1.05 g/Kg/d	QUS	63 18-18 year old women	63	BUS, SOS
Weikert et al, 2005, Germany[146]	CS	67.9g/d	QUS	F 35-67y	8178	Os calcis
Whiting et al, 2002, Canada[147]	CS	1.15 g/Kg/d	DXA	M 39-42y	57	BMD
Yazdenpanah et al, 2007, The Netherlands [148]	CS	81.3g/d , 1.1g/Kg/d	DXA	Men and Women aged 55 years and over	5304	BMD
Zhang et al. 2010, China[149]	LS	1.7 g/Kg/d	DXA	757 Girls (Mean age 10 years)	757	BMC

AP, animal protein; BA, Bone area; BAP, Bone Alkaline Phosphatase; BMC, bone mineral content; BMD, bone mineral density; BTM, Bone turnover markers; BUA, Broadband Ultrasound Attenuation; CS, Cross-sectional Study; CT, Computer Tomography; DPA, Dual Photon Absorptiometry; DPYD, Deoxypyridinoline; DXA, Dual energy x-ray absorptiometry; EW, Endosteal Width; HPO, Hydroxyproline; HR-pQCT, High resolution Peripheral Computed Tomography; LS, Longitudinal Study; NTX, N-terminal Telopeptide of Collagen; pQCT, Peripheral Quantitative Computer Tomography; OC, Osteocalcin; P1NP, Procollagen type 1 N-Terminal Propeptide; PW, Periosteal Width; PYD,

Pyridinoline; QUS, Quantitative Ultrasound; SOS, Speed of Sound; SP, soy protein; SPA, Single photon Absorptiometry; SSI, Strength-Strain Index; TP, total protein; VP, vegetable protein.

Table 2: Characteristics of the 29 studies reporting fracture or osteoporosis diagnosis data (6 of which also in table 1): Cohort and cross-cultural studies.

Study[a]	Mean Protein	Population	Length	Total n	Fracture/ BMD site	Protein type	Parameter	Confounder Adjustments
Abelow et al, 1992, USA cross cultural[150]	10.4g/d-77.8g/d AP	F aged over 50y	-	34 studies, 16 countries	Hip fracture	AP	Fracture Incidence	Age
Beasley et al. 2014, USA[119]	<13.3% to ≥15.6% of energy intake from protein	Women aged 50-79 y at baseline	6y	144,580	Any, Hip, Spine, Forearm	TP	HR	Age, BMI, race-ethnicity, calibrated energy intake, general health, physical activity, history of fracture at age 55 y, history of parental fracture, current smoking, corticosteroid use, glucocorticoid use, treated diabetes, rheumatoid arthritis, and hormone use
Dargent-Molina et al, 2008, France[57]	TP:46(7.5) g/d, AP:29 (8.8) g/d, VP:12(3.0) g/d	POM women	8.37y	36217 (2408 with fracture)	Any low impact fracture	TP AP VP	RR	Adjusted for BMI, physical activity, parity, maternal history of hip fracture, HT use, smoking status, and alcohol intake
Feskanich et al, 1996, USA[59]	79.6 g/d median	F, 35-59y	12y	85,900	Forearm, Hip, Fracture	AP TP VP	RR	Age, BMI, vigorous activity per week; menopausal status, use of postmenopausal hormones; cigarette smoking; use of thyroid hormone medication, thiazide diuretics and alcohol/caffeine.
Frassetto et al, 2000, USA cross cultural[151]	48 to 110.9 g/d	F aged over 50y		33 countries	Hip fracture	TP AP VP	Hip Fracture Incidence	-
Gunn et al, 2014, New Zealand[28]	79 g/d	POM women, 60 years of age DXA	Cross-sectional	142	Osteoporosis diagnosis	TP	Protein intake (g) by BMD diagnosis	-

Study[a]	Mean Protein	Population	Length	Total n	Fracture/ BMD site	Protein type	Parameter	Confounder Adjustments
Key et al, 2007, UK[152]	Women: 73.1 (21.6) g/d 77.8(22.6) g/d	Men and Women aged 20-89 years	5.2y	26 749 women and 7947 men	All sites, fractures (including high trauma)	TP	Incident Rate Ratio	Method of recruitment and adjusted for age, smoking, intakes of energy and each other nutrient, alcohol consumption, body mass index, walking, cycling, vigorous exercise, other exercise, physical activity at work, marital status and, for women, parity and use of hormone replacement therapy
Langsetmo et al, 2015, Canada[62]	TP: 0.79(0.60-1.03). AP:17.6(12.8-23)g/d VP:24.3(18.8-31.0)g/d	M and F, aged 25-49 and ≥50 years	5y	6510	Fragility fracture: n=4543 Main fracture: n=4570	TP	HR	Age, height, TEI, centre (women only), education, smoking, alcohol intake, physical activity, sedentary hours, calcium and vitamin D supplement use, hormone therapy (women only), bisphosphonate use (women only), and diagnosis of osteoporosis (women only)
Meyer et al, 1997, Norway[61]	0.8	M/F (mean age 47.1y)	11.4y	19752 F 20035 M	HF- F HF-M	AP	RR	Age at screening, body height, body mass index, serf-reported physical activity at work and during leisure time, diabetes, disability pension, marital status, and smoking
Misra et al, 2011, USA[63]	64g/d (energy adjusted)	M/F mean age=75 years	11.6y	946 (n=100 HF)	HF	TP	HR	Age, sex, weight, height and total energy intake
Munger et al, 1999, USA[58]	1.2 units g/Kg/d	Postmenopausal F (55-69y)	1-3y	32 050	HF	AP, TP, VP	RR	Age, body mass index, number of pregnancies, smoking, alcohol use, estrogen use, and physical activity.
Mussolino et al, 1998, USA[60]	<56g/d - >98g/d	Caucasian M (45-74y)	22y	2879	HF	TP	RR	BMI, previous fracture, smoker, physical activity, alcohol, chronic health condition, calcium intake, weight loss.
Sahni et al, 2010, USA[64]	Men TP: 75-79.0g/d	Men and women aged	7 to 14 years	3656	HF	TP, AP VP, AP:VP ratio	HR	Sex and menopause status (group 1: men; group 2: premenopausal women; group 3: postmenopausal women), age(years), weight at baseline (kg), height at baseline (m), physical activity index, intake of energy (MJ/day) and total

Study[a]	Mean Protein	Population	Length	Total n	Fracture/ BMD site	Protein type	Parameter	Confounder Adjustments
	AP: 52-54 g/d , VP: 23-25g/d	mean= 55 (9.9)years						vitamin D (IU/day), and smoking status (current versus former/never) and calcium intake
Sellmeyer et al, 2001, USA[153]	49.8g/d	F > 65y old	7.0y +/- 1.5y	1035	HF	VP, AP, AP:VP	RR	Age and body weight
Zhong et al, 2009 USA[154]	Mean(SE)= 61+/-0.8 g/d	POM women 50+ y old	<7y	2006	All fragility fractures	TP	OR	Age, race, body mass index (underweight/normal, overweight, obese), physical activity level, smoking status, alcohol use (heavy, moderate/none), hormone use, general health status, osteoporosis, arthritis, vision impairment, and stroke.
Zhang 2005[155]	SP: 9.6g/d Non Soy: 134g/d	Women aged 40-70 years old	4.5 y	24403	All fractures	SP	RR	Age, body mass index, hours of exercise per week, cigarette smoking, alcohol consumption, history of diabetes mellitus, level of education, family income, season of recruitment, and intakes of total calories, calcium, non-soy protein, fruits, and vegetables

[a]All studies are cohorts unless otherwise stated. AP, Animal Protein; BMD, Bone Mineral Density; HR, Hazard Ratio; OR, Odds Ratio; POM, Postmenopausal; RR, Relative Risk; SP, Soy Protein; TEI, Total Energy Intake; TP, Total Protein; VP, Vegetable Protein.

Table 3: Characteristics of the 29 studies reporting fracture or osteoporosis diagnosis data (6 of which also in table 1): 13 Case-control studies

Study	Protein intake	Measure	n	Group/outcome	Confounder Adjustments
Alissa et al, 2011, Saudi Arabia, Non-Prospective [117]	77g/d	DXA	122 POM Women, aged 50-60 years	Normal BMD vs. Osteopenic	Non adjusted for confounders
Chevalley et al. 2011, Switzerland Prospective[156]	47-63 g/d	DXA	176 boys- measured during pre-puberty and adolescence	Fracture vs. No Fracture	Non adjusted for confounders
Chiu et al, 1997, Taiwan Non-Prospective[23]	1.09 g/Kg/d	DPA (BMD)	258 Older post F	OR for Osteopenia diagnosis: LS and FN	Age, BMI, physical activity, calcium intake, non-protein energy intake, long term vegan/vegetarianism
Coin et al, Italy, 2008 Non-Prospective[25]	1.02 g/Kg/d	DXA	136 Males, mean age 73.9+/-5.6 years	OR for low THBMD	BMI
Farrin et al. 2008, Iran Non-prospective[157]	81.4g/d	DXA	58 POM women	LSBMD based diagnosis: Normal/Osteopenic/ Osteoporotic	Unadjusted
Kim et al, 2008, Korea Non-prospective[158]	TP= 60g/d, AP= 19g/d, VP= 40g/d	DXA	271 POM women: 134 cases and 137 controls	Osteoporotic vs. Non-Osteoporotic TP, AP, VP	Age, smoking, alcohol drinking, BMI, exercise, family history of osteoporosis, and energy intakes
Martinez-Ramirez et al, 2012, Spain Non-Prospective[65]	TP:105 (1.0) g/d. AP:66-70 (1.3) g/d. VP: 38 (0.63)g/d,	Aged 65 years or over, cases	167 cases and 167 controls	OR : All low energy fractures. TP, AP, VP, AP:VP ratio	Age, sex, energy intake, vegetable protein intake or animal protein intake, serum vitamin C, calcium intake, underlying chronic disease, home access, Katz's index, physical activity, HDL cholesterol and MUFA/PUFA intake.
Nieves et al, 1992, USA Non-prospective[66]	<24g/d to >55g/d	F 50 to 103y	329 (161 cases, 168 controls)	OR Hip fracture	Hospital, age, BMI, oestrogen use, chronic disease status

Park et al, 2014, Korea Non-prospective[159]	81.93+/-52.31 g/d	DXA	1157 PRE women	Z-Score ≥0 , Z-score<0	Non-adjusted
Perez-Durillo et al, 2011, Spain Non-prospective[160]	Cases 60 (19)g/d; controls 94 (19) g/d	Women > 65 y	44 cases and 42 controls	TP, Hip fracture c	BMI, carbohydrate intake and calcium intake
Preisinger et al, 1995, Austria Non-prospective[161]	15 % total energy, 45-96 g/d	Osteoporosis	23 POM women 50-70 years old	TP, AP, VP, Osteoporotic vs. Non Osteoporotic	Not required as just intake data
Samieri et al, 2013, France Prospective [162]	70-76 g/d	Men and women >65y	1482	Incident fracture of hip, spine or wrist	Not adjusted for confounders
Wengreen et al, 2004, USA Non-prospective[67]	1.2g/Kg/d	50-89y M/F	2501 (1157 cases, 1334 controls)	Cases Controls, Hip (OR), TP AP VP	BMI, smoking, alcohol, physical activity, oestrogen use, gender, total Calcium and Vitamin D intakes (diet and supplements), potassium intake, age. AP model also adjusted for VP intake, VP model also adjusted for AP intake.

AP, Animal Protein; BMD, Bone Mineral Density; DPA, Dual Photon Absorptiometry; DXA, Dual X-Ray Absorptiometry; LSBMD, Lumbar Spine Bone Mineral Density; OR, Odds Ratio; POM, Postmenopausal; PRE, Premenopausal; THBMD, Total Hip Bone Mineral Density; TP, total protein; VP, vegetable protein

Table 4: Characteristics of the 30 intervention studies reporting randomised control trials of dietary protein supplementation on bone health outcomes

Study, Country, Duration	Baseline protein intake	Supplement vs control	Change in protein intake	Subject Total n	Outcomes Measured
Alekel et al, 2000, USA, 24wks[73]	No information in paper	40g/d Soy vs Whey	+ 40g/d	N = 24 SPI+; N = 24 SPI-, N = 21 whey protein (control) PERI F	BMC, BMD, BAP
Aoe et al, 2001, Japan[96]	No information in paper	40mg/d MBP vs Placebo	+ 40mg/d (MBP)	32 PRE F	% change data only: Calcaneal BMD
Aoe et al 2005, Japan, 6mo[69]	No information in paper	40mg/d MBP vs Inactive placebo	+40mg/d (MBP)	27 PERI F	NTX, OC, BMD
Arjmandi et al, 2003, USA, 3mo[87]	Mean (SE): Soy group – 60(6)g/d, MBP group (75(9) g/d	40g/d Soy protein vs MBP	+40g/d	42 POM F	BAP, DPYD
Cao et al, 2011, USA Crossover study 14wks (7 weeks each arm)[1]	No information in paper	61g/d diet ('lower protein control-US daily recommendation) vs. 118g/d ('higher protein' group) diet	+/-57g/d	N=16 40-75 year old POM F,	NTX, DPYD
Ceglia et al, 2009 Crossover study 41 d[82]	Mean (SD): 69.1 (22.1) g/d	Mean (SD)2.1(2.02) Low vs. 96.7(5.7) High	-37.0 g/d (low) +27.6 g/d (high)	M/F 54-82 years old N=10	OC, NTX
Cuneo et al, 2010, Brazil[88]	Mean (SD) : 67(18.8) g/d (placebo), 61.9(24) g/d (collagen)	Hydrolysed collagen (10g/d protein) vs. maltodextrin placebo	+10 g/d	N=36 collagen, N=35 placebo, 45-65 year old POM F	BAP, CTX, OC
Dalais et al, 2003, AUS, 3mo[89]	109(7) g/d Soy group, 112(6)g/d Placebo	40g Soy protein vs casein placebo	+40g/d	106 POM F 50-75 y	PYD, DPYD
Dawson-Hughes et al 2004,USA, 63d[91]	No information in g (17-18% of total energy)	Mean(SD): High: 57.6(8.2) g/d protein , Low: 2.8(0.5) g/d protein	No baseline data in grams	32 Elderly M/F	NTX, OC
Evans et al, 2007, USA Crossover study, 9 months[78]	No information in paper	25.6g Soy protein isolate (I) vs. Milk protein isolate (p),	+25.6g/d	Postmenopausal women N=22, Mean age 63 years	BMD, BAP, CTX

Study, Country, Duration	Baseline protein intake	Supplement vs control	Change in protein intake	Subject Total n	Outcomes Measured
		exercise counterbalanced across groups (1/2 in each group exercise, ½ in each group no exercise)			
Hunt et al, 2009, USA, 7 wks Cross over study [83]	63(15) g/d LC 69(17) g/d HC	Protein g/d: LCLP 58, LCHP 112, HCLP 59, HCHP 115	LCLP -5g/d, LCHP +49g/d, HCLP -10g/d, HCHP +46g/d	N=13 in two LC arms, n=14 in two HC arms Post F	DPYD, OC, BAP, TRAP
Ince et al 2004, USA, 2wks Crossover study[94]	1.1 g/kg/d	Change to low (0.8g/Kg/d) protein diet	-0.2g/Kg/d	39 Pre F, 22-39y	NTX, OC
Jenkins et al, 2003, USA, 2mo Crossover study[85]	No information in g (18% of total energy)	Vegetable diet: 189g/d protein, (16% total energy) vs Control diet: 111g/d protein (27% total energy)	+78g/d	20 Middle aged M/F	NTX, BAP
Kenny et al, 2009, USA, 1 year [74]	Mean +/-SD : Soy group- 62.5 (13.7) g/d, Mixed control group- 57.0(21.9)	18g Soy protein (I) vs. 18g Mixed control protein (Casein, Whey and Egg) (p). No isoflavones in these two study arms	+18g/d	Women over 60 years old (mean=71y)	BMD, BAP, NTX
Kerstetter et al, 1999, USA, 4d Crossover study[80]	1.0g/Kg/d	High (2.1g/kg/d)vs low (0.7g/kg/d) protein	+1.1g/Kg/d High -0.3g/Kg/d Low	16 Pre F, 20-40g	OC,BAP, NTX
Kerstsetter et al, 2015, USA, 18mo[68]	Mean (SEM): 72.9(1.8) Maltodextrin Group, 73.9(1.9) Whey Group	45g Whey protein or Isocaloric maltodextrin	+45g/d	Men over 70 y and women over 60 years, n=121	BMD, P1NP, CTX, OC
Khalil et al, 2002, US, 3mo[86]	No information in paper	Soy vs Milk protein (40g)	+40g/d	64 M, 59.2+/-17.6y	BAP, DPYD

Study, Country, Duration	Baseline protein intake	Supplement vs control	Change in protein intake	Subject Total n	Outcomes Measured
Lampl et al. 1978, New Guinea, 8 mo[92]	11g/d	Normal diet (11g/d)vs. normal diet plus 20g/d milk protein supplement	31g/d	7-13 year old Bundi children, male and female, with low protein intakes	Periosteal breadth , Endosteal breadth, Compact bone breadth
Martin-Bautista et al. 2011, Spain, 4 mo[93]	Mean(SD): 34.1(26.0)g/d Collagen , 33.0(18.1)g/d Placebo	Drink containing partially hydrolysed collagen vs. placebo drink	+4.25g/d	60 children aged 6-11 years	BAP, OC, TRAP, CTX
Roughead et al, 2003, USA, 8wk Cross over study[81]	No information in paper	High meat diet: 117g/d (20% of energy) versus low meat diet: 68g/d (12% of energy)	+49g/d	15 POM F	HPO, OC, NTX, BAP
Schurch et al, 1998 Switzerland, 6mo[95]	Mean (SD) 45.0 (15.2) g/d protein group vs. 51.0 (19.0) g/d control group	Total protein (20g/d) vs placebo	+20g/d	82 Elderly M/F 80.7y+/-7.4	%Change data only: DPYD, FSBMD, OC, PFBMD, PYD, BMD
Shapses et al, 1995, USA, crossover study, 5d[84]	0.99 g/Kg/d (no information in g)	LPHC(0.44g/Kg/D protein, p) vs. HPHC (2.71g/kg/d, I) Calcium in both groups=1600mg/d	-0.55g/Kg/d Low +1.70g/Kg/d High	21-42 year old males and females	HPO
Spence et al, 2005, USA, crossover study 28d per phase[79]	No information in paper	Soy protein isolate without isoflavones diet (96g/d) vs. casein-whey protein diet (91g/d)	+0 (as comparing protein type not dose, although 5g/d difference in actuality)	N=15 Post F	BAP ,OC ,NTX
Tkatch et al, 1992, Switzerland, 38days[72]	No information in paper	20.4g/d Protein in nutritional supplement vs. the same nutritional supplement without protein	+20.4g/d	62 M/F elderly, mean age 82y	BMD,OC
Toba et al 2001, Japan, 16d[90]	No information in paper	MBP (300mg/d) vs inactive placebo	+300 mg/d MBP	30 M, 36.2y+/-8.5	NTX,OC

Study, Country, Duration	Baseline protein intake	Supplement vs control	Change in protein intake	Subject Total n	Outcomes Measured
Uenishi et al, 2007, Japan, 6mo[70]	No information in paper	40mg/d MBP vs inactive placebo	+40mg/d MBP	35 Pre F	BMD
Vupadhyahula et al, 2009, USA[75]	Mean (SD): 62.9(1.9) Soy protein group, 61.3(1.6) Milk Protein group	25g soy protein (no isoflavones), 25g milk (casein, whey) protein	+25g/d	203 Post F Mean (SE) age 64 0.6)y	BMD, NTX
Yamamura et al, 2002, Japan[77]	No information in paper	MBP(40mg) vs inactive placebo	+40mg/d MBP	33 Pre F	BMD
Zhu et al, 2011, AUS, 2y[76]	Mean(SD): 76(18)g/d High protein group, 76(16) Low protein placebo group	High protein drink 30g vs. low protein drink (placebo) 2.1g	+27.9g/d	219 70-80 year old women	vBMD, BMD
Zou et al 2009, China, 8 mo[71]	No information in paper	Milk with 40mg MBP vs. Milk without MBP	+40mg/d MBP	57 women, 20 years old	BMD

AUS, Australia; BAP, Bone Alkaline Phosphatase; BMC, Bone Mineral Content; BMD, Bone Mineral Density; CTX, C-Terminal Peptide of Collagen; DPYD, Deoxypyridinoline; FSBMD, Femoral Shaft Bone Mineral Density; HCHP, High Calcium High Protein; HCLP, High Calcium Low Protein; HPO, Hydroxyproline; LCHP, Low Calcium High Protein; LCLP, Low Calcium Low Protein; MBP, Milk Basic Protein; NTX, N-Terminal Peptide of Collagen; OC, Osteocalcin; P1NP, Procollagen type 1 N-Terminal Propeptide; PERI, Perimenopausal; PFBMD, Proximal Femoral Bone Mineral Density; POM, Postmenopausal; PRE, Premenopausal; PYD, Pyridinoline; SPI, Soy Protein Isolate; TRAP, Tartrate Resistant Alkaline Phosphatase; vBMD, volumetric Bone Mineral Density

Figure Captions

Fig. 1 PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses flow diagram to illustrate search results

Fig. 2 Protein intake and all low trauma fractures (lowest intake category: RR=1) Animal (top) Vegetable (middle) Total Protein (bottom)

Online Resource Caption

Electronic Supplementary File containing further information on study methods and results as well as all Supplementary Figures and Tables.