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Evaluation of Dietary Protein and Amino Acid Requirements: A Systematic Review

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

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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. 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 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.

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The authors gratefully acknowledge the following individuals for their contributions to this project: [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.]

Evaluation of Dietary Protein and Amino Acid Requirements: A Systematic Review

Structured Abstract

Objective: This review assesses the evidence on requirements for average daily dietary protein and individual indispensable amino acid intake for healthy individuals by life stage and sex. The results will inform future updates to the Dietary Reference Intakes (DRI) for protein.

Data sources: We searched Medline, EMBASE, AGRICOLA, and Scopus from January 2000 through March 2024 for studies from peer-reviewed published literature and supplemented with citation searching of relevant systematic reviews and original research.

Review methods: Two reviewers independently screened titles, abstracts, and full-text publications. We included randomized and nonrandomized controlled trials, prospective cohort, and nested case-control studies that enrolled infants through older adults and investigated total protein and amino acid requirements using a variety of methods (i.e., nitrogen balance, indicator amino acid oxidation, etc.) We extracted data, assessed risk of bias, synthesized results from low to moderate risk of bias studies in a narrative manner, and evaluated the strength of the evidence supporting the conclusions. The protocol was registered on PROSPERO (CRD42023446618).

Results: We identified 11,344 studies of which 68 articles reporting on 66 unique studies were eligible for the review and 45 studies were assessed as low or moderate risk of bias. For most populations, one or two studies of higher methodological rigor were available for both protein and amino acid requirements. For infants, six studies examined requirements for isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. For children and adolescents, seven studies examined requirements for protein, lysine, methionine, phenylalanine, and total branched chain amino acids. For pregnant people, four studies examined requirements for protein, lysine, and phenylalanine. For adults aged 19-50 years, 16 studies examined requirements for protein, leucine, lysine, methionine, phenylalanine, threonine, and valine and for adults aged 51->70 years, six studies examined requirements for protein, leucine, and phenylalanine. Both males and females were studied for all requirements except pregnant people (females only), valine requirement estimates for infants (males only), phenylalanine requirements for children and adolescents (not reported), and protein, leucine, methionine, phenylalanine, threonine, and valine requirements for adults aged 19-50 years (males only). Commonly used methods included indicator amino acid oxidation, 24-hour indicator amino acid oxidation, 24-hour indicator amino acid balance, and nitrogen balance.

Conclusions: Overall, evidence from January 2000 to March 2024 is inconclusive across populations to determine the average daily dietary protein and indispensable amino acid requirements.

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Executive Summary

Main Points

- Overall, evidence from January 2000 to March 2024 was inconclusive across populations to determine the average daily dietary protein and indispensable amino acid requirements across populations.
- For most populations, one or two studies of higher methodological rigor examined protein or indispensable amino acid requirement estimates. When more than two studies were available, different designs and/or methods to calculate the estimate requirements were used, making comparisons between studies difficult.
- We identified a critical need for the development of a single standardized and validated method to assess protein and amino acid requirement estimates. Future studies, using this new method, should focus on population groups including infants, children, adolescents, pregnant people, and lactating people where protein intake is essential for appropriate growth and development.

Background and Purpose

Protein is essential for the growth, development, function, and maintenance of human health.[1] Protein is a complex structure made up of various amino acids; some amino acids are considered "indispensable" because the body cannot produce them and therefore, they must be consumed in the diet. The current Dietary Reference Intakes for protein and amino acids were established in 2005.[2]

This report reviews the evidence on the average daily dietary protein and individual indispensable amino acid intake requirement of apparently healthy individuals by life stage and sex. We considered evidence published since the current DRIs were set and our findings will serve as a key reference for future updates to the DRIs for protein and amino acids.

Methods

The methods for this systematic review followed the Agency for Healthcare Research and Quality Methods Guide for Effectiveness and Comparative Effectiveness Reviews, which we describe in the full report. Our searches covered publication dates from January 2000 through March 2024. We included randomized and nonrandomized controlled trials, prospective cohort, and nested case-control studies that investigated total protein and amino acid requirements using a variety of methods (i.e., nitrogen balance, indicator amino acid oxidation, etc.). We extracted data, assessed risk of bias, narratively summarized and synthesized results, and evaluated the strength of the evidence supporting the conclusions.

For full details on the methods, see the full report [https://effectivehealthcare.ahrq.gov/products/dietary-protein-intake/protocol] of this review.

Results

We identified 11,344 studies, of which 68 articles reporting on 66 unique studies were eligible for the review and 45 studies were assessed as low or moderate risk of bias and therefore included in the synthesis of results. Overall, the evidence was insufficient to draw conclusions

for average daily dietary protein and indispensable amino acid requirements. Estimated requirements for protein and indispensable amino acids varied both by population and by method used to calculate the requirement. For most populations, one or two studies of higher methodological rigor were available for both protein and amino acid requirements. For infants, six studies examined requirements for isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine using the indicator amino acid oxidation method. Both males and females were studied for all requirements except valine where only males were studied. For children and adolescents, seven studies examined requirements for protein, lysine, methionine, phenylalanine, and total branched chain amino acids using the indicator amino acid oxidation method. Both males and females were studied for all requirements except phenylalanine, for which the sex of participants was not reported. For pregnant people, four studies examined requirements for protein, lysine, and phenylalanine using the direct amino acid oxidation and indicator amino acid oxidation methods. For adults aged 19-50 years, 16 studies examined requirements for protein, leucine, lysine, methionine, phenylalanine, threonine, and valine using the indicator amino acid oxidation, 24-hour indicator amino acid oxidation/24-hour indicator amino acid balance, nitrogen balance and plasma amino acid response methods. Only males were studied for all requirements except lysine, where one study enrolled only females. For adults aged 51->70 years, six studies examined requirements for protein, leucine, and phenylalanine using the direct amino acid oxidation, indicator amino acid oxidation, and nitrogen balance methods. Even when more than two studies were available for protein or amino acids, they used different designs and/or methods to calculate the requirement estimates, making synthesis impossible. Studies without a protein or amino acid requirement estimate (k=6) reported outcomes such as growth, nitrogen balance, leucine oxidation, phenylalanine oxidation, rate of $^{13}CO_2$ released from tracer oxidation ($F^{13}CO_2$), and 24-hour whole body lysine balance and oxidation.

Strengths and Limitations

The current systematic review has several strengths and limitations. One strength is that we captured a wide array of methodologies used to calculate protein and amino acid requirements, which expands on the previous evidence used to inform the 2005 Dietary Reference Intakes. We also included a range of populations, from infants to older adults and pregnant people. And we performed risk of bias assessments and graded the strength of evidence, which provides context about the methodological rigor and confidence in the findings of the included studies.

However, our review is limited by our decision not to capture or review evidence on the protein content of human milk, which was used in the previous Dietary Reference Intakes to determine the requirements. We made this decision to avoid duplicating other ongoing efforts by the U.S. federal government to document the nutrient composition of human milk.[3-6] Also, since our review sought to examine on the quantity of dietary protein, we did not examine how protein quality impacts requirements.

Implications and Conclusions

Our review expands on the previous evidence used to inform the 2005 Dietary Reference Intakes and offers valuable insights for future research. We found very few studies for protein or indispensable amino acids across all populations. Overall, evidence from January 2000 to March 2024 is insufficient across populations to determine the average daily dietary protein and

indispensable amino acid requirements. This field critically needs a single standardized and validated method to assess protein and amino acid requirement estimates with future studies focused specifically on life stages where protein intake is vital to growth and development.

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Chapter 1. Introduction

Importance of Protein for Health

Protein is essential for growth, development, function, and maintenance of human health. [1] Inadequate protein intake can lead to negative health effects. [2] Aside from its widely appreciated role in building and developing bone and muscle, [3-5] protein also serves many other functions including providing structural elements to cells/tissues, transporting nutrients, and containing antibodies and cytokines that support immune response. [6, 7] More information on protein intake in relation to chronic conditions can be found in the concurrent systematic review on the total dietary protein intake and related bone disease, kidney disease, and sarcopenia outcomes (https://effectivehealthcare.ahrq.gov/products/effect-protein-intake/protocol).

Protein Composition and Quality

Protein is a complex structure made up of amino acids. There are 21 genetically encoded amino acids used for protein synthesis in humans: alanine, arginine, asparagine, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, and selenocysteine. Amino acids are grouped based on the body's ability to produce them. [8, 9] Nine amino acids are considered "indispensable" (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) because the body cannot make them, and people must therefore consume these in their diets. The remaining amino acids are considered "dispensable" because the body can produce them on its own.

Protein-rich foods come from both plant and animal sources such as meat, poultry, seafood, eggs, beans, legumes, and nuts, and these sources differ based on the amount and type of protein and amino acids they contain. Quality, or how easily the body can access and use the amino acids should be considered when creating recommendations for protein and amino acid requirements. [10-12] Protein quality can be measured using the protein digestibility corrected amino acid score (PDCAAS), digestible indispensable amino acid score (DIAAS), biological value (BV), and net protein utilization (NPU) etc. [13] Protein from animal sources (i.e., eggs, milk, meat) is considered higher quality while protein from plant sources (i.e., grains, legumes, nuts) is considered lower quality due to amino acid content and digestibility. [10, 11, 13, 14]

Measurement of Protein and Amino Acid Requirements

Total protein and amino acid requirements across the lifespan can be measured in a variety of ways. In all methods, the amount of energy provided must be adequate to assess true protein requirements. [15, 16] The nitrogen balance and factorial method have been used to estimate protein requirements and the nitrogen balance method is considered to be the most valid approach. [16-18] Within the last few decades, the indicator amino acid oxidation (IAAO) technique has emerged as another method to estimate protein requirements, but this method has not been validated for this purpose. [19] IAAO, direct amino acid oxidation (DAAO), 24-h IAAO/24-h indicator amino acid balance (IAAB), and the plasma amino acid response method have been used to estimate amino acid requirements and in the 2005 DRIs, the 24-h IAAO/24-h IAAB methods were established as the most appropriate method to estimate requirements when available. [16]All

methods have inherent advantages and disadvantages in estimating both protein and amino acid requirements. [20] We briefly describe each technique below.

Nitrogen balance method

The nitrogen balance method is designed to measure nitrogen intakes and nitrogen excretions (e.g., urine, feces, and miscellaneous losses) during both fed and fasted states across multiple levels of protein intake. Nitrogen excretions are subtracted from nitrogen intakes to determine the protein amount required to achieve nitrogen equilibrium (nitrogen intake = nitrogen excretion). This represents the point at which the protein requirement is met.

Factorial method

The factorial method estimates the protein requirement by taking into consideration nitrogen estimates of the maintenance requirement, rates of protein deposition, and efficiency of protein use. [16]

IAAO and DAAO method

The IAAO method measures the oxidation and/or rate of $^{13}CO_2$ released from tracer oxidation ($F^{13}CO_2$) of the isotopically labeled indicator amino acid during the fed state across multiple protein or amino acid intakes to estimate protein or amino acid requirements. [21-23] As described by Elango et al., [22] an indicator amino acid is an indispensable amino acid with well-regulated body pools that is irreversibly oxidized. As test protein or amino acid intakes increase oxidation of the indicator amino acid decreases until the breakpoint or the point at which the protein or amino acid requirement is met. [21-23]

The DAAO method is similar to the IAAO method in which the rate of $^{13}CO_2$ released from tracer oxidation ($F^{13}CO_2$) is measured in the fed state across multiple amino acid intakes to determine the amino acid requirements. However, the test amino acid is isotopically labeled rather than an indicator amino acid and an inverse oxidation response to the IAAO method occurs. [16, 22, 23]

24-h IAAO/24-h IAAB method

Like the IAAO method, the 24-h IAAO method measures the oxidation of an indicator amino acid across multiple amino acid intakes over a 24-h period and a breakpoint is determined to estimate the amino acid requirement. [18, 22] Similarly, a breakpoint is determined using the 24-h IAAB, which measures the balance of the indicator amino acid, taking indicator amino acid oxidation and subtracting from indicator amino acid intake. [18, 22] These methods are generally performed following an adaptation period of 6 days and take into account both fed and fasted states. [24]

Plasma amino acid response method

The plasma amino acid response method assumes that once intake of the test amino acid meets the requirement, the concentration of the test amino acid will increase in the plasma. [16] However, many factors can influence changes to plasma concentrations, making this method challenging to interpret.

Growth Measurement

Growth potential is determined by genetics and other factors such as nutritional status. Amino acids are required not only for accretion of tissue but also for the production of growth hormones and the development of matrix for bone growth. Inadequate intake of protein/amino acids has been associated with short stature and failure to grow, while excessive intake in early childhood may increase adiposity and growth factors.[25-27] Therefore, determining the amount of dietary protein intake to achieve appropriate linear growth from birth through adolescence could be another approach to estimate protein requirements during these stages of life.

Protein and Amino Acid Dietary Reference Intakes

The Dietary Reference Intakes (DRIs) are a set of reference values established (by life stage and sex) for micro and macronutrients based on available scientific evidence.[28] The DRIs are made up of four reference values: the estimated average requirement (EAR), recommended dietary allowance (RDA), adequate intake (AI), and tolerable upper intake level (UL). The EAR is intended to cover the minimum nutrient requirements for 50 percent of the healthy population whereas the RDA is intended to be sufficient for 97.5 percent of the healthy population.[28] An AI is established only when an RDA cannot be determined and is an approximation or estimate of a nutrient intake assumed to be adequate. A UL is the highest possible nutrient intake likely to pose no adverse health effects.[28] Generally, for protein, a higher EAR and RDA is required for both males and females during vital periods of growth and development such as infancy, childhood, and adolescence (EAR 0.71-1.0 g/kg/d, RDA: 0.85-1.2 g/kg/d). The EAR and RDA during these stages of life are the same for both males and females except from age 14 to 18 years, where the EAR for males is slightly higher (EAR: 0.73 g/kg/d and 0.71 g/kg/d).[16] A higher EAR and RDA is also observed during pregnancy and lactation (EAR: 0.88-1.05 g/kg/d, RDA: 1.1-1.3 g/kg/d).[16] Protein EARs and RDAs decrease slightly for adults but are the same for males and females (19->70 years, EAR: 0.66 g/kg/d, RDA: 0.8 g/kg/d).[16] Individual amino acid intake requirements follow a similar pattern.

Importantly, the DRIs for protein and amino acids were published in 2005 and have not been updated for nearly two decades. Since then, new evidence has become available on requirements for average daily protein and individual indispensable amino acids. Our systematic review was commissioned by the Joint Canada-U.S. Dietary Reference Intakes Working Group, who were informed by a series of evidence scans conducted in collaboration with the U.S. Department of Agriculture Nutrition Evidence Systematic Review team, which helped determine areas where systematic review of the evidence was needed.[29] We undertook this review on the recommendation of the Joint Canada-U.S. Dietary Reference Intakes Working Group to inform the update of the protein and amino acid DRIs by the National Academies of Sciences, Engineering, and Medicine. It will serve as a key reference for a future expert panel who will be tasked with updating the DRIs for all macronutrients, including protein.

Scope and Key Questions

The goal of this review is to assess the evidence on requirements for average daily dietary protein and individual indispensable amino acid intake for healthy individuals by life stage and sex.

Key Questions

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

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

Report Organization

The remainder of this report presents the methods used to conduct the literature searches, data abstraction, and analysis; the results of the review organized by Key Question and population; and 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 conclusions.

Chapter 2. Methods

Review Approach

Our review methods 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). [30]

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 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 provided content and methodological expertise on protein, amino acids, and nutrition 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/dietary-protein-intake/protocol). We registered the protocol on PROSPERO (CRD42023446618).

We invited experts in protein, amino acids, and nutrition throughout the life course 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 description of the review search processes applies to all Key Questions. Our librarian team member developed multiple search strategies for 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).[31] 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 screened titles and abstracts of results using predefined criteria. When the machine learning system was trained, we moved to one screener as soon as we reached a 90 percent recall rate of citations eligible for full-text review. We stopped screening citations remaining past a 95 percent

recall rate of citations eligible for full-text review. Using the same online system, two independent investigators performed full-text screening to determine if inclusion criteria were met. 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 require 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.

Chapter 2. Methods

Table 1. Inclusion and exclusion criteria by population, intervention, comparator, outcome, timing, setting/study design (PICOTS)

Term	Inclusion Criteria	Exclusion Criteria
Population KQ1 & 2	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 at intervention exposure: • Infants, children, adolescents (0-18 yr) • Adults (≥19 yr)	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 (i.e., below the recommended dietary allowance of protein (RDA) per age). 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. Non-human participants (e.g., animal studies, in-vitro models)
Interventions KQ1 & 2	Total daily protein intake level Total daily intake of indispensable AAs (Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, Valine)	Studies that only assess protein intake via parenteral nutrition or intravenous nutrition support Studies that examine food products or dietary supplements not widely available to U.S. consumers. Multi-component interventions that do not isolate the effect or association of protein (including protein and exercise combinations).
Comparison KQ1 & 2	Different total daily protein intake level Different total daily intake of indispensable AAs	No comparator
Outcomes KQ1	Total protein requirement* as defined by the following indicators or criterion of adequacy, including but not limited to: • Nitrogen balance method • Factorial method • Indicator AA oxidation method • Linear growth for infants, children, adolescents (0-18 yr)	No relevant exclusion criteria

Term	Inclusion Criteria	Exclusion Criteria
Outcomes KQ2	Indispensable AA requirement* as defined by the following indicators of adequacy, including but not limited to: • Plasma AA response method • Direct AA oxidation method • 24-h AA balance method • Indicator AA oxidation method	No relevant exclusion criteria
Timing KQ1 & 2	All duration and follow up	No relevant exclusion criteria
Setting KQ1 & 2	All settings	No relevant exclusion criteria
Study Design KQ1 & 2	Randomized controlled trials Nonrandomized controlled trials, including quasi-experimental and controlled before-and-after studies Prospective cohort studies Nested case-control studies	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 All other study designs
Study Size KQ1 & 2	Any sample size for metabolic studies Any sample size for studies involving women who are pregnant or lactating	For studies other than metabolic studies and studies with women who are pregnant and lactating: • 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	English only (due to resource limitations)	Non-English publications
Geographic Location KQ1 & 2	Locations with food products or dietary supplements widely available to U.S. consumers, including those rated high and very high on the HDI. No HDI specification for highly controlled metabolic studies that examine protein or amino acid requirements.	Locations with less than high HDI
Publication Date KQ1 & 2	2000 to present	Prior to 2000
Publication Status KQ1 & 2	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: AA = amino acid; h = hour; HDI = Human Development Index; KQ = key question; N = number, RCT = randomized controlled trial; RDA = recommended dietary allowance; U.S. = United States; yr = year

Note: For this systematic review, the term "metabolic studies" refers to nitrogen balance studies, indicator amino acid oxidation, 24-hour indicator amino acid oxidation/24-hour indicator amino acid balance, direct amino acid oxidation, and plasma amino acid response studies.

*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.

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. [32] 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, [33] because these are generalizable to the United States and Canada. We excluded studies conducted in countries classified as medium or low on HDI. However, no HDI specification was required for highly controlled metabolic studies that examined protein or amino acid requirements because requirements are not likely to vary considerably between individuals with different countries of origin. We applied the HDI classification based on the year the intervention was conducted or the exposure data were collected. If the study did not report the year(s) in which the intervention/exposure data were collected, the HDI classification for the year of publication was applied. If the year of publication was more recent than the available HDI values, then we used the most recent HDI classifications. If a country was not listed in the HDI, then we used the current country classification from the World Bank, and included those studies conducted in countries grouped as upper-middle or high income. [34] For multinational studies, we applied the HDI classification for the majority of the countries.

Data Extraction and Data Management

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). Review 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 E and F). A summary of the basic characteristics of all eligible studies can be found in Appendix D. All eligible studies underwent risk of bias assessment (see section below). 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 studies assessed as low or moderate risk of bias (these studies make up the 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, pregnancy, lactation, and menopausal status, health status/co-morbidities, medication use, supplement use), 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, comparisons, outcomes, intervention duration, and risk of bias assessments.

In addition, findings for outcomes were extracted from studies included in the analytic set (i.e., studies rated as low to moderate risk of bias) (Appendix H). Our analytic set consist of studies with higher methodological rigor (i.e., studies rated as low to moderate risk of bias). We based the findings of this review on these studies because they are less susceptible to biases that can reduce the robustness of findings. We also extracted findings for protein and amino acid requirement estimates for those studies not included in the analytic set (i.e., studies rated as high or very high risk of bias) (Appendix G).

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

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 the study design (Appendix C).

We used the Cochrane Risk of Bias tool 2.0 parallel design version[35] to assess risk of bias of parallel RCTs, rating them 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 cross over design version[35] to assess risk of bias of cross over 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 nonrandomized controlled trials (including quasi-experimental and controlled before-and-after studies), risk of bias by outcomes was rated using the Risk of Bias In Nonrandomized Studies - of Interventions (ROBINS-I) tool[36] 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), we rated risk of bias by outcomes using the Risk Of Bias In Nonrandomized Studies - of Exposure (ROBINS-E) tool[37] 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, 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 domains 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 included studies, we chose to use the two-step process to proceed with our ROBINS-E risk of bias assessment in a timely manner.

One investigator independently assessed 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, the overall risk of bias assessments for each study outcome were classified as low risk, moderate risk, or high risk. For observational studies, the overall risk of bias assessments for each study outcome were classified 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/Analysis

We organized our results by Key Question, then population and outcome. We provided a narrative qualitative synthesis due to the diverse methodologies and data heterogeneity and sparsity across studies, precluding a meta-analysis.

Our findings were grouped by population: infants, children and adolescents, adults (19-50 years), adults (51->70 years), and adults (19-50 years and 51->70 years). We categorized adults by these age ranges because the 2005 DRIs for protein used similar age range categories. [16] Generally, the literature aligned well with this categorization. We show values equivalent to the EAR (i.e., mean maintenance requirement or breakpoint) and RDA (i.e., safe protein allowance or upper 95% CI) when available. For each comparison, we present narrative summary of findings tables for the outcomes in the Results section.

Grading the Strength of Evidence

Strength of evidence is the extent of our confidence in drawing a specific conclusion and is based on causal inference criteria. The overall strength of evidence for identified outcomes for Key Questions 1 and 2 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. [38]

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 trial studies with both 1) a low risk of bias and 2) domains that were consistent, direct, and precise. 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 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 judgement.

Notably, an assessment of insufficient 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 Appendix I.

Chapter 3. Results

Introduction

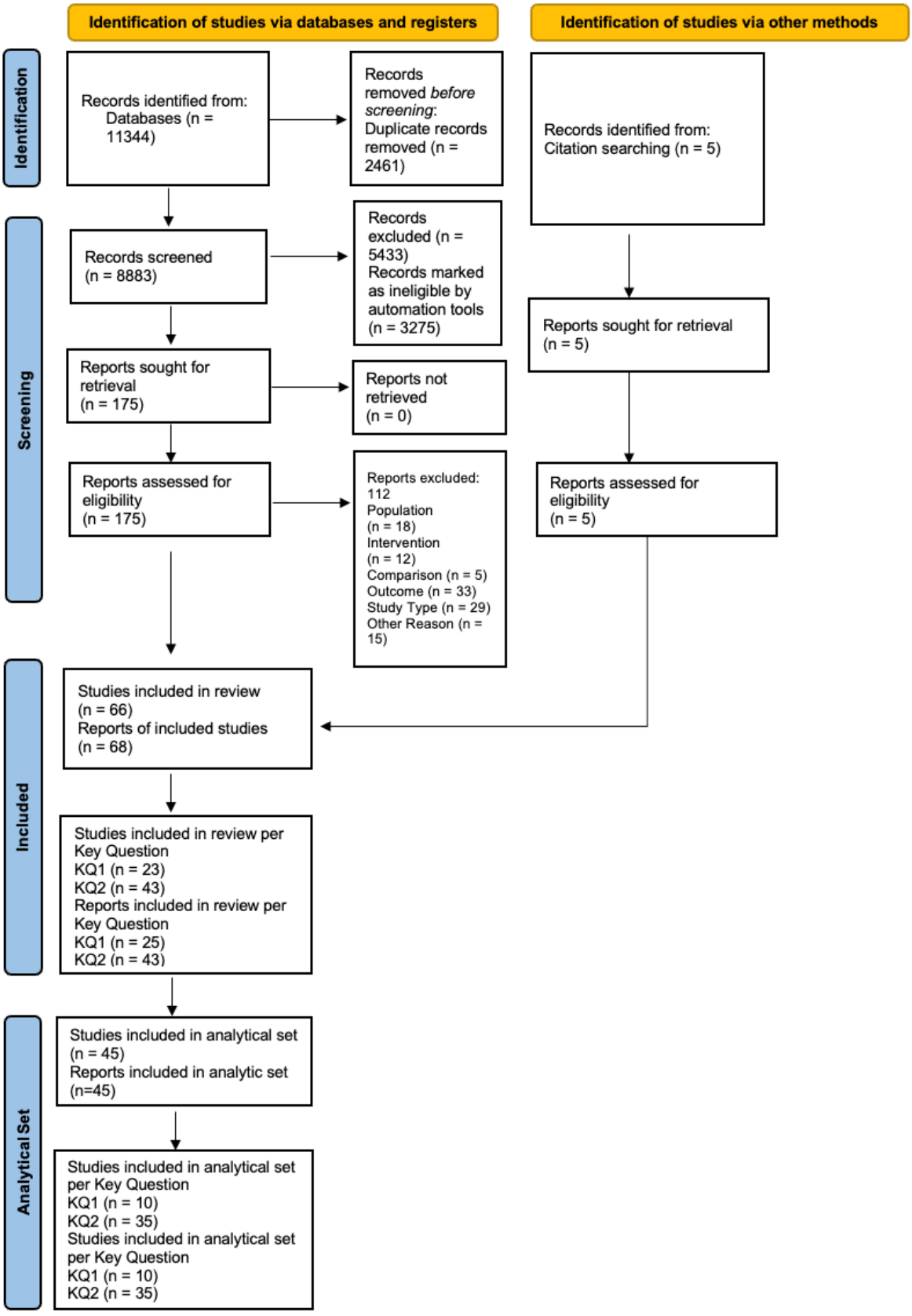
This section describes the results of the literature search, followed by study characteristics and the reported findings of included studies that had low to moderate risk of bias (analytic set). Results for Key Question 1 are presented by population whereas results for Key Question 2 are presented by each individual amino acid and population. A summary of study characteristics of all eligible studies can be found in Appendix D. Risk of bias assessment and detailed study characteristics tables of all eligible studies can be found in Appendix C, E, and F. Detailed findings tables and strength of evidence for studies in the analytic set can be found in Appendix H and I. Appendix G provides a narrative summary and findings tables for studies not included in the analytic set (i.e., studies that were high/very high risk of bias) that report protein and amino acid requirement estimates.

Results of Literature Searches

Figure 1 presents the literature flow of the search results. Database searches of published literature resulted in 11344 potentially relevant articles. After dual review of abstracts and titles, we screened the full text of 175 articles; of these, 63 articles reporting on 61 unique studies met the inclusion criteria. Additionally, five articles reporting on five studies were identified through supplemented searches of relevant systematic reviews and original research. Thus, 68 articles reporting on 66 studies were included in the systematic review. [39-76] [77-106] Among these, 45 studies were rated as low to moderate risk of bias and comprise our analytic set. [40, 44-63, 65, 67-75, 78, 79, 82, 86, 90-93, 96, 99, 100, 103-105] A breakdown per Key Question is shown in Figure 1 below. We list studies excluded at full text screening and reasons for exclusion in Appendix B.

Chapter 3. Results

Figure 1. Literature flow diagram



From: 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 average daily dietary protein intake requirements of apparently healthy individuals by life stage and sex?

Key Points

- Ten of 25 studies were assessed as low or moderate risk of bias and were included in the analytic set.
    - Protein requirement estimates were examined in eight studies, including children/adolescents (1 RCT), pregnant people (1 RCT), adults 19-50 years (1 RCT, 1 non-RCT), and adults 51->70 years (4 RCTs).
- Of these, both males and females were enrolled in two studies (children/adolescents, adults 51->70 years), whereas only males were enrolled in three studies (adults 19-50 years and adults 51->70 years) and only females in three studies (pregnant people, adults 51->70 years).
- Overall, evidence was insufficient to draw conclusions for average daily dietary protein intake requirements for any population.

Infants

Study Characteristics and Findings of Analytic Set

Table 2 summarizes the study characteristics of the analytic set. One study addressed the average daily protein requirement for infants and reports the effect of protein intake on growth but does not report a protein requirement estimate. [65]

Table 2. Basic characteristics of non-RCT analytic set: infants

Characteristic	Information
Total studies	1 study
Location of studies	1 study in Thailand
Design of studies	1 study non-RCT
Settings	1 study outpatient/community-dwelling
Age	Start: 6 mo End:12 mo
Sex of study participants	1 study with both females and males
Sample size	145
Follow up Duration	6 mo
Outcomes evaluated	1 study length-for-age z score 1 study conditional growth length-for-age z score
Risk of bias	1 study moderate risk

Abbreviations: mo = month; RCT = randomized controlled trial

Diet Intervention and Experimental Methods

In this study, [65] dietary assessment and growth measures were taken at 6, 9, and 12 months of age. Dietary assessment was performed using food frequency questionnaires, 24-h recalls, and 3-day food records. The average percent of energy from protein was 7.8 percent, 12.6 percent, and 15.6 percent at 6, 9, and 12 months and was provided in the form of breast milk, formula, and/or complimentary feeding. Body weight and recumbent length were measured, and length-for-age z score (LAZ) were calculated using WHO Anthro version 3.2.2. Conditional growth status was calculated as z-score deviation from average size of the study population. Baseline size at 6 months was controlled for when determining conditional growth status. To compare the effects of protein intake and growth, infants were divided into 3 protein intake categories, low protein (≤10.9% energy from protein), median protein (11-12.8% of energy from protein), and high protein (≥ 12.9% of energy from protein). [65]

Findings

Other (Growth)

Evidence was insufficient to draw conclusions about the effects of protein intake on growth, specifically LAZ and conditional LAZ. Table 3 provides a summary of findings.

Table 3. Summary of findings for infants (non-RCT): LAZ and conditional LAZ

Outcome Comparisons	Study(PMID) Study Design (n analyzed) Timing	Country Ethnicity/ Race Age Sex (% female)	Protein amount (% energy from protein)	Findings	Direction of effect	Strength of Evidence**
***LAZ 6 mo** **1. HPro vs LPro** **2. HPro vs MPro** **3. MPro vs LPro** **LAZ 9 mo** **1. HPro vs LPro** **2. HPro vs MPro** **3. MPro vs LPro** **LAZ 12 mo** **1. HPro vs LPro** **2. HPro vs MPro** **3. MPro vs LPro** **Conditional LAZ 12 mo** **1. HPro vs LPro** **2. HPro vs MPro** **3. MPro vs LPro**	Kittisakmontri, 2022[65] (36235599) non-RCT (n=145 (n=36 HPro, n=73 MPro, n=36 LPro)) 6, 9, and 12 mo	Thailand NR Start: 6 mo End: 12 mo 49.7%	LPro: ≤10.9% MPro: 11-12.8% HPro: ≥12.9%	LAZ 6 mo: HPro: -0.15 MPro: -0.55 LPro: -0.66 1. 0.50(-0.01, 1.01) 2. -0.40(-0.05, 0.84) 3. 0.11(-0.34, 0.55) LAZ 9 mo: HPro: -0.17 MPro: -0.48 LPro: -0.69 1. 0.52(-0.01, 1.05) 2. 0.32(-0.14, 0.77) 3. 0.20(-0.24, 0.65) LAZ 12 mo: HPro: -0.19 MPro: -0.55 LPro: -0.64 1. 0.45(-0.07, 0.96) 2. 0.35(-0.09, 0.80) 3. 0.10(-0.35, 0.54) Conditional LAZ 12 mo: HPro: 0.07 MPro: -0.01 LPro: -0.04	LAZ 6 mo: 1. no difference 2. no difference 3. no difference LAZ 9 mo: 1. no difference 2. no difference 3. no difference LAZ 12 mo: 1. no difference 2. no difference 3. no difference Conditional LAZ 12 mo: 1. no difference 2. no difference 3. no difference	LAZ: Insufficient Conditional LAZ: insufficient

Abbreviations: HPro = high protein (≥ 12.9% of energy from protein); LAZ = length-for-age z score; LPro = low protein (≤10.9% energy from protein); mo = months; MPro = median protein (11-12.8% of energy from protein); N = number; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial

*Data are shown as LAZ at 6, 9, and 12 months followed by the mean difference between groups and 95% confidence interval of the mean difference in parentheses.

**Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.7, the main reasons for this insufficient rating were that the evidence was derived from a single study, making it impossible to assess consistency.

Children and Adolescents

Study Characteristics and Findings of Analytic Set

Table 4 summarizes the study characteristics of the analytic set. One study addressed the question of the average daily protein requirements for children and adolescents. [50]

Table 4. Basic characteristics of RCT analytic set: children and adolescents

Characteristic	Information
Total studies	1 study
Location of studies	1 study in Canada
Design of studies	1 study RCT cross over
Settings	1 study outpatient/community-dwelling
Age range (average)	8.4 yr
Sex of study participants	1 study with both females and males
Sample size	7
Intervention Duration	1 study 2 adaptation d, 1 study d (3 d total) per test intake
Outcomes evaluated	1 study protein requirement estimate ($F^{13}CO_2$, phenylalanine oxidation)
Risk of bias	1 study moderate risk

Abbreviations: d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; RCT = randomized controlled trial; yr = year

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Diet Intervention and Experimental Methods

In this study, [50] participants underwent 2 adaptation days (1.5 g/kg/d protein) and 1 study day for each test intake. Participants received seven different protein intake levels ranging from 0.1-2.56 g/kg/d. The IAAO method was performed, and the protein requirement estimate was calculated using a 2-phase linear regression crossover model on the $F^{13}CO_2$ and phenylalanine oxidation data.

Protein Requirement Estimate

Evidence was insufficient to draw conclusions about the protein requirement estimate for children and adolescents. Table 5 provides a summary of findings.

Table 5. Summary of findings for children and adolescents (RCT): protein requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Test Protein Amount	Findings	Strength of Evidence*
Protein requirement estimate **1. Phenylalanine oxidation** **2. $F^{13}CO_2$**	Elango, 2011[50] (22049165) RCT (n=7; 56) Data obtained following each study d (d 3)	Canada NR 8.4 yr 28.6%	0.1-2.56 g/kg/d	1. Breakpoint: 1.25 g/kg/d, upper 95% CI: 1.5 g/kg/d 2. Breakpoint: 1.3 g/kg/d, upper 95% CI: 1.55 g/kg/d	Insufficient

Abbreviations: d = day; CI = confidence interval; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; N = number; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year

*Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.1, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Pregnancy

Study Characteristics and Findings of Analytic Set

Table 6 summarizes the study characteristics of the analytic set. One study addressed the question of the average daily protein requirements for pregnant people (early gestation 11-20 weeks, late gestation 32-38 weeks).[99]

Table 6. Basic characteristics of RCT analytic set: pregnant people

Characteristic	Information
Total studies	1 study
Location of studies	1 study in Canada
Design of studies	1 study RCT cross over
Settings	1 study outpatient/community-dwelling
Age range (average)	EG: 30.6 yr LG: 30.3 yr
Sex of study participants	1 study with only females
Sample size	29 (EG: n=17, LG: n=19)*
Intervention Duration	1 study 2 adaptation d, 1 study d (3 d total) per test intake
Outcomes evaluated	1 study protein requirement estimate ($F^{13}CO_2$)
Risk of bias	1 study moderate risk

Abbreviations: d = day; EG = early gestation; $F^{13}CO_2$ = rate of tracer oxidation to $^{13}CO_2$ [tracer; phenylalanine]; LG = late gestation; N = number; RCT = randomized controlled trial; yr = year

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

*Seven women were studied in both early and late gestation

Diet Intervention and Experimental Methods

In this study, [99] participants underwent 2 adaptation days (1.5 g/kg/d protein) and 1 study day per test intake. Participants received 1-4 different protein intake levels ranging from 0.22-2.56 g/kg/d. The IAAO method was performed, and the protein requirement estimate was calculated using a 2-phase linear regression crossover model on the $F^{13}CO_2$ data.

Findings

Protein Requirement Estimate

Evidence was insufficient to draw conclusions about the protein requirement estimate for pregnant people. Table 7 provides a summary of findings.

Table 7. Summary of findings for pregnant people (RCT): protein requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Test Protein Amount	Findings	Strength of Evidence*
Protein requirement estimate **1. $F^{13}CO2$ (EG)** **2. $F^{13}CO2$ (LG)**	Stephens, 2015[99] (25527661) RCT (EG: n=17; 35) (LG: n=19; 43) Data obtained following each study d (d 3)	Canada NR EG: 30.6 yr LG: 30.3 yr 100%	0.22-2.56 g/kg/d	1. Breakpoint: 1.22 g/kg/d, upper 95% CI: 1.66 g/kg/d 2. Breakpoint: 1.52 g/kg/d, upper 95% CI: 1.77 g/kg/d	Insufficient

Abbreviations: d = day; CI = confidence interval; EG = early gestation; $F^{13}CO_2$ = rate of tracer oxidation to $^{13}CO_2$ [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; LG = late gestation; N = number; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year

*Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.2, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Adults 19-50 years

Study Characteristics and Findings of Analytic Set

Tables 8 and 9 summarizes the study characteristics of the analytic set for RCTs and non-RCTs. Two studies (one RCT[61] and one non-RCT[40]) addressed the question of the average daily protein requirements for adults 19-50 years. [40, 61]

Table 8. Basic characteristics of RCT analytic set: adults 19-50 years

Characteristic	Information
Total studies	1 study
Location of studies	1 study in Canada
Design of studies	1 study RCT cross over
Settings	1 study outpatient/community-dwelling

Characteristic	Information
Age range (average)	26.8 yr
Sex of study participants	1 study with only males
Sample size	8
Intervention Duration	1 study 2 adaptation d, 1 study d (3 d total) per test intake
Outcomes evaluated	1 study protein requirement estimate ($F^{13}CO_2$)
Risk of bias	1 study moderate risk

Abbreviations: d = day; $F^{13}CO_2$ = rate $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; RCT = randomized controlled trial; yr = year

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Table 9. Basic characteristics of non-RCT analytic set: adults 19-50 years

Characteristic	Information
Total studies	1 study
Location of studies	1 study in Nigeria
Design of studies	1 study non-RCT, cross over
Settings	1 study outpatient/community-dwelling
Age range (average)	23.2 yr
Sex of study participants	1 study with only males
Sample size	18
Intervention Duration	1 study 1 adaptation d followed by 10 d on the study diet per test intake
Outcomes evaluated	1 study protein requirement estimate (nitrogen balance)
Risk of bias	1 study low risk

Abbreviations: d = day; RCT = randomized controlled trial; yr = year

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Diet Intervention and Experimental Methods

Protein intake ranges and methods used to calculate the protein requirement estimates differed between studies.

In the RCT, [61] participants underwent 2 adaptation days (1.0 g/kg/d protein) and 1 study day per test intake. Participants received seven different protein intake levels ranging from 0.1-1.8 g/kg/d. The IAAO method was performed, and the protein requirement estimate was calculated using a bi-phase linear regression crossover model on the $F^{13}CO_2$ data.

In the non-RCT[40] participants underwent 1 adaptation day (<0.1 g/kg/d protein) followed by 10 days on the study diet per test intake. Participants received four different protein intake levels ranging from 0.4-0.9 g/kg/d. Nitrogen balance was measured on days 5-10 and the protein requirement estimate was calculated using a linear regression equation relating nitrogen intake to balance.

Findings

Protein Requirement Estimate

Evidence was insufficient to draw conclusions about the protein requirement estimate for adults 19-50 years. Table 10 and 11 provides a summary of findings from the RCT and non-RCT.

Table 10. Summary of findings for adults 19-50 years (RCT): protein requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Test Protein Amount	Findings	Strength of Evidence*
Protein requirement estimate **1. $F^{13}CO_2$**	Humayun, 2007[61] (17921376) RCT (n=8; 56) Data obtained following each study d (d 3)	Canada 25% South Asian, 37.5% East Asian, 12.5% African, 25% White 26.8 yr 0%	0.1-1.8 g/kg/d	1. Breakpoint: 0.93 g/kg/d, upper 95% CI: 1.24 g/kg/d	Insufficient

Abbreviations: CI = confidence interval; d=day; $F^{13}CO_2$ = rate $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; N = number; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year

*Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.3, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Table 11. Summary of findings for adults 19-50 years (non-RCT): protein requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Test Protein Amount	Findings	Strength of Evidence***
Protein requirement estimate **1. Nitrogen balance (Northern Nigeria arm)** **2. Nitrogen balance (South Eastern Nigeria arm)** **3. Nitrogen balance (both arms)**	Atinmo, 2010[40] (NA) non-RCT (n=7; 28 Northern Nigeria arm (n=11; 44 South Eastern Nigeria arm) Data obtained following each study period (d 5-10)	Nigeria NR 23.3 yr 0%	I: 0.4-0.9 g/kg/d *A: 63.92-156.23 mg/kg/d nitrogen	1. Mean maintenance requirement: 108.01 mg/kg/d nitrogen; SD: 9.45 mg/kg/d nitrogen 2. Mean maintenance requirement: 110.82 mg/kg/d nitrogen; SD: 14.53 mg/kg/d nitrogen 3. Mean maintenance requirement: 109.73 mg/kg/d nitrogen; SD: 12.56 mg/kg/d nitrogen**	Insufficient

Abbreviations: A = actual; d = day; g/kg/d = grams per kilogram per day; I = intended; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized control trial; SD = standard deviation; yr = year

*Average protein intake range is shown.

**Combined the mean requirement and protein allowance was calculated to be 0.686 g/kg/d and 0.843 g/kg/d protein

***Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.5, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Adults 51->70 years

Study Characteristics and Findings of Analytic Set

Table 12 summarizes the study characteristics of the analytic set. Four RCTs addressed the question of the average daily protein requirements for adults 51->70 years. [79, 86, 92, 93]

Table 12. Basic characteristics of RCT analytic set: adults 51->70 years

Characteristic	Information
Total studies	4 studies
Location of studies	1 study in the United States 2 studies in Canada 1 study in China
Design of studies	4 studies RCT cross over
Settings	4 studies outpatient/community-dwelling
Age range (average)	70.9 to 75 yr
Sex of study participants	2 studies with only females 1 study with only males 1 study with both females and males
Sample size range	6 to 14
Intervention Duration	3 studies 2 adaptation d, 1 study d (3 d total) per test intake 1 study 1 adaptation d followed by 17 d on the study diet (18 d total) per test intake
Outcomes evaluated	4 studies protein requirement estimate ($F^{13}CO_2$, nitrogen balance)
Risk of bias	4 studies moderate risk

Abbreviations: d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; RCT = randomized controlled trial; yr = year

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Diet Intervention and Experimental Methods

Protein intake ranges and methods used to calculate the protein requirement estimates differed between studies.

In one RCT, [86] participants underwent 1 adaptation day (0.18 g/kg/d protein) followed by 17 days on the study diet per test intake. Participants received 3 different protein intakes designed to provide low protein (0.5 g/kg/d), medium protein (0.75 g/kg/d) and high protein (1.0 g/kg/d). Nitrogen balance was measured at week 2 (days 7-10) and week 3 (days 14-17) and the protein requirement estimate was calculated using a linear regression equation between protein intake and nitrogen balance.

In the other three RCTs, [79, 92, 93] participants underwent 2 adaptation days (1.0 g/kg/d protein) and 1 study day per test intake. In one study[79] participants received 6 different protein intakes except for 2 participants who received 4 ranging from 0.3-1.8 g/kg/d. In the other two studies[92, 93] participants received either 2-11 different protein intakes[93] or 7 different protein intakes[92]

ranging from 0.2-2.0 g/kg/d. In all three studies, [79, 92, 93] the IAAO method was performed, and the protein requirement estimate was calculated using a nonlinear mixed-effects model on the $F^{13}CO_2$ data.

Findings

Protein Requirement Estimate

Evidence was insufficient to draw conclusions about the protein requirement estimate for adults 51->70 years. Table 13 provides a summary of findings.

Table 13. Summary of findings for adults 51->70 years: protein requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Test Protein Amount	Findings	Strength of Evidence***
Protein requirement estimate **1. $F^{13}CO_2$**	Mao, 2020[79] (32140711) RCT (n=14; 80) Data obtained following each study d (d 3)	China NR F: 73.1 yr M: 70.9 yr 50%	0.3-1.8 g/kg/d	1. Breakpoint: 0.91 g/kg/d; upper 95% CI: 1.17 g/kg/d	Insufficient
Protein requirement estimate **1. $F^{13}CO_2$**	Rafii, 2015[93] (25320185) RCT (n=12; 83) Data obtained following each study d (d 3)	Canada 91.67% Caucasian, 8.33% Asian 74.3 yr 100%	0.2-2.0 g/kg/d	1. Breakpoint: 0.96 g/kg/d; upper 95% CI: 1.29 g/kg/d	Insufficient
Protein requirement estimate **1. $F^{13}CO_2$**	Rafii, 2015[92] (26962173) RCT (n=6; 42) Data obtained following each study d (d 3)	Canada 83% White, 17% African Canadian 71.3 yr 0%	0.2-2.0 g/kg/d	1. Breakpoint: 0.94 g/kg/d; upper 95% CI: 1.24 g/kg/d	Insufficient

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Test Protein Amount	Findings	Strength of Evidence***
***Protein requirement estimate** **1. Nitrogen balance (week 2)** **2. Nitrogen balance (week 3)**	Morse, 2001[86] (11682582) RCT (n=11; 33) 2 and 3 wk	United States NR 75 yr 100%	I: 0.5-1.0 g/kg/d **A: 0.53-1.06 g/kg/d	1. Mean protein requirement: 0.70 g/kg/d; protein allowance: 0.90 g/kg/d 2. Mean protein requirement: 0.56 g/kg/d; protein allowance: 0.76 g/kg/d	Insufficient

Abbreviations: A = actual; CI = confidence interval; d = day; F= female; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; I=intended; M= male; N = number; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; wk = week; yr = year

*The mean protein requirement was significantly different between week 2 and week 3.

**Average protein intake range is shown.

***Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.4, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Adults 19-50 and 51->70 years

Study Characteristics and Findings of Analytic Set

Table 14 summarizes the study characteristics of the analytic set. One study addressed the question of the average daily protein requirement for adults in both age groups 19-50 and 51->70 years; [104] however, this study reports nitrogen balance and leucine oxidation rather than a protein requirement estimate.

Table 14. Study characteristics of RCT analytic set: adults 19-50 and 51->70 years

Characteristic	Information
Total studies	1 study
Location of studies	1 study in the United States
Design of studies	1 study RCT cross over
Settings	1 study outpatient/community-dwelling
Age range	24.3 to 70 yr (average)
Sex of study participants	1 study with both females and males
Sample size	19
Intervention Duration	1 study 10 d per test intake
Outcomes evaluated	1 study nitrogen balance 1 study leucine oxidation
Risk of bias	1 study moderate risk

Abbreviations: d = day; RCT = randomized controlled trial; yr = year

Diet Intervention and Experimental Methods

In this study, [104] participants were assigned to a usual protein (1.5 g/kg FFM/d) and high protein (3.0 g/kg FFM/d) diet. Nitrogen balance was calculated from subtracting the 24-h urinary nitrogen excretion from nitrogen intakes (days 9-11) and amino acid kinetics were performed following infusion of a leucine tracer (L-[1-13C]leucine) to calculate leucine oxidation on day 11.

Findings

Other (Nitrogen balance, leucine oxidation)

Evidence was insufficient to draw conclusions about nitrogen balance or leucine oxidation for adults 19-50 and 51->70 years. Table 15 provides a summary of findings.

Table 15. Summary of findings for adults 19-50 and 51->70 years: nitrogen balance and leucine oxidation

Outcome comparisons	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Test Protein Amount	Findings	Direction of effect	Strength of Evidence*
Nitrogen balance **1. YA vs OA** **2. UP, YA vs HP, YA** **3. UP, OA vs HP, OA** **Leucine oxidation** **1. YA vs OA** **2. UP, YA vs HP, YA** **3. UP, OA vs HP, OA**	Walrand, 2008[104] (18697911) RCT (n=19; 38) 10 d	United States NR YA: 24.3 yr OA: 70.0 yr YA: 50% OA: 44%	UP: 1.5 g/kg FFM/d HP: 3.0 g/kg FFM/d	Nitrogen balance (g/kg FFM/d): UP, YA: 2.77 ± 0.11 HP, YA: 5.42 ± 0.45 UP, OA: 2.48 ± 0.12 HP, OA: 5.32 ± 0.18 Leucine oxidation (µmol/kg FFM/min): UP, YA: 27.4 ± 1.8 HP, YA: 38.2 ± 2.1 UP, OA: 31.2 ± 1.5 HP, OA: 37.3 ± 1.3	Nitrogen balance: 1. No difference 2. Significantly increased on the HP diet 3. Significantly increased on the HP diet Leucine oxidation: 1. No difference 2. Significantly increased on the HP diet 3. Significantly increased on the HP diet	Nitrogen balance: Insufficient Leucine oxidation: Insufficient

Abbreviations: d = day; g/kg FFM/d = grams per kilogram fat free mass per day; HP = high protein; N = number; NR = not reported; OA = older adults; PMID = PubMed Identification Number; RCT = randomized controlled trial; µmol/kg FFM/min = micromole per kilogram fat free mass per minute; UP = usual protein; YA = younger adults; yr = year

Note: Values are reported as mean ± standard error

*Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.6, the main reasons for this insufficient rating were that 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 average daily individual indispensable amino acid intake requirements of apparently healthy individuals by life stage and sex?

Key Points

- Thirty-five of 43 studies were assessed as low or moderate risk of bias and were included in the analytic set. Of these:
    - Isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine requirement estimates were examined in six RCTs for infants. Both males and females were studied for all requirements except valine where only males were studied.
    - Lysine, methionine, phenylalanine, and total branched chain amino acids requirement estimates were examined in six RCTs for children and adolescents. Both males and females were studied for all requirements except phenylalanine in which the sex of participants is not reported.
    - Lysine and phenylalanine requirement estimates were examined in three RCTs for females who were pregnant.
    - Leucine, lysine, methionine, phenylalanine, threonine, and valine requirement estimates were examined in 14 RCTs for adults 19-50 years. Only males were studied for all requirement estimates except lysine where one study enrolled only females.
    - Leucine and phenylalanine requirement estimates were examined in two RCTs for adults 51->70 years. Both males and females were studied for all requirements.
    - Histidine requirement estimates were not examined for any population.
- Overall, evidence was insufficient to draw conclusions for the average daily dietary individual indispensable amino acid requirements for any population.

Histidine

No studies in the analytic set examined the histidine requirement estimate for infants, children and adolescents, pregnant people, adults 19-50 years, and adults 51->70 years.

Isoleucine

Infants

Study Characteristics and Findings of Analytic Set

Table 16 summarizes the study characteristics of the analytic set. One study addressed the question of the average daily isoleucine requirement in infants. [44]

Table 16. Basic characteristics of RCT analytic set: infants

Characteristic	Information
Total studies	1 study
Location of studies	1 study in China
Design of studies	1 study RCT parallel
Settings	1 study initially admitted to the hospital
Age range (average)	12 d (average gestational age 39.5 wk)
Sex of study participants	1 study with both females and males
Sample size	22
Intervention Duration	1 study 1 adaptation d, 1 study d (2 d total)
Outcomes evaluated	1 study isoleucine requirement estimate ($F^{13}CO_2$)
Risk of bias	1 study low risk

Abbreviations: d = day; $F^{13}CO_2$ = the fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; RCT = randomized controlled trial; wk = week

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Diet Intervention and Experimental Methods

In this study, [44] participants underwent a 24-h adaptation period and 1 study day (2.96 g/kg/d average protein intake). Participants received one isoleucine intake ranging from 5-216 mg/kg/d. The IAAO method was performed, and the isoleucine requirement estimate was calculated using a 2-phase regression model on the $F^{13}CO_2$ data.

Findings

Isoleucine Requirement Estimate

Evidence was insufficient to draw conclusions about the isoleucine requirement estimate for infants. Table 17 provides a summary of findings.

Table 17. Summary of findings for infants: isoleucine requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence*
Isoleucine requirement estimate **1. $F^{13}CO_2$**	de Groof, 2014[44] (24284437) RCT	China 100% Asian 12 d	2.96 g/kg/d 5-216 mg/kg/d isoleucine	1. Breakpoint: 105 mg/kg/d, upper 95% CI 150 mg/kg/d	Insufficient

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence*
	(n=22; 22) Data obtained following each study d (d 2)	59%			

Abbreviations: CI = confidence interval; d = day; $F^{13}CO_2$ = the fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; mg/kg/d = milligram per kilogram per day; N = number; PMID = PubMed Identification Number; RCT = randomized controlled trial

*Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.8, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Children and Adolescents

No studies in the analytic set examined the isoleucine requirement estimate. However, one study[78] examined the total branched chain amino acids requirement estimate for children and adolescents as described in the section on "Total Branched Chain Amino Acids" below.

Pregnancy

No studies in the analytic set examined the isoleucine requirement estimate.

Adults 19-50 years

No studies in the analytic set examined the isoleucine requirement estimate.

Adults 51->70 years

No studies in the analytic set examined the isoleucine requirement estimate.

Leucine

Infants

Study Characteristics and Findings of Analytic Set

Table 18 summarizes the characteristics of the analytic set. One study addressed the question of the average daily leucine requirement in infants. [44]

Table 18. Basic characteristics of RCT analytic set: infants

Characteristic	Information
Total studies	1 study
Location of studies	1 study in China
Design of studies	1 study RCT parallel
Settings	1 study initially admitted to the hospital
Age range (average)	11 d (average gestational age 39.4 wk)
Sex of study participants	1 study with both females and males

Characteristic	Information
Sample size	33
Intervention Duration	1 study 1 adaptation d, 1 study d (2 d total)
Outcomes evaluated	1 study leucine requirement estimate ($F^{13}CO_2$)
Risk of bias	1 study low risk

Abbreviations: d = day; $F^{13}CO_2$ = The fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; RCT = randomized controlled trial; wk = week

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Diet Intervention and Experimental Methods

In this study, [44] participants underwent a 24-h adaptation period and 1 study day (2.98 g/kg/d average protein intake). Participants received one leucine intake ranging from 5-370 mg/kg/d. The IAAO method was performed, and the leucine requirement estimate was calculated using a 2-phase regression model on the $F^{13}CO_2$ data.

Findings

Leucine Requirement Estimate

Evidence was insufficient to draw conclusions about the leucine requirement estimate for infants. Table 19 provides a summary of findings.

Table 19. Summary of findings for infants: leucine requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence*
Leucine requirement estimate **1. $F^{13}CO_2$**	de Groof, 2014[44] (24284437) RCT (n=33; 33) Data obtained following each study d (d 2)	China 100% Asian 11 d 51.5%	2.98 g/kg/d 5-370 mg/kg/d leucine	1. Breakpoint: 140 mg/kg/d, upper 95% CI 245 mg/kg/d	Insufficient

Abbreviation: CI = confidence interval; d = day; $F^{13}CO_2$ = The fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; mg/kg/d = milligram per kilogram per day; N = number; PMID = PubMed Identification Number; RCT = randomized controlled trial

*Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.9, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Children and Adolescents

No studies in the analytic set examined the leucine requirement estimate. However, one study[78] examined the total branched chain amino acids requirement estimate for children and adolescents as described in section 3.13 “Total Branched Chain Amino Acids” below.

Pregnancy

No studies in the analytic set examined the leucine requirement estimate.

Adults 19-50 years

Study Characteristics and Findings of Analytic Set

Table 20 summarizes the characteristics of the analytic set. One study addressed the question of the average daily leucine requirement in adults 19-50 years. [68]

Table 20. Basic characteristics of RCT analytic set: adults 19-50 years

Characteristic	Information
Total studies	1 study
Location of studies	1 study in India
Design of studies	1 study RCT cross over
Settings	1 study outpatient/community-dwelling
Age range (average)	21.35 yr
Sex of study participants	1 study with only males
Sample size	20
Intervention Duration	1 study 6 adaptation d, 1 study d (7 d total) per test intake
Outcomes evaluated	1 study leucine requirement estimate (24-h IAAB, nitrogen balance)
Risk of bias	1 study moderate risk

Abbreviations: d = day; h = hour; IAAB = indicator amino acid balance; RCT = randomized controlled trial; yr = year

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Diet Intervention and Experimental Methods

In this study, [68] participants underwent 6 adaptation days and 1 study day (160 mg/kg/d nitrogen, 1.0 g/kg/d protein) per test intake. Participants received two different leucine intakes ranging from 14-40 mg/kg/d. The 24-h IAAB method and nitrogen balance method were measured, and the leucine requirement estimate was calculated using a zero-balance intercept analysis on the 24-h IAAB and nitrogen balance data with leucine intake as a continuous covariate.

Findings

Leucine Requirement Estimate

Evidence was insufficient to draw conclusions about the leucine requirement estimate for adults 19-50 years. Table 21 provides a summary of findings.

Table 21. Summary of findings for adults 19-50 years: leucine requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence*
Leucine requirement estimate **1. 24-h IAAB** **2. Nitrogen balance**	Kurpad, 2001[68] (11722955) RCT (n=20; 40 leucine balance; 36 nitrogen balance) Data obtained following each study d (d 7)	India NR 21.35 yr 0%	1.0 g/kg/d 14-40 mg/kg/d leucine	1. Zero-balance estimate: 37.3 mg/kg/d; upper 95% CI: 50 mg/kg/d 2. Zero-balance estimate: 37.6; upper 95% CI: ND	insufficient

Abbreviations: d = day; CI = confidence interval; h = hour; IAAB = indicator amino acid balance; g/kg/d = grams per kilogram per day; mg/kg/d = milligram per kilogram per day; N = number; ND = unable to be determined; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year

*Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.10, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Adults 51->70 years

Study Characteristics and Findings of Analytic Set

Table 22 summarizes the characteristics of the analytic set. One study addressed the question of the average daily leucine requirement in adults 51->70 years. [100]

Table 22. Basic characteristics of RCT analytic set: adults 51->70 years

Characteristic	Information
Total studies	1 study
Location of studies	1 study in Canada
Design of studies	1 study RCT cross over
Settings	1 study outpatient/community-dwelling
Age range (average)	70.4 to 70.7 yr
Sex of study participants	1 study with both females and males
Sample size	16
Intervention Duration	1 study 2 adaptation d, 1 study d (3 d total) per test intake
Outcomes evaluated	1 study leucine requirement estimate ($F^{13}CO_2$)
Risk of bias	1 study moderate risk

Abbreviations: d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ release from tracer oxidation [tracer; phenylalanine]; RCT = randomized controlled trial; yr = year

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Diet Intervention and Experimental Methods

In this study, [100] participants underwent 2 adaptation days and 1 study day (1.0 g/kg/d protein) per test intake. Participants received 3-7 different leucine intakes ranging from 20-120 mg/kg/d. The IAAO method was performed, and the leucine requirement estimate was calculated using a biphasic linear mixed-effects model on the $F^{13}CO_2$ data.

Findings

Leucine Requirement Estimate

Evidence was insufficient to draw conclusions about the leucine requirement estimate for adults 51->70 years. Table 23 provides a summary of findings.

Table 23. Summary of findings for adults (51->70 years): leucine requirement estimate

Outcome Data used to calculate requirement	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein amount Test Amino Acid Amount	Findings	Strength of Evidence**
***Leucine requirement estimate** **1. $F^{13}CO_2$ (males)** **2. $F^{13}CO_2$ (females)** **3. $F^{13}CO_2$ (combined)**	Szwiega, 2021[100] (33330915) RCT (n=16; 93) Data obtained following each study d (d 3)	Canada NR M: 70.4 yr F: 70.7 yr 56.3%	1.0 g/kg/d 20-120 mg/kg/d leucine	1. BW basis: Breakpoint: 77.8 mg/kg/d, upper 95% CI: 81 mg/kg/d 1.FFM basis: Mean requirement: 115 ± 3.2 mg/kg FFM/d, upper 95% CI: 125.4 mg/kg FFM/d 2. BW basis: Breakpoint: 78.2 mg/kg/d, upper 95% CI: 82 mg/kg/d 2. FFM basis: Mean requirement: 127.6 ± 2.4 mg/kg FFM/d, upper 95% CI: 133.7 mg/kg FFM/d 3. Breakpoint: 78.5 mg/kg/d, upper 95% CI: 81 mg/kg/d	Insufficient

Abbreviations: BW = body weight; CI = confidence interval; d = day; F = female; FFM = fat free mass; $F^{13}CO_2$ = rate of $^{13}CO_2$ release from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; M = male; mg/kg/d = milligram per kilogram per day; N = number; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year

*No difference in the breakpoint was found between males and females on a body weight basis but a difference in the mean requirement was observed on a fat free mass basis. Mean requirement ± standard error of the mean

**Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.11, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Lysine

Infants

Table 24 summarizes the study characteristics of the analytic set. One study addressed the question of the average daily lysine requirement for infants. [58]

Table 24. Basic characteristics of RCT analytic set: infants

Characteristic	Information
Total studies	1 study
Location of studies	1 study in China
Design of studies	1 study RCT parallel
Settings	1 study initially admitted to the hospital
Age range (average)	12 d (average gestational age 39 wk)
Sex of study participants	1 study with both females and males
Sample size	21
Intervention Duration	1 study 1 adaptation d, 1 study d (2 d total)
Outcomes evaluated	1 study lysine requirement estimate ($F^{13}CO_2$)
Risk of bias	1 study low risk

Abbreviations: d = day; $F^{13}CO_2$ = The fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; RCT = randomized controlled trial; wk = week

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Diet Intervention and Experimental Methods

In this study, [58] participants underwent a 24-h adaptation period and 1 study day (2.99 g/kg/d average protein intake). Participants received one lysine intake ranging from 15-240 mg/kg/d. The IAAO method was performed, and the lysine requirement estimate was calculated using a biphasic linear regression crossover model on the $F^{13}CO_2$ and phenylalanine oxidation (urinary and plasma enrichment) data.

Findings

Lysine Requirement Estimate

Evidence was insufficient to draw conclusions about the lysine requirement estimate for infants. Table 25 provides a summary of findings.

Table 25. Summary of findings for infants: lysine requirement estimate

Outcome Data used to calculate requirement estimate	Study(PIMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence*
Lysine requirement estimate **1. $F^{13}CO_2$ (first isotopic plateau)** **2. $F^{13}CO_2$ (second isotopic plateau)** **3. Phenylalanine oxidation (urinary enrichment)** **4. Phenylalanine oxidation (plasma enrichment)**	Huang, 2011[58] (22049162) RCT (n=21; 21) Data obtained following each study d (d 2)	China NR 12 d 43%	2.99 g/kg/d 15-240 mg/kg/d lysine	1. Breakpoint: 130 mg/kg/d, upper 95% CI: 188.4 mg/kg/d 2. Breakpoint: 130 mg/kg/d, upper 95% CI: 183.7 mg/kg/d 3. Breakpoint: 130 mg/kg/d, upper 95% CI: 183.2 mg/kg/d 4. Breakpoint: 130 mg/kg/d, upper 95% CI: 185.6 mg/kg/d	Insufficient

Abbreviations: CI = confidence interval; d = day; $F^{13}CO_2$ = The fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; mg/kg/d = milligrams per kilogram per day; N = number; NR = not reported; PMID = PubMed Identification; RCT = randomized controlled trial

*Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.12, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Children and Adolescents

Study Characteristics and Findings of Analytic Set

Table 26 summarizes the study characteristics of the analytic set. Two studies addressed the question of the average daily lysine requirement for children and adolescents. [49, 91]

Table 26. Basic characteristics of RCT analytic set: children and adolescents

Characteristic	Information
Total studies	2 studies
Location of studies	1 study in Canada 1 study in India
Design of studies	2 studies RCT cross over
Settings	2 studies outpatient/community-dwelling
Age range (average)	8.4 yr
Sex of study participants	2 studies with both females and males
Sample size range	5 to 6

Characteristic	Information
Intervention Duration	2 studies 2 adaptation d, 1 study d (3 d total) per test intake
Outcomes evaluated	2 studies lysine requirement estimate ($F^{13}CO_2$)
Risk of bias	2 studies moderate risk

Abbreviations: d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; RCT = randomized controlled trial; yr = year

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Diet Intervention and Experimental Methods

In both studies,[49, 91] participants underwent 2 adaptation days and 1 study day (1.5 g/kg/d protein) per test intake. Participants received seven different lysine intakes ranging from 5-80 mg/kg/d. The IAAO method was performed, and the lysine requirement was calculated for both studies using a 2-phase linear regression crossover model on the $F^{13}CO_2$ data.

Findings

Lysine Requirement Estimate

Evidence was insufficient to draw conclusions about the lysine requirement estimate for children and adolescents. Table 27 provides a summary of findings.

Table 27. Summary of findings for children and adolescents: lysine requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence*
Lysine requirement estimate 1. $F^{13}CO_2$	Elango, 2007[49] (17684206) RCT (n= 5; 35) Data obtained following each study d (d 3)	Canada NR 8.4 yr 20%	1.5 g/kg/d 5-80 mg/kg/d lysine	1. Breakpoint: 35 mg/kg/d, upper 95% CI: 58 mg/kg/d	Insufficient
Lysine requirement estimate 1. $F^{13}CO_2$	Pillai, 2010[91] (19923398) RCT (n= 6; 42) Data obtained following each study d (d 3)	India NR 8.4 yr 50%	1.5 g/kg/d 5-80 mg/kg/d lysine	1. Breakpoint: 33.5 mg/kg/d, upper 95% CI: 46.6 mg/kg/d	Insufficient

Abbreviations: CI = confidence interval; d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; mg/kg/d = milligram per kilogram per day; N = number; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year

*Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.13, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Pregnancy

Study Characteristics and Findings of Analytic Set

Table 28 summarizes the study characteristics of the analytic set. One study addressed the question of the average daily lysine requirements for pregnant people (early gestation 12-19 weeks, late gestation 33-39 weeks). [90]

Table 28. Basic characteristics of RCT analytic set: pregnant people

Characteristic	Information
Total studies	1 study
Location of studies	1 study in Canada
Design of studies	1 study RCT cross over
Settings	1 study outpatient/community-dwelling
Age range (average)	EG: 29.5 yr LG: 30.5 yr
Sex of study participants	1 study with only females
Sample size	33 (EG: n=14, LG: n=19)*
Intervention Duration	1 study 2 adaptation d, 1 study d (3 d total) per test intake
Outcomes evaluated	1 study lysine requirement estimate ($F^{13}CO_2$)
Risk of bias	1 study moderate risk

Abbreviations: d = day; EG = early gestation; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; LG = late gestation; N = number; RCT = randomized controlled trial; yr = year

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

*One woman was studied in both early and late gestation

Diet Intervention and Experimental Methods

In this study, [90] participants underwent 2 adaptation days and 1 study day (1.5 g/kg/d protein) per test intake. Participants received ≤5 lysine intakes ranging from 6-84 mg/kg/d. The IAAO method was performed, and the lysine requirement estimate was calculated using a bi-phase linear regression crossover model on the $F^{13}CO_2$ data.

Findings

Lysine Requirement Estimate

Evidence was insufficient to draw conclusions about the lysine requirement estimate for pregnant people. Table 29 provides a summary of the findings.

Table 29. Summary of findings for pregnant people: lysine requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence*
Lysine requirement estimate	Payne, 2018[90] (29378056) RCT	Canada NR	1.5 g/kg/d 6-84 mg/kg/d lysine	1. Breakpoint: 36.6 mg/kg/d, upper 95% CI: 46.2 mg/kg/d	Insufficient

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence*
1. $F^{13}CO_2$ (EG) 2. $F^{13}CO_2$ (LG)	(EG: n= 14; 27) (LG: n= 19; 36) Data obtained following each study d (d 3)	EG: 29.5 yr LG: 30.5 yr 100%		2. Breakpoint: 50.3 mg/kg/d, upper 95% CI: 60.4 mg/kg/d	

Abbreviations: CI = confidence interval; d = day; EG = early gestation; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; LG = late gestation; mg/kg/d = milligram per kilogram per day; N = number; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year

*Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.14, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Adults 19-50 years

Study Characteristics and Findings of Analytic Set

Tables 30, 31 and 32 summarize the study characteristics of the analytic set. Five studies addressed the question of the average daily lysine requirements in adults 19-50 years. [47, 48, 67, 69, 70] Of these, three studies do report a lysine requirement estimate[67, 69, 70] and two do not, and will be summarized separately. [47, 48]

Table 30. Basic characteristics of RCTs in the analytic set: adults 19-50 years; that report a lysine requirement estimate

Characteristic	Information
Total studies	3 studies
Location of studies	1 study in Canada 2 studies in India
Design of studies	3 studies RCT cross over
Settings	3 studies outpatient/community-dwelling
Age range (average)	19.12 to 33.6 yr
Sex of study participants	1 study with only females 2 studies with only males
Sample size range	5 to 18
Intervention Duration	1 study 2 adaptation d, 1 study d (3 d total) per test intake 1 study 8 adaptation d, 1 study d (9 d total) per test intake 1 study, 6 adaptation d followed by 1 study d then 13 adaptation d followed by 1 study d (21 d total) per test intake
Outcomes evaluated	3 studies lysine requirement estimate (24-h IAAO, 12-h fed IAAO, 24-h IAAB, $F^{13}CO_2$)
Risk of bias	1 study low risk 2 studies moderate risk

Abbreviations: d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; h = hour; IAAB = indicator amino acid balance; IAAO = indicator amino acid oxidation; RCT = randomized controlled trial; yr = year

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Table 31. Basic characteristics of RCT analytic set: adults 19-50 years; that do not report a lysine requirement estimate

Characteristic	Information
Total studies	1 study
Location of studies	1 study in Canada
Design of studies	1 study RCT cross over
Settings	1 study outpatient/community-dwelling
Age range (average)	23.6 yr
Sex of study participants	1 study with only males
Sample size	5
Intervention Duration	1 study 2-d adaptation period prior to 7-d experimental period which consisted of 8-h, 3-d, and 7-d adaptation followed by a study d on d 1, 3, and 7 per test intake
Outcomes evaluated	1 study $F^{13}CO_2$
Risk of bias	1 study moderate risk

Abbreviations: d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; h = hour; RCT = randomized controlled trial; yr = year

Table 32. Basic characteristics of non-RCT analytic set: adults 19-50 years; that do not report a lysine requirement estimate

Characteristic	Information
Total studies	1 study
Location of studies	1 study in the United States
Design of studies	1 study non-RCT parallel intervention
Settings	1 study outpatient/community-dwelling
Age range (average)	21 to 23 yr
Sex of study participants	1 study with both females and males
Sample size	11
Intervention Duration	1 study 6 adaptation d, 1 study d (7 d total) per test intake
Outcomes evaluated	1 study 24-h whole body lysine[1-13C] oxidation 1 study 24-h whole body lysine balance
Risk of bias	1 study low risk

Abbreviations: d = day; h = hour; RCT = randomized controlled trial; yr = year

Diet Intervention and Experimental Methods

Lysine intake ranges and methods used to calculate the lysine requirement estimates differed between studies.

In one RCT, [69] participants underwent 8 adaptation days and 1 study day (160 mg/kg/d nitrogen, 1.0 g/kg/d protein) per test intake. Participants received two lysine intakes ranging from 12-36 mg/kg/d. The 24-h IAAO and 24-h IAAB method were measured, and the lysine requirement estimate was calculated using a two-phase linear regression model on the 24-h IAAO, 12-h fed IAAO, and 24-h IAAB data.

Similarly, in another RCT, [70] participants underwent a 21-day study with 2 adaptation periods (6 days and 13 days) and 2 study days (day 7 and day 21, 160 mg/kg/d nitrogen, 1.0 g/kg/d protein) per test intake. Participants received two lysine intakes ranging from 12-36 mg/kg/d. The 24-h IAAO and 24-h IAAB method were measured, and the lysine requirement estimate was calculated using a two-phase linear regression model on the 24-h IAAO, 12-h fed IAAO, and 24-h IAAB data.

In a different RCT, [67] participants underwent 2 adaptation days and 1 study day (1.0 g/kg/d protein) per test intake. Participants received seven lysine intakes per menstrual phase (follicular and luteal) ranging from 10-60 mg/kg/d lysine. The IAAO method was performed, and the lysine requirement estimate was calculated using a two-phase linear regression crossover model on $F^{13}CO_2$ data adjusted for subject and sex hormones.

For studies that do not report a lysine requirement estimate, one RCT[48] calculated the effect of adaptation period timing on $F^{13}CO_2$ in which participants were adapted to lysine intakes ranging from 5-70 mg/kg/d for either 8-hours, 3-days or 7-days using the IAAO method. In the non-RCT, [47] the effect of either 14-15 mg/kg/d or 28-29 mg/kg/d lysine (average lysine intake: 15.53 mg/kg/d and 29.1 mg/kg/d) was calculated on 24-h whole body lysine[1-13C] oxidation and 24-h whole body lysine balance.

Findings

Lysine Requirement Estimate

Evidence was insufficient to draw conclusions about the lysine requirement estimate for adults 19-50 years. Table 33 provides a summary of findings.

Table 33. Summary of findings for adults 19-50 years: lysine requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence**
Lysine requirement estimate **1. 24-h IAAO** **2. 12-h fed IAAO** **3. 24-h IAAB**	Kurpad, 2001[69] (11333843) RCT (n= 16; 32) Data obtained following each study d (d 9)	India NR 19.78 yr 0%	1.0 g/kg/d 12-36 mg/kg/d lysine	1. Breakpoint: 28.7 mg/kg/d, upper 95% CI: 48 mg/kg/d 2. Breakpoint: 28.2 mg/kg/d, upper 95% CI: 48 mg/kg/d 3. Breakpoint: 29.7 mg/kg/d, upper 95% CI: 49 mg/kg/d	Insufficient
Lysine requirement estimate **1. 24-h IAAO (d 7)** **2. 24-h IAAB (d 7)** **3. 24-h IAAO (d 21)** **4. 24-h IAAB (d 21)**	Kurpad, 2002[70] (12145014) RCT (n= 18; 36) Data obtained following each study d (d 7 and d 21)	India NR 19.12 yr 0%	1.0 g/kg/d 12-36 mg/kg/d lysine	1. Breakpoint: 31 mg/kg/d, upper 95% CI: 40 mg/kg/d 2. Breakpoint: 31 mg/kg/d, upper 95% CI: 40 mg/kg/d 3. Breakpoint: 31 mg/kg/d, upper 95% CI: 48 mg/kg/d 4. Breakpoint: 31 mg/kg/d,	Insufficient

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence**
5. 24-h IAAO (d 7 and 21) **6. 12-h fed IAAO (d 7 and 21)** **7. 24-h IAAB (d 7 and 21)**				upper 95% CI: 47 mg/kg/d 5. Breakpoint: 31 mg/kg/d, upper 95% CI: 38 mg/kg/d 6. Breakpoint: 26 mg/kg/d, upper 95% CI: 72 mg/kg/d 7. Breakpoint: 31 mg/kg/d, upper 95% CI: 38 mg/kg/d	
***Lysine requirement estimate** **1. $F^{13}CO_2$ (follicular)** **2. $F^{13}CO_2$ (luteal)**	Kriengsinyos, 2004[67] (15308475) RCT (n= 5; 35 follicular phase) (n=5; 35 luteal phase) Data obtained following each study d (d 3)	Canada NR 33.6 yr 100%	1.0 g/kg/d 10-60 mg/kg/d lysine	1. Breakpoint: 35 mg/kg/d, upper 95% CI: 47.9 mg/kg/d 2. Breakpoint: 37.7 mg/kg/d, upper 95% CI: 43.6 mg/kg/d	Insufficient

Abbreviations: CI = confidence interval; d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; h = hour; IAAB = indicator amino acid balance; IAAO = indicator amino acid oxidation; mg/kg/d = milligram per kilogram per day; N = number; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year

*The breakpoint was significantly different between the luteal and follicular phase.

**Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.15, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Other ($F^{13}CO_2$, Lysine Balance, Lysine Oxidation)

Evidence was insufficient to draw conclusions about the $F^{13}CO_2$, lysine balance, and lysine oxidation data. Table 34 provides a summary of findings.

Table 34. Summary of findings for adults 19-50 years (RCT): $F^{13}CO_2$, lysine balance, lysine oxidation

Outcome Comparisons	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Direction of effect	Strength of Evidence*
$F^{13}CO_2$ **1. 8-h adaptation vs 3-d adaptation vs 7-d adaptation** **2. 5 mg/kg/d lysine vs 20 mg/kg/d lysine vs 35 mg/kg/d lysine vs 70 mg/kg/d lysine**	Elango, 2009[48] (193639367) RCT (n= 5; 60) 7-d period with 8-h adaptation, 3-d adaptation, 7-d adaptation	Canada NR 23.6 yr 0%	1.0 g/kg/d 5-70 mg/kg/d lysine	8-h (µmol/kg/h): 5: 0.465 ± 0.093 20: 0.365 ± 0.129 35: 0.412 ± 0.097 70: 0.316 ± 0.086 3-d (µmol/kg/h): 5: 0.495 ± 0.096 20: 0.338 ± 0.030 35: 0.346 ± 0.084 70: 0.305 ± 0.123 7-d (µmol/kg/h): 5: 0.511 ± 0.039 20: 0.317 ± 0.096 35: 0.358 ± 0.086 70: 0.280 ± 0.086 8h-7d (µmol/kg/h): 5: 0.490 ± 0.077 20: 0.340 ± 0.090 35: 0.372 ± 0.088 70: 0.300 ± 0.094	1. No difference 2. Higher oxidation at lower lysine intake	Insufficient
24-h Lysine balance 1. **LL vs IL** **24-h Lysine oxidation**	El-Khoury, 2000[47] (10871570) non-RCT	United States NR LL: 21 yr IL: 23 yr	1.0 g/kg/d LL: I: 14-15 mg/kg/d lysine	24-h Lysine balance (mg/kg/d): LL: -12.4 ± 9.2 IL: 1.8 ± 17.7	24-h Lysine balance: 1. Significant difference between IL and LL and LL was	24-h Lysine balance: insufficient 24-h Lysine oxidation: insufficient

Outcome Comparisons	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Direction of effect	Strength of Evidence*
1. LL vs IL	(n= 11 (LL: n=5 , IL: n=6) Data obtained following study d (d 7)	LL: 60% IL: 0%	A: 15.53 ± 0.53 mg/kg/d lysine IL: I: 28-29 mg/kg/d lysine A: 29.1 ± 0.24 mg/kg/d lysine	24-h Lysine oxidation (mg/kg/d) LL: 27.9 ± 8.8 IL: 27.3 ± 17.6	significantly different from zero 24-h Lysine oxidation: 1. No difference	

Abbreviations: A = actual; d = day; $F^{13}CO_2$ = rate of $F^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; h = hour; I = intended; IL = intermediate lysine; LL = low lysine; mg/kg/d = milligram per kilogram per day; N = number; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year

Note: Values reported as mean ± standard deviation

*Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.16 and I.17, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was, in some instances, imprecise due to challenges with evaluating precision.

Adults 51->70 years

No studies in the analytic set examined the lysine requirement estimate.

Methionine

Infants

Study Characteristics and Findings of Analytic Set

Table 35 summarizes the study characteristics of the analytic set. One study addressed the question of the average daily methionine requirement for infants. [59]

Table 35. Basic characteristics of RCT analytic set: infants

Characteristic	Information
Total studies	1 study
Location of studies	1 study in China
Design of studies	1 study RCT parallel
Settings	1 study initially admitted to the hospital
Age range (average)	13 d (average gestational age 39 wk)
Sex of study participants	1 study with both females and males
Sample size	33
Intervention Duration	1 study 1 adaptation d, 1 study d (2 d total)
Outcomes evaluated	1 study methionine requirement estimate ($F^{13}CO_2$)

Characteristic	Information
Risk of bias	1 study moderate risk

Abbreviations: d = day; $F^{13}CO_2$ = the fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; RCT = randomized controlled trial; wk = week

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Diet Intervention and Experimental Methods

In this study, [59] participants underwent a 24-hour adaptation period and 1 study day (3.0 g/kg/d average protein intake). Participants received one methionine intake ranging from 3-59 mg/kg/d and cysteine was provided in excess (91 mg/kg/d). The IAAO method was performed, and the methionine requirement estimate was calculated using a biphasic linear regression analysis on the $F^{13}CO_2$ data.

Findings

Methionine Requirement Estimate

Evidence was insufficient to draw conclusions about the methionine requirement estimate for infants. Table 36 provides a summary of findings.

Table 36. Summary of findings for infants: methionine requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence*
Methionine requirement estimate **1. $F^{13}CO_2$**	Huang, 2012[59] (22492372) RCT (n=33; 33) Data obtained following each study d (d 2)	China NR 13 d 27%	3.0 g/kg/d 3-59 mg/kg/d methionine; 91 mg/kg/d cysteine	1. Breakpoint: 38 mg/kg/d, upper 95% CI: 48 mg/kg/d	Insufficient

Abbreviations: CI = confidence interval; d = day; $F^{13}CO_2$ = The fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; mg/kg/d = milligram per kilogram per day; N = number; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial

*Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.18, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Children and Adolescents

Study Characteristics and Findings of Analytic Set

Table 37 summarizes the study characteristics of the analytic set. Two studies addressed the question of the average daily methionine requirement in children and adolescents. [63, 103]

Table 37. Basic characteristics of RCT analytic set: children and adolescents

Characteristic	Information
Total studies	2 studies
Location of studies	2 studies in Canada
Design of studies	2 studies RCT cross over
Settings	2 studies outpatient/community-dwelling
Age range (average)	9.1 to 9.4 yr
Sex of study participants	2 studies with both females and males
Sample size	6
Intervention Duration	2 studies 2 adaptation d, 1 study d (3 d total) per test intake
Outcomes evaluated	1 study methionine requirement estimate ($F^{13}CO_2$) 1 study total sulfur amino acid requirement estimate ($F^{13}CO_2$)
Risk of bias	2 studies moderate risk

Abbreviations: d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; NR = not reported; RCT = randomized controlled trial; yr = year

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Diet Intervention and Experimental Methods

Methionine intake ranges and cysteine intake provided differed between studies.

In both studies, [63, 103] participants underwent 2 adaptation days and 1 study day (1.5 g/kg/d protein) per test intake. In one study, [63] participants received six different methionine intakes ranging from 0-15 mg/kg/d and 21 mg/kg/d cysteine and in the other study, [103] participants received six different methionine intakes ranging from 0-35 mg/kg/d and 0 mg/kg/d cysteine. In both studies, [63, 103] the IAAO method was performed, and the methionine requirement estimate was calculated using a biphasic linear regression crossover model on the $F^{13}CO_2$ data.

Findings

Methionine Requirement Estimate

Evidence was insufficient to draw conclusions about the methionine requirement estimate for children and adolescents. Table 38 provides a summary of the findings.

Table 38. Summary of findings for children and adolescents: methionine requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence*
Methionine requirement estimate **1. $F^{13}CO_2$**	Humayun, 2006[63] (17093160) RCT (n=6; 36) Data obtained each study d (d 3)	Canada NR 9.4 yr 16.6%	1.5 g/kg/d 0-15 mg/kg/d methionine; 12 mg/kg/d cysteine	1. Breakpoint: 5.8 mg/kg/d, upper 95% CI 7.3 mg/kg/d	Insufficient

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence*
Total sulfur amino acid requirement estimate **1. $F^{13}CO_2$**	Turner, 2006[103] (16522909) RCT (n=6; 36) Data obtained following each study d (d 3)	Canada NR 9.1 yr 17%	1.5 g/kg/d 0-35 mg/kg/d methionine; 0 mg/kg/d cysteine	1. Breakpoint: 12.9 mg/kg/d, upper 95% CI: 17.2 mg/kg/d	Insufficient

Abbreviations: CI = confidence interval; d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; mg/kg/d milligram per kilogram per day; N = number; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year

*Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.19, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Pregnancy

No studies in the analytic set examined the methionine requirement estimate.

Adults 19-50 years

Study Characteristics and Findings of Analytic Set

Table 39 and 40 summarizes the characteristics of the analytic set. Five studies addressed the question of the average daily methionine requirement for adults 19-50 years. [45, 46, 62, 74, 75] Of these, four studies[45, 46, 74, 75] reported a methionine requirement estimate and one did not and is summarized separately. [62]

Table 39. Basic characteristics of RCT analytic set: adults 19-50 years; that report a methionine requirement estimate

Characteristic	Information
Total studies	4 studies
Location of studies	2 studies in Canada 2 studies in India
Design of studies	4 studies RCT cross over
Settings	4 studies outpatient/community-dwelling
Age range (average)	20.6 to 29 yr
Sex of study participants	4 studies with only males
Sample size range	6 to 42
Intervention Duration	2 studies 2 adaptation d, 1 study d (3 d total) per test intake 2 studies 6 adaptation d, 1 study d (7 d total) per test intake
Outcomes evaluated	2 studies methionine requirement estimate ($F^{13}CO_2$, 24-h IAAO, 24-h IAAB) 2 studies total sulfur amino acid requirement estimate ($F^{13}CO_2$, 24-h IAAO, 24-h IAAB)
Risk of bias	4 studies moderate risk

Abbreviations: d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ release from tracer oxidation [tracer; phenylalanine]; h = hour; IAAB = indicator amino acid balance; IAAO = indicator amino acid oxidation; RCT = randomized controlled trial; yr = year

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Table 40. Basic characteristics of RCT analytic set: adults 19-50 years; that do not report a methionine requirement estimate

Characteristic	Information
Total studies	1 study
Location of studies	1 study in Canada
Design of studies	1 study RCT cross over
Settings	1 study outpatient/community-dwelling
Age range (average)	26.5 yr
Sex of study participants	1 study with only males
Sample size	7
Intervention Duration	1 study 2 adaptation d, 1 study d (3 d total) per test intake
Outcomes evaluated	1 study phenylalanine oxidation
Risk of bias	1 study moderate risk

Abbreviations: d = day; RCT = randomized controlled trial; yr = year

Diet Intervention and Experimental Methods

Methionine intake ranges, amount of cysteine provided, and methods used to calculate the methionine requirement estimates differed between studies. Two RCTs[45, 46] used the IAAO method to calculate the methionine or total sulfur amino acid requirement estimate whereas two other RCTs used the 24-h IAAO and 24-h IAAB. [74, 75]

In two RCTs, [45, 46] participants underwent 2 adaptation days and 1 study day (1.0 g/kg/d) per test intake. In one RCT, [45] participants received six different methionine intakes ranging from 0-13 mg/kg/d and 21 mg/kg/d cysteine. In the other RCT, [46] participants received six different methionine intakes ranging from 0-32 mg/kg/d and 0 mg/kg/d cysteine. In both studies, [45, 46] the IAAO method was performed, and the methionine and total sulfur amino acid requirement estimates were calculated using a 2-phase linear regression crossover model on the $F^{13}CO_2$ data.

In two other RCTs, [74, 75] participants underwent 6 adaptation days and 1 study day (1.0 g/kg/d protein) per test intake. In one RCT, [75] participants received three different methionine intakes ranging from 3-24 mg/kg/d and either 5 or 12 mg/kg/d cysteine. In the other RCT, [74] participants received three different methionine intakes ranging from 3-24 mg/kg/d and 0 mg/kg/d cysteine. In both studies, the 24-h IAAO and 24-h IAAB method were measured, and the methionine and total sulfur amino acid requirement estimate was calculated using a 2-phase linear regression model on the 24-h IAAO and 24-h IAAB data.

The one RCT[62] that did not report a methionine requirement estimate, used the IAAO method to calculate the phenylalanine oxidation of various methionine intakes from a crystalline amino acid mixture (20-70% of the total sulfur amino acid requirement provided), casein and soy protein isolate (40-70% of the total sulfur amino acid requirement provided).

Findings

Methionine Requirement Estimate

Evidence was insufficient to draw conclusions about the methionine or total sulfur amino acid requirement estimate for adults 19-50 years. Table 41 provides a summary of findings.

Table 41. Summary of findings for adults 19-50 years: methionine requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence**
Methionine requirement estimate **1. $F^{13}CO_2$**	Di Buono, 2001[45] (11722957) RCT (n=6; 36) Data obtained following each study d (d 3)	Canada NR 26.3 yr 0%	1.0 g/kg/d 0-13 mg/kg/d methionine; 21 mg/kg/d cysteine	1. Breakpoint: 4.5 mg/kg/d, upper 95% CI: 10.1 mg/kg/d	Insufficient
***Methionine requirement estimate** **1. 24-h IAAO (5 mg/kg/d cysteine)** **2. 24-h IAAB (5 mg/kg/d cysteine)** **3. 24-h IAAO (12 mg/kg/d cysteine)** **4. 24-h IAAB (12 mg/kg/d cysteine)**	Kurpad, 2004[75] (15585764) RCT (5 mg/kg/d cysteine: n=21; 63) (12 mg/kg/d cysteine: n=21; 63) Data obtained following each study d (d 7)	India NR 5 mg/kg/d cysteine: 22 yr 12 mg/kg/d cysteine: 21.7 yr 0%	1.0 g/kg/d 5 mg/kg/d cysteine: 3-24 mg/kg/d methionine 12 mg/kg/d cysteine: 3-24 mg/kg/d methionine	1. Breakpoint: 20 mg/kg/d, upper 95% CI: 26 mg/kg/d 2. Breakpoint: 20 mg/kg/d, upper 95% CI: 25 mg/kg/d 3. Breakpoint: 10 mg/kg/d, upper 95% CI: 16 mg/kg/d 4. Breakpoint: 10 mg/kg/d, upper 95% CI: 16 mg/kg/d	Insufficient
Total sulfur amino acid requirement estimate **1. $F^{13}CO_2$**	Di Buono, 2001[46] (11722956) RCT (n=6; 36) Data obtained following each study d (d 3)	Canada NR 29 yr 0%	1.0 g/kg/d 0-32 mg/kg/d methionine; 0 mg/kg/d cysteine	1. Breakpoint: 12.6 mg/kg/d, upper 95% CI: 21 mg/kg/d	Insufficient
Total sulfur amino acid requirement estimate **1. 24-h IAAO**	Kurpad, 2003[74] (12716672) RCT (n=21; 63)	India NR 20.6 yr 0%	1.0 g/kg/d 3-24 mg/kg/d methionine; 0 mg/kg/d cysteine	1. Breakpoint: 14 mg/kg/d, upper 95% CI: 23 mg/kg/d 2. Breakpoint: 15 mg/kg/d,	Insufficient

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence**
2. 24-h IAAB	Data obtained following each study d (d 7)			upper 95% CI 27 mg/kg/d	

Abbreviations: CI = confidence interval; d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ release from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; h = hour; IAAB = indicator amino acid balance; IAAO = indicator amino acid oxidation; mg/kg/d = milligram per kilogram per day; N = number; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year

*Estimated requirements significantly differed between the 5 mg/kg/d and 12 mg/kg/d cysteine groups[75] but not from the 0 mg/kg/d group. [74]

**Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.20, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Other (Phenylalanine Oxidation)

Evidence was insufficient to draw conclusions about the phenylalanine oxidation data. Table 42 provides a summary of the findings.

Table 42. Summary of findings for adults 19-50 years (RCT): phenylalanine oxidation

Outcome Comparisons	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/RaceMean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Direction of effect	Strength of Evidence*
Phenylalanine oxidation **1. Intakes of crystalline AA mix at 20-70% TSAA requirement** **2. Intakes of casein at 40-70% TSAA requirement** **3. Intakes of SPI at 40-70% TSAA requirement**	Humayun, 2007[62] (17634258) RCT (n= 7; 91) Data obtained following each study d (d 3)	Canada NR 26.5 yr 0%	1.0 g/kg/d AA mix at 20-70% TSAA requirement Casein at 40-70% TSAA requirement SPI at 40-70% TSAA requirement	¥	1. Linear decrease 2. No change 3. No change	Insufficient

Abbreviations: AA = amino acid; d = day; g/kg/d = grams per kilogram per day; N = number; PMID = PubMed Identification Number; RCT = randomized controlled trial; SPI = soy protein isolate; TSAA = total sulfur amino acids; yr = year

¥Data reported in figures of original paper.

*Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.21, the main reasons for this insufficient rating were that 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.

Adults 51->70 years

No studies in the analytic set examined the methionine requirement estimate.

Phenylalanine

Infants

Study Characteristics and Findings of Analytic Set

Table 43 summarizes the study characteristics of the analytic set. One study addressed the question of the average daily phenylalanine requirement in infants. [54]

Table 43. Basic characteristics of RCT analytic set: infants

Characteristic	Information
Total studies	1 study
Location of studies	1 study in China
Design of studies	1 study RCT parallel
Settings	1 study initially admitted to the hospital
Age range (average)	13 d (average gestational age 38.9 wk)
Sex of study participants	1 study with both females and males
Sample size	20
Intervention Duration	1 study 1 adaptation d, 1 study d (2 d total)
Outcomes evaluated	1 study phenylalanine requirement estimate ($F^{13}CO_2$)
Risk of bias	1 study moderate risk

Abbreviations: d = day; $F^{13}CO_2$ = the fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; lysine]; RCT = randomized controlled trial; wk = week

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Diet Intervention and Experimental Methods

In this study, [54] participants underwent a 24-hour adaptation period and 1 study day (2.96 g/kg/d protein). Participants received one phenylalanine intake ranging from 5-166 mg/kg/d and excess tyrosine (166 mg/kg/d). The IAAO method was performed, and the phenylalanine requirement estimate was calculated using a nonlinear regression model on the $F^{13}CO_2$ data.

Findings

Phenylalanine Requirement Estimate

Evidence was insufficient to draw conclusions about the phenylalanine requirement estimate for infants. Table 44 provides a summary of findings.

Table 44. Summary of findings for infants: phenylalanine requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence*
Phenylalanine requirement estimate **1. $F^{13}CO_2$**	Hogewind-Schoonenboom, 2015[54] (25926506) RCT (n=20; 20) Data obtained following each study d (d 2)	Canada NR 13 d 55%	2.96 g/kg/d 5-166 mg/kg/d phenylalanine; 166 mg/kg/d tyrosine	1. Breakpoint: 58 mg/kg/d, upper 95% CI: 78 mg/kg/d	Insufficient

Abbreviations: CI = confidence interval; d = day; $F^{13}CO_2$ = fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; lysine]; g/kg/d = grams per kilogram per day; mg/kg/d = milligram per kilogram per day; N = number; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial

*Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.22, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Children and Adolescents

Study Characteristics and Findings of Analytic Set

Table 45 summarizes the study characteristics of the analytic set. One study addressed the question of the average daily aromatic amino acid requirement for children and adolescents. [55]

Table 45. Basic characteristics of RCT analytic set: children and adolescents

Characteristic	Information
Total studies	1 study
Location of studies	1 study in Canada
Design of studies	1 study RCT cross over
Settings	1 study outpatient/community-dwelling
Age range (average)	9.1 yr
Sex of study participants	NR
Sample size	5
Intervention Duration	1 study 2 adaptation d, 1 study d (3 d total) per test intake
Outcomes evaluated	1 study aromatic AA requirement estimate ($F^{13}CO_2$)
Risk of bias	1 study moderate risk

Abbreviations: AA = amino acid; d = day; $F^{13}CO_2$=rate of $^{13}CO_2$ released from tracer oxidation [tracer; lysine]; NR = not reported; RCT = randomized controlled trial; yr = year

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Diet Intervention and Experimental Methods

In this study, [55] participants underwent 2 adaptation days and 1 study day (1.5 g/kg/d) per test intake. Participants received eight different phenylalanine intakes ranging from 5-70 mg/kg/d and 0 mg/kg/d tyrosine. The IAAO method was performed, and the aromatic amino acid requirement estimate was calculated using a two-phase linear regression crossover model on the $F^{13}CO_2$ data.

Findings

Phenylalanine Requirement Estimate

Evidence was insufficient to draw conclusions about the aromatic amino acid requirement estimate for children and adolescents. Table 46 provides a summary of findings.

Table 46. Summary of findings for the aromatic amino acid requirements for children and adolescents

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence**
***Aromatic amino acid requirement estimate 1. $F^{13}CO_2$**	Hsu, 2007[55] (17314698) RCT (n=5; 40) Data obtained following each study d (d 3)	Canada NR 9.1 yr NR	1.5 g/kg/d 5-70 mg/kg/d phenylalanine; 0 mg/kg/d tyrosine	1. Breakpoint: 28 mg/kg/d, upper 95% CI: NR	Insufficient

Abbreviations: CI = confidence interval; d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; lysine]; g/kg/d = grams per kilogram per day; mg/kg/d = milligram per kilogram per day; N = number; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year

*Hsu et al. [55] note that this is a biologically implausible value.

**Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.23, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Pregnancy

Study Characteristics and Findings of Analytic Set

Table 47 summarizes the study characteristics of the analytic set. Two studies addressed the question of the average daily phenylalanine requirement for pregnant people (13-19 weeks early gestation, 33-39 weeks late gestation). [51, 52] Of these, one calculated the phenylalanine requirement estimate[52] while the other calculated the aromatic amino acid requirement estimate. [51]

Table 47. Basic characteristics of RCT analytic set: pregnant people

Characteristic	Information
Total studies	2 studies
Location of studies	2 studies in Canada
Design of studies	2 studies RCT cross over
Settings	2 studies outpatient/community-dwelling
Age range (average)	29.3 to 32.3 yr
Sex of study participants	2 studies with only females
Sample size range	19 to 23*
Intervention Duration	2 studies 2 adaptation d, 1 study d (3 d total) per test intake
Outcomes evaluated	1 study phenylalanine requirement estimate ($F^{13}CO_2$) 1 study aromatic AA requirement estimate ($F^{13}CO_2$)
Risk of bias	2 studies low risk

Abbreviations: AA = amino acid; d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; leucine or phenylalanine]; RCT = randomized controlled trial; yr = year

*One participant was studied in both early and late gestation in one study[51] and five participants were studied in both early and late gestation in another study. [52]

Diet Intervention and Experimental Methods

Phenylalanine intake ranges, amount of tyrosine provided, and methods used to calculate the phenylalanine requirement estimates differed between studies.

In one RCT, [52] participants underwent 2 adaptation days and 1 study day (1.5 g/kg/d protein) per test intake. Participants received ≤ 6 different phenylalanine intakes ranging from 5.5-30.5 mg/kg/d (DAAO) and 2.5-30.5 mg/kg/d (IAAO) and excess tyrosine (61 mg/kg/d). The DAAO method was performed in both early and late gestation and the IAAO method was performed in late gestation and the phenylalanine requirement estimate was calculated using a bi-phase linear regression crossover analysis on the $F^{13}CO_2$ data.

In the other RCT, [51] participants underwent 2 adaptation days and 1 study day (1.5 g/kg/d protein) per test intake. Participants received ≤ 6 different phenylalanine intakes ranging from 5-100 mg/kg/d and 0 mg/kg/d tyrosine. The IAAO method was performed, and the aromatic amino acid requirement estimate was calculated using a bi-phase linear regression crossover analysis on the $F^{13}CO_2$ data.

Findings

Phenylalanine Requirement Estimate

Evidence was insufficient to draw conclusions about the phenylalanine or aromatic amino acid requirement estimates for pregnant people. Table 48 provides a summary of findings.

Table 48. Summary of findings for pregnant people: phenylalanine requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence**
***Phenylalanine requirement estimate**	Ennis, 2020[52] (31758682)	Canada NR	1.5 g/kg/d	1. Breakpoint: 15.14 mg/kg/d,	Insufficient

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence**
1. $F^{13}CO_2$ (EG, DAAO) **2. $F^{13}CO_2$ (LG, DAAO)** **3. $F^{13}CO_2$ (LG, IAAO)**	RCT (EG, DAAO: n= 9; 26) (LG, DAAO: n= 9; 25) (LG, IAAO: n= 13; 25) Data obtained following each study d (d 3)	 EG, DAAO: 29.3 yr LG, DAAO: 29.5 yr LG, IAAO: 30.9 yr 100%	EG and LG, DAAO: 5.5-30.5 mg/kg/d phenylalanine; 61 mg/kg/d tyrosine LG, IAAO: 2.5-30.5 mg/kg/d phenylalanine, 61 mg/kg/d tyrosine	upper 95% CI: 19.9 mg/kg/d 2. Breakpoint: 21.05 mg/kg/d, upper 95% CI: 24.7 mg/kg/d 3. Breakpoint: 21.36 mg/kg/d, upper 95% CI: 32.2 mg/kg/d	
***Aromatic amino acid requirement estimate** **1. $F^{13}CO_2$ (EG)** **2. $F^{13}CO_2$ (LG)**	Ennis, 2020[51] (33188409) RCT (EG: n= 10; 24) (LG: n= 10; 27) Data obtained following each study d (d 3)	Canada NR EG: 32.3 yr LG: 30.0 yr 100%	1.5 g/kg/d 5-100 mg/kg/d phenylalanine; 0 mg/kg/d tyrosine	1. Breakpoint: 43.57 mg/kg/d, upper 95% CI: 58.8 mg/kg/d 2. Breakpoint: 49.56 mg/kg/d, upper 95% CI: 63.1 mg/kg/d	Insufficient

Abbreviations: CI = confidence interval; d = day; DAAO = direct amino acid oxidation; EG = early gestation; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; leucine or phenylalanine]; g/kg/d = grams per kilogram per day; IAAO = indicator amino acid oxidation; LG = late gestation; mg/kg/d = milligram per kilogram per day; N = number; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year

*There was a significant difference in the mean requirement estimate by gestational stage in both studies.

**Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.24, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Adults 19-50 years

Study Characteristics and Findings of Analytic Set

Table 49 summarizes the study characteristics of the analytic set. Three studies addressed the question of the average daily phenylalanine requirements in adults 19-50 years. [56, 57, 73] All were performed with the absence of tyrosine and thus calculate the aromatic amino acid requirement estimates.

Table 49. Basic characteristics of RCT analytic set: adults 19-50 years

Characteristic	Information
Total studies	3 studies
Location of studies	2 studies in Canada 1 study in India
Design of studies	3 studies RCT cross over
Settings	3 studies outpatient/community-dwelling

Characteristic	Information
Age range (average)	21.8 to 30.4 yr
Sex of study participants	3 studies with only males
Sample size range	5 to 32
Intervention Duration	1 study 6 adaptation d, 1 study d (7 d total) per test intake 2 studies 2 adaptation d, 1 study d (3 d total) per test intake
Outcomes evaluated	3 studies aromatic amino acid requirement estimate (24-h IAAO, 12-h fed IAAO, 24-h IAAB, $F^{13}CO_2$)
Risk of bias	3 studies moderate risk

Abbreviations: d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer leucine or lysine]; h = hour; IAAB = indicator amino acid balance; IAAO = indicator amino acid oxidation; RCT = randomized controlled trial; yr = year

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Diet Intervention and Experimental Methods

Phenylalanine intake ranges and methods used to calculate the phenylalanine requirement estimates differed between studies.

In one RCT, [73] participants underwent 6 adaptation days and 1 study day (160 mg/kg/d nitrogen, 1.0 g/kg/d protein) per test intake. Participants received two different phenylalanine intakes ranging from 19-47 mg/kg/d and 0 mg/kg/d tyrosine. The 24-h IAAO and 24-h IAAB methods were measured, and the aromatic amino acid requirement was calculated using a 2-phase linear random-effects regression model on the 24-h IAAO, 12-h fed IAAO, and 24-h IAAB data.

In another RCT, [56] participants underwent 2 adaptation days and 1 study day (1.0 g/kg/d protein) per test intake. Participants received eight different phenylalanine intakes except one who received seven ranging from 5-70 mg/kg/d and 0 mg/kg/d tyrosine. The IAAO method was performed, and the aromatic amino acid requirement was calculated using a 2-phase linear regression crossover model on the $F^{13}CO_2$ data.

In the other RCT, [57] participants underwent 2 adaptation days and 1 study day (1.0 g/kg/d protein) per test intake. Participants received seven different phenylalanine intakes ranging from 5-60 mg/kg/d in part A and 5-65 mg/kg/d in part B with 0 mg/kg/d tyrosine. The IAAO method was performed, and the aromatic amino acid requirement was calculated using a 2-phase linear regression crossover model on the $F^{13}CO_2$ data.

Findings

Phenylalanine Requirement Estimate

Evidence was insufficient to draw conclusions about the aromatic amino acid requirement estimate for adults 19-50 years. Table 50 provides a summary of findings.

Table 50. Summary of findings for adults 19-50 years: aromatic amino acid requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence**
Aromatic amino acid requirement estimate	Hsu, 2006[56] (16400054) RCT	Canada NR	1.0 g/kg/d	1. Breakpoint: 47.73 mg/kg/d, upper 95% CI: NR	Insufficient

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence**
1. $F^{13}CO_2$ (model 5vs 3)* **2. $F^{13}CO_2$ (model 6vs 2)***	(n=5; 39) Data obtained following each study d (d 3)	29.4 yr 0%	5-70 mg/kg/d phenylalanine; 0 mg/kg/d tyrosine	2. Breakpoint: 51.71 mg/kg/d, upper 95% CI: NR	
Aromatic amino acid requirement estimate **1. $F^{13}CO_2$ (part A)** **2. $F^{13}CO_2$ (part B)**	Hsu, 2006[57] (16549457) RCT (Part A: n=5; 35) (Part B: n=5; 35) Data obtained following each study d (d 3)	Canada NR Part A: 29.4 yr Part B: 30.4 yr 0%	1.0 g/kg/d Part A: 5-60 mg/kg/d phenylalanine; 0 mg/kg/d tyrosine Part B: 5-65 mg/kg/d phenylalanine; 0 mg/kg/d tyrosine	1. Breakpoint: ND mg/kg/d, upper 95% CI: ND 2. Breakpoint: 41.9 mg/kg/d, upper 95% CI: NR	Insufficient
Aromatic amino acid requirement estimate **1. 24-h IAAO** **2. 12-h fed IAAO** **3. 24-h IAAB**	Kurpad, 2006[73] (16762944) RCT (n=32; 64) Data obtained following each study d (d 7)	India NR 21.8 yr 0%	1.0 g/kg/d 19-47 mg/kg/d phenylalanine; 0 mg/kg/d tyrosine	1. Breakpoint: 37 mg/kg/d, upper 95% CI: >47 mg/kg/d 2. Breakpoint: 36 mg/kg/d, upper 95% CI: >47 mg/kg/d 3. Breakpoint: 38 mg/kg/d, upper 95% CI: >47 mg/kg/d	Insufficient

Abbreviations: CI = confidence interval; d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer leucine or lysine]; g/kg/d = grams per kilogram per day; h = hour; IAAB = indicator amino acid balance; IAAO = indicator amino acid oxidation; mg/kg/d = milligram per kilogram per day; N = number; NR = not reported; ND = unable to be determined; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year

*Model 5vs3=5 phenylalanine intakes on one line and 3 phenylalanine intakes on the other, Model 6vs2= 6 phenylalanine intakes on one line and 2 phenylalanine intakes on the other

**Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.25, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Adults 51->70 years

Study Characteristics and Findings of Analytic Set

Table 51 summarizes the study characteristics of the analytic set. One study addressed the question of the average daily phenylalanine requirements in adults 51->70 years. [82]

Table 51. Basic characteristics of RCT analytic set: adults 51->70 years

Characteristic	Information
Total studies	1 study
Location of studies	1 study in Canada
Design of studies	1 study RCT cross over
Settings	1 study outpatient/community-dwelling
Age range (average)	70.8 to 76.7 yr
Sex of study participants	1 study with both females and males
Sample size	12
Intervention Duration	1 study 2 adaptation d, 1 study d (3 d total) per test intake
Outcomes evaluated	1 study phenylalanine requirement estimate ($F^{13}CO_2$)
Risk of bias	1 study moderate risk

Abbreviations: d = day; $F^{13}CO_2$ = Rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; RCT = randomized controlled trial; yr = year

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Diet Intervention and Experimental Methods

In this study, [82] participants underwent 2 adaptation days and 1 study day (1.0 g/kg/d protein) per test intake. Participants received 3-7 different phenylalanine intakes ranging from 7.2-40 mg/kg/d and excess tyrosine (40 mg/kg/d). The DAAO method was performed, and the phenylalanine requirement estimate was calculated using a mixed model 2-phase linear regression on the $F^{13}CO_2$ data.

Findings

Phenylalanine Requirement Estimate

Evidence was insufficient to draw conclusions about the phenylalanine requirement estimate for adults 51->70 years. Table 52 provides a summary of findings.

Table 52. Summary of findings for adults 51->70 years: phenylalanine requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence**
***Phenylalanine requirement estimate** **1. $F^{13}CO_2$ (men)** **2. $F^{13}CO_2$ (women)** **3. $F^{13}CO_2$ (combined)**	Martin, 2019[82] (31271193) RCT (n= 12; 66) Data obtained following each study d (d 3)	Canada NR M: 70.8 yr F: 76.7 yr 50%	1.0 g/kg/d 7.2-40 mg/kg/d phenylalanine; 40 mg/kg/d tyrosine	1. BW basis: Breakpoint: 9.3 mg/kg/d and 11.9 mg/kg FFM/d, upper 95% CI: NR 1. FFM basis: Mean requirement: 11.9 ± 0.94 mg/kg FFM/d 2. BW basis: Breakpoint: 8.4	Insufficient

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence**
				mg/kg/d and 12.8 mg/kg FFM/d, upper 95% CI: NR 2. FFM basis: Mean requirement: 12.8 ± 0.94 mg/kg FFM/d 3. Breakpoint: 9.03 mg/kg/d, upper 95% CI: 15.9 mg/kg/d	

Abbreviations: CI = confidence interval; d = day; FFM = fat free mass; $F^{13}CO_2$ = Rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; mg/kg/d = milligram per kilogram per day; N = number; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year

*There was no difference in the breakpoint between men and women on a body weight or fat free mass basis. Mean requirement ± standard deviation

**Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.26, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Threonine

Infants

Study Characteristics and Findings of Analytic Set

Table 53 summarizes the study characteristics of the analytic set. One study addressed the question of the average daily threonine requirement for infants. [53]

Table 53. Basic characteristics of RCT analytic set: infants

Characteristic	Information
Total studies	1 study
Location of studies	1 study in China
Design of studies	1 study RCT parallel
Settings	1 study initially admitted to the hospital
Age range (average)	10 d (average gestational age 39 wk)
Sex of study participants	1 study with both females and males
Sample size	32
Intervention Duration	1 study 1 adaptation d, 1 study d (2 d total)
Outcomes evaluated	1 study threonine requirement estimate ($F^{13}CO_2$)

Characteristic	Information
Risk of bias	1 study moderate risk

Abbreviations: d = day; $F^{13}CO_2$ = the fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; RCT = randomized controlled trial; wk = week

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Diet Intervention and Experimental Methods

In this study, [53] participants underwent a 24-h adaptation period and 1 study day (2.96 g/kg/d protein). Participant received one threonine intake ranging from 5-182 mg/kg/d. The IAAO method was performed, and the threonine requirement estimate was calculated using a biphasic linear regression crossover model on the $F^{13}CO_2$ data.

Findings

Threonine Requirement Estimate

Evidence was insufficient to draw conclusions about the threonine requirement estimate for infants. Table 54 provides a summary of findings.

Table 54. Summary of findings for infants: threonine requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence*
Threonine requirement estimate **1. $F^{13}CO_2$**	Hogewind-Schoonenboom, 2015[53] (25844708) RCT (n=32; 32) Data obtained following each study d (d 2)	China NR 10 d 40.6%	2.96 g/kg/d 5-182 mg/kg/d threonine	1. Breakpoint: 68 mg/kg/d, upper 95% CI: 104 mg/kg/d	Insufficient

Abbreviations: CI = confidence interval; d = day; $F^{13}CO_2$ = the fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; mg/kg/d = milligram per kilogram per day; N = number; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial

*Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.27, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Children and Adolescents

No studies in the analytic set examined the threonine requirement estimate.

Pregnancy

No studies in the analytic set examined the threonine requirement estimate.

Adults 19-50 years

Study Characteristics and Findings of Analytic Set

Table 55 summarizes the study characteristics of the analytic set. Two studies addressed the question of the average daily threonine requirements for adults 19-50 years. [71, 105]

Table 55. Basic characteristics of RCT analytic set: adults 19-50 years

Characteristic	Information
Total studies	2 studies
Location of studies	1 study in Canada 1 study in India
Design of studies	2 studies RCT cross over
Settings	2 studies outpatient/community-dwelling
Age range (average)	19.6 to 26.5 yr
Sex of study participants	2 studies with only males
Sample size range	6 to 16
Intervention Duration	1 study 6 adaptation d, 1 study d (7 d total) per test intake 1 study 2 adaptation d (d 1-2, 4-5, and 7-8), 1 study d (d 3, 6, and 9) over 9 d
Outcomes evaluated	2 studies threonine requirement estimate (fasted and fed plasma AA response, 24-h IAAO, 12-h fed IAAO, 24-h IAAB, $F^{13}CO_2$)
Risk of bias	2 studies moderate risk

Abbreviations: AA = amino acid; d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; h = hour; IAAB = indicator amino acid balance; IAAO = indicator amino acid oxidation; RCT = randomized controlled trial; yr = year

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Diet Intervention and Experimental Methods

Threonine intake ranges and methods used to calculate the threonine requirement estimates differed between studies.

In one RCT[71] participants underwent 6 adaptation days and 1 study day (160 mg/kg/d nitrogen, 1.0 g/kg/d protein) per test intake. Participants received three different threonine intakes ranging from 7-27 mg/kg/d. Plasma amino acid response, 24-h IAAO, and 24-h IAAB methods were measured, and the threonine requirement was calculated by using a two-phase linear regression model on the fasted and fed plasma amino acid response, 24-h IAAO, 12-h fed IAAO, and 24-h IAAB data.

In the other RCT[105] participants underwent two 9-day periods consisting of 2 adaptation days (days 1-2, 4-5, 7-8, 47 mg/kg/d threonine, 1.0 g/kg/d protein) and 1 study day (days 3, 6, and 9, 1.0 g/kg/d protein) per test intake. Participants received six different threonine intakes ranging from 5-35 mg/kg/d. The IAAO method was performed, and the threonine requirement was calculated by using a two-phase linear regression crossover model on the $F^{13}CO_2$ data.

Findings

Threonine Requirement Estimate

Evidence was insufficient to draw conclusions about the threonine requirement estimate for adults 19-50 years. Table 56 provides a summary of findings.

Table 56. Summary of findings for adults 19-50 years: threonine requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence*
Threonine requirement estimate **1. Fasted plasma AA response** **2. 3-h fed plasma AA response** **3. 6-h fed plasma AA response** **4. 12-h fed IAAO** **5. 24-h IAAO** **6. 24-h IAAB**	Kurpad, 2002[71] (12324292) RCT (n=16; 48) Data obtained following each study d (d 7)	India NR 19.6 yr 0%	1.0 g/kg/d 7-27 mg/kg/d threonine	1. Breakpoint: 15 mg/kg/d, upper 95% CI: ND 2. Breakpoint: 13 mg/kg/d, upper 95% CI: 18 mg/kg/d 3. Breakpoint: 13 mg/kg/d, upper 95% CI: 19 mg/kg/d 4. Breakpoint: 15 mg/kg/d, upper 95% CI: ND mg/kg/d 5. Breakpoint: 15 mg/kg/d, upper 95% CI: 25 mg/kg/d 6. Breakpoint: 15 mg/kg/d, upper 95% CI: 27 mg/kg/d	Insufficient
Threonine requirement estimate **1. $F^{13}CO_2$**	Wilson, 2000[105] (1070217) RCT (n= 6; 36) Data obtained following each study d (d 3)	Canada NR 26.5 yr 0%	1.0 g/kg/d 5-35 mg/kg/d threonine	1. Breakpoint: 19 mg/kg/d, upper 95% CI: 26.2 mg/kg/d	Insufficient

Abbreviations: AA = amino acid; d = day; CI = confidence interval; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; h = hour; IAAB = indicator amino acid balance; IAAO = indicator amino acid oxidation; mg/kg/d = milligram per kilogram per day; N = number; ND = unable to be determined; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year

*Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.28, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Adults 51->70 years

No studies in the analytic set examined the threonine requirement estimate.

Tryptophan

Infants

Study Characteristics and Findings of Analytic Set

Table 57 summarizes the study characteristics of the analytic set. One study addressed the question of the average daily tryptophan requirement in infants. [60]

Table 57. Basic characteristics of RCT analytic set: infants

Characteristic	Information
Total studies	1 study
Location of studies	1 study in China
Design of studies	1 study RCT parallel
Settings	1 study initially admitted to the hospital
Age range (average)	9 d (average gestational age 39 wk)
Sex of study participants	1 study with both females and males
Sample size	30
Intervention Duration	1 study 1 adaptation d, 1 study d (2 d total)
Outcomes evaluated	1 study tryptophan requirement estimate ($F^{13}CO_2$)
Risk of bias	1 study moderate risk

Abbreviations: d = day; $F^{13C}O_2$ = the fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; RCT = randomized controlled trial; wk = week

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Diet Intervention and Experimental Methods

In this study, [60] participants underwent a 24-h adaptation period and 1 study day (2.96 g/kg/d protein). Participants received one tryptophan intake ranging from 0.5-73 mg/kg/d. The IAAO method was performed, and the tryptophan requirement estimate was calculated using biphasic linear regression crossover model on the $F^{13}CO_2$ data.

Findings

Tryptophan Requirement Estimate

Evidence was insufficient to draw conclusions about the tryptophan requirement estimate for infants. Table 58 provides a summary of findings.

Table 58. Summary of findings for infants: tryptophan requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence*
Tryptophan requirement estimate **1. $F^{13}CO_2$**	Huang, 2014[60] (24824360) RCT (n=30; 30) Data obtained following each study d (d 2)	China NR 9 d 43%	2.96 g/kg/d 0.5-73 mg/kg/d tryptophan	1. Breakpoint: 15 mg/kg/d, upper 95% CI: 31 mg/kg/d	Insufficient

Abbreviations: CI = confidence interval; d = day; $F^{13}CO_2$ = the fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; mg/kg/d = milligram per kilogram per day; N = number; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial

*Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.31, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Children and Adolescents

No studies in the analytic set examined the tryptophan requirement estimate.

Pregnancy

No studies in the analytic set examined the tryptophan requirement estimate.

Adults 19-50 years

No studies in the analytic set examined the tryptophan requirement estimate.

Adults 51->70 years

No studies in the analytic set examined the tryptophan requirement estimate.

Total Branched Chain Amino Acids

Infants

No studies in the analytic set examined the total branched chain amino acids requirement estimate.

Children and Adolescents

Study Characteristics and Findings of Analytic Set

Table 59 summarizes the study characteristics of the analytic set. One study addressed the question of the average daily total branched chain amino acids requirements for children and adolescents. [78]

Table 59. Basic characteristics of RCT analytic set: children and adolescents

Characteristic	Information
Total studies	1 study
Location of studies	1 study in Canada
Design of studies	1 study RCT cross over
Settings	1 study outpatient/community-dwelling
Age range (average)	8.5 yr
Sex of study participants	1 study with both females and males
Sample size	5
Intervention Duration	1 study 2 adaptation d, 1 study d (3 d total) per test intake
Outcomes evaluated	1 study total branched chain amino acid requirement estimate ($F^{13}CO_2$)
Risk of bias	1 study moderate risk

Abbreviations: d = day; $F^{13}CO_2$ = rate of release of $^{13}CO_2$ from tracer oxidation [tracer; phenylalanine]; RCT = randomized controlled trial; yr = year

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Diet Intervention and Experimental Methods

In this study, [78] participants underwent 2 adaptation days and 1 study day (~10% of energy from protein) per test intake. Participants received seven different total branched chain amino acids intakes ranging from 75-225 mg/kg/d. Each intake consisted of 29 percent isoleucine, 38.5 percent leucine, and 32.5 percent valine. The IAAO method was performed, and the total branched chain amino acids requirement estimate was calculated using a two-phase linear regression crossover model on the $F^{13}CO_2$ data.

Findings

Total Branched Chain Amino Acids Requirement Estimate

Evidence was insufficient to draw conclusions about the total branched chain amino acids requirement estimate for children and adolescents. Table 60 provides a summary of findings.

Table 60. Summary of findings for children and adolescents: total branched chain amino acids requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence*
Total branched chain AA requirement estimate **1. $F^{13}CO_2$**	Mager, 2003[78] (14608071) RCT (n=5; 35) Data obtained following each study d (d 3)	Canada NR 8.5 yr 80%	~10% of energy from protein 75-225 mg/kg/d BCAA	1. Breakpoint: 147.3 mg/kg/d, upper 95% CI: 191.5 mg/kg/d	Insufficient

Abbreviations: AA = amino acid; BCAA = branched chain amino acids; CI = confidence interval; d = day; $F^{13}CO_2$=rate of release of $^{13}CO_2$ from tracer oxidation [tracer; phenylalanine]; mg/kg/d = milligram per kilogram per day; N = number; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year

*Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.29, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Pregnancy

No studies in the analytic set examined the total branched chain amino acids requirement estimate.

Adults 19-50 years

Table 61 summarizes the study characteristics of the analytic set. One study addressed the question of the average daily total branched chain amino acids requirements for adults 19-50 years. [96] This study does not report total branched chain amino acids requirement estimates but does report phenylalanine oxidation and $F^{13}CO_2$.

Table 61. Basic characteristics of RCT analytic set: adults 19-50 years

Characteristic	Information
Total studies	1 study
Location of studies	1 study in Canada
Design of studies	1 study RCT cross over
Settings	1 study outpatient/community-dwelling
Age range (average)	26.8 yr
Sex of study participants	1 study with only males
Sample size	5
Intervention Duration	1 study 2 adaptation d, 1 study d (3 d total) per test intake
Outcomes evaluated	1 study $F^{13}CO_2$ 1 study phenylalanine oxidation
Risk of bias	1 study moderate risk

Abbreviations: d = day; $F^{13}CO_2$ = rate of release of $^{13}CO_2$ from tracer oxidation [tracer; phenylalanine]; RCT = randomized controlled trial; yr = year

Diet Intervention and Experimental Methods

In this study, [96] participants underwent 2 adaptation days and 1 study day (1.0 g/kg/d protein) per test intake. Participants received six different total branched amino acids intakes in which isoleucine was held constant and leucine and valine were reduced by 10 and 20 percent, leucine was held constant and isoleucine and valine were reduced by 10 and 20 percent, and valine was held constant and isoleucine and leucine were reduced by 10 and 20 percent. The IAAO method was performed and the effect of varying branched chain amino acids intakes on $F^{13}CO_2$ and phenylalanine oxidation in comparison to the visual breakpoint determined for each participant in a separate study (rated high risk of bias in our systematic review) [97] were compared.

Findings

Other ($F^{13}CO_2$, phenylalanine oxidation)

Evidence was insufficient to draw conclusions about the $F^{13}CO_2$ and phenylalanine oxidation data. Table 62 provides a summary of findings.

Table 62. Summary of findings for adults 19-50 years: $F^{13}CO_2$ and phenylalanine oxidation

Outcome Comparison	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Direction of effect	Strength of Evidence **
$F^{13}CO_2$ **1. Ile con. -10% vs VBP** **2. Ile con. -20% vs VBP** **3. Ile constant-10% vs –20%** **4. Leu con. -10% vs VBP** **5. Leu con. -20% vs VBP** **6. Leu con. -10% vs –20%** **7. Val con. -10% vs VBP** **8. Val con. -20% vs VBP** **9. Val con. -10% vs –20%** **Phenylalanine oxidation** **1. Ile con. -10% vs VBP** **2. Ile con. -20% vs VBP** **3. Ile constant-10% vs –20%**	Riazi, 2003[96] (14608070) RCT (5; 35)* Data obtained following each study d (d 3)	Canada NR 26.8 yr 0%	1.0 g/kg/d Isoleucine provided at requirement and leucine and valine provided at 10 and 20% less than the requirement Leucine provided at requirement and isoleucine and valine provided at 10 and 20% less than the requirement Valine provided at requirement and isoleucine and leucine provided at 10 and 20% less than the requirement	$F^{13}CO_2$(µmol/kg/h) 1. 0.58 ± 0.10; 27.3% MD 2. 0.65 ± 0.08; 42.7% MD 3. NA 4. 0.60 ± 0.09; 31.3% MD 5. 0.61 ± 0.13; 35.2% MD 6. NA 7. 0.58 ± 0.16; 27.3% MD 8. 0.59 ± 0.21; 30.4% MD 9. NA Phenylalanine oxidation (µmol/kg/h) 1. 3.98 ± 0.94; 37.7% MD 2. 4.93 ± 1.11; 70.5% MD 3. NA 4. 4.08 ± 1.70; 41.2% MD 5. 4.79 ± 1.23; 65.7% MD 6. NA 7. 3.94 ± 1.53; 36.3% MD 8. 4.02 ± 0.70; 39% MD 9. NA	$F^{13}CO_2$ 1. No difference 2. Significant increase 3. No difference 4. No difference 5. Significant increase 6. No difference 7. No difference 8. No difference 9. No difference Phenylalanine oxidation 1. No difference 2. Significant increase 3. No difference 4. No difference 5. Significant increase 6. No difference 7. No difference	Insufficient

Outcome Comparison	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Direction of effect	Strength of Evidence **
4. Leu con. -10% vs VBP **5. Leu con. -20% vs VBP** **6. Leu con. -10% vs –20%** **7. Val con. -10% vs VBP** **8. Val con. -20% vs VBP** **9. Val con. -10% vs –20%**					8. No difference 9. No difference	

Abbreviations: d = day; $F^{13}CO_2$ = rate of release of $^{13}CO_2$ from tracer oxidation [tracer; phenylalanine]; Ile = isoleucine; leu = leucine; MD = mean difference; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; val = valine; VBP = visual breakpoint; yr = year

Note: Values shown as mean ± standard deviation

*n=30 total observations were observed in the current study[96] and n=5 observations were from the previous study[97] for the visual breakpoint.

**Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.30, the main reasons for this insufficient rating were that 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.

Adults 51->70 years

No studies in the analytic set examined the total branched chain amino acids requirement estimate.

Valine

Infants

Study Characteristics and Findings of Analytic Set

Table 63 summarizes the study characteristics of the analytic set. One study addressed the question of the average daily valine requirement in infants. [44]

Table 63. Basic characteristics of RCT analytic set: infants

Characteristic	Information
Total studies	1 study
Location of studies	1 study in China
Design of studies	1 study RCT parallel
Settings	1 study initially admitted to the hospital
Age range (average)	15 d
Sex of study participants	1 study with only males
Sample size	28
Intervention Duration	1 study 1 adaptation d, 1 study d (2 d total) per test intake
Outcomes evaluated	1 study valine requirement estimate ($F^{13}CO_2$)
Risk of bias	1 study low risk

Abbreviations: d = day; $F^{13}CO_2$= the fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; RCT = randomized controlled trial

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Diet Intervention and Experimental Methods

In this study, [44] participants underwent a 24-hour adaptation period and one study day (2.96 g/kg/d). Participants received 1 valine intake except for 1 participant who received 2 different intakes ranging from 5-236 mg/kg/d. The IAAO method was performed, and the valine requirement estimate was calculated using a 2-phase regression model on the $F^{13}CO_2$ data.

Findings

Valine Requirement Estimate

Evidence was insufficient to draw conclusions about the valine requirement estimate for infants. Table 64 provides a summary of findings.

Table 64. Summary of findings for infants: valine requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence*
Valine requirement estimate **1. $F^{13}CO_2$**	de Groof, 2014[44] (24284437) RCT (n=28; 29) Data obtained following each study d (d 2)	China 100% Asian 15 d 0%	2.96 g/kg/d 5-236 mg/kg/d valine	1. Breakpoint: 110 mg/kg/d, upper 95% CI: 164 mg/kg/d	Insufficient

Abbreviations: CI = confidence interval; d = day; $F^{13}CO_2$= The fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; mg/kg/d = milligram per kilogram per day; N = number; PMID = PubMed Identification Number; RCT = randomized controlled trial

*Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.32, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible

to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Children and Adolescents

No studies in the analytic set examined the valine requirement estimate. However, one study[78] examined the total branched chain amino acids requirement estimate for children as described in section 3.13 "Total Branched Chain Amino Acids" above.

Pregnancy

No studies in the analytic set examined the valine requirement estimate.

Adults 19-50 years

Study Characteristics and Findings of Analytic Set

Table 65 summarizes the study characteristics of the analytic set. One study addressed the question of the average daily valine requirements in adults 19-50 years. [72]

Table 65. Basic characteristics of RCT analytic set: adults 19-50 years

Characteristic	Information
Total studies	1 study
Location of studies	1 study in India
Design of studies	1 study RCT cross over
Settings	1 study outpatient/community-dwelling
Age range (average)	21.5 yr
Sex of study participants	1 study with only males
Sample size	18
Intervention Duration	1 study 6 adaptation d, 1 study d (7 d total) per test intake
Outcomes evaluated	Valine requirement estimate (24-h IAAO, 12-h fed IAAO, 24-h IAAB, $F^{13}CO_2$)
Risk of bias	1 study moderate risk

Abbreviations: d = day; $F^{13}CO_2$ = proportion of tracer oxidized [tracer; phenylalanine]; h = hour; IAAB = indicator amino acid balance; IAAO = indicator amino acid oxidation; NR = not reported; RCT = randomized controlled trial; yr = year

Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Diet Intervention and Experimental Methods

In this study, [72] participants underwent 6 adaptation days and 1 study day (160 mg/kg/d nitrogen, 1.0 g/kg/d protein) per test intake. Participants received 3 different valine intakes ranging from 5-35 mg/kg/d. The 24-h IAAO and 24-h IAAB methods were measured, and the valine requirement estimate was calculated using a two-phase linear regression model on the 24-h IAAO, 12-h fed IAAO, 24-h IAAB, and $F^{13}CO_2$ data.

Findings

Valine Requirement Estimate

Evidence was insufficient to draw conclusions about the valine requirement estimate for adults 19-50 years. Table 66 provides a summary of findings.

Table 66. Summary of findings for adults 19-50 years: valine requirement estimate

Outcome Data used to calculate requirement estimate	Study(PMID) Study Design (n analyzed; total observations) Timing	Country Ethnicity/Race Mean age Sex (% female)	Protein Amount Test Amino Acid Amount	Findings	Strength of Evidence*
Valine requirement estimate **1. 24-h IAAO** **2. 12-h fed IAAO** **3. 24-h IAAB** **4. $F^{13}CO_2$**	Kurpad, 2005[72] (16087981) RCT[68] (n=18; 54) Data obtained following each study d (d 7)	India NR 21.5 yr 0%	1.0 g/kg/d 5-35 mg/kg/d valine	1. Breakpoint: 17 mg/kg/d, upper 95% CI: ≥35 mg/kg/d 2. Breakpoint: 18 mg/kg/d, upper 95% CI: ≥35 mg/kg/d 3. Breakpoint: 17 mg/kg/d, upper 95% CI: 28 mg/kg/d 4. Breakpoint: 20 mg/kg/d, upper 95% CI: ≥35 mg/kg/d	Insufficient

Abbreviations: CI = confidence interval; d = day; $F^{13}CO_2$ = proportion of tracer oxidized [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; h = hour; IAAB = indicator amino acid balance; IAAO = indicator amino acid oxidation; mg/kg/d = milligram per kilogram per day; N = number; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year

*Strength of evidence was evaluated based on five designated domains outlined in the Methods section. As provided in Appendix Table I.33, the main reasons for this insufficient rating were that evidence was derived from a single study, making it impossible to assess consistency, and that the outcome effect estimate was unable to be determined due to challenges with evaluating precision.

Adults 51->70 years

No studies in the analytic set examined the valine requirement estimate.

Chapter 4. Discussion

Overview

Our review sought to assess the body of evidence from January 2000 to March 2024 on requirements for average daily dietary protein and individual indispensable amino acid intake among apparently healthy individuals by life stage and sex. To accomplish this, we identified and synthesized findings from studies with higher methodological rigor (low to moderate risk of bias) because these are less likely influenced by factors lessening the reliability of the outcomes. Of 66 eligible studies, 45 were assessed as low to moderate risk of bias and enrolled a broad range of populations using a variety of methods to calculate the requirement estimates.

Overall, we found the evidence insufficient to draw conclusions for average daily dietary protein intake requirements for any population, regardless of age. We found no evidence that calculated the protein requirement estimates for infants (although human milk protein was outside our scope). Rather, we found a single study that determined the effect of protein intake on linear growth. For the indispensable amino acids, we found no studies for histidine, and a single study each for isoleucine and tryptophan. The remaining amino acids had some literature representing the population groups, but again, evidence was insufficient to allow for conclusions.

For protein requirement estimates among adults aged 19-50 years and 51->70 years, our findings reflect those of the prior published meta-analyses and scoping review. [61, 107-109] Our review covered literature from 2000 and thus overlaps marginally with prior published meta-analyses of protein intake and nitrogen balance, including the meta-analysis that informed current DRIs. [61, 107, 108] Our review overlaps more with a 2023 scoping review on protein requirements derived from the IAAO method. [109] But the studies in our review included a wider array of experimental methods. The evidence we reviewed reported protein requirements (EAR and RDA) for adults aged 19-50 years and 51->70 years from 0.91-0.96 g/kg/d (breakpoint) and 1.17-1.29 g/kg/d (upper 95% CI) when the IAAO method was used, and a bi-phase linear regression or nonlinear mixed effects model was applied. [61, 79, 92, 93] These findings reflect those of a meta-analysis by Humayun and colleagues[61] and a scoping review by Matsumoto and colleagues. [109] When nitrogen balance was used and a linear regression analysis was applied, the mean protein requirement ranged from 0.56-0.70 g/kg/d and the protein allowance (equivalent to the RDA) ranged from 0.76-0.90 g/kg/d. [40, 86] which also reflects the findings by Rand and colleagues[107] and by Li and colleagues. [108] However, neither these meta-analyses nor the one by Humayun and colleagues assessed risk of bias or strength of evidence of the contributing studies, and the scoping review only assessed risk of bias.

The evidence we reviewed for children and pregnant people reported higher protein requirements than current DRIs. Reported protein requirements (EAR and RDA) for children ranged from 1.25-1.3 g/kg/d (breakpoint) and 1.5-1.55 g/kg/d. [50] This is ~58-71 percent higher than current recommendations for children 4-8 and 9-13 years old (EAR: 0.76 g/kg/d, RDA: 0.95 g/kg/d). [16] Similarly, protein requirement estimates varied by stage of gestation (early vs. late) [99] and were higher than current DRIs, which are not separated by gestational stage. However, these data should be interpreted with caution because findings for these populations are based on a single study each, and whether these observed values are consistent across multiple studies remains to be seen.

Across population groups, the findings reported for individual indispensable amino acid requirements in our review both reflect and depart from current DRIs. Because our literature search was conducted from 2000, prior to the publication of the DRI in 2005, we found some

overlap for studies that evaluated leucine, lysine, methionine, and threonine requirements. This might, in part, explain some similarities between our findings and the current DRIs. Other similarities exist for studies that do not overlap the current DRIs. For instance, studies in this review reported a lysine requirement for children similar to the current EAR and RDA recommendations, but with wide ranges for RDA estimates. [49, 91] Notably, for adults 51->70 years, leucine requirement estimates are much higher (breakpoint: 78.5 mg/kg/d, upper 95% CI: 81 mg/kg/d) [100] than current recommendations (EAR: 34 mg/kg/d, RDA: 42 mg/kg/d). [16] In addition, included studies varied from the current DRIs for phenylalanine and methionine depending on the amount of tyrosine or cysteine provided. For pregnant women, phenylalanine requirement in the presence of excess tyrosine (61 mg/kg/d) ranged from 15.14-21.36 mg/kg/d (breakpoint) and 19.9-32.2 mg/kg/d (upper 95% CI) for early and late gestation, [52] but in the absence of tyrosine ranged from 43.57-49.56 (breakpoint) and 58.8-63.1 mg/kg/d (upper 95% CI). [51] This differs from current recommendations, which are set for phenylalanine plus tyrosine (EAR: 36 mg/kg/d, RDA: 44 mg/kg/d). [16] Regarding infants, comparing this review's findings for indispensable amino acids with the DRIs is challenging. This is because the DRIs established an AI for infants based on the quantity of indispensable amino acids and volume consumed from human milk, whereas the studies in this systematic review use the IAAO method to calculate the breakpoint and upper 95 percent CI. We did not find any other systematic reviews that examined amino acid requirements and therefore, could not determine how the findings of this systematic review compares to others. Notably, we found sparse literature on the individual indispensable amino acid requirements across populations; therefore, consistency of these findings is unknown.

Limitations of the Evidence Base

Over the past 20 years, some progress has been made to address the questions of the daily protein and indispensable amino acid requirements; however, sufficient evidence has not been generated across populations. For most populations, we found one study of higher methodological rigor examined protein requirements, except for adults 51->70 years, where we found four studies. We saw a similar pattern for individual indispensable amino acid requirements, with many populations having either no evidence or one or two studies of higher methodological rigor. Evidence was especially sparse for populations such as infants, children, adolescents, and pregnant people. For infants, we found no studies that examined the protein requirement estimates. Rather, available studies assessed the effect of protein intake on growth, but most were of high or very high risk of bias. Few studies addressed requirements for adolescents, with most conducted in younger children (6-11 years). We also found no available evidence on requirements for protein or individual indispensable amino acids during lactation. Furthermore, for adults 19-50 years, most studies enrolled male participants but not female. This is a major limitation because we could not determine whether protein and amino acid requirements differ by sex. In addition, study sample sizes were relatively small and may not represent the general population.

When multiple studies for protein or individual indispensable amino acids were available, their designs and methods varied widely, making comparisons between studies difficult. Commonly used methods were nitrogen balance, IAAO, 24-h IAAO, and 24-h IAAB. In comparison to studies conducted prior to 2000, we saw an increased use of the IAAO method to estimate protein requirements. This may be attributed to the shorter study duration and minimally invasive nature of the IAAO method, making it easier to conduct in understudied populations.

However, the validity of this method for estimating protein requirements must still be established. Evidence from adults, presented above, suggests protein requirement estimates from the IAAO method are higher than findings from nitrogen balance. In addition, most studies using the IAAO method found higher protein requirement estimates than the current DRIs for protein, but there is not sufficient data using the IAAO method across populations to determine the consistency of these findings. Importantly, each method to assess requirements has inherent advantages and disadvantages[16, 17, 22, 110, 111] that may contribute to differences in findings for protein or amino acid requirements across studies.

Conducting nutrition research is complex and presents unique challenges that can affect study quality and risk of bias. Further, study quality and risk of bias are two distinct concepts that must be distinguished. "Quality" refers to how well the research was conducted according to the chosen study design, whereas risk of bias refers to the potential for the study's design and conduct to introduce systematic errors which can lead to under 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 of high quality and still have significant risk of bias. Research challenges in the field of nutrition range from attrition, adherence, and blinding to limitations of dietary assessment tools/nutrient databases and plausibility of assessing long-term effects of interventions on health outcomes. [112, 113] If future studies are to be improved and the state of evidence better understood, assessing the risk of bias that may arise due to these challenges is essential.

In our systematic review the most common reason for high risk of bias was high attrition rate, which can affect study findings and compromise study validity. The ability to estimate protein and amino acid requirements could be improved by addressing this key research limitation. Employing techniques to reduce dropout rates could include engaging in more frequent communication and sending a greater number of reminders regarding study visits, initiating incentive programs, or arranging transportation to and from the study facility. Additionally, using appropriate statistical analysis to account for loss of participants during follow up (e.g., using an intention to treat approach) is another way to address this limitation. Other factors also affected risk of bias assessments, such as lack of reporting on allocation sequence concealment and on length of washout period and not using intention to treat analyses. To note, studies required to assess protein and amino acid requirements are cumbersome, requiring lengthy adaptation and/or study days, willingness of participants to be studied over multiple test intakes, and intensive resource and time commitments. Establishing ways to reduce the burden of conducting these studies would be instrumental in the field.

Strengths and Limitations of This Review

Our review has strengths and limitations. A major strength is that ours was the first review to capture such a wide array of methodologies used to calculate protein and amino acid requirements. This was not done in the previous meta-analyses or in the scoping review. Rather, the prior meta-analyses used nitrogen balance studies, and the scoping review used IAAO studies to inform protein requirement estimates. Our systematic review further expands on the previous meta-analyses by including population groups from infants to older adults and pregnant people. And we captured literature that addressed individual indispensable amino acid requirements in addition to protein requirements. Moreover, unlike previous meta-analyses, we performed risk of

bias assessments and graded the strength of evidence, which provides context about the methodological rigor and confidence in the findings of the included studies. Another strength was the extensive search algorithm and strategy used to capture all relevant literature in this topic area since the publication of the previous DRIs in 2005. To the best of our knowledge, this search algorithm captured all relevant literature to answer the Key Questions of the daily average requirement for dietary protein and individual indispensable amino acid intake of apparently healthy individuals. Additionally, most studies in our review were of a higher methodological rigor (low or moderate risk of bias) and were RCTs. Yet, despite our expansive search strategy, the evidence base continues to be sparse and was insufficient for drawing conclusions.

Our review is limited by the fact that we did not capture or review evidence on the protein content of human milk. Human milk is appropriate as the sole source of nutrition for infants for at least the first 6 months of life. [16] Therefore, the amount of protein in human milk is assumed adequate to meet the requirements for this population. Because the U.S. government is already seeking to document the nutrient composition of human milk, [114-117] we deemed it unnecessary to capture and review this information.

Research Gaps Identified and Suggestions for Future Research

Our systematic review highlights the state of the evidence for protein and indispensable amino acid requirements across the lifespan. Currently, multiple available methods are used to assess protein or amino acid requirements. However, the heterogeneity across these methods makes conducting a meta-analysis impossible. If the field is to move forward, a single standardized and validated method must be established—one that can be performed with high methodological rigor across all populations. Focus should be placed on population groups including infants, children, adolescents, pregnant people, and lactating people where protein intake is essential for appropriate growth and development. An adequate sample of both males and females should be enrolled and a stratified analysis used to determine if requirements for protein or indispensable amino acids differ by sex.

As described in Chapter 1, protein source/quality and energy intake directly affect the availability of protein/amino acids to meet the body's protein needs. Protein source impacts the amino acid content and digestibility of a protein which has implications for how easily the body can access and use these amino acids. Additionally, if energy intake is inadequate, energy will be used from protein to support the body's energy needs rather than the body's protein needs (e.g., muscle building). [15, 16] Therefore, requirements for protein and amino acids should be considered in the context of the entire diet, including dietary patterns and energy requirements. In many of the studies using IAAO, 24-hour IAAO, and 24-hour IAAB methods, a eucaloric liquid diet with an amino acid mixture was provided, but a liquid diet is atypical. Future studies should focus on developing standardized meals with known protein and amino acid amounts. This would allow researchers to examine whether a more typical diet pattern, with varying quantity and quality of protein, impacts requirement estimates when the IAAO, 24-hour IAAO, and 24-hour IAAB methods are used. Importantly, how the findings from these acute, highly controlled studies translate to direct health outcomes should be determined. Evidence from longer-term feeding trials and observational studies on protein intake and health should be used to improve our understanding of adequate protein intake to improve specific health outcomes.

Future systematic reviews in this area could focus on protein quality and evidence on the tolerable upper intake level (UL). Since our review sought to examine the quantity of dietary protein, we did not look specifically at protein quality. However, as explained, protein quality could substantially impact requirements and the extent to which requirements are impacted by protein quality should be determined. Additionally, our review's scope did not include the tolerable upper intake level (UL), but the available evidence on the UL should be examined. Briefly, evidence on the UL for amino acids appears sparse but has been slowly growing over the past ~2 decades. [118-125]

Conclusions

Overall, evidence from January 2000 to March 2024 is insufficient across populations to determine the average daily protein and indispensable amino acid requirements of apparently healthy individuals by life stage and sex. We could not draw conclusions because of an overall lack of literature across populations and the fact that methods varied widely over the past 20 years. Our review highlights a critical need for the development of a single standardized and validated method to determine protein and amino acid requirement estimates, especially for life stages where protein intake is vital to growth and development.

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Abbreviations and Acronyms

A	Actual
AA	Amino Acid
AHRQ	Agency for Healthcare Research and Quality
AI	Adequate Intake
BCAA	Branched Chain Amino Acids
BM	Breast Milk
BV	Biological Value
CI	Confidence Interval
D	Day
DAAO	Direct Amino Acid Oxidation
DIAAS	Digestible Indispensable Amino Acid Score
DRI	Dietary Reference Intakes
EAR	Estimated Average Requirement
EG	Early Gestation
EPC	Evidence-based Practice Center
F	Female
FFM	Fat Free Mass
$F^{13}CO_2$	Rate of $^{13}CO_2$ Released from Tracer Oxidation
G	Gram
H	Hour
HDI	Human Development Index
HHS	Health and Human Services
HPro	High Protein
I	Intended
IL	Intermediate Lysine
Ile	Isoleucine
IAAB	Indicator Amino Acid Balance
IAAO	Indicator Amino Acid Oxidation
KG	Kilogram
KQ	Key Question
LAZ	Length-For-Age Z Score
Leu	Leucine
LG	Late Gestation
LL	Low Lysine
LPro	Low Protein
M	Male

MD	Mean Difference
MG	Milligram
MO	Month
MPro	Median Protein
N	Number
NASEM	National Academies of Sciences, Engineering, and Medicine
ND	Unable to be Determined
NESR	Nutrition Evidence Systematic Review
NPU	Net Protein Utilization
NA	Not Applicable
NR	Not Reported
OA	Older Adult
PDCAAS	Protein Digestibility Corrected Amino Acid Score
PICOTS	Population, Intervention, Comparison, Outcomes and Timing
PRISMA	Preferred Reporting Items for Systematic Review and Meta-Analysis
PMID	PubMed Identification Number
RCT	Randomized Controlled Trial
RDA	Recommended Dietary Allowance
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
SE	Standard Error of the Mean
SMD	Standardized Mean Difference
SPI	Soy Protein Isolate
SRDR	Systematic Review Data Repository
UL	Tolerable Upper Intake Level
US	United States
UP	Usual Protein
USDA	United States Department of Agriculture
µMOL	Micromoles