



Nutrition Society Live 2020 was held virtually on 14–15 July 2020

Symposium three: Physiological determinants for protein requirements

Dietary protein and bone health: towards a synthesised view

Andrea L. Darling* , D. Joe Millward and Susan A. Lanham-New

Nutrition, Food & Exercise Sciences Department, School of Biosciences and Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford GU2 7XH, UK

The present paper reviews published literature on the relationship between dietary protein and bone health. It will include arguments both for and against the anabolic and catabolic effects of dietary protein on bone health. Adequate protein intake provides the amino acids used in building and maintaining bone tissue, as well as stimulating the action of insulin-like growth factor 1, which in turn promotes bone growth and increases calcium absorption. However, the metabolism of dietary sulphur amino acids, mainly from animal protein, can lead to increased physiological acidity, which may be detrimental for bone health in the long term. Similarly, cereal foods contain dietary phytate, which in turn contains phosphate. It is known that phosphate consumption can also lead to increased physiological acidity. Therefore, cereal products may produce as much acid as do animal proteins that contain sulphur amino acids. The overall effect of dietary protein on physiological acidity, and its consequent impact on bone health, is extremely complex and somewhat controversial. The consensus is now moving towards a synthesised approach. Particularly, how anabolic and catabolic mechanisms interact; as well as how the context of the whole diet and the type of protein consumed is important.

Protein: Bone health: Acid–base: Insulin-like growth factor-1

Bone mass changes over the life cycle⁽¹⁾. In childhood and adolescence, there is a rapid increase in bone size, with a relatively large need for dietary calcium and protein. Peak bone mass (PBM) is achieved after age 20 years and is maintained through the mid-years (i.e. thirties to forties)⁽¹⁾. In women, there is a rapid loss of bone during the menopause due to loss of oestrogen⁽²⁾, which leads to lower bone strength post-menopause (osteoporosis)⁽³⁾ and increased fracture risk⁽⁴⁾. In men, there is a slower decline in bone mass⁽¹⁾, leading to osteoporosis at a more advanced age⁽⁵⁾. It is important to achieve a good PBM in early life in order to prepare for the loss of bone with ageing⁽⁶⁾. Apart from rare cases of pathologically high bone mass, generally, the higher the PBM, the less the likelihood of the occurrence of

osteoporosis and bone fracture in later life⁽⁷⁾. Suboptimal lifestyle factors may reduce the initial PBM and predispose the individual to increased risk of osteoporosis⁽¹⁾ (Fig. 1).

Hormonal factors (sex hormones and insulin-like growth factor 1 (IGF-1)); nutritional factors (dietary nutrients such as calcium, protein, phosphorus and potassium) and mechanical factors (physical activity, bone shape and bone material properties) affect PBM⁽⁸⁾ (Fig. 2). Genetic factors are also important but are unmodifiable^(9,10). It is therefore important for public health interventions to focus on modifiable factors such as achieving adequate intakes of bone-supporting nutrients, ensuring adequate exercise and a healthy body weight.

Abbreviations: BMC, bone mineral content; BMD, bone mineral density; IGF-1, insulin-like growth factor 1; PBM, peak bone mass.

*Corresponding author: Andrea L. Darling, email a.l.darling@surrey.ac.uk

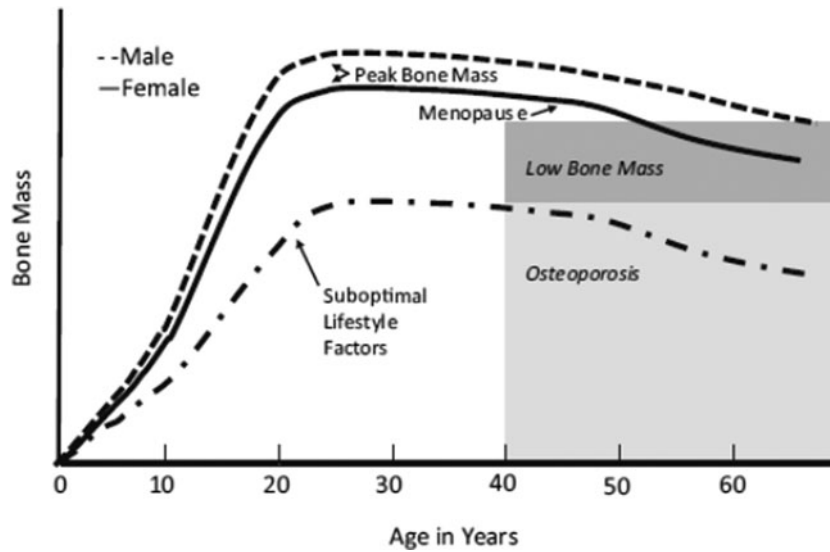


Fig. 1. Bone mass across the lifespan with optimal and suboptimal lifestyle choices. Source: Reproduced unmodified from Weaver *et al.*⁽¹⁾. Creative Commons Attribution-Non Commercial 4.0 International License (<http://creativecommons.org/licenses/by-nc/4.0/>).

Dietary protein is crucial for the maintenance of bone tissue as well as for bone growth. Bone is 35 % protein and requires a supply of amino acids to be used for protein turnover. The mechanostat is a process whereby bone remodels itself in response to elastic deformation acting on it, one large provider of this force is muscle mass⁽¹⁾. This explains observations that higher muscle mass is associated with increased bone mass⁽¹²⁾. Adequate protein ensures an adequate muscle mass, so is an important determinant of bone health. The current internationally recommended protein intake guideline for adults of all ages is 0.83 g/kg/d⁽¹³⁾ although values of 0.8 g/d are used by many agencies with some recommending higher values for the elderly^(14–18). Requirements for infants, children and adolescents vary by country, but are higher than that in adults due to the need for growth. For example, in the UK, the recommended nutrient intake is 12.5 g/d in those aged 0–3 months⁽¹⁷⁾ which translates to over 2 g/kg/d. Western populations are generally dietary protein sufficient. For example, in the UK National Diet and Nutrition Survey (2014–2016, aged 19–64 years), the median protein intake was 74 g/d. Based on a UK average body weight of 72 kg for women and 85 kg for men⁽¹⁸⁾, this suggests a median intake of over 1 g/kg/d.

There are some groups in western societies, such as frail older people, who are at risk of a low-protein intake. For example, in one study, 32 % of frail older people did not meet the 0.8 g/kg/d requirement⁽¹⁹⁾. Conversely, in another study older care home residents had sufficient protein intake, with 95 % attaining 0.8–1 g/kg/d⁽²⁰⁾, and an analysis of the UK National Diet and Nutrition Survey of protein intakes of the elderly after trimming for under-reporting indicated median intakes of 1.24 g/kg/d with a negligible prevalence of deficiency⁽²¹⁾. Low-protein intakes are important due

to the association of low-protein intakes and frailty in older people⁽²²⁾.

Also, protein-energy malnutrition is still very common throughout the developing world. For example, 22.2 % of children aged 0–59 months globally have stunted growth and 7.5 % of children have wasting⁽²³⁾, although actual protein deficiency *per se* is rare with growth deficits more likely to reflect enteric infections from a poor environment⁽²⁴⁾.

The present paper will now discuss the proposed anabolic and catabolic actions of protein on bone health. It will exclude discussion of weight-loss studies as protein metabolism may differ in this situation.

Anabolic associations of dietary protein with bone health

Protein intake stimulates the release of the hormone IGF-1⁽²⁵⁾, which increases muscle mass⁽²⁵⁾ and bone growth⁽²⁶⁾. Accordingly, lower protein intake leads to lower IGF-1^(25,27) which in turn leads to a lower bone mass^(25–28). This could result in a higher fracture risk, with studies finding a negative association between IGF-1 concentration and predicted fracture risk^(25,29,30). Correcting low-protein intake theoretically leads to a variety of musculoskeletal health benefits in older individuals (Fig. 3)⁽²⁵⁾.

Observational studies have shown a beneficial association between a higher protein intake and improved bone health. For example, in children and adolescents, cross-sectional analyses have associated a higher protein intake with a higher bone mineral content (BMC)^(31,32) and a larger bone area^(32,33). In longitudinal research, studying children with high physical activity levels, a higher protein intake was associated with an increase in femoral neck bone mineral density (BMD) *z* score

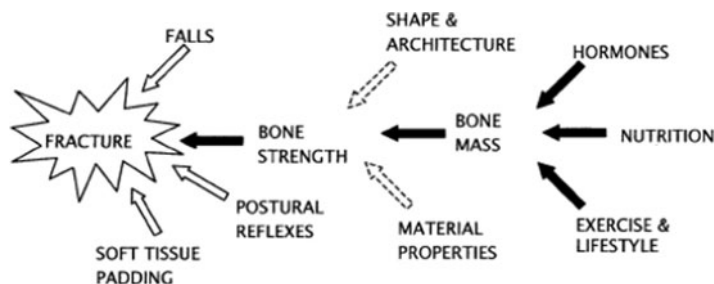


Fig. 2. Factors contributing to osteoporosis fracture risk. Source: Reprinted from Heaney⁽⁶⁾. Copyright (2020), with permission from Elsevier. <https://www.sciencedirect.com/science/article/pii/S8756328203002369?via%3Dihub>

between age 7 and 15 years⁽³⁴⁾. However, lower protein intake was associated with a reduction in femoral neck BMD *z* score during the same time period⁽³⁴⁾. In older adults (over 60 years), higher protein intake has been associated, in cross-sectional studies, with higher spinal BMD^(35,36), total body BMD⁽³⁶⁾ and femoral neck BMD in women⁽³⁷⁾. Higher protein intake has also been associated with higher total hip BMD in men and women^(37,38). Conversely, studies have found no difference in protein intake between women with normal BMD and women with osteopenia or osteoporosis⁽³⁹⁾, and no association between protein intake and spinal or femoral neck BMD in older women⁽⁴⁰⁾.

In premenopausal women, some studies have found that increased protein intake is associated with higher hip or spine BMD^(41–43) or BMC^(41,44). However, other studies have found no association with radial, spinal or femoral neck BMC⁽⁴⁵⁾ or lumbar spine or femoral neck BMD^(42,43,45,46). The few studies assessing younger to middle-aged men have found a positive association between protein intake and BMD in black men⁽⁴⁷⁾ and vertebral BMC in all men⁽⁴⁸⁾. However, studies have also found no association between protein intake and BMD in white men⁽⁴⁷⁾ and no association for all men for total hip and spine BMD⁽⁴⁹⁾ or radial BMC⁽⁴⁸⁾. However, it must be borne in mind that not all observational analyses are multivariate adjusted. Some associations between dietary protein and bone health will be due to confounding from dietary, lifestyle and demographic factors. The type of protein consumed, and the adequacy of calcium intake may also vary between studies. These factors could explain differing results.

Protein supplementation studies have shown an improvement in BMD, BMC or other indices or bone size or strength in some studies but not others. For example, one study found improved bone growth after protein supplementation in malnourished children⁽⁵⁰⁾. However, there have been no trials to date in non-malnourished children. In terms of older people, in a study of hospitalised adults with a hip fracture, there was a reduced femoral shaft bone loss in those supplemented with 20 g/d protein⁽⁵¹⁾. Similarly, a study of older patients' post-hip fracture found that 20 g/d protein supplementation was associated with reduced proximal femur bone loss⁽⁵²⁾. However, a study in community-

dwelling adults aged 70–80 years, found no effect of whey protein supplementation (30 g/d) on bone mass or strength⁽⁵³⁾. Therefore, benefits of supplemental protein on bone may be confined to frailer older people post-hip fracture.

In terms of bone markers, over all age groups, evidence from trials is also mixed. Some studies have found no difference in bone markers in participants allocated to high- or low-protein diets⁽⁵⁴⁾ or participants allocated to a protein supplement compared to placebo⁽⁵⁵⁾. However, some studies have found lower bone resorption in those supplemented with protein^(56,57).

Catabolic associations of dietary protein with bone health

To maintain life, extracellular fluid must strictly stay within the limits of pH 7.35–7.45 (hydrogen ions between 0.035 and 0.045 mEq). Each day, human subjects on a typical western diet produce 1 mEq/kg body weight⁽⁵⁸⁾. This increased physiological acidity leads to a series of physiological responses to neutralise the acid (Fig. 4)⁽⁵⁹⁾. The body instigates buffering of body fluids, including increased bicarbonate production. The lungs increase carbon dioxide loss, the kidneys excrete more acid and bone loses alkaline mineral into the body fluids⁽⁵⁹⁾. The latter is achieved via increased activity of osteoclast cells⁽⁶⁰⁾, which break down and remodel bone tissue. There is also evidence for a direct dissolution of bone calcium carbonate under exposure to acidity⁽⁶¹⁾. Studies of acidic states such as ammonium chloride ingestion⁽⁶²⁾ and starvation⁽⁶³⁾ have demonstrated a negative calcium balance and increased calciuria^(59,64). This negative calcium balance could have a negative impact on bone health if it occurs over the long term.

Dietary composition influences the acid–base status of the body. The consumption of sulphur amino acids from animal protein increases physiological acidity, as does phosphate from dietary phytates in grains. This means some cereal proteins produce as much, or more physiological acidity than animal proteins. For example, oatmeal, walnuts and whole wheat are higher producers of acidity than are chicken, beef and cheddar⁽⁶⁵⁾.

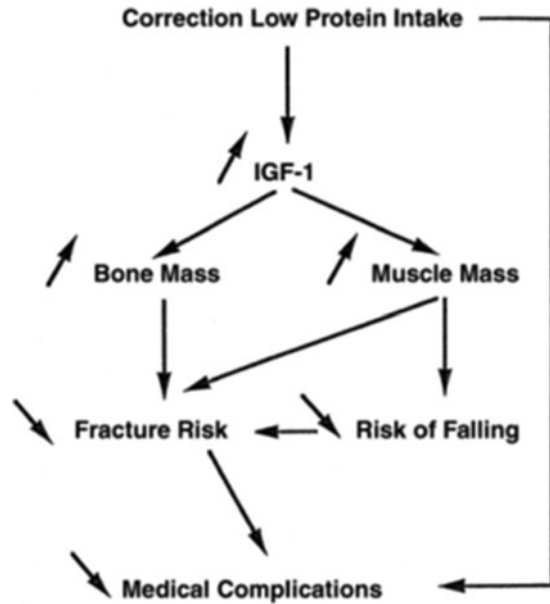


Fig. 3. Effects of correcting protein deficiency in older individuals. Source: Reprinted from Rizzoli *et al.*⁽²⁹⁾. Copyright (2020), with permission from Elsevier. <https://www.sciencedirect.com/science/article/pii/S1297319X01002950?via%3Dihub>. IGF-1, insulin-like growth factor 1.

Consumption of green vegetables and fruit leads to increased alkalinity. This is because they contain alkaline potassium salts of weak organic acids such as citrate, lactate and malate.

A higher protein:potassium ratio is undesirable, as demonstrated by the finding that it is associated with increased higher renal net acid excretion⁽⁶⁶⁾. A higher protein:potassium ratio is associated with higher potential renal acid load⁽⁶⁶⁾. Therefore, high protein, without adequate protective potassium, will increase physiological acidity. The net endogenous acid production in modern western diets could have negative implications for bone health, if the acidity is large enough and for long enough. An analysis of the net endogenous acid production of modern and preagricultural diets found that modern diets had an average of +48 mEq/d compared with -88 mEq/d for the preagricultural diets⁽⁶⁷⁾. Therefore, today we consume more acidic diets than was previously the case.

In terms of epidemiology, some ecological studies in the 1990s have suggested that higher protein intakes are associated with a detriment to bone health. For example, two studies found a positive association between animal protein intake per capita and hip fracture incidence^(68,69). However, ecological studies are prone to bias due to the methodology used. Moreover, few, if any, cross-sectional, cohort studies or randomised controlled trials have found an association between higher protein intake and poorer indices of bone health.

It is known that calcium excretion may rise with increased protein intake suggesting a detriment to bone mass. However, evidence shows that calcium absorption may increase, offsetting calcium loss. One study, using a

within-subjects study design, gave research participants a low-protein diet (0.7 g/kg/d) and a high-protein diet (2.1 g/kg/d). They found increased urinary calcium during the high-protein diet, but calcium absorption also increased⁽⁷⁰⁾. However, another intervention trial showed no difference in calcium absorption, urinary calcium excretion or level of bone resorption markers when consuming the RDA of protein compared with consuming three times the RDA⁽⁷¹⁾. This suggests no detrimental effect of higher protein intake on calcium metabolism and bone markers. However, this was only a short-term trial in only a small sample size, and it is unclear what the effect would be on bone metabolism in the long term.

Baseline calcium intake may also be important. For example, in the Framingham study, the increased fracture risk associated with higher animal protein intake was only present in the participants with lower calcium intake (<800 mg/d)⁽⁷²⁾. There was no association between higher animal protein intake and fracture risk when calcium intake was sufficient (\geq 800 mg/d)⁽⁷²⁾. This suggests adequate calcium intake may offset any detrimental effects of a high animal protein diet.

Systematic reviews and meta-analyses on protein intake and bone

There are conflicting findings from systematic reviews and meta-analyses on dietary protein and bone health. Meta-analyses of protein supplementation have found either no overall effect⁽⁷³⁾ or a tiny beneficial effect^(74,75) on bone health, with no evidence of a detrimental effect in any of the systematic review and meta-analyses published to date. Meta-analyses of cross-sectional studies assessing the relationship between dietary protein and bone health generally show a positive association^(73,74), although the association is often not present when analysing only multivariate-adjusted studies⁽⁷³⁾.

Meta-analyses of cohort studies have found either a beneficial association with fracture risk^(76,77) or no association with fracture risk^(73,74). Therefore, any small gains in BMD may not translate into fracture risk in the long term⁽⁷³⁾. The association between protein intake and bone health in observational studies is stronger in case-control studies compared with cohort studies⁽⁷³⁾. This could be due to case-control studies having significant inherent bias⁽⁷⁸⁾. Overall, the message across these meta-analyses is that there is no evidence of a detrimental association between protein intake and bone health. As evidenced earlier, some meta-analyses suggest a benefit of protein to bone health, but others suggest no association.

Towards a synthesised view of dietary protein and bone health

There have been recent efforts to synthesise the anabolic and catabolic mechanisms of dietary protein on bone health. A key review⁽⁷⁹⁾ discusses how the positive aspects of dietary protein intake, including increased

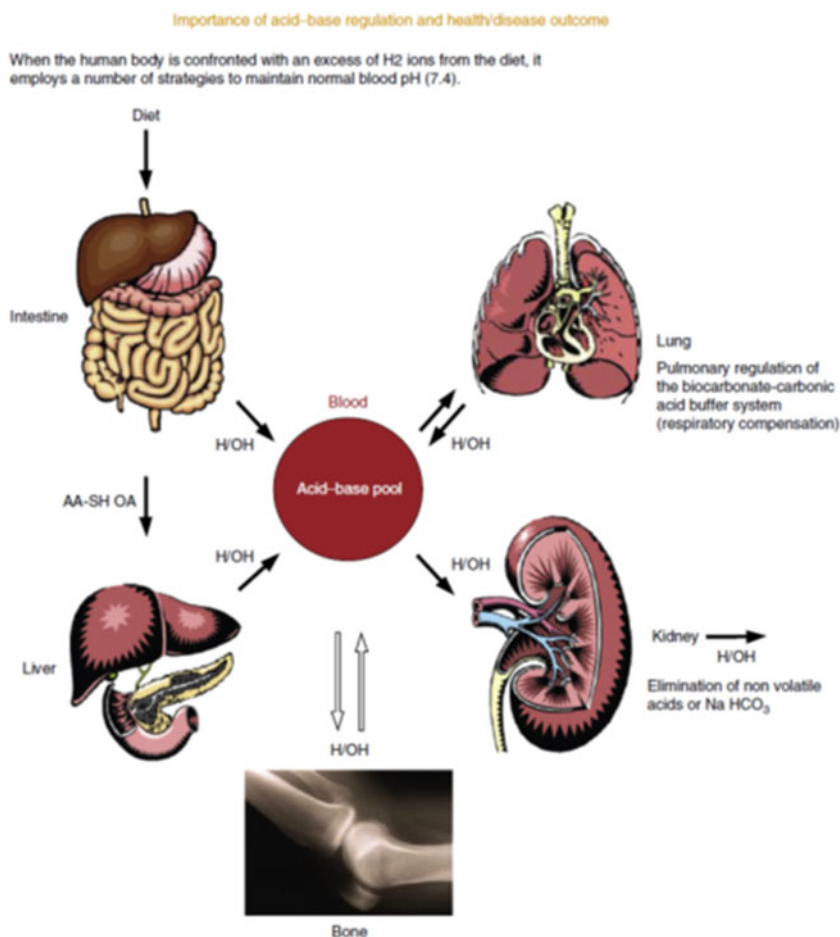


Fig. 4. (Colour online) Acid-base regulation. Source: Lanham-New *et al.*⁽⁵⁹⁾. Reproduced with permission from Elsevier. <https://www.sciencedirect.com/science/article/pii/B9780123750839000295>. AA-SH OA, sulphur amino acids-organic acids; H₂, hydrogen; OH, hydroxide; NaHCO₃, sodium bicarbonate.

calcium absorption and IGF-1 induced bone formation, work in tandem with the negative effects. Particularly, they discuss how protein may benefit bone health if consumed as part of a diet containing enough dietary calcium, and alkalisng fruit and vegetables⁽⁷⁹⁾.

This synthesised approach may explain some complex findings of research studies. For example, in one study, higher dietary protein was associated with larger bone size (periosteal circumference and cortical area), and higher BMC and polar strength strain index⁽⁸⁰⁾. However, children in the same study with a high dietary potential renal acid load had a lower BMC and cortical area than those with a lower dietary potential renal acid load⁽⁸⁰⁾.

A low protein:potassium ratio is likely to be ensured by consuming a balanced diet. Indeed, there is an argument for a whole diet approach for bone health⁽⁶⁵⁾, which includes a balanced intake of nutrients such as protein, potassium, calcium and phosphate. As discussed earlier, one way of increasing potassium intake is to consume more fruit and vegetables. Adequate calcium intake may also help compensate for any sulphur amino acid-induced bone loss⁽⁸¹⁾. Adequate protein intake ensures

enough amino acids for growth and repair of body tissues but should not be in excess. Other food constituents such as soya isoflavones and caffeine may also have potential effects on bone health⁽⁶⁵⁾. Soya isoflavones are known to have oestrogen-like effects on the body. Therefore, theoretically they may have beneficial effects on bone. Some studies have found a benefit of soya isoflavone supplementation on BMD^(82,83), but most studies have found no benefit^(84–86). Higher caffeine intake has been associated with poorer bone health⁽⁸⁷⁾, which could be due to a small caffeine-induced reduction in calcium absorption⁽⁸⁸⁾. However, this could also be due to consumption of caffeinated beverages being higher in individuals who have low calcium intakes⁽⁸⁹⁾.

Conclusion

There is a long-standing debate as to whether high dietary protein intakes are beneficial or detrimental for bone health. We know that adequate dietary protein intake is essential to provide amino acids for building and maintaining bone tissue. It also has anabolic effects

on bone by stimulating the release of IGF-1 and calcium absorption from the gut. However, some forms of dietary proteins may increase net physiological acidity because of their sulphur amino acid or phytate content. This could lead to increased bone loss in the long term in order to provide a source of alkaline mineral.

Research over the past 40 years has supported both anabolic and catabolic associations between protein intake and bone health. Data from cross-sectional studies support a positive association. However, cohort studies assessing fracture risk show both positive and negative associations, leading to null associations in meta-analyses. Intervention studies assessing BMD show no effect (or a tiny benefit) of protein intake for bone health in adults. There is a lack of research on this topic assessing children and adolescents, as well as adults with very low or very high intakes of dietary protein.

To make sense of the opposing effects of dietary protein on bone we are moving towards a synthesised view whereby dietary protein has both anabolic and catabolic effects on bone. The overall effect depends on the whole diet, as food components modify the net physiological pH. For example, calcium-containing foods, or the consumption of fruit and vegetables, may contribute to reduced physiological acidity from a higher protein diet.

Acknowledgements

A. L. D. is very grateful to the UK Nutrition Society for the opportunity to present at the Nutrition Society Live 2020 virtual conference.

Financial Support

None.

Conflict of Interest

None.

Authorship

The authors had joint responsibility for all aspects of preparation of the paper.

References

1. Weaver CM, Gordon CM, Janz KF, *et al.* (2016) The National Osteoporosis Foundation's Position statement on peak bone mass development and lifestyle factors: a systematic review and implementation recommendations. *Osteoporos Int* **27**, 1281–1386.
2. Karlamangla AS, Burnett-Bowie SM & Crandall CJ (2018) Bone health during the menopause transition and beyond. *Obstet Gynecol Clin North Am* **45**, 695–708.
3. Ishii S, Cauley JA, Greendale GA *et al.* (2013) Trajectories of femoral neck strength in relation to the final menstrual period in a multi-ethnic cohort. *Osteoporos Int* **24**, 2471–2481.
4. Jones CM & Boelaert K (2015) The endocrinology of ageing: a mini-review. *Gerontology* **61**, 291–300.
5. Adler RA (2014) Osteoporosis in men: a review. *Bone Res* **2**, 14001.
6. Bachrach LK (2001) Acquisition of optimal bone mass in childhood and adolescence. *Trends Endocrinol Metab* **12**, 22–28.
7. Ferretti JL, Cointry GR, Capozza RF *et al.* (2003) Bone mass, bone strength, muscle-bone interactions, osteopenias and osteoporoses. *Mech Ageing Dev* **124**, 269–279.
8. Heaney RP (2003) Is the paradigm shifting? *Bone* **33**, 457–465.
9. Bay CP, Levy SM, Janz KF *et al.* (2019) Genome-wide association analysis of longitudinal bone mineral content data from the Iowa bone development study. *J Clin Densitom* **19**, S1094–S6950.
10. Chanpaisaeng K, Reyes Fernandez PC & Fleet JC (2019) Dietary calcium intake and genetics have site-specific effects on peak trabecular bone mass and microarchitecture in male mice. *Bone* **125**, 46–53.
11. Frost HM (2003) Bone's mechanostat: a 2003 update. *Anat Rec A Discov Mol Cell Evol Biol* **275**, 1081–1101.
12. Chalhoub D, Boudreau R, Greenspan S *et al.* (2018) Associations between lean mass, muscle strength and power, and skeletal size, density and strength in older men. *J Bone Miner Res* **33**, 1612–1621.
13. WHO (2007) *Protein and amino acid requirements in human nutrition*. https://apps.who.int/iris/bitstream/handle/10665/43411/WHO_TRS_935_eng.pdf?ua=1 (accessed August 2020).
14. Australian National Health and Medical Research Council (NHMRC) and the New Zealand Ministry of Health (MoH) (2006) *Nutrient Reference Values for Australia and New Zealand: Protein*. https://www.nrv.gov.au/sites/default/files/content/n35-protein_0.pdf (accessed August 2020).
15. Institute of Medicine (2005) *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*. <https://www.nap.edu/catalog/10490/dietary-reference-intakes-for-energy-carbohydrate-fiber-fat-fatty-acids-cholesterol-protein-and-amino-acids> (accessed August 2020).
16. European Food Safety Authority (2012) Scientific Opinion on Dietary Reference Values for protein. <https://www.efsa.europa.eu/en/efsajournal/pub/2557> (accessed August 2020).
17. COMA (1991) *Dietary Reference Values of Food Energy and Nutrients for the United Kingdom: Report 41*: The Stationery Office Ltd.
18. Health Survey for England (2018) Health Survey for England 2018: Overweight and obesity in adults and children. <https://files.digital.nhs.uk/52/FD7E18/HSE18-Adult-Child-Obesity-rep.pdf> (accessed August 2020).
19. Mendonca N, Kingston A, Granic A *et al.* (2019) Protein intake and transitions between frailty states and to death in very old adults: the Newcastle 85+ study. *Age Ageing* **49**, 32–38.
20. Engelheart S, Brummer RJ & Berteus Forslund H (2020) Meal patterns in relation to energy and protein intake in older adults in home health care. *Clin Nutr ESPEN* **35**, 180–187.
21. Millward DJ (2012) Nutrition and sarcopenia: evidence for an interaction. *Proc Nutr Soc* **71**, 566–575.
22. Rahi B, Colombet Z, Gonzalez-Colaco Harmand M *et al.* (2016) Higher protein but not energy intake is associated with a lower prevalence of frailty Among community-dwelling older adults in the French three-city cohort. *J Am Med Dir Assoc* **17**, 672 e677–672 e611.
23. WHO (2018) 2018 Global Nutrition Report: Executive Summary. <https://www.who.int/nutrition/globalnutrition>

- report/2018_Global_Nutrition_Report_Executive_Summary-en.pdf?ua=1 (accessed August 2020).
24. Millward DJ (2017) Nutrition, infection and stunting: the roles of deficiencies of individual nutrients and foods, and of inflammation, as determinants of reduced linear growth of children. *Nutr Res Rev* **30**, 50–72.
 25. Rizzoli R, Ammann P, Chevalley T *et al.* (2001) Protein intake and bone disorders in the elderly. *Joint Bone Spine* **68**, 383–392.
 26. Rizzoli R, Bonjour JP & Chevalley T (2007) Dietary protein intakes and bone growth. *International Congress Series*, **1297**, 50–59.
 27. Switkowski KM, Jacques PF, Must A *et al.* (2019) Associations of protein intake in early childhood with body composition, height, and insulin-like growth factor I in mid-childhood and early adolescence. *Am J Clin Nutr* **109**, 1154–1163.
 28. Yakar S, Werner H & Rosen CJ (2018) Insulin-like growth factors: actions on the skeleton. *J Mol Endocrinol* **61**, T115–T137.
 29. Boker J, Volzke H, Nauck M *et al.* (2018) Associations of insulin-like growth factor-I and insulin-like growth factor binding protein-3 with bone quality in the general adult population. *Clin Endocrinol (Oxf)* **88**, 830–837.
 30. Ohlsson C, Mellstrom D, Carlzon D *et al.* (2011) Older men with low serum IGF-1 have an increased risk of incident fractures: the MrOS Sweden study. *J Bone Miner Res* **26**, 865–872.
 31. Bounds W, Skinner J, Carruth BR *et al.* (2005) The relationship of dietary and lifestyle factors to bone mineral indexes in children. *J Am Diet Assoc* **105**, 735–741.
 32. Ekbote VH, Khadilkar AV, Chiplonkar SA *et al.* (2011) Determinants of bone mineral content and bone area in Indian preschool children. *J Bone Miner Metab* **29**, 334–341.
 33. Hoppe C, Molgaard C & Michaelsen KF (2000) Bone size and bone mass in 10-year-old Danish children: effect of current diet. *Osteoporos Int* **11**, 1024–1030.
 34. Chevalley T, Bonjour JP, van Rietbergen B *et al.* (2014) Tracking of environmental determinants of bone structure and strength development in healthy boys: an eight-year follow up study on the positive interaction between physical activity and protein intake from prepuberty to mid-late adolescence. *J Bone Miner Res* **29**, 2182–2192.
 35. Chiu JF, Lan SJ, Yang CY *et al.* (1997) Long-term vegetarian diet and bone mineral density in postmenopausal Taiwanese women. *Calcif Tissue Int* **60**, 245–249.
 36. Rapuri PB, Gallagher JC & Haynatzka V (2003) Protein intake: effects on bone mineral density and the rate of bone loss in elderly women. *Am J Clin Nutr* **77**, 1517–1525.
 37. Devine A, Dick IM, Islam AF *et al.* (2005) Protein consumption is an important predictor of lower limb bone mass in elderly women. *Am J Clin Nutr* **81**, 1423–1428.
 38. Coin A, Perissinotto E, Enzi G *et al.* (2008) Predictors of low bone mineral density in the elderly: the role of dietary intake, nutritional status and sarcopenia. *Eur J Clin Nutr* **62**, 802–809.
 39. Gunn CA, Weber JL & Kruger MC (2014) Diet, weight, cytokines and bone health in postmenopausal women. *J Nutr Health Aging* **18**, 479–486.
 40. Lau EM, Kwok T, Woo J *et al.* (1998) Bone mineral density in Chinese elderly female vegetarians, vegans, lacto-vegetarians and omnivores. *Eur J Clin Nutr* **52**, 60–64.
 41. Quintas ME, Ortega RM, Lopez-Sobaler AM *et al.* (2003) Influence of dietetic and anthropometric factors and of the type of sport practised on bone density in different groups of women. *Eur J Clin Nutr* **57**(Suppl. 1), S58–S62.
 42. Henderson NK, Price RI, Cole JH *et al.* (1995) Bone density in young women is associated with body weight and muscle strength but not dietary intakes. *J Bone Miner Res* **10**, 384–393.
 43. Chan R, Woo J, Lau W *et al.* (2009) Effects of lifestyle and diet on bone health in young adult Chinese women living in Hong Kong and Beijing. *Food Nutr Bull* **30**, 370–378.
 44. Lacey JM, Anderson JJ, Fujita T *et al.* (1991) Correlates of cortical bone mass among premenopausal and postmenopausal Japanese women. *J Bone Miner Res* **6**, 651–659.
 45. Freudenheim JL, Johnson NE & Smith EL (1986) Relationships between usual nutrient intake and bone-mineral content of women 35–65 years of age: longitudinal and cross-sectional analysis. *Am J Clin Nutr* **44**, 863–876.
 46. New SA, Bolton-Smith C, Grubb DA *et al.* (1997) Nutritional influences on bone mineral density: a cross-sectional study in premenopausal women. *Am J Clin Nutr* **65**, 1831–1839.
 47. Jaime PC, Latorre Mdo R, Florindo AA *et al.* (2006) Dietary intake of Brazilian black and white men and its relationship to the bone mineral density of the femoral neck. *Sao Paulo Med J* **124**, 267–270.
 48. Orwoll ES, Weigel RM, Oviatt SK *et al.* (1987) Serum protein concentrations and bone mineral content in aging normal men. *Am J Clin Nutr* **46**, 614–621.
 49. Whiting SJ, Boyle JL, Thompson A *et al.* (2002) Dietary protein, phosphorus and potassium are beneficial to bone mineral density in adult men consuming adequate dietary calcium. *J Am Coll Nutr* **21**, 402–409.
 50. Lampl M & Johnston FE (1978) The effects of protein supplementation on the growth and skeletal maturation of New Guinean school children. *Ann Hum Biol* **5**, 219–227.
 51. Tkatch L, Rapin CH, Rizzoli R *et al.* (1992) Benefits of oral protein supplementation in elderly patients with fracture of the proximal femur. *J Am Coll Nutr* **11**, 519–525.
 52. Schurch MA, Rizzoli R, Slosman D *et al.* (1998) Protein supplements increase serum insulin-like growth factor-I levels and attenuate proximal femur bone loss in patients with recent hip fracture. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med* **128**, 801–809.
 53. Zhu K, Meng X, Kerr DA *et al.* (2011) The effects of a two-year randomized, controlled trial of whey protein supplementation on bone structure, IGF-1, and urinary calcium excretion in older postmenopausal women. *J Bone Miner Res* **26**, 2298–2306.
 54. Cao JJ, Johnson LK & Hunt JR (2011) A diet high in meat protein and potential renal acid load increases fractional calcium absorption and urinary calcium excretion without affecting markers of bone resorption or formation in postmenopausal women. *J Nutr* **141**, 391–397.
 55. Cuneo F, Costa-Paiva L, Pinto-Neto AM *et al.* (2010) Effect of dietary supplementation with collagen hydrolysates on bone metabolism of postmenopausal women with low mineral density. *Maturitas* **65**, 253–257.
 56. Dawson-Hughes B, Harris SS, Rasmussen H *et al.* (2004) Effect of dietary protein supplements on calcium excretion in healthy older men and women. *J Clin Endocrinol Metab* **89**, 1169–1173.
 57. Hunt JR, Johnson LK & Fariba Roughead ZK (2009) Dietary protein and calcium interact to influence calcium retention: a controlled feeding study. *Am J Clin Nutr* **89**, 1357–1365.
 58. Lemann J Jr (1999) Relationship between urinary calcium and net acid excretion as determined by dietary protein and potassium: a review. *Nephron* **81**(Suppl. 1), 18–25.
 59. Lanham-New SA, Alghamdi M & Jalal J (2013) Nutritional aspects of bone. In *Encyclopedia of Human Nutrition (Third Edition)*, pp. 220–226 [B Caballero, L H Allen, A Prentice, editors]. London : Elsevier.



60. Arnett TR & Dempster DW (1986) Effect of pH on bone resorption by rat osteoclasts in vitro. *Endocrinology* **119**, 119–124.
61. Bushinsky DA & Lechleider RJ (1987) Mechanism of proton-induced bone calcium release: calcium carbonate-dissolution. *Am J Physiol* **253**, F998–1005.
62. Osther PJ (2006) Effect of acute acid loading on acid-base and calcium metabolism. *Scand J Urol Nephrol* **40**, 35–44.
63. Grinspoon SK, Baum HB, Kim V *et al.* (1995) Decreased bone formation and increased mineral dissolution during acute fasting in young women. *J Clin Endocrinol Metab* **80**, 3628–3633.
64. New SA (2002) Nutrition society medal lecture. The role of the skeleton in acid-base homeostasis. *Proc Nutr Soc* **61**, 151–164.
65. Massey LK (2003) Dietary animal and plant protein and human bone health: a whole foods approach. *J Nutr* **133**, 862S–865S.
66. Frassetto LA, Todd KM, Morris RC Jr *et al.* (1998) Estimation of net endogenous noncarbonic acid production in humans from diet potassium and protein contents. *Am J Clin Nutr* **68**, 576–583.
67. Sebastian A, Frassetto LA, Sellmeyer DE *et al.* (2002) Estimation of the net acid load of the diet of ancestral pre-agricultural Homo sapiens and their hominid ancestors. *Am J Clin Nutr* **76**, 1308–1316.
68. Abelow BJ, Holford TR & Insogna KL (1992) Cross-cultural association between dietary animal protein and hip fracture: a hypothesis. *Calcif Tissue Int* **50**, 14–18.
69. Frassetto LA, Todd KM, Morris RC Jr *et al.* (2000) Worldwide incidence of hip fracture in elderly women: relation to consumption of animal and vegetable foods. *J Gerontol A Biol Sci Med Sci* **55**, M585–M592.
70. Kerstetter JE, O'Brien KO & Insogna KL (2003) Dietary protein, calcium metabolism, and skeletal homeostasis revisited. *Am J Clin Nutr* **78**, 584S–592S.
71. Cao JJ, Pasiakos SM, Margolis LM *et al.* (2014) Calcium homeostasis and bone metabolic responses to high-protein diets during energy deficit in healthy young adults: a randomized controlled trial. *Am J Clin Nutr* **99**, 400–407.
72. Sahni S, Cupples LA, McLean RR *et al.* (2010) Protective effect of high protein and calcium intake on the risk of hip fracture in the Framingham offspring cohort. *J Bone Miner Res* **25**, 2770–2776.
73. Darling AL, Manders RJF, Sahni S *et al.* (2019) Dietary protein and bone health across the life-course: an updated systematic review and meta-analysis over 40 years. *Osteoporos Int* **30**, 741–761.
74. 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.
75. 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.
76. Groenendijk I, den Boeft L, van Loon LJC *et al.* (2019) High versus low dietary protein intake and bone health in older adults: a systematic review and meta-analysis. *Comput Struct Biotechnol J* **17**, 1101–1112.
77. 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.
78. Kopec JA & Esdaile JM (1990) Bias in case-control studies. A review. *J Epidemiol Community Health* **44**, 179–186.
79. Thorpe MP & Evans EM (2011) Dietary protein and bone health: harmonizing conflicting theories. *Nutr Rev* **69**, 215–230.
80. Alexy U, Remer T, Manz F *et al.* (2005) Long-term protein intake and dietary potential renal acid load are associated with bone modeling and remodeling at the proximal radius in healthy children. *Am J Clin Nutr* **82**, 1107–1114.
81. Dawson-Hughes B & Harris SS (2002) Calcium intake influences the association of protein intake with rates of bone loss in elderly men and women. *Am J Clin Nutr* **75**, 773–779.
82. Taku K, Melby MK, Takebayashi J *et al.* (2010) Effect of soy isoflavone extract supplements on bone mineral density in menopausal women: meta-analysis of randomized controlled trials. *Asia Pac J Clin Nutr* **19**, 33–42.
83. Wei P, Liu M, Chen Y *et al.* (2012) Systematic review of soy isoflavone supplements on osteoporosis in women. *Asian Pac J Trop Med* **5**, 243–248.
84. Liu J, Ho SC, Su YX *et al.* (2009) Effect of long-term intervention of soy isoflavones on bone mineral density in women: a meta-analysis of randomized controlled trials. *Bone* **44**, 948–953.
85. Levis S, Strickman-Stein N, Ganjei-Azar P *et al.* (2011) Soy isoflavones in the prevention of menopausal bone loss and menopausal symptoms: a randomized, double-blind trial. *Arch Intern Med* **171**, 1363–1369.
86. Tai TY, Tsai KS, Tu ST *et al.* (2012) The effect of soy isoflavone on bone mineral density in postmenopausal Taiwanese women with bone loss: a 2-year randomized double-blind placebo-controlled study. *Osteoporos Int* **23**, 1571–1580.
87. Poole R, Kennedy OJ, Roderick P *et al.* (2017) Coffee consumption and health: umbrella review of meta-analyses of multiple health outcomes. *BMJ* **359**, j5024.
88. Barger-Lux MJ & Heaney RP (1995) Caffeine and the calcium economy revisited. *Osteoporos Int* **5**, 97–102.
89. Heaney RP (2002) Effects of caffeine on bone and the calcium economy. *Food Chem Toxicol* **40**, 1263–1270.