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Review

Exploring Opportunities to Better Characterize the Effects of Dietary Protein on Health across the Lifespan

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A B S T R A C T

Remarkable advances have been made over the last 30 y in understanding the role of dietary protein in optimizing muscle health across the lifespan. That is, acute (<24 h) stable isotope-derived measures of muscle protein synthesis have led to established recommendations for protein quantity, quality, source, and timing of protein ingestion to support muscle health at rest, post exercise, and to overcome age-related anabolic resistance in older adults. Although muscle health is undoubtedly important, moving from muscle to other associated or disease-specific outcomes is a critical next step for the field, given the mounting evidence documenting the effects of dietary protein on measures of chronic disease and age-related decline (for example, cardiovascular disease, type 2 diabetes mellitus, obesity, frailty, and osteoporosis). In this narrative review, we posit that future studies evaluating the potential role of dietary protein build off of the existing knowledge base generated from decades of past research and focus their efforts on closing unanswered knowledge gaps pertaining to dietary protein and health across the lifespan. Throughout this review, we highlight potential methodologies and novel outcome measures that researchers may consider as starting points to facilitate the next 30 y of advances in the field of dietary protein and health.

Keywords: low and high protein diets, strength, sarcopenia, cardiovascular disease, type 2 diabetes mellitus, physical function, bone fracture risk, satiety, sleep, obesity

Statement of significance

In this narrative review, we make the case that tremendous gains have been made in our understanding of dietary protein and muscle over the last three decades, but that future studies evaluating the potential effects of dietary protein should emphasize outcomes critical to optimizing health across the lifespan, focusing their efforts on closing the unanswered gaps in knowledge pertaining to cardiometabolic health, frailty, bone accrual and maintenance, and weight management. We provide recommendations for the methodologies and outcome measures to help facilitate the next 30 y of advances in the field of dietary protein and health.

Introduction

Data generated from nearly three decades of highly controlled laboratory studies evaluating the effects of dietary protein intake on isotopic measures of whole-body or muscle protein turnover serve as the basis for dietary interventions in long-term clinical trials and for many contemporary dietary protein recommendations to optimize muscle health across the lifespan. For example, recommendations that advocate for equally distributing dietary protein intake across meals [1], how much protein to consume

Abbreviations: aBMD, areal bone mineral density; CVD, cardiovascular disease; DISST, dynamic insulin sensitivity and secretion test; GI, glycemic index; HbA1c, hemoglobin A1c; HOMA-IR, homeostatic model assessment for insulin resistance; HR-pQCT, high-resolution peripheral quantitative computed tomography; IGF-1, insulin-like growth factor 1; IGF-1 BP3, insulin-like growth factor binding protein-3; RDA, recommended dietary allowance; REE, resting energy expenditure; RET, resistance exercise training; SNP, single-nucleotide polymorphism; T2DM, type 2 diabetes mellitus; TC, total cholesterol.

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within each meal [2], post exercise [3], and the amount necessary to overcome age-related anabolic resistance [4] were derived from tracer studies quantifying muscle protein synthesis or net whole-body protein balance in response to ingesting free-form amino acids, isolated intact proteins, protein-containing foods, or mixed meals.

Although undoubtedly important, manipulating protein intake beyond the recommended dietary allowance (RDA; 0.8 g/kg/d) may also confer other meaningful health benefits. For example, altering dietary protein quantity, quality (that is, essential amino acid content, digestibility, and bioavailability), and source (that is, amino acids, isolated protein, protein-rich foods, or protein in mixed meals) may modulate insulin sensitivity, cardiovascular disease (CVD), frailty, osteoporosis and fracture risk, appetite, and obesity. However, although quality research continues to expand our understanding of how dietary protein intake plays an important role in mediating a variety of health outcomes, critical gaps in knowledge remain. As such, future research on dietary protein should consider extending beyond commonly used assessments of muscle protein synthesis and whole-body protein turnover and identify appropriate outcomes and credible biomarkers needed to clearly define how increasing dietary protein beyond the RDA or altering the source of protein consumed may or may not optimize health across the lifespan (Table 1).

Recognizing that the broad range of health conditions affected by diet is beyond the scope of any one review, and given the state of the science, we chose to focus on how dietary protein affects cardiometabolic health, frailty, bone, and weight management. Throughout our review, we emphasize research methodologies and data collection techniques that researchers in this field should consider as a foundation for future studies to best assess how dietary protein intake affects health (Table 2).

Cardiometabolic Health

Cardiometabolic health encompasses known risk factors that increase the likelihood of impaired metabolism (for example, insulin resistance) and cardiovascular events (for example, myocardial infarction and stroke). Dietary protein intake can influence measures that are useful for assessing, and reflective of, cardiometabolic health, such as blood pressure and plasma lipid concentrations. Similarly, dietary protein intake has been associated with risk of insulin resistance, a known comorbidity and contributor to the development of CVD. The potential influences of dietary protein on cardiometabolic health are examined here.

Insulin resistance

As the foundational building block for muscle, dietary protein intake and its constituent amino acids have been theorized to enhance glucose disposal through greater muscle uptake. Indeed, greater skeletal muscle mass is associated with improved metabolic function (for example, glucose disposal, protein metabolism, mitochondrial function, decreased arterial stiffness, and lower oxidative stress) that lowers the risk of emergent CVD [5,6].

In contrast, select epidemiological research has shown associations between higher relative dietary protein intakes (for example, quartile 4 compared with quartile 1) and the development of insulin resistance [7]. Similarly, dietary protein restriction has been reported to improve insulin sensitivity, as measured via hyperinsulinemic–euglycemic clamp [8]. This observation, however, was in a small number of individuals with metabolic syndrome who lost 6.6% of initial body weight after a dietary intervention. Weight loss is a well-known confounder when assessing the effect of dietary interventions on insulin resistance [9].

The dynamic insulin sensitivity and secretion test (DISST) can be used to assess changes in insulin sensitivity in response to dietary interventions [10–12]. DISST measures in women with overweight and obesity with a family history of type 2 diabetes mellitus (T2DM) reveal beneficial changes in cardiometabolic biomarkers paired with a reduction in insulin sensitivity after weight loss on a high-protein (30% daily kcal), high-fiber diet (>30 g/d) [11]. DISST assessments may not always align with static measures of glucose utilization [for example, fasting blood glucose and insulin concentrations, homeostatic model assessment for insulin resistance (HOMA-IR)]. The study authors described these seemingly opposing results as the potential for metabolic adaptations to illustrate the physiological need to regulate glucose homeostasis, as opposed to as an indicator of metabolic dysregulation. Regardless, markers of CVD risk were improved after a dietary intervention emphasizing energy restriction and higher fiber and protein intakes.

This relationship between dietary protein intake and insulin sensitivity may be mediated by impaired BCAA catabolism [13–16]. Based on clinical data, enhancing BCAA disposal can resolve insulin resistance [17]. Related controlled interventions have shown remission of prediabetes with a high-protein diet (30% daily kcal) [18,19], but care must be taken when considering whether to ascribe this effect to high-protein intakes compared with displacing carbohydrates in the diet with more protein. Similarly, a diet containing 30% energy intake from protein reduced insulin resistance and improved daily blood glucose concentration variability—a known risk factor for T2DM—in insulin-resistant women with obesity in a crossover study, relative to a Mediterranean diet [20]. Total red meat intake was not associated with changes in T2DM biomarkers, including fasting blood glucose, insulin, HOMA-IR, and hemoglobin A1c (HbA1c) [21]. These observations again highlight the importance of the food matrix and overall dietary quality, as well as the need to utilize sound scientific research when building evidence-based dietary interventions.

Genetic and microbiome influences on T2DM

Genetic risk scores, calculated via the identification of single-nucleotide polymorphisms (SNPs) associated with T2DM, suggest that increased protein intake (25% daily kcal) may reduce fasting insulin in White individuals with higher risk scores [22]. Those with lower risk scores, however, experienced reduced fasting insulin and HOMA-IR on a protein-adequate diet (15% daily kcal). It is important to note, therefore, that genetic variations may also influence the individual cardiometabolic response to dietary protein intake, with significant interactions relating to glucose metabolism and insulin sensitivity.

Protein-rich foods generally have a lower glycemic index (GI) than their carbohydrate-rich counterparts, which can be beneficial in moderating postprandial glucose uptake and insulin secretion [23]. There are noted sex differences in postprandial glycemic control, however, with high GI foods tending to induce

TABLE 1
Measures for consideration of use in future research to assess the effects of dietary protein intake on selected health outcomes (listed alphabetically by domain).

Outcome measures	Benefit
Cardiometabolic	
BCAA catabolism	Associated with the development of insulin resistance
Blood Lipids (for example, TC, LDL, HDL, and TG)	CVD risk profile
Blood pressure	Indicator of overall cardiovascular health
Computed tomography (CT)	Capable of differentiating skeletal muscle from other lean mass and assessing change over time
D_3-creatine	Associated with functional capacity, insulin resistance, and mortality
DXA	Easily obtained body composition measures, including trunk and appendicular lean mass, fat mass, and bone mineral content
Genetic Risk Scores based on SNPs associated with T2DM	Greater individualized precision in identifying both disease risk and method of dietary intervention most likely to mitigate that risk
Insulin sensitivity (for example, hyperinsulinemic–euglycemic clamp, DISST, HOMA-IR) and blood glucose control [for example, plasma (glucose), HbA1c]	Predictors of insulin resistance and T2DM development
MRI	Capable of differentiating skeletal muscle from other lean mass and assessing change over time
Microbiome profiling	Determine the balance between microbes known to negatively compared with positively affect glucose metabolism and insulin sensitivity
Nutrimetabolomics	Identification of food components and metabolites linked to cardiometabolic health
Serum trimethylamine N-oxide	Predictor of future cardiovascular events (for example, myocardial infarction and stroke) and mortality
Frailty	
CT	Capable of differentiating skeletal muscle from other lean mass and assessing change over time
D_3-creatine	Associated with functional capacity, insulin resistance, and mortality
DXA	Easily obtained body composition measures, including trunk and appendicular lean mass, fat mass, and bone mineral content
MRI	Capable of differentiating skeletal muscle from other lean mass and assessing change over time
Mobility assessments (for example, gait length and speed, 30-s sit-to-stand test, 4-stage balance test)	Direct indicators of muscle functionality
Bone health	
Biomarkers (for example, parathyroid hormone, osteocalcin, sclerostin, phosphorous, and ionized calcium)	Easily measured indicators of bone formation and resorption, though less accurate and not specific to areas of higher fracture risk
Calcium stable isotopes	Measure calcium absorption, excretion, exchange with bone, and whole-body retention
DXA	Measure bone mineral content and density; associated with fracture risk
High-resolution peripheral quantitative computed tomography	Determine bone volume and differentiate between trabecular and cortical bone
Serum IGF-1 and IGF-1 BP3	Predictors of bone calcium accretion in adolescence
Weight management	
Appetite-regulating hormones (for example, GLP-1, PYY, CCK, and leptin)	Related to energy intake and changes in body mass
Body weight	Determine changes in body mass over time
DXA	Determine changes in %BF over time
Genetic risk scores based on SNPs associated with the microbiome	Related to changes in fat mass and adipose distribution
Resting energy expenditure	Typically represents the greatest contributor to daily energy expenditure
Sleep measures (for example, actigraphy, sleep time, and sleep onset latency)	Associated with the risk of weight gain and regain

Abbreviations: %BF, percent body fat; CCK, cholecystokinin; CVD, cardiovascular disease; DISST, dynamic insulin sensitivity and secretion test; DXA; GLP-1, glucagon-like peptide-1; HbA1c, hemoglobin A1c; HOMA-IR, homeostatic model assessment for insulin resistance; IGF-1, insulin-like growth factor 1; IGF-1 BP3, insulin-like growth factor binding protein-3; PYY, peptide YY; SNP, single-nucleotide polymorphism; TC, total cholesterol; T2DM, type 2 diabetes mellitus.

a greater glycemic response in women than men [24]. As such, future investigations with dietary protein interventions should account for sex-related disparities while continuing to evaluate glucose utilization, including assessment of fasting and postprandial blood glucose and insulin concentrations, 24-h continuous glucose monitoring, and, for studies of sufficient duration (that is, ≥12 wk), pre- and postintervention HbA1c and HOMA-IR assessments [25].

The gut microbiome likely also influences glycemic control and CVD risk [26,27]. Certain gut bacteria (for example, genus *Lachnoclostridium*) have the potential to generate metabolic byproducts that negatively affect glucose metabolism and lower insulin sensitivity [20], whereas others (for example, genus *Coprococcus*) may play a beneficial role in maintaining plasma glucose homeostasis. Higher genetic risk scores, based on 20 SNPs associated with the relative abundance of gut microbes, were associated with greater reductions in fat mass and percentages of both fat mass and trunk fat after 6 m of energy restriction (~83% baseline kcal intake) with adequate protein intake (15% daily kcal) [28]. Genetic risk scores were not

TABLE 2
Suggested outcome measures and recommendations for their use in future dietary protein-related research (listed alphabetically by domain).

Outcome measures	Effect of dietary protein	Recommendations
Cardiometabolic		
Blood Lipids (for example, TC, LDL, HDL, and TG)	=	Control for confounding effects of the food matrix, particularly fat and carbohydrate amount and type
Blood pressure	↑, =	Determine whether protein source and food matrix affect observed changes
Insulin sensitivity	↑, =, ↓	Control for confounding effects of weight loss/gain and assess efficiency of plasma BCAA catabolism to delineate the effect of dietary protein intake from impaired metabolism
Microbiome	?	Determine how protein source (that is, amino acid profile, food matrix) affects relative abundance of beneficial microbes
Muscle mass	↑, =	Evaluate protein intake in the context of overall energy intake; relate changes to glucose disposal and insulin sensitivity
Frailty		
Mobility and functionality assessments	↑, =	Utilize longitudinal measures to relate changes over time to protein intake; preintervention supplementation trials (for example, cancer and surgery) to assess differences relative to current standard care
Muscle mass	↑, =	Evaluate influence of protein intervention in isolation or when combined with physical activity; when available, use tools to differentiate skeletal muscle from lean mass (for example, CT, MRI and D_3-creatine)
Bone		
Bone mineral content	↑	Use of HR-pQCT to assess incremental changes and differences in trabecular compared with cortical bone; DXA suitable for larger and longer assessments
IGF-1, osteokines	↑	Continued exploration of protein's impact on muscle–bone crosstalk
Parathyroid hormone	↓	Confirm dietary protein's role in suppressing increased bone turnover
Weight management		
Appetite-regulating hormones (for example, GLP-1, PYY, CCK, and leptin)	↑	Continued assessment to differentiate effect of protein source and food matrix on satiety signals
Body mass and % body fat	?	Reconciliation of short- compared with long-term study findings; control for dietary intervention compliance
Microbiome	?	Determine potential influence of bacterial amino acid metabolism on satiety signals
Sleep	↑	Continued examination of the effect of dietary protein on quantity and quality of sleep, particularly the potential role for presleep protein ingestion

Abbreviations: ↑, improved by dietary protein; ↓, worsened by dietary protein; =, no observed effect from dietary protein; ?, unknown effect of dietary protein; CCK, cholecystokinin; CT, computed tomography; DXA; GLP-1, glucagon-like peptide-1; HbA1c, hemoglobin A1c; HR-pQCT, high-resolution peripheral quantitative computed tomography; IGF-1, insulin-like growth factor 1; PYY, peptide YY; TC, total cholesterol.

associated with body composition changes for those consuming protein at 25% daily kcal intake at 6 mo, and all associations disappeared by 2 y post baseline, regardless of genetic risk score and assigned protein intake, likely due to reduced dietary compliance of study volunteers. In the near term, however, the gut microbiome may serve a role in mediating the impact of dietary interventions on body composition and cardiometabolic health.

Changes in the gut microbiome were associated with improved CVD risk factors [for example, total cholesterol (TC), LDL, TG, HDL, TC/HDL ratio] in those after a U.S. Healthy Dietary pattern, again highlighting the importance of the food matrix and overall dietary quality [29]. Much remains to be learned regarding the microbiome and its potential impact on cardiometabolic disease risk [30]. As such, future investigations aimed at better understanding and modifying the diet-induced influence of gut microbes on chronic disease risk will be beneficial (Table 2).

CVD markers

In clinical research, a higher-protein, lower-fat meal (higher protein: 30 g protein, 17 g fat, 52 g carbohydrate compared with lower protein: 13 g protein, 25 g fat, 54 g carbohydrate) increased plasma arginine concentrations, a known vasodilator, though biomarkers associated with blood pressure were unaffected, as was blood pressure itself in response to exercise [31]. In adults with overweight and obesity, reductions in red meat intake were associated with lower serum trimethylamine N-oxide concentrations, a biomarker for atherosclerosis risk [32]. Similarly, with the exception of isoflavone-containing soy, protein-rich foods did not independently lower plasma total- and LDL-cholesterol concentrations [30]. Total red meat intake also did not influence inflammatory biomarkers associated with CVD [21]. In contrast, the incorporation of milk protein isolate (0.7 g/kg/d) as a component of an energy-restricted diet (750 kcal/d below estimated requirements) improved blood pressure and plasma triglyceride concentrations, when compared with the addition of a carbohydrate control (0.7 g/kg/d; primarily maltodextrin) [33].

Clearly, much remains to be learned regarding how dietary protein intake may influence blood pressure and plasma lipid concentrations. Discerning the impact of the protein component compared with the overall food matrix will be important to identify future dietary interventions.

Nutrimetabolomics

A useful tool in evaluating food makeup and quality is nutrimetabolomics, the determination of the inherent chemical composition of food items and their post-consumption metabolite production [34,35]. Nutrimetabolomic profiles have been

used to identify bioactive compounds in the food matrix beyond traditional classifications of animal- compared with plant-protein sources, including BCAAs, phospholipids, citric acid cycle intermediates, polyphenols, and gut microbiota metabolites [36,37]. The differences between protein sources illustrated via nutrimetabolomic profiles may underlie noted disparities in health outcomes, which have typically been ascribed more generally to animal- or plant-protein intakes. Continued use of these tools will allow for a greater appreciation of the impact of the protein-rich food matrix on disease risk beyond just categorizing protein source (that is, animal compared with plant). This point is of particular importance when considering protein isolates, which may retain the amino acid profiles of their sources but are without potential beneficial or detrimental additional components of the native food matrix.

Considerations for observational research

The applicability of observational research is often limited by the inability to determine causality [38]. A recent umbrella review utilized Bradford Hill criteria to examine potential causal relationships between dietary protein intake and both CVD and T2DM [39]. The Bradford Hill method considers 9 viewpoints—strength, consistency, specificity, temporality, biological gradient (that is, dose–response curve), plausibility, coherence, experiment, and analogy—when seeking to identify potential causal relationships in non-experimental data [40]. This current work [39] assessed different protein sources, illustrating a potential link between red and processed meat intakes and a higher risk of T2DM, but not to increased likelihood of CVD. Lean chicken and beef have been associated with increased total and fat mass loss during energy restriction, increased lean body mass when combined with resistance exercise training (RET) during energy balance, and lower plasma total and LDL-cholesterol concentrations [41]. Notably, protein-rich foods (that is, high protein density per kcal, such as poultry, eggs, seafood, red meat, dairy, nuts, and lentils) are often not well-described in the research literature and, when included, descriptions are not standardized (for example, “lean” and “processed”), which can make it difficult to reconcile findings across studies [42]. Similarly, the overall macronutrient and micronutrient composition of these protein-rich foods is critical in mediating the health effects associated with their intake. As such, the composition and nutrient profiles of protein foods used in experimental research should be identified and clearly described in future reports [43]. These factors, along with the use of Bradford criteria and other similar assessment practices (for example, STROBE [Strengthening the Reporting of Observational studies in Epidemiology], TRIPOD [Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis], and STARD [Standards for Reporting of Diagnostic Accuracy Studies] criteria) [38], will be valuable for increasing the utility of observational research.

In total, continued investigation of the effects of dietary protein intake on insulin sensitivity, gut microbiome modulation, and CVD markers will help to expand the current knowledge base. The assessment of relevant biomarkers (Table 1) is critical for understanding these effects and for creating future opportunities for targeted recommendations to enhance health and well-being.

Frailty Prevention

Aging is associated with declines in muscle mass, strength, and physical function, collectively known as sarcopenia. A fundamental question in aging biology is the degree to which these declines are intractable or subject to mitigation through diet and physical activity intervention [44]. There is little doubt that RET is the most potent stimulus to ameliorate sarcopenia [45], yet numerous attempts have been made to investigate how supplemental dietary protein can augment RET-induced adaptations in aging adults [46–49]. The consensus is that protein plays a small part in improving lean mass (note this is a proxy for skeletal muscle) with RET [47]; however, clinically meaningful enhancements in strength and, importantly, physical function are far less commonly observed [46–49]. The small effects of protein in augmenting lean mass, strength, and physical function are likely due to the anabolic potency of RET, which is far more robust compared with protein as a stimulus for anti-sarcopenic adaptation [50].

In the absence of RET, no interventional trials, to date, have shown an effect of increased dietary protein intake or protein supplementation on improving body composition, strength, or physical function [46,48]. This observation may be because no trial has ever been analyzed with sufficient power (participants or duration) to see such an effect with currently available methods for quantifying skeletal muscle mass, let alone strength and physical function. Further discussion is warranted, however, because systematic reviews of observational trials have noted an association between dietary protein intake above the RDA and lower incidence of sarcopenia [51,52], preservation of lean mass [53,54], and improved physical function [53].

Dietary and physiological stressors associated with frailty

Certain stressors, including weight loss, disuse, and disease, affect the aging process and, relevant to this discussion, can impact protein requirements. During energy restriction, increasing dietary protein intake enhances fat mass loss and lean mass retention in older adults [55,56]. Disuse and unloading precipitously reduce muscle mass and strength, which, in humans, is mainly due to a reduction in muscle protein synthesis [57]. In middle-aged adults, mixtures of amino acids enriched in leucine may mitigate the decline in muscle protein synthesis seen with disuse and thus ameliorate disuse atrophy [58,59]. Although plausible, to the best of our knowledge, there is still no clear evidence to support the role of leucine-enriched mixtures of amino acids on disuse atrophy in older adults [60,61]. Regardless, when considering that older adults: *1*) may experience disuse events more frequently than middle-aged or younger adults; *2*) are likely in a state of anabolic resistance [62,63]; and *3*) have higher requirements for the essential amino acid leucine than their younger counterparts [64], it is sensible to recommend that older adults prioritize high-quality protein ingestion anytime they experience a disuse event [65]. This thesis is prudent because the recovery from disuse events is often delayed in older compared with younger adults and is possibly absent without loading activity and high leucine-containing protein ingestion [66].

Frailty outcomes measures

Future work in older adults needs to focus on determining protein needs and optimal intake levels to mitigate declines in mobility. Disuse events and diseases pose a particular risk for older individuals, as age is a leading risk factor for many chronic diseases. Disuse events are particularly deleterious in older individuals and can begin a sharp downward spiral in muscle mass, strength, and physical mobility that could precipitate a step toward the development of mobility disability and frailty [44]. Importantly, we need to use gold-standard measures (Table 1), including limb computed tomography or MRI, to capture changes in skeletal muscle (as the biological substrate of mobility), not lean mass, with aging or stress. Another alternative is using stable isotope-labeled creatine (that is, D_3-creatine) to measure the body creatinine pool and estimate skeletal muscle mass, a non-invasive measure associated with functional capacity, insulin resistance, and mortality [67,68]. When access to, or feasibility in using, such measures are limited (that is, cost, equipment, and analytical capacity), bioelectrical impedance analysis and bioelectrical impedance spectroscopy may be effectively employed to estimate skeletal muscle mass [69].

Assessing frailty on a continuum, using validated measures [70], can allow for the identification of intervention points in older adults who present with indicators of vulnerability despite no obvious signs of functional limitation. Along these lines, it will be valuable to begin incorporating multiple domains of mobility assessment, including self-reporting, laboratory-based measures, and real-life measures from wearable devices (Table 2). The integration of these three domains of mobility can provide a much more complete picture of how any dietary protein, activity-based, or combination intervention affects functionality.

Bone Health

Considerable growth in the understanding of bone biology, combined with recent advances in novel research methods for assessing bone metabolism, tissue adaptation, and microstructural changes in humans skeleton, yields an opportune time to study the effects of dietary protein on bone health and osteoporosis risk.

There are currently two evidence-based dietary protein-related strategies for minimizing risk of low bone mass and fracture. The first is to build the highest peak mass and strength that is genetically possible during adolescent growth, when bones are most sensitive to mechanical stress [71] and when the rate of peak bone mineral accrual is greatest [72]. The second is to continue to build bone mass and maintain those benefits throughout adulthood, while also preventing the loss of bone mass and strength that can accompany menopause in women and advancing age in all sexes [73].

Dietary protein and bone mass

Nutrient-dense diets and physical activity are the non-pharmacologic cornerstones of building, maintaining, and preventing loss of bone mass and strength throughout life. Nutrition recommendations for bone health have focused on micronutrients, especially calcium and vitamin D, which are frequently deficient in populations around the world [74]. Despite the important structural role of protein in bone, in that protein comprises half of bone volume and one-third of bone mass, dietary protein intake has received much less focus than calcium and vitamin D for bone health. This relative lack of attention may be because protein intake is generally perceived as meeting or exceeding the RDA, although this is not always true for older adults [75]. Longitudinal observational and retrospective studies report a critical role of total dietary protein, as well as the combination of higher-protein intakes and increased physical activity, in building a strong skeleton during childhood [76,77] and in maintaining skeletal health during adulthood and old age [78].

There are a number of established mechanisms of dietary protein effects on the skeleton. Dietary protein stimulates bone collagen synthesis [79], suppresses serum concentrations of parathyroid hormone [80], and regulates serum insulin-like growth factor-1 (IGF-1). Serum IGF-1 and its binding protein, insulin-like growth factor binding protein-3, are predictors of bone calcium accretion [81]. Urinary calcium excretion increases with protein intake but is offset by an increase in fractional calcium absorption and a decrease in endogenous fecal excretion of calcium. Net calcium and bone mineralization are unaffected and not negatively impacted by increasing dietary protein intake [82,83]. Loss of bone because of protein-induced calciuria through the acid–base balance theory has largely been debunked [84]. Individual L-amino acids may also activate the calcium-sensing receptor, which regulates fractional calcium absorption and retention when calcium intakes are low [85], thereby leading to higher calcium and bone retention.

Recent advances in bone biology reveal novel roles for dietary protein in the pursuit of lifelong bone health. For example, there is mounting evidence that the skeleton is an endocrine organ unto itself, regulating other organs throughout the body, including skeletal muscle, through secretion of osteokines such as sclerostin, osteocalcin, and fibroblast growth factor-23, from the resident cells of bone—osteocytes [86,87]. Reciprocally, other organ systems not traditionally thought to exert endocrine influences on bone health are now known to do so. For example, myokines secreted from muscle cells during exercise, such as irisin, IL-6, and myostatin, have been shown to influence bone metabolism, providing a novel muscle–bone connection outside of the classically recognized mechanical relationship [88,89]. Limited evidence exists that dietary protein can stimulate myokine expression and secretion from muscle cells [90], which in turn favorably affects bone tissue [91]. These data highlight a promising hypothesis demanding more research that could add an endocrine foundation to the existing mechanical narrative, that dietary protein exerts positive effects on muscle, particularly with exercise [92,93], that is then transferred to bone via mechanical loading and subsequent bone functional adaptation [94,95].

Bone outcomes measures

Innovative bone metabolism research methods (Table 1), including the use of novel biomarkers of bone formation, resorption, myokine, and osteokine concentrations in biological tissues, may continue to reveal the relationships among dietary protein, muscle–bone crosstalk, and bone health. Another research method that continues to provide insight into bone biology is the use of calcium isotope tracers, which can measure calcium absorption, excretion, exchange with bone, and whole-

body retention. Tracers can be used to calculate calcium body pool sizes and rates of exchange when combined with compartmental modeling. Using this method and a crossover design, whole-body retention of ^{47}Ca at the midpoints of interventions of moderate and low-protein diets (20% and 12% of daily energy, respectively) consumed for 8 wk each in 13 healthy postmenopausal women were not different because of protein intake [83]. Similarly, crossover feeding trials in premenopausal women using dual-stable calcium isotopes demonstrated no benefit to supplementing a low-protein diet (0.7 g/kg/d) with dibasic amino acids [96], nor a high-protein diet (2.1 g/kg/d) on net bone balance [97]. Together, these studies confirm that high-protein diets are not detrimental to bone but large and long clinical trials are needed to clarify if protein provides benefit on calcium absorption and bone health. Such methods could be used to answer important questions about the influence of individual amino acids on calcium utilization from bone, the impact of plant-based diets on bone, and understanding diet, gut, muscle, and bone interactions.

Demonstrating cause and effect between any intervention, including dietary protein intake and bone health, lies in measuring actual changes in the bone tissue. Traditionally, the planar nature of DXA scans and lack of imaging resolution have limited the ability of DXA to delineate small but mechanically meaningful changes in bone tissue and structure. Nevertheless, bone imaging via DXA has been used to demonstrate increases in areal bone mineral density (aBMD) over 18 mo in 82 pubertal girls [98] and decreases in aBMD over 2 y in 173 postmenopausal women [99] with a milk intervention. These studies illustrate the sample sizes and durations required to discern dietary protein effects in humans on meaningful bone outcomes using DXA.

Some of the limitations of DXA can be overcome with the addition of second-generation high-resolution peripheral quantitative computed tomography (HR-pQCT), which can be used in longitudinal assessments to capture small changes in bone [100,101] (Table 2). HR-pQCT also yields volumetric assessment and differentiation of trabecular and cortical bone, as well as assessment of trabecular microarchitecture, although not necessarily at the most relevant sites for fracture. In a cross-sectional study using HR-pQCT to study distal tibial bone microarchitecture and finite-element analyses to study mechanical properties in 746 postmenopausal women, total, animal, and dairy protein but not plant-protein intake, measured via FFQ, were positively associated with bone microarchitecture, stiffness, and failure load at the distal tibia and radius [102]. In another cross-sectional analysis of 1016 men with a mean age of 84 y, using an FFQ and HR-pQCT, higher dairy protein intake was associated with higher estimated failure load at the distal radius and tibia; higher non-dairy animal protein was associated with higher failure load at the distal radius; and plant-protein intake was not associated with failure load at any site [103]. Collectively, these studies suggest that dietary protein is favorable for bone microarchitecture and strength of the distal limbs.

Intervention studies are needed to more firmly establish a causal relationship between protein intake and bone health. High-resolution imaging technology holds promise to reveal the magnitude and mechanical consequences of dietary protein-derived benefits to bone.

Weight Management

In the context of energy restriction for the purposes of losing body fat mass, higher-protein diets on the order of 1.2–1.6 g/kg/d have repeatedly been associated with greater weight loss, adipose loss, and lean mass preservation [104,105]. These results, however, appear to be time-dependent. Well-controlled interventions of 3–4 wk show consistent benefits toward enhancing fat mass loss and muscle retention. In contrast, longer duration free-living studies provide increasingly conflicting results in terms of the effect of prescribed dietary protein intake on muscle and fat mass changes [104,106,107]. The POUNDS Lost study noted greater weight loss over 2 y with a higher-protein intake (25% compared with 15% daily kcal), though the weight loss trajectory and total mass lost was greater in year 1 for all diet groups, with weight regain observed in year 2 [108]. In the free-living environment, dietary prescription compliance typically decreases over time, serving as a contributor to the disparity in research findings between short- and long-term studies.

Dietary protein and satiety and energy expenditure

Protein intake augments postprandial satiety via enhanced secretion of appetite-regulating hormones (for example, glucagon-like peptide-1, peptide YY, cholecystokinin, leptin) [104,109,110], which rise in a graded fashion with increased dietary protein ingestion [104]. Specific amino acids (that is, leucine, lysine, threonine, and tryptophan) may exert greater effects on appetite hormones than others [109], though these alterations do not appear to modulate energy intake at subsequent meals [104,107]. The protein source (for example, whey, casein, soy, and pea) may also affect satiety, again suggesting a role for specific amino acids in the underlying appetite response to feeding [104,111]. Similarly, the food matrix matters in that solid foods typically elicit a greater satiety response than do beverages, including protein-rich beverages [104].

Higher-than-RDA (that is, 1.2–1.6 g/kg/d) protein intakes also enhance postprandial diet-induced thermogenesis and may help to preserve resting energy expenditure (REE) during weight loss [104]. The muscle-sparing role of higher-protein intakes during weight loss likely contributes to this REE maintenance [104,105]. Sex may also influence the appetite-related response to higher-protein intakes, with differences observed between men and women in both the appetite sensation response and appetite-regulating hormones [110]. Estrogen is a known regulator of these factors, so great care should be taken to account for sex-based differences in future diet interventions and appetite regulation studies. Although likely a minor influence, studies using soy-based protein interventions should recognize and account for the potential phytoestrogen influence of soy isoflavones, more so in women than men [112–114].

Given that excess adiposity is a known risk factor for the development of chronic kidney disease [115], it is prudent to assess kidney function before initiating higher dietary protein intakes in individuals with obesity. Past assertions that higher-protein diets are damaging to otherwise healthy kidneys are not supported by the research literature [116,117], but established kidney disease still necessitates a dietary protein restriction.

Microbiome and dietary protein influences on weight management

The gut microbiome likely plays a role in mediating the effects of dietary protein on weight management. Calculated microbiome genetic risk scores, based on 20 SNPs associated with relative abundance of gut microbes, reveal interactions between dietary protein intake and changes in fat mass and adipose distribution [28,108]. As dietary protein intake increases, more peptides and free amino acids reach the colonic bacteria and influence microbiome metabolite production [118]. A better understanding of the role of colonic bacterial amino acid fermentation and metabolism is required to fully understand the microbiome-mediated effects of dietary protein on weight management and health outcomes. Similarly, the importance of the food matrix and eating pattern must again be stressed, as dietary fiber content, micronutrient load, and antinutritive factors influence the microbiome.

Sleep and dietary protein influences on weight management

Lack of adequate duration and quality sleep is a known risk factor for weight gain, with short-term sleep restriction increasing weight gain and chronic sleep patterns of $\leq$6 h per night associated with increased BMI [119]. Moderate and high-protein (20% and 33% daily kcal, respectively), energy-deficient (−750 kcal/d) diets resulting in 6 kg weight loss over 12 wk were shown to improve both subjective sleep and daytime sleepiness scores, as well as sleep efficiency measured via actigraphy [120]. These improvements were independent of objective sleep measures (for example, sleep time, sleep onset latency, and time in bed), which did not change in response to the dietary intervention. After successful weight loss, greater sleep disturbances are associated with a higher likelihood of weight regain after loss [108,119].

Presleep protein consumption, specifically casein ingestion, has been revealed to support whole-body and muscle protein synthesis during sleep, exercise recovery and adaptation, and resting metabolic rate [121,122]. Eating before sleep has long been discouraged over concerns that it can enhance adipose deposition, yet presleep casein ingestion, as opposed to carbohydrate, actually maintained overnight lipolysis and fat oxidation and blunted the morning hunger response in an overweight population [121]. Similarly, presleep protein ingestion has not been reported to increase the risk of gastroesophageal reflux, which is often a concern when sleeping soon after eating [122]. The relationship among sleep, dietary protein, and underlying mechanisms affecting satiety and metabolic rate deserves additional attention.

Future research should continue to evaluate the impact of dietary protein on weight management (Table 2). In addition to anthropometrics (for example, weight, percent body fat, and muscle mass), attention should be paid to subjective hunger sensations, microbiome influences, appetite-regulating hormones, sleep influences, and subsequent energy intakes (Table 1).

Summary and Recommendations

Scientific research investigating the effects of dietary protein intake on human health has expanded greatly over the last three decades. Although our related understanding has increased dramatically, translating decades of acute laboratory studies focused on muscle or whole-body protein turnover to long-term health is challenging. We encourage future investigations to utilize study designs and methodologies that best allow for the determination of meaningful health outcome measures. We also recommend that research distinguish the effects of dietary protein and its constituent amino acids from matrix effects of the whole food or composition of the meal in which the protein source is fed. As the field shifts emphasis to elucidating the role of dietary protein in supporting and sustaining human health throughout the lifespan, past research accomplishments provide a foundation for innovative thinking and an approach for the continued creation of an evidence base to best support future public health policy guidance and nutrition initiatives.

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Author contributions

The authors' responsibilities were as follows – JWC and SMPasiakos: designed the project; JWC and SMPasiakos: had primary responsibility for the final content of the manuscript; and all authors: wrote the manuscript, read and approved the manuscript.

Conflict of interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: JWC reports financial support, administrative support, and article publishing charges were provided by Institute for the Advancement of Food and Nutrition Sciences (IAFNS). SMPhillips reports a relationship with Enhanced Recovery that includes non-financial support, a relationship with Nestle Health Sciences that includes consulting or advisory, a relationship with Exerkine that includes equity or stocks, patent #3052324 issued to Exerkine, and patent #16/182891 issued to Exerkine. SMPasiakos is a past government liaison to the IAFNS Protein Committee. CMW and JMH declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. This work was supported by IAFNS. IAFNS is a nonprofit science organization that pools funding from industry and advances science through the in-kind and financial contributions from private and public sector members.

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References

[1] M.M. Mamerow, J.A. Mettler, K.L. English, S.L. Casperson, E. Arentson-Lantz, M. Sheffield-Moore, et al., Dietary protein distribution positively influences 24-h muscle protein synthesis in healthy adults, J. Nutr. 144 (6) (2014) 876–880.

[2] D.R. Moore, Maximizing post-exercise anabolism: the case for relative protein intakes, Front. Nutr. 6 (2019) 147.

[3] D.R. Moore, M.J. Robinson, J.L. Fry, J.E. Tang, E.I. Glover, S.B. Wilkinson, et al., Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men, Am. J. Clin. Nutr. 89 (1) (2009) 161–168.
[4] D.R. Moore, T.A. Churchward-Venne, O. Witard, L. Breen, N.A. Burd, K.D. Tipton, et al., Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men, J. Gerontol. A Biol, Sci. Med. Sci. 70 (1) (2015) 57–62.
[5] E. Damigou, M. Kouvari, D. Panagiotakos, The role of skeletal muscle mass on cardiovascular disease risk: an emerging role on modulating lipid profile, Curr. Opin. Cardiol. 38 (4) (2023) 352–357.
[6] H.E. Park, G.E. Chung, H. Lee, M.J. Kim, S.-Y. Choi, W. Lee, et al., Significance of low muscle mass on arterial stiffness as measured by cardio-ankle vascular index, Front Cardiovasc. Med. 9 (2022) 857871.
[7] A. Rietman, J. Schwarz, D. Tomé, F.J. Kok, M. Mensink, High dietary protein intake, reducing or eliciting insulin resistance? Eur. J. Clin. Nutr. 68 (9) (2014) 973–979.
[8] R. Ferraz-Bannitz, R.A. Beraldo, A.A. Peluso, M. Dall, P. Babaei, R.C. Foglietti, et al., Dietary protein restriction improves metabolic dysfunction in patients with metabolic syndrome in a randomized, controlled trial, Nutrients 14 (13) (2022) 2670.
[9] L.D. Clamp, D.J. Hume, E.V. Lambert, J. Kroff, Enhanced insulin sensitivity in successful, long-term weight loss maintainers compared with matched controls with no weight loss history, Nutr, Diabetes 7 (6) (2017) e282.
[10] K.A. McAuley, J.E. Berkeley, P.D. Docherty, T.F. Lotz, L.A. Te Morenga, G.M. Shaw, et al., The dynamic insulin sensitivity and secretion test—a novel measure of insulin sensitivity, Metabolism 60 (12) (2011) 1748–1756.
[11] L. Te Morenga, P. Docherty, S. Williams, J. Mann, The effect of a diet moderately high in protein and fiber on insulin sensitivity measured using the dynamic insulin sensitivity and secretion test (DISST), Nutrients 9 (12) (2017) 1291.
[12] J. Wilson, P. Docherty, R. Stubbs, J.G. Chase, J. Krebs, Assessment of the dynamic insulin secretion and sensitivity test (DISST) pre and post gastric bypass surgery, Exp. Clin. Endocrinol, Diabetes 128 (3) (2020) 164–169.
[13] C. Jang, S.F. Oh, S. Wada, G.C. Rowe, L. Liu, M.C. Chan, et al., A branched-chain amino acid metabolite drives vascular fatty acid transport and causes insulin resistance, Nat. Med. 22 (4) (2016) 421–426.
[14] C. Lerin, A.B. Goldfine, T. Boes, M. Liu, S. Kasif, J.M. Dreyfuss, et al., Defects in muscle branched-chain amino acid oxidation contribute to impaired lipid metabolism, Mol. Metab. 5 (10) (2016) 926–936.
[15] M. Zhou, J. Shao, C.-Y. Wu, L. Shu, W. Dong, Y. Liu, et al., Targeting BCAA catabolism to treat obesity-associated insulin resistance, Diabetes 68 (9) (2019) 1730–1746.
[16] J. Zhang, R. Chi, Y. Zhang, Y. Xie, Y. Liu, Q. Bao, et al., Preoperative administration of branched-chain amino acids reduces postoperative insulin resistance in rats by reducing liver gluconeogenesis, Nutr. Metab. 19 (1) (2022) 78.
[17] F. Vanweert, M. Neinast, E.E. Tapia, T. van de Weijer, J. Hoeks, V.B. Schrauwen-Hinderling, et al., A randomized placebo-controlled clinical trial for pharmacological activation of BCAA catabolism in patients with type 2 diabetes, Nat. Commun. 13 (1) (2022) 3508.
[18] F.B. Stentz, A. Mikhael, O. Kineish, J. Christman, C. Sands, High protein diet leads to prediabetes remission and positive changes in incretins and cardiovascular risk factors, Nutr. Metab. Cardiovasc. Dis. 31 (4) (2021) 1227–1237.
[19] F.B. Stentz, A. Brewer, J. Wan, C. Garber, B. Daniels, C. Sands, et al., Remission of pre-diabetes to normal glucose tolerance in obese adults with high protein versus high carbohydrate diet: randomized control trial, BMJ Open Diabetes Res. Care 4 (1) (2016) e000258.
[20] F. Tettamanzi, V. Bagnardi, P. Louca, A. Nogal, G.S. Monti, S.P. Mambrini, et al., A high protein diet is more effective in improving insulin resistance and glycemic variability compared to a Mediterranean diet—A cross-over controlled inpatient dietary study, Nutrients 13 (12) (2021) 4380.
[21] L.E. O'Connor, J.E. Kim, C.M. Clark, W. Zhu, W.W. Campbell, Effects of total red meat intake on glycemic control and inflammatory biomarkers: a meta-analysis of randomized controlled trials, Adv. Nutr. 12 (1) (2021) 115–127.
[22] T. Huang, S.H. Ley, Y. Zheng, T. Wang, G.A. Bray, F.M. Sacks, et al., Genetic susceptibility to diabetes and long-term improvement of insulin resistance and β cell function during weight loss: the preventing overweight using novel dietary strategies (POUNDS LOST) trial, Am. J. Clin. Nutr. 104 (1) (2016) 198–204.
[23] R.E. Bergia, R. Giacco, T. Hjorth, I. Biskup, W. Zhu, G. Costabile, et al., Differential glycemic effects of low- versus high-glycemic index Mediterranean-style eating patterns in adults at risk for type 2 diabetes: the MEDGI-carb randomized controlled trial, Nutrients 14 (3) (2022) 706.
[24] M. Vitale, G. Costabile, R.E. Bergia, T. Hjorth, W.W. Campbell, R. Landberg, et al., The effects of Mediterranean diets with low or high glycemic index on plasma glucose and insulin profiles are different in adult men and women: data from MEDGI-Carb randomized clinical trial, Clin. Nutr. 42 (10) (2023) 2022–2028.
[25] R.E. Bergia, I. Biskup, R. Giacco, G. Costabile, S. Gray, A. Wright, et al., The MEDGICarb-study: design of a multi-center randomized controlled trial to determine the differential health-promoting effects of low- and high-glycemic index Mediterranean-style eating patterns, Contemp. Clin. Trials Commun. 19 (2020) 100640.
[26] D. Zeevi, T. Korem, N. Zmora, D. Israeli, D. Rothschild, A. Weinberger, et al., Personalized nutrition by prediction of glycemic responses, Cell 163 (5) (2015) 1079–1094.
[27] S.E. Berry, A.M. Valdes, D.A. Drew, F. Asnicar, M. Mazidi, J. Wolf, et al., Human postprandial responses to food and potential for precision nutrition, Nat. Med. 26 (6) (2020) 964–973.
[28] X. Li, Q. Xue, H. Ma, C.M. Champagne, G.A. Bray, F.M. Sacks, et al., Genetically determined gut microbial abundance and 2-year changes in central adiposity and body composition: the POUNDS lost trial, Clin. Nutr. 41 (12) (2022) 2817–2824.
[29] Y. Wang, S.R. Lindemann, T.L. Cross, M. Tang, C.M. Clark, W.W. Campbell, Effects of adding lean red meat to a U.S.-style healthy vegetarian dietary pattern on gut microbiota and cardiovascular risk factors in young adults: a crossover randomized controlled trial, J. Nutr. 153 (5) (2023) 1439–1452.
[30] Y. Wang, E.R. Hill, W.W. Campbell, L.E. O'Connor, Plant- and animal-based protein-rich foods and cardiovascular health, Curr. Atheroscler Rep. 24 (4) (2022) 197–213.
[31] R.E. Bergia, W.W. Campbell, B.T. Roseguini, J.E. Kim, A high-protein meal does not improve blood pressure or vasoactive biomarker responses to acute exercise in humans, Nutr. Res. 81 (2020) 97–107.
[32] S. Krishnan, L.E. O'Connor, Y. Wang, E.R. Gertz, W.W. Campbell, B.J. Bennett, Adopting a Mediterranean-style eating pattern with low, but not moderate, unprocessed, lean red meat intake reduces fasting serum trimethylamine N-oxide (TMAO) in adults who are overweight or obese, Br. J. Nutr. 128 (9) (2021) 1–21.
[33] J.L. Hudson, J. Zhou, J.E. Kim, W.W. Campbell, Incorporating milk protein isolate into an energy-restricted western-style eating pattern augments improvements in blood pressure and triglycerides, but not body composition changes in adults classified as overweight or obese: a randomized controlled trial, Nutrients 12 (3) (2020) 851.
[34] C.N. Uffelman, K.A. Doenges, M.L. Armstrong, K. Quinn, R.M. Reisdorph, M. Tang, et al., Metabolomics profiling of white button, crimini, portabella, lion's mane, maitake, oyster, and shiitake mushrooms using untargeted metabolomics and targeted amino acid analysis, Foods 12 (16) (2023) 2985.
[35] N.A. Reisdorph, A.E. Hendricks, M. Tang, K.A. Doenges, R.M. Reisdorph, B.C. Tooker, et al., Nutrimetabolomics reveals food-specific compounds in urine of adults consuming a DASH-style diet, Sci. Rep. 10 (1) (2020) 1157.
[36] M. Gueugneau, F. Capel, L.-E. Monfoulet, S. Polakof, Metabolomics signatures of plant protein intake: effects of amino acids and compounds associated with plant protein on cardiometabolic health, Curr. Opin. Clin. Nutr. Metab. Care. 26 (2) (2023) 189–194.
[37] G. Lépine, H. Fouillet, D. Rémond, J.-F. Huneau, F. Mariotti, S. Polakof, A scoping review: metabolomics signatures associated with animal and plant protein intake and their potential relation with cardiometabolic risk, Adv. Nutr. 12 (6) (2021) 2112–2131.
[38] C.L. Ramspek, E.W. Steyerberg, R.D. Riley, F.R. Rosendaal, O.M. Dekkers, F.W. Dekker, et al., Prediction or causality? A scoping review of their conflation within current observational research, Eur. J. Epidemiol. 36 (9) (2021) 889–898.
[39] E.R. Hill, L.E. O'Connor, Y. Wang, C.M. Clark, B.S. McGowan, M.R. Forman, et al., Red and processed meat intakes and cardiovascular disease and type 2 diabetes mellitus: an umbrella systematic review and assessment of causal relations using Bradford Hill's criteria, Crit. Rev. Food Sci. Nutr. 64 (9) (2024) 2423–2440.
[40] A.B. Hill, The environment and disease: association or causation? Proc. R. Soc. Med. 58 (5) (1965) 295–300.

[41] G. Connolly, W.W. Campbell, Poultry consumption and human cardiometabolic health-related outcomes: a narrative review, Nutrients 15 (16) (2023) 3550.
[42] L.E. O'Connor, C.L. Gifford, D.R. Woerner, J.L. Sharp, K.E. Belk, W.W. Campbell, Dietary meat categories and descriptions in chronic disease research are substantively different within and between experimental and observational studies: a systematic review and landscape analysis, Adv. Nutr. 11 (1) (2020) 41–51.
[43] W.W. Campbell, Animal-based and plant-based protein-rich foods and cardiovascular health: a complex conundrum, Am. J. Clin. Nutr. 110 (1) (2019) 8–9.
[44] G. Coletta, S.M. Phillips, An elusive consensus definition of sarcopenia impedes research and clinical treatment: a narrative review, Age Res. Rev. 86 (2023) 101883.
[45] M. Izquierdo, R.A. Merchant, J.E. Morley, S.D. Anker, I. Aprahamian, H. Arai, et al., International exercise recommendations in older adults (ICFSR): expert consensus guidelines, J. Nutr. Health Aging 25 (7) (2021) 824–853.
[46] E.A. Nunes, L. Colenso-Semple, S.R. McKellar, T. Yau, M.U. Ali, D. Fitzpatrick-Lewis, et al., Systematic review and meta-analysis of protein intake to support muscle mass and function in healthy adults, J. Cachexia Sarcopenia Muscle 13 (2) (2022) 795–810.
[47] R.P. Kirwan, M. Mazidi, C. Rodríguez García, K.E. Lane, A. Jafari, T. Butler, et al., Protein interventions augment the effect of resistance exercise on appendicular lean mass and handgrip strength in older adults: a systematic review and meta-analysis of randomized controlled trials, Am. J. Clin. Nutr. 115 (3) (2022) 897–913.
[48] DSM Ten Haaf, M.A.H. Nuijten, M.F.H. Maessen, A.M.H. Horstman, T.M.H. Eijsvogels, M.T.E. Hopman, Effects of protein supplementation on lean body mass, muscle strength, and physical performance in nonfrail community-dwelling older adults: a systematic review and meta-analysis, Am. J. Clin. Nutr. 108 (5) (2018) 1043–1059.
[49] J. Wirth, E. Hillesheim, L. Brennan, The role of protein intake and its timing on body composition and muscle function in healthy adults: a systematic review and meta-analysis of randomized controlled trials, J. Nutr. 150 (6) (2020) 1443–1460.
[50] R.W. Morton, K.T. Murphy, S.R. McKellar, B.J. Schoenfeld, M. Henselmans, E. Helms, et al., A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults, Br. J. Sports Med. 52 (6) (2018) 376–384.
[51] A. Granic, L. Dismore, C. Hurst, S.M. Robinson, A.A. Sayer, Myoprotective whole foods, muscle health and sarcopenia: a systematic review of observational and intervention studies in older adults, Nutrients 12 (8) (2020) 2257.
[52] I. Bloom, C. Shand, C. Cooper, S. Robinson, J. Baird, Diet quality and sarcopenia in older adults: a systematic review, Nutrients 10 (3) (2018) 308.
[53] H.J. Coelho-Júnior, R. Calvani, M. Tosato, F. Landi, A. Picca, E. Marzetti, Protein intake and physical function in older adults: a systematic review and meta-analysis, Ageing Res. Rev. 81 (2022) 101731.
[54] A. Yaegashi, T. Kimura, T. Hirata, A. Tamakoshi, Association of dietary protein intake with skeletal muscle mass in older adults: a systematic review, Geriatr. Gerontol. Int. 21 (12) (2021) 1077–1083.
[55] E.M. Weinheimer, L.P. Sands, W.W. Campbell, A systematic review of the separate and combined effects of energy restriction and exercise on fat-free mass in middle-aged and older adults: implications for sarcopenic obesity, Nutr. Rev. 68 (7) (2010) 375–388.
[56] J.E. Kim, L.E. O'Connor, L.P. Sands, M.B. Slebodnik, W.W. Campbell, Effects of dietary protein intake on body composition changes after weight loss in older adults: a systematic review and meta-analysis, Nutr. Rev. 74 (3) (2016) 210–224.
[57] S.M. Phillips, E.I. Glover, M.J. Rennie, Alterations of protein turnover underlying disuse atrophy in human skeletal muscle, J. Appl. Physiol. 107 (3) (2009) 645–654.
[58] K.L. English, J.A. Mettler, J.B. Ellison, M.M. Mamerow, E. Arentson-Lantz, J.M. Pattarini, et al., Leucine partially protects muscle mass and function during bed rest in middle-aged adults, Am. J. Clin. Nutr. 103 (2) (2016) 465–473.
[59] T.M. Holloway, C. McGlory, S. McKellar, A. Morgan, M. Hamill, R. Afeyan, et al., A novel amino acid composition ameliorates short-term muscle disuse atrophy in healthy young men, Front Nutr 6 (2019) 105.
[60] H. Ye, J.-M. Yang, Y. Luo, Y. Long, J.-H. Zhang, Y.-B. Zhong, et al., Do dietary supplements prevent loss of muscle mass and strength during muscle disuse? A systematic review and meta-analysis of randomized controlled trials, Front Nutr 10 (2023) 1093988.
[61] M.L. Dirks, B.T. Wall, R. Nilwik, D.H.J.M. Weerts, L.B. Verdijk, L.J.C. van Loon, Skeletal muscle disuse atrophy is not attenuated by dietary protein supplementation in healthy older men, J. Nutr. 144 (8) (2014) 1196–1203.
[62] B.T. Wall, M.L. Dirks, T. Snijders, J.-W. van Dijk, M. Fritsch, L.B. Verdijk, et al., Short-term muscle disuse lowers myofibrillar protein synthesis rates and induces anabolic resistance to protein ingestion, Am. J. Physiol. Endocrinol. Metab. 310 (2) (2016) E137–E147.
[63] B.T. Wall, T. Snijders, J.M.G. Senden, C.L.P. Ottenbros, A.P. Gijsen, L.B. Verdijk, et al., Disuse impairs the muscle protein synthetic response to protein ingestion in healthy men, J. Clin. Endocrinol. Metab. 98 (12) (2013) 4872–4881.
[64] S. Szwiega, P.B. Pencharz, M. Rafii, M. Lebarron, J. Chang, R.O. Ball, et al., Dietary leucine requirement of older men and women is higher than current recommendations, Am. J. Clin. Nutr. 113 (2) (2021) 410–419.
[65] C. McGlory, M.T. von Allmen, T. Stokes, R.W. Morton, A.J. Hector, B.A. Lago, et al., Failed recovery of glycemic control and myofibrillar protein synthesis with 2 wk of physical inactivity in overweight, prediabetic older adults, J. Gerontol. A Biol. Sci. Med. Sci. 73 (8) (2018) 1070–1077.
[66] S.Y. Oikawa, T.M. Holloway, S.M. Phillips, The impact of step reduction on muscle health in aging: protein and exercise as countermeasures, Front Nutr 6 (2019) 75.
[67] P.M. Cawthon, T. Blackwell, S.R. Cummings, E.S. Orwoll, K.A. Duchowny, D.M. Kado, et al., Muscle mass assessed by the D3-creatine dilution method and incident self-reported disability and mortality in a prospective observational study of community-dwelling older men, J. Gerontol. A Biol, Sci. Med. Sci. 76 (1) (2021) 123–130.
[68] W.J. Evans, P.M. Cawthon, D3Creatine dilution as a direct, non-invasive and accurate measurement of muscle mass for aging research, Calcif Tissue Int 114 (1) (2023) 3–8.
[69] H. Sagayama, E. Kondo, Y. Tanabe, A. Uchizawa, W.J. Evans, M. Shankaran, et al., Comparison of bioelectrical impedance indices for skeletal muscle mass and intracellular water measurements of physically active young men and athletes, J. Nutr. 153 (9) (2023) 2543–2551.
[70] A.B. Newman, T.L. Blackwell, T. Mau, P.M. Cawthon, P.M. Coen, S.R. Cummings, et al., Vigor to frailty as a continuum - a new approach in the study of muscle, mobility and aging (SOMMA) cohort, J. Gerontol. A Biol, Sci. Med. Sci. 79 (2023) glad244.
[71] S. Srinivasan, T.S. Gross, S.D. Bain, Bone mechanotransduction may require augmentation in order to strengthen the senescent skeleton, Ageing Res. Rev. 11 (3) (2012) 353–360.
[72] L. Gabel, L. Nettlefold, P.M. Brasher, S.A. Moore, Y. Ahamed, H.M. Macdonald, et al., Reexamining the surfaces of bone in boys and girls during adolescent growth: a 12-year mixed longitudinal pQCT study, J. Bone Miner. Res. 30 (12) (2015) 2158–2167.
[73] J.M. Hughes, N. Charkoudian, J.N. Barnes, B.J. Morgan, Revisiting the debate: does exercise build strong bones in the mature and senescent skeleton? Front Physiol 7 (2016) 369.
[74] N.W. Smith, A.J. Fletcher, L.A. Dave, J.P. Hill, W.C. McNabb, Use of the DELTA model to understand the food system and global nutrition, J. Nutr. 151 (10) (2021) 3253–3261.
[75] C.E. Berryman, H.R. Lieberman, V.L. Fulgoni, S.M. Pasiakos, Protein intake trends and conformity with the dietary reference intakes in the United States: analysis of the National Health and Nutrition Examination Survey, 2001-2014, Am. J. Clin. Nutr. 108 (2) (2018) 405–413.
[76] U. Alexy, T. Remer, F. Manz, C.M. Neu, E. Schoenau, 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 (5) (2005) 1107–1114.
[77] T. Chevalley, J.-P. Bonjour, S. Ferrari, R. Rizzoli, High-protein intake enhances the positive impact of physical activity on BMC in prepubertal boys, J. Bone Miner. Res. 23 (1) (2008) 131–142.
[78] M.T. Hannan, K.L. Tucker, B. Dawson-Hughes, L.A. Cupples, D.T. Felson, D.P. Kiel, Effect of dietary protein on bone loss in elderly men and women: the Framingham Osteoporosis Study, J. Bone Miner. Res. 15 (12) (2000) 2504–2512.
[79] J.A. Babraj, K. Smith, D.J.R. Cuthbertson, P. Rickhuss, J.S. Dorling, M.J. Rennie, Human bone collagen synthesis is a rapid, nutritionally modulated process, J. Bone Miner. Res. 20 (6) (2005) 930–937.
[80] E. Gaffney-Stomberg, J.J. Cao, G.G. Lin, C.R. Wulff, N.E. Murphy, A.J. Young, et al., Dietary protein level and source differentially affect

bone metabolism, strength, and intestinal calcium transporter expression during ad libitum and food-restricted conditions in male rats, J. Nutr. 144 (6) (2014) 821–829.
[81] K.M. Hill, M. Braun, M. Kern, B.R. Martin, J.W. Navalta, D.A. Sedlock, et al., Predictors of calcium retention in adolescent boys, J. Clin. Endocrinol. Metab. 93 (12) (2008) 4743–4748.
[82] L.A. Spence, E.R. Lipscomb, J. Cadogan, B. Martin, M.E. Wastney, M. Peacock, et al., The effect of soy protein and soy isoflavones on calcium metabolism in postmenopausal women: a randomized crossover study, Am. J. Clin. Nutr. 81 (4) (2005) 916–922.
[83] Z.K. Roughead, L.K. Johnson, G.I. Lykken, J.R. Hunt, Controlled high meat diets do not affect calcium retention or indices of bone status in healthy postmenopausal women, J. Nutr. 133 (4) (2003) 1020–1026.
[84] J.-P. Bonjour, Nutritional disturbance in acid-base balance and osteoporosis: a hypothesis that disregards the essential homeostatic role of the kidney, Br. J. Nutr. 110 (7) (2013) 1168–1177.
[85] A.D. Conigrave, A.H. Franks, E.M. Brown, S.J. Quinn, L-amino acid sensing by the calcium-sensing receptor: a general mechanism for coupling protein and calcium metabolism? Eur. J. Clin. Nutr. 56 (11) (2002) 1072–1080.
[86] S.L. Dallas, M. Prideaux, L.F. Bonewald, The osteocyte: an endocrine cell ... and more, Endocr. Rev. 34 (5) (2013) 658–690.
[87] A. Shimonty, L.F. Bonewald, J.R. Huot, Metabolic health and disease: a role of osteokines? Calcif Tissue Int 113 (1) (2023) 21–38.
[88] Z. Zhao, K. Yan, Q. Guan, Q. Guo, C. Zhao, Mechanism and physical activities in bone-skeletal muscle crosstalk, Front Endocrinol 14 (2023) 1287972.
[89] M. Herrmann, K. Engelke, R. Ebert, S. Müller-Deubert, M. Rudert, F. Ziouti, et al., Interactions between muscle and bone-where physics meets biology, Biomolecules 10 (3) (2020) 432.
[90] L.E. Neidert, C.B. Mobley, W.C. Kephart, M.D. Roberts, H.A. Kluess, The serine protease, dipeptidyl peptidase IV as a myokine: dietary protein and exercise mimetics as a stimulus for transcription and release, Physiol. Rep. 4 (12) (2016) e12827.
[91] H. Suresh Kumar, E.N. Barnett, J.L. Fowlkes, E. Kalaitzoglou, R.T. Annamalai, Biomechanical stimulation of muscle constructs influences phenotype of bone constructs by modulating myokine secretion, JBMR Plus 7 (11) (2023) e10804.
[92] J.W. Carbone, S.M. Pasiakos, Dietary protein and muscle mass: translating science to application and health benefit, Nutrients 11 (5) (2019) 1136.
[93] S.M. Phillips, W. Martinson, Nutrient-rich, high-quality, protein-containing dairy foods in combination with exercise in aging persons to mitigate sarcopenia, Nutr. Rev. 77 (4) (2019) 216–229.
[94] C.M. Turcotte, K.N. Rabey, D.J. Green, S.C. McFarlin, Muscle attachment sites and behavioral reconstruction: an experimental test of muscle-bone structural response to habitual activity, Am. J. Biol. Anthropol. 177 (1) (2022) 63–82.
[95] J. Rittweger, Ten years muscle-bone hypothesis: what have we learned so far?–almost a festschrift–, J. Musculoskelet. Neuronal, Interact 8 (2) (2008) 174–178.
[96] J.D. Bihuniak, R.R. Sullivan, C.A. Simpson, D.M. Caseria, T.B. Huedo-Medina, K.O. O'Brien, et al., Supplementing a low-protein diet with dibasic amino acids increases urinary calcium excretion in young women, J. Nutr. 144 (3) (2014) 282–288.
[97] J.E. Kerstetter, K.O. O'Brien, D.M. Caseria, D.E. Wall, K.L. Insogna, The impact of dietary protein on calcium absorption and kinetic measures of bone turnover in women, J. Clin. Endocrinol. Metab. 90 (1) (2005) 26–31.
[98] J. Cadogan, R. Eastell, N. Jones, M.E. Barker, Milk intake and bone mineral acquisition in adolescent girls: randomised, controlled intervention trial, BMJ 315 (7118) (1997) 1255–1260.
[99] W.S.S. Chee, A.R. Suriah, S.P. Chan, Y. Zaitun, Y.M. Chan, The effect of milk supplementation on bone mineral density in postmenopausal Chinese women in Malaysia, Osteoporos Int 14 (10) (2003) 828–834.
[100] K.L. Troy, M.E. Mancuso, J.E. Johnson, Z. Wu, T.J. Schnitzer, T.A. Butler, Bone adaptation in adult women is related to loading dose: a 12-month randomized controlled trial, J. Bone Miner. Res. 35 (7) (2020) 1300–1312.
[101] J.M. Hughes, K.M. Taylor, K.I. Guerriere, N.I. Smith, J.S. Staab, L.A. Walker, et al., Changes in distal tibial microarchitecture during eight weeks of U.S. army basic combat training differ by sex and race, JBMR Plus 7 (4) (2023) e10719.
[102] C. Durosier-Izart, E. Biver, F. Merminod, B. van Rietbergen, T. Chevalley, F.R. Herrmann, et al., Peripheral skeleton bone strength is positively correlated with total and dairy protein intakes in healthy postmenopausal women, Am. J. Clin. Nutr. 105 (2) (2017) 513–525.
[103] L. Langsetmo, J.M. Shikany, A.J. Burghardt, P.M. Cawthon, E.S. Orwoll, J.A. Cauley, et al., High dairy protein intake is associated with greater bone strength parameters at the distal radius and tibia in older men: a cross-sectional study, Osteoporos Int 29 (1) (2018) 69–77.
[104] H.J. Leidy, P.M. Clifton, A. Astrup, T.P. Wycherley, M.S. Westerterp-Plantenga, N.D. Luscombe-Marsh, et al., The role of protein in weight loss and maintenance, Am. J. Clin. Nutr. 101 (6) (2015) 1320S–1329S.
[105] S.M. Pasiakos, J.J. Cao, L.M. Margolis, E.R. Sauter, L.D. Whigham, J.P. McClung, et al., Effects of high-protein diets on fat-free mass and muscle protein synthesis following weight loss: a randomized controlled trial, FASEB J 27 (9) (2013) 3837–3847.
[106] J.A. Gwin, K.C. Maki, H.J. Leidy, Increased protein consumption during the day from an energy-restricted diet augments satiety but does not reduce daily fat or carbohydrate intake on a free-living test day in overweight women, J. Nutr. 147 (12) (2017) 2338–2346.
[107] S.M. Douglas, A.W. Byers, H.J. Leidy, Habitual breakfast patterns do not influence appetite and satiety responses in normal vs. high-protein breakfasts in overweight adolescent girls, Nutrients 11 (6) (2019) 1223.
[108] L. Qi, Y. Heianza, X. Li, F.M. Sacks, G.A. Bray, Toward precision weight-loss dietary interventions: findings from the POUNDS lost trial, Nutrients 15 (16) (2023) 3665.
[109] B.M. Giglio, P.C.B. Lobo, G.D. Pimentel, Effects of whey protein supplementation on adiposity, body weight, and glycemic parameters: a synthesis of evidence, Nutr. Metab. Cardiovasc. Dis. 33 (2) (2023) 258–274.
[110] C.L.P. Oliveira, N.G. Boulé, S.A. Elliott, A.M. Sharma, M. Siervo, A. Berg, et al., A high-protein total diet replacement alters the regulation of food intake and energy homeostasis in healthy, normal-weight adults, Eur. J. Nutr. 61 (4) (2022) 1849–1861.
[111] M.L. Braden, J.A. Gwin, H.J. Leidy, Protein source influences acute appetite and satiety but not subsequent food intake in healthy adults, J. Nutr. 153 (6) (2023) 1825–1833.
[112] M.S. Kurzer, Hormonal effects of soy in premenopausal women and men, J. Nutr. 132 (3) (2002) 570S–573S.
[113] M. Messina, Soy foods, isoflavones, and the health of postmenopausal women, Am. J. Clin. Nutr. 100 (Suppl 1) (2014) 423S–430S.
[114] K.E. Reed, J. Camargo, J. Hamilton-Reeves, M. Kurzer, M. Messina, Neither soy nor isoflavone intake affects male reproductive hormones: an expanded and updated meta-analysis of clinical studies, Reprod. Toxicol. 100 (2021) 60–67.
[115] C.P. Kovesdy, S.L. Furth, C. Zoccali, World Kidney Day Steering Committee, Obesity and kidney disease: hidden consequences of the epidemic, Can J. Kidney Health Dis. 4 (2017) 2054358117698669.
[116] M.C. Devries, A. Sithamparapillai, K.S. Brimble, L. Banfield, R.W. Morton, S.M. Phillips, Changes in kidney function do not differ between healthy adults consuming higher- compared with lower- or normal-protein diets: a systematic review and meta-analysis, J. Nutr. 148 (11) (2018) 1760–1775.
[117] C.E. Berryman, S. Agarwal, H.R. Lieberman, V.L. Fulgoni, S.M. Pasiakos, Diets higher in animal and plant protein are associated with lower adiposity and do not impair kidney function in US adults, Am. J. Clin. Nutr. 104 (3) (2016) 743–749.
[118] K. Prokopidis, M.M. Cervo, A. Gandham, D. Scott, Impact of protein intake in older adults with sarcopenia and obesity: a gut microbiota perspective, Nutrients 12 (8) (2020) 2285.
[119] E. Papatriantafyllou, D. Efthymiou, E. Zoumbaneas, C.A. Popescu, E. Vassilopoulou, Sleep deprivation: effects on weight loss and weight loss maintenance, Nutrients 14 (8) (2022) 1549.
[120] J.L. Hudson, J. Zhou, W.W. Campbell, Adults who are overweight or obese and consuming an energy-restricted healthy US-style eating pattern at either the recommended or a higher protein quantity perceive a shift from "poor" to "good" sleep: a randomized controlled trial, J. Nutr. 150 (12) (2020) 3216–3223.
[121] R. Jäger, C.M. Kerksick, B.I. Campbell, P.J. Cribb, S.D. Wells, T.M. Skwiat, et al., International Society of Sports Nutrition Position Stand: protein and exercise, J. Int. Soc. Sports Nutr. 14 (2017) 20.
[122] C.E.G. Reis, L.M.R. Loureiro, H. Roschel, T.H.M. da Costa, Effects of pre-sleep protein consumption on muscle-related outcomes – a systematic review, J. Sci. Med. Sport. 24 (2) (2021) 177–182.