Optimizing Dietary Protein for Lifelong Bone Health

A Paradox Unraveled

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Bone undergoes continuous remodeling; therefore, an adequate supply of amino acid and mineral substrate is needed to support the formation and maintenance of bone across the life span. Although a considerable amount of research has been given to the type and amount of dietary protein intake necessary to achieve optimal bone health, authoritative bodies have varying recommendations around intake, largely established on nonbone health outcomes and/or early nitrogen-balance studies. The relationship of dietary protein intake and bone health has sparked intense debate for many decades, and there are inconsistencies in how healthcare providers counsel patients about protein in relation to bone health and prevention of osteoporosis. However, a recent series of investigations from various researches and leading bone health societies have contradicted these early hypotheses and led to a clearer understanding of the role dietary protein plays in optimizing bone health across the life span. This article reviews the existing evidence to date and summarizes a recent webinar cohosted by the American Society for Nutrition and American Bone Health. Nutr Today. 2019;54(3):107–115

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Dietary protein is the major structural component of all cells (including bone cells) in the body. Proteins function as enzymes, in membranes, as transport carriers, and as hormones, and their component amino acids serve as precursors for nucleic acids, hormones, vitamins, and other important molecules. Dietary Reference Intakes (DRIs) for protein and amino acids were established by the National Academy of Medicine in 2005, using the available nitrogen-balance studies as a proxy for protein synthesis. The Estimated Average Requirement (EAR) was calculated using this methodology to be the average minimal amount of protein (nitrogen) intake to maintain nitrogen equilibrium. In theory, if intake matches excretion, there will be no change in protein levels in the body over time. The Recommended Dietary Allowance (RDA) was calculated as 2 SDs above the EAR to be 0.8 g of good-quality protein (ie, balanced in all 9 essential amino acids) per kilogram of body weight per day for adult men and women (0.85 g/kg per day for children). The National Academy of Medicine also defined an acceptable macronutrient distribution range or range of intake for a particular energy source that is associated with a reduced risk of chronic disease, while providing intakes of all essential nutrients, for protein to be 10% to 35% of total calories per day. While the upper range for total protein in the diet as a percent of total energy intake was set to no more than 35% to decrease risk of chronic disease, there were insufficient data to provide dose-response relationships to establish a tolerable upper intake level for total protein or for any of the amino acids.[1]

Numerous groups have debated whether the current RDA for protein is sufficient to meet the needs of the entire population.

A more accurate and technologically advanced method than traditional nitrogen-balance studies, the indicator amino acid oxidation technique, has been developed since establishment of the DRIs for protein in 2005. This technique has been used to show that male and female adults 65 years or older require protein intakes in excess of 35% of the current RDA to remain in nitrogen balance.[2,3] Levels between 1.2 and 1.5 g/kg per day have been proposed for preserving muscle function in older adults.[4] Pregnant women have also been shown to have a greater requirement than their current RDA (0.88 mg/kg per day) that increases from about 1.22 to 1.52 mg/kg per day from early to late gestation.[5] Requirements for athletes have recently been shown to be approximately 1.83 mg/kg per day,[6] which is well above current recommendations from the American Dietetic Association, Dietitians of Canada, and American College of Sports Medicine.[7] The above findings bring attention to the need for additional health outcomes research with a focus on musculoskeletal health as this system is likely to be affected directly.

Osteoporosis and low bone mass are currently estimated to be a major public health risk for 53.6 million US adults 50 years or older.[8] In addition to calcium in the presence of adequate vitamin D, dietary protein is a key nutrient for bone health across the life span and therefore has a function in the prevention of osteoporosis.[9] Protein makes up roughly 50% of the volume of bone and about one-third of its mass.[10] The bone protein matrix undergoes continuous turnover and remodeling; therefore, an adequate supply of amino acid and mineral substrate is needed to support the formation and maintenance of bone across the life span. Because of the cross-linking of collagen molecules in bone that involves posttranslational modifications of amino acids (including hydroxylation of lysine and proline), many of the collagen fragments released during proteolysis as part of bone remodeling cannot be reutilized to build new bone matrix.[11] Accordingly, a daily supply of dietary protein is necessary for continual bone accretion prior to attaining peak bone mass and maintenance thereafter. However, the relationship between dietary protein intake and bone health has sparked intense debate for many decades, and there are still inconsistencies between how healthcare providers counsel patients about protein intake in relation to bone health and prevention of osteoporosis. Protein has been reported to be both detrimental and beneficial to bone health, depending on a variety of factors, including the amount of protein in the diet, the protein source, calcium intake, weight loss, and the acid-base balance of the diet.[11] Early studies reported that higher protein intakes increased urinary calcium, leading to the assumption that continuous higher intakes were detrimental to long-term bone health. Balance studies were correct in that protein did not affect net balance, but they were misinterpreted. Balance studies are not well suited to determine fractional calcium absorption. Intake of animal-derived proteins has been further hypothesized to add additional detriment to bone due to the postulated increased presence of acidic sulfur–containing amino acids compared with plant-based proteins. Recently, a series of investigations from various researchers and leading bone health societies including the International Osteoporosis Foundation (IOF), National Osteoporosis Foundation (NOF), American Bone Health, and American Society for Nutrition (ASN) have led to a clearer understanding of the role dietary protein plays in optimizing bone health across the life span. The IOF and European Society for Clinical and Economical Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases now advise that dietary protein levels above the current RDA in the United States and Canada, regardless of the source, may be beneficial in reducing bone loss and hip fracture risk, provided calcium intakes are adequate. This article reviews the existing evidence to date and summarizes a recent webinar hosted by the ASN and American Bone Health in September of 2018.[12] Continuing Education credits for dietitians are available for viewing the on-demand webinar recording that can be found on the ASN website (https://nutrition.org/meetings/continuing-education/protein-bone-health/).

How Much Protein Does the US Population Consume?

The US Dietary Guidelines for Americans recommends consuming a mixed-protein diet, consisting of high-quality animal and plant-based foods.[13] The percent of men who consume less than the current EAR has been shown to be low (<3%) using estimates from the National Health and Nutrition Examination Survey (NHANES) 2003–2004 data sets. Although most age/sex groups appear to consume more protein than their respective EAR, a portion of adolescent females (7.7%) and older women (7.2%–8.6%) report consuming protein levels below the current EAR,[14] which may lead to insufficient protein intake to maintain musculoskeletal health. However, there is no significant difference in protein intake as percent energy intake among adults of all ages (Figure 1).[15] There is a trend toward decreased protein intake with advancing age. Protein intake averaged 56 ± 14 g/d in young children, increased to a high of 91 ± 22 g/d in adults aged 19 to 30 years, and decreased to 66 ± 17 g/d in the elderly. The median intake of protein on a percentage of calories basis ranged from 13.4% in children aged 4 to 8 years to 16.0% in men aged 51 to 70 years. Even the 95th percentile of protein intake did not approach the highest acceptable macronutrient distribution range of 35% for an age/sex group. The highest 95th percentile of protein intake was 20.8% of calories in men aged 51 to 70 years.[14]

Daily patterns of protein intake among younger and older adults indicate the majority of protein is consumed at dinner (43.0 and 35.8 g, respectively), followed by lunch

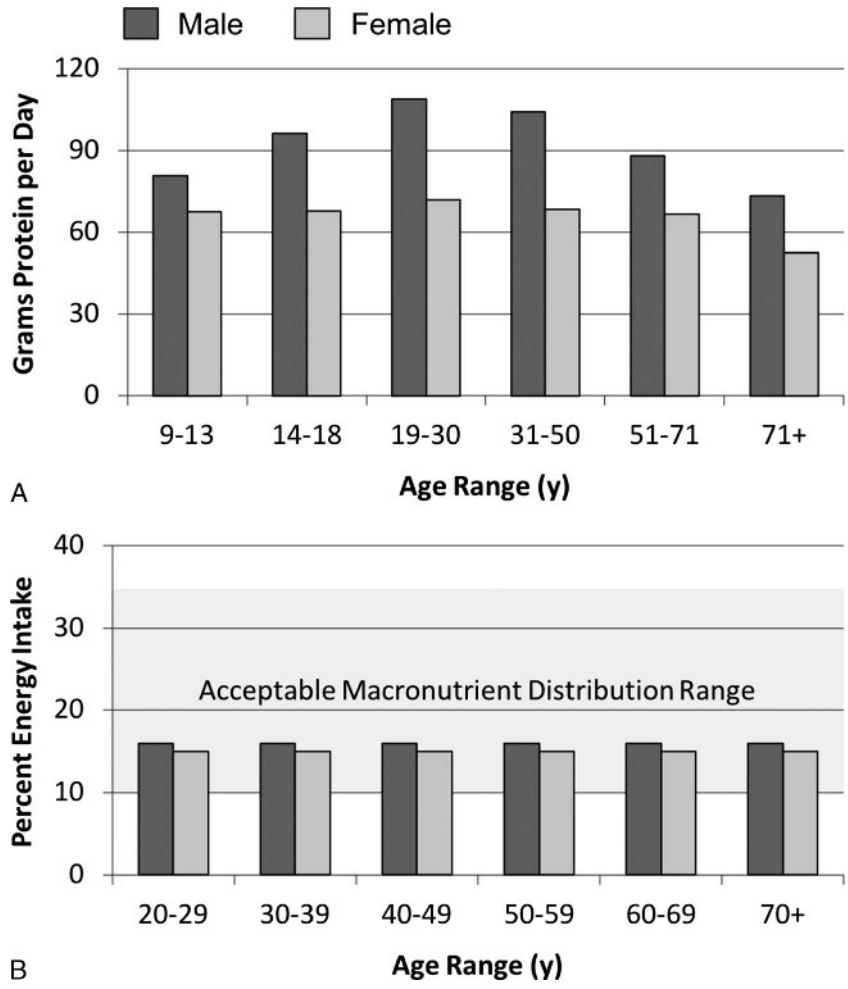

FIGURE 1. Dietary protein intakes by age (A) and as percent of total calories (B) from the National Health and Nutrition Examination Survey, 2003–2004. Adapted with permission from Fulgoni.[14]

(25.4 and 20.2 g, respectively), breakfast (11.1 and 13.3 g, respectively), and as snacks (8.8 and 7.2 g, respectively).[16] The percentages of total protein intakes derived from animal, dairy, and plant protein were shown to be 46%, 16%, and 30% (8% could not be classified), respectively, among adults 19 years or older enrolled in the NHANES 2007–2010. Chicken and beef were the primary food sources of animal protein intake. Cheese, reduced-fat milk, and ice cream/dairy desserts were the primary sources of dairy protein intake. Yeast breads, rolls/buns, and nuts/seeds were the primary sources of plant-based protein intake.[17] Among older individuals enrolled in the NHANES 2005–2006, 5% to 12% of men and 20% to 24% of women were found to be inadequate relative to the current EAR when intakes were adjusted for body weight.[18] Researchers should consider adjustment for body weight when assessing protein adequacy as the current DRIs reflect "reference" weights that reflect the median body mass index of the subpopulation of interest.[19] Age-related causes of protein shortfall include inadequate intake of dietary protein (eg, loss of appetite), a reduction in the utilization of available protein (eg, anabolic resistance), and a higher basal requirement (eg, increased oxidation of protein and increases in the prevalence of acute and chronic diseases).[20]

Because osteoporosis is a skeletal disorder characterized by low bone mineral density (BMD) with compromised bone strength predisposing an individual to increased fracture risk, 2 separate concerns arise to prevent fracture: (1) attainment of peak bone mass in early life and (2) prevention of adult bone loss, particularly in midlife to late life. In both instances, low dietary protein induces hypercatabolism and negative nitrogen balance.

EFFECT OF PROTEIN INTAKE ON PEDIATRIC BONE ACCRUAL AND PEAK BONE MASS

Dietary protein has a significant influence on bone throughout the life cycle that likely begins at some point

during the prenatal period. Findings from mother-offspring cohort studies demonstrate the impact of maternal diet during pregnancy on bone health outcomes in children.[21] During growth and development, skeletal growth proceeds through the coordinated action of bone deposition and resorption to allow bones to expand (periosteal apposition of cortical bone) and lengthen (endochondral ossification) into their adult form.[22–24] This process of bone modeling begins during fetal growth and continues until epiphyseal fusion, usually by the end of the second decade of life. Some skeletal characteristics, such as cortical density and structural strength, determined by bone dimensions and thickness, continue to increase after epiphyseal fusion and into the third decade of life,[22,23] a stage known as peak bone mass (Figure 1). Peak bone mass is generally described as the amount of bone gained by the time a stable skeletal state has been attained during young adulthood. The concept more broadly captures peak bone strength, which is characterized by mass, density, microarchitecture, microrepair mechanisms, and geometric properties that provide structural strength.[23] The frequency of fractures is actually higher among children, particularly young adolescents, as compared with young and middle-aged adults,[25] reflecting the vulnerability of the growing skeleton.

The NOF recently published a comprehensive position statement and systematic review on lifestyle factors that influence peak bone mass development. This systematic review concluded C-level or "limited" evidence for the benefit of protein on bone. This grade was based on beneficial findings from 4 prospective studies and 1 null short-duration randomized controlled trial (RCT).[23] It is important to note that this grade does not discredit the potential role protein may play in pediatric bone development in regard to achieving peak bone mass, but rather emphasizes the lack of current human evidence to date. Interestingly, protein intakes in periadolescent females were positively associated with total body BMD and bone mineral content over 5 years, but only in those with calcium intakes of greater than 1000 mg/d.[26] The NOF assigned B-level or "moderate" evidence for the benefit of dairy on bone,[23] a primary source of protein particularly in younger children. Data from the NOF systematic review demonstrate a need for both calcium and protein adequacy to fully realize the benefit of each nutrient on bone. A copy of the detailed open-access NOF position statement and materials for health providers and patients are available for free at www.nof.org.[23]

TOTAL PROTEIN INTAKE INCREASES BMD AND REDUCES FRACTURES IN OLDER ADULTS

Bone appears to plateau for a number of years during the third and into the fourth decades of life,[23] after which it is gradually lost during middle age to older adulthood (Figure 2).[23] An estimated 60% to 80% of the variability in bone mass and osteoporosis risk has been suggested to be explained by heritable lifestyle factors.[23] However, there is mounting evidence that older adults need more dietary protein as compared to their younger counterparts to support good health and promote recovery from illness and maintain functionality. For the past 50 years or more, there has been significant controversy over whether higher intakes of dietary protein are beneficial or detrimental to long-term bone health in adults. Since 1920, higher dietary protein intakes have consistently been shown to increase urinary calcium.[27,28] Early metabolic balance studies reported that higher protein intakes did not affect intestinal calcium absorption,[29–36] suggesting that the additional urinary calcium must be derived from bone tissue. However, contrary to early metabolic balance studies, more recent contemporary and sensitive dual stable calcium isotope studies have found higher protein intakes to increase intestinal calcium absorption, such that the increase in urinary calcium can be accounted for by the improved absorption efficiency.[37–39] Four published meta-analyses have attempted to address the longstanding controversy surrounding the effectiveness of dietary protein intake on bone health in adults. Darling et al[40] found no effect of higher protein intake on fracture outcomes, whereas the more recent study by Wu et al[41] found slight beneficial associations for high versus low intake on hip fracture risk. Shams-White et al[42] found a beneficial relationship of high versus low protein intake and BMD and bone mineral content for nearly all bone sites; however, statistical significance was present only at the lumbar spine. Wallace and Frankenfeld[43] found that dietary protein intakes above the current RDA (0.8 g/kg per day) may be beneficial in preventing hip fractures (hazard ratio, 0.84; 95% confidence interval, 0.73–0.95) after excluding studies that were designed for weight loss, as well as those that did not correct for or exclude those individuals using hormone replacement therapy (HRT), 2 strong drivers of bone density in postmenopausal women with the ability to mask the effects any nutritional intervention. At the very least, contrary to the longstanding hypothesis, these 4 systematic reviews suggest that higher intakes of dietary protein do not have any detrimental effect on bone and may pose a substantial beneficial effect among older adults.[40–43] What should dietitians consider when comparing the 2 most recent meta-analyses on dietary protein and bone health? Shams-White et al[42] illustrate what we would expect if recommendations around dietary protein intakes were increased globally across the aging population (eg, effects on healthcare cost savings). These data are extremely relevant given that older individuals often substitute other macronutrients for extra protein for various reasons such as weight loss and muscle maintenance. Bone mineral density has been shown to be affected by

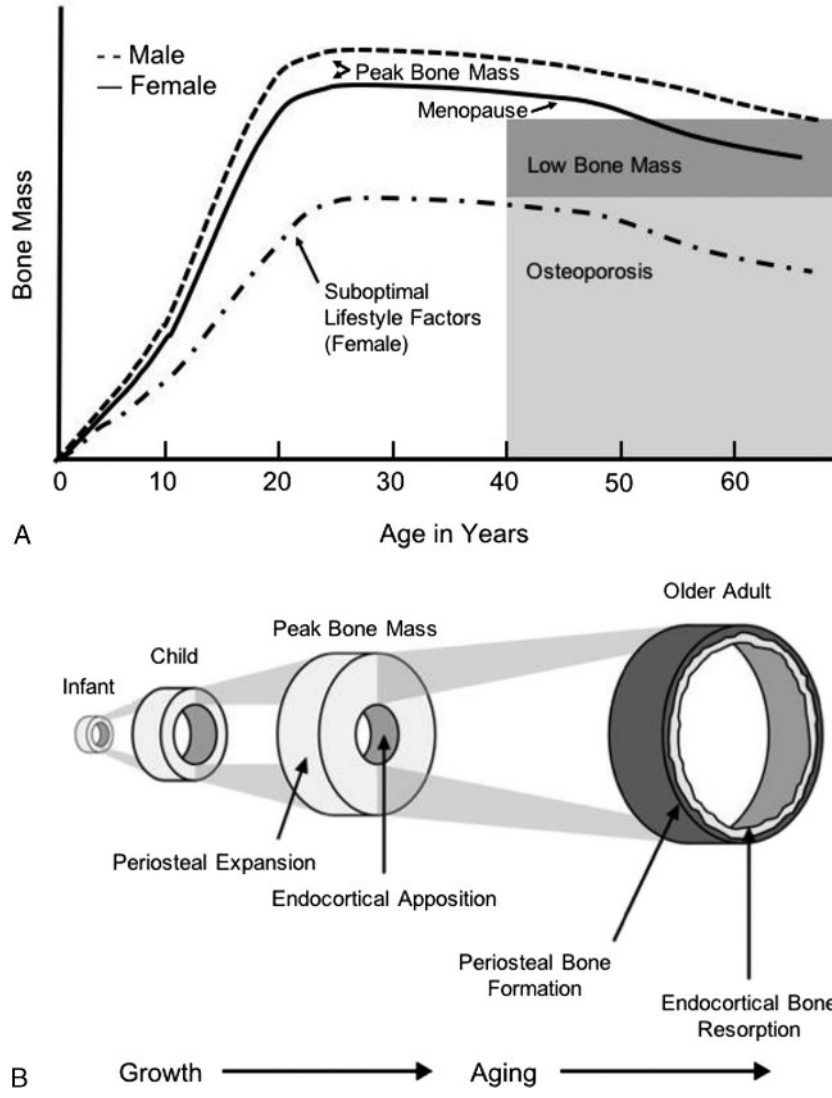

FIGURE 2. (A) Bone mass across the life span with optimal and suboptimal lifestyle factors. (B) Changes in structural composition of bone throughout the life span. Adapted with permission from Weaver et al.[23]

protein intakes at 24% (ie, 1.1 g/kg per day) versus 18% (0.8 g/kg per day) of total calories. High dietary protein intake may primarily influence trabecular bone loss as compared with a normal protein diet.[44] Some postmenopausal women commonly use HRT for treatment of menopause and to reduce the risk of osteoporosis; although the number of women using HRT has greatly decreased over the past decade, older intervention and population studies utilized in systematic reviews still contain significant amounts of women on HRT. Although these 2 factors likely mask the true effect of protein on adult bone, this systematic review gives insight into the population effect of increasing intake recommendations. Wallace and Frankenfeld,[43] correcting for these factors, illustrate what is likely to be the real effect of increasing protein intake within an individual patient's diet. The strengths and limitations of both systematic reviews should be considered in the bone health context.

A recent review and position statement by the IOF and European Society for Clinical and Economical Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases found no adverse effect of higher protein intakes on bone.

The groups noted that increased protein benefits attenuation of age-related bone loss and reduces hip fracture risk. Furthermore, they found that the proposed causal link between dietary acid load due to increased protein intake and osteoporosis is not supported by clinical evidence.[45] No RCT has examined the effect of dietary protein on fracture risk, irrespective of fracture site. Rather, evidence in regard to fractures is limited to prospective cohort studies. In a randomized placebo-controlled trial, conducted among calcium- and vitamin D–replete patients with a recent hip fracture, a protein supplement of 20 g/d led to a 50% reduction in proximal BMD decrease within 1 year.[46] Bone mineral density, which is an important determinant of bone strength, appears to be positively associated with dietary protein intakes.[45] When assessing fracture risk, 3 studies noted in the IOF review found a synergistic interaction between dietary protein and calcium intakes in relation to fracture risk.[47–49] One study found a significant interaction between calcium with vitamin D supplementation and higher protein intake on femoral neck and total body BMD. Positive effects of calcium with vitamin D supplementation on femoral neck BMD were most evident in the highest dietary protein tertile; there was an estimated +2.8% difference in femoral neck BMD between higher and lower protein tertiles.[50]

Animal Versus Plant Protein—Debunking the Acid-Base Hypothesis

There has been much debate on the "acid-base hypothesis," which theorizes that metabolism of high protein intake, particularly from animal origin with sulfur-containing amino acids, leads to increased acid production and resultant bone resorption, as reviewed by Bonjour.[51] This hypothesis is supported by evidence from healthy subjects or in patients with chronic renal failure, indicating that the administration of large doses of ammonium chloride led to a marked decrease in serum bicarbonate and an increase in urinary calcium excretion attributed to mobilization from calcium carbonate from bone to buffer the acid load.[52] Conversely, supplementation with basic potassium bicarbonate in healthy individuals and/or those with chronic renal failure is associated with improved calcium balance.[52] Alkali administration is associated with a reduction in net acid excretion with no change in bone formation markers. In 25 analyzed studies, diet-derived acid load was manipulated by dietary intakes, such as sulfur-containing amino acids, protein, meat, grain, or fruits and vegetables, or acidic or alkaline salts such as ammonium chloride, potassium bicarbonate, or potassium citrate. A positive linear relationship was found between changes in urinary calcium excretion and changes in net acid excretion in urine over a wide range of acidic and alkaline urine samples. It should be noted that food-related variations in urinary acid excretion represent a physiological and homeostatic response to dietary acid load.[53] However, an association between urinary calcium and acid excretion does not imply that the source of calcium is primary because of increased bone resorption, thereby contributing to the development of osteoporosis. An alternative possibility is that acidosis or alkalosis due to dietary factors, such as increased sulfur-containing amino acid intake, alters renal tubular reabsorption of calcium. Thus, resulting hypercalciuria may represent more of an increase in calcium throughput versus mobilization of bone mineral. If this acid-base hypothesis were to hold true, we would expect there to be a loss in BMD over time due to chronic elevated bone resorption. As previously discussed, higher-protein diets with predominantly animal protein seem to increase BMD and reduce fractures.[40–43] Furthermore, a strict vegetarian diet with protein derived from grains and legumes would deliver as many millimoles of sulfur per gram of protein as would a purely meat-based diet, and it is unlikely that bone is exposed to small changes in extracellular pH in relation to animal or plant protein consumption within the limits of a balanced diet.[45,54]

In the Osteoporotic Fractures in Men Study cohort, the hazard ratios were 0.84, 0.80, and 0.84 for total, dairy, and nondairy animal proteins, respectively, whereas it was 0.99 for vegetable protein.[55] A prospective investigation of the Iowa Women's Health Study showed higher protein intake to be associated with a reduced incidence of hip fracture among more than 40 000 postmenopausal women. The protective effect was mostly observed with dietary protein intake from animal origin.[56] In the Framingham Offspring Study, lower relative hip fracture risk was noted in those with higher protein intakes when calcium intake was greater than 800 mg/d,[47] illustrating the potential synergy between the 2 nutrients. Similarly, among participants enrolled in the European Investigation Into Cancer cohort, fracture risk did not differ among those who consumed equal to or greater than 525 mg/d of calcium. Vegans with low intake of calcium (<525 mg/d) had an increased risk of total fractures as compared with meat eaters.[57] Numerous RCTs have assessed the influence of both protein and calcium supplementation on bone health variables, through administration of dairy products. Although small in size and statistical power, altogether, dairy products (some being fortified with calcium and/or vitamin D) were consistently associated with a decrease in circulating parathyroid hormone, an increase in insulin growth factor 1, and a decrease in bone resorption markers. Ten of 13 studies reviewed by the IOF reported reduced decreases or improvements in BMD in response to dairy products.[45] It is clear that a diet low in fruits and vegetables appears to be associated with increased fracture risk[58]; however,

nutrient density of the diet seems to have an even stronger correlation.[59]

SAFETY CONCERNS WITH HIGHER PROTEIN INTAKES

Although it is believed that there is no risk of adverse effects when healthy people consume high-protein diets, the lack of long-term human studies should be considered.[60] Glomerular filtration rate of the kidney rises after protein consumption is increased,[61] but this response declines with age. However, although it is a fact that higher protein intakes are harmful to individuals with existing kidney dysfunction, there is little supportive evidence that it is dangerous to generally healthy individuals.[60,61]

A fairly large body of evidence demonstrates that high-portion diets providing 2 to 3 times the RDA do not appear to increase the risk of adverse health outcomes, including renal stones, dehydration, compromised renal function, reduced bone health, or when consumed for months, altered glomerular filtration rate or blood levels of lipids, glucose, creatinine, or blood urea nitrogen in healthy individuals.[1,62–65]

Characterizing the relationship between high protein intake and hydration is of great importance given the increase in excretion of urea and other nitrogenous wastes requires more water to avoid dehydration. A recent systematic review found limited and inconsistent evidence with regard to the role of protein intake and risk of kidney stones. Increased protein intake did not have clinically significant effects on blood markers of kidney function.[66] It should be noted that low-risk-of-bias, long-term human clinical studies assessing the effect of higher protein intakes on renal health are absent, and further research is warranted.

Although there are no clear renal-related contraindications to high-protein diets in individuals with healthy kidney functions, theoretical risks should be reviewed carefully with the patient. Because chronic kidney disease (CKD) is often a common and silent disease that increases in prevalence with age, screening serum creatinine measurement and urinary dipstick test for proteinuria before initiation of a high-protein diet is practical in middle-aged to older adults. Individuals with diabetes are at a higher risk of kidney disease,[67] and these recommendations also apply to them.

Conditions for Limiting Protein Intake

The National Kidney Foundation recommends protein intakes below 0.6 to 0.75 g/kg per day for nondialyzed individuals with CKD.[68] High protein intakes present the potential for significant harm in individuals with CKD and should be avoided when possible. High-protein diets have also been associated with an increased risk of renal stone formation in some but not all large population studies,[60] and therefore protein levels should remain modest among individuals who have experienced prior or recurrent renal stones. Increased renal stone prevalence has been attributed to increases in obesity and diabetes and is likely to be highly influenced by an individual's genome. It should be noted that the literature assessing effects of higher protein intakes on renal stone incidence is weak, at best. Human intervention studies designed to assess renal stone incidence as a primary outcome are greatly needed as a recent systematic review of the peer-reviewed literature found insufficient evidence to determine if increased protein intakes influence kidney health outcomes.[66]

Assessing the Role of Protein—Study Design Matters

Randomized controlled trials are considered the criterion standard from a clinical research paradigm[69]; however, there is a scarcity of high-quality diet-related intervention trials assessing protein intake with bone as a primary outcome. Randomized controlled trials with health-related outcomes (including bone) are often challenging to conduct and interpret because of a number of factors including cost, time commitment, and difficulties in maintaining compliance, health problems or medication changes, and ethical issues associated with assigning people to a nonintervention control comparison group.[70–72] For trials with bone as an outcome, a long-term follow-up period is required because the entire bone remodeling cycle typically ranges from 6 to 9 months in duration.[72] As with most dietary interventions, it is challenging to use a placebo-controlled design because blinding is difficult or impossible to achieve and the absence of nutrition is not a practical or ethical study arm.[71,72] A group of internationally recognized scientists attending the 2017 Interdisciplinary Symposium on Nutritional Aspects of Osteoporosis recently reviewed pertinent factors to consider when designing research, methodologies, dietary assessment, statistical analyses, and other special considerations for nutrition and

bone relationships.[72] Strongly related to the protein and bone discussion is the failure of many studies to account for and document all potential confounding factors and assumptions, which limit our ability to adequately synthesize research in a meta-analysis.[72]

CONCLUSIONS

The role of protein appears to be complex and is likely dependent on the presence of other nutrients available in a mixed diet. At the very least, contrary to the longstanding hypothesis, fairly compelling evidence to date suggests that higher intakes of dietary protein do not have any detrimental effect on bone and likely pose a beneficial effect. There is currently no direct evidence of detriment to BMD or fractures resulting from consumption of animal versus plant protein, although evidence is limited because of low intake of plant protein across observational studies and interventions in relation to total protein intake. Diets high in plant-based foods are important for health and disease prevention. However, we should not underscore the importance of animal-derived foods that contribute higher levels of protein (and certain micronutrients), particularly as we age and bone loss becomes more apparent.

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