



Evidence-Based Practice Center Systematic Review Protocol

Project Title: Evaluation of Dietary Protein Intake Requirements

I. Background and Objectives for the Systematic Review

Protein, a major macronutrient, is essential for optimal growth, development, function, and maintenance of human health.¹ Protein is critical for building and developing bone and muscle, required for locomotion and strength.²⁻⁴ In addition, protein serves many other functions including providing structural elements to cells/tissues, transporting nutrients, and comprising antibodies and cytokines that aid in the immune response.^{5,6} Protein is made up of various amino acids, 9 indispensable (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) and 11 dispensable (alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine, and tyrosine), which are characterized based on the body's ability to produce them.⁷ Protein-rich foods include meat, poultry, seafood, eggs, beans, legumes, and nuts. If protein intake is inadequate, it can lead to detrimental health effects.⁸ Notably, the 2020–2025 Dietary Guidelines for Americans do not list protein as a nutrient of concern based on the fact that most Americans consume enough already.⁹

The minimum amount of protein and amino acids required for health are described by the dietary reference intakes (DRIs), a set of reference values established for nutrients.¹⁰ The RDA, adjusted by life stage, is intended to cover minimum protein and amino acids needs for 97.5% of the healthy population.¹¹ Generally, a higher RDA is required during vital periods of growth and development such as infancy (older infants (7-12 mo) 1.0g/kg/d), childhood through adolescents (1-18 years, 0.85-1.0 g/kg/d), and pregnancy and lactation (1.1-1.3 g/kg/d).¹⁰ Protein RDAs decrease slightly to 0.8 g/kg/d for adults (19 to >70 years). A similar pattern can also be observed for individual amino acid intake requirements.

Importantly, the DRIs for protein and amino acids were published in 2005, and have not been updated for nearly 2 decades. Moreover, some nutrition experts consider DRIs for adults and children somewhat arbitrary because they were largely derived from studies that examined primarily healthy young men.¹¹ Over the last 20 years, novel scientific research using more advanced technology and methodology has been published on the optimal protein and amino acid requirements across the life stages. Current DRIs were established based on analysis of available nitrogen balance studies, which tend to underestimate protein requirements.¹²⁻¹⁴ More recently, several studies using the indicator amino acid oxidation (IAAO) method found requirements for protein to be higher than current EAR and RDA values for children (6-11 years), pregnant women, young men, and adults >65.¹⁵⁻¹⁹ For example, Stephens et al.¹⁶ observed a need for 39 percent and 73 percent higher protein intake in early and late stage gestation than current EAR recommendations. Ultimately, as new evidence is available on optimal protein intake requirements for health across life stages, re-evaluation of the average daily dietary protein and individual amino acid intake requirements is warranted.

Purpose of the Review

This systematic review will examine the Key Questions (KQs) as outlined below. The review seeks to provide an up-to-date and comprehensive key summary of the evidence for protein and amino acid requirements for a future U.S. and Canadian government DRI panel review of DRIs for optimal protein and amino acid intake.

II. Key Questions

Key Question 1: What is the average daily dietary protein intake requirements of apparently healthy individuals by life stage and sex?

Key Question 2: What is the average daily dietary individual indispensable amino acid intake requirements of apparently healthy individuals by life stage and sex?

Please see Table 1 for Inclusion and Exclusion criteria by PICOTS

III. Methods

A. Criteria for Inclusion/Exclusion of Studies

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

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

Element	Inclusion Criteria	Exclusion Criteria
<p>Population KQ1 & 2</p>	<ul style="list-style-type: none"> ● Participants who are healthy and/or have chronic diseases or chronic disease risk factors, including those with obesity ● Studies that enroll some participants diagnosed with a disease or hospitalized or in a long-term care facility with an illness or injury ● Studies that enroll some participants diagnosed with a disease or with the health outcome of interest ● Participants who are pregnant and lactating ● Age at intervention exposure: <ul style="list-style-type: none"> ○ Infants, children, adolescents (0-18 years) ○ Adults (19-64) ○ Older adults (65 years and older) 	<ul style="list-style-type: none"> ● Studies that exclusively enroll participants diagnosed with a disease, hospitalized, or in a long-term care facility with an illness or injury (for this criterion, studies that exclusively enroll participants with obesity will not be excluded) ● Studies that aim to treat participants who have already been diagnosed with the outcome of interest (except weight loss interventions in overweight or obese subjects) ● Studies that exclusively enroll undernourished participants ● Studies that exclusively enroll participants with a baseline diet deficient in protein ● Studies that exclusively enroll preterm infants ● Studies that exclusively enroll post-bariatric surgery subjects ● Studies that exclusively recruit elite athletes ● Participants with existing conditions that clearly are known to alter nutrient metabolism or requirements, or those being treated with medications that alter nutrient metabolism
<p>Interventions KQ1 & 2</p>	<ul style="list-style-type: none"> ● Total daily protein intake level ● Total daily intake of indispensable AAs (Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, 	<ul style="list-style-type: none"> ● Studies that only assess protein intake via infusions (rather than the GI tract) ● Studies that examine food products or dietary supplements not widely available to U.S.

	Valine)	<p>consumers</p> <ul style="list-style-type: none"> Multi-component interventions that do not isolate the effect or association of protein (including protein and exercise combinations)
Comparison KQ1 & 2	<ul style="list-style-type: none"> Different total daily protein intake level Different total daily intake of indispensable AAs 	<ul style="list-style-type: none"> No comparator
Outcomes KQ1	<p>Total protein requirement* as defined by the following indicators or criterion of adequacy, including but not limited to:</p> <ul style="list-style-type: none"> Nitrogen balance method Factorial method Indicator AA oxidation method Mean protein intake of infants fed principally human milk (0-6 months) Mean protein content of human milk (0-6 months) Body composition (lean mass) Linear growth for infants, children, adolescents (0-18 years) Activities of daily living for older adults (65 years and older) 	
Outcomes KQ2	<p>Indispensable AA requirement* as defined by the following indicators of adequacy, including but not limited to:</p> <ul style="list-style-type: none"> Plasma AA response method Direct AA oxidation method 24-hour AA balance 	

	<p>method</p> <ul style="list-style-type: none"> • Indicator AA oxidation method • Mean AA intake of infants fed principally human milk (0-6 months) • Mean protein content of human milk (0-6 months) 	
Timing KQ1 & 2	<ul style="list-style-type: none"> • All duration and follow up 	
Setting KQ1 & 2	<ul style="list-style-type: none"> • All settings 	
Study Design KQ1 & 2	<ul style="list-style-type: none"> • Randomized controlled trials • Non-randomized controlled trials, including quasi-experimental and controlled before-and-after studies • Prospective cohort studies • Nested case-control studies 	<ul style="list-style-type: none"> • International and government reports • Narrative reviews • Systematic reviews, meta-analyses, umbrella reviews, scoping reviews • Uncontrolled trials • Case-control studies • Uncontrolled before-and-after studies • Retrospective cohort studies
Study Size KQ1 & 2		<ul style="list-style-type: none"> • N < 6 participants and without power for crossover studies • Other studies with N < 50 participants (for RCTs - 25 participants analyzed per study arm), and without power calculations
Language KQ1 & 2	<ul style="list-style-type: none"> • English only (due to resource limitations) 	
Geographic Location KQ1 & 2	<ul style="list-style-type: none"> • Locations with food products or dietary supplements widely available to U.S. consumers, including those 	

	rated very high on the Human Development Index	
Publication Date KQ1 & 2	<ul style="list-style-type: none"> • 2000 to present 	
Publication Status KQ1 & 2	<ul style="list-style-type: none"> • Articles published in peer-reviewed journals 	<ul style="list-style-type: none"> • 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)

*Requirement is defined as the lowest daily intake value for a nutrient that will meet the need as defined by a specified indicator or criterion of adequacy, of apparently healthy individuals

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 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 DRIs for protein and amino acids 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).²⁰ 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.

Throughout the screening process to ensure consistency in interpreting eligibility criteria, team members will meet regularly to discuss questions and issues arising from screening. 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 a 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), protein source, energy balance status (i.e., studies that examine protein intake in the context of energy imbalance states, such as a calorie restricted diet), 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 randomized controlled trials (RCTs) by outcomes will be rated using the Cochrane Risk of Bias tool 2.0.²¹ Risk will be assessed as low, moderate,

or high 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 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.²² Risk will be assessed 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 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) tool²³ 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 to 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 a given outcome are believable given the study's limitations.

E. Data Synthesis

Results will be organized first by key questions followed 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 and outcome with meta-analysis.²⁴ 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)²⁵ random effects model in Comprehensive Meta-Analysis version 3 (Biostat, Englewood, New Jersey) or R.²⁶ 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 and can lead to Type I error.²⁵

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;²⁷ we will interpret the I^2 statistic as described below.²⁸ When we find heterogeneity, we will attempt to determine possible reasons for it by examining individual study and subgroup characteristics.

- 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

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.

F. Grading the Evidence Quality for Major Comparisons and Outcomes

The overall strength of evidence for outcomes for 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.²⁹ For each comparison, one investigator will rate the strength 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; the body of evidence, if one exists, precludes the ability to draw a judgment.

IV. References

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V. Definition of Terms

Abbreviations:

AA	Amino Acid
AHRQ	Agency for Healthcare Research and Quality
CI	Confidence intervals

DRI	Dietary Reference Intake
EPC	Evidence-based Practice Center
EAR	Estimated Average Requirement
GRADE	Grading of Recommendations Assessment, Development and Evaluation
HHS	Health and Human Services
HKSJ	Hartung, Knapp, Sidik, and Jonkman Random Effects Model
IAAO	Indicator Amino Acid Oxidation
IOM	Institute of Medicine
KQ	Key Question
PICOTS	Population, Intervention, Comparator, Outcome, Timing, Setting/Study Design
RCT	Randomized Controlled Trial
RoB	Risk of Bias
RR	Risk Ratios
RD	Absolute Risk Differences
RDA	Recommended Dietary Allowance
SMD	Standardized Mean Differences
SR	Systematic Review
TOO	Task Order Officer
U.S.	United States
USDA	United States Department of Agriculture
WMD	Weighted Mean Difference

VI. Summary of Protocol Amendments

If we need to amend this protocol, we will give the date of each amendment, describe the change and provide the rationale in this section. Changes will not be incorporated into the protocol. See example table below:

Date	Section	Original Protocol	Revised Protocol	Rationale
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This should be the effective date of the change in protocol.	Specify where the change would be found in the protocol.	Describe the language of the original protocol.	Describe the change in protocol.	Justify why the change will improve the report. If necessary, describe why the change does not introduce bias. Do not use justification as “because the AE/TOO/TEP/Peer reviewer told us to” but explain what the change hopes to accomplish.
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VII. Review of Key Questions

The Joint Canada-U.S. 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 healthy 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 endorsements 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 content 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 lacto-vegetarian or macronutrient? or meat? or milk or nut? or nutrition* or nutrient* or pea or peas or pescatarian or pescavegan or plant? or poultry or recommend* or soy? or supplement* or vegan or vegetable? or vegetarian or whey or yolk?)).ti,ab. 336131
 - 2 amino acids, essential/ or exp arginine/ or histidine/ or isoleucine/ or leucine/ or lysine/ or exp methionine/ or exp phenylalanine/ or exp threonine/ or tryptophan/ or exp valine/ or (arginine or histidine or isoleucine or leucine or lysine or methionine or phenylalanine or threonine or tryptophan or valine).ti,ab,kf. or (amino acid* adj3 (balance* or content or essential or indispensable or intake or oxidation or response)).ti,ab. 587253
 - 3 1 or 2 894336
 - 4 nutritional requirements/ or recommended dietary allowances/ or nutritional status/ or (daily intake or dietary reference intake* or nutrition* require* or recommend* dietary allowance* or acceptable macronutrient distribution or nutrition* status).ti,ab. 110910
 - 5 3 and 413921
 - 6 (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. 3701829
 - 7 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
 - 8 6 or 7 7109069
 - 9 5 and 85965
 - 10 limit 9 to (english language and yr="2000 -Current") 4346
 - 11 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 rabbit or rabbits or rat or rats or ruminant? or sow or sows or swine).ti. 2229720
 - 12 10 not 11 3853
 - 13 comment/ or editorial/ or letter/ 2151008
 - 14 12 not 13 **3843**