Protein and Bone Health across the Lifespan

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Abstract

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

1. *Introduction*

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

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

22 million women and 5.5 million men in the EU[8] are affected by osteoporosis, which, in itself, is not necessarily a major clinical problem, but does increase the risk of developing an osteoporotic fracture; a major clinical problem affecting both the quality and quantity of one's life[9]. There were 3.5 M osteoporotic fractures in the EU in 2010; 620 000 of which were hip fractures, 520 000 of which were vertebral fractures, 560 000 of which were forearm fractures and 1 800 000 of which were classified as 'other fractures'[8]. The UK

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

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

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

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

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

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

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

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

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

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

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

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

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

206 to play in the processes linking dietary intake and growth[53], and exert multiple influences on
207 bone[48,49]. These influences include chondrocyte proliferation and differentiation, as well as
208 the stimulation of osteoblast activity[42]. Additionally, IGF-1 is purported to exert an
209 influence on bone resorption[54], by mediating the stromal cell expression of osteoprotegerin
210 (OPG) and its ligand[55]. 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[56,57], 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[58]. 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[59]. 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[58]. Dietary protein intake has been
219 reported to be inversely related to sex hormone binding globulin (SHBG) concentration[60].
220 SHBG is a plasma glycoprotein whose primary biological action is to bind, and thereby
221 inactivate, many of the androgens and estrogens[61]. Both androgens and estrogens are
222 recognised as exerting pleiotropic osteogenic effects[46,47], 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[62,63].
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[64], and in determining the response of muscle to exercise and training[45,65]. In
231 turn, lean body mass is recognised as one of the strongest predictors of bone mass[66].
232 Additionally, physical loading is recognised as the primary determinant of bone mass and
233 architecture[14,15], with both gravitational and muscular loading known to stimulate the bone
234 remodelling cycle, and ultimately to enhance bone[67]. 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

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

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

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

Meta-analyses directly investigating the acid-ash hypothesis

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

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

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

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

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

higher protein intakes on fracture risk[77,78], while two others concluded that there was some evidence that higher protein intakes could reduce hip fracture risk[79,80].

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

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

As described above, there is no evidence of an adverse effect of higher protein intakes on bone, while some evidence of a positive influence on fracture risk and BMD exists. Recommendations related to the optimal protein intake to support bone health is an ever-evolving topic, and a myriad of factors must be considered when assessing the protein requirements of any one individual. Notwithstanding this complexity, there is some evidence to support an osteogenic influence of protein intakes above the current RDA of $0.8 g \cdot day^{-1}$ in certain situations; namely childhood, adolescence and old age, and in situations characterised by reduced energy availability.

Lifespan

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

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

Reduced Energy Availability and Weight Loss

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

6. Concluding remarks and perspectives

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

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

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

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

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REFERENCES:

1. Henry YM, Fatayerji D, Eastell R (2004) Attainment of peak bone mass at the lumbar spine, femoral neck and radius in men and women: Relative contributions of bone size and volumetric bone mineral density. *Osteoporos Int* **15**, 263–273.
2. Recker R, Davies K, Hinders S *et al.* (1992) Bone gain in young adult women. *J Am Med Assoc* **268**, 2403–2408.
3. Hendrickx G, Boudin E, Van Hul W (2015) A look behind the scenes: The risk and pathogenesis of primary osteoporosis. *Nat Rev Rheumatol* **11**, 462–474.
4. Demontiero O, Vidal C, Duque G (2012) Aging and bone loss: New insights for the clinician. *Ther Adv Musculoskelet Dis* **4**, 61–76.
5. World Health Organization (1994) Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: Report of a WHO study group [meeting held in Rome from 22 to 25 June 1992]. Geneva: World Health Organization. http://www.who.int/iris/handle/10665/39142
6. Blake G, Fogelman I (2007) The role of DXA bone density scans in the diagnosis and treatment of osteoporosis. *Postgrad Med* J **83**, 509–517.
7. Ammann P, Rizzoli R (2003) Bone strength and its determinants. *Osteoporos Int* **14**, 13–18.
8. Hernlund E, Svedbom A, Ivergård M *et al.* (2013) Osteoporosis in the European Union: Medical management, epidemiology and economic burden: A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). *Arch Osteoporos* **8**, 136.
9. Cauley JA (2013) Public health impact of osteoporosis. *Gerontol - Ser A Biol Sci Med Sci* **68**, 1243–1251.

10. Park N (2016) Population estimates for UK, England and Wales, Scotland and Northern Ireland: Mid 2016 [Internet]. Office for National Statistics. Available from: https://www.ons.gov.uk/peoplepopulationandcommunity/populationandmigration/populationestimates/bulletins/annualmidyearpopulationestimates/mid2016
11. Blair S, Sallis R, Hutber A *et al.* (2012) Exercise therapy - the public health message. *Scand J Med Sci Sport* **22**, 24–28.
12. Chastin S, Mandrichenko O, Helbostadt J *et al.* (2014) Associations between objectively-measured sedentary behaviour and physical activity with bone mineral density in adults and older adults, the NHANES study. *Bone* **64**, 254–262.
13. Langsetmo L, Hitchcock CL, Kingwell EJ *et al.* (2012) Physical activity, body mass index and bone mineral density-associations in a prospective population-based cohort of women and men: The Canadian Multicentre Osteoporosis Study (CaMos). *Bone* **50**, 401–408.
14. Frost H (2004) A 2003 update of bone physiology and Wolff's Law for clinicians. *Angle Orthod* **74**, 3–15.
15. Lima F, De Falco V, Baima J *et al.* (2001) Effect of impact load and active load on bone metabolism and body composition of adolescent athletes. *Med Sci Sport Exerc* **33**, 1318–1323.
16. Wong PKK, Christie JJ, Wark JD (2007) The effects of smoking on bone health. *Clin Sci* **113**, 233–241.
17. Ilich JZ, Kerstetter J (2000) Nutrition in bone health revisited: A story beyond calcium. *J Am Coll Nutr* **19**, 715–737.
18. Heaney R (2007) Bone Health. *Am J Clin Nutr* **85**, 300–303.
19. Zimmerman E, Busse B, Ritchie R (2015) The fracture mechanics of human bone: influence of disease and treatment. *Bonekey Rep* **4**, 743.
20. Sherman H (1920) Calcium requirement of maintenance in man. *J Biol Chem* **44**, 21–7.
21. Kraut J, Coburn J (1998) Bone, acid and, osteoporosis. *N Engl J Med* **330**, 1821–1822.
22. Barzel U, Massey L (1998) Excess dietary protein can adversely effect bone. *J Nutr* **128**, 1051–1053.
23. Robergs RA (2004) Biochemistry of exercise-induced metabolic acidosis. *AJP Regul Integr Comp Physiol* **287**, 502–16.
24. Aoi W, Marunaka Y (2014) Importance of pH homeostasis in metabolic health and diseases: Crucial role of membrane proton transport. *Biomed Res Int* 598986.

25. Levitsky M (2018) Acid-base balance. In: Pulmonary Physiology. 9th ed. London: McGraw-Hill Education; p. 179–205.
26. Hamm L, Nakhoul N, Hering-Smith K (2015) Acid-base homeostasis. *Clin J Am Soc Nephrol* **10**, 2232–2242.
27. Remer T, Dimitriou T, Manz F (2003) Dietary potential renal acid load and renal net acid excretion in healthy, free-living children and adolescents. *Am J Clin Nutr* **77**, 1255-1260.
28. Remer T, Manz F (1994) Estimation of the renal net acid excretion by adults consuming diets containing variable amounts of protein. *Am J Clin Nutr* **59**, 1356–1361.
29. Remer T, Manz F (1995) Potential renal acid load of foods and its influence on urine pH. *J Am Diet Assoc* **95**, 791–797.
30. Carnauba R, Baptistella A, Paschoal V *et al.* (2017) Diet-induced low-grade metabolic acidosis and clinical outcomes: A review. *Nutrients* **25**, E538.
31. Krieger NS, Frick KK, Bushinsky DA (2004) Mechanism of acid-induced bone resorption. *Curr Opin Nephrol Hypertens* **13**, 423–436.
32. Fenton T, Eliasziw M, Lyon A *et al.* (2008) Meta-analysis of the quantity of calcium excretion associated with the net acid excretion of the modern diet under the acid ash diet hypothesis. *Am J Clin Nutr* **88**, 1159–1166.
33. New SA, MacDonald HM, Campbell MK *et al.* (2004) Lower estimates of net endogenous noncarbonic acid production are positively associated with indexes of bone health in premenopausal and perimenopausal women. *Am J Clin Nutr* **79**, 131–138.
34. Macdonald HM, New SA, Fraser WD *et al.* (2005) Low dietary potassium intakes and high dietary estimates of net endogenous acid production are associated with low bone mineral density in premenopausal women and increased markers of bone resorption in postmenopausal women. *Am J Clin Nutr* **81**, 923–933.
35. Buclin T, Cosma M, Appenzeller M *et al.* (2001) Diet acids and alkalis influence calcium retention in bone. *Osteoporos Int* **12**, 493–499.
36. Brandao-Burch A, Utting JC, Orriss IR *et al.* (2005) Acidosis inhibits bone formation by osteoblasts in vitro by preventing mineralization. *Calcif Tissue Int* **77**, 167–174.
37. Yuan FL, Xu MH, Li X *et al.* (2016) The roles of acidosis in osteoclast biology. *Front Physiol* **7**, 1–8.
38. Kerstetter J, O'Brien K, Caseria D *et al.* (2005) The impact of dietary protein on

612 calcium absorption and kinetic measures of bone turnover in women. *J Clin*
613 *Endocrinol Metab* **90**, 26–31.
614 39. Calvez J, Poupin N, Chesnau C *et al.* (2012). Protein intake, calcium balance and
615 health consequences. *Eur J Clin Nutr* **66**, 281–295.
616 40. Mangano KM, Walsh SJ, Kenny AM *et al.* (2014) Dietary acid load is associated with
617 lower bone mineral density in men with low intake of dietary calcium. *J Bone Miner*
618 *Res* **29**, 500–506.
619 41. Dawson-Hughes B, Harris SS (2002) Calcium intake influences the association of
620 protein intake with rates of bone loss in elderly men and women. *Am J Clin Nutr* **75**,
621 773-779.
622 42. Bonjour J, Ammann P, Chevalley T *et al.* (2001) Protein intake and bone growth. Can
623 *J Appl Physiol* **26**, 153–166.
624 43. Insel P, Turner E, Ross D (2010) Proteins and amino acids: Function follows form. In:
625 Discovering Nutrition. Third. London: Jones & Bartlett Publishers, p. 211–45.
626 44. Bonjour J, Schuren M, Chevalley T *et al.* (1997) Protein intake, IGF-1 and
627 osteoporosis. *Osteoporos Int* **7**, 36–42.
628 45. Atherton P, Smith K (2012) Muscle protein synthesis in response to nutrition and
629 exercise. *J Physiol* **590**, 1049–1057.
630 46. Manolagas S, O'Brien C, Almeida M (2013) The role of estrogen and androgen
631 receptors in bone health and disease. *Nat Rev Endocrinol* **9**, 699–712.
632 47. Almeida M, Laurent M, Dubois V *et al.* (2017) Estrogens and androgens in skeletal
633 physiology and pathophysiology. *Physiol Rev* **97**, 135–187.
634 48. Kawai M, Rosen C (2012) The insulin-like growth factor system in bone. Basic and
635 clinical implications. *Endocrinol Metab Clin North Am* **41**, 323–333.
636 49. Guntur A, Rosen C (2013) IGF-1 regulation of key signalling pathways in bone.
637 *Bonekey Rep* **2**, 437.
638 50. Thissen JP, Ketelslegers JM, Underwood LE (1994) Nutritional regulation of the
639 insulin-like growth factors. *Endocr Rev* **15**, 80–101.
640 51. Woelfle J, Chia D, Rotwein P (2003) Mechanisms of growth hormone (GH) action:
641 Identification of conserved stat3 binding sites that mediate GH induced insulin like
642 growth factor-1 gene activation. *J Biol Chem* **278**, 1261–1266.
643 52. Kaplan SA, Cohen P (2007) Review: The somatomedin hypothesis 2007: 50 Years
644 later. *J Clin Endocrinol Metab* **92**, 4529–4235.
645 53. Frystyk J, Delhanty P, Skjerbek C *et al.* (1999) Changes in the circulating IGF system

during short term fasting and refeeding. *Am J Physiol Endocrinol Metab* **40**, 245–252.
54. Hofbauer L, Khosla S, Dunstan C *et al.* (2000) The roles of osteoprotegerin and osteoprotegerin ligand in the paractine regulation of bone resorption. *J Bone Miner Res* **15**, 2–12.
55. Mrak E, Lanzi R, Losa M *et al.* (2007) Growth hormone stimulates osteoprotegerin expression and secretion in human osteoblast-like cells. *J Endrocrinology* **192**, 639–645.
56. Rubin J, Ackert-Bicknell C, Zhu L *et al.* (2002) IGF-1 regulates osteoprotegerin (OPG) and receptor activator of nuclear factor-kB ligand in vitro and OPG in vivo. *J Clin Endocrinol Metab* **87**, 4273–4279.
57. Ueland T (2004) Bone metabolism in relation to alterations in systemic growth hormone. *Growth Horm IGF Res* **22**, 329–338.
58. Fleet JC, Schoch RD (2010) Molecular mechanisms for regulation of intestinal calcium absorption by vitamin D and other factors. *Crit Rev Clin Lab Sci* **47**, 181–195.
59. Zoidis E, Gosteli-Peter M, Ghirlanda-Keller C *et al.* (2002) IGF-I and GH stimulate Phex mRNA expression in lungs and bones and 1,25-dihydroxyvitamin D(3) production in hypophysectomized rats. *Eur J Endocrinol* **146**, 97–105.
60. Longcope C, Feldman H, McKinlay J *et al.* (2000) Diet and sex hormone-binding globulin. *J Clin Endocrinol Metab* **85**, 293–296.
61. Kahn S, Hryb D, Nakhla A *et al.* (2002) Sex hormone binding globulin is synthesized in target cells. *J Endocrinol* **175**, 113–120.
62. Dolan E, McGoldrick A, Davenport C *et al.* (2012) An altered hormonal profile and elevated rate of bone loss are associated with low bone mass in professional horse-racing jockeys. *J Bone Miner Metab* **30**, 534–542.
63. Slemenda C, Longcope C, Peacock M *et al.* (1996) Sex steroids, bone mass, and bone loss. A prospective study of pre-, peri-, and postmenopausal women. *J Clin Invest* **97**, 14–21.
64. Witard O, Wardle S, MacNaughton L *et al.* (2016) Protein considerations for optimising skeletal muscle mass in healthy young and older adults. *Nutrients* **8**, 181.
65. Biolo G, Tipton K, Klein S *et al.* (1997) An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein. *Am J Physiol* **273**, 122–129.
66. Ho-Pham LT, Nguyen UDT, Nguyen TV (2014) Association between lean mass, fat mass, and bone mineral density: A meta-analysis. *J Clin Endocrinol Metab* **99**, 30–38.
67. Kohrt WM, Barry DW, Schwartz RS (2009) Muscle forces or gravity: What

predominates mechanical loading on bone? *Med Sci Sport Exerc* **41**, 2050–2055.
68. MacDonell R, Hamrick MW, Isales CM (2016) Protein/amino-acid modulation of bone cell function. *Bonekey Rep* **5**, 827.
69. Rizzoli R, Biver E, Bonjour J *et al.* Benefits and safety of dietary protein for bone health. *Osteoporos Int* Published online 8 May 2018. doi. 10.1007/s00198-018-4534-5.
70. Fenton TR, Lyon AW, Eliasziw M *et al.* (2009) Meta-analysis of the effect of the acid-ash hypothesis of osteoporosis on calcium balance. *J Bone Miner Res* **24**, 1835–1840.
71. Fenton TR, Lyon AW, Eliasziw M (2009) Phosphate decreases urine calcium and increases calcium balance: A meta-analysis of the osteoporosis acid-ash diet hypothesis. *Nutr J* **8**, 41.
72. Fenton T, Tough S, Lyon A *et al.* (2011) Causal assessment of dietary acid load and bone disease: A systematic review & meta-analysis applying Hill's epidemiologic criteria for causality. *Nutr J* **10**, 41.
73. Shams-White MM, Chung M, Fu Z *et al.* (2018) Animal versus plant protein and adult bone health : A systematic review and meta-analysis from the National Osteoporosis Foundation. *PLoS One* **13**, e0192459.
74. Bonjour JP (2013) Nutritional disturbance in acid-base balance and osteoporosis: A hypothesis that disregards the essential homeostatic role of the kidney. *Br J Nutr* **110**, 1168–1177.
75. Smith H (1961) From Fish to Philosopher. New York: Anchor Books Doubleday.
76. Kanis JA, Borgstrom F, De Laet C *et al.* (2005) Assessment of fracture risk. *Osteoporos Int* **16**, 581–589.
77. Darling AL, Millward DJ, Torgerson DJ *et al.* (2009) Dietary protein and bone health: A systematic review and meta-analysis. *Am J Clin Nutr* **90**, 1674–1692.
78. Shams-White MM, Chung M, Du M *et al.* (2017) Dietary protein and bone health: A systematic review and meta-analysis from the National Osteoporosis Foundation. *Am J Clin Nutr* **105**, 1528-1543.
79. Wallace TC, Frankenfeld CL (2017) Dietary protein intake above the current RDA and bone health: A systematic review and meta-analysis. *J Am Coll Nutr* **36**, 481–496.
80. Wu AM, Sun XL, Lv QB *et al.* (2015) The relationship between dietary protein consumption and risk of fracture: A subgroup and dose-response meta-analysis of prospective cohort studies. *Sci Rep* **5**, 9151.
81. Antonio J, Ellerbroek A, Evans C *et al.* (2018) High protein consumption in trained women: Bad to the bone? *J Int Soc Sports Nutr* **15**, 6.

82. Rosen C. Primer on the metabolic bone diseases and disorders of mineral metabolism. 8th ed. Rosen C, Bouillon R, Compston J, Rosen V, editors. United States: Wiley-Blackwell; 2013. 1078 p.
83. Rizzoli R, Bianchi M, Garabedian M *et al.* (2010) Maximizing bone mineral mass gain during growth for the prevention of fractures in the adolescents and the elderly. *Bone* **46**, 294–305.
84. Santos L, Elliott-Sale KJ, Sale C (2017) Exercise and bone health across the lifespan. *Biogerontology* **18**, 931–946.
85. Chevalley T, Bonjour J, Ferrari S *et al.* (2008) High-protein intake enhances the positive impact of physical activity on BMC in prepubertal boys. *J Bone Miner Res* **23**, 131–142.
86. National Research Council Subcommittee (1989) Protein and amino acids. In: Recommended Dietary Allowances. 10th ed. Washington (DC): National Academies Press (US), p. 52–78.
87. Heaney R (2009) Dairy and bone health. *J Am Coll Nutr* **28**, 82–90.
88. Gaucheron F (2011) Milk and dairy products: A unique micronutrient combination. *J Am Coll Nutr* **30**, 400–409.
89. Drake MT, Clarke BL, Khosla S (2008) Bisphosphonates: Mechanism of action and role in clinical practice. *Mayo Clin Proc* **83**, 1032–45.
90. Surdykowski AK, Kenny AM, Insogna KL *et al.* (2011) Optimizing bone health in older adults: The importance of dietary protein. *Aging health* **6**, 345–357.
91. Traylor DA, Gorissen SHM, Phillips SM (2018) Protein requirements and optimal intakes in aging: Are we ready to recommend more than the recommended daily allowance? *Adv Nutr* **9**, 171-182.
92. Papageorgiou M, Dolan E, Elliott KJ *et al.* (2017) Reduced energy availability : implications for bone health in physically active populations. *Eur J Nutr* **57**, 847-859.
93. Ihle R, Loucks AB (2004) Dose-response relationships between energy availability and bone turnover in young exercising women. *J Bone Miner Res* **19**, 1231–1240.
94. Papageorgiou M, Elliott-Sale KJ, Parsons A *et al.* (2017) Effects of reduced energy availability on bone metabolism in women and men. *Bone* **105**, 191–9.
95. Loucks AB, Kiens B, Wright HH (2011) Energy availability in athletes. *J Sports Sci* **29**, 37–41.
96. Mountjoy M, Burke L, Ackerman KE *et al.* International Olympic Committee (IOC) consensus statement on Relative Energy Deficiency in Sport (RED-S): 2018 Update.

Int J Sport Nutr Exerc Metab. Published online 17 May 2018. doi. 10.1123/ijsnem.2018-0136.

97. Robinson L, Aldridge V, Clark EM *et al.* (2016) A systematic review and meta-analysis of the association between eating disorders and bone density. *Osteoporos Int* **27**, 1953–1966.

98. Michaelsson K, Bergstrom R, Mallmin H *et al.* (1996) Screening for osteopenia and osteoporosis: Selection by body composition. *Osteoporos Int* **6**, 120–126.

99. Gerdem P, Ringsberg K, Akesson K *et al.* (2003) Influence of muscle strength, physical activity and weight on bone mass in a population-based sample of 1004 elderly women. *Osteoporos Int* **14**, 768–772.

100. Hunter G, Plaisance E, Fisher G (2014) Weight loss and bone mineral density. *Curr Opin Endocrinol Diabetes Obes* **21**, 358–362.

101. Dolan E, Swinton PA, Sale C, *et al.* (2017). Influence of adipose tissue mass on bone mass in an overweight or obese population: Systematic review and meta-analysis. *Nutr Rev* **75**, 858–870.

102. Josse AR, Atkinson SA, Tarnopolsky MA *et al.* (2014) Diets higher in dairy foods and dietary protein support bone health during diet- and exercise-induced weight loss in overweight and obese premenopausal women. *J Clin Endocrinol Metab* **97**, 251-260.

103. Phillips S, Van Loon L (2011) Dietary protein requirements for athletes: From requirements to optimum adaptation. *J Sports Sci* **29**, 29–38.

104. Swinton P, Stephens Hemingway B, Saunders B *et al.* (2018) A statistical framework to interpret individual response to intervention: Paving the way for personalised nutrition and exercise prescription. *Front Nutr* **5**, 41.

Figure Legends:

Figure 1: Mechanisms through which protein may impact bone.

Pathways: 1) Dietary protein upregulates the activity of various anabolic hormones and growth factors (e.g., IGF-1; androgens; oestrogens or incretins), which in turn exert an osteogenic influence. 2) Dietary protein positively impacts muscle mass and functionality, with indirect benefit to bone through the increased mechanical loading that this provides. 3) Dietary protein increased the renal acid load, inducing a state of low grade metabolic acidosis. Ca^{2+}, and other alkaline minerals are leached from the bone in order to neutralise pH, thus reducing acid load. Ca2+ is subsequently lost through an increased urinary excretion, thus causing bone demineralisation. 4) Dietary protein increases dietary calcium absorption, thus increasing serum calcium availability, allowing for pH neutralisation, without undue detriment to bone.