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The Effect of Protein Intake on Health: A Systematic Review

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U.S. Department of Health and Human Services
5600 Fishers Lane
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www.ahrq.gov

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Prepared by:
[To be included in the final version of the report.]

Investigators:
[To be included in the final version of the report.]

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None of the investigators have any affiliations or financial involvement that conflicts with the material presented in this report.

The information in this report is intended to help healthcare decisionmakers—patients and clinicians, health system leaders, and policymakers, among others—make well-informed decisions and thereby improve the quality of healthcare services. This report is not intended to be a substitute for the application of clinical judgment. Anyone who makes decisions concerning the provision of clinical care should consider this report in the same way as any medical reference and in conjunction with all other pertinent information, i.e., in the context of available resources and circumstances presented by individual patients.

This report may periodically be assessed for the currency of conclusions. If an assessment is done, the resulting surveillance report describing the methodology and findings will be found on the Effective Health Care Program Web site at www.effectivehealthcare.ahrq.gov. Search on the title of the report.

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Preface

The Agency for Healthcare Research and Quality (AHRQ), through its Evidence-based Practice Centers (EPCs), sponsors the development of systematic reviews to assist public- and private-sector organizations in their efforts to improve the quality of healthcare in the United States. These reviews provide comprehensive, science-based information on common, costly medical conditions, and new healthcare technologies and strategies. This review was requested by the Joint U.S.-Canadian Dietary Reference Intakes (DRI) Working group from the Agency for Healthcare Research and Quality (AHRQ) Evidence-based Practice Center (EPC) program to provide a key summary of evidence in the development of a new reference value for chronic disease risk reduction to provide a foundation for a future National Academies of Sciences, Engineering, and Medicine (NASEM) review of the DRIs for protein.

The reports and assessments provide organizations with comprehensive, evidence-based information on common medical conditions and new healthcare technologies and strategies. They also identify research gaps in the selected scientific area, identify methodological and scientific weaknesses, suggest research needs, and move the field forward through an unbiased, evidence-based assessment of the available literature. The EPCs systematically review the relevant scientific literature on topics assigned to them by AHRQ and conduct additional analyses when appropriate prior to developing their reports and assessments.

To bring the broadest range of experts into the development of evidence reports and health technology assessments, AHRQ encourages the EPCs to form partnerships and enter into collaborations with other medical and research organizations. The EPCs work with these partner organizations to ensure that the evidence reports and technology assessments they produce will become building blocks for healthcare quality improvement projects throughout the Nation. The reports undergo peer review and public comment prior to their release as a final report.

AHRQ expects that these systematic reviews will be helpful to health plans, providers, purchasers, government programs, and the healthcare system as a whole. Transparency and stakeholder input are essential to the Effective Health Care Program.

If you have comments on this systematic review, they may be sent by mail to the Task Order Officer named below at: Agency for Healthcare Research and Quality, 5600 Fishers Lane, Rockville, MD 20857, or by email to epc@ahrq.hhs.gov.

Robert Otto Valdez, Ph.D., M.H.S.A.
Director
Agency for Healthcare Research and Quality

Therese Miller, Dr.P.H.
Director
Center for Evidence and Practice Improvement
Agency for Healthcare Research and Quality

Christine Chang, M.D., M.P.H.
Director, Evidence-based Practice Center Program
Center for Evidence and Practice Improvement
Agency for Healthcare Research and Quality

David W. Niebuhr, M.D., M.P.H., M.Sc,,
Task Order Officer
Holly Wethington, Ph.D., Task Order Officer
Center for Evidence and Practice Improvement
Agency for Healthcare Research and Quality

Acknowledgments

[To be included in the final version of the report.]

Technical Expert Panel

In designing the study questions and methodology at the outset of this report, the EPC consulted several technical and content experts. Broad expertise and perspectives were sought. Divergent and conflicting opinions are common and perceived as healthy scientific discourse that results in a thoughtful, relevant systematic review. Therefore, in the end, study questions, design, methodologic approaches, and/or conclusions do not necessarily represent the views of individual technical and content experts.

Technical Experts must disclose any financial conflicts of interest greater than $5,000 and any other relevant business or professional conflicts of interest. Because of their unique clinical or content expertise, individuals with potential conflicts may be retained. The TOO and the EPC work to balance, manage, or mitigate any potential conflicts of interest identified.

The list of Technical Experts who provided input to this report follows:
[To be included in the final version of the report.]

Peer Reviewers

Prior to publication of the final evidence report, EPCs sought input from independent Peer Reviewers without financial conflicts of interest. However, the conclusions and synthesis of the scientific literature presented in this report do not necessarily represent the views of individual reviewers.

Peer Reviewers must disclose any financial conflicts of interest greater than $5,000 and any other relevant business or professional conflicts of interest. Because of their unique clinical or content expertise, individuals with potential nonfinancial conflicts may be retained. The Task Order Officer and the EPC work to balance, manage, or mitigate any potential nonfinancial conflicts of interest identified.

The list of Peer Reviewers follows:
[To be included in the final version of the report.]

The Effect of Protein Intake on Health: A Systematic Review

Structured Abstract

Objective. This review examines the association between dietary protein intake and the risk of bone disease, kidney disease, and sarcopenia, aiming to inform future Dietary Reference Intakes (DRIs) updates, including the development of a Chronic Disease Risk Reduction (CDRR) reference value for protein.

Data sources. We searched Medline, EMBASE, AGRICOLA, and Scopus from January 2000 to March 2024, and supplemented by citation searching of relevant reviews and original research.

Review methods. Following the AHRQ Methods Guide for Effectiveness and Comparative Effectiveness Reviews and registering the protocol on PROSPERO (CRD42023446621), we included randomized and non-randomized controlled trials, prospective cohort studies, and nested case-control studies that enrolled healthy participants and examined dietary protein intake without exercise. We assessed the risk of bias, performed a qualitative synthesis of studies rated as low to moderate risk of bias, and evaluated the strength of evidence.

Results. Of 10,949 studies, 82 articles detailing 81 unique studies met our inclusion criteria. Thirteen of these, rated as low to moderate risk of bias, were included in our synthesis. These included set comprised studies on bone disease (4 randomized controlled trials [RCTs] and 1 prospective cohort study), kidney disease (1 RCT), and sarcopenia (9 RCTs). The overall evidence was deemed insufficient to address the Key Questions, primarily due to a limited number of studies rated as low to moderate risk, the diversity of dietary protein interventions, and the wide range of outcomes which made synthesizing results and comparing studies challenging. Additionally, studies used intermediate markers or sarcopenia diagnostic components rather than direct outcomes to assess disease risk. Notably, we found very scant literature addressing children and adolescents. Our analysis was informed by only one study each of the impact of dietary protein intake on bone disease risk (mixed findings) in children and adolescents, and the impact of dietary protein on kidney disease risk (no significant effects) in adults. The findings related to adult bone disease were inconsistent, with some studies indicating no effect and others suggesting benefits on bone health metrics. Studies on sarcopenia risk also reported inconsistent results concerning muscle mass, physical performance, and muscle strength.

Conclusions. The evidence gathered since 2000 on associations between dietary protein intake and the risks of bone disease, kidney disease, and sarcopenia is unclear, indicating a need for more rigorous research in these areas.

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Executive Summary

Main Points

- Research conducted since 2000 on the association between dietary protein intake and bone disease, kidney disease, and sarcopenia risks is insufficient and inconclusive. Improving this evidence base will require more robust long-term studies.
- To assess chronic disease risk, studies used intermediate markers, including surrogate markers for bone, kidney disease, and sarcopenia diagnostic components. However, these markers may not fully represent the conditions' complexity, presence, and progression. Sarcopenia's absence as a study endpoint marks a significant research gap.
- Varied methods and outcome measures made it hard to compare results across studies.
- A notable research gap regarding the impact of dietary protein intake on bone health in children and adolescents highlights the urgent need for further investigation.

Background and Purpose

Since the publication of the protein Dietary Reference Intakes (DRIs) in 2005, no update has been made.[1] Protein is essential for optimal growth, development, function, and maintenance of human health.[2] It significantly influences bone health across all life stages and is essential for the development of peak bone mass in children and adolescents.[3,4] In adults, dietary protein has a complex effect on bone health, described as both beneficial[5,6] and potentially harmful.[7,8] The relationship between protein intake and kidney health is still under debate, particularly in those without existing kidney issues.[9-12] For sarcopenia, protein is considered potentially important in slowing its progression, underscoring protein's role in addressing age-related health concerns.[13,14]

This report reviews the association between dietary protein intake and bone disease, kidney disease, and sarcopenia risks, aiming to inform updates to the protein DRIs, including a new reference value for chronic disease risk reduction.

Methods

Following the Agency for Healthcare Research and Quality's guidelines, our systematic review (PROSPERO registration CRD42023446621) assessed literature from January 2000 to March 2024, searching Medline, EMBASE, AGRICOLA, and citations of reviews and original research. We included randomized and non-randomized trials, prospective cohorts, and nested case-controls in healthy individuals, exploring dietary protein intake without exercise. We assessed risk of bias using Cochrane Risk of Bias 2.0 and ROBINS-E, extracted data, qualitatively synthesized findings from studies rated as low to moderate risk of bias (studies less prone to biases affecting the robustness of their findings), and evaluated the strength of evidence.

For further details on the methods, see the full report [include a hyperlink/URL to the full report on the AHRQ website].

Results

Of 10,949 identified studies, 82 articles detailing 81 distinct studies met our inclusion criteria. Among these, 13 studies rated as low to moderate risk of bias were synthesized. This analytic set included five bone disease studies (3 randomized controlled trials in adults [2 low

and 1 moderate risk of bias], 1 prospective cohort study in adults [moderate risk of bias], and 1 randomized controlled trial in children and adolescents [low risk of bias and the only eligible child study]); one kidney disease study (1 randomized controlled trial [moderate risk of bias]); and nine sarcopenia studies (9 randomized controlled trials [7 low and 2 moderate risk of bias]).

The evidence was insufficient to address the Key Questions, with only a few studies rated as low to moderate risk of bias. Particularly scarce was the literature pertaining to children and adolescents. A single study assessing dietary protein's association with bone disease risk in children and adolescents showed mixed results on bone health measures such as turnover markers, and lumbar spine bone mineral density, content, and bone area. Additionally, just one study informed dietary protein's association with adult kidney disease risk, and it reported no significant effects on kidney function, assessed by creatinine clearance. Findings on the effects of protein intake on adult bone disease were inconsistent, showing both no difference and benefit on outcomes such as bone turnover markers, bone mineral density of the lumbar spine, total hip, and femoral neck, as well as total body bone mineral density and content. Similarly, the studies on sarcopenia risk showed inconsistent results regarding muscle mass, physical performance, and muscle strength. The variety of outcome measures, the differences in dietary protein intake levels, and sparse outcome data distribution across studies made it challenging to synthesize and compare findings. Furthermore, studies used established intermediate markers to evaluate disease risk, such as surrogate markers for bone and kidney health and diagnostic components of sarcopenia, instead of direct chronic condition outcomes.

Strengths and Limitations

Our systematic review had several strengths, including a unique emphasis on multiple chronic diseases, and the inclusion of all relevant outcomes. Additionally, our review is notable for examining the relationship between dietary protein intake and bone disease risk in children and adolescents, and for isolating the effects of protein intake without exercise.

However, our exclusion of pre-2000 studies might have missed crucial foundational research, though this is unlikely to significantly affect our findings. Further, by focusing only on studies rated low to moderate risk of bias, we limited the size of our body of evidence, but including high risk of bias studies would likely have lessened the robustness of our findings and the strength of evidence.

Our review identifies evidence base limitations, including reliance on recognized intermediate markers for bone and kidney disease and sarcopenia, which may not reflect the full complexity, presence, and progression of chronic diseases, diverse outcome measurement methods with inherent limitations, the absence of sarcopenia as a study endpoint, lack of randomized controlled trials, and the challenges in assessing risk of bias, particularly in non-randomized studies, such as prospective cohort studies. These limitations could pose challenges in accurately assessing health effects.

Implications and Conclusions

Studies conducted since 2000 on the association between dietary protein intake and the risks of bone disease, kidney disease, and sarcopenia have yielded unclear yet potentially significant findings. Ambiguities stem from study limitations, lack of studies on vital demographics such as children and adolescents for bone health, varying protein intake levels in studies, and inconsistent outcome measures across studies. This underlines the crucial need for more comprehensive, high quality, long-term research to strengthen the evidence base. Such

improvements will be essential for assessing dietary protein's impact on these chronic conditions.

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Chapter 1. Introduction

Background

Protein is essential for optimal growth, development, function, and maintenance of human health.[1] Bone disease, kidney disease, and sarcopenia, considered important chronic conditions relevant to protein intake and chronic disease risk, have been studied for decades. Dietary protein intake plays a crucial role in maintaining bone health throughout all stages of life. Adequate protein intake during childhood and adolescence is crucial for supporting robust growth and development. It helps achieve peak bone mass, establishing a strong foundation for bone health later in life.[2, 3] In adults, the impact of protein on bone health is more nuanced, and has been described as having both positive[4, 5] and detrimental effects,[6, 7] suggesting a complex and somewhat paradoxical relationship between dietary protein intake and bone health. Concerns also exist around dietary protein intake and its modulating impact on kidney health. Whether dietary protein intake worsens kidney health in the general population is unknown.[8-11] Sarcopenia is an age-related condition, characterized by progressive loss of muscle strength and muscle mass, and/or physical performance, as defined by the European Working Group for Sarcopenia in Older People (EWGSOP2).[12] Although sarcopenia can occur earlier in life,[12] it is most common among older adults and most concerning at older ages, given that the condition's progression is associated with malnutrition, anorexia, frailty, disability, reduced cardiopulmonary function, metabolic syndrome, insulin resistance, cognitive impairment, falls and fractures, depressive symptoms, hospitalization, and death.[12-14] Dietary protein intake might be a factor in slowing down the progression of sarcopenia.[15, 16]

Dietary Reference Intakes

Dietary reference intakes (DRIs) are a set of scientifically developed reference values for nutrients. DRIs expand on the periodic reports called Recommended Dietary Allowances (RDAs), which have been published since 1941 by the National Academy of Sciences. The Governments of the United States and Canada have jointly developed DRIs since the mid-1990s.[17] DRI values are specific for each nutrient, each of which has special uses.[17] The values related to meeting nutritional requirements are: RDA, estimated average requirement (EAR), and adequate intake. The value for preventing excessive intakes and the risk of adverse effects in the general population is the tolerable upper intake level. The value related to supporting the importance of high protein intakes when calorie intake is low is the acceptable macronutrient distribution range. The value for reducing risk of chronic disease is the chronic disease risk reduction intake (CDRR).[17]

The DRIs are intended for the general healthy population. DRI values can be used by nutrition experts, governments, non-governmental organizations, and academic institutions for a variety of activities, including developing dietary guidelines and food guides (including the *Dietary Guidelines for Americans*), developing nutrition labels, informing dietary counseling and educational materials for consumers and patients, and surveillance of safe levels of nutrients in foods and supplements for the nutritional health of the population. DRIs for protein were first published in 2005[17] as part of a comprehensive report on energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids.

Protein Dietary Reference Intakes

In 2005, the Dietary Reference Intakes for protein were published[17] and they have not been updated since. In the DRI publication, the protein intake recommendations for the general population were set at 0.66 and 0.8 g/kg/day as the EAR and RDA, respectively. The EAR is set at a point that meets the needs of half the population, and RDA values are set to meet the needs of the vast majority (97-98%) of the target healthy population.[17] However, data was insufficient to establish a tolerable upper intake level for protein. The acceptable macronutrient distribution range for protein was set at 10 to 35 percent of calorie intake.[17] The evidence to support these values came from analyses of available nitrogen balance studies which mostly enrolled primarily healthy young men. Some nutrition experts consider the protein DRIs somewhat lacking because studies that used other methods to derive protein requirements in a generalizable population were not included. Further, current DRIs for protein have no set reference value for CDRR, because the CDRR was developed after the most recent protein DRI values were established.

More information on the DRIs for protein requirements can be found in the concurrent systematic review on the daily dietary protein intake and amino acid requirement throughout the life course [https://effectivehealthcare.ahrq.gov/products/dietary-protein-intake/protocol].

Chronic Disease Endpoints in Dietary Reference Intakes

Overall, the effort to update DRIs seeks to incorporate evidence on chronic disease in order to include a new category of values specific to CDRR.[18] CDRR is established through a comprehensive, multi-step process that includes a critical assessment of the strength of scientific evidence regarding specific nutrients and the risk of chronic diseases. Evidence on chronic disease was first included in the development of a DRI for a reference value for chronic disease risk reduction intake in the 2019 updated review of DRIs for sodium and potassium.[18, 19]

Since the last protein DRIs were developed, new and relevant scientific research has emerged on the relationship between dietary protein intake and chronic disease risk. The Joint Canada-US Dietary Reference Intakes Working Group collaborated with the U.S. Department of Agriculture (USDA) Nutrition Evidence Systematic Review team to conduct a series of evidence scans on acute adverse health effects, chronic disease risk, and daily requirements.[20] The scans described the volume and characteristics of research available and helped to determine the focus of the reviews that will be conducted and will serve as a key evidence source that will inform future updates to the DRIs for macronutrients by the National Academies of Sciences, Engineering, and Medicine (NASEM).

The current review stems from this exercise and was undertaken at the recommendation of the Joint Canada-U.S. Dietary Reference Intakes Working Group.

Scope and Key Questions

The goal of this report is to review the evidence on the association between dietary protein intake and risk of bone disease, kidney disease, and sarcopenia.

Key Questions

Key Question 1: What is the association between dietary protein intake and risk of bone disease?

Key Question 2: What is the association between dietary protein intake and risk of kidney disease?

Key Question 3: What is the association between dietary protein intake and risk of sarcopenia?

Report Organization

The remainder of this report presents the methods used to conduct the literature searches, data abstraction, and analysis for this review; the results (organized by Key Question); and a discussion of the findings within the context of what is already known, the limitations of the evidence base and the review, suggestions for future research, and the conclusions.

Chapter 2. Methods

Review Approach

The methods for this systematic review followed the Agency for Healthcare Research and Quality (AHRQ) Methods Guide for Effectiveness and Comparative Effectiveness Reviews (available at https://effectivehealthcare.ahrq.gov/topics/cer-methods-guide/overview). This systematic review also reports in accordance with the Preferred Items for Reporting in Systematic Reviews and Meta-Analyses (PRISMA).[21]

The Joint Canada-U.S. Dietary Reference Intakes Working Group prioritized areas for systematic review and developed the questions for this systematic review. AHRQ and Partners (HHS and USDA) finalized the Key Questions. The Evidence-based Practice Center (EPC) confirmed the Key Questions with input from AHRQ and Partners to ensure that the Key Questions were specific and relevant. A panel of technical experts of front-line clinicians and researchers provided content and methodological experience in protein, nutrition, epidemiology, bone disease, kidney disease, and sarcopenia throughout the development of the review protocol. The protocol was posted online June 2, 2023, with amendments posted online December 6, 2023. (https://effectivehealthcare.ahrq.gov/products/effect-protein-intake/protocol). We registered the protocol on PROSPERO (CRD42023446621).

We invited experts in protein, nutrition, epidemiology, bone disease, kidney disease, and sarcopenia to provide external peer review of this systematic review; AHRQ and an associate editor also provided comments. The draft report will be posted on the AHRQ website for 4 weeks to elicit public comments. We will address all reviewer comments, revising the text as appropriate. A disposition of comments table of public comments will be posted on the AHRQ website 3 months after the Agency posts the final systematic review.

Literature Search Strategies

The following discussion about the review search processes applies to all Key Questions. Our librarian team member developed multiple search strategies for different relevant databases, including Medline, EMBASE, AGRICOLA, and Scopus, incorporating vocabulary and natural language relevant to the Key Questions (Appendix A). We reviewed and agreed on the search strategies through a consensus among team members. Searches were conducted from January 2000 through March 2024 to capture all relevant published literature since the current DRIs for protein were established in 2005. Search strategies were peer reviewed by a reference librarian who was not a team member.

Study Selection

We reviewed bibliographic database search results for studies relevant to our PICOTS framework and study-specific inclusion criteria described in Table 1.

Search results were downloaded to EndNote X9 and screened in PICO Portal software (www.picoportal.net).[22] PICO portal is a web-based screening tool that improves efficiency and accuracy in the screening process and management of the process by using machine learning to sort and present first the citations most likely to be eligible. Two independent investigators screened titles and abstracts of results using predefined criteria. When the machine learning system was trained, we moved on to one screener as soon as we reached a 90 percent recall rate of citations eligible for full-text screen. We stopped screening citations remaining past a 95

percent recall rate of citations eligible for full-text screen. Two independent investigators performed full-text screening to determine if inclusion criteria were met, using the same online system. Differences in screening decisions were resolved by consultation between reviewers, and, if necessary, consultation with a third reviewer. We documented the inclusion and exclusion status of citations that underwent full-text screening. Throughout the screening process, team members met regularly to discuss training material and any issues that arose to ensure consistent application of inclusion criteria. A complete list of publications excluded after full-text review appears in Appendix B.

Additionally, during screening, we tagged studies in PICO portal (using certain identifiers, such as small sample size, study design) to help us sort the literature and track study characteristics that could necessitate revisiting based on review findings. Multiple publications relating to the same study were mapped to a unique study.

We supplemented our bibliographic database searches with citation searching of relevant systematic reviews and original research. We solicited literature through a notice in the Federal Register and Supplementary Evidence and Data for Systematic Review submission portal and other information solicited through the AHRQ Effective Health Care website. We used information from these sources to assess publication and reporting bias and inform future research needs.

We will update searches while the draft report is under public review.

Table 1. Inclusion and Exclusion Criteria by Population, Intervention, Comparator, Outcome, Timing, Setting/Study Design (PICOTS)

Element	Inclusion	Exclusion

Population KQ1	• Participants who are healthy and/or have chronic diseases or chronic disease risk factors, including those with obesity.
	• Participants who are pregnant and lactating
	• Age of participants (at intervention or exposure):
	  ○ Infants, children, and adolescents (0-18 years)
	  ○ Adults (19-64 years)
	  ○ Older adults (65 years and older)
		• Participants sample exclusively diagnosed with a disease or hospitalized or in a long-term care facility with an illness or injury
		• Participants who have already been diagnosed with bone disease
		• Participants with existing conditions that clearly are known to alter nutrient metabolism or requirements, or those being treated with medications that alter nutrient metabolism
		• Participant sample exclusively undernourished
		• Participant sample exclusively with a baseline diet deficient in protein (i.e below the recommended daily allowance of protein (RDA) per age)
		• Participant sample exclusively pre-term infant
		• Participant sample exclusively post-bariatric surgery subjects
		• Participant sample exclusively elite athletes
		• Non-human participants (e.g., animal studies, in-vitro models)

Population KQ2&3	• Participants who are healthy and/or have chronic diseases or chronic disease risk factors, including those with obesity. • Participants who are pregnant and lactating	• Participants sample exclusively diagnosed with a disease or hospitalized or in a long-term care facility with an illness or injury • Participants who have already been diagnosed with kidney disease and/or sarcopenia

Element	Inclusion	Exclusion

	• Age of participants (at intervention or exposure):
	  ○ Adults (19-64 years)
	  ○ Older adults (65 years and older)
		• Participants with existing conditions that clearly are known to alter nutrient metabolism or requirements, or those being treated with medications that alter nutrient metabolism
		• Participant sample exclusively undernourished
		• Participant sample exclusively with a baseline diet deficient in protein (i.e below the recommended dietary allowance of protein (RDA) per age)
		• Participant sample exclusively post-bariatric surgery subjects
		• Participant sample exclusively elite athletes
		• Non-human participants (e.g., animal studies, in-vitro models)

Interventions KQ1-3	• Total dietary protein intake from food, beverages, and dietary supplements with or without energy restriction • Assessment of % AMDR for protein with or without the % from the other macronutrients (carbohydrate and fat)	• No specification on the amount of protein intake (e.g., only the type of protein or source of protein reported) • Protein intake via parenteral nutrition or intravenous nutrition support • Food products or dietary supplements not widely available to U.S. consumers • Protein intake evaluated with exercise
Comparison KQ1-3	• Consumption of different levels of total dietary protein intake • No comparator	Comparison of different sources of protein (i.e., animal versus plant protein) without specification on the levels of total dietary protein intake
Outcomes KQ1	Bone outcomes, including but not limited to: • Osteoporosis • Osteopenia • Fracture • Bone mass including bone mineral density, bone mineral content etc.	No relevant exclusion criteria
Outcomes KQ2	Kidney outcomes including but not limited to: • Incidence of kidney stones or ureteral stones • Incidence of CKD (including evaluations from estimated glomerular filtration (eGFR) rate with or without a parameter for race) • Kidney insufficiency	No relevant exclusion criteria
Outcomes KQ3	Aging associated sarcopenia (any definition) and its diagnostic indicators, including but not limited to: • Muscle mass (such as skeletal muscle mass, lean body mass, and fat free mass) • Physical performance (such as Timed Up-and-Go [TUG], gait speed, and Short Physical Performance Battery [SPPB] etc.) • Muscle strength	No relevant exclusion criteria
Timing KQ1-3	All duration and followup	No relevant exclusion criteria
Setting KQ1-3	All settings	No relevant exclusion criteria

Element	Inclusion	Exclusion
Study design KQ1-3	• Randomized controlled trials (RCTs) • Non-randomized controlled trials (Non-RCTs), including quasi-experimental and controlled before-and-after studies • Prospective cohort studies with or without comparison group with appropriate analytic technique • Nested case-control studies	• Narrative reviews • Systematic reviews, meta-analyses, umbrella reviews, scoping reviews • Systematic reviews or meta-analyses that exclusively include cross-sectional and/or uncontrolled studies • Retrospective cohort studies • All other study designs
Language KQ1-3	English only (due to resource limitations)	Non-English publications
Geographic Location KQ1-3	Locations with food products or dietary supplements widely available to U.S. consumers, including those rated high and very high on the Human Development Index	Locations with less than high HDI
Study size KQ1-3	Studies with N > 50 participants (for RCTs – 25 participants analyzed per study arm)	Studies with N < 50 participants (for RCTs – 25 participants analyzed per study arm), and without power calculation
Publication date KQ1-3	2000 to present	Prior to 2000
Publication status KQ1-3	Articles published in peer-reviewed journals	Articles that have not been peer reviewed and are not published in peer-reviewed journals (e.g., unpublished data, manuscripts, pre-prints, reports, abstracts, conference proceedings)

Abbreviations: AMDR = Acceptable macronutrient distribution range; CKD = chronic kidney disease; eGFR = estimated glomerular filtration rate; GI = gastrointestinal; HDI = human development index; SPBB = Short Physical Performance Battery; KQ = key question; N = number; RCT = randomized controlled trial; U.S. = United States

The Human Development Index

Introduced by the United Nations in 1990, the Human Development Index (HDI) classifies countries based on a summary measure of average achievement in three key dimensions of human development: health, education and economics.[23] We used USDA Nutrition Evidence Systematic Review's (NESR's) standard criteria to include studies conducted in countries classified as high or very high on the HDI,[24] which are most generalizable to the United States and Canada, and to exclude studies conducted in countries classified as medium or low on the HDI. We applied HDI classification based on the year the intervention was conducted or the exposure data were collected. If studies did not report the year(s) in which the intervention/exposure data were collected, we applied the HDI classification for the year of publication. If the study year was more recent than the available HDI values, we used the most recent HDI classifications. If a country was not listed in the HDI, we used the current country classification from the World Bank and included those studies conducted in countries grouped as upper-middle or high income.[25] For multinational studies, we applied the HDI classification for the majority of the countries.

Data Extraction

The Systematic Review Data Repository (SRDR) online system (http://srdr.ahrq.gov) (a detailed and standardized web-based customizable data extraction form) was used for study-level data extraction. The SRDR form was pilot-tested and refined within the review team.

Studies that met inclusion criteria were distributed to EPC reviewers for data extraction. Data were extracted by one reviewer, and to ensure accuracy, a second, senior systematic reviewer

conducted quality checks on randomly selected studies (20% of the literature set). Team members met at least weekly to discuss questions about data extraction and to ensure consistency in abstraction.

We extracted data from all eligible studies into evidence tables (Appendix C). In addition, we presented a summary of the basic characteristics of all eligible studies in Appendix D. All eligible studies underwent risk of bias assessment (see section below). For studies that addressed more than one Key Question, we extracted the relevant data separately for each Key Question. We used a two-step process for our data extraction: 1) we extracted study characteristics for all eligible studies, and 2) we extracted findings for outcomes from only studies rated as low to moderate risk of bias (these studies make up our analytic set). For all eligible studies, data elements on study characteristics extracted include: author, year of publication, sponsorship, setting, study design, population (including sample size, age, sex, race/ethnicity, socioeconomic status, physical activity level, body mass index, obesity status, menopausal status, health status/co-morbidities, medication use, supplement use (such as calcium, vitamin D, etc.), energy balance status (i.e., studies that examine protein intake in the context of energy imbalance states), intervention and control characteristics, comparisons, outcomes, intervention duration, and risk of bias assessments.

In addition to the above data elements on study characteristics extracted for all eligible studies, findings for outcomes were extracted from studies included in the analytic set (i.e., studies rated as low to moderate risk of bias) (Appendix E).

We used only studies with higher methodological rigor (i.e., studies rated as low to moderate risk of bias) to comprise our analytic set. We based the findings of this review on these studies because they are less prone to biases that can reduce the robustness of their findings.

At the end of the project, all data will be available in SRDR online system (http://srdr.ahrq.gov) for full public access.

Assessing Methodological Risk of Bias of Individual Studies

Risk of bias is the extent to which the design and conduct of a study are unlikely to have prevented bias in the results. We assessed the methodological risk of bias of each included original study based on study design (Appendix G).

We implemented the Cochrane Risk of Bias tool 2.0 parallel design version[26, 27] to assess risk of bias of parallel RCTs, as low risk, some concerns (moderate risk), or high risk for each of the following domains: 1) Bias arising from randomization process; 2) Bias due to deviations from intended interventions; 3) Bias due to missing outcome data; 4) Bias in measurement of the outcome; 5) Bias in selection of reported result. In addition, we used the Cochrane Risk of Bias tool 2.0 crossover design version[26] to assess risk of bias of crossover RCTs, as low risk, some concerns (moderate risk), or high risk for each of the following domains: 1) Bias arising from randomization process; 2) Bias from period and carryover effects; 3). Bias due to deviations from intended interventions; 4) Bias due to missing outcome data; 5) Bias in measurement of the outcome; 6) Bias in selection of reported result.

For observational studies (including prospective cohort studies with or without comparison group and nested case-control studies), risk of bias by outcomes were rated using the Risk Of Bias In Non-randomized Studies – of Exposure (ROBINS-E) tool[28] as low, moderate, serious, critical, or no information for each of the following domains: 1) Bias due to confounding; 2) Bias in selection of participants into the study; 3) Bias due exposure classification; 4) Bias due to deviations from intended interventions; 5) Bias due to missing data; 6) Bias in measurement of

outcomes; 7) Bias in selection of the reported result; and an overall risk of bias judgment option low, moderate, high (serious) or very high (critical).

When using the ROBINS-E tool, we carried out our assessment using a two-step process for all eligible studies. We first assessed only domain 3 – 7. When at least one domain was assessed as high risk or very high risk of bias, we determined that a study had an overall risk of bias judgement of high risk or very high risk of bias (based on the ROBINS-E algorithm for reaching overall risk of bias judgement); and we decided that no further assessment was required using domains 1 – 2. When domains 3 – 7 were not assessed as high risk or very high risk of bias (i.e., where the domains were either low or moderate risk of bias), we decided to carry out further assessment using domains 1– 2. Given the number of eligible studies, we chose to use the two-step process to proceed with our ROBINS-E risk of bias assessment in a timely manner.

One reviewer independently assessed the risk of bias for eligible studies by outcome; a second investigator reviewed each risk of bias assessment. Investigators consulted to reconcile any discrepancies in the risk of bias assessments. For RCTs, we classified the overall risk of bias assessments for each study outcome as low risk, moderate risk, or high risk. For observational studies, we classified the overall risk of bias assessments for each study outcome as low, moderate, high (serious) or very high (critical).

We based overall risk of bias assessments on the collective risk of bias across components and confidence that the study results for given outcomes were believable given the study's limitations. When determining the overall strength of evidence, we considered any quality issues pertinent to the specific outcomes of interest.

Data Synthesis

We organized our findings by Key Question, then population (adults or children and adolescents), study design, outcomes, and comparisons. We provided a qualitative synthesis due to heterogenous methodologies and outcomes data present across the studies, precluding a meta-analysis.

To better summarize our findings, we grouped our outcomes into broad categories. For Key Question 1, we grouped outcomes into six categories: bone turnover markers (such as overall turnover markers, bone resorption markers, and bone formation markers), bone mineral density (BMD) and bone mineral content (BMC) of the axial skeleton (such as lumbar spine), BMD and BMC of the appendicular skeleton (such as total hip, femoral neck, trochanter, intertrochanter, total forearm, radius, etc.), total body BMD and BMC, osteoporotic fractures and fracture risk (such as fragility fracture [including, osteoporotic and low-trauma fractures], fracture at specific sites [such as hip, spine, forearm, etc.], and bone geometry and strength indices. For Key Question 2, we grouped outcomes into five categories: kidney function, kidney stones, electrolytes, proteinuria, and hyperfiltration. For Key Question 3, we grouped outcomes into three categories: muscle mass (such as skeletal muscle mass, lean body mass, and fat free mass etc.), physical performance (such as Timed Up-and-Go [TUG], gait speed, and Short Physical Performance Battery [SPPB] etc.), and muscle strength. As noted above, the term "muscle mass" is used throughout this report and includes measures such as skeletal muscle mass, lean body mass and fat free mass depending on the technology used by the investigators.

For each comparison, we presented summary of findings tables for the outcomes in the Results section.

Grading the Strength of Evidence for Major Comparisons and Outcomes

The strength of evidence (SoE) is the extent of our confidence in drawing a specific conclusion, and is based on causal inference criteria.

The overall SoE for comparisons and outcomes identified for Key Questions 1 – 3 were evaluated based on five required domains: 1) study limitations (risk of bias); 2) consistency (similarity of effect direction and size); 3) directness (single, direct link between intervention and outcome); 4) precision (degree of certainty around an estimate); and 5) reporting bias.[29]

Based on study design and risk of bias, we rated study limitations as low, moderate, high, or very high. Consistency was rated as consistent, inconsistent, or unknown/not applicable (e.g., single study) based on whether intervention effects were similar in direction and magnitude, and statistical significance of all studies. Directness was rated as either direct or indirect based on the need for indirect comparisons when inference requires observations across studies (i.e., more than one step was needed to reach the conclusion). Precision was rated as precise or imprecise based on the degree of certainty surrounding each effect estimate or qualitative finding. An imprecise estimate is one for which the confidence interval is wide enough to include clinically distinct conclusions.

An outcome with an overall rating of "high strength of evidence" implies that the included contributing studies were randomized controlled trials with both a low risk of bias, and with consistent, direct, and precise domains. If we had found any outcome to have at least moderate or high strength of evidence, we would have evaluated reporting bias by the potential for publication bias, selective outcome reporting bias, and selective analysis reporting bias. We would have done this by comparing reported results with those mentioned in the methods section and an assessment of the grey literature to assess potentially unpublished studies. However, no findings rose to this level. Other factors considered in assessing strength of evidence included dose-response relationship, the presence of confounders, and strength of association.

Based on these factors, we rated the overall strength of evidence for each outcome as:

High: Very confident that estimate of effect lies close to true effect. Few or no deficiencies in body of evidence, findings are believed to be stable.

Moderate: Moderately confident that estimate of effect lies close to true effect. Some deficiencies in body of evidence; findings likely to be stable, but some doubt.

Low: Limited confidence that estimate of effect lies close to true effect; major or numerous deficiencies in body of evidence. Additional evidence necessary before concluding that findings are stable or that estimate of effect is close to true effect.

Insufficient: No evidence, unable to estimate an effect, or no confidence in estimate of effect. Available evidence or lack of evidence precludes judgment.

Notably, an assessment of insufficient evidence does not mean that the intervention is ineffective. Rather, it means that due to the uncertainty of the evidence, we could not draw meaningful conclusions about its effectiveness at this time.

For each comparison, we presented the strength of evidence for the outcomes in a SoE table (Appendix H).

Chapter 3. Results

Introduction

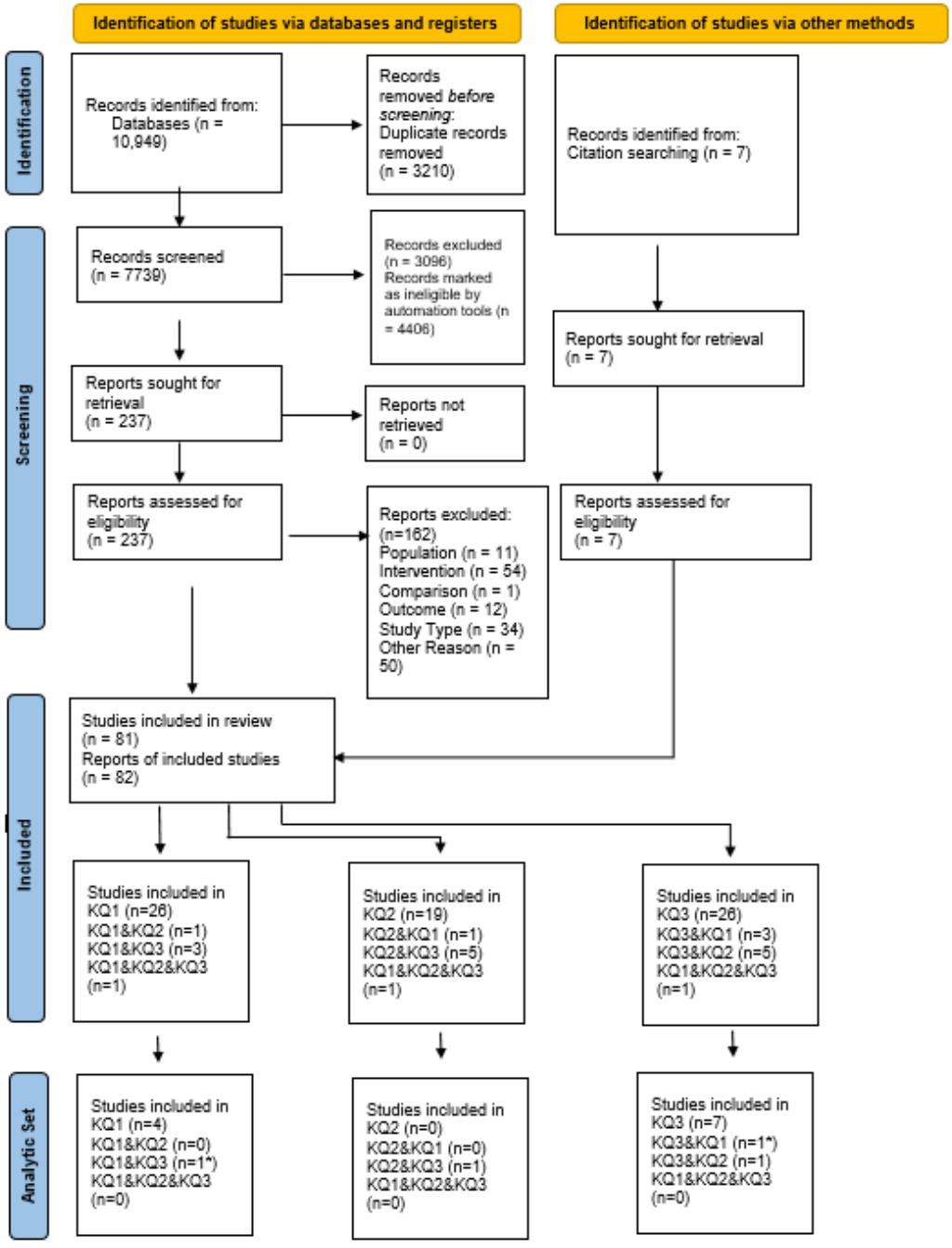
This section describes the results of the literature search, followed by study characteristics and the reported findings of included studies rated as low to moderate risk of bias (analytic set) for each of the Key Questions.

Results of Literature Searches

Figure 1 presents the literature flow of the search results. Database searches of published literature resulted in 10,949 potentially relevant articles. After dual review of abstracts and titles, we assessed 237 articles for eligibility at full text. Of these, we determined that 82 articles reporting on 81 unique studies met the inclusion criteria.[14, 30-110] Thirteen of the eligible studies were rated as low to moderate risk of bias and comprise our analytic set.[33, 37, 45, 51, 65, 74, 91, 92, 99, 100, 102, 106, 110] A breakdown per Key Question is shown in Figure 1 below.

We list studies excluded at full-text screening, sorted by the reason for exclusion, in Appendix B.

Figure 1. Literature flow diagram



*Only studies rated as low to moderate risk of bias were included in the analytic set. One study reported data on KQ1, KQ2, and KQ3; but only the KQ1 and KQ3 outcomes had low risk of bias and were included in analytic set.

The PRISMA process is outlined in Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. Doi: 10.1136/bmj.n71. For more information, visit: http://www.prisma-statement.org/

Key Question 1. What is the association between dietary protein intake and risk of bone disease?

Adults

Key Points

- Evidence was insufficient to draw conclusions for any outcome for adults related to risk of bone disease.
- The variety of outcome measures, differences in dietary protein intake levels, and the limited spread of outcome data across studies made synthesizing and comparing findings significantly challenging.

Study Characteristics

Four (3 RCTs and 1 non-RCT) of thirty eligible studies of adult participants were rated as low to moderate risk of bias and were included in the analytic set.[37, 65, 92, 99] Table 2 summarizes the basic characteristics of the analytic set for studies of adult participants by study design.

Table 2. Basic characteristics of analytic set for risk of bone disease: adults

Characteristic	Information (RCT)	Information (Non-RCT)
Total Studies	3 studies	1 study
U.S studies	1 study	0 studies
Non-U.S. studies	2 studies – France and Denmark	1 study – Mexico
Settings	Community dwelling: 1 study NR: 2 studies	Community dwelling: 1 study
Study design	RCT (parallel): 3 studies	Non-RCT (prospective cohort study without comparison arm): 1 study
Sex of study participants	Female only: 1 study Female and Male: 2 studies	Female only: 1 study
Age range	40 to 75 years	45 to 92 years
Sample size	65 to 208 (range)	317
Follow-up duration range	6 weeks – 1.5 years	6.4 years
Outcomes Evaluated:	Bone Turnover Marker (Overall Turnover) – Osteoclacin: 1 study Bone Formation Marker – P1NP: 1 study Bone Resorption Marker – CTX: 1 study Bone Resorption Marker – TRAP: 1 study Bone Formation Marker – BAP: 1 study BMD of the Appendicular Skeleton (femoral neck): 1 study BMD of the Appendicular Skeleton (hip, total): 1 study BMD of the Axial Skeleton (lumbar spine): 2 studies Total Body/Whole Body BMC (total body): 2 studies Total Body/Whole Body BMD (whole body): 1 study	BMD of the Appendicular Skeleton (femoral neck): 1 study BMD of the Appendicular Skeleton (hip, total): 1 study BMD of the Axial Skeleton (lumbar spine): 1 study
Menopausal status	Post-menopausal: 1 study NR: 2 studies	Post-menopausal: 1 study

Characteristic	Information (RCT)	Information (Non-RCT)
Risk of bias	2 Low 1 Moderate	1 Moderate

Abbreviations: BAP = Bone specific alkaline phosphatase; BMC = bone mineral content; BMD = bone mineral density; CTX = C-terminal peptide of collagen; NA = not applicable; non-RCT = non-randomized controlled trial; NR = not reported; P1NP = procollagen type 1 N-terminal propeptide; RCT = randomized controlled trial; TRAP = 5b, tartrate resistant alkaline phosphatase, isoform 5; U.S. = United States

Dietary Protein Intake Intervention

Dietary protein intake interventions and comparators were different across RCTs (Table 3). Different cut-off points (such as high and low) were used to describe protein intake levels.

The RCTs assessed dietary protein intake using different methods, including food frequency questionnaire,[37] food records,[65] and a shop computer system.[99] The prospective cohort study assessed dietary protein intake using a food frequency questionnaire. Absolute protein intake (g) was used as the protein measurement unit.[92]

Protein measurement units also differed, including absolute protein intake (g) or protein intake per energy intake (%).

Compliance to the dietary protein intake intervention was measured by urinary nitrogen excretion,[65, 99] and self-rating diary.[37] Studies reported good compliance.

Table 3. Protein intake interventions and comparators for risk of bone disease in adults

Study	Intervention	Comparator
Bonjour (2012)[37]	I: Treated group (test food – 13.8 g protein) A: Mean change (SD) 11.4 (18.5) g/d	I: Usual diet - NR A: Mean change (SD) 0.9 (16.5) g/d
Kerstetter (2015)[65]	I: High Protein (45g whey protein supplement isolate) A: Mean (SEM) 90.7 (3.3) g/d	I: Low Protein (carbohydrate isocaloric maltodextrin control supplement) - NR A: Mean (SEM) 72.7 (2.4) g/d
Skov (2002)[99]	I: High protein diet (protein – 25% of total energy) A: Mean (SEM) 102.5 (6.6) g/d	I: Low protein diet (protein – 12% of total energy) A: Mean (SEM)70.5 (6.7) g/d

Abbreviations: A = actual dietary protein intake; d = day; g = gram; I = intended dietary protein intake; NR = not reported; SEM = standard error of mean; SD = standard deviation

Outcomes Assessment

RCT

Bone turnover markers (overall turnover, resorption markers, and formation markers)

One study reported bone outcome measures using bone turnover markers including overall turnover marker (osteocalcin [OC, mg/L]), bone resorption markers (C-terminal peptide of collagen [CTX, nmol/L] and tartrate resistant alkaline phosphatase [TRAP, U/L]), and bone formation markers (bone alkaline phosphatase [BAP, mg/L] and procollagen type 1 N-terminal propeptide [P1NP, mg/L]) assessed by serum automated immunoassay on the Elecsys platform (Roche Diagnostics).[37]

BMD of the axial skeleton (lumbar spine)

Two studies[65, 99] reported bone outcome measures using lumbar spine BMD (g/cm^2) measured by dual-energy X-ray absorptiometry (DXA) Hologic 4500W machine or a Lunar Prodigy DPX-IQ[65] and Hologic 1000/W machine.[99]

BMD of the appendicular skeleton (total hip and femoral neck)

One study reported bone outcome measures using BMD total hip and femoral neck (g/cm^2) measured by DXA Hologic 4500W machine or a Lunar Prodigy DPX-IQ.[65]

Total body BMD and BMC

One study reported bone outcome measures using total body BMD and BMC (g/cm^2) measured by DXA Hologic 1000/W machine.[99]

Non-RCT

BMD of the axial skeleton and BMD of the appendicular skeleton

The prospective cohort study reported bone outcome measures using lumbar spine BMD (L1-L4) (g/cm2) and BMD total hip and femoral neck (g/cm2) assessed using a DXA Lunar DPX NT instrument (Lunar Radiation Corp) machine.[92]

Confounding factors

Non-RCT

The prospective cohort study reported adjustment for important confounders, including energy intake and physical activity. However, calcium intake, an important confounder, was not considered.[92]

Findings

The variations in dietary protein intake interventions and comparisons across the studies, particularly when evaluating identical outcomes, required individualized analysis and assessment of the results and strength of evidence strength for each study.

Bone turnover markers

Based on the results from one French based RCT[37] (n=71) that enrolled post-menopausal females with a 6-week intervention, evidence was insufficient to conclude whether protein intake is associated with changes in bone turnover markers. The study reported findings of no effects of protein intake on osteocalcin, CTX, BAP and P1NP, and the inverse effect of protein intake on TRAP. Table 4 provides a summary of findings.

Table 4. Summary of findings for bone turnover markers outcomes in adults

Outcome Comparisons	#Studies/ Design (n analyzed) Study Duration	Country Age Sex of study participants (% females)	Baseline Protein	Outcome Baseline	Outcome Followup	Direction of Effect	Strength of the Evidence*
OC **I: Treated group (test food – 13.8 g protein) vs C: usual diet**	**Bonjour (2012)** [37] 1 RCT (n=71) 6 weeks	France Mean age (SD): I: 57.1 (3.9) y C: 56.1 (3.9) y 100% females	I: Mean (SD) 72 (17) g/d C: Mean (SD) 199 (79) g/d	I: Mean (SD): 25.9 (9.7) mg/L C: Mean (SD): 26.9 (9.6) mg/L	I: Change in OC Mean (SD): -0.39 (3.6) mg/L C: Change in OC Mean (SD): 0.77 (3.4) mg/L	No difference	Insufficient
CTX **I: Treated group (test food – 13.8 g protein) vs C: usual diet**	**Bonjour (2012)** [37] 1 RCT (n=71) 6 weeks	France Mean age (SD): I: 57.1 (3.9) y C: 56.1 (3.9) y 100% females	I: Mean (SD) 72 (17) g/d C: Mean (SD) 199 (79) g/d	I: Mean (SD) 3.56 (1.6) nmol/L C: Mean (SD) 3.56 (1.58) nmol/L	I: (Change in CTX): Mean (SD) -0.18 (0.70) nmol/L C: (Change in CTX) Mean (SD): 0.06 (0.85) nmol/L	No difference	Insufficient
TRAP **I: Treated group (test food – 13.8 g protein) vs C: usual diet**	**Bonjour (2012)** [37] 1 RCT (n=71) 6 weeks	France Mean age (SD): I: 57.1 (3.9) y C: 56.1 (3.9) y 100% females	I: Mean (SD) 72 (17) g/d C: Mean (SD) 199 (79) g/d	I; Mean (SD) 5.49 (1.42) U/L C: Mean (SD) 5.35 (1.38) U/L	I: (Change in TRAP): Mean (SD) -0.64 (0.56) U/L C: (Change in TRAP): Mean (SD) -0.34 (0.59) U/L	Found benefit	Insufficient
BAP **I: Treated group (test food – 13.8 g protein) vs C: usual diet**	**Bonjour (2012)** [37] 1 RCT (n=71) 6 weeks	France Mean age (SD): I: 57.1 (3.9) y C: 56.1 (3.9) y 100% females	I: Mean (SD) 72 (17) g/d C: Mean (SD) 199 (79) g/d	I: Mean (SD) 11.3 (3.8) mg/L C: Mean (SD) 10.8 (3.2) mg/L	I: Mean (SD) -1.2 (1.8) mg/L C: Mean (SD) -0.9 (1.2) mg/L	No difference	Insufficient
P1NP **I: Treated group (test food – 13.8 g protein) vs C: usual diet**	**Bonjour (2012)** [37] 1 RCT (n=71) 6 weeks	France Mean age (SD): I: 57.1 (3.9) y C: 56.1 (3.9) y 100% females	I: Mean (SD) 72 (17) g/d C: Mean (SD) 199 (79) g/d	I: Mean (SD) 52.0 (19.7) mg/L C: Mean (SD) 54.2 (20.3) mg/L	I: Mean (SD) 0.25 (9.3) mg/L C: Mean (SD) 2.8 (10.8) mg/L	No difference	Insufficient

Abbreviations: BAP = Bone alkaline phosphatase; C = control; CTX = C-terminal peptide of collagen; d = day; g = gram; I = intervention; mg/L = milligrams per liter; n = number; nmol/L = nanomols per liter; OC = osteocalcin; P1NP = procollagen type 1 N-terminal propeptide; RCT = randomized controlled trial; SD = standard deviation; TRAP = tartrate resistant alkaline phosphatase; U/L = Units per; y = years

***:** Strength of the evidence was evaluated based on five designated domains outlined in the Methods section, and was insufficient. As provided in Appendix Table H1, the main reasons for this insufficient rating were that the evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was imprecise due to challenges with evaluating precision.

BMD of the Axial skeleton (lumbar spine)

Evidence from two RCTs was insufficient to conclude whether protein intake was associated with changes in lumbar spine BMD.[65, 99] One U.S.-based RCT (n=171) that enrolled about 85 percent females reported no difference in the lumbar spine BMD between the intervention and comparator group at the end of the 18-month intervention.[65] A Danish RCT (n=50) that enrolled about 76 percent females also reported no difference in the lumbar spine BMD at the end of the 6-month intervention.[99]

In addition, evidence from a prospective cohort study based in Mexico that enrolled post-menopausal female participants (n=317) was insufficient to conclude whether protein intake was associated with changes in lumbar spine BMD. The study reported no difference in lumbar spine BMD at the end of the 6.4 years study.[92] Table 5 provides a summary of findings.

Table 5. Summary of findings for lumbar spine BMD outcomes in adults

Outcome Comparisons	#Studies/ Design (n analyzed) Study Duration	Country Age Sex of study participants (% females)	Baseline Protein	Outcome Baseline	Outcome Followup	Direction of Effect	Strength of the Evidence*
Lumbar spine BMD **I: High protein (45g whey protein supplement isolate) vs** **C: low protein (carbohydrate - isocaloric maltodextrin control supplement)**	**Kerstetter (2015)[65]** 1 RCT (n=171) 18 months	U.S. Mean (SD) age: I: 69.9 (6.1) y C: 70.5 (6.4) y I: 84% females C: 87.3% females	I: Mean (SEM) 73.8 (1.9) g/d C: Mean (SEM) 72.9 (1.8) g/d	I: Mean (SD) 1.09 (0.01) g/cm^2 C: Mean (SD) 1.10 (0.01) g/cm^2	I: Mean (SD) 1.10 (0.01) g/cm^2 C: Mean (SD) 1.11 (0.02) g/cm^2	No difference	Insufficient
Lumbar spine BMD **I: High protein diet (protein – 25% of total energy) vs** **C: low protein diet (protein – 12% of total energy)**	**Skov (2002)[99]** 1 RCT (n=50) 6 months	Denmark Mean age (SD): I: 39.4 (2.0) y C: 39.8 (1.9) y I: 76% females C: 76% females	I: Mean (SEM) 89.1 (3.9) g/d C: Mean (SEM) 87.8 (5.0) g/d	I: Mean (SEM)1.03 (0.02) g/cm^2 C: Mean (SEM) 1.17 (0.01) g/cm^2	I: Mean (SEM) 1.04 (0.02) g/cm^2 C: Mean (SEM) 1.01 (0.03) g/cm^2	No difference	Insufficient
Lumbar spine BMD (L1-L4) **No comparison arm**	**Rivera-Paredez (2021)[92]** 1 non-RCT (n=317) 6.4 years	Mexico Mean age (SD): 57 y 100% females	Whole cohort: Median (IQR): 66.4 (51.1-86.0) g/d	Whole cohort: Mean (SD): 1.035 (0.171) g/cm^2	Whole cohort: Mean (SD): 0.999 (0.893) g/cm^2	No difference	Insufficient

Abbreviations: BMD = bone mineral density; C = control; d = day; g = gram; ; g/cm^2 = grams per centimeter squared; I = intervention; IQR = inter quartile range; L1 = lumbar vertebrae 1; L4 = lumbar vertebrae 4; n = number; RCT = randomized controlled trial; non-RCT = non-randomized controlled trial; SD = standard deviation; SEM = standard error of mean; U.S. = United States; y = years

*: Strength of the evidence was evaluated based on five designated domains outlined in the Methods section, and was insufficient. As provided in Appendix Table H1, the main reasons for this insufficient rating were that the evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was imprecise due to challenges with evaluating precision.

BMD of the Appendicular skeleton (total hip and femoral neck)

Based on the results from one U.S.-based RCT (n=171) that enrolled about 85 percent females, the evidence was insufficient to conclude whether protein intake is associated with changes in BMD total hip and femoral neck. The study reported no difference in the total hip BMD and femoral neck BMD between the intervention and comparator group at the end of the 18-month intervention.[65]

In addition, evidence from a prospective cohort study based in Mexico that enrolled post-menopausal female participants (n=317) was insufficient to conclude whether protein intake was associated with changes in total hip and femoral neck BMD. The study reported no association between protein intake and total hip BMD, and positive association between protein intake and femoral neck BMD at the end of the 6.4 years study.[92] Table 6 provides a summary of findings.

Table 6. Summary of findings for BMD total hip and femoral neck outcomes in adults

Outcome Comparisons	#Studies/ Design (n analyzed) Study Duration	Country Age Sex of study participants (% females)	Baseline protein Mean (SD)	Outcome Baseline	Outcome Followup	Direction of Effect	Strength of the Evidence*
Total hip BMD **I: High protein (45g whey protein supplement isolate) vs C: low protein (carbohydrate - isocaloric maltodextrin control supplement)**	**Kerstetter (2015)[65]** 1 RCT (n=171) 18 months	U.S. Mean age (SD) age: I: 69.9 (6.1) y C: 70.5 (6.4) y I: 84% females C: 87.3% females	I: Mean (SEM) 73.8 (1.9) g/d C: Mean (SEM) 72.9 (1.8) g/d	I: LSM (SEM) 0.89 (0.01) g/cm^2 C: LSM (SEM) 0.90 (0.01) g/cm^2	I: LSM (SEM) 0.88 (0.01) g/cm2 C: LSM (SEM) 0.89 (0.01) g/cm^2	No difference	Insufficient
Femoral neck BMD **I: High protein (45g whey protein supplement isolate) vs C: low protein (carbohydrate - isocaloric maltodextrin control supplement)**	**Kerstetter (2015)[65]** 1 RCT (n=171) 18 months	U.S. Mean age (SD): I: 69.9 (6.1) y C: 70.5 (6.4) y I: 84% females C: 87.3% females	I: Mean (SEM) 73.8 (1.9) g/d C: Mean (SEM) 72.9 (1.8) g/d	I: LSM (SEM) 0.81 (0.01) cm^2 C: LSM (SEM) 0.82 (0.01) cm^2	I: LMS (SEM) 0.80 (0.01) cm^2 C: LSM (SEM) 0.82 (0.01) g/cm^2	No difference	Insufficient
Total hip BMD **No comparison arm**	**Rivera-Paredez (2021)[92]** 1 non-RCT (n=317) 6.4 years	Mexico Mean age (SD): 57 y 100% females	Whole cohort: Median (IQR): 66.4 (51.1-86.0) g/d	Whole cohort: Mean (SD): 0.959 (0.140) g/cm^2	Whole cohort: Mean (SD): 0.917 (0.137) g/cm^2	No difference	Insufficient
Femoral neck BMD **No comparison arm**	**Rivera-Paredez (2021)[92]** 1 non-RCT (n=317) 6.4 years	Mexico Mean age (SD): 57 y 100% females	Whole cohort: Median (IQR): 66.4 (51.1-86.0) g/d	Whole cohort: Mean (SD): 0.921 (0.135) g/cm^2	Whole cohort: Mean (SD): 0.873 (0.127) g/cm^2	Found benefit	Insufficient

Abbreviations: BMD = bone mineral density**;** C = control; d = day; g/cm^2 = grams per centimeter squared; g = gram; I = intervention; IQR = inter quartile range; LSM = least square mean; n = number; non-RCT = non-randomized controlled trial; RCT = randomized controlled trial; SD = standard deviation; SEM = standard error of mean; U.S. = United States; y = years

*: Strength of the evidence was evaluated based on five designated domains outlined in the Methods section, and was insufficient. As provided in Appendix Table H1, the main reasons for this insufficient rating were that the evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was imprecise due to challenges with evaluating precision.

Total body BMD and BMC

Based on the results from one Danish study (n=50) that enrolled about 76 percent females, the evidence was insufficient to conclude whether protein intake is associated with changes in total body BMD and BMC. The study reported finding of no effect of protein intake on total body BMD and a positive effect of protein on total body BMC. [99] Table 7 provides a summary of findings.

Table 7. Summary of findings for total body BMD and BMC in adults

Outcome Comparisons	#Studies/ Design (n analyzed) Study Duration	Country Age Sex of study participants (% females)	Baseline Protein Mean (SD)	Outcome Baseline	Outcome Followup	Direction of Effect	Strength of the Evidence*
Total body BMD **I: High protein diet (protein – 25% of total energy) vs C: low protein diet (protein – 12% of total energy)**	**Skov (2002)**[99] 1 RCT (n=50) 6 months	Denmark Mean (SD) age: I: 39.4 (2.0) y C: 39.8 (1.9) y I: 76% females C: 76% females	I: Mean (SEM) 89.1 (3.9) g/d C: Mean (SEM) 87.8 (5.0) g/d	I: Mean (SEM)1.17 (0.01) g/cm^2 C: Mean (SEM) 1.18 (0.01) g/cm^2	I: Mean (SEM) 1.17 (0.01) g/cm^2 C: Mean (SEM) 1.17 (0.01) g/cm^2	No difference	Insufficient
Total body BMC **I: High protein diet (protein – 25% of total energy) vs C: low protein diet (protein – 12% of total energy)**	**Skov (2002)**[99] 1 RCT (n=50) 6 months	Denmark Mean age (SD): I: 39.4 (2.0) y C: 39.8 (1.9) y I: 76% females C: 76% females	I: Mean (SEM) 89.1 (3.9) g/d C: Mean (SEM) 87.8 (5.0) g/d	I: M (SEM) 2828 (71) g C: M (SEM) 2760 (72) g	I: M (SEM) 2713 (75) g C: M (SEM) 2660 (75) g	Found benefit	Insufficient

Abbreviations: BMC = bone mineral content; BMD = bone mineral density; C = control; d = day; g = gram; g/cm^2 = grams per centimeter squared; I = intervention; n = number; RCT = randomized controlled trial; SD = standard deviation; SEM = standard error mean; U.S. = United States; y = years

*: Strength of the evidence was evaluated based on five designated domains outlined in the Methods section, and was insufficient. As provided in Appendix Table H1, the main reasons for this insufficient rating were that the evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was imprecise due to challenges with evaluating precision.

Additional Information on Clinical Endpoint Outcomes

Because key clinical endpoint outcomes such as fragility fracture (including, osteoporotic and low-trauma fractures) and fracture at specific sites (such as hip, spine, forearm, etc.) were not captured in our analytic set (i.e., studies rated as low to moderate risk of bias) for bone disease risk in adults, and with the goal of providing clinically relevant information for future reference, we made efforts to revisit our eligible studies that were rated as high risk of bias to separately capture these outcomes, and they are available in Appendix F.

Children and Adolescents

Key Points

- Evidence was insufficient to draw conclusions for any outcome related to risk of bone disease in children and adolescents, based on a single study.

Study Characteristics

We identified one unique eligible study (1 RCT) based in Denmark that examined the association between dietary protein intake and risk of bone disease in children and adolescents.[102] The study enrolled children and adolescents aged 6 to 8 years (about 52 percent females) with a 24-week intervention. The study was rated as low risk of bias and was included in the analytic set.

Dietary Protein Intake Intervention

Four arms (—two intervention arms and two comparator arms) were reported.[102] The two intervention arms had a high protein intake with Vitamin D or placebo intervention, and the two comparator arms had normal protein intake with Vitamin D or placebo intervention (Table 8). Study characteristics were presented for each of the four arms; however, the study authors performed combined analyses of the two high protein intake arms, as well as for the two normal protein intake arms. The study authors independently evaluated the effects of protein intake separate from Vitamin D by employing a 2 × 2 factorial trial design.

Dietary protein intake was assessed using a food frequency questionnaire.[102] Absolute protein intake (g), and protein intake per energy intake (%) were used as the protein measurement unit.

Compliance to the dietary protein intake intervention was assessed using recording sheets and dietary records.[102] The study reported good compliance.

Table 8. Protein intervention intake and comparator for risk of bone disease in children/adolescents

Study	Intervention 1	Intervention 2	Comparator 1	Comparator 2
Stounbjerg (2021)[102]	I: Placebo-HP (placebo plus drained low-fat yogurt with a high protein content of 9-11 g protein/100 g) A: 17.7 (3.3) % of energy	I: Vitamin D-HP (vitamin D plus drained low-fat yogurt with a high protein content of 9-11 g protein/100 g) A: 19.0 (3.4) % of energy	I: Placebo-NP (placebo plus regular yogurt with a normal protein content of 3.0-3.9 g protein/100 g) A: 15.8 (2.7) % of energy	I: Vitamin D-NP (vitamin D plus regular yogurt with a normal protein content of 3.0-3.9 g protein/100 g) A: 16.0 (2.2) % of energy

Abbreviations: A = actual dietary protein intake; g = gram; HP = high protein; I = intended dietary protein intake; NP = normal protein

Outcomes Assessment

Bone turnover markers (overall turnover)

Osteocalcin (OC, mg/L) was measured by immunoassay on an Immulite 2000 Xpi Systems Analyzer (Siemens Healthcare GmbH).[102]

BMD and BMC of the axial skeleton

BMD of the lumbar spine, L1-L4 (g/cm^2), BMD of the lumbar spine, L1-L4 zscore, and BMC of the lumbar spine, L1-L4 (g) were measured by DXA GE Lunar Prodigy (GE Healthcare) scanner.[102]

Bone geometry and strength indices

Bone area (BA) of the lumbar spine, L1-L4 (cm^2) was measured by DXA machine – GE Lunar Prodigy (GE Healthcare) scanner.[102]

Findings

Based on the results from just one study, the evidence was insufficient to conclude whether protein intake is associated with changes in bone outcomes among children and adolescents. The study reported findings of inverse effect of protein intake on osteocalcin (a bone turnover marker), positive effects of protein intake on lumbar spine BMD (L1-L4) and lumbar spine BMD (L1-L4) zscore, no effect of protein intake on lumbar spine BMC (L1-L4), and no effect of protein intake on lumbar spine BA (L1-L4). Table 9 provides a summary of findings.

Table 9. Summary of findings for bone disease outcomes in children/adolescents

Outcome Comparisons	#Studies/ Design (n analyzed) Study duration	Country Age Sex of study participants (% females)	Baseline Protein	Outcome Baseline	Outcome Followup	Direction of Effect	Strength of the Evidence*
OC **I: High protein (9-11 g protein/100 g) vs C: normal protein (3.0-3.9 g protein/100 g)**	**Stounbjerg (2021)[102]** 1 RCT (n=152) 24 weeks	Denmark Median age: I1: 7.8y I2: 7.8 y C1: 7.6 y C2: 7.6 y I1: 48% females I2: 44% females C1: 53% females C2: 61% females	I1: Mean (SD) 15.4 (2.4) % of energy I2: Mean (SD) 15.7 (2.3) % of energy C1: Mean (SD) 15.0 (2.2) % of energy C2: Mean (SD) 15.7 (2.6) % of energy	I1: Mean (SD): 38.3 (9.1) µg/L I2: Mean (SD): 37.1 (10.8) µg/L C1: Mean (SD): 38.1 (11.9) µg/L C2: Mean (SD) 37.1 (9.5) µg/L	I1: Mean (SD): 38.3 (9.1) µg/L I2: Mean (SD): 38.2 (10.0) µg/L C1: Mean (SD): 5.3 (8.5) µg/L C2: Mean (SD): 39.8 (9.8) µg/L	Found benefit	Insufficient
Lumbar spine BMD (L1-L4) **I: High protein (9-11 g protein/100 g) vs C: normal protein (3.0-3.9 g protein/100 g)**	**Stounbjerg (2021)[102]** 1 RCT (n=184) 24 weeks	Denmark Median age: I1: 7.8y I2: 7.8 y C1: 7.6 y C2: 7.6 y I1: 48% females I2: 44% females C1: 53% females C2: 61% females	I1: Mean (SD) 15.4 (2.4) % of energy I2: Mean (SD) 15.7 (2.3) % of energy C1: Mean (SD) 15.0 (2.2) % of energy C2: Mean (SD) 15.7 (2.6) % of energy	I1: Mean (SD): 0.681 (0.074) g/cm^2 I2: Mean (SD): 0.682 (0.084) g/cm^2 C1: Mean (SD): 0.691 (0.078) g/cm^2 C2: Mean (SD): 0.679 (0.074) g/cm^2	I1: Mean (SD): 0.681 (0.074) g/cm^2 I2: Mean (SD): 0.692 (0.082) g/cm^2 C1: Mean (SD): 0.702 (0.086) g/cm^2 C2: Mean (SD): 0.695 (0.078) g/cm^2	Found benefit	Insufficient
Lumbar spine BMD (L1-L4) zscore **I: High protein (9-11 g protein/100 g) vs C: normal protein (3.0-3.9 g protein/100 g)**	**Stounbjerg (2021)[102]** 1 RCT (n=184) 24 weeks	Denmark Median age: I1: 7.8y I2: 7.8 y C1: 7.6 y C2: 7.6 y I1: 48% females I2: 44% females C1: 53% females C2: 61% females	I1: Mean (SD) 15.4 (2.4) % of energy I2: Mean (SD) 15.7 (2.3) % of energy C1: Mean (SD) 15.0 (2.2) % of energy C2: Mean (SD) 15.7 (2.6) % of energy	I1: Mean (SD): 0.056 (0.807) I2: Mean (SD): 0.077 (0.955) C1: Mean (SD): 0.152 (0.918) C2: Mean (SD): 0.022 (0.836)	I1: Mean (SD): 0.056 (0.807) I2: Mean (SD): 0.066 (0.908 C1: Mean (SD): 0.145 (0.980) C2: Mean (SD): 0.073 (0.852)	Found benefit	Insufficient

Outcome Comparisons	#Studies/ Design (n analyzed) Study duration	Country Age Sex of study participants (% females)	Baseline Protein	Outcome Baseline	Outcome Followup	Direction of Effect	Strength of the Evidence*
Lumbar spine BMC (L1-L4) **I: High protein (9-11 g protein/100 g) vs C: normal protein (3.0-3.9 g protein/100 g)**	**Stounbjerg (2021)**[102] 1 RCT (n=184) 24 weeks	Denmark Median age: I1: 7.8y I2: 7.8 y C1: 7.6 y C2: 7.6 y I1: 48% females I2: 44% females C1: 53% females C2: 61% females	I1: Mean (SD) 15.4 (2.4) % of energy I2: Mean (SD) 15.7 (2.3) % of energy C1: Mean (SD) 15.0 (2.2) % of energy C2: Mean (SD) 15.7 (2.6) % of energy	I1: Mean (SD): 21.5 (4.4) g I2: Mean (SD): 21.8 (4.2) g C1: Mean (SD): 22.4 (4.6) g C2: Mean (SD): 22.3 (4.1) g	I1: Mean (SD): 21.5 (4.4) g I2: Mean (SD): 23.2 (4.3) g C1: Mean (SD): 23.8 (5.2) g C2: Mean (SD): 23.6 (4.5) g	No difference	Insufficient
Lumbar spine BA (L1-L4) **I: High protein (9-11 g protein/100 g) vs C: normal protein (3.0-3.9 g protein/100 g)**	**Stounbjerg (2021)**[102] 1 RCT (n=184) 24 weeks	Denmark Median age: I1: 7.8y I2: 7.8 y C1: 7.6 y C2: 7.6 y I1: 48% females I2: 44% females C1: 53% females C2: 61% females	I1: Mean (SD) 15.4 (2.4) % of energy I2: Mean (SD) 15.7 (2.3) % of energy C1: Mean (SD) 15.0 (2.2) % of energy C2: Mean (SD) 15.7 (2.6) % of energy	I1: Mean (SD): 1.3 (4.2) cm2 I2: Mean (SD): 31.9 (3.7) cm2 C1: Mean (SD): 32.2 (3.8) cm2 C2: Mean (SD): 32.7 (3.4) cm2	I1: Mean (SD): 1.3 (4.2) cm2 I2: Mean (SD): 33.3 (3.8) cm2 C1: Mean (SD): 33.8 (4.3) cm2 C2: Mean (SD): 33.8 (3.6) cm2	No difference	Insufficient

Abbreviations: BMC = bone mineral content; BMD = bone mineral density; C = control; cm^2 = centimeter squared; g = gram; g/cm^2 = grams per centimeter squared; OC = osteocalcin; I = intervention; n = number; RCT = randomized controlled trial; SD = standard deviation;; µg/L = micro grams per liter; y = years

*: Strength of the evidence was evaluated based on five designated domains outlined in the Methods section, and was insufficient. As provided in Appendix Table H2, the main reasons for this insufficient rating were that the evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was imprecise due to challenges with evaluating precision..

Key Question 2. What is the association between dietary protein intake and risk of kidney disease?

Key Points

- Evidence was insufficient to draw conclusions on the effect of protein intake on kidney function (determined by creatinine clearance), based on a single study.

Study Characteristics

Only one RCT (of 26 eligible studies) that examined the association between dietary protein intake and risk of kidney disease was rated as low to moderate risk of bias and was included in the analytic set.[110] Specifically, the study was rated as moderate risk of bias. The study was based in Australia and enrolled males with overweight or obesity aged 20 to 65 years with a 52-week intervention.

Dietary Protein Intake intervention

High protein intake intervention to a low protein (high carbohydrate) intervention was compared (Table 10). Dietary protein intake was assessed using a daily food record.[110] Protein intake per body weight (g/kg body weight) and protein intake per energy intake (%) were used as the protein measurement units. Compliance to the dietary protein intake intervention was assessed by a food checklist. The study reported good compliance.

Table 10. Protein intake intervention and comparator for risk of kidney disease

Study	Intervention	Comparator
Wycherley (2012)[110]	I: High Protein (35% energy from protein; ~1.3 g/kg/d) A: Mean ~1.24 g/kg/d	I: Low Protein (high carbohydrate – 17% energy from protein; ~0.85 g/kg/d) A: Mean ~0.82 g/kg/d

Abbreviations: A = actual dietary protein intake; d = day; g = gram; I = intended dietary protein intake; kg = kilogram

~: Approximately

Outcomes Assessment

Kidney function

Kidney function was measured using creatinine clearance (ml min^{-1} 1.73 m^{-2}). Creatinine clearance was calculated as (urine creatinine ($mmol^{-1}$) x urine volume (ml))/(plasma creatinine ($mmol^{-1}$) x minutes) and corrected for body surface.[110]

Findings

Kidney Function: Creatinine clearance Outcome

Based on the results from just one study, the evidence was insufficient to conclude whether protein intake is associated with changes in kidney function. The study reported findings of no effect of protein intake on kidney function determined by creatinine clearance.[110] Table 11 provides a summary of findings.

Table 11. Summary of findings for creatinine clearance

Outcome Comparisons	#Studies/ Design (n analyzed) Timing	Country Age Sex of study participants (% females)	Baseline Protein	Outcome Baseline	Outcome Followup	Direction of Effect	Strength of the Evidence**
Creatinine clearance **I: High protein (35% energy from protein) vs C: low protein (high carbohydrate – 17% energy from protein)**	**Wycherley (2012)[110]** 1 RCT (n=120) 52 weeks	Australia Mean age (SD): I: 51.3 (9.4) y C: 50.2 (9.3) y 0% females	I: *NR C: *NR	I: *NR C: *NR	I: *NR C: *NR	No difference	Insufficient

Abbreviations: C = control; I = intervention; M = mean; n = number; NR = not reported; RCT = randomized controlled trial; SD = standard deviation; y = years

*: Baseline characteristics and followup information were presented for participants who completed the 52-week intervention; but intention-to-treat evaluation was conducted for the full sample (n=120).

**: Strength of the evidence was evaluated based on five designated domains outlined in the Methods section and was insufficient. As provided in Appendix Table H3, the main reasons for this insufficient rating were that the evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was imprecise due to challenges with evaluating precision.

Additional Information on Clinical Endpoint Outcomes

Given the absence of significant clinical endpoint outcomes, such as chronic kidney disease and end-stage renal disease in our analyzed studies (i.e., studies rated as low to moderate risk of bias) for kidney disease risk, and with the objective to provide data of clinical significance for future use, we undertook additional steps to separately capture these outcomes from our eligible studies that were rated as high risk of bias. They are presented in Appendix F.

Key Question 3. What is the association between dietary protein intake and risk of sarcopenia?

Key Points

- Evidence was insufficient to draw conclusions for any outcome related to risk of sarcopenia.
- Diversity in outcome measures, measurement methods, and dietary protein intake levels across studies presented considerable challenges in aggregating and comparing findings.

Study Characteristics

Nine RCTs of 35 unique eligible studies from 36 publications were rated as low to moderate risk of bias and were included in the analytic set.[33, 45, 51, 65, 74, 91, 100, 106, 110] Table 12 summarizes the basic characteristics of the analytic set.

Table 12. Basic characteristics of analytic set for risk of sarcopenia

Characteristic	Information
Total Studies	9 studies

Characteristic	Information
U.S studies	2 studies
Non-U.S. studies	2 studies – Australia 1 study – Netherlands 1 study – Netherlands and Finland 1 study – China 1 study – Germany 1 study – Iran
Settings	Community dwelling: 7 studies NR: 2 studies
Study design	RCT (parallel): 9 studies
Sex of study participants	Female only: 4 studies Male only: 1 study Female and Male: 4 studies
Age range	24 to 80 years
Sample size	52 to 208 (range)
Follow-up duration range	12 weeks – 2 years
Outcomes Evaluated:	Muscle mass – Appendicular skeletal muscle index (ASMi): 2 studies Muscle mass – Whole skeletal muscle mass estimated by BIA: 1 study Muscle mass – Total lean body mass estimated by DXA: 4 studies Muscle mass – Appendicular lean body mass/skeletal muscle mass estimated by DXA: 3 studies Muscle mass – Fat Free Mass estimated by DXA: 2 studies Muscle mass – Fat Free Mass estimated by BIA: 2 studies Physical Performance – Timed Up-and-Go (TUG) [Timed: start in sitting position, get up and walk 3-meters, turn around come back and sit down]: 1 study Physical Performance – 4 m walk gait speed- Walk 8m: 1 study Physical Performance – 400m walk speed: 3 studies Physical Performance – Short Physical Performance Battery (SPPB) (includes sit-to-stand test; 3- or 4-meter timed walk; balance): 4 studies Muscle Strength – Grip strength, hand grip: 5 studies Muscle Strength – Leg/Knee extension (including 1-RM leg extension): 3 studies Muscle Strength – Knee flexion: 1 study Muscle Strength – 1-RM leg press: 1 study Muscle Strength – Sum 1-RM strength: 1 study Muscle Strength – Sum knee extension peak torque: 1 study Muscle Strength – Sum knee flexion peak torque: 1 study Muscle Strength – Chair stand test: 1 study
Menopausal status	Post-menopausal: 4 studies Pre-menopausal: 1 study NA: 1 study NR: 3 studies
Risk of bias	7 Low 2 Moderate

Abbreviations: BIA = bioelectrical impedance analysis; DXA = dual-energy x-ray absorptiometry; LTC = long-term care; m = meter; NA = not applicable; non-RCT = non-randomized controlled trial; NR = not reported; RM = repetition maximum; U.S. = United States

Dietary Protein Intake intervention

Dietary protein intake interventions and comparators were different across RCTs (Table 13). Different cut-off points (such as low, normal and high) were used to describe protein intake levels. Studies assessed dietary protein intake using different methods, including food frequency questionnaire,[74] chemical analysis of the duplicate meals,[33] 3-day food records;[65, 106] 2-week food records,[110] 1-week food records,[100] food diaries and 24 food dietary recall questionnaire,[91] food diaries and food checklists,[45] and 24 food dietary recall questionnaire.[51]

Protein measurement units also differed, including absolute protein intake (g), protein intake per body weight (g/kg body weight), or protein intake per energy intake (%).

Compliance to the dietary protein intake intervention was measured by urinary nitrogen excretion,[65, 100, 106] blood urea nitrogen concentration,[100] diet record,[100] direct provision of meals to study participants,[100] food checklist,[110] percentage of participants reaching a certain dietary protein intake,[91] number of empty test containers returned by the participants,[74, 106] laboratory visits and phone calls and chats,[51] training sessions and phone interview,[45] and a daily contact with the investigators and dietitians.[33] Studies reported good compliance.

Table 13. Protein intake interventions and comparators for risk of sarcopenia

Study	Intervention	Comparator	Comparator	Comparator
Backx (2016)[33]	I: High protein diet (contain 1.7 g/kg/d) A: Mean (SD) 1.69 g/kg/d	I: Normal Protein diet (contain 0.9 g/kg/d) A: Mean (SD) 0.92 g/kg/d	NA	NA
Englert (2021)[45]	I: High Protein (1.5 g/kg/d) A: Mean (SD) 1.4 (0.1) g/kg/d	I: Normal Protein (0.8 g/kg/d) A: Mean (SD) 0.8 (0.1) g/kg/d	NA	NA
Haghighat (2021)[51]	I: High protein (high protein snack (50g of soybeans, protein: 18.2 g)) A: Mean (SD) 1.28 (0.2) g/kg/d	I: Low protein (~3.5 servings of fruit, protein: <2 g) A: Mean (SD) 0.87 (0.12) g/kg/d	NA	NA
Kerstetter (2015)[65]	I: High Protein (45g whey protein supplement isolate: 40 g of protein) A: Mean (SEM) 1.30 (0.05) g/kg/d	I: Low Protein (carbohydrate - isocaloric maltodextrin control supplement) A: Mean (SEM) 1.05 (0.04) g/kg/d	NA	NA
Li (2021)[74]	I: Whey Protein (whey protein blended supplement twice daily: 7.98 g protein per supplement; total protein 1.5 g/kg/d) A: Mean (SD) 1.39 (0.24) g/kg/d	I: *Soy protein (soy protein blended supplement twice daily: 8.80 g protein per supplement; total protein 1.5 g/kg/d) A: Mean (SD) 1.51 (0.41) g/kg/d	I: *Whey-Soy protein group (1:1 ratio of whey and soy blended supplement: 8.39 g protein per supplement; total protein 1.5 g/kg/d) A: Mean (SD) 1.49 (0.34) g/kg/d	I: Control (no supplementation) A: Mean (SD) 1.11 (0.25) g/kg/d
Reinders (2022)[91]	I: Protein advice (advised to increase protein intake to ≥1.2 g/kg aBW/d) A: Mean (SD) 1.21 (0.03) g/kg aBW/d	I: Control (no advice to increase protein consumption) A: Mean (SD) 0.86 (0.02) g/kg aBW/d	NA	NA

Study	Intervention	Comparator	Comparator	Comparator
Smith (2018)[100]	I: Weight loss plus whey protein supplement (hypocaloric diet with increased protein intake 1.2 g/kg/d) A: Mean (SD) 1.22 (0.03) g/kg/d	I: Weight loss plus recommended protein (hypocaloric diet with 0.8 g/kg/d protein) A: Mean (SD) 0.86 (0.03) g/kg/d	NA	NA
Wycherley (2021)[110]	I: High Protein (35% energy from protein; ~1.3 g/kg/d) A: Mean ~1.24 g/kg/d	I: Low Protein (high carbohydrate – 17% energy from protein; ~0.85 g/kg/d) A: Mean ~0.82 g/kg/d	NA	NA
Zhu (2015)[106]	I: High Protein (supplement drink – 30 g/d) A: Mean (SD) 95.9 (19.9) g/d	I: Placebo supplement (high-carbohydrate drink supplement drink – 2.1 g/d) A: Mean (SD) 73.1 (16.9) g/d	NA	NA

Abbreviations: A = actual dietary protein intake; aBW = adjusted body weight; d = day; g = gram; kg = kilogram; I = intended dietary protein intake; NA = not applicable; SD = standard deviation; SEM = standard error of the mean

*: Intervention arm

Outcomes Assessment

Muscle mass

Four studies[33, 65, 74, 100] reported muscle mass measures using total body lean mass (kg) estimated by dual-energy X-ray absorptiometry DXA DPX-L, Lunar Radiation Corp, Madison, [33,100] Hologic 4500W machine or a Lunar Prodigy DPX-IQ,[65] and Discovery W, Hologic machine.[74]

Three studies[33, 74, 106] reported muscle mass measures using appendicular lean mass/skeletal muscle mass (kg) estimated by DXA DPX-L,[33] Lunar Radiation Corp, Madison,[106] and Discovery W, Hologic machine.[74]

Two studies[74, 106] reported muscle mass measures using appendicular skeletal muscle index (ASMi) (kg/m^2) calculated as appendicular skeletal muscle mass (kg) divided by height (m^2).

One study reported muscle mass measures using whole skeletal muscle mass (kg) measured by Bioelectrical impedance analysis (BIA) In-body 270, Biospace, Korea.[51]

Four studies[45, 91, 100, 110] reported muscle mass measures using fat free mass (FFM) (kg) estimated by DXA model DPX-L, Lunar Radiation Corp, Madison,[100] DXA Lunar Prodigy, General Electric, Madison,[110] BIA model Seca medical body composition analyzer (mBCA) 515/514,[45] and BIA BodyStat 1500MDD, United Kingdom.[91]

Physical Performance

One study reported physical performance using Timed Up-and-Go (TUG).[106] One study reported physical performance using gait speed — 4m gait speed.[74] Three studies[33, 45, 91] reported physical performance using 400m walk speed.

Four studies[33, 45, 74, 91] reported physical performance using Short Physical Performance Battery (SPPB). SPPB consists of three measures categories: balance, gait speed, and strength.

Muscle strength

Five studies[33, 45, 74, 91, 106] reported muscle strength using handgrip strength (kg) measured by a hand dynamometer.

One study reported muscle strength using 1-RM leg press (kg) measured by a leg strength machine (Technogym, Rotterdam, The Netherlands).[33]

One study reported muscle strength using knee flexor strength (kg) measured by strain gauge.[106]

Three studies[33, 91, 106] reported muscle strength using knee extensor strength (kg) expressed as 1-RM leg extension (kg) measured by a leg strength machine (Technogym, Rotterdam, The Netherlands);[33] leg extension strength (N) (assessment tool not reported)[91] and knee extensor strength (kg) measured by strain gauge.[106]

One study reported muscle strength using sum 1-RM strength (kg) (sum of leg press, knee extension, and knee flexion) measured by a Hoist multi-station weight machine (Hoist Fitness Systems, Poway, California).[100]

One study reported muscle strength using sum knee extension peak torque (Nm) and sum knee flexion peak torque (Nm) measured by a Biodex 3 dynamometer (Biodex Medical Systems, Shirley, New York).[100]

One study reported muscle strength using a chair stand test.[74]

Findings

The differences in dietary protein intake interventions and comparisons across the studies, particularly when evaluating the same outcomes, required that the results and the strength of evidence be examined and assessed individually for each study. Additionally, the use of different technologies to measure the same outcomes further underscored the need for individual study analysis.

Muscle Mass

Table 14 provides a summary of findings for all muscle mass outcomes. Evidence from four RCTs was insufficient to conclude whether protein intake was associated with changes in total body lean mass.[33, 65, 74, 100] One study based in Netherlands that enrolled males and females with overweight or obesity reported no difference in the total body lean mass between the intervention and comparator.[33] A U.S.-based study (n=207) that enrolled about 85 percent females reported no difference in the total body lean mass between the intervention and comparator.[65] Another U.S.-based study (n=52) that enrolled postmenopausal females with obesity reported no difference in the total body lean mass between the intervention and comparator.[100] However, one China based study (n=123) that enrolled about 50 percent females reported maintained total body lean mass for the intervention groups and a reduction in the control group.[74]

Evidence from three RCTs[33, 74, 106] was insufficient to conclude whether protein intake was associated with changes in appendicular lean mass/skeletal muscle mass. One China based study (n=123) that enrolled about 50 percent females reported a maintained appendicular lean mass for the intervention groups and a reduction in the control group at the end of the 6-month intervention.[74] However, a Netherlands study that enrolled males and females with overweight or obesity reported no difference in the appendicular lean mass/skeletal muscle mass for the

intervention group at the end of the 12-week intervention.[33] An Australian study (n=181) that enrolled postmenopausal females also reported no difference in the appendicular lean mass/skeletal muscle mass between the intervention and comparator group at the end of the 2-year intervention.[106]

Evidence from two RCTs was insufficient to conclude whether protein intake was associated with changes in appendicular skeletal muscle mass index.[74, 106] One China based study (n=123) that enrolled about 50 percent females reported a maintained appendicular skeletal muscle mass index for the intervention groups and a reduction in the control group at the end of the 6-month intervention.[74] However, an Australian study (n=181) that enrolled postmenopausal females reported no difference in the appendicular skeletal muscle mass index between the intervention and comparator group at the end of the 2-year intervention.[106]

Evidence from one RCT based in Iran (n=107)[51] that enrolled only females with a 6-month intervention, was insufficient to conclude whether protein intake was associated with changes in whole skeletal muscle mass. The study reported findings of positive effect of protein intake on whole skeletal muscle mass.

Evidence from four RCTs was insufficient to conclude whether protein intake was associated with changes in FFM. [45, 91, 100, 110] One German study (n=54) that enrolled postmenopausal females reported no difference in the FFM between the intervention and comparator group at the end of the 12-week intervention.[45] A study based in Finland and Netherlands (n=187) of about 53 percent females also reported no difference in the FFM between the intervention and comparator group at the end of the 6-month intervention.[91] In addition, one Australian study (n=120) of males with overweight or obesity reported no difference in the FFM between the intervention and comparator group at the end of the 52-week intervention.[110] Further, a U.S. based study (n=52) that enrolled postmenopausal females with obesity reported no difference in the FFM between the intervention and comparator group at the end of the 6-month intervention.[100]

Table 14. Summary of findings for muscle mass

Outcome Comparisons	#Studies/ Design (n analyzed) Study Duration	Country Age Sex of study participants (% females)	Baseline Protein	Outcome Baseline	Outcome Followup	Direction of Effect	Strength of the Evidence**
Total body lean mass by DXA **I: High protein diet (contain 1.7g of protein/kg/day) vs C: normal protein diet (contain 0.9 g protein/kg/day)**	**Backx (2016)[33]** 1 RCT (n=NR) 12 weeks	Netherlands Mean age (SD): I: 63 (4.8) y C: 62 (4.8) y I: 41.9% females C: 40% females	I: Mean (SD) 1.1 (0.4) g/kg/d C: Mean (SD) 1.1 (0.4) g/kg/d	I: Mean (SD) 54.8 (12.2) kg C: Mean (SD) 54.5 (9.3) kg	I: Mean (SD) 53.1 (11.4) kg C: Mean (SD) 52.4 (9.1) kg	No difference	Insufficient
Total body lean mass by DXA **I: High protein (45g whey protein supplement isolate) vs C: low protein (carbohydrate -isocaloric maltodextrin control supplement)**	**Kerstetter (2015)[65]** 1 RCT (n=207) 18 months	U.S. Mean age (SD): I: 69.9 (6.1) y C: 70.5(6.4) y I: 84% females C: 87.3% females	I: Mean (SEM) 73.8 (1.9) g/d C: Mean (SEM) 72.9 (1.8) g/d	I: Mean (SEM) 42.6 (0.8) kg C: Mean (SEM) 42.0 (0.8) kg	I: Mean (SEM) 42.6 (0.8) kg C: Mean (SEM) 41.5 (0.8) kg	No difference	Insufficient
Total body lean mass by DXA **I: Weight loss plus whey protein supplement (hypocaloric diet with increased protein intake 1.2 g/kg/d) vs C: weight loss plus recommended protein (hypocaloric diet with 0.8 g/kg/d protein)**	**Smith (2018)[100]** 1 RCT (n=52) 6 months	U.S. Mean age: NR 100% females	I: NR C: NR	I: Mean (SEM) 44.4 (1.0) kg C: Mean (SEM) 45.7 (0.9) kg	I: Mean (SEM) 43.3 (1.0) kg C: Mean (SEM) 44.2 (1.0) kg	No difference	Insufficient

Outcome Comparisons	#Studies/ Design (n analyzed) Study Duration	Country Age Sex of study participants (% females)	Baseline Protein	Outcome Baseline	Outcome Followup	Direction of Effect	Strength of the Evidence**
Total body lean mass by DXA **I1: Whey Protein (whey protein blended supplement), I2: soy protein (soy protein blended supplement), I3: whey-Soy protein group (1:1 ratio of whey and soy blended supplement) vs C: control (no supplementation)**	**Li (2021)**[74] 1 RCT (n=123) 6 months	China Mean age (SD): I1: 71 (4) y I2: 69 (4) y I3: 70 (4) y C: 71 (4) y I1: 48.4% females I2: 51.6% females I3: 45.2% females C: 56.7% females	I1: Mean (SD) 1.14 (0.36) g/kg/d I2: Mean (SD) 1.11 (0.33) g/kg/d I3: Mean (SD) 1.14 (0.37) g/kg/d C: Mean (SD) 1.17 (0.30) g/kg/d	I1: Mean (SD) 34.96 (6.75) kg I2: Mean (SD) 34.66 (6.83) kg I3: Mean (SD) 35.49 (6.49) kg C: Mean (SD) 33.69 (6.17) kg	I1: Mean (SD) 35.13 (6.4) kg I2: Mean (SD) 34.84 (6.78) kg I3: Mean (SD) 35.77 (6.57) kg C: Mean (SD) 33.32 (6.0) kg	Found benefit	Insufficient
Appendicular lean mass/skeletal muscle mass by DXA **I1: Whey Protein (whey protein blended supplement), I2: soy protein (soy protein blended supplement), I3: whey-Soy protein group (1:1 ratio of whey and soy blended supplement) vs C: control (no supplementation)**	**Li (2021)**[74] 1 RCT (n=123) 6 months	China Mean age (SD): I1: 71 (4) y I2: 69 (4) y I3: 70 (4) y C: 71 (4) y I1: 48.4% females I2: 51.6% females I3: 45.2% females C: 56.7% females	I1: Mean (SD) 1.14 (0.36) g/kg/d I2: Mean (SD) 1.11 (0.33) g/kg/d I3: Mean (SD) 1.14 (0.37) g/kg/d C: Mean (SD) 1.17 (0.30) g/kg/d	I1: Mean (SD) 14.47 (3.34) kg I2: Mean (SD) 14.46 (3.27) kg I3: Mean (SD) 15.07 (3.33) kg C: Mean (SD) 14.13 (3.03) kg	I1: Mean (SD) 14.62 (3.10) kg I2: Mean (SD) 14.54 (3.27) kg I3: Mean (SD) 15.26 (3.38) kg C: Mean (SD) 13.76 (2.98) kg	Found benefit	Insufficient
Appendicular lean mass/skeletal muscle mass by DXA **I: High protein diet (contain 1.7g of protein/kg/day) vs C: normal protein diet (contain 0.9 g protein/kg/day)**	**Backx (2016)**[33] 1 RCT (n=NR) 12 weeks	Netherlands Mean age (SD): I: 63 (4.8) y C: 62 (4.8) y I: 41.9% females C: 40% females	I: Mean (SD) 1.1 (0.4) g/kg/d C: Mean (SD) 1.1 (0.4) g/kg/d	I: Mean (SD) 23.8 (5.5) kg C: Mean (SD) 23.8 (4.8) kg	I: Mean (SD) 23.1 (5.4) kg C: Mean (SD) 22.8 (4.6) kg	No difference	Insufficient

Outcome Comparisons	#Studies/ Design (n analyzed) Study Duration	Country Age Sex of study participants (% females)	Baseline Protein	Outcome Baseline	Outcome Followup	Direction of Effect	Strength of the Evidence**
Appendicular lean mass/skeletal muscle mass by DXA **I: High Protein (supplement drink – 30 g of protein per day) vs C: placebo supplement (high-carbohydrate drink supplement drink – 2.1 g of protein per day)**	**Zhu (2015)[106]** 1 RCT (n=181) 2 years	Australia Mean age (SD): I: 74.2 (2.8) y C: 74.3 (2.6) y 100% females	I: Mean (SD) 1.2 (0.3) g/kg/d C: Mean (SD) 1.1 (0.3) g/kg/d	I: Mean (SD)16.2 (2.4) kg C: Mean (SD) 16.6 (2.4) kg	I (Change at 2 y): Mean (SEM) -0.03 (0.07) kg C (Change at 2 y): Mean (SEM) 0.03 (0.08) kg	No difference	Insufficient
Appendicular skeletal muscle mass index **I1: Whey Protein (whey protein blended supplement), I2: soy protein (soy protein blended supplement), I3: whey-Soy protein group (1:1 ratio of whey and soy blended supplement) vs C: control (no supplementation)**	**Li (2021)[74]** 1 RCT (n=123) 6 months	China Mean age (SD): I1: 71 (4) y I2: 69 (4) y I3: 70 (4) y C: 71 (4) y I1: 48.4% females I2: 51.6% females I3: 45.2% females C: 56.7% females	I1: Mean (SD) 1.14 (0.36) g/kg/d I2: Mean (SD) 1.11 (0.33) g/kg/d I3: Mean (SD) 1.14 (0.37) g/kg/d C: Mean (SD) 1.17 (0.30) g/kg/d	I1: Mean (SD) 5.70 (0.92) kg/m^2 I2: Mean (SD) 5.62 (0.83) kg/m^2 I3: Mean (SD) 5.68 (0.81) kg/m^2 C: Mean (SD) 5.65 (0.84) kg/m^2	I1: Mean (SD) 5.76 (0.81) kg/m^2 I2: Mean (SD) 5.65 (0.84) kg/m^2 I3: Mean (SD) 5.75 (0.80) kg/m^2 C: Mean (SD) 5.50 (0.81) kg/m^2	Found benefit	Insufficient
Appendicular skeletal muscle mass index **I: High Protein (supplement drink – 30 g of protein per day) vs C: placebo supplement (high-carbohydrate drink supplement drink – 2.1 g of protein per day)**	**Zhu (2015)[106]** 1 RCT (n=181) 2 years	Australia Mean age (SD): I: 74.2 (2.8) y C: 74.3 (2.6) y 100% females	I: Mean (SD) 1.2 (0.3) g/kg/d C: Mean (SD) 1.1 (0.3) g/kg/d	I: Mean (SD) 6.3 (0.7) kg/m^2 C: Mean (SD) 6.5 (0.8) kg/m^2	I (Change at 2 y): Mean (SEM) 0.02 (0.03) kg/m^2 C (Change at 2 y): Mean (SEM) 0.05 (0.03) kg/m^2	No difference	Insufficient

Outcome Comparisons	#Studies/ Design (n analyzed) Study Duration	Country Age Sex of study participants (% females)	Baseline Protein	Outcome Baseline	Outcome Followup	Direction of Effect	Strength of the Evidence**
Whole skeletal muscle mass by BIA **I: High protein (high protein snack (50g of soybeans, protein: 18.2 g)) vs C: low protein (~3.5 servings of fruit, protein: <2 g)**	**Haghighat (2021)**[51] 1 RCT (n=107) 6 months	Iran Mean age: NR 100% females	I: Mean (SD) 0.84 (0.15) g/kg/d C: Mean (SD) 0.79 (0.14) g/kg/d	I: NR C: NR	I (Increase): Mean 1.2 kg; 95% CI= 1.5 to 1 C (Increase): Mean 0.3 kg. 95% CI=0.7 to 0.02	Found benefit	Insufficient
FFM by BIA **I: High Protein (1.5 g/kg body weight/day) vs C: normal protein (0.8 g/kg body weight/day)**	**Englert (2021)**[45] 1 RCT (n=54) 12 weeks	Germany Mean age: I: 59.0 (6) y C: 58.7 (6) y I: 100% females C: 100% females	I: NR C: NR	I: Mean (SD) 46.8 (6.9) kg C: Mean (SD) 46.7 (5.0) kg	I (Change at 12 weeks): Mean (SD) -0.9 (1.1) kg C (Change at 12 weeks): Mean (SD) -1.0 (1.3) kg	No difference	Insufficient
FFM by BIA **I: Protein advice (advised to increase protein intake to ≥1.2 g/kg aBW/d) vs C: control (no advice to increase protein consumption)**	**Reinders (2022)**[91] 1 RCT (n=187) 6 months	Finland and Netherlands Mean age (SD): I: 75.9 (5.0) y C: 75.0 (4.4) y I: 52.1% females C: 54.9% females	I: Mean (SD) 0.82 (0.01) g/kg aBW/d C: Mean (SD) 0.82 (0.01) g/kg aBW/d	I: Mean (SE) 52.0 (1.06) kg C: Mean (SE) 51.8 (0.97) kg	I: Mean (SE) 52.6 (1.15) kg C: Mean (SE) 52.1 (0.99) kg	No difference	Insufficient
FFM by DXA **I: Weight loss plus whey protein supplement (hypocaloric diet with increased protein intake 1.2 g/kg/d) vs C: weight loss plus recommended protein (hypocaloric diet with 0.8 g/kg/d protein)**	**Smith (2018)**[100] 1 RCT (n=52) 6 months	U.S. Mean age: NR 100% females	I: NR C: NR	I: Mean (SEM) 46.9 (1.0) kg C: Mean (SEM) 48.2 (1.0) kg	I: Mean (SEM) 45.8 (1.0) kg C: Mean (SEM) 46.7 (1.0) kg	No difference	Insufficient

Outcome Comparisons	#Studies/ Design (n analyzed) Study Duration	Country Age Sex of study participants (% females)	Baseline Protein	Outcome Baseline	Outcome Followup	Direction of Effect	Strength of the Evidence**
FFM by DXA **I: High protein (35% energy from protein) vs C: low protein (high carbohydrate – 17% energy from protein)**	**Wycherley (2012)[110]** 1 RCT (n=120) 52 weeks	Australia Mean age (SD): I: 51.3 (9.4) y C: 50.2 (9.3) y 0% females	I: *NR C: *NR	I: *NR C: *NR	I: *NR C: *NR	No difference	Insufficient

Abbreviations: aBW= adjusted body weight; BIA = bioelectrical impedance analysis; C = control; CI = confidence interval**;** d = day; DXA = dual-energy X-ray absorptiometry; FFM = fat free mass; g = gram; I = intervention; kg = kilogram; kg/m^2 = kilograms per square meter; M = mean; n = number; NR = not reported; RCT = randomized controlled trial**;** SD = standard deviation; SEM = standard error of the mean; y = years; U.S. = United States

*: Baseline characteristics and followup information were presented for participants who completed the 52-week intervention; but intention-to-treat evaluation was conducted for the full sample (n=120).

**: Strength of the evidence was evaluated based on five designated domains outlined in the Methods section, and was insufficient. As provided in Appendix Table H4, the main reasons for this insufficient rating were that the evidence was derived from a single study, making it impossible to assess consistency, and in some instances the outcome effect estimate was imprecise due to challenges with evaluating precision.

Physical Performance

Table 15 provides a summary of findings for all physical performance outcomes. Evidence from one Australia based RCT (n=181)[106] was insufficient to conclude whether protein intake is associated with changes in Timed Up-and-Go. The study enrolled postmenopausal females with a 2-year intervention and reported findings of no effect of protein intake on Timed Up-and-Go.

Evidence from one China based RCT (n=123) that enrolled about 50 percent females with a 6-month intervention, was insufficient to conclude whether protein intake is associated with changes in 4m gait speed.[74] The study reported maintained 4m gait speed for the intervention groups and a reduction in the control group at the end of the intervention.

Evidence from three RCTs was insufficient to conclude whether protein intake was associated with changes in 400m walk speed.[33, 45, 91] One study based in Netherlands (n= 59) that enrolled males and females with overweight or obesity reported no difference in the 400m walk speed between the intervention and comparator group up at the end of the 12-week intervention.[33] A German study (n=54) that enrolled postmenopausal females also reported no difference in 400m walk speed between the intervention and comparator group at the end of the 12-week intervention.[45] However, a study based in Finland and Netherlands (n=187) that enrolled about 53 percent females reported an improvement in 400m walk speed between the intervention and comparator group at the end of the 6-month intervention.[91]

Evidence from four RCTs was insufficient to conclude whether protein intake was associated with changes in SPPB.[33, 45, 74, 91] One study based in Netherlands (n=60) that enrolled males and females with overweight or obesity reported no difference in the SPPB between the intervention and comparator group at the end of the 12-week intervention.[33] A German study (n=54) that enrolled postmenopausal females reported no difference in the SPPB between the intervention and comparator group at the end of the 12-week intervention.[45] In addition, a study based in Finland and Netherlands (n=187) that enrolled about 53 percent females reported no difference in SPPB between the intervention and comparator group at the end of the 6-month intervention.[91] However, one study based in China (n=123) that enrolled about 50 percent females reported a maintained SPPB for the intervention groups and a reduction in the control group at the end of the 6-month intervention.[74]

Table 15. Summary of findings for physical performance

Outcome Comparisons	#Studies/ Design (n analyzed) Study Duration	Country Age Sex of study participants (% females)	Baseline Protein	Outcome Baseline	Outcomes Followup	Direction of Effect	Strength of the Evidence*
TUG **I: High Protein (supplement drink – 30 g of protein per day) vs C: placebo supplement (high-carbohydrate drink supplement drink – 2.1 g of protein per day)**	**Zhu (2015)**[106] 1 RCT (n=181) 2 years	Australia Mean age (SD): I: 74.2 (2.8) y C: 74.3 (2.6) y 100% females	I: Mean (SD) 1.2 (0.3) g/kg/d C: Mean (SD) 1.1 (0.3) g/kg/d	I: Mean (SD) 7.9 (1.3) s C: Mean (SD) 8.0 (1.5) s	I (Change at 2 y): Mean (SEM) 0.46 (0.12) s C (Change at 2 y): Mean (SEM) -0.55(0.12) s	No difference	Insufficient
4m gait speed **I1: Whey Protein (whey protein blended supplement), I2: soy protein (soy protein blended supplement), I3: whey-Soy protein group (1:1 ratio of whey and soy blended supplement) vs C: control (no supplementation)**	**Li (2021)**[74] 1 RCT (n=123) 6 months	China Mean age (SD): I1: 71 (4) y I2: 69 (4) y I3: 70 (4) y C: 71 (4) y I1: 48.4% females I2: 51.6% females I3: 45.2% females C: 56.7% females	I1: Mean (SD) 1.14 (0.36) g/kg/d I2: Mean (SD) 1.11 (0.33) g/kg/d I3: Mean (SD) 1.14 (0.37) g/kg/d C: Mean (SD) 1.17 (0.30) g/kg/d	I1: Mean (SD) 1.12 (0.2) m/s I2: Mean (SD) 1.16 (0.16) m/s I3: Mean (SD) 1.15 (0.20) m/s C: Mean (SD) 1.12 (0.1) m/s	I1: Mean (SD) 1.14 (0.12) m/s I2: Mean (SD) 1.15 (0.14) m/s I3: Mean (SD) 1.13 (0.17) m/s C: Mean (SD) 0.96 (0.16) m/s	Found benefit	Insufficient
400m walk speed **I: High protein diet (contain 1.7g of protein/kg/day) vs C: normal protein diet (contain 0.9 g protein/kg/day)**	**Backx (2016)**[33] 1 RCT (n=59) 12 weeks	Netherlands Mean age (SD): I: 63 (4.8) y C: 62 (4.8) y I: 41.9% females C: 40% females	I: Mean (SD) 1.1 (0.4) g/kg/d C: Mean (SD) 1.1 (0.4) g/kg/d	I: Mean (SD) 1.46 (0.19) m/s C: Mean (SD) 1.45 (0.19) m/s	I: Mean (SD) 1.5 (0.2) m/s C: Mean (SD) 1.47 (0.22) m/s	No difference	Insufficient
400m walk speed **I: High Protein (1.5 g/kg body weight/day) vs C: normal protein (0.8 g/kg body weight/day)**	**Englert (2021)**[45] 1 RCT (n=54) 12 weeks	Germany Mean age (SD): I: 59.0 (6) y C: 58.7 (6) y 100% females	I: NR C: NR	I: Mean (SD) 4:10 (0:33) min: sec C: Mean (SD) 4:11 (0:31) min: sec	I (Change at 12 weeks): Mean (SD) -0:00 (0:07) min: sec C (Change at 12 weeks): Mean (SD) -0:05 (0:12) min: sec	No difference	Insufficient

Outcome Comparisons	#Studies/ Design (n analyzed) Study Duration	Country Age Sex of study participants (% females)	Baseline Protein	Outcome Baseline	Outcomes Followup	Direction of Effect	Strength of the Evidence*
400m walk speed **I: Protein advice (advised to increase protein intake to ≥1.2 g/kg aBW/d) vs C: control (no advice to increase protein consumption)**	**Reinders (2022)[91]** 1 RCT (n=187) 6 months	Finland and Netherlands Mean age (SD): I: 75.9 (5.0) y C: 75.0 (4.4) y I: 52.1% females C: 54.9% females	I: Mean (SD) 0.82 (0.01) g/kg aBW/d C: Mean (SD) 0.82 (0.01) g/kg aBW/d	I: Mean (SE) 311.3 (7.2) s C: Mean (SE) 311.1 (9.3) s	I: Mean (SE) 306.0 (6.85) s C: Mean (SE) 318.2 (11.0) s	Found benefit	Insufficient
SPPB **I: High protein diet (contain 1.7g of protein/kg/day) vs C: normal protein diet (contain 0.9 g protein/kg/day)**	**Backx (2016)[33]** 1 RCT (n=60) 12 weeks	Netherlands Mean age (SD): I: 63 (4.8) y C: 62 (4.8) y I: 41.9% females C: 40% females	I: Mean (SD) 1.1 (0.4) g/kg/d C: Mean (SD) 1.1 (0.4) g/kg/d	I: Mean (SD) 11.6 (0.7) C: Mean (SD) 11.4 (0.9)	I: Mean (SD) 11.7 (0.5) C: Mean (SD) 11.6 (0.6)	No difference	Insufficient
SPPB **I: High Protein (1.5 g/kg body weight/day) vs C: normal protein (0.8 g/kg body weight/day)**	**Englert (2021)[45]** 1 RCT (n=54) 12 weeks	Germany Mean age (SD): I: 59.0 (6) y C: 58.7 (6) y 100% females	I: NR C: NR	I: Mean (SD) 9.4 (1.1) C: Mean (SD) 9.9 (1.0)	I (Change at 12 weeks): Mean (SD) +0.4 (0.09) C (Change at 12 weeks): Mean (SD) +0.6 (0.8)	No difference	Insufficient
SPPB **I: Protein advice (advised to increase protein intake to ≥1.2 g/kg aBW/d) vs C: control (no advice to increase protein consumption)**	**Reinders (2022)[91]** 1 RCT (n=187) 6 months	Finland and Netherlands Mean age (SD): I: 75.9 (5.0) y C: 75.0 (4.4) y I: 52.1% females C: 54.9% females	I: Mean (SD) 0.82 (0.01) g/kg aBW/d C: Mean (SD) 0.82 (0.01) g/kg aBW/d	I: Mean (SE) 9.8 (0.14) C: Mean (SE) 9.7 (0.17)	I: Mean (SE) 10.0 (0.14) C: Mean (SE) 10.0 (0.17)	No difference	Insufficient

Outcome Comparisons	#Studies/ Design (n analyzed) Study Duration	Country Age Sex of study participants (% females)	Baseline Protein	Outcome Baseline	Outcomes Followup	Direction of Effect	Strength of the Evidence*
SPPB **I1: Whey Protein (whey protein blended supplement), I2: soy protein (soy protein blended supplement), I3: whey-Soy protein group (1:1 ratio of whey and soy blended supplement) vs C: control (no supplementation)**	**Li (2021)**[74] 1 RCT (n=123) 6 months	China Mean age (SD): I1: 71 (4) y I2: 69 (4) y I3: 70 (4) y C: 71 (4) y I1: 48.4% females I2: 51.6% females I3: 45.2% females C: 56.7% females	I1: Mean (SD) 1.14 (0.36) g/kg/d I2: Mean (SD) 1.11 (0.33) g/kg/d I3: Mean (SD) 1.14 (0.37) g/kg/d C: Mean (SD) 1.17 (0.30) g/kg/d	I1: Mean (SD) 11.23 (0.8) I2: Mean (SD) 11.58 (0.56) I3: Mean (SD) 11.39 (0.88) C: Mean (SD) 11.51 (0.62)	I1: Mean (SD) 11.65 (0.61) I2: Mean (SD) 11.52 (0.63) I3: Mean (SD) 11.71 (0.78) C: Mean (SD) 10.61 (1.28)	Found benefit	Insufficient

Abbreviations: aBW = adjusted body weigh; C = control**;** d = day; g = gram; I = intervention; kg = kilogram; m = meter; M = mean; min = minutes; n = number; NR = not reported; s/secs = seconds; RCT = randomized controlled trial; SPPB = short physical performance battery; SD = standard deviation; SEM = standard error of the mean; TUG = timed up and go; y = years

*: Strength of the evidence was evaluated based on five designated domains outlined in the Methods section, and was insufficient. As provided in Appendix Table H4, the main reasons for this insufficient rating were that the evidence was derived from a single study, making it impossible to assess consistency, and in some instances the outcome effect estimate was imprecise due to challenges with evaluating precision.

Muscle Strength

Table 16 provides a summary of findings for all muscle strength outcomes. Evidence from five RCTs was insufficient to conclude whether protein intake was associated with changes in hand grip strength.[33, 45, 74, 91, 106] One study based in Netherlands (n=60) that enrolled 40 percent females reported no difference in handgrip strength between the intervention and comparator group at the end of the 12-week intervention.[33] One study based in China (n=123) that enrolled about 50 percent females also reported no difference in handgrip strength between the intervention groups and control group at the end of the 6-month intervention.[74] In addition, a study based in Finland and Netherlands (n=187) that enrolled about 53 percent females reported no difference in handgrip strength between the intervention and comparator group at the end of the 6-month intervention.[91] Further, an Australian study (n=181) that enrolled postmenopausal females reported no difference in handgrip strength between the intervention and comparator group at the end of the 2-year intervention.[106] However, a German study (n=54) that enrolled postmenopausal females reported an improvement in handgrip strength between the intervention and comparator group at the end of the 12-week intervention.[45]

Based on the results from one RCT[33] (n=53) that enrolled males and females with overweight or obesity based in Netherlands with a 12-week intervention, the evidence was insufficient to conclude whether protein intake is associated with changes in 1-RM leg press. The study reported findings of no effect of protein intake on 1-RM leg press.

Based on the results from a 2-year Australian RCT (n=181)[106] that enrolled postmenopausal females, the evidence was insufficient to conclude whether protein intake is associated with changes in knee flexor strength. The study reported findings of no effect of protein intake on knee flexor strength.

Evidence from three RCTs was insufficient to conclude whether protein intake was associated with changes in leg extensor strength.[33, 91, 106] One study based in Netherlands (n=53) that enrolled males and females with overweight or obesity reported no difference in the leg extensor strength expressed as 1-RM leg extension between the intervention and comparator group at the end of the 12-week intervention.[33] An Australian study (n=181) that enrolled postmenopausal females also reported no difference in the leg extensor strength expressed as knee extensor strength between the intervention and comparator group at the end of the 2-year intervention.[106] However, a study based in Finland and Netherlands (n=187) that enrolled about 53 percent females reported improvement in the leg extensor strength between the intervention and comparator group at the end of the 6-month intervention.[91]

One U.S. based 6-month RCT (n=52)[100] that enrolled postmenopausal females with obesity provided insufficient evidence to draw conclusions on its findings of no effect of protein intake on sum 1-RM strength, sum knee extension peak torque, and sum knee flexion peak torque.

One China based 6-month RCT (n=123)[74] that enrolled about 50 percent females provided insufficient evidence to draw conclusions about the inverse effect of protein intake on chair stand test.

Table 16. Summary of findings for muscle strength

Outcome Comparisons	#Studies /Design (n analyzed) Study Duration	Country Age Sex of study participants (% females)	Baseline Protein	Outcome Baseline	Outcome Followup	Direction of Effect	Strength of the Evidence*
Handgrip strength **I: High protein diet (contain 1.7g of protein/kg/day) vs C: normal protein diet (contain 0.9 g protein/kg/day)**	**Backx (2016)**[33] 1 RCT (n=60) 12 weeks	Netherlands Mean age (SD): I: 63 (4.8) y C: 62 (4.8) y I: 41.9% females C: 40% females	I: Mean (SD) 1.1 (0.4) g/kg/d C: Mean (SD) 1.1 (0.4) g/kg/d	I: Mean (SD) 40 (11) kg C: Mean (SD) 41 (10) kg	I: Mean (SD) 37 (9) kg C: Mean (SD) 40 (11) kg	No difference	Insufficient
Handgrip strength **I1: Whey Protein (whey protein blended supplement), I2: soy protein (soy protein blended supplement), I3: whey-Soy protein group (1:1 ratio of whey and soy blended supplement) vs C: control (no supplementation)**	**Li (2021)**[74] 1 RCT (n=123) 6 months	China Mean age (SD): I1: 71 (4) y I2: 69 (4) y I3: 70 (4) y C: 71 (4) y I1: 48.4% females I2: 51.6% females I3: 45.2% females C: 56.7% females	I1: Mean (SD) 1.14 (0.36) g/kg/d I2: Mean (SD) 1.11 (0.33) g/kg/d I3: Mean (SD) 1.14 (0.37) g/kg/d C: Mean (SD) 1.17 (0.30) g/kg/d	I1: Mean (SD) 27.06 (7.78) kg I2: Mean (SD) 26.88 (6.93) kg I3: Mean (SD) 28.42 (8.81) kg C: Mean (SD) 24.90 (7.33) kg	I1: Mean (SD) 26.78 (7.93) kg I2: Mean (SD) 27.48 (7.03) kg I3: Mean (SD) 28.45 (8.17) kg C: Mean (SD) 25.33 (6.63) kg	No difference	Insufficient
Handgrip strength **Protein advice (advised to increase protein intake to ≥1.2 g/kg aBW/d) vs control (no advice to increase protein consumption)**	**Reinders (2022)**[91] 1 RCT (n=187) 6 months	Finland and Netherlands Mean age (SD): I: 75.9 (5.0) y C: 75.0 (4.4) y I: 52.1% females C: 54.9% females	I: Mean (SD) 0.82 (0.01) g/kg aBW/d C: Mean (SD) 0.82 (0.01) g/kg aBW/d	I: Mean (SE) 30.2 (1.04) kg C: Mean (SE) 29.2 (0.96) kg	I: Mean (SE) 29.3 (1.05) kg C: Mean (SE) 27.8 (0.93) kg	No difference	Insufficient

Outcome Comparisons	#Studies /Design (n analyzed) Study Duration	Country Age Sex of study participants (% females)	Baseline Protein	Outcome Baseline	Outcome Followup	Direction of Effect	Strength of the Evidence*
Handgrip strength **I: High Protein (supplement drink – 30 g of protein per day) vs C: placebo supplement (high-carbohydrate drink supplement drink – 2.1 g of protein per day)**	**Zhu (2015)**[106] 1 RCT (n=181) 2 years	Australia Mean age (SD): I: 74.2 (2.8) y C: 74.3 (2.6) y 100% females	I: Mean (SD) 1.2 (0.3) g/kg/d C: Mean (SD) 1.1 (0.3) g/kg/d	I: Mean (SD) 21.7 (5.2) kg C: Mean (SD) 21.7 (5.5) kg	I (Change at 2 y): Mean (SD) -1.09 (0.41) kg C (Change at 2 y): Mean (SEM) -1.53 (0.42) kg	No difference	Insufficient
Handgrip strength **I: High Protein (1.5 g/kg body weight/day) vs C: normal protein (0.8 g/kg body weight/day)**	**Englert (2021)**[45] 1 RCT (n=54) 12 weeks	Germany Mean age (SD): I: 59.0 (6) y C: 58.7 (6) y 100% females	I: NR C: NR	I: Mean (SD) 28.7 (7.2) kg C: Mean (SD) 29.0 (4.9) kg	I (Change at 12 weeks): Mean (SD) +0.01 (2.6) kg C (Change at 12 weeks): Mean (SD) -1.6 (3.3) kg	Found benefit	Insufficient
1-RM leg press **I: High protein diet (contain 1.7g of protein/kg/day) vs C: normal protein diet (contain 0.9 g protein/kg/day)**	**Backx (2016)**[33] 1 RCT (n=53) 12 weeks	Netherlands Mean age (SD): I: 63 (4.8) y C: 62 (4.8) y I: 41.9% females C: 40% females	I: Mean (SD) 1.1 (0.4) g/kg/d C: Mean (SD) 1.1 (0.4) g/kg/d	I: Mean (SD) 142 (44) kg C: Mean (SD) 157 (33) kg	I: Mean (SD) 143 (39) kg C: Mean (SD)148 (30) kg	No difference	Insufficient
Knee flexor strength **I: High Protein (supplement drink – 30 g of protein per day) vs C: placebo supplement (high-carbohydrate drink supplement drink – 2.1 g of protein per day)**	**Zhu (2015)**[106] 1 RCT (n=181) 2 years	Australia Mean age (SD): I: 74.2 (2.8) y C: 74.3 (2.6) y 100% females	I: Mean (SD) 1.2 (0.3) g/kg/d C: Mean (SD) 1.1 (0.3) g/kg/d	I: Mean (SD) 9.1 (3.6) kg C: Mean (SD) 9.7 (3.7) kg	I (Change at 2 y): Mean (SEM) 3.18 (0.38) kg C (Change at 2 y): Mean (SEM) 2.36 (0.49) kg	No difference	Insufficient

Outcome Comparisons	#Studies /Design (n analyzed) Study Duration	Country Age Sex of study participants (% females)	Baseline Protein	Outcome Baseline	Outcome Followup	Direction of Effect	Strength of the Evidence*
Leg extensor strength (1-REM leg extension) **I: High protein diet (contain 1.7g of protein/kg/day) vs C: normal protein diet (contain 0.9 g protein/kg/day)**	**Backx (2016)[33]** 1 RCT (n=53) 12 weeks	Netherlands Mean age (SD): I: 63 (4.8) y C: 62 (4.8) y I: 41.9% females C: 40% females	I: Mean (SD) 1.1 (0.4) g/kg/d C: Mean (SD) 1.1 (0.4) g/kg/d	I: Mean (SD) 93 (31) kg C: Mean (SD) 98 (25) kg	I: Mean (SD) 91 (29) kg C: Mean (SD) 94 (25) kg	No difference	Insufficient
Leg extensor strength (knee extensor strength) **I: High Protein (supplement drink – 30 g of protein per day) vs C: placebo supplement (high-carbohydrate drink supplement drink – 2.1 g of protein per day)**	**Zhu (2015)[106]** 1 RCT (n=181) 2 years	Australia Mean age (SD): I: 74.2 (2.8) y C: 74.3 (2.6) y 100% females	I: Mean (SD) 1.2 (0.3) g/kg/d C: Mean (SD) 1.1 (0.3) g/kg/d	I: Mean (SD) 15.4 (5.3) kg C: Mean (SD) 16.1 (7.2) kg	I (Change at 2 y): Mean (SEM) 3.36 (0.68) kg C (Change at 2 y): Mean (SEM) 3.17 (0.80) kg	No difference	Insufficient
Leg extensor strength **I: Protein advice (advised to increase protein intake to ≥1.2 g/kg aBW/d) vs C: control (no advice to increase protein consumption)**	**Reinders (2022)[91]** 1 RCT (n=187) 6 months	Finland and Netherlands Mean age (SD): I: 75.9 (5.0) y C: 75.0 (4.4) y I: 52.1% females C: 54.9% females	I: Mean (SD) 0.82 (0.01) g/kg aBW/d C: Mean (SD) 0.82 (0.01) g/kg aBW/d	I: Mean (SE) 309.4 (14.5) N C: Mean (SE) 311.4 (12.9) N	I: Mean (SE) 326.1 (14.2) N C: Mean (SE) 295.5 (12.4) N	Found benefit	Insufficient
Sum 1-RM strength **I: Weight loss plus whey protein supplement (hypocaloric diet with increased protein intake 1.2 g/kg/d) vs C: weight loss plus recommended protein (hypocaloric diet with 0.8 g/kg/d protein)**	**Smith (2018)[100]** 1 RCT (n=52) 6 months	U.S. Mean age: NR 100% females	I: NR C: NR	I: Mean (SEM) 170 (6) kg C: Mean (SEM) 163 (6) kg	I: Mean (SEM) 173 (6) kg C: Mean (SEM) 164 (6) kg	No difference	Insufficient

Outcome Comparisons	#Studies /Design (n analyzed) Study Duration	Country Age Sex of study participants (% females)	Baseline Protein	Outcome Baseline	Outcome Followup	Direction of Effect	Strength of the Evidence*
Sum knee extension peak torque **I: Weight loss plus whey protein supplement (hypocaloric diet with increased protein intake 1.2 g/kg/d) vs C: weight loss plus recommended protein (hypocaloric diet with 0.8 g/kg/d protein)**	**Smith (2018)[100]** 1 RCT (n=52) 6 months	U.S. Mean age: NR 100% females	I: NR C: NR	I: Mean (SEM) 326 (14) Nm C: Mean (SEM) 305 (13) Nm	I: Mean (SEM) 309 (13) Nm C: Mean (SEM) 303 (13) Nm	No difference	Insufficient
Sum knee flexion peak torque **I: Weight loss plus whey protein supplement (hypocaloric diet with increased protein intake 1.2 g/kg/d) vs C: weight loss plus recommended protein (hypocaloric diet with 0.8 g/kg/d protein)**	**Smith (2018)[100]** 1 RCT (n=52) 6 months	U.S. Mean age: NR 100% females	I: NR C: NR	I: Mean (SEM) 188 (7) Nm C: Mean (SEM) 178 (7) Nm	I: Mean (SEM) 183 (6) Nm C: Mean (SEM) 177 (7) Nm	No difference	Insufficient
Chair stand test **I1: Whey Protein (whey protein blended supplement), I2: soy protein (soy protein blended supplement), I3: whey-Soy protein group (1:1 ratio of whey and soy blended supplement) vs C: control (no supplementation**	**Li (2021)[74]** 1 RCT (n=123) 6 months	China Mean age (SD): I1: 71 (4) y I2: 69 (4) y I3: 70 (4) y C: 71 (4) y I1: 48.4% females I2: 51.6% females I3: 45.2% females C: 56.7% females	I1: Mean (SD) 1.14 (0.36) g/kg/d I2: Mean (SD) 1.11 (0.33) g/kg/d I3: Mean (SD) 1.14 (0.37) g/kg/d C: Mean (SD) 1.17 (0.30) g/kg/d	I1: Mean (SD) 8.95 (1.54) s I2: Mean (SD) 8.43 (1.63) s I3: Mean (SD) 8.68 (1.37) s C: Mean (SD) 8.32 (1.32) s	I1: Mean (SD) 8.22 (1.48) s I2: Mean (SD) 7.60 (1.71) s I3: Mean (SD) 8.25 (1.36) s C: Mean (SD) 9.72 (1.89) s	Found benefit	Insufficient

Abbreviations: aBW = adjusted body weight; C = control; d = day; g = gram; I = intervention; kg = kilogram; n = number; M = mean; N = newtons; Nm = newton meters; NR = not reported; RCT = randomized controlled trial; s = seconds; SD = standard deviation; SE = standard error; SEM= standard error of the mean; U.S. = United States; y = years

*: Strength of the evidence was evaluated based on five designated domains outlined in the Methods section, and was insufficient. As provided in Appendix Table H4, the main reasons for this insufficient rating were that the evidence was derived from a single study, making it impossible to assess consistency, and in some instances the outcome effect estimate was imprecise due to challenges with evaluating precision.

Additional Information on Clinical Endpoint Outcomes

Since sarcopenia was not captured as a clinical endpoint in our analytic set of studies rated as low to moderate risk of bias, and with the goal to provide clinically valuable data for future insights, we returned to our eligible studies that were rated as high risk of bias to gather information on sarcopenia separately. We found that sarcopenia had not been reported as an endpoint in any of the reviewed studies.

Chapter 4. Discussion

Overview

Our review sought to assess evidence from 2000 onwards regarding the association between dietary protein intake and the risks of bone disease, kidney disease, and sarcopenia. To achieve this, we focused on identifying and synthesizing data from studies rated as having low to moderate risk of bias, meaning these studies were conducted with higher methodological rigor and were less likely to be influenced by factors that could compromise the reliability of their findings.

Our search yielded 10,949 studies, from which we identified 82 articles detailing 81 unique studies that met our inclusion criteria. Among these, 13 studies were rated as low to moderate risk of bias and were synthesized. This analytic set comprised five studies focused on bone disease, including three RCTs on adults (2 low risk of bias and 1 moderate risk of bias), one prospective cohort study on adults (moderate risk of bias), and one RCT on children and adolescents (low risk of bias and the only eligible study on children and adolescents). The set also included one RCT on kidney disease (moderate risk of bias), and nine RCTs examining sarcopenia (7 low risk of bias and 2 moderate risk of bias).

Overall, the evidence was insufficient to address the Key Questions. We found few studies rated as low to moderate risk of bias. Research focusing on children and adolescents is notably sparse. Our review on the association between dietary protein intake and the risk of bone disease in children and adolescents was based on a single study with mixed findings on bone health measures including, bone turnover marker (osteocalcin), bone mineral density, content and bone area of the lumbar spine. Additionally, our findings on the association between dietary protein intake and the risk of kidney disease in adults were informed by another single study that found no significant effects on kidney function, as measured by creatinine clearance. Studies investigating the impact of dietary protein intake on adult bone disease yielded inconsistent results, with studies reporting both no difference and beneficial effects on various outcomes, including bone turnover markers (overall turnover marker [osteocalcin], bone resorption markers [CTX and TRAP], and bone formation markers [BAP and P1NP]), BMD of the lumbar spine, total hip, and femoral neck, as well as total body BMD and BMC. The assessment of sarcopenia risk also revealed inconsistent findings concerning muscle mass, physical performance, and muscle strength.

We were largely not able to collate and compare findings across the studies due to heterogeneity in outcome measures and dietary protein intake interventions and comparisons, and sparse distribution of outcome data across the studies. Furthermore, rather than directly investigating the presence and progression of the chronic conditions of interest as endpoint outcomes, studies used established intermediate markers for disease risk assessment, which included different surrogate markers for bone and kidney health and components of a sarcopenia diagnosis. This made it difficult to discern the impact of dietary protein intake on health.

For adults, the study in our review that reported bone turnover markers had results similar to prior reviews by Wallace and Frankenfeld[111], Groenendijk and colleagues,[108] and Tsagari,[110] who found no effect of dietary protein intake on overall turnover markers, bone formation markers and bone resorption markers as reflected by osteocalcin, CTX and P1NP, respectively. With respect to the BMD changes, our studies results are consistent with previous systematic review and meta-analyses by Darling and colleagues[112] who found no association between protein intake

and BMD of the lumbar spine, and a review by Wallace and Frankenfeld[111] which reported inconsistent study findings for BMD of the femoral neck. Darling and colleagues[112] conducted meta-analyses and included studies regardless of their risk of bias assessment, while results from Wallace and Frankenfeld[111] were based on qualitative evaluations without reporting on risk of bias of included studies. In two previous reviews by Darling and colleagues[112] and Tsagari,[110] we observed similar findings to reported results in our review, supporting no effect of dietary protein intake on total body BMD; but neither of these reviews focused their analyses on studies with higher methodological rigor.

For children and adolescents, our review's one included study reported mixed effects of dietary protein intake on bone health outcomes including, bone turnover marker (osteocalcin), bone mineral density, content and bone area of the lumbar spine. We identified no previous reviews with similar findings in this age group.

Given the significant role of dietary protein intake in kidney disease, this relationship was also examined. Our review's findings on this question come from a single study of apparently healthy adults, which found no significant effects of dietary protein intake on kidney function as measured by creatinine clearance. This provides crucial perspective on the role of dietary protein in kidney health, especially when considering the potential variability in effects between individuals with pre-existing kidney conditions and those without. We identified no prior reviews with kidney health findings similar to those of this single study.

Sarcopenia, characterized by the decline in muscle strength, muscle mass and physical performance associated with aging, poses a considerable public health challenge. Therefore, identifying dietary factors that can mitigate these declines is crucial for developing dietary guidelines and establishing nutrient recommendations. The studies in our review reported results consistent with a previous systematic review and meta-analyses by Hanach and colleagues[113] who found no effects of dietary protein intake on muscle strength determined by 1-RM leg press and inconsistent study findings for the association between dietary protein intake and physical performance evaluated by Short Physical Performance Battery. Similar to our review, Hanach and colleagues[113] presented their findings qualitatively. However, they included all studies regardless of the risk of bias assessment. Reported results on the association between dietary protein intake and appendicular lean mass/skeletal muscle mass in our review reflects inconsistent study findings reported in a systematic review of observational studies by Yaeghashi and colleagues.[114] But while our review examined randomized controlled trials and prospective cohort studies, Yaeghashi and colleagues[114] examined mostly cross-sectional studies, which have significant methodological limitations in determining causal relationships.

Limitations of the Evidence Base

This evidence base has several limitations, a major one being the sparse literature for bone disease outcomes in children and adolescents. This gap highlights a critical need for more research for these populations. Skeletal biology in early life differs distinctly from that of older age as do nutritional needs at these stages. Additionally, many studies focused on post-menopausal women potentially due to their increased risk of bone disease and sarcopenia. Future research, however, should consider including older men to increase knowledge for this group and applicability of findings. Studies analyzed protein intake at different levels, using various cut-off points to define high and low protein intake, and some did not specify the targeted dietary protein intake levels for their interventions, such as aiming for 1.5 g/kg/day of protein. This

heterogeneity made it impossible for us to conduct a combined synthesis of studies. It also raises questions about whether differences in achieved protein intake can accurately predict changes in outcomes of interest. In addition, the baseline diets of the populations generally showed protein intakes that meet (and often slightly exceed) protein intake recommendations. Results could differ if people with a low protein intake (or very high protein intake) were included. Further, many studies compared non-interventional protein intakes of 0.8 g/kg/day (representing a value similar to the current RDA), against higher intakes, often between 1.2-1.7 g/kg/day. This raises concerns about potentially overlooking a plateau effect for the outcomes of interest.

Studies used different parameters to evaluate bone disease, kidney disease, and sarcopenia outcomes (for example, studies used about 30 different types of bone outcomes). This, too, made combined synthesis of studies difficult. Further, due to the chronic nature of the disease conditions, many studies did not concentrate on actual occurrence and progression of these chronic diseases, and instead relied on recognized intermediate markers for evaluating disease risk, including alterations in surrogate markers related to bone and kidney health and the diagnostic components of sarcopenia. Importantly, these intermediate outcomes reviewed are acknowledged surrogate markers for disease risk assessment or formal diagnostic guidelines for diseases. However, these measures have limitations. For instance, bone markers can be affected by gender, ethnicity, fasting or feeding condition, circadian rhythm, exercise level and certain medications.[115] Moreover, intra-and inter-laboratory assay variability can influence the degree of bone turnover levels. Although bone mineral density is currently the gold standard for diagnosis of osteoporosis and predicts the risk of fracture, its significant changes could be limited by study duration precision error and reference database.[116]

Studies did not report sarcopenia as an endpoint outcome. However, sarcopenia is expected to rise along with the aging population worldwide. Despite its growing occurrence, no universally accepted diagnostic criterion exists for sarcopenia, as evidenced by various operational definitions. Recent efforts have produced two notable definitions: one from the European Working Group on Sarcopenia in Older People's second meeting (EWGSOP2)[12] and another by the Sarcopenia Definition and Outcomes Consortium (SDOC).[117] The diagnostic challenges of sarcopenia include prognostic inaccuracy, diverse diagnostic tools, and the absence of a precise muscle mass measurement method.[118] Studies often use DXA and BIA to “estimate” muscle mass; more precise measurements may come from methods such as Computed Tomography (CT), Magnetic Resonance Imaging (MRI), and D3-creatine dilution. However, all body composition assessment technologies each have their unique methodology, operational principles, and potential sources of error.[119]

Conducting nutrition research is complex and comes with unique challenges that can affect both the quality of the study and its risk of bias. These two concepts differ in important ways. “Quality” refers to how well the research was conducted as a given study design, whereas risk of bias focuses on the potential of the study’s design and conduct to introduce systematic errors which can lead to underestimation or overestimation of either the true effect of an intervention on an outcome or the true association between an exposure and outcome. A study can be conducted with high quality yet still have significant risk of bias. Schwingshackl and colleagues[120] highlighted the significance of applying risk of bias assessments in nutritional studies to boost the credibility of systematic reviews, including using rigorous methods in nutrition research. They pointed out challenges akin to those we observed in our review. Our review rated many prospective cohort studies as high risk of bias, primarily due to unreported followup protein intake and high dropout rates, leading to potential misclassification and attrition

biases. Ways to address these challenges might include scheduling regular followup assessments (e.g., monthly, quarterly, or annually), using consistent dietary data collection methods, leveraging technology for data submission (such as mobile apps or web-based platforms), employing analytical strategies that can handle the complexity of longitudinal dietary (such as mixed-effects models, to analyze changes in diet over time and their potential impacts on health outcomes), offering appropriate incentives (such as financial compensation, free health screenings, or access to nutritional products or services), and maintaining transparent communication to keep participants engaged.

Despite that our review focused on studies of higher methodological rigor, predominantly RCTs, we encountered several challenges. Notably, some RCTs were rated as high risk of bias due to attrition, where significant participant loss may have skewed results and compromised study validity. This issue affects both the study's statistical power and the balance of confounders, threatening the reliability of findings. To mitigate this, studies should incorporate regular quality control checks and include dropout participants in analyses via intention-to-treat (ITT) approach, ensuring analyses reflect the initial allocation of participants, thus minimizing attrition bias. Additionally, achieving adequate blinding in nutrition-related RCTs, including those we reviewed, is often challenging due to the complex nature of food-based interventions and the difficulty in creating a suitable placebo. However, minimizing bias requires blinding study participants, outcome assessors, and data collectors. To facilitate more controlled trials, employing specific designs such as well-controlled feeding trials and supermarket models, alongside objective biomarkers for compliance, is beneficial. Further, the typical duration of RCTs, which ranges from 6 weeks to 2 years, generally falls short for assessing long-term outcomes. Chronic diseases take years or even decades to develop, and longer followup periods are needed to assess the effects of dietary protein intake on these conditions.

Strengths and Limitations of This Review

Our systematic review has several strengths. We focused on several chronic disease conditions rather than just one, as with prior reviews, and we included all relevant outcomes for these conditions. Additionally, our review is one of only a few that specifically examines the association between dietary protein intake and risk of bone disease in children and adolescents. Finally, we examined studies that assessed the isolated effects of protein intake without exercise on the outcomes of interest to ensure that the findings are specific to the effects of protein, and not confounded by other co-interventions.

Our review is limited by the fact that we neither captured nor reviewed evidence prior to 2000. Since research methodologies typically advance over time, we can reasonably assume that studies from before 2000 could have faced greater challenges in rigor than those we examined. Therefore, our exclusion of these earlier studies did not likely affect our findings in a significant way. Additionally, although our search was comprehensive, we focused on studies rated as low to moderate risk of bias, which resulted in a smaller body of evidence. But including high risk bias studies would likely have diminished the robustness of our findings and the strength of evidence and made it more difficult to draw conclusions.

Research Gaps Identified by This Review

Our review offers valuable insights for further research, including the need for randomized controlled trials and prospective cohort studies with higher methodological rigor, particularly in understudied populations such as children and adolescents. This evidence base needs more

studies that specifically examine dietary protein intake and the risk of kidney disease; and studies that report on similar bone disease, kidney disease, and sarcopenia outcomes. Future studies should prioritize prospective cohort designs that feature multiple assessments of dietary protein intake over time. This would allow for a nuanced understanding of how changes in protein intake relate to health outcomes by capturing dietary variations and their longitudinal effects.

Investigators should focus on standardizing the dietary protein intake levels used as interventions and comparators alongside longer intervention followup period in RCTs. Studies that address broader implications of the role of other key nutrients, overall diet quality, and dietary patterns with sound methodological rigor are needed. Future research should focus on treating sarcopenia as a distinct endpoint. Establishing a consensus on the definition of sarcopenia is crucial to driving forward research in this area.

Conclusions

Studies conducted since 2000 on the association between dietary protein intake and the risks of bone disease, kidney disease, and sarcopenia have yielded unclear findings. Nevertheless, these inconclusive results do not negate the potential role of dietary protein intake on the risk of these chronic conditions. Reasons for lack of clarity include limitations in the original research, lack of focus on crucial groups such as children and adolescents for understanding bone health from a young age, variations in the amounts of protein intake analyzed in randomized controlled trials, and inconsistency in outcomes measured across different studies. Our review highlights the important need for more rigorous, generalizable, long-term, and high quality studies to enhance the current evidence base and more effectively evaluate the impact of dietary protein intake on the risk of these chronic conditions.

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Abbreviations and Acronyms

A	Actual dietary protein intake
aBW	Adjusted Body Weight
AHRQ	Agency for Healthcare Research and Quality
AI	Adequate Intake
AMDR	Acceptable Macronutrient Distribution Range
AMSTAR	Assessment of Multiple Systematic Reviews
ASM	Appendicular Skeletal Muscle Mass
ASMi	Appendicular Skeletal Muscle Index
BAP	Bone Alkaline Phosphatase
BIA	Bioelectrical Impedance Analysis
BMC	Bone Mineral Content
BMD	Bone Mineral Density
BW	Body Wight
C	Control
CI	Confidence Interval
CKD	Chronic Kidney Disease
CT	Computerized Tomography
CTX	C-terminal Peptide of Collagen
D	Day
DXA	Dual-Energy X-ray Absorptiometry
DRI	Dietary Reference Intakes
EAR	Estimated Average Requirement
eGFR	Estimated Glomerular Filtration Rate
EPC	Evidence-based Practice Center
ESRD	End-Stage Renal Disease
EWGSOP2	European Working Group for Sarcopenia in Older People
FFM	Fat Free Mass
G	Gram
G/CM^2	Gram Per Centimeter Squared
GI	Gastrointestinal
HDI	Human Development Index
HHS	Health and Human Services
HP	High Protein
IQR	Inter Quartile Range
I	Interventions
I	Intended dietary protein intake

KG	Kilogram’
KG/M^2	Kilograms Per Square Meter
KQ	Key Question
LSM	Least Square Mean
LTC	Long-term Care
M	Meter
MRI	Magnetic Resonance Imaging
n	Number
N	Newtons
NA	Not Applicable
NP	Normal Protein
NR	Not Reported
NASEM	National Academies of Sciences, Engineering, and Medicine
NESR	Nutrition Evidence Systematic Review
NIH	National Institutes of Health
Nm	Newton Meters
Non-RCT	Non-randomized Controlled Trial
OC	Osteocalcin
P1NP	Procollagen Type 1 N-terminal Propeptide
PICOTS	Population, Intervention, Comparison, Outcomes and Timing
PRISMA	Preferred Reporting Items for Systematic Review and Meta-Analysis
RCT	Randomized Controlled Trial
RDA	Recommended Dietary Allowance
RM	Repetition Maximum
RoB	Risk of Bias
ROBINS-E	Risk of Bias in Non-randomized Studies of Exposure
ROBINS-I	Risk of Bias in Non-randomized Studies of Intervention
S	Seconds
SD	Standard Deviation
SE	Standard Error
SEM	Standard Error of the Mean
SMD	Standardized Mean Difference
SPPB	Short Physical Performance Battery
SOE	Strength of Evidence
SRDR	Systematic Review Data Repository
TBBA	Total Body Bone Area
TRAP	Tartrate Resistant Alkaline Phosphatase
TUG	Timed Up-and-Go

UL	Upper Level
U/L	Units Per Liter
US	United States
USDA	United States Department of Agriculture
y	Years
µg/L	Micro Grams Per Liter