Evidence-based Practice Center Systematic Review Protocol
Project Title: *The Effect of Protein Intake on Health*

Publication Date: June 2, 2023
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(Amendments Details–see Section VI)

I. Background and Objectives for the Systematic Review

Protein is a major macronutrient essential for optimal growth, development, function, and maintenance of human health.[1] Dietary Reference Intakes (DRIs) are a set of quantitative reference values for essential nutrients (including protein) developed jointly for the United States (U.S.) and Canada. DRIs for protein were first published in 2005[2] and have not been updated since. Some nutrition experts consider protein DRIs for adults and children somewhat lacking because they were largely derived from studies that examined primarily healthy young men,[2] and there has been continuous emphasis on the relationship between protein intake and health, including chronic disease risk. Overall, the planning for new DRIs update include efforts to incorporate evidence on chronic disease in developing DRI values to include a new category of values specific to chronic disease risk reduction.[3] The first and only DRI update where evidence on chronic disease has been applied is in the development of a new reference value for chronic disease risk reduction (CDRR) in the 2019 updated review of DRIs for sodium and potassium.[4,5] Bone disease, kidney disease, and sarcopenia are thought to be some of the important chronic conditions of relevance to protein intake and chronic disease risk, and have been extensively researched for decades.

Although dietary protein is fundamental for optimal bone health across all life stages, protein consumption has been, somewhat paradoxically, described as both beneficial and damaging to bone health. Protein's bone benefits are thought to stem from 1) its ability to increase secretion of insulin-like growth factor 1(IGF 1), a growth hormone that promotes normal growth in bones and tissues,[6] and 2) intestinal calcium

absorption, which promotes bone mineralization, a process by which bone matrix becomes filled with calcium.[7] However, high protein intake has also been suggested as damaging to bone health based on in vitro study showing increased osteoclast activity and body acidity, which predicts subsequent bone demineralization.[8] While significant attention has been given to the question of how much protein people should eat for optimal bone health, recommendations vary widely. Several systematic reviews have suggested bone health benefits from increased protein intake,[9-12] but other investigators have reported no effect of protein intake on bone health[13] along with mixed association between different sources of animal-based dietary protein and risk of rheumatoid arthritis.[14]

Concerns also exist around dietary protein intake and long-term kidney health. Some researchers have suggested that dietary protein intake may result in kidney disease through increased glomerular filtration rate (GFR), which impairs kidney function and can result in eventual kidney damage and failure.[15,16] However, some investigators have argued otherwise, stating that the capacity to increase GFR in response to protein feeding is a normal adaptive function of the kidney, and that this adaptive response does not present a risk factor for the development of chronic kidney disease (CKD).[17,18] Recent systematic reviews examining the effect of dietary protein intake on kidney health have come to differing conclusions, including no effect[19] and insufficient evidence.[20]

Sarcopenia is a progressive decline in muscle mass and strength with aging.[21] Aging muscle tissue may require more protein to help repair and maintain itself,[22,23] and failure to properly stimulate muscle protein can lead to loss of muscle mass, reduced strength, and poorer physical function.[24] In general, healthy people between the ages of 20 and 30 experience negligible loss of muscle mass annually, but that loss increases between the ages of 30 and 50 and accelerates even further after age 50.[25] Although sarcopenia tends to occur gradually in men,[26] women experience a sharp decline in muscle mass after menopause.[27] Loss in muscle mass plays an important role in the most common chronic conditions (such as heart disease and cancer),[28] and progressive sarcopenia is central to the development of frailty, increased fall risk, physical disabilities, and fractures.[28] While current systematic reviews do suggest a positive

association between dietary protein intake and prevention of sarcopenia,[29,30] other investigators have stated that the literature on the topic is inconclusive.[31]

Ultimately, evidence on dietary protein's causal role, if any, in bone disease, kidney disease and sarcopenia need to be thoroughly examined as part of the development of new DRIs for protein intake.

Purpose of the Review

This systematic review will examine the Key Questions (KQs) as outlined below. The review is aimed to provide an up-to-date and comprehensive key summary of evidence for dietary protein intake and risk of bone disease, kidney disease, and sarcopenia for a future U.S. and Canadian government protein DRI panel review of DRIs for an optimal protein intake.

II. The 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?

III. Methods

A. Criteria for Inclusion/Exclusion of Studies in the Review

Studies will be included in the review based on the study-specific inclusion and exclusion criteria described in Table 1.

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 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 Age of participants (at intervention or exposure): ○ 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 kidney disease and/or sarcopenia 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 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	No specification on the amount of protein intake (e.g., only the type of protein or source of protein reported) Assessment of %AMDR, but no description of the entire macronutrient distribution of the diet (i.e., examination a single macronutrient in relation to outcomes) Protein intake via infusions (rather than the GI tract) 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: ○ Osteoporosis	

	○ Osteopenia ○ Fracture ○ Bone mass including bone mineral density, bone mineral content	
Outcomes KQ2	Kidney outcomes: ○ 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	
Outcomes KQ3	Aging associated sarcopenia and its diagnostic indicators, including but not limited to muscle mass, muscle function, muscle strength	
Timing KQ1-3	All duration and follow up	
Setting KQ1-3	All settings	
Study design KQ1-3	● Randomized controlled trials (RCTs) ● Non-randomized controlled trials, 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)	
Geographic Location KQ1-3	Locations with food products or dietary supplements widely available to U.S. consumers, including those rated very high on the Human Development Index	
Study size KQ1-3		Studies with N < 50 participants (for RCTs - 25 participants analyzed per study arm), and without power calculation
Publication date KQ1-3	2000 to present	
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; GI=gastrointestinal; U.S.=United States; KQ=key question; CKD=chronic kidney disease; eGFR=estimated glomerular filtration rate; RCT=randomized controlled trial

B. Searching for the Evidence: Literature Search Strategies for Identification of Relevant Studies to Answer the Key Questions

Our librarian team member will develop multiple search strategies for different relevant databases, including Medline, EMBASE, AGRICOLA, ADA Evidence Library, Scopus, and Science Citation Index Expanded (Web of Science), incorporating vocabulary and natural language relevant to the KQs (Appendix A). We will review and agree on the search strategies through a consensus by the team members. Searches will be conducted from 2000 to present to capture all relevant published literature since the current DRIs for protein were established in 2005. We will also use previously published reviews to confirm search algorithm adequacy.

Search results will be downloaded to EndNote X9 and screened in PICO Portal software (www.picoportal.net).[32] 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 those citations most likely to be eligible. Two independent investigators will screen titles and abstracts of results using predefined criteria. As the machine learning system is trained, we will move to one screener when we reach a 90% recall rate of citations eligible for full-text screen. We will stop screening citations remaining past a 95% recall rate of citations eligible for full-text screen. Two independent investigators will perform full-text screening to determine if inclusion criteria are met, using the same online system. Differences in screening decisions will be resolved by consultation and consensus with a third investigator. Additionally, during screening, we will tag studies in PICO portal (using certain identifiers, such as small sample size) to help us sort the literature and track study characteristics that may require revisiting based on review findings. Multiple publications relating to the same study will be mapped to unique study.

We will supplement our bibliographic database searches with citation searching of relevant systematic reviews and original research. Additionally, we will search ClinicalTrials.gov to identify completed and ongoing studies. Literature will also be solicited 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. Information from these sources will be used to assess publication and reporting bias and inform future research needs.

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

C. Data Abstraction and Data Management

Studies meeting inclusion criteria will be distributed among investigators for data extraction. These data fields will include author, year of publication, sponsorship, setting, study design, population (including sample size, age, gender/sex, race/ethnicity, socioeconomic status, physical activity level, health status, type of diet (e.g., vegan, vegetarian), energy balance status (i.e., studies that examine protein intake in the context of energy imbalance states), intervention and control characteristics, comparison, outcomes cited, results of outcomes and adverse effects, intervention duration and study followup, and risk of bias elements.

Relevant data will be extracted into extraction forms created in Microsoft Excel. Data will be extracted to evidence and outcomes tables by one investigator and reviewed and verified for accuracy by a second investigator. We will rely on studies with high methodological rigor. We will not extract data from high risk of bias studies.

D. Assessment of Methodological Risk of Bias of Individual Studies

Risk of bias of eligible RCTs by outcomes will be rated using the Cochrane Risk of Bias tool 2.0[33] 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. For non-randomized controlled trials (including quasi-experimental and controlled before-and-after studies), risk of bias by outcomes will be rated using the Risk Of Bias In Non-randomized Studies - of Interventions (ROBINS-I) tool[34] 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 in classification of interventions; 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, or high (serious or critical).

For observational studies (including prospective cohort studies with or without comparison group and nested case-control studies), risk of bias by outcomes will be rated using the Risk Of Bias In Non-randomized Studies - of Exposure (ROBINS-E) tool[35] 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, or high (serious or critical).

One investigator will independently assess risk of bias for eligible studies by outcome; a second investigator will review each risk of bias assessment. Investigators will consult to reconcile any discrepancies in risk of bias assessments. For RCTs, the overall risk of bias assessments for each study outcome will be classified as low risk, moderate risk, or high risk. For non-randomized controlled trials and observational studies, the overall risk of bias assessments for each study outcome will be classified as low, moderate, or high (serious or critical). Overall risk of bias assessments will be based upon the collective risk of bias across components and confidence that the study results for given outcomes are believable given the study's limitations.

E. Data Synthesis

Results will be organized first by key question. The results will be organized by life stage, intervention, and then by targeted outcome. We will first describe the results in evidence tables, and then assess the clinical and methodological heterogeneity (including study design) and variation in effect size to determine appropriateness of pooling data for each unique comparison with meta-analysis.[36] When meta-analysis is not possible, we will provide a qualitative synthesis. When meta-analysis is possible, we will synthesize data using a Hartung, Knapp, Sidik, and Jonkman (HKSJ)[37] random effects model in Comprehensive Meta-Analysis version 3 (Biostat, Englewood, New Jersey) or R.[38] We will calculate risk ratios (RR) and absolute risk differences (RD) with the corresponding

95 percent confidence intervals (CI) for binary outcomes and weighted mean differences (WMD) and/or standardized mean differences (SMD) with the corresponding 95 percent CIs for continuous outcomes if combining similar outcomes measured with different instruments. The HKSJ method is more conservative than the commonly used DerSimonian-Laird approach which may result in overly narrow confidence intervals that can lead to Type 1 error.[37]

We will identify heterogeneity (inconsistency) through visual inspection of the forest plots to assess the amount of overlap of CIs, and the I^2 statistic, which quantifies inconsistency across studies to assess the impact of heterogeneity on the meta-analysis;[39] we will interpret the I^2 statistic as follows.[40]

- 0% to 40%: may not be important
- 30% to 60%: may indicate moderate heterogeneity
- 50% to 90%: may indicate substantial heterogeneity
- 75% to 100%: considerable heterogeneity

When we find heterogeneity, we will attempt to determine possible reasons for it by examining individual study and subgroup characteristics.

F. Grading the Evidence Quality for Major Comparisons and Outcomes

The overall strength of evidence for outcomes within each comparison will be evaluated based on five required domains: (1) study limitations (risk of bias); (2) directness (single, direct link between intervention and outcome); (3) consistency (similarity of effect direction and size); (4) precision (degree of certainty around an estimate); and (5) reporting bias.[41] For each comparison, one investigator will rate the strength quality of evidence for each outcome as high, moderate, low or insufficient. These ratings will then be reviewed by a second investigator and confirmed by team consensus. An assessment of high indicates strong confidence that the estimate of effect lies close to true effect and that there are few or no deficiencies in the body of evidence such that findings are believed to be stable. An assessment of insufficient indicates no evidence was located, and we were unable to estimate an effect or had no confidence in the estimate of effect in the body of evidence, if one exists, and precludes the ability to draw a judgment.

For each comparison, we will present a summary of the evidence for the outcomes in a Summary of Findings table. If meta-analysis is not possible, we will present results in a narrative Summary of Findings table.

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V. Definition of Terms

Abbreviations:

AHRQ	Agency for Healthcare Research and Quality
AMDR	Acceptable Macronutrient Distribution Range
CDRR	Chronic Disease Risk Reduction
CKD	Chronic Kidney Disease
CI	Confidence Interval
DRI	Dietary Reference Intake
EPC	Evidence-based Practice Center
GFR	Glomerular Filtration Rate
GI	Gastrointestinal
GRADE	Grading of Recommendations Assessment, Development and Evaluation
HKSJ	Hartung, Knapp, Sidik, and Jonkman
IGF 1	Insulin-like Growth Factor 1
KQ	Key Question
PICOTS	Population, Intervention, Comparator, Outcomes, Timing, and Study design/setting
RCT	Randomized Controlled Trial
RR	Relative Risk
RoB	Risk of Bias
TOO	Task Order Officer

U.S. United States

WMD Weighted Mean Difference

VI. Summary of Protocol Amendments

Date	Section	Original Protocol	Revised Protocol	Rationale
11/17/23	Table 1: Inclusion/Exclusion Criteria by Population, Intervention, Comparator, Outcome, Timing, Setting/Study Design (PICOTS) Geographic Location KQ1-3	Inclusion criteria: Locations with food products or dietary supplements widely available to U.S. consumers, including those rated very high on the Human Development Index	Inclusion criteria: Locations with food products or dietary supplements widely available to U.S. consumers, including those rated high or very high on the Human Development Index	Including studies that were conducted in countries rated high and very high HDI allows for a more expansive body of literature to be considered and may provide greater evidence to answer the key questions of this systematic review.
11/17/23	Table 1: Inclusion/Exclusion Criteria by Population, Intervention, Comparator, Outcome, Timing, Setting/Study Design (PICOTS) Interventions KQ1-3	Exclusion criteria: No specification on the amount of protein intake (e.g., only the type of protein or source of protein reported) Assessment of %AMDR, but no description of the entire macronutrient distribution of the diet (i.e., examination a single macronutrient in relation to outcomes) Protein intake via infusions (rather than the GI tract) Food products or dietary supplements not widely available to U.S. consumers Protein intake evaluated with exercise	Exclusion criteria: No specification on the amount of protein intake (e.g., only the type of protein or source of protein reported) Protein intake via infusions (rather than the GI tract) Food products or dietary supplements not widely available to U.S. consumers Protein intake evaluated with exercise	This systematic review focuses on dietary protein; and a report on only the % calories from protein in the included studies is sufficient for our findings.

11/29/23	Table 1: Inclusion/Exclusion Criteria by Population, Intervention, Comparator, Outcome, Timing, Setting/Study Design (PICOTS) Interventions KQ1-3	Inclusion criteria: Total dietary protein intake from food, beverages, and dietary supplements	Inclusion criteria: Total dietary protein intake from food, beverages, and dietary supplements Assessment of % AMDR for protein with or without the % from the other macronutrients (carbohydrate and fat)	This systematic review focuses on dietary protein; and a report on only the % calories from protein in the included studies is sufficient for our findings.
11/28/23	Table 1: Inclusion/Exclusion Criteria by Population, Intervention, Comparator, Outcome, Timing, Setting/Study Design (PICOTS) Outcomes KQ1	Inclusion criteria: Bone outcomes: o Osteoporosis o Osteopenia o Fracture o Bone mass including bone mineral density, bone mineral content	Inclusion criteria: Bone outcomes including but not limited to: o Osteoporosis o Osteopenia o Fracture o Bone mass including bone mineral density, bone mineral content	For a comprehensive systematic review, all relevant bone outcomes identified in the literature set will need to be covered.
11/28/23	Table 1: Inclusion/Exclusion Criteria by Population, Intervention, Comparator, Outcome, Timing, Setting/Study Design (PICOTS) Outcomes KQ2	Inclusion criteria: Kidney outcomes: o Incidence of kidney stones or ureteral stones o Incidence of CKD (including evaluations from estimated glomerular filtration (eGFR) rate with or without a parameter for race) o Kidney insufficiency	Inclusion criteria: Kidney outcomes including but not limited to: o Incidence of kidney stones or ureteral stones o Incidence of CKD (including evaluations from estimated glomerular filtration (eGFR) rate with or without a parameter for race) o Kidney insufficiency	For a comprehensive systematic review, all relevant kidney outcomes identified in the literature set will need to be covered.
11/28/23	III Methods. B. Searching for the Evidence: Literature Search	Our librarian team member will develop multiple	Our librarian team member will develop multiple search strategies	ADA Evidence Library was removed because it is not a bibliographic

	Strategies for Identification of Relevant Studies to Answer the Key Question	search strategies for different relevant databases, including Medline, EMBASE, AGRICOLA, ADA Evidence Library, Scopus and Science Citation Index Expanded (Web of Science), incorporating vocabulary and natural language relevant to the KQs (Appendix A).	for different relevant databases, including Medline, EMBASE, AGRICOLA, and Scopus, incorporating vocabulary and natural language relevant to the KQs (Appendix A).	database. Science Citation Index Expanded (Web of Science) was removed because Scopus was searched, which is a larger, more comprehensive database that is multidisciplinary in nature and covers a broad number of subjects. Additionally, including 4 bibliographic databases is typically considered a satisfactory number for a systematic review.

VII. Review of Key Questions

The Joint Canada-US Dietary Reference Intakes Working Group prioritized areas for systematic review and developed the questions for the systematic review. AHRQ and Partners (HHS and USDA) finalized the key questions. The EPC confirmed the key questions with input from AHRQ and Partners to ensure that the key questions are specific and relevant.

VIII. Technical Experts

Technical Experts constitute a multi-disciplinary group of clinical, content, and methodological experts who provide input in defining populations, interventions, comparisons, or outcomes and identify particular studies or databases to search. They are selected to provide broad expertise and perspectives specific to the topic under development. Divergent and conflicting opinions are common and perceived as health scientific discourse that results in a thoughtful, relevant systematic review. Therefore, study questions, design, and methodological approaches do not necessarily represent the views of individual technical and content experts. Technical Experts provide information to the EPC to identify literature search strategies and recommend approaches to specific

issues as requested by the EPC. Technical Experts do not do analysis of any kind nor do they contribute to the writing of the report. They have not reviewed the report, except as given the opportunity to do so through the peer or public review mechanism.

Technical Experts must disclose any financial conflicts of interest greater than $10,000 and any other relevant business or professional conflicts of interest. Because of their unique clinical or content expertise, individuals are invited to serve as Technical Experts and those who present with potential conflicts may be retained. The TOO and the EPC work to balance, manage, or mitigate any potential conflicts of interest identified.

IX. Peer Reviewers

Peer reviewers are invited to provide written comments on the draft report based on their clinical, content, or methodological expertise. The EPC considers all peer review comments on the draft report in preparation of the final report. Peer reviewers do not participate in writing or editing of the final report or other products. The final report does not necessarily represent the views of individual reviewers. The EPC will complete a disposition of all peer review comments. The disposition of comments for systematic reviews and technical briefs will be published three months after the publication of the evidence report.

Potential Peer Reviewers must disclose any financial conflicts of interest greater than $10,000 and any other relevant business or professional conflicts of interest. Invited Peer Reviewers may not have any financial conflict of interest greater than $10,000. Peer reviewers who disclose potential business or professional conflicts of interest may submit comments on draft reports through the public comment mechanism.

X. EPC Team Disclosures

EPC core team members must disclose any financial conflicts of interest greater than $1,000 and any other relevant business or professional conflicts of interest. Related financial conflicts of interest that cumulatively total greater than $1,000 will usually disqualify EPC core team investigators.

XI. Role of the Funder

This project was funded under Contract No. 75Q80120D00008 from the Agency for Healthcare Research and Quality, U.S. Department of Health and Human Services. The Task Order Officer reviewed contract deliverables for adherence to contract requirements and quality. The authors of this report are responsible for its content. Statements in the report should not be construed as endorsement by the Agency for Healthcare Research and Quality or the U.S. Department of Health and Human Services.

Appendix A: Search Strategy

Database: Ovid MEDLINE(R) ALL <1946 to April 18, 2023>

Search Strategy:

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1 exp Dietary Proteins/ or Diet, High-Protein/ or diet, high-protein low-carbohydrate/ or (protein* adj3 (ate or animal? or bean? or consume* or consumption or dairy or diet* or eat or eating or egg? or fed or feed or fish or food or foods or fruit? or grain? or high* or increase* or intake* or meat? or milk or nut? or nutrition or nutrient* or pea or peas or plant? or poultry or recommend* or soy? or supplement* or vegan or vegetable? or whey or yolk?)).ti,ab. 351405

2 "Bone and Bones"/ or Bone Density/ or bone diseases/ or bone diseases, metabolic/ or bone demineralization, pathologic/ or bone resorption/ or Fractures, Bone/ or (bone disease? or bone densit* or bone demineralization or bone health or bone mass or bone resorption or fracture* or osteoporosis or osteopenia).ti,ab. 526554

3 kidney calculi/ or Kidney Diseases/ or kidney failure, chronic/ or nephrolithiasis/ or renal insufficiency/ or renal insufficiency, chronic/ or ureterolithiasis/ or ureteral calculi/ or (kidney disease? or kidney function or kidney stone? or nephrolithiasis or ureteral calculi or ureteral stone? or ureterolithiasis or renal calculi or renal disease? or renal function or renal insufficiency).ti,ab. 426914

4 Muscular Atrophy/ or exp Muscle Strength/ or muscle weakness/ or sarcopenia/ or (muscle adj3 (atrophy or loss or mass or strength or wasting or weak* or sarcopenia)).ti,ab. 131084

5 or/2-4 1064075

6 1 and 517642

7 ((randomized controlled trial or controlled clinical trial).pt. or randomi?ed.ti,ab. or placebo.ti,ab. or randomly.ti,ab. or trial.ti,ab. or groups.ti,ab.) not (animals/ not humans/) 3196210

8 clinical trial/ or pragmatic clinical trial/ or case-control studies/ or cohort studies/ or prospective studies/ or controlled before-after studies/ or (before-after or between group* or nested case-control* or prospective or quasi-experimental or risk*).mp. 4785988

9 7 or 8 6649176

10 6 and 97113

11 limit 10 to (english language and yr="2000 -Current") 5748

12 Animal Feed/ or Diet/ve or exp Observational Study, Veterinary/ or exp Randomized Controlled Trial, Veterinary/ or (bovine or broiler* or bulls or calf or calves or chicken or chickens or cattle or cow or cows or dog or dogs or fingerlings or hens or mice or mouse or monkey* or murine or pig or piglets or pigs or rat or rats or sow or sows or swine).ti. 2107822

13 11 not 12 5437

14	comment/ or letter/ or editorial/	2151008
15	13 not 14	**5404**