

1 **Protein and Bone Health across the Lifespan**

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18 **Abstract**

19 Bone health is determined by the rate of accrual in early life, followed by the rate of age  
20 associated bone loss. Dietary protein intake might have a role in bone health across both of  
21 these phases via pleiotropic mechanistic pathways. Herein we summarise the pathways  
22 through which protein may exert either a positive or negative influence on bone. In *Section 1*,  
23 we describe the acid-ash hypothesis, which states that a high protein intake may lead to an  
24 acidic residue that must be neutralised through the leaching of calcium and other minerals  
25 from the bone, subsequently leading to demineralisation and bone weakening. Conversely,  
26 and as described in *Section 2*, protein intake may act to strengthen bone by stimulating the  
27 activity of various anabolic hormones and growth factors, or by optimising muscle mass and  
28 functionality, which itself has an osteogenic influence. The net effect of these contrasting  
29 pathways is described in *Section 3*, where a number of meta-analyses have demonstrated that  
30 higher protein intakes have a small positive impact on bone mass and fracture risk.  
31 Sometimes higher than recommended protein intakes are advised, *e.g.*, during the earlier and  
32 later phases of the lifespan or during reduced energy availability. We conclude that protein is  
33 an essential nutrient for bone health, although further research is required to clarify the  
34 mechanistic pathways through which it exerts its influence, along with clarification of the  
35 quantities, food sources and timing to allow for the optimisation of this protective influence  
36 and ultimately a reduction in fracture risk.

## 37 **1. Introduction**

38 During childhood and adolescence bone mass rapidly increases, such that approximately 90%  
39 of bone mass is acquired by the age of 20 years<sup>(1,2)</sup>. Thereafter, bone mass enters a period of  
40 relative stability before beginning an age related decline as we enter later middle age. This  
41 response occurs in both men and women but, in general, men have greater bone mineral  
42 density (BMD) than women, while women also have a slightly higher rate of age-related  
43 BMD decline, particularly during the early postmenopausal period<sup>(3)</sup>. A normal rate of bone  
44 loss does not tend to present a major clinical problem unless the individual did not generate a  
45 high enough peak bone mass during childhood and adolescence; under these circumstances  
46 the development of osteopenia or osteoporosis can become clinically relevant issues. Even  
47 with a reasonable degree of bone accrual during childhood and adolescence, these conditions  
48 can still develop during older age with an accelerated rate of bone loss, which can occur as a  
49 result of an imbalance between osteoclast-mediated bone resorption and osteoblast-mediated  
50 bone formation; whereby the rate of bone resorption exceeds the rate of bone formation<sup>(4)</sup>.

51

52 Osteoporosis is “a progressive systemic skeletal disease characterised by low bone mass and  
53 micro-architectural deterioration of bone tissue, with consequent increase in bone fragility  
54 and susceptibility of fracture”<sup>(5)</sup> and is usually indicated by comparing BMD values to young  
55 healthy individuals of the same sex, thus generating a T-score. To standardise the diagnosis  
56 of osteoporosis, the WHO categorised a T-score of -1 or more as normal, with a score of  
57 between -1 and -2.5 being indicative of osteopenia and a score of -2.5 or below defining  
58 osteoporosis<sup>(5)</sup>. A z-score can also be calculated, usually in older individuals to indicate a  
59 severity of osteoporosis, by comparing an individual’s BMD to that of age-matched  
60 individuals with normal bone mass<sup>(6)</sup>. Areal BMD (aBMD), as generated using dual-energy  
61 X-ray absorptiometry (DXA), only accounts for around 60 to 70% of the variance in bone  
62 strength<sup>(7)</sup>, however, and there is a need to consider volumetric BMD, bone geometry and  
63 bone architecture in the context of bone strength, as highlighted by the WHO definition.

64

65 22 million women and 5.5 million men in the EU<sup>(8)</sup> are affected by osteoporosis, which, in  
66 itself, is not necessarily a major clinical problem, but does increase the risk of developing an  
67 osteoporotic fracture; a major clinical problem affecting both the quality and quantity of  
68 one’s life<sup>(9)</sup>. There were 3.5 M osteoporotic fractures in the EU in 2010; 620 000 of which  
69 were hip fractures, 520 000 of which were vertebral fractures, 560 000 of which were  
70 forearm fractures and 1 800 000 of which were classified as ‘other fractures’<sup>(8)</sup>. The UK

71 Office for National Statistics predicted, in 2016, that the prevalence of osteoporosis will  
72 increase in the coming decades as a direct result of population ageing, with over a third of the  
73 UK population being over 50 years of age<sup>(10)</sup>. Additionally, failure to meet physical activity  
74 guidelines is common-place in today's society, with negative implications for numerous  
75 chronic health conditions<sup>(11)</sup>, including reduced bone mineral density<sup>(12,13)</sup>. Should current  
76 societal trends toward reduced physical activity, and increased sedentary behaviours  
77 continue, the prevalence of lifestyle associated conditions, such as osteoporosis, might also  
78 increase.

79

80 There are a number of non-modifiable (*e.g.*, genetics, age, sex and race) and modifiable (*e.g.*,  
81 exercise, diet and smoking) factors that influence both bone accrual and loss. Among the  
82 modifiable risk factors, the mechanical loading achieved through some types of exercise  
83 undoubtedly has the largest positive effect on the bone, with high-impact, multi-directional  
84 type activities generally considered to provide the greatest osteogenic stimulus<sup>(14,15)</sup>. In  
85 contrast, smoking is clearly deleterious<sup>(16)</sup>. With regards to nutrition, the macronutrients (*e.g.*,  
86 carbohydrate, fat and protein) and many micronutrients (*e.g.*, calcium, vitamin D, vitamin K,  
87 magnesium, potassium, phosphorus, etc) are known to modulate bone<sup>(17)</sup>. Of these, perhaps  
88 one of the most interesting nutrients is protein, partly because it has been suggested to exert  
89 both positive and negative effects.

90

91 Protein makes up around half of the bones volume and around 33% of its mass<sup>(18)</sup> and the  
92 structural matrix of bone consists of protein encased in a crystalline mineral<sup>(19)</sup>. Given this  
93 and the fact that collagen and non-collagenous proteins form the organic matrix of bone, it  
94 would seem logical to suppose that there might be an important role for dietary protein intake  
95 on bone accrual during childhood and adolescence and in the maintenance of bone health in  
96 older age. In contrast, however, early findings<sup>(20)</sup> have suggested that there might be a  
97 negative impact of a high dietary protein intake on bone, largely due to a greater loss of  
98 calcium from the skeleton in order to offset an increase in acid load.

99

100 Theoretical evidence exists to support the fact that there might be both positive and negative  
101 effects of protein on the bone, but there is limited consensus on whether protein is, in fact, a  
102 bone protective or harming nutrient. The aim of this review is to summarise the potential  
103 mechanisms that may lead to either a positive or a negative influence of protein on bone. We  
104 will subsequently consider evidence on the influence of dietary or supplementary protein

105 intake on indicators of bone health, thus evaluating the net effect of these, at times  
106 conflicting, pathways. Finally, we will consider situations whereby higher than recommended  
107 protein intakes may be advisable, as well as making recommendations for on-going research  
108 and practice in this area.

109

## 110 ***2. AGAINST – Mechanisms through which protein may negatively impact bone***

111 For many years, the role of protein in bone health has been questioned, with many postulating  
112 that high dietary protein intakes could be detrimental to bone, due to the acidic load that this  
113 may impose on the body<sup>(21,22)</sup>. This has been termed the “acid-ash hypothesis” and is  
114 summarised in Figure 1. The body requires a close to neutral pH for optimal function, and  
115 deviations from this homeostatic set-point can have widespread metabolic and physiological  
116 consequences<sup>(23,24)</sup>. Accordingly, the body has a wide range of mechanisms designed to  
117 regulate pH and to prevent large deviations toward either an acidic or alkaline  
118 environment<sup>(25,26)</sup>. It has long been recognised that the metabolism of foods results in the  
119 production of an acidic or alkaline residue, and therefore usual dietary intake can  
120 theoretically influence the pH of the body. The potential renal acid load (PRAL) of an  
121 individual’s habitual dietary intake can be calculated using validated algorithms<sup>(27-29)</sup>, and  
122 this calculation provides an indication of the net endogenous acid production within the body.  
123 PRAL is proportional to acid producing elements, including protein and phosphorus, and  
124 inversely related to alkaline elements, including potassium, calcium and magnesium. It has  
125 long been suggested that protein, and mainly animal proteins that have a high content of  
126 sulphur containing amino acids, have an acidic effect on the body, while fruits and vegetables  
127 generally have an alkaline influence. Thus, a diet high in animal proteins, and low in fruits  
128 and vegetables, has been proposed to induce a state of low-grade metabolic acidosis, with  
129 wide-ranging consequences for various metabolic processes<sup>(30)</sup>. One of the main  
130 physiological processes thought to be impacted by low-grade metabolic acidosis is bone  
131 metabolism<sup>(31)</sup>. The reason for this is that an excess intake of acid-producing foods requires a  
132 proportionate amount of alkaline substances in order to neutralise this effect. If these alkaline  
133 substances are not present in the diet, they must be attained from another source. Bone tissue  
134 has numerous physiological roles within the body, one of which is to act as a reservoir of  
135 minerals, most of which have alkaline properties. It has been proposed, therefore, that during  
136 a state of low-grade metabolic acidosis, as may occur with high dietary protein intakes,  
137 minerals such as magnesium, potassium and calcium will be excreted from the bone into the  
138 blood stream, thus allowing for neutralisation of excess acid and a return to neutral pH<sup>(30,31)</sup>.

139 A large body of evidence exists that theoretically supports the acid-ash hypothesis. A meta-  
140 analysis provided strong evidence that diets with high PRAL are indeed associated with  
141 higher urinary calcium excretion rates<sup>(32)</sup>. Indeed, if these losses continued unchecked over  
142 time, reported calcium losses of 66mg·day<sup>-1</sup>, would lead to a loss of 24g, or approximately  
143 2% of total skeletal mineral mass per year<sup>(32)</sup>. Large cross-sectional studies have reported an  
144 inverse relationship between net endogenous acid production (NEAP) and BMD, and a  
145 positive association between NEAP and indicators of bone resorption<sup>(33,34)</sup>, thus strengthening  
146 the belief that an acidic diet may be detrimental to bone. In further support of the acid-ash  
147 hypothesis, was a 4 day acute, cross-over trial, which reported that an alkaline diet inhibited  
148 bone resorption, while an acidic diet promoted urinary calcium and c-telopeptide of type 1  
149 collagen ( $\beta$ -CTX) excretion, demonstrating that an acidic diet may disrupt bone metabolism  
150 toward a resorptive state<sup>(35)</sup>. The findings of these human studies are supported by *in vitro*  
151 evidence, which indicated that osteoblasts cultured at a pH of 7.4 are capable of abundant  
152 mineralisation that progressively declined with reduced pH until mineralisation halted at a pH  
153 of approximately 6.9<sup>(36)</sup>. Similarly, osteoclast activity is stimulated by an acidic environment,  
154 thus elevating bone resorption<sup>(37)</sup>.

155

156 The acid-ash hypothesis has led to wide-spread belief that an increased calcium excretion as a  
157 result of high protein intakes, will lead to subsequent bone demineralisation. Accordingly,  
158 traditional dietary advice has suggested that high dietary protein intake should be avoided in  
159 order to protect the structural integrity of the bone tissue. The acid-ash hypothesis is,  
160 however, based upon the assumption that the excess calcium excreted when individuals  
161 consume a high-protein diet derives from skeletal demineralisation. Kerstetter *et al.*  
162 investigated this by administering doubly labelled calcium isotopes in conjunction with a  
163 moderate and high protein diet for 10 days; showing that the hypercalciuria induced by the  
164 high-protein diet actually derived from dietary calcium intake, and not, as previously  
165 assumed, from the bone<sup>(38)</sup>. Increased calcium excretion during periods of high protein intake  
166 may, in fact, derive from other sources, including a modulation of calcium renal handling, or  
167 an increase in gastrointestinal calcium absorption<sup>(39)</sup>. Mangano *et al.* demonstrated the  
168 importance of nutrient to nutrient interactions between protein and calcium intakes and  
169 kinetics by investigating the relationship between dietary acid load, supplemental calcium  
170 and BMD in 1,218 men aged >60 years. They showed an inverse relationship between PRAL  
171 and proximal femur BMD in men consuming <800mg of calcium per day, but no association

172 between dietary acid load and BMD in men consuming >800mg of calcium per day<sup>(40)</sup>.  
173 Similarly, Dawson-Hughes *et al.* showed that higher total protein intake was associated with  
174 improved BMD in a group that were supplemented with calcium and vitamin D, but not in  
175 those who were not supplemented. Consideration of the proportion of protein intake obtained  
176 from animal or plant sources did not alter these results, demonstrating that it was the total  
177 amount, and not the source, of protein that was related to the identified BMD changes<sup>(41)</sup>.  
178 Thus, it appears that, although the acid-ash hypothesis has mechanistic merit, the actual  
179 influence of dietary acid load, and more specifically animal protein intake, on bone may be  
180 moderated by factors such as calcium availability and kidney function.

181

### 182 ***3. FOR – Mechanisms through which protein may positively impact bone***

183 In contrast to the widely held belief that high protein intake may be detrimental to bone, is  
184 evidence of various mechanisms, both direct and indirect, through which protein may be  
185 protective of bone<sup>(18,42)</sup>. Proteins are carbon, hydrogen, oxygen and nitrogen containing  
186 molecules, comprising polymers of amino acids, of which there are 20. The complexity of  
187 protein structure allows fulfilment of multiple and wide-ranging physiological roles,  
188 including functions in structural (collagen), contractile (myosin and actin), immune  
189 (antibodies) and regulatory (enzymes and hormones) processes<sup>(43)</sup>. Many of these processes  
190 are essential to the maintenance of bone structure and functionality, and thus adequate protein  
191 intake may be essential to the development and maintenance of a healthy bone. Bone  
192 comprises a protein matrix encased in a crystalline mineral, and bone has been estimated to  
193 comprise approximately 50% protein and 50% mineral<sup>(19)</sup>. Thus, bone strength is not solely  
194 dependent upon mineralisation, but will also depend upon the integrity of its protein  
195 components. As such, protein has an essential and direct structural function to fulfil in bone  
196 metabolism.

197

198 In addition to its structural role, adequate protein intake is essential to stimulate the activity  
199 of anabolic hormones and growth factors<sup>(44,45)</sup>, most of which have essential roles in the  
200 regulation of bone mass and micro-architecture<sup>(46–49)</sup>. For example, dietary protein intake  
201 contributes to the regulation of the insulin like growth factor 1 (IGF-1)<sup>(50)</sup>, although given the  
202 effect of protein intake on circulating insulin concentrations, the independent effects are  
203 somewhat tricky to determine. The IGFs are a group of pleiotropic growth factors, whose  
204 effects are in many ways mediated through the action of growth hormone<sup>(51)</sup>, but which also  
205 exert direct anabolic influences<sup>(52)</sup>. These factors are widely recognised as having a key role

206 to play in the processes linking dietary intake and growth<sup>(53)</sup>, and exert multiple influences on  
207 bone<sup>(48,49)</sup>. These influences include chondrocyte proliferation and differentiation, as well as  
208 the stimulation of osteoblast activity<sup>(42)</sup>. Additionally, IGF-1 is purported to exert an  
209 influence on bone resorption<sup>(54)</sup>, by mediating the stromal cell expression of osteoprotegerin  
210 (OPG) and its ligand<sup>(55)</sup>. Given its potential role in the regulation of both bone formation and  
211 resorption, it has been suggested that IGF-1 may aid in the mediation of the complex  
212 coupling processes of bone remodelling<sup>(56,57)</sup>, thus directly modulating the influence of  
213 nutritional intake on bone metabolism. IGF-1 may also indirectly act to regulate bone through  
214 a role in the moderation of calcium absorption<sup>(58)</sup>. This influence may occur, at least in part,  
215 due to an increased renal conversion of the inactive 25 hydroxyvitamin D3 to its active form,  
216 1, 25 dihydroxy-vitamin D3<sup>(59)</sup>. It has also been suggested, however, that other, non-Vitamin-  
217 D related pathways, may contribute to the influence of IGF-1 on calcium absorption,  
218 although research is ongoing to more fully elucidate these<sup>(58)</sup>. Dietary protein intake has been  
219 reported to be inversely related to sex hormone binding globulin (SHBG) concentration<sup>(60)</sup>.  
220 SHBG is a plasma glycoprotein whose primary biological action is to bind, and thereby  
221 inactivate, many of the androgens and estrogens<sup>(61)</sup>. Both androgens and estrogens are  
222 recognised as exerting pleiotropic osteogenic effects<sup>(46,47)</sup>, and thus their bio-availability, as  
223 determined by SHBG concentration, will exert multiple influences on bone metabolism.  
224 Indeed, SHBG content has previously been reported to predict bone mass in a number of  
225 populations<sup>(62,63)</sup>.

226

227 Lean body mass exerts an important moderating influence on bone; thus dietary protein  
228 intake may indirectly influence bone through its impact on lean muscle mass. It is widely  
229 recognised that protein intake is an essential component governing lean muscle mass and  
230 functionality<sup>(64)</sup>, and in determining the response of muscle to exercise and training<sup>(45,65)</sup>. In  
231 turn, lean body mass is recognised as one of the strongest predictors of bone mass<sup>(66)</sup>.  
232 Additionally, physical loading is recognised as the primary determinant of bone mass and  
233 architecture<sup>(14,15)</sup>, with both gravitational and muscular loading known to stimulate the bone  
234 remodelling cycle, and ultimately to enhance bone<sup>(67)</sup>. The strong body of evidence  
235 supporting a positive influence of protein intake on muscle mass and function is therefore  
236 likely to indirectly and positively influence bone.

237

238 In fact, a myriad of mechanistic pathways exist, which may govern the influence of dietary  
239 protein intake on bone. These include the influence of protein on the calcium/vitamin



240 D/parathyroid axis, moderation of various nutrient-regulated hormones, including the  
241 androgens, estrogens and incretins, along with its influence on the absorption and action of  
242 other nutrients, *e.g.*, calcium, that directly impact bone. Additionally, the individual protein  
243 components, namely isolated amino acids, also act to regulate bone metabolism through a  
244 wide range of mechanisms<sup>(68)</sup>. An in-depth discussion of all of these factors is beyond the  
245 scope of this review, but the examples provided herein do, however, serve to highlight how  
246 dietary protein intake may act to mediate the actions of hormones and growth factors that  
247 regulate bone metabolism, and ultimately, its strength and functionality.

248

#### 249 ***4. The influence of dietary or supplementary protein intake on bone***

250 It is clear from the information described in the previous sections, that protein intake has the  
251 capacity to influence bone through a wide range of mechanisms, and that this influence may  
252 theoretically be either positive or negative. But what is the net effect of these pleiotropic, and  
253 at times conflicting, mechanisms on bone? A significant body of literature, based on diverse  
254 designs and populations, has evaluated the net effect of dietary or supplemental protein intake  
255 on bone. In the interest of conciseness, and to focus on studies that have been deemed to be  
256 of high quality, and with low risk of bias, we will focus our discussion on the results of meta-  
257 analyses that have been conducted to synthesise and evaluate the influence of dietary or  
258 supplemental protein intake on bone. For further information on this topic area, readers are  
259 referred to the recent comprehensive summary by Rizzoli *et al.*<sup>(69)</sup>.

260

#### 261 *Meta-analyses directly investigating the acid-ash hypothesis*

262 A number of meta-analyses have been conducted to specifically test elements of the acid-ash  
263 hypothesis<sup>(32,70–72)</sup>. Briefly, and as described in Section 2, this hypothesis states that a  
264 prolonged and high intake of acid forming foods, such as animal proteins, may cause a state  
265 of low-grade metabolic acidosis within the body. This may subsequently lead to bone  
266 demineralisation, as calcium and other minerals are excreted from the bone in order to  
267 neutralise excess dietary acid, and restore the neutral pH, which the body requires for optimal  
268 function. In support of this hypothesis Fenton *et al.* conducted a meta-analysis to assess the  
269 relationship between net acid and calcium excretion. The authors identified a linear  
270 relationship between urinary acid and calcium excretion, consistent with proponents of the  
271 acid-ash hypothesis<sup>(32)</sup>. They also raised an important point, however, in that the linear  
272 relationship identified between net acid and calcium excretion, does not provide any evidence  
273 related to the source of excess calcium excretion, and therefore the results of that particular

274 meta-analysis could not be taken to infer bone loss as a result of a high acid-producing  
275 diet<sup>(32)</sup>. Indeed, the same group subsequently conducted investigations regarding the  
276 influence of diet acid load on calcium balance<sup>(70)</sup>, and on the influence of supplemental  
277 dietary phosphate on indicators of calcium balance and bone metabolism<sup>(71)</sup>. Despite the  
278 linear relationship between diet acid load and calcium excretion reported in their first meta-  
279 analysis, Fenton *et al.* subsequently reported that diet acid load had no influence on net  
280 calcium balance, nor on bone resorption, as assessed by N-telopeptides<sup>(70)</sup>, demonstrating  
281 that, although an increased dietary acid load did cause increased calcium excretion, this did  
282 not influence overall net calcium balance. This likely occurred due to other influences of  
283 protein on bone, such as in increase in dietary calcium absorption<sup>(39)</sup>. Additionally, meta-  
284 analysis of all data that reported the effect of manipulated dietary phosphate on bone  
285 outcomes indicated that dietary phosphate consumption caused a reduction of urinary calcium  
286 excretion, even when the phosphate salt used had a high acid load<sup>(71)</sup>. This finding was in  
287 direct opposition to the acid-ash hypothesis, given that it considers phosphate to be one of the  
288 main acid forming components of our diets, suggesting that this should have led to an  
289 increase in calcium excretion and bone demineralisation. Further disputing the acid-ash  
290 hypothesis, were meta-analytic data from Shams-White *et al.*, who investigated the  
291 differential impact of soy versus animal based proteins on calcium balance and bone  
292 outcomes, reporting no difference between these dietary protein sources<sup>(73)</sup>, thus disproving  
293 the widely held belief that animal proteins convey a greater acidic load, and subsequently, a  
294 higher degree of bone demineralisation, than plant based proteins. Finally, Fenton *et al.*  
295 published a comprehensive meta-analysis, in which they applied Hill's epidemiological  
296 criteria for causality model to conclusively evaluate the state of science regarding the  
297 influence of dietary acid load on bone outcomes<sup>(72)</sup>. Hill's model considers causality in  
298 relation to 5 criteria, namely temporality, strength, biological gradient, plausibility,  
299 consistency and experiment. The authors considered 55 studies of varying designs, all of  
300 which were deemed to be of high quality and with low risk of bias. They concluded that there  
301 was no causal association between dietary acid load and osteoporotic disease and, as such,  
302 that an alkaline diet was not protective of bone health<sup>(72)</sup>. Indeed, pH regulation is essential  
303 for usual metabolic function, and accordingly, the body has a wide range of mechanisms  
304 designed to maintain the internal environment of the body fluids, with the kidneys having an  
305 essential role in regulating the acid-base environment of the body<sup>(74)</sup>. Homer W. Smith<sup>(75)</sup>  
306 stated that "*the composition of the body fluids is determined not by what the mouth takes in,*  
307 *but what the kidneys keep*", and the scientific evidence collectively indicates that the

308 maintenance of acid-base balance can be achieved without undue detriment to the bone, due  
309 to the wide range of regulatory mechanisms that have evolved in order to protect the neutral  
310 environment of our bodies.

311

312 *Meta-analyses investigating the influence of protein on BMD and fracture risk:*

313 The meta-analyses described above indicate that dietary acid load is unlikely to lead to bone  
314 demineralisation, as postulated by the acid-ash hypothesis. These investigations do not,  
315 however, describe the potential of protein to influence bone mineral density, or fracture risk,  
316 both of which are important indicators of bone strength and functionality. Although it has its  
317 limitations, bone mineral density (BMD) assessed by dual energy x-ray absorptiometry  
318 (DXA) scanning is commonly accepted as the principal diagnostic tool for bone disorders  
319 such as osteoporosis<sup>(76)</sup>. Meta-analyses investigating the influence of dietary protein intake  
320 collectively indicate a positive, albeit small, effect of higher dietary protein intakes on BMD  
321 at various sites<sup>(77-79)</sup>. Darling *et al.* reported a positive association between dietary protein  
322 intake and BMD at all sites, although the estimated effect was small, with dietary protein  
323 intake only accounting for 1-2% of the total variation in bone density<sup>(77)</sup>. In relation to studies  
324 investigating the influence of supplemental protein, an effect was identified at the lumbar  
325 spine site only<sup>(77)</sup>. More recently, Shams-White *et al.* conducted a comprehensive meta-  
326 analysis of 16 high-quality RCT's and 20 prospective cohort studies, and reported a positive  
327 effect of higher protein intake on BMD at the lumbar spine, but not at the other sites  
328 investigated (total hip, femoral neck and total body). In addition, they did not show any effect  
329 of higher protein intake on bone turnover marker concentrations<sup>(78)</sup>. In agreement with the  
330 findings of Darling *et al.*<sup>(77)</sup>, the effect of protein on BMD was small, with a net percentage  
331 change of 0.52% (95%CI: 0.06 – 0.97%<sup>(78)</sup>). Collectively, these meta-analyses indicate a  
332 beneficial, albeit small, influence of higher protein intakes on BMD. Ultimately, however, the  
333 main outcome of interest when assessing the influence of dietary protein on bone health is the  
334 susceptibility of the individual to fracture. Fracture risk is a complex and multi-factorial  
335 phenomena, and there is no one outcome measure that can conclusively indicate who will  
336 fracture and who will not. As such, randomised controlled trials investigating the influence of  
337 supplemental or increased dietary protein are not available, and meta-analyses in this area  
338 have focused their attention on prospective cohort studies that have investigated the  
339 relationship between dietary protein intake and the occurrence of fracture<sup>(77-80)</sup>. These meta-  
340 analyses have reported mixed results, with two large meta-analyses reporting no influence of

341 higher protein intakes on fracture risk<sup>(77,78)</sup>, while two others concluded that there was some  
342 evidence that higher protein intakes could reduce hip fracture risk<sup>(79,80)</sup>.

343

344 Collectively, the available meta-analyses, which represent the highest level of evidence  
345 currently available, indicate no adverse effect of higher protein intakes on bone. Conversely,  
346 the available evidence appears to indicate a small but beneficial influence of higher protein  
347 intakes on BMD, along with a potential reduction in hip fracture risk. It is important to  
348 identify that the meta-analyses described herein, generally focused on variation in protein  
349 intake within recommended ranges. As such, they were not designed to identify whether  
350 higher protein intakes, above the recommended daily intakes, are protective or harmful to  
351 bone? This is important, as it is generally recognised that most nutrients tend to exert a  
352 biphasic response, whereby optimal intakes exert a stimulatory and beneficial response, while  
353 lower or higher intakes may be harmful or inhibitory. Wallace *et al.* investigated this topic,  
354 by conducting a meta-analysis of those randomised controlled trials, and prospective cohort  
355 studies, that specifically investigated the influence of dietary protein intake above the current  
356 US recommended daily allowance (RDA) of 0.8 g·kg·day<sup>-1</sup><sup>(79)</sup>. The authors critically  
357 synthesised the evidence from 16 randomised controlled trials (RCTs) and 13 prospective  
358 cohort studies, and concluded that protein intakes above the current RDA could be beneficial  
359 in reducing fracture risk and preventing bone loss. No adverse effect of protein intakes above  
360 the current RDA was identified. Further disputing the notion that very high protein intakes  
361 may be harmful to bone, was evidence from a recent original study, that reported no influence  
362 of 6 months of dietary protein intakes far in excess of the current RDA (>2.2g·kg·day<sup>-1</sup>) on  
363 total body or lumbar spine BMD in well-trained women<sup>(81)</sup>.

364

##### 365 ***5. Situations in which bone potentially requires higher protein intakes: The influence of*** 366 ***lifespan, reduced energy availability and weight loss***

367 As described above, there is no evidence of an adverse effect of higher protein intakes on  
368 bone, while some evidence of a positive influence on fracture risk and BMD exists.  
369 Recommendations related to the optimal protein intake to support bone health is an ever-  
370 evolving topic, and a myriad of factors must be considered when assessing the protein  
371 requirements of any one individual. Notwithstanding this complexity, there is some evidence  
372 to support an osteogenic influence of protein intakes above the current RDA of 0.8g·day<sup>-1</sup> in  
373 certain situations; namely childhood, adolescence and old age, and in situations characterised  
374 by reduced energy availability.

375 *Lifespan*

376 It is generally recognised that there are three distinct phases of bone development throughout  
377 the lifespan, namely: 1) Bone accrual (birth - ~30 years); 2) Relative bone stability (~30 –  
378 ~45 years) and 3) Bone loss (~>45 years)<sup>(82)</sup>. Phases 1) and 3) are critical points in the overall  
379 maintenance of bone health, and optimisation of bone accrual, followed by minimisation of  
380 age-related bone losses, are essential to prevent subsequent development of bone disorders,  
381 such as osteoporosis<sup>(83)</sup>. Physical activity, and the subsequent muscular and gravitational  
382 loads that it conveys on bone<sup>(14)</sup>, is recognised as an essential determinant of bone accrual  
383 and maintenance throughout the lifespan<sup>(84)</sup>. Additionally, it seems that higher protein intakes  
384 may support these processes. Chevalley *et al.* reported that higher than median protein  
385 intakes enhanced the positive impact of physical activity on bone accrual in prepubertal  
386 boys<sup>(85)</sup>. Accordingly, children and adolescents have higher RDA's for protein than adults,  
387 namely, 1 – 3yrs: 1.2g·kg·day<sup>-1</sup>; 7 – 14yrs: 1g·kg·day<sup>-1</sup>; 15 – 18yrs: 0.9g·kg·day<sup>-1</sup>, with all other  
388 groups, apart from infants and athletes, recommended to intake 0.8g·kg·day<sup>-1</sup><sup>(86)</sup>. Dairy  
389 products are often promoted as an ideal whole food to promote bone accrual in early years<sup>(87)</sup>  
390 due to their nutritional composition, which comprises a high proportion of high-quality  
391 protein, with the term “high-quality” referring to a protein source containing all essential  
392 amino acids. Additionally, dairy foods are abundant in micronutrients deemed essential to  
393 bone, including calcium, magnesium and phosphorus<sup>(88)</sup>. Indeed an adequate intake of dairy  
394 products, typically defined as 2 - 3 servings of dairy per day, along with weight-bearing  
395 activity, have been recommended as important strategies to optimise bone accrual in the  
396 earlier stages of the lifespan<sup>(83)</sup>.

397

398 Bone loss and a subsequent increase in fracture risk is a well-known complication of ageing.  
399 Indeed osteoporotic fractures are associated with a wide range of adverse social and  
400 economic consequences<sup>(9)</sup>. Many of the pharmacological interventions intended to prevent or  
401 reverse bone loss have numerous adverse effects, limiting their long-term use<sup>(89)</sup>.  
402 Accordingly, lifestyle strategies to protect and maintain bone throughout the lifespan are  
403 desirable. Exercise and physical activity habits are considered important to this process.  
404 Protein intakes may be particularly relevant for older adults to negate the negative  
405 consequences of senescence, and higher than the currently recommended daily protein  
406 intakes have been suggested to be required to protect bone in older adults<sup>(90)</sup>, as well as to  
407 enhance muscle mass and function<sup>(91)</sup>.

408

409 *Reduced Energy Availability and Weight Loss*

410 A key factor in the regulation of bone is the amount of available energy for this process.  
411 Strong evidence exists supporting a negative impact of both acute and chronic exposure to  
412 reduced energy availability on bone health<sup>(92)</sup>. Markers of bone formation have been reported  
413 to be reduced in response to low energy availability (defined as  $<30 \text{ kcal}\cdot\text{kgLBM}\cdot\text{day}^{-1}$ <sup>(93,94)</sup>),  
414 and this is thought to occur in an attempt to preserve energy for more immediately essential  
415 functions, such as respiration, thermoregulation and necessary movement<sup>(95)</sup>. Although the  
416 negative bone consequences of this phenomena have primarily been investigated in athletes  
417 who have very high levels of training related energy expenditure<sup>(96)</sup>, or individuals suffering  
418 from chronic eating disorders<sup>(97)</sup>, it may also have relevance for those undergoing weight loss  
419 interventions. There is a long-held belief that obesity may be protective of bone health, which  
420 is based on the positive associations reported between absolute body mass and bone  
421 mass<sup>(98,99)</sup>, along with evidence that some weight loss interventions may also lead to bone  
422 loss<sup>(100)</sup>. This likely occurs as a result of reduced energy availability, along with a concurrent  
423 loss of lean muscle mass. Accordingly, strategies to protect both bone and lean mass during  
424 weight loss are essential. Recently, we reported that increased adipose mass in overweight or  
425 obese populations is negatively correlated with bone mass, but only when accompanied by a  
426 relative reduction in lean mass, highlighting the importance of optimizing the relative  
427 proportion between adipose and lean mass when considering interventions to protect bone  
428 during weight loss<sup>(101)</sup>. Exercise based interventions appear to be the most logical way to  
429 achieve this. Importantly, evidence supports the efficacy of higher protein intakes to protect  
430 bone during exercise and diet induced weight loss<sup>(102)</sup>. Josse *et al.* investigated the influence  
431 of a higher intake of dairy foods, dietary calcium and protein during diet and exercise-  
432 induced weight loss on a range of bone metabolic markers<sup>(102)</sup>. They reported that higher  
433 protein and calcium intakes were protective of bone health, while still allowing equivalent  
434 weight loss due to the hypocaloric diet under investigation. This study did not allow isolation  
435 of the independent effects of protein and calcium, although it is widely recognised that these  
436 nutrients are likely to have interactive osteogenic effects. Additionally, higher protein intakes  
437 are recognised as being protective of muscle mass during periods of reduced energy  
438 availability<sup>(103)</sup>. As described earlier, muscle mass is an important mediator of bone  
439 remodelling, which occurs due to the mechanical loads that muscle conveys to bone.

440  
441  
442

## 443 **6. Concluding remarks and perspectives**

444 Even though evidence exists supporting pleiotropic mechanistic pathways through which  
445 protein intake may positively or negatively impact bone, the highest level of evidence  
446 available supports a net osteogenic influence of dietary protein intake on bone health. In the  
447 presence of adequate calcium intake along with normal kidney function, it appears that the  
448 potential renal acid load induced by a diet high in protein, be it animal or plant, does not lead  
449 to bone demineralisation, as purported by the acid-ash hypothesis. In contrast, evidence exists  
450 to support a positive, albeit small, effect of protein intake on bone mass and fracture risk,  
451 which likely occurs due to the influence of protein on anabolic hormones and growth factors,  
452 which themselves directly mediate bone metabolism, in addition to the indirect influence of  
453 high protein intake on lean muscle mass and function. Despite this, a number of important  
454 research questions remain, which must be answered before consensus regarding the optimal  
455 protein intake required to optimise bone health can be reached. Higher than recommended  
456 protein intakes appear to be supported in some situations, such as in athletes who have high,  
457 training related energy expenditure, and a high requirement for musculoskeletal repair and  
458 adaptation, individuals who have reduced energy availability, with and without the need to  
459 reduce body mass, or those in the earlier or later stages of the lifespan. Although higher than  
460 the current recommended protein intake of  $0.8\text{g}\cdot\text{kg}\cdot\text{day}^{-1}$  may be required in these situations,  
461 just how high these protein intakes should be is not clear. It is important that higher protein  
462 intakes do not occur at the expense of the adequacy of other nutrients, nor that they result in  
463 an inadvertent energy surplus, which may in itself negatively impact bone, particularly in  
464 sedentary individuals. It is widely recognised that physical loading is the main modifying  
465 variable that determines bone mass, strength and functionality. Surprisingly, very little is  
466 known about how protein intakes may moderate this effect, and this is an important area of  
467 future research. This may be particularly relevant in the earlier and latter stages of the  
468 lifespan. It is widely recognised that optimal bone accrual in the early years, and thus  
469 developing a homeostatic reserve to subsequently protect against age related bone loss, is a  
470 key factor determining the subsequent development (or otherwise) of osteoporosis and  
471 associated fractures. The combined influence of activity programs with protein intake in  
472 children and younger adults are therefore of importance. This assertion is supported by data  
473 from Chevalley *et al.*, who reported that higher protein intakes were associated with  
474 enhanced benefits of physical activity on BMD in a group of prepubertal boys<sup>(85)</sup>. Similarly,  
475 bone loss and fracture typically present themselves in the latter third of the lifespan, meaning

476 that strategies to protect bone in older adults, including the adequacy of protein intake, are  
477 highly important in the older population.

478

479 Importantly, and as described in this review, protein intakes do not impact bone health in  
480 isolation, and their ultimate impact may depend upon interactions with a wide range of other  
481 nutrients and metabolic factors. Acknowledgement of the complexity of these processes is  
482 important. Well-designed and rigorously controlled studies are required to isolate the  
483 mechanistic pathways through which protein may act to influence bone remodelling.  
484 Additionally, it is widely recognised that individual variation exists in response to virtually  
485 all nutritional interventions. Consideration of the individual response to controlled  
486 interventions that investigate the influence of protein on bone<sup>(104)</sup>, may allow for elucidation  
487 of factors that moderate this response, thus enhancing our understanding of the complex and  
488 potentially multifaceted influence of dietary or supplemental protein on bone. The results of  
489 these studies should be interpreted within the context in which they were investigated,  
490 however, and wider extrapolations avoided. Additionally, all proteins are not equal, nor  
491 should recommendation based research focus solely on the quantity of protein required. We  
492 support a whole-food approach to nutrition and whole foods comprise a combination of  
493 macronutrients, micronutrients and phytochemicals, the combination of which may ultimately  
494 impact their net effect on bone. Therefore research is needed to elucidate the influence of  
495 protein *per se*, as well as to investigate the potentially disparate influence of various whole-  
496 food protein sources. More recently, research attention has investigated the differential  
497 influence of the timing of protein intake, along with its distribution throughout the day. To  
498 date, little is known about how these factors may act to moderate the bone response to protein  
499 intake, which represents another exciting area of on-going research.

500

501 Knowledge related to the influence of protein intake on bone has exponentially increased in  
502 recent years, and it seems to be time to abandon the long-held belief that higher protein  
503 intakes lead to bone demineralisation, particularly in healthy individuals who have an  
504 adequate calcium intake. Ultimately, it seems clear that protein has the capacity to exert a  
505 protective influence on bone, and on-going research, designed to more fully investigate  
506 mechanistic pathways through which this occurs, along with clarification of optimal  
507 quantities, sources and timing, will allow for the optimisation of this protective influence,  
508 thus providing an effective, non-pharmacological and lifestyle orientated strategy to protect  
509 bone health.



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514

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516 ED has no conflict of interest to declare. CS has received a small honorarium from The Dairy  
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518

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782 **Figure Legends:**

783 **Figure 1:** Mechanisms through which protein may impact bone.

784 *Pathways: 1) Dietary protein upregulates the activity of various anabolic hormones and*  
785 *growth factors (e.g., IGF-1; androgens; oestrogens or incretins), which in turn exert an*  
786 *osteogenic influence. 2) Dietary protein positively impacts muscle mass and functionality,*  
787 *with indirect benefit to bone through the increased mechanical loading that this provides. 3)*  
788 *Dietary protein increased the renal acid load, inducing a state of low grade metabolic*  
789 *acidosis.  $Ca^{2+}$ , and other alkaline minerals are leached from the bone in order to neutralise*  
790 *pH, thus reducing acid load.  $Ca^{2+}$  is subsequently lost through an increased urinary*  
791 *excretion, thus causing bone demineralisation. 4) Dietary protein increases dietary calcium*  
792 *absorption, thus increasing serum calcium availability, allowing for pH neutralisation,*  
793 *without undue detriment to bone.*