Appendices

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Appendix A. Search Strategy and Data Sources

Search Details and Sources

The search strategy was designed and conducted by an experienced systematic review Librarian with input from the investigators. Another Librarian peer reviewed the draft MEDLINE search strategy using the PRESS Checklist. The MEDLINE search included a combination of relevant keywords and MeSH search terms, the search was translated in each database's controlled vocabulary. To find additional relevant studies, included studies from relevant systematic reviews were manually screened. We applied the following limits or filters to the database searches:

- *Date*. We considered a literature search starting in 2000 sufficient for the purpose of this review.
- *Language*. Publications were excluded if they were written in a language other than English. This was due to resource constraints.
- *Publication status.* We searched for published studies.
- *Human or organism*. The search was limited to human studies.
- *Study design*. Filters were created to limit by study design based on inclusion criteria.
- *Filers.* For Embase (Ovid), we created a modified filter based on a EMBASE RCT filter for OVID April 30, 2023 revision at: https://sites.google.com/a/york.ac.uk/issg-search-filters-resource/home/rcts/embase-rct-filter#h.6ctlh4t8tat Accessed 2023-12-05
- *Filters*. For Scopus, we created a modified filter based on a CADTH search filter for
- All Clinical Trials - Scopus. https://searchfilters.cadth.ca/link/106 Accessed 2023-12-05.
- *Filters*. We created a modified filter based on a CADTH search filter to remove Embase and
- MEDLINE records in Scopus. (https://searchfilters.cadth.ca/link/97 Accessed 2023-12-05)

We conducted a comprehensive literature search in May 2023. We searched the following databases:

- Ovid MEDLINE(R) ALL <1946 to May 24, 2023>. Date searched: May 23, 2023
- Embase (Ovid) Date searched: May 23, 2023
- Agricola (Ovid) Date searched: May 26, 2023
- Scopus (Elsevier) Date searched: May 26, 2023

We conducted an updated search on January 17, 2024 and March 26, 2024. We will conduct another updated search during public review.

Ovid MEDLINE(R) ALL <1946 to May 24, 2023>

1 animal proteins, dietary/ or dietary proteins/ or egg proteins, dietary/ or fish proteins, dietary/ or fruit proteins/ or grain proteins/ or meat proteins/ or milk proteins/ or nut proteins/ or plant proteins, dietary/ or pea proteins/ or poultry proteins/ or shellfish proteins/ or Soybean Proteins/ or whey proteins/ or Diet, High-Protein/ or diet, high-protein low-carbohydrate/ or (protein? adj3 (ate or animal? or bean? or beef or cheese? or consume* or consumption or

content or dairy or diet* or eat or eating or egg? or fish or food or foods or fruit? or goat or grain? or high or increase* or intake* or lacto-vegetarian or lamb or legume? or lentils or macronutrient? or meat? or milk or miso or nut? or nutrition* or nutrient* or pea or peas or pescatarian or pescavegan or plant? or poultry or pork or recommend* or seed? or shellfish? or soy? or soybean? or supplement* or tofu or tempeh or veal or vegan or vegetable? or vegetarian or whey or yog?urt or yolk?)).ti,ab.

2 amino acids, essential/ or exp arginine/ or histidine/ or isoleucine/ or leucine/ or lysine/ or exp methionine/ or exp phenylalanine/ or exp threonine/ or tryptophan/ or exp valine/ or (arginine or histidine or isoleucine or leucine or lysine or methionine or phenylalanine or threonine or tryptophan or valine).ti,ab,kf. or (amino acid* adj3 (balance* or content or essential or indispensable or intake or oxidation or response)).ti,ab.

3 1 or 2

4 nutritional requirements/ or recommended dietary allowances/ or nutritional status/ or (daily intake or dietary reference intake* or nutrition* require* or recommend* dietary allowance* or acceptable macronutrient distribution or nutrition* status).ti,ab.

5 3 and 4

6 (randomized controlled trial or controlled clinical trial).pt. or randomi?ed.ti,ab. or placebo.ti,ab. or randomly.ab. or trial.ab. or groups.ab. or control*.ab. or matched.ab.

7 allocated.ti,ab,hw.

8 ((singl* or doubl* or triple) adj (blind* or dumm* or mask*)).ti,ab,hw,kf.

9 ((equivalence or superiority or non-inferiority or noninferiority) adj3 (study or studies or trial*)).ti,ab,hw,kf.

10 (Nonrandom* or non random* or non-random* or quasi-random* or quasirandom*).ti,ab,hw,kf.

11 or/6-10

12 clinical trial/ or cross-over studies/ or pragmatic clinical trial/ or case-control studies/ or cohort studies/ or prospective studies/ or controlled before-after studies/ or (before-after or between group* or crossover or cross-over or nested case-control* or prospectiv* or quasi-experiment*).mp.

13 Cohort analy*.tw.

14 (Follow up adj (study or studies)).tw.

15 (observational adj (study or studies)).tw.

16 or/12-15

17 11 or 16

18 5 and 17

19 limit 18 to (english language and yr="2000 -Current")

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

21 19 not 20

22 comment/ or editorial/ or letter/

23 21 not 22

Embase <1974 to 2023 May 24>

1 animal protein/ or avian protein/ or fish protein/ or meat protein/ or milk protein/ or pea protein/ or plant protein/ or protein diet/ or protein intake/ or shellfish protein/ or soybean protein/ or whey protein/ or high-protein low-carbohydrate diet/ or (protein? adj3 (ate or animal? or bean? or beef or cheese? or consume* or consumption or content or dairy or diet* or eat or eating or egg? or fish or food or foods or fruit? or goat or grain? or high or increase* or intake* or lacto-vegetarian or lamb or legume? or lentils or macronutrient? or meat? or milk or miso or nut? or nutrition* or nutrient* or pea or peas or pescatarian or pescavegan or plant? or pork or poultry or recommend* or seed? or shellfish? or soy? or soybean? or supplement* or tofu or tempeh or veal or vegan or vegetable? or vegetarian or whey or yog?urt or yolk?)).ti,ab.
2 amino acid intake/ or exp essential amino acid/ or (arginine or histidine or isoleucine or leucine or lysine or methionine or phenylalanine or threonine or tryptophan or valine).ti,ab,kf. or (amino acid* adj3 (balance* or content or essential or indispensable or intake or oxidation or response)).ti,ab.
3 1 or 2
4 dietary reference intake/ or macronutrient intake/ or nutritional requirement/ or nutritional status/ or (daily intake or dietary reference intake* or nutrition* require* or recommend* dietary allowance* or acceptable macronutrient distribution or nutrition* status).ti,ab.
5 3 and 4
6 exp randomized controlled trial/
7 controlled clinical trial/
8 randomization/
9 intermethod comparison/
10 random*.ti,ab.
11 placebo.ti,ab.
12 (compare or compared or comparison).ti,ab.
13 ((evaluated or evaluate or evaluating or assessed or assess) and (compare or compared or comparing or comparison)).ti,ab.
14 ((double or single or doubly or singly) adj (blind or blinded or blindly)).ti,ab.
15 double blind procedure/
16 parallel group?.ti,ab.
17 (crossover or cross over).ti,ab.
18 ((assign* or match or matched or allocation) adj5 (alternate or group? or intervention? or patient? or subject? or participant?)).ti,ab.
19 (controlled adj7 (study or design or trial)).ti,ab.
20 (volunteer or volunteers).ti,ab.
21 human experiment/
22 trial.ti.
23 Controlled study/ or cohort analysis/ or controlled clinical trial/ or pretest posttest control group design/ or prospective study/ or quasi experimental study/ or (cohort or compared or groups or nested case control or multivariate).ti,ab.
24 or/6-23
25 5 and 24
26 (exp animal/ or animal experiment/ or nonhuman/) not (exp human/ or human experiment/)

27 25 not 26
28 limit 27 to (english language and yr="2000 -Current")
29 (Conference Abstract or Conference Review).pt.
30 28 not 29
31 limit 30 to (books or chapter or conference abstract or conference paper or "conference review" or editorial or letter or note or "preprint (unpublished, non-peer reviewed)")
32 30 not 31

AGRICOLA <1970 to May 2023>

1 animal source protein/ or dairy protein/ or egg source protein/ or high protein foods/ or legume protein/ or meat protein/ or exp high protein diet/ or exp plant source protein/ or soy protein/ or textured proteins/ or (protein? adj3 (ate or animal? or bean? or beef or consume* or consumption or content or dairy or diet* or eat or eating or egg? or fish or food or foods or fruit? or goat or grain? or high or increase* or intake* or lacto-vegetarian or lamb or legume? or lentil? or macronutrient? or meat? or milk or nut? or nutrition* or nutrient* or pea or peas or pescatarian or pescavegan or plant? or poultry or pork or recommend* or seafood or seed? or shellfish or soy? or soybean? or supplement* or tempeh or tofu or veal or vegan or vegetable? or vegetarian or whey or yog?urt or yolk?)).ti,ab.
2 essential amino acids/ or arginine/ or histidine/ or isoleucine/ or leucine/ or lysine/ or exp methionine/ or phenylalanine/ or threonine/ or tryptophan/ or valine/ or (arginine or histidine or isoleucine or L-arginine or leucine or lysine or methionine or phenylalanine or threonine or tryptophan or valine or (amino acid* adj3 (balance* or content or essential or indicator or indispensable or intake or oxidation or response))).ti,ab.
3 1 or 2
4 exp estimated average requirement/ or dietary recommendations/ or dietary reference intakes/ or nutrient intake/ or nutritional status/ or "u.s. recommended daily allowances"/ or (daily intake or dietary reference intake* or nutrition* require* or recommend* dietary allowance* or RDA or acceptable macronutrient distribution or nutrition* status or protein require*).ti,ab.
5 3 and 4
6 clinical trials/ or randomized clinical trials/ or (nonrandom* or non random* or non-random* or quasi-random* or quasirandom* or random* or placebo or trial or groups).ti,ab,hw.
7 allocated.ti,ab,hw.
8 ((singl* or doubl*) adj (blind* or dumm* or mask*)).ti,ab,hw.
9 ((equivalence or superiority or non-inferiority or noninferiority) adj3 (study or studies or trial*)).ti,ab,hw.
10 cohort studies/ or cross over studies/ or nutritional intervention/ or observational studies/ or prospective studies/ or (before-after or cohort study or cross-over or crossover or nested case-control or nutrition* intervention or prospective*).ti,ab,hw,id.
11 (observational adj (study or studies)).tw.
12 (Follow up adj (study or studies)).tw.
13 Cohort analy*.tw.
14 or/6-13
15 5 and 14

16 humans/ or exp human nutrition/ or exp people/ or (adult? or adolescen* or boys? or breast-feeding or child or children or elderly or girl? or human? or infant? or man or men or pediatric? or teenager? or wom?n or youth?).ti,ab.
17 15 and 16
18 limit 17 to (english language and yr="2000 -Current")
19 agriculture/ or agricultural products/ or animal experimentation/ or exp animal feeding/ or animal feeding operations/ or animal models/ or animal nutrition/ or animal production/ or animal science/ or animal welfare/ or dogs/ or dog breeds/ or grazing trials/ or greenhouse experimentation/ or herd replacement rate/ or livestock production/ or (agriculture or cat or cats or cattle or dog or dogs or heifer? or herd or herds or livestock or pet food?).ti.
20 laboratory animals/ or rodentia/ or rodents/ or (laboratory animal? or mice or mouse or rat or rats or rodents or rodentia).ti.
21 Animals/ not (Animals/ and Humans/)
22 or/19-21
23 18 not 22

Scopus

INDEXTERMS ("dietary proteins") OR INDEXTERMS ("animal proteins, dietary") OR INDEXTERMS ("egg proteins, dietary") OR INDEXTERMS ("meat proteins") OR INDEXTERMS ("fish proteins, dietary") OR INDEXTERMS ("milk proteins") OR INDEXTERMS ("whey proteins") OR INDEXTERMS ("grain proteins") OR INDEXTERMS ("plant proteins, dietary") OR INDEXTERMS ("Diet, High-Protein") OR INDEXTERMS ("diet, high-protein low-carbohydrate") OR INDEXTERMS ("Diet, High-Protein") OR INDEXTERMS ("diet, high-protein low-carbohydrate") OR INDEXTERMS ("diet, high-protein low-carbohydrate") OR TITLE-ABS ((protein*) W/3 (ate OR animal? OR bean? OR beef OR consume* OR consumption OR content OR dairy OR diet* OR eat OR eating OR egg? OR fish OR food OR foods OR fruit? OR goat OR grain? OR high OR increase* OR intake* OR lacto-vegetarian OR lamb OR legume OR lentil? OR macronutrient? OR meat? OR milk OR nut? OR nutrition* OR nutrient* OR pea OR peas OR pescatarian OR pescavegan OR plant? OR pork OR poultry OR recommend* OR soy? OR supplement* OR tempeh OR tofu OR veal OR vegan OR vegetable? OR vegetarian OR whey OR yogurt OR yolk?)) OR INDEXTERMS ("amino acids, essential") OR INDEXTERMS (arginine) OR INDEXTERMS (histidine) OR INDEXTERMS (isoleucine) OR INDEXTERMS (leucine) OR INDEXTERMS (lysine) OR INDEXTERMS (methionine) OR INDEXTERMS (phenylalanine) OR INDEXTERMS (threonine) OR INDEXTERMS (tryptophan) OR INDEXTERMS (valine) OR TITLE-ABS ("essential amino acids") OR TITLE-ABS ((amino) W/3 (balance* OR content OR essential OR indispensable OR intake OR oxidation OR response)) OR TITLE-ABS (arginine) OR TITLE-ABS (histidine) OR TITLE-ABS (isoleucine) OR TITLE-ABS (leucine) OR TITLE-ABS (lysine) OR TITLE-ABS (methionine) OR TITLE-ABS (phenylalanine) OR TITLE-ABS (threonine) OR TITLE-ABS (tryptophan) OR TITLE-ABS (valine) AND INDEXTERMS ("nutritional requirements") OR INDEXTERMS ("recommended dietary allowances") OR INDEXTERMS ("nutritional status") OR TITLE-ABS ("daily intake" OR "dietary reference intake*" OR "nutrition* require*" OR "recommend* dietary allowance*" OR "acceptable macronutrient distribution" OR "nutrition* status") OR TITLE-ABS ("acceptable macronutrient distribution") OR TITLE-ABS ("daily intake") OR TITLE-ABS ("dietary recommendations") OR TITLE-ABS ("dietary reference intakes") OR TITLE-ABS-KEY ("estimated average requirement") OR TITLE-ABS ("nutrient intake") OR TITLE-ABS ("nutritional requirements") OR TITLE-ABS ("nutritional status") AND PUBYEAR > 1999 AND PUBYEAR < 2024 AND NOT INDEX (medline) AND NOT (PMID (0* OR 1* OR 2* OR 3* OR 4* OR 5* OR 6* OR 7* OR 8* OR 9*)) AND NOT INDEX (embase) AND ORIG-LOAD-DATE AFT 20230724 AND (LIMIT-TO (SRCTYPE , "j")) AND (LIMIT-TO (PUBSTAGE , "final")) AND (LIMIT-TO (DOCTYPE , "ar")) AND (EXCLUDE (SUBJAREA , "AGRI") OR EXCLUDE (SUBJAREA , "VETE")) AND (LIMIT-TO (LANGUAGE , "English"))

Appendix B. References Excluded at Full Text

P=Population
I=Intervention
C=Comparison
O=Outcome
S=Study Design
X=Other Reasons

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Appendix C. Risk of Bias Assessment

Table C.1. Risk of Bias Assessments of Randomized Controlled Trials (Parallel Design) with RoB-2

Study (PMID)	Bias from randomization process	Bias from deviation from intended interventions (assignment)	Bias from missing outcome data	Bias in measurement of outcome	Bias in selection of reported result	Overall risk of bias (Low, Moderate, High)
Borgonha, 2022[1] (11916756)	Moderate	Low	High	Low	Low	High
de Groof, 2014[2] (24284437)	Low risk	Low	Low	Low	Low	Low
Hogewind-Schoonenboom, 2015[3] (25844708)	Moderate	Moderate	Low	Low	Low	Moderate
Hogewind-Schoonenboom, 2015[4] (25926506)	Moderate	Moderate	Low	Low	Low	Moderate
Huang, 2011[5] (22049162)	Low	Low	Low	Low	Low	Low
Huang, 2012[6] (22492372)	Moderate	Low	Low	Low	Low	Moderate
Huang, 2014[7] (24824360)	Moderate	Low	Low	Low	Low	Moderate
Jakobsen, 2010[8] (21239090)	Low	Moderate	High	Low	Low	High
Koletzko, 2009[9] (19386747)	Low	High	High	Low	Low	High
Larnkjaer, 2009[10] (19174829)	Moderate	High	High	Low	Moderate	High
Meckling, 2007[11] (17622289)	Moderate	High	High	Low	Low	High
Räihä, 2002[12] (12352513)	Low	High	High	Low	Low	High

Abbreviations: PMID = PubMed Identification Number; RoB-2 = risk of bias tool for randomized trials

Note: When at least one domain is at a high risk of bias, we determined that a study had an overall risk of bias judgement of high risk of bias (based on the RoB-2 algorithm for reaching overall risk of bias judgement)

Table C.2. Risk of Bias Assessments of Randomized Controlled Trials (Cross over Design) with RoB-2

PMID, Author, Year	Bias from randomization process	Bias from period and carryover effects	Bias from deviation from intended interventions (assignment)	Bias from missing outcome data	Bias in measurement of outcome	Bias in selection of reported result	Overall risk of bias (Low, Moderate, High)
Al-Mokbel, 2019[13] (30753549)	Moderate	Low	Low	High	Low	Low	High
Campbell, 2008[14] (18996869)	Moderate	Low	High	High	Low	Low	High
Conley, 2013[15] (22841544)	Moderate	Low	High	High	Low	Low	High
Di Buono, 2001[16] (11722957)	Moderate	Low	Low	Low	Low	Low	Moderate
Di Buono, 2001[17] (11722956)	Moderate	Low	Low	Low	Low	Low	Moderate
Elango, 2009[18] (19369367)	Moderate	Low	Low	Low	Low	Low	Moderate
Elango, 2007[19] (17684206)	Moderate	Low	Low	Low	Low	Low	Moderate
Elango, 2011[20] (22049165)	Moderate	Low	Low	Low	Low	Low	Moderate
Ennis, 2020[21] (33188409)	Low	Low	Low	Low	Low	Low	Low
Ennis, 2020[22] (31758682)	Low	Low	Low	Low	Low	Low	Low
Hsu, 2007[23] (17314698)	Moderate	Moderate	Low	Low	Low	Low	Moderate
Hsu, 2006[24] (16400054)	Moderate	Moderate	Low	Low	Low	Low	Moderate
Hsu, 2006[25] (16549457)	Moderate	Moderate	Low	Low	Low	Low	Moderate
Humayun, 2007[26] (17634258)	Moderate	Low	Low	Low	Low	Low	Moderate
Humayun, 2007[27] (17921376)	Moderate	Low	Low	Low	Low	Low	Moderate
Humayun, 2006[28] (17093160)	Moderate	Low	Low	Low	Low	Low	Moderate
Kriengsinyos, 2004[29] (15308475)	Moderate	Moderate	Low	Low	Low	Low	Moderate
Kurpad, 2001[30] (11722955)	Moderate	Low	Low	Low	Low	Low	Moderate

PMID, Author, Year	Bias from randomization process	Bias from period and carryover effects	Bias from deviation from intended interventions (assignment)	Bias from missing outcome data	Bias in measurement of outcome	Bias in selection of reported result	Overall risk of bias (Low, Moderate, High)
Kurpad, 2001[31] (11333843)	Moderate	Low	Low	Low	low	low	Moderate
Kurpad, 2002[32] (12324292)	Moderate	Moderate	Low	Low	Low	Low	Moderate
Kurpad, 2002[33] (12145014)	Low	Low	Low	Low	Low	Low	Low
Kurpad, 2005[34] (16087981)	Moderate	Moderate	Low	Low	Low	Low	Moderate
Kurpad, 2006[35] (16762944)	Moderate	Moderate	Low	Low	Low	Low	Moderate
Kurpad, 2003[36] (12716672)	Moderate	Moderate	Low	Low	Low	Low	Moderate
Kurpad, 2004[37] (15585764)	Moderate	Moderate	Low	Low	Low	Low	Moderate
Tian, 2011[38] (21859657)	Moderate	Moderate	Moderate	High	Moderate	Low	High
Mager, 2003[39] (14608071)	Moderate	Low	Low	Low	Low	Low	Moderate
Mao, 2020[40] (32140711)	Moderate	Low	Low	Low	Moderate	Moderate	Moderate
Martens, 2013[41] (23221572)	Moderate	Low	High	High	Low	Moderate	High
Martens, 2014[42] (24760974)	Moderate	Low	High	High	Low	Moderate	High
Martin, 2019[43] (31271193)	Moderate	Low	Low	Moderate	Moderate	Low	Moderate
Morse, 2001[44] (11682582)	Moderate	Low	Moderate	Low	Low	Low	Moderate
Paoletti, 2022[45] (34871427)	Moderate	Low	High	High	Moderate	Low	High
Paoletti, 2023[46] (37356549)	Moderate	Low	Moderate	High	Low	Low	High
Payne, 2018[47] (29378056)	Moderate	Low	Low	Low	Low	Low	Moderate
Pillai, 2010[48] (19923398)	Moderate	Low	Low	Low	Low	Low	Moderate
Rafii, 2015[49] (25320185)	Moderate	Moderate	Low	Low	Low	Low	Moderate

PMID, Author, Year	Bias from randomization process	Bias from period and carryover effects	Bias from deviation from intended interventions (assignment)	Bias from missing outcome data	Bias in measurement of outcome	Bias in selection of reported result	Overall risk of bias (Low, Moderate, High)
Rafii, 2015[50] (26962173)	Moderate	Low	Low	Low	Low	Low	Moderate
Raguso, 2000[51] (10648263)	Moderate	Low	Low	High	Low	Low	High
Riazi, 2003[52] (12730426)	Moderate	Moderate	Moderate	Low	Low	Moderate	High
Riazai, 2003[53] (14608070)	Moderate	Moderate	Low	Low	Low	Low	Moderate
Stephens, 2015[54] (25527661)	Moderate	Low	Low	Low	Low	Low	Moderate
Szwiega, 2021[55] (33330915)	Moderate	Low	Low	Low	Low	Low	Moderate
Tang, 2014[56] (24429540)	Moderate	Low	High	High	Moderate	Low	High
Turner, 2006[57] (16522909)	Moderate	Low	Low	Low	Moderate	Low	Moderate
Walrand, 2008[58] (18697911)	Moderate	Low	Moderate	Low	Moderate	Low	Moderate
Wilson, 2000[59] (10702170)	Moderate	Low	Low	Low	Low	Low	Moderate
Wu, 2023[60] (38073288)	Moderate	Low	Low	High	Moderate	Low	High

Abbreviations: PMID = PubMed Identification Number; RoB-2 = risk of bias tool for randomized trials

Note: When at least one domain is at a high risk of bias, we determined that a study had an overall risk of bias judgement of high risk of bias (based on the RoB-2 algorithm for reaching overall risk of bias judgement)

Table C.3. Risk of Bias Assessments of Non-Randomized Comparison Studies with ROBINS-I

PMID, Author, Year	Bias due to confounding	Bias in selection of participants into the study	Bias in classification of interventions	Bias from deviation from intended interventions (assignment)	Bias due to missing data	Bias in measurement of outcome	Bias in selection of reported result	Overall risk of bias (Low, Moderate, High)
Atinmo, 2010[61] (NA)	Low	Low	Low	Low	Low	Low	Low	Low
El-Khoury, 2000[62] (10871570)	Low	Low	Low	Low	Low	Low	Low	Low
Li, 2013[63] (23981551)	Moderate	Low	Low	Low	High	Low	Low	High
Millward, 2002[64] (12450900)	Low	Low	Low	High	Low	Low	Low	High
Millward, 2000[65] (10871569)	Low	Low	Low	High	Low	Low	Low	High

Abbreviations: NA = not applicable; PMID = PubMed Identification Number; ROBINS-I = risk of bias in non-randomized studies of interventions
Note: When at least one domain is at a 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-I algorithm for reaching overall risk of bias judgement).

Table C.4. Risk of Bias Assessments of Non-Randomized Comparison Studies with ROBINS-E

PMID, Author, Year	Bias in selection of participants into the study (or into the analysis)	Bias due to post-exposure interventions	Bias due to missing data	Bias from measurement of the outcome	Bias in selection of the reported result	Bias due to confounding	Bias from measurement of the exposure	Overall risk of bias (Low, Moderate, High)
*§Olga, 2022[66] (36259139)	-	-	-	-	-	-	-	Very High
*Sims, 2020[67] (32401302)	Low	Low	High	Low	Low	-	-	High
Kittisakmontri, 2022[68] (36235599)	Low	Low	Low	Low	Low	Moderate	Low	Moderate

Abbreviations: PMID = PubMed Identification Number; ROBINS-E = risk of bias in non-randomized studies of exposures
*Note: Some characteristics of a study or a result led directly to the RoB result being at very high risk of bias. Detailed risk-of-bias assessment was unnecessary. When at least one domain is at a 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).
§ Olga, 2022[66] (36259139): In section B (preliminary considerations) of the ROBINS-E assessment, it was determined that this study was very high risk of bias because the method of measuring the exposure was inappropriate. No further assessment was required.

Appendix D. Summary of Study Characteristics of All Eligible Studies

Protein

Infants

Table D.1 and D.2 summarize the characteristics of the entire literature set for studies (RCTs and non-RCTs) in infants. Detailed study characteristics for all studies can be found in Appendix E and F.

For RCTs, three studies addressed the average daily protein requirement. [9, 10, 12] The first study that met the inclusion criteria was published in 2002. [12] One study was conducted in Denmark, [10] one in Italy, [12] and one was a multinational study conducted in Belgium, Germany, Italy, Poland and Spain. [9] All studies were RCTs with an intervention duration ranging from 3 months to 2 years. Studies had a sample size ranging from 83 to 1679. All studies were assessed as high risk of bias. [9, 10, 12]

For non-RCTs, three studies addressed the average daily protein requirement. [66, 67, 68] The first study that met the inclusion criteria was published in 2020. [67] One study was conducted in Thailand, [68] one in the United Kingdom, [66] and one in the United States. [67] All were non-RCTs with a follow up duration ranging from 6 to 12 months. Studies had a sample size ranging from 70 to 174. One study was assessed as very high risk of bias, [66] one was assessed as high risk, [67] and one was assessed as moderate risk and was included in the analytic set. [68]

D.1. Protein RCT literature set: infants

Characteristic	Information
Total studies	3 studies
Location of studies	1 study in Denmark 1 study in Italy 1 study in Belgium, Germany, Italy, Poland and Spain
Design of studies	3 studies RCT
Settings	3 studies outpatient/community-dwelling
Age range (average)	5.3 days to 9.07 months; 1 study reported median age which ranged from 12-16 days depending on intervention group
Sex of studies	3 studies with both females and males
Sample size range	83 to 1679
Intervention Duration	1 study 3 months 1 study 4 months 1 study 2 years
Outcomes Evaluated	1 study length-for-age z score 1 study length gain 2 studies change in length

Characteristic	Information
Risk of bias	3 studies high risk
Analytic set	0 studies

Abbreviations: RCT = randomized controlled trial

Table D.2. Protein non-RCT literature set: infants

Characteristic	Information
Total studies	3 studies
Location of studies	1 study in the United States 1 study in Thailand 1 study in the United Kingdom
Design of studies	3 studies non-RCT
Settings	3 studies outpatient/community-dwelling
Age range	Start: Birth to 6 months End; 9 months to 12 months
Sex of studies	3 studies with both females and males
Sample size range	70 to 174
Follow up Duration	6 to 12 months
Outcomes Evaluated	2 studies length-for-age z score 1 study length SDS 1 study length gain 1 study conditional growth length-for-age z score 1 study association between BM protein intake and length gain 1 study association of length-for-age z score and daily protein intake
Risk of bias	1 study moderate risk (analytic set) 1 study high risk 1 study very high risk
Analytic set	1 study

Abbreviations: BM = breast milk; RCT = randomized controlled trial; SDS = standard deviation score

Children and Adolescents

Table D.3 summarizes the characteristics of the entire literature set for studies in children and adolescents. Detailed study characteristics for all studies can be found in Appendix E and F. One study addressed the average daily protein requirement for children. [20] This RCT was published in 2011, used a cross over design, was conducted in Canada, and enrolled seven participants. It was assessed as moderate risk of bias and was included in the analytic set. [20]

Table D.3. Protein RCT literature 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 years
Sex of studies	1 study with both females and males
Sample size range	7
Intervention Duration	1 study 2 adaptation days, 1 study day (3 days total) per test intake
Outcomes Evaluated	1 study protein requirement estimate ($F^{13}CO_2$, phenylalanine oxidation)
Risk of bias	1 study moderate risk (analytic set)
Analytic set	1 study

Abbreviations: $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; RCT = randomized controlled trial
Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Pregnant People

Table D.4 summarizes the characteristics of the entire literature set for studies in pregnant people. Detailed study characteristics for all studies can be found in Appendix E and F. One study addressed the average daily protein requirement for pregnant people. [54] It was published in 2015, was an RCT with a cross over design, and conducted in Canada. Of the 29 participants, seven were studied at both early and late gestation, and average gestational stage was 16.5 weeks in early gestation and was 35.4 weeks in late gestation. This study was assessed as moderate risk of bias and was included in the analytic set. [54]

Table D.4. Protein RCT literature 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)	Early gestation: 30.6 years Late gestation: 30.3 years
Sex of studies	1 study with only females

Characteristic	Information
Sample size range*	Early gestation: 17 Late gestation: 19
Intervention Duration	1 study 2 adaptation days, 1 study day (3 days total) per test intake
Outcomes Evaluated	1 study protein requirement estimate ($F^{13}CO_2$)
Risk of bias	1 study moderate risk (analytic set)
Analytic set	1 study

Abbreviations: $F^{13}CO_2$ = rate of tracer oxidation to $^{13}CO_2$ [tracer; phenylalanine]; RCT = randomized controlled trial
Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.
*Seven women were studied in both early and late gestation

Adults 19-50 years

Table D.5 and D.6 summarize the characteristics of the entire literature set for studies (RCTs and non-RCTs) in adults 19-50 years. Detailed study characteristics for all studies can be found in Appendix E and F.

For RCTs, three studies addressed the average daily protein requirements for adults (19-50 years). [8, 27, 38] The first study that met the inclusion criteria was published in 2007. [27] One study was conducted in Canada, [27] one in China, [38] and one in Denmark. [8] Two used a cross over design[27, 38] and one a parallel design. [8] Studies had a sample size ranging from 8 to 23. Two studies were assessed as high risk of bias[8, 38] and one was assessed as moderate risk of bias and was included in the analytic set. [27]

For non-RCTs, two studies addressed the average daily protein requirements for adults 19-50 years. [61, 63] The first study that met the inclusion criteria was published in 2010. [61] One study was conducted in China[63] and one in Nigeria. [61] Both used a cross over design. The studies had sample sizes of 18 and 19. One study was assessed as high risk of bias[63] and one was assessed as low risk of bias and was included in the analytic set. [61]

Table D.5. Protein RCT literature set: adults (19-50 years)

Characteristic	Information
Total studies	3 studies
Location of studies	1 study in Canada 1 study in China 1 study in Denmark
Design of studies	1 study RCT parallel 2 studies RCT cross over
Settings	3 studies outpatient/community-dwelling
Age range (average)	21.6 to 26.8 years
Sex of studies	1 study with only females 2 studies with only males

Characteristic	Information
Sample size range	8 to 23
Intervention Duration	1 study 2 adaptation days, 1 study day (3 days total) per test intake 1 study 6 adaptation days, 1 study day (7 days total) per test intake 1 study 3 weeks
Outcomes Evaluated	2 studies protein requirement estimate ($F^{13}CO_2$, leucine oxidation) 1 study nitrogen balance
Risk of bias	1 study moderate risk (analytic set) 2 studies high risk
Analytic set	1 study

Abbreviations: $F^{13}CO_2$ = rate $^{13}CO_2$ released from tracer oxidation [tracer; leucine or phenylalanine]; RCT = randomized controlled trial
Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Table D.6. Protein non-RCT literature set: adults (19-50 years)

Characteristic	Information
Total studies	2 studies
Location of studies	1 study in China 1 study in Nigeria
Design of studies	2 studies non-RCT
Settings	2 studies outpatient/community-dwelling
Age range (average)	21.1 to 23.2 years
Sex of studies	1 study with only males 1 study with both females and males
Sample size range	18 to 19
Intervention Duration	1 study 5 adaptation days, 1 study day (6 days total) per test intake 1 study 10 days per test intake
Outcomes Evaluated	2 studies protein requirement estimate ($F^{13}CO_2$, nitrogen balance)
Risk of bias	1 study low risk (analytic set) 1 study high risk
Analytic set	1 study

Abbreviations: $F^{13}CO_2$ = rate $^{13}CO_2$ released from tracer oxidation [tracer; leucine]; RCT = randomized controlled trial
Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Adults 51->70 years

Table D.7 summarizes the characteristics of the entire literature set for studies in adults 51->70 years. Detailed study characteristics for all studies can be found in Appendix E and F. Six RCTs addressed the average daily protein requirement for adults

51->70 years.[40, 44, 49, 50, 56, 60] The first study to meet the inclusion criteria was published in 2001.[44] Two studies were conducted in Canada,[49, 50] two in China,[40, 60] and two in the United States.[44, 56] All six used a cross over design and had a sample size ranging from 6 to 16. Two studies was assessed as high risk of bias[56, 60] while the other four were assessed as moderate risk and were included in the analytic set.[40, 44, 49, 50] Of note, the publication by Morse and colleagues[44] is one of three publications (Campbell et al.[14] and Conley et al.,[15] presented in adults 19-50 and 51->70 years section below) from the same study. The publication by Morse et al.[44] reports findings for older women, which is a subset of the entire population. The findings of the entire population are reported by Campbell et al.[14] and Conley et al.[15] and include younger men, younger women, older men, and older women. The Morse et al.[44] publication reports findings for protein requirement estimates from nitrogen balance data both at week 2 and week 3. The Campbell et al.[14] publication reports findings for protein requirement estimates from nitrogen balance data at week 3 and the Conley et al.[15] publication report whole-body leucine kinetics data. The publication by Morse et al.[44] received a moderate risk of bias and the publications by Campbell et al.[14] and Conley et al.[15] received high risk of bias because of the attrition rate of the population being reported on (i.e., Morse et al.[44] subset of population [older women] vs Campbell et al.[14] and Conley et al.[15] entire population [younger men, younger women, older men, and older women]).

Table D.7. Protein RCT literature set: adults (51->70 years)

Characteristic	Information
Total studies	6 studies
Location of studies	2 studies in the United States 2 studies in Canada 2 studies in China
Design of studies	6 studies RCT cross over
Settings	6 studies outpatient/community-dwelling
Age range (average)	70.6 to 82 years
Sex of studies	3 studies with only females 1 study with only males 2 studies with both females and males
Sample size range	6 to 16
Intervention Duration	5 studies 2 adaptation days, 1 study day (3 days total) per test intake 1 study 1 adaptation day followed by 17 days on the study diet (18 days total) per test intake
Outcomes Evaluated	6 studies protein requirement estimate ($F^{13}CO_2$, nitrogen balance)
Risk of bias	4 studies moderate risk (analytic set) 2 studies high risk
Analytic set	4 studies

Abbreviations: $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; RCT = randomized controlled trial
Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Adults 19-50 and 51->70 years

Table D.8 summarizes the characteristics of the entire literature set for studies in adults 19-50 and 51->70 years. Detailed study characteristics for all studies can be found in Appendix E and F. Five studies reported in 6 publications addressed the average daily protein requirement in both adults 19-50 years and 51->70 years. [11, 14, 15, 41, 42, 58] The first study that met the inclusion criteria was published in 2007. [11] One study was conducted in Canada, [11] one in Netherlands, [41] one in Netherlands and the United States, [42] and two studies reported in three publications in the United States. [14, 15, 58] One study used a parallel RCT design[11] and five studies reported six publications used a crossover RCT design. [14, 15, 41, 42, 58] Studies had a sample size ranging from 19 to 79. Four studies reported in five publications were assessed as high risk of bias[11, 14, 15, 41, 42] and one was assessed as moderate risk of bias and was included in the analytic set. [58]

Table D.8. Protein RCT literature set: adults (19-50 years and 51->70 years)

Characteristic	Information
Total studies	5 studies reported in 6 publications
Location of studies	3 studies reported in 4 publications in the United States* 1 study in Canada 2 studies in Netherlands*
Design of studies	1 study RCT parallel 4 studies reported in 5 publications RCT cross over
Settings	5 studies reported in 6 publications outpatient/community-dwelling
Age range (average)	24.3 to 75 years
Sex of studies	1 study with only females 4 studies reported in 5 publications with both females and males
Sample size range	19 to 79
Intervention Duration	1 study reported in 2 publications 1 adaptation day followed by 17 days on the study diet (18 days total) per test intake 2 studies 12 days per intake 1 study 10 days per test intake 1 study 12 weeks
Outcomes Evaluated	1 study protein requirement estimate (nitrogen balance) 4 studies nitrogen balance 2 studies leucine oxidation/whole body leucine kinetics
Risk of bias	1 study moderate risk (analytic set) 4 studies reported in 5 publications high risk
Analytic set	1 study

Abbreviations: RCT = randomized controlled trial
Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.
*One study[42] was conducted in both the Netherlands and the United States.

Indispensable Amino Acids

Infants

Table D.9 summarizes the characteristics of the entire literature set for studies in infants. Detailed study characteristics for all studies can be found in Appendix F. Six RCTs addressed the average daily individual indispensable amino acid requirement for infants. [2, 3, 4, 5, 6, 7] The first study that met the inclusion criteria was published in 2011. [5] All were conducted in China and had a sample size ranging from 20 to 33. Two studies were assessed as low risk of bias[2, 5] and the other four were assessed as moderate risk of bias. [3, 4, 6, 7] All six studies were included in the analytic set. [2, 3, 4, 5, 6, 7]

Table D.9. Indispensable amino acid RCT literature set: infants

Characteristic	Information
Total studies	6 studies
Location of studies	6 studies in China
Design of studies	6 studies RCT parallel
Settings	6 studies initially admitted to the hospital
Age range (average)	9 to 15 days
Sex of studies	1 study with only males* 6 studies with both females and males*
Sample size range	20 to 33
Intervention Duration	6 studies 1 adaptation day, 1 study day (2 days total)
Outcomes Evaluated	1 study isoleucine requirement estimate ($F^{13}CO_2$) 1 study leucine requirement estimate ($F^{13}CO_2$) 1 study valine requirement estimate ($F^{13}CO_2$) 1 study threonine requirement estimate ($F^{13}CO_2$) 1 study tryptophan requirement estimate ($F^{13}CO_2$) 1 study methionine requirement estimate ($F^{13}CO_2$) 1 study phenylalanine requirement estimate ($F^{13}CO_2$) 1 study lysine requirement estimate ($F^{13}CO_2$)
Risk of bias	2 studies low risk (analytic set) 4 studies moderate risk (analytic set)
Analytic set	6 studies

Abbreviations: $F^{13}CO_2$ = the fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; lysine or phenylalanine]; RCT = randomized controlled trial
Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.
*de Groof et al. [2] calculated the isoleucine, leucine and valine requirements in which the isoleucine and leucine requirements were calculated in both females and males but the valine requirements were calculated in only males.

Children and Adolescents

Table D.10 summarizes the characteristics of the entire literature set for studies in children and adolescents. Detailed study characteristics for all studies can be found in Appendix E and F. Seven studies addressed the average daily individual indispensable amino acid requirement for children and adolescents. [13, 19, 23, 28, 39, 48, 57] The first study that met the inclusion criteria was published in 2003. [39] One study was conducted in India[48] and the other six in Canada. [13, 19, 23, 28, 39, 57] All were RCTs with a cross over design and sample size ranging from 5 to 7 participants. One was assessed as high risk of bias[13] and six were assessed as moderate risk of bias and were included in the analytic set. [19, 23, 28, 39, 48, 57]

Table D.10. Indispensable amino acid RCT literature set: children and adolescents

Characteristic	Information
Total studies	7 studies
Location of studies	6 studies in Canada 1 study in India
Design of studies	7 studies RCT cross over
Settings	7 studies outpatient/community-dwelling
Age range (average)	8.4 to 10.2 years
Sex of studies	6 studies with both females and males 1 study NR
Sample size range	5 to 7
Intervention Duration	7 studies 2 adaptation days, 1 study day (3 days total) per test intake
Outcomes Evaluated	1 study total branch chain amino acid requirement estimate ($F^{13}CO_2$) 2 studies lysine requirement estimate ($F^{13}CO_2$) 1 study methionine requirement estimate ($F^{13}CO_2$) 1 study total sulfur amino acid requirement estimate ($F^{13}CO_2$) 1 study aromatic amino acid requirement estimate ($F^{13}CO_2$) 1 study tryptophan requirement estimate ($F^{13}CO_2$)
Risk of bias	6 studies moderate risk (analytic set) 1 study high risk
Analytic set	6 studies

Abbreviations: $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; lysine or phenylalanine]; NR = not reported; RCT = randomized controlled trial
Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Pregnant People

Table D.11 summarizes the characteristics of the entire literature set for studies in pregnant people. Detailed study characteristics for all studies can be found in Appendix F. Three RCTs addressed the average daily individual indispensable amino acid requirement for pregnant people.[21, 22, 47] The first study that met the inclusion criteria was published in 2018.[47] All were conducted in Canada and used a cross over design. The sample size ranged from 9 to 14 in early gestation and 9 to 19 in late gestation. In all studies, some women were studied during both early and late gestation. Average gestational stage ranged from 16.3 to 17.5 weeks in early gestation and 34.1 to 36.1 weeks in late gestation. One study was assessed as moderate risk[47] and two were assessed as low risk of bias.[21, 22] All three were included in the analytic set.[21, 22, 47]

Table D.11. Indispensable amino acid RCT literature set: pregnant people

Characteristic	Information
Total studies	3 studies
Location of studies	3 studies in Canada
Design of studies	3 studies RCT cross over
Settings	3 studies outpatient/community-dwelling
Age range (average)	Early gestation: 29.3 to 32.3 years Late gestation: 29.5 to 30.5 years
Sex of studies	3 studies with only females
Sample size range	Early gestation: 9 to 14 Late gestation: 9 to 19
Intervention Duration	3 studies 2 adaptation days, 1 study day (3 days total) per test intake
Outcomes Evaluated	1 study lysine requirement estimate ($F^{13}CO_2$) 1 study aromatic amino acid requirement estimate ($F^{13}CO_2$) 1 study phenylalanine requirement estimate ($F^{13}CO_2$)
Risk of bias	2 studies low risk (analytic set) 1 study moderate risk (analytic set)
Analytic set	3 studies

Abbreviations: $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; leucine or phenylalanine]; RCT = randomized controlled trial
Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Adults 19-50 years

Table D.12 and D.13 summarize the characteristics of the entire literature set for studies (RCTs and non-RCTs) in adults 19-50 years. Detailed study characteristics for all studies can be found in Appendix E and F.

For RCTs, 21 studies addressed the average daily individual indispensable amino acid requirements for adults (19-50 years).[1, 16-18, 24-26, 29-37, 45, 51-53, 59] The first studies that met the inclusion criteria were published in 2000.[51, 59] Eleven studies were conducted in Canada,[16-18, 24-26, 29, 45, 52, 53, 59] eight in India,[30-37] and two in the United States.[1, 51] All were RCTs with a cross over design except one that used a parallel design.[1] Studies had a sample size ranging from 5 to 32. Four studies were assessed as high risk of bias,[1, 45, 51, 52]

16 were assessed as moderate risk, [16-18, 24-26, 29-32, 34-37, 53, 59] and one was assessed as low risk. [33] Therefore, 17 studies were included in the analytic set. [16-18, 24-26, 29-37, 53, 59]

For non-RCTs, three studies addressed the average daily individual indispensable amino acid requirements for adults 19-50 years. [62, 64, 65] The first studies that met the inclusion criteria were published in 2000. [62, 65] Two were conducted in the United Kingdom, [64, 65] and one in the United States. [62] Two used a cross over design[64, 65] and one used a parallel design. [62] Studies had a sample size ranging from 5 to 11. Two studies were assessed as high risk of bias[64, 65] and one was assessed as low risk and was included in the analytic set. [62]

Table D.12. Indispensable amino acid RCT literature set: adults (19-50 years)

Characteristic	Information
Total studies	21 studies
Location of studies	2 studies in the United States 11 studies in Canada 8 studies in India
Design of studies	1 study RCT parallel 20 studies RCT cross over
Settings	21 studies outpatient/community-dwelling
Age range (average)	19.12 to 33.6 years
Sex of studies	1 study with only females 19 studies with only males 1 study with both females and males
Sample size range	5 to 32
Intervention Duration	7 studies 6 adaptation days, 1 study day (7 days total) per test intake 9 studies 2 adaptation days, 1 study day (3 days total) per test intake 1 study 2-day adaptation period prior to 7-day experimental period which consisted of 8-hour, 3-day, and 7-day adaptation followed by a study day on day 1, 3, and 7 per test intake 1 study 8 adaptation days, 1 study day (9 days total) per test intake 1 study, 6 adaptation days followed by 1 study day then 13 adaptation days followed by 1 study day (21 days total) per test intake 1 study 2 adaptation days (days 1-2, 4-5, and 7-8), 1 study day (days 3, 6, and 9) over 9 days 1 study 14 days

Characteristic	Information
Outcomes Evaluated	1 study leucine requirement estimate (24-h IAAB, nitrogen balance) 3 studies lysine requirement estimate (24-h IAAO, 12-h fed IAAO, 24-h IAAB, $F^{13}CO_2$) 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) 3 studies aromatic amino acid requirement estimate (24-h IAAO, 12-h fed IAAO, 24-h IAAB, $F^{13}CO_2$) 2 studies threonine requirement estimate (fasted plasma amino acid response, fed plasma amino acid response, 24-h IAAO, 12-h fed IAAO, 24-h IAAB, $F^{13}CO_2$) 1 study valine requirement estimate (24-h IAAO, 12-h fed IAAO, 24-h IAAB, $F^{13}CO_2$*) 1 study total branched chain amino acid requirement estimate ($F^{13}CO_2$, phenylalanine oxidation, phenylalanine balance for 9 h of intake) 2 studies phenylalanine oxidation 3 studies $F^{13}CO_2$ 1 study sulfur amino acid kinetic balance 1 study leucine balance 1 study leucine oxidation
Risk of bias	1 study low risk (analytic set) 16 studies moderate risk (analytic set) 4 studies high risk
Analytic set	17 studies

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

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

*$F^{13}CO_2$ = proportion of tracer oxidized [tracer; phenylalanine]

Table D.13. Indispensable amino acid non-RCT literature set: adults (19-50 years)

Characteristic	Information
Total studies	3 studies
Location of studies	1 study in the United States 2 studies in the United Kingdom
Design of studies	3 studies non-RCT
Settings	3 studies outpatient/community-dwelling
Age range (average)	21 to 33.2 years
Sex of studies	3 studies with both females and males
Sample size range	5 to 11
Intervention Duration	1 study 6 adaptation days, 1 study day (7 days total) 2 studies 9-h infusions

Characteristic	Information
Outcomes Evaluated	2 studies lysine requirement estimate (lysine content of the EAR for wheat protein) 1 study 24-h whole body lysine[1-13C] oxidation 1 study 24-h whole body lysine balance 2 studies leucine oxidation 2 studies leucine, lysine and nitrogen balance
Risk of bias	1 study low risk (analytic set) 2 studies high risk
Analytic set	1 study

Abbreviations: EAR = estimated average requirement; h = hour; RCT = randomized controlled trial
Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Adults 51->70 years

Table D.14 summarizes the characteristics of the entire literature set for studies in adults 51->70 years. Detailed study characteristics for all studies can be found in Appendix E and F. Three RCTs addressed the average daily individual indispensable amino acid requirements. [43, 46, 55] The first study that met the inclusion criteria was published in 2019. [43] All three were conducted in Canada and used a cross over design. The sample size ranged from 12 to 16. One study was assessed as high risk of bias[46] and two were assessed as moderate risk of bias and were included in the analytic set. [43, 55]

Table D.14. Indispensable amino acid RCT literature set: adults (51->70 years)

Characteristic	Information
Total studies	3 studies
Location of studies	3 studies in Canada
Design of studies	3 studies RCT cross over
Settings	3 studies outpatient/community-dwelling
Age range (average)	67.3 to 76.7 years
Sex of studies	3 studies with both females and males
Sample size range	12 to 16
Intervention Duration	3 studies 2 adaptation days, 1 study day (3 days total) per test intake
Outcomes Evaluated	1 study leucine requirement estimate ($F^{13}CO_2$) 1 study phenylalanine 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 (analytic set) 1 study high risk
Analytic set	2 studies

Abbreviations: $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; RCT = randomized controlled trial
Note: For outcomes evaluated, parentheses indicate data used to calculate requirement estimates.

Appendix E. Study Characteristics (High or Very High RoB)

Protein

Table E.1. Protein RCTs infants

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and methods of assessment)
Koletzko, 2009[9] (19386747) Belgium, Germany, Italy, Poland and Spain Very high HDI Outpatient RCT Government; Non-profit High ROB	Population: Infants Total sample N: 1679 **Intervention: Lower Protein** N: 540 % Female: 49.3% Mean Age/Range/Age at Baseline: Median (25^{th} and 75^{th} percentile) 16 (2, 30) d Race: NR Mean BMI at baseline: 13.6; SD 1.6 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: Mother's educational level: 32.2% low, 51.9% middle, 16% high Physical activity level: NA Medication use: NR Supplement use: NR **Intervention: Higher Protein**	**Intervention: Lower Protein** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: Infant formula: 7.1% of energy from protein Follow-on formula: 8.8% of energy from protein Intended amino acid intake: NR Intended carbohydrate intake: Infant formula: 7.5 g/100 mL Follow-on formula: 7.6 g/100 mL Intended fat intake: Infant formula: 3.9 g/100 mL Follow-on formula: 4.0 g/100 mL *Actual protein intake: Infant formula: Median (range) 8.9 (7.2-10.6) % of energy from protein Follow-on formula: Median (range) 10.7 (8.9-15.9) % of	**Intervention: Lower Protein** Diet type: Infant formula Protein source: Animal Energy status: NR Dietary assessment method: 3 d weighed food records at 3, 6, 12, and 24 mo of age. Intakes of energy and macronutrients were calculated by using a database that was based on the German BLS II.3. Food items and recipes not identified were added according to information from the manufacturers, other databases or ingredients. How protein was administered: Formula provided to participants. Protein assessment method: Same as dietary assessment methods. **Intervention: Higher Protein** Diet type: Infant formula Protein source: Animal Energy status: NR Dietary assessment method:	Outcome measure: Length-for-age z score Measure/Method of Assessment: Recumbent length and standing height were measured twice to the nearest 0.1 cm and the mean value was used. Anthropometric results were expressed as z scores relative to the growth standards of the WHO for breastfed children. Isotope used: NA

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and methods of assessment)
	N: 550 % Female: 47.3% Mean Age/Range/Age at Baseline: Median (25^{th} and 75^{th} percentile) 15 (2, 28) d Race: NR Mean BMI at baseline: 13.5; SD 1.5 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: Mother's education level: 31.4% low, 52% middle, 16.6% high Physical activity level: NA Medication use: NR Supplement use: NR **Comparator: Breastfed** N: 589 % Female: 51.4% Mean Age/Range/Age at Baseline: Median (25^{th} and 75^{th} percentile) 12 (3, 21) d Race: NR Mean BMI at baseline: 13.2; SD 1.4 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA	energy from protein *Actual amino acid intake: NR *Actual carbohydrate intake: Infant formula: 7.5 (6.8-8.9) g/100 mL Follow-on formula: Median (range) 8.5 (6.6-10.2) g/100 mL *Actual fat intake: Infant formula: Median (range) 3.6 (3.1-4.0) g/100 mL Follow-on formula: Median (range) 3.3 (2.6-4.4) g/100 mL Study duration: 2 yr **Intervention: Higher Protein** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: Infant formula: 11.7% of energy from protein Follow-on formula: 17.6% of energy from protein Intended amino acid intake: NR Intended carbohydrate intake: Infant formula: 7.5 g/100 mL Follow-on formula: 7.6 g/100 mL Intended fat intake: Infant formula: 3.5 g/100mL Follow-on formula: 3.27 g/100	3 d weighed food records at 3, 6, 12, and 24 mo of age. Intakes of energy and macronutrients were calculated by using a database that was based on the German BLS II.3. Food items and recipes not identified were added according to information from the manufacturers, other databases or ingredients. How protein was administered: Formula provided to participants. Protein assessment method: Same as dietary assessment methods. **Comparator: Breastfed** Diet type: Breast milk Protein source: Human milk Energy status: NR Dietary assessment method: Energy intake not calculated for food records with any breastfeeding, because breastfeeding as intake of breast milk was only measured in a subgroup of infants. How protein was administered: Breast milk consumption Protein assessment method: Same as dietary assessment method.	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and methods of assessment)
	Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: Mother's education level: 13.3% low, 46.8% middle, 39.8% high Physical activity level: NA Medication use: NR Supplement use: NR	mL *Actual protein intake: Infant formula: Median (range) 8.9 (7.2-10.6) % of energy from protein Follow-on formula: 10.7 (8.9-15.9) % of energy from protein *Actual amino acid intake: NR *Actual carbohydrate intake: Infant formula: Median (range) 7.5 (6.8-8.9) g/100 mL Follow-on formula: Median (range) 8.5 (6.6-10.2) g/100 mL *Actual fat intake: Infant formula: Median (range) 3.6 (3.1-4.0) g/100 mL Follow-on formula: Median (range) 3.3 (2.6-4.4) g/100 mL Study duration: 2 yr **Comparator: Breastfed** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: NA Intended amino acid intake: NA Intended carbohydrate intake: NA Intended fat intake: NA		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and methods of assessment)
		**Actual protein intake: 1.2; SD 0.2 g/100 mL **Actual amino acid intake: NR **Actual carbohydrate intake: 7.4; SD 0.2 g/100 mL **Actual fat intake: 3.6; SD 0.7 g/100 mL Study duration: 2 yr Crossover details: Number of intakes per participant: NA Total intake observations: NA Wash out period: NA		
Larnkjaer, 2009[10] (19174829) Denmark Very high HDI Outpatient RCT Government High ROB	Population: Infants Total sample N: 83 **Intervention: Infant Formula** N: 45 % Female: 44% Mean Age/Range/Age at Baseline: 9.07; SD 0.31 mo Race: NR Mean BMI at baseline: NR Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR	**Intervention: Infant Formula** Baseline protein intake: 11.8; SD 1.7% of energy from protein Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: NR Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: 11.4; SD 1.3% of energy from protein Actual amino acid intake: NR Actual carbohydrate intake:	**Intervention: Infant Formula** Diet type: Infant formula Protein source: Infant formula Energy status: NR Dietary assessment method: 7 consecutive day diet record recorded by parents. Portion sizes estimated and nutritional calculations made with GIES software (version 0.993B, the Danish Institute for Food and Veterinary Research) How protein was administered: Any infant formula on the Danish market with a protein content ≤ 1.5 g/100 mL was considered acceptable.	Outcome measure: Change in length 9-12 months Measure/Method of Assessment: Recumbent length was measured 3 times to the nearest millimeter on an electronic measuring board and the mean of the measurements was used. Isotope used: NA

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and methods of assessment)
	Physical activity level: NA Medication use: NR Supplement use: Received either a daily fish oil supplement (5 mL/d) or no supplement. Iron supplementation recommended to infants consuming <400 mL/d **Comparator: Whole Milk** N: 38 % Female: 58% Mean Age/Range/Age at Baseline: 9.14; SD 0.30 mo Race: NR Mean BMI at baseline: NR Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: Received either a daily fish oil supplement (5 mL/d) or no supplement. Iron supplementation recommended to infants consuming <400 mL/d	NR Actual fat intake: NR Study duration: 3 mo **Comparator: Whole Milk** Baseline protein intake: 11.9; SD 2.5% of energy from protein Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: NR Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: 14.2; SD 2.2% of energy from protein Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 3 mo Crossover details: Number of intakes per participant: NA Total intake observations: NA Wash out period: NA	Common infant formulas used contained 1.2 and 1.5 g/100 mL Protein assessment method: Same as diet assessment method. **Comparator: Whole Milk** Diet type: Whole milk Protein source: Animal Energy status: NR Dietary assessment method: 7 consecutive day diet record recorded by parents. Portion sizes estimated and nutritional calculations made with GIES software (version 0.993B, the Danish Institute for Food and Veterinary Research) How protein was administered: NR Protein assessment method: Same as diet assessment method.	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and methods of assessment)
Räihä, 2002[12] (12352513) Italy Very high HDI Outpatient RCT Industry High ROB	Population: Infants Total sample N: 113 **Intervention: Formula 2.2** N: 29 % Female: 62% Mean Age/Range/Age at Baseline: Gestational age: 39.0; SD 1.1 wk, Started formula at: 7.2; SD 6.7 d Race: NR Mean BMI at baseline: 13.5; SD 0.9 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: Mother's education: 9.0; SD 2.9 yr Physical activity level: NA Medication use: NR Supplement use: NR **Intervention: Formula 1.8 MSW** N: 29 % Female: 49% Mean Age/Range/Age at Baseline: Gestational age: 39.1; SD 1.4 wk, Started formula at: 5.3; SD 3.9 d Race: NR	**Intervention: Formula 2.2** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 2.2 g/100kcal Intended amino acid intake: See table 1 in original paper for more information Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: **30 d:** 2.74; SD 0.5 g/kg/d **60 d:** 2.58; SD 0.56 g/kg/d **90 d:** 2.22; SD 0.55 g/kg/d **120 d:** 2.00; SD 0.34 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 4 mo **Intervention: Formula 1.8 MSW** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA	**Intervention: Formula 2.2** Diet type: Infant formula Protein source: Whey/casein ratio 60/40 Energy status: NR Dietary assessment method: Parents completed a dietary logbook of all formula consumed for 3 d prior to study visits (d 30, 60, 90, 120). Formula intakes were calculated per kg BW and averaged over the 3 d. Daily protein and energy intakes were derived from the volumes consumed and analyzed values of protein and energy, based on instruction for formula reconstitution (129 g powder/l). How protein was administered: Provided in the formula. Protein assessment method: Same as dietary assessment method. **Intervention: Formula 1.8 MSW** Diet type: Infant formula Protein source: Whey/casein ratio 70/30 (modified sweet whey) Energy status: NR Dietary assessment method: Parents completed a dietary logbook of all formula	Outcome measure: Length gains, change in length for age Measure/Method of Assessment: Length was measured using an infant measuring board with a built-in millimeter ruler. Isotope used: NA

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and methods of assessment)
	Mean BMI at baseline: 13.4; SD 1.1 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: Mother's education: 7.7; SD 2.8 yr Physical activity level: NA Medication use: NR Supplement use: NR **Intervention: Formula 1.8 AW** N: 27 % Female: 32% Mean Age/Range/Age at Baseline: Gestational age: 39.3; SD 1.2 wk, Started formula at: 5.3; SD 4.2 d Race: NR Mean BMI at baseline: 13.4; SD 1.0 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: Mother's	Intended protein intake: 1.8 g/100 kcal Intended amino acid intake: See table 1 in original paper for more information Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: **30 d:** 2.32; SD 0.45 g/kg/d **60 d:** 1.91; SD 0.35 g/kg/d **90 d:** 1.76; SD 0.34 g/kg/d **120 d:** 1.68; SD 0.44 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 4 mo **Intervention: Formula 1.8 AW** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.8 g/100kcal Intended amino acid intake: See table 1 in original paper for more information Intended carbohydrate intake: NR Intended fat intake: NR	consumed for 3 d prior to study visits (d 30, 60, 90, 120). Formula intakes were calculated per kg BW and averaged over the 3 d. Daily protein and energy intakes were derived from the volumes consumed and analyzed values of protein and energy, based on instruction for formula reconstitution (129 g powder/l). How protein was administered: Provided in the formula. Protein assessment method: Same as dietary assessment method. **Intervention: Formula 1.8 AW** Diet type: Infant formula Protein source: Whey/casein ratio 70/30 (acid whey) Energy status: NR Dietary assessment method: Parents completed a dietary logbook of all formula consumed for 3 d prior to study visits (d 30, 60, 90, 120). Formula intakes were calculated per kg BW and averaged over the 3 d. Daily protein and energy intakes were derived from the volumes consumed and analyzed values of protein	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and methods of assessment)
	education: 10.7; SD 3.8 yr Physical activity level: NA Medication use: NR Supplement use: NR **Comparator: Breast Milk** N: 28 % Female: 39% Mean Age/Range/Age at Baseline: Gestational age: 39.1; SD 1.0 wk Race: NR Mean BMI at baseline: 13.9; SD 1.1 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: Mother's education: 11.4; SD 3.9 yr Physical activity level: NA Medication use: NR Supplement use: NR	Actual protein intake: **30 d:** 2.13; SD 0.39 g/kg/d **60 d:** 1.88; SD 0.40 g/kg/d **90 d:** 1.76; SD 0.40 g/kg/d **120 d:** 1.71; SD 0.29 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 4 mo **Comparator: Breast Milk** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: NA Intended amino acid intake: See table 1 in original paper for more information Intended carbohydrate intake: NA Intended fat intake: NA Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 4 mo Crossover details:	and energy, based on instruction for formula reconstitution (129 g powder/l). How protein was administered: Provided in the formula. Protein assessment method: Same as dietary assessment method. **Comparator: Breast Milk** Diet type: Breast milk Protein source: Human milk Energy status: NR Dietary assessment method: NR How protein was administered: Breast milk consumption Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and methods of assessment)
		Number of intakes per participant: NA Total intake observations: NA Wash out period: NA		

Abbreviations: AW = acid whey; cm = centimeter; d = day; g/d = gram per day; HDI = human development index; mo = month; kg/m^2 = kilogram per square meter; l = liter; mL/d = milliliter per day; MSW = modified sweet whey; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; SDS = standard deviation score; WHO = World Health Organization; wk = week, yr = year
*Values reported for all formulas (lower and higher protein) based on study participants 3-d weighted food protocols, n=45 for infant formulas and n=94 for follow-on formulas.
**Values for human milk macronutrient content obtained from the Darling study, n=58.

Table E.2. Protein RCTs adults (19-50 years)

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Jakobsen, 2011[8] (21239090) Denmark Very High HDI Outpatient RCT, parallel design Industry High ROB	Population: Adults (19-50 yr) Total sample N: 23 **Usual Protein Diet (Arm 1):** N: 12 % Female: 0% Mean Age/Range/Age at Baseline: 23.7; SD 3.5 yr Race: NR Mean BMI at baseline: 22.1; SD 1.8 kg/m^2 Health status/comorbidities: Healthy Obesity status: Excluded overweight subjects (BMI ≥ 25 kg/m^2) Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA	**Usual Protein Diet (Arm 1):** Baseline protein intake: 1.5; SD 0.3 g/kg/d Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.5 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: 109.6; SD 0.7 g	**Usual Protein Diet (Arm 1):** Diet type: Mixed Protein source: Mixed, primarily animal Energy status: Eucaloric Dietary assessment method: Diet samples collected and analyzed for nutrients using a national database (Dankost3000; National Food Agency) How protein was administered: Participants received all food and beverages from the department and were instructed not to consume anything else but water and salt.	Outcome measure: Nitrogen balance Measure/Method of Assessment: Nitrogen balance method Equation: Balance = Protein intake – 6.25 x (Urinary Nitrogen + Faecal Nitrogen + Miscellaneous Nitrogen) Isotope used: NA

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Lactation stage: NA Menopausal status: NA Income level: NR Education level: Mean level was a bachelor's degree Physical activity level: Subjects instructed not to change their habitual physical activity levels. Strenuous physical exercise (> 4-h/wk) was excluded. Medication use: Use of any medication was excluded. Supplement use: NR **High Protein Diet (Arm 2):** N: 11 % Female: 0% Mean Age/Range/Age at Baseline: 24.7; SD 3.6 yr Race: NR Mean BMI at baseline: 22.3; SD 1.2 kg/m^2 Health status/comorbidities: Healthy Obesity status: Excluded overweight subjects (BMI ≥ 25 kg/m^2) Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: Mean level was a bachelor's degree Physical activity level: Subjects instructed not to	Actual amino acid intake: NR Actual carbohydrate intake: 427.9; SD 2.3 g Actual fat intake: 97.1; SD 0.5 g Study duration: 1 wk run-in period; 3 wk intervention period **High Protein Diet (Arm 2):** Baseline protein intake: 1.7; SD 0.2 g/kg/d Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 3.0 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: 230.6; SD 0.8 g Actual amino acid intake: NR Actual carbohydrate intake: 331.4; SD 1.7 g Actual fat intake: 102.7; SD 0.7 g Study duration: 1 wk run-in period; 3 wk intervention period	Protein assessment method: Diet samples collected and analyzed for nutrients using a national database (Dankost3000; National Food Agency) and urine content of nitrogen was analyzed using an Elementar Vario Max CN analyzer. **High Protein Diet (Arm 2):** Same as above	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	change their habitual physical activity levels. Strenuous physical exercise (> 4-h/wk) was excluded. Medication use: Use of any medication was excluded. Supplement use: NR	Crossover details: Number of intakes per participant: NA Total intake observations: NA Wash out period: NA		
Tian, 2011[38] (21859657) China High HDI Outpatient RCT, cross over design NR High ROB	Population: Adults (19-50 yr) Total sample N: 20 **Intervention: Varied Protein Intakes** N: 20 % Female: 100% Mean Age/Range/Age at Baseline: 21.6; SD 0.9 yr Race: NR Mean BMI at baseline: NR Health status/comorbidities: Considered healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: Excluded if participants had an irregular menstrual cycle Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: Instructed to maintain light physical activity Medication use: NR Supplement use: Excluded supplement use	**Intervention: Varied Protein Intakes** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 0.7, 0.78, 0.86, 0.94, 1.02, 1.10 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR **0.70 g/kg/d protein** Actual protein intake: 0.79; SD 0.04 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 5.0; SD 0.5 g/kg/d Actual fat intake: 1.2; SD 0.3 g/kg/d **0.78 g/kg/d protein** Actual protein intake: 0.91; SD 0.07 g/kg/d Actual amino acid intake: NR	**Intervention: Varied Protein Intakes** Diet type: Mixed (same diet on adaptation and study day) Protein source: Mixed Energy status: Eucaloric Dietary assessment method: Meals were weighed and recorded before and after consumption for actual intake. Samples of each food were analyzed for total nitrogen, fat, carbohydrate, water and ash How protein was administered: Diets provided to participants in a three-day rotation of menus. Protein was distributed into three meals with the ratio of 3:4:3 throughout the day. Protein assessment method: Protein contents in foods were calculated based on the China Food Composition for food quantity and samples of each food were analyzed for nitrogen/protein content by Kjeldahl analysis	Outcome measure: Protein requirement estimate calculated from $F^{13}CO_2$ and leucine oxidation Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C] leucine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Actual carbohydrate intake: 5.4; SD 0.8 g/kg/d Actual fat intake: 1.2; SD 0.4 g/kg/d **0.86 g/kg/d protein** Actual protein intake: 0.92; SD 0.05 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 4.6; SD 0.5 g/kg/d Actual fat intake: 1.0; SD 0.2 g/kg/d **0.94 g/kg/d protein** Actual protein intake: 0.99; SD 0.05 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 5.1; SD 0.4 g/kg/d Actual fat intake: 1.1; SD 0.2 g/kg/d **1.02 g/kg/d protein** Actual protein intake: 1.07; SD 0.05 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 4.5; SD 0.3 g/kg/d Actual fat intake: 0.9; SD 0.2 g/kg/d **1.10 g/kg/d protein** Actual protein intake: 1.17; SD 0.06 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 5.0; SD 0.4 g/kg/d		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Actual fat intake: 1.0; SD 0.2 g/kg/d Study duration: 7 d (d 1-6 adaptation period, d 7 study d) **Crossover details:** **Number of intakes per participant: 3** ***Total intake observations: 60** Wash out period: Study was carried out for three consecutive periods		

Abbreviations: BMI = body mass index; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; leucine]; g = gram; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; h/w = hours per week; IAAO = indicator amino acid oxidation; kg/m^2 = kilogram per square meter; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; SD = standard deviation; wk = week; yr = year
*N values for each outcome not provided. Initially 20 subjects were recruited and received 3 intakes each (60 total observations). However, it is unclear if this is the number of total participants and observations analyzed.

Table E.3. Protein RCTs adults (51->70 years)

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Tang, 2014[56] (24429540) United States Very High HDI Outpatient RCT, cross over design Government, academic High ROB	Population: Adults (51->70 yr) Total sample N: 6 **Intervention: Varied Protein Intakes** N: 6 % Female: 100% Mean Age/Range/Age at Baseline: 82; SE 1 yr	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.0	**Adaptation Period:** Diet type: Mixed Protein source: Mixed Energy status: Eucaloric Dietary assessment method: Diets developed using Pronutra and prepared and distributed at the Indiana Clinical Research Center bio	Outcome measure: Protein requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: [1-13C] phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Race: NR Mean BMI at baseline: 26; SE 2 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NR Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: Multivitamin supplement	g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: Variable amounts of carbohydrate Intended fat intake: 30% energy from fat Example menu shown in table 1 of original paper Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 0.1, 0.3, 0.6, 0.9, 1.2, 1.5, and 1.8 g/kg/d Intended amino acid intake: AA composition was the same as egg protein, but phenylalanine was kept constant at 30.5 mg/kg/d and tyrosine was kept constant at 40.7 mg/kg/d; Table 2 in original paper has more	nutrition facility at Purdue University How protein was administered: Each participant was provided all pre-prepared foods and beverages. Participants were instructed to consume all of the foods and beverages provided and to not consume any other items. Protein assessment method: Same as above **Study Day:** Diet type: Drinks that contained a protein- and amino acid free- diet powder and a crystalline amino acid mixture Protein source: Crystalline AA mixture based on egg protein Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Each participant was provided 8 isoenergetic testing day drinks at hourly intervals. Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		information Intended carbohydrate intake: 70% energy from carbohydrate Intended fat intake: 30% of energy from fat Actual protein intake: 0.1, 0.3, 0.6, 0.9, 1.2, 1.5, and 1.8 g/kg/d Actual amino acid intake: AA composition was the same as egg protein, but phenylalanine was kept constant at 30.5 mg/kg/d and tyrosine was kept constant at 40.7 mg/kg/d; Table 2 in original paper has more information Actual carbohydrate intake: 70% energy from carbohydrate Actual fat intake: 30% of energy from fat Study duration: 1 d (d 3) Crossover details: Number of intakes per participant: 7 Total intake observations: 42 Wash out period: ≥ 1 wk		
Wu, 2023[60] (38073288) China High HDI Outpatient RCT, cross over design	Population: Adults (51->70 yr) Total sample N: 16 **Intervention: Varied Protein Intake (Men)**	**Adaptation Period: (Men)** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR	**Adaptation Period (Same for Men and Women)** Diet type: Standard Chinese diet Protein source: NR Energy status: Eucaloric	Outcome measure: Protein requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Government High ROB	N: 9 % Female: 0% Mean Age/Range/Age at Baseline: 70.6; SD 2.8 yr Race: NR Mean BMI at baseline: 26.1; SD 1.4 kg/m^2 Health status/comorbidities: Healthy; Participants with hypertension were not excluded if their blood pressure was well controlled, and their antihypertensive medications were taken as prescribed. Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: Daily multivitamin and mineral tablet **Intervention: Varied Protein Intake (Women)** N: 7 % Female: 100% Mean Age/Range/Age at Baseline: 71; SD 4.2 yr Race: NR Mean BMI at baseline: 25.5; SD 3.4 kg/m^2	Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: 0.993; SD 0.052 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 5.445; SD 0.394 g/kg/d Actual fat intake: 0.792; SD 0.037 g/kg/d Study duration: 2 d **Adaptation Period: (Women)** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: 1.017;	Dietary assessment method: Weights of each food item were taken prior to and following consumption. Type and quantity of food consumed and detection of macronutrient concentrations were determined. How protein was administered: Diet was weighted in daily portions and provided to participants. Protein assessment method: Same as dietary assessment method. **Study Day: (Same for Men and Women)** Diet type: Lactalbumin powder, protein-free biscuits, protein-free and fried starch slices and protein-free lotus root starch Protein source: Lactalbumin powder Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Each participant received 8 hourly isoenergetic meals. Protein assessment method: Amino acid composition of the same batch of marketable lactalbumin powder was determined	 Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Health status/comorbidities: Healthy; Participants with hypertension were not excluded if their blood pressure was well controlled, and their antihypertensive medications were taken as prescribed. Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NR Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: Daily multivitamin and mineral tablet	SD 0.050 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 5.460; SD 0.403 g/kg/d Actual fat intake: 0.852; SD 0.072 g/kg/d Study duration: 2 d **Study Day: (Same for Men and Women)** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 0.1, 0.3, 0.6, 0.9, 1.2, 1.5 and 1.8 g/kg/d Intended amino acid intake: 54.4 mg/kg/d phenylalanine, 58.0 mg/kg/d tyrosine. See Table 1 in original paper for more information. Intended carbohydrate intake: 40.8-65.3% of energy from carbohydrate Intended fat intake: 33% of energy from fat Actual protein intake: 0.1, 0.3, 0.6, 0.9, 1.2, 1.5 and 1.8 g/kg/d Actual amino acid intake: 54.4 mg/kg/d phenylalanine, 58.0 mg/kg/d tyrosine. See	according to the Chinese standard GB 5009.124-2016.	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Table 1 in original paper for more information. Actual carbohydrate intake: 40.8-65.3% of energy from carbohydrate Actual fat intake: 33% of energy from fat Study duration: 1 d (d 3) Crossover details: Number of intakes per participant: 5-7 Total intake observations: 106 Wash out period: 1 wk		

Abbreviations: AA = amino acid; BMI = body mass index; d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation[tracer; phenylalanine]; g = gram; g/kg/d = grams per kilogram per day; HDI = human development index; IAAO = indicator amino acid oxidation; kg/m^2 = kilogram per square meter; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; SD = standard deviation; SE = standard error; wk = week; yr = year.

Table E.4. Protein RCTs adults (19-50 years and 51->70 years)

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Campbell, 2008[14] (18996869) United States Very High HDI Outpatient RCT, cross over design Government High ROB	Population: Adults (19-50 and 51-70 yr) Total sample N: 42 **Younger Men (YM) (LPro, MPro, HPro):** N: 11 % Female: 0%	**YM, YW, OM, OW (All Arms):** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR	**YM, YW, OM, OW (All Arms):** Diet type: 3-d rotation of menus Protein source: Highly digestible, animal proteins void of meats Energy status: Eucaloric	Outcome measure: Protein requirement estimate calculated from nitrogen balance Measure/Method of Assessment: Nitrogen balance method

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Mean Age/Range/Age at Baseline: 29; SD 7 yr Race: 90.9% White, 9.1% Asian Mean BMI at baseline: 24.8; SD 4.4 kg/m^2 Health status/comorbidities: Healthy, no diabetes mellitus Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: A multivitamin was provided to the subjects daily. **Younger Women (YW) (LPro, MPro, HPro):** N: 12 % Female: 100% Mean Age/Range/Age at Baseline: 30; SD 8 yr Race: 83.3% white, 16.7% African Americans Mean BMI at baseline: 22.8; SD 2.5 kg/m^2 Health status/comorbidities: Healthy, no diabetes mellitus Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA	Intended protein intake: Day 1: <0.2 g/kg/d; Day 2-18: LPro: 0.50 g/kg/d, 63% of RDA; MPro: 0.75 g/kg/d, 94% of RDA; HPro: 1.00 g/kg/d, 125% of RDA Intended amino acid intake: NR Intended carbohydrate intake: 65% of energy from carbohydrate Intended fat intake: 35% energy from fat **YM LPro:** Actual protein intake: 0.51; SD 0.01 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 6.43; SD 0.84 g/kg/d Actual fat intake: 1.54; SD 0.22 g/kg/d **YM MPro:** Actual protein intake: 0.77; SD 0.02 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 6.20; SD 0.91 g/kg/d Actual fat intake: 1.46; SD 0.21 g/kg/d **YM HPro:** Actual protein intake: 1.02; SD 0.02 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 6.02; SD 0.86 g/kg/d	Dietary assessment method: The energy and macronutrient contents of the menus were calculated by using Nutritionist Pro computer software. How protein was administered: Meals provided to participants. Participants were regularly counseled to completely consume all foods and beverages provided to them and to not consume any non-protocol food items. Participants agreed to scrape and rinse all utensils, dishes, and glassware with water and to consume the rinsing. Protein assessment method: Diet samples collected and analyzed for total nitrogen content (Leco model FP-528 analyzer); protein content calculated using conversion factor of 6.25 g protein/g nitrogen	Equation: $I_N - (U_N + F_N + M_N)$ Isotope used: NA

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Lactation stage: NA Menopausal status: All of the young women began each trial 5-7 d after the onset of their menstrual cycle Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: A multivitamin was provided to the subjects daily. **Older Men (OM) (LPro, MPro, HPro):** N: 8 % Female: 0% Mean Age/Range/Age at Baseline: 72; SD 6 yr Race: 100% white Mean BMI at baseline: 26.2; SD 3.5 kg/m^2 Health status/comorbidities: Healthy, no diabetes mellitus Obesity status: NR Pubertal status: NR Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: A multivitamin was provided to the subjects daily.	Actual fat intake: 1.41; SD 0.19 g/kg/d **YW LPro:** Actual protein intake: 0.50; SD 0.04 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 5.96; SD 0.54 g/kg/d Actual fat intake: 1.42; SD 0.13 g/kg/d **YW MPro:** Actual protein intake: 0.74; SD 0.02 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 5.76; SD 0.52 g/kg/d Actual fat intake: 1.38; SD 0.13 g/kg/d **YW HPro:** Actual protein intake: 0.98; SD 0.02 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 5.57; SD 0.51 g/kg/d Actual fat intake: 1.34; SD 0.12 g/kg/d **OM LPro:** Actual protein intake: 0.51; SD 0.01 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 5.40; SD 0.94 g/kg/d Actual fat intake: 1.28; SD 0.22 g/kg/d		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Older Women (OW) (LPro, MPro, HPro): N: 11 % Female: 100% Mean Age/Range/Age at Baseline: 75; SD 4 yr Race: 100% white Mean BMI at baseline: 27.8; SD 4.1 kg/m^2 Health status/comorbidities: Healthy, no diabetes mellitus Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NR Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: A multivitamin was provided to the subjects daily.	**OM MPro:** Actual protein intake: 0.77; SD 0.02 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 5.22; SD 0.60 g/kg/d Actual fat intake: 1.24; SD 0.15 g/kg/d **OM HPro:** Actual protein intake: 1.01; SD 0.02 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 5.27; SD 1.28 g/kg/d Actual fat intake: 1.25; SD 0.26 g/kg/d **OW LPro:** Actual protein intake: 0.50; SD 0.02 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 4.61; SD 0.47 g/kg/d Actual fat intake: 1.08; SD 0.12 g/kg/d **OW MPro:** Actual protein intake: 0.76; SD 0.03 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 4.36; SD 0.46 g/kg/d Actual fat intake: 1.08; SD 0.13 g/kg/d **OW HPro:**		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Actual protein intake: 1.01; SD 0.03 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 4.22; SD 0.47 g/kg/d Actual fat intake: 1.03; SD 0.12 g/kg/d Study duration: Three 18-d trials Crossover details: Number of intakes per participant: 3 Total intake observations: 126 Wash out period: Minimum of 1 wk		
Conley, 2013[15] (22841544) United States Very High HDI Outpatient RCT, cross over design Government, academic High ROB	Population: Adults (19-50 and 51->70 yr) Total sample N: 40 **Younger Men (YM) (LPro, MPro, HPro):** N: 11 % Female: 0% Mean Age/Range/Age at Baseline: 29.5; SE 2.0 yr Race: NR Mean BMI at baseline: 25.0; SE 1.3 kg/m^2 Health status/comorbidities: Healthy, no diabetes mellitus Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA	**YM, YW, OM, OW (All Arms):** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: Day 1: <0.2 g/kg/d; Day 2-18: LPro: 0.50 g/kg/d, 63% of RDA; MPro: 0.75 g/kg/d, 94% of RDA; HPro: 1.00 g/kg/d, 125% of RDA Intended amino acid intake: NR Intended carbohydrate intake: 65% of energy from carbohydrate Intended fat intake: 35% energy from fat	**YM, YW, OM, OW (All Arms):** Diet type: 3-day rotation of menus; d 12 subjects consumed a formula beverage for whole-body leucine kinetics Protein source: Highly digestible, animal proteins void of meats Energy status: Eucaloric Dietary assessment method: Energy and macronutrient contents of menus calculated using Nutritionist Pro computer software How protein was administered: Meals provided to participants throughout the trial; On d 12, participants received a formula beverage	Outcome measure: Leucine balance, oxidation, synthesis, breakdown, and turnover and nitrogen balance* Measure/Method of Assessment: Whole-body leucine kinetics (8-h infusion in the fasted (3-h) and fed (5-h) state) *Nitrogen balance as calculated in Campbell et al.,[14] included in this systematic review Isotope used: L-[1-13C] leucine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: NR **Older Men (OM) (LPro, MPro, HPro):** N: 9 % Female: 0% Mean Age/Range/Age at Baseline: 72.6; SE 2.2 yr Race: NR Mean BMI at baseline: 25.7; SE 0.9 kg/m^2 Health status/comorbidities: Healthy, no diabetes mellitus Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: NR **Younger Women (YW) (LPro, MPro, HPro):** N: 11 % Female: 100% Mean Age/Range/Age at Baseline: 28.9; SE 2.0 yr Race: NR Mean BMI at baseline: 22.2; SE 0.9 kg/m^2	Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: Three 18-d trials Crossover details: Number of intakes per participant: 3 Total intake observations: 120 Wash out period: Minimum of 1 wk	that contained one twelfth of daily protein and energy intakes set for that trial. Protein assessment method: Diet samples collected and analyzed for total nitrogen content (Leco model FP-528 analyzer); protein content calculated using conversion factor of 6.25 g protein/d nitrogen	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Health status/comorbidities: Healthy, no diabetes mellitus Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: NR **Older Women (OW) (LPro, MPro, HPro):** N: 9 % Female: 100% Mean Age/Range/Age at Baseline: 74.4; SE 1.4 yr Race: NR Mean BMI at baseline: 27.4; SE 1.1 kg/m^2 Health status/comorbidities: Healthy, no diabetes mellitus Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: NR			
Meckling, 2007[11] (17622289) Canada	Population: Adults (19-50 and 51->70 yr)	**High Protein Diet:** Baseline protein intake: 71 g	**High Protein Diet and Control:**	Outcome measure: Nitrogen balance

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Very High HDI Community-dwelling RCT, parallel design NR High ROB	Total sample N: 30 **High Protein Diet:** N: 15 % Female: 100% Mean Age/Range/Age at Baseline: 45; SD 16 yr Race: NR Mean BMI at baseline: 31.2; SD 3.5 kg/m^2 Health status/comorbidities: No major clinical disease requiring treatment with drugs known to affect blood pressure, protein metabolism, CVD or diabetes risk factors. Obesity status: Overweight, obese Pubertal status: NA Pregnant or lactating: NR Gestation stage: NR Lactation stage: NR Menopausal status: Premenopausal Income level: NR Education level: NR Physical activity level: NR Medication use: Subjects were instructed to refrain from medications 24-h before measurements. Supplement use: NR **Control:** N: 15 % Female: 100% Mean Age/Range/Age at	Baseline amino acid intake: NR Baseline carbohydrate intake: 225 g Baseline fat intake: 68 g Baseline data shown for all groups combined Intended protein intake: 1.0 g of protein to 1.0 g of carbohydrate Intended amino acid intake: NR Intended carbohydrate intake: 1.0 g of protein to 1.0 g of carbohydrate Intended fat intake: target fat intake to ≤ 30% Actual protein intake: 84 g Actual amino acid intake: NR Actual carbohydrate intake: 127 g Actual fat intake: 60 g Study duration: 12 wk **Control:** Baseline protein intake: 71 g Baseline amino acid intake: NR Baseline carbohydrate intake: 225 g Baseline fat intake: 68 g Baseline data shown for all groups combined Intended protein intake: 1.0 g	Diet type: Mixed Protein source: Mixed Energy status: Hypocaloric Dietary assessment method: Baseline intake was analyzed using the nutrient analysis software Foodworks version 3 and post-study 7-d records from wk 3 and 9 were chosen for full nutrient analysis. How protein was administered: All subjects were provided with recipes, food tables, and lists of high-protein foods. Participants were also provided counseling suggestions. Protein assessment method: Assessment of 7 d records from both wk 3 and 9 for full nutrient analysis	Measure/Method of Assessment: Nitrogen balance = nitrogen in the diet - (nitrogen content of urine sample + 0.12) where 0.12 is the estimated daily loss of nitrogen. Isotope used: NA

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Baseline: 47; SD 12 yr Race: NR Mean BMI at baseline: 28.7; SD 2.3 kg/m^2 Health status/comorbidities: No major clinical disease requiring treatment with drugs known to affect blood pressure, protein metabolism, CVD or diabetes risk factors. Obesity status: Overweight, obese Pubertal status: NA Pregnant or lactating: NR Gestation stage: NR Lactation stage: NR Menopausal status: Premenopausal Income level: NR Education level: NR Physical activity level: NR Medication use: Subjects were instructed to refrain from medications 24-h before measurements. Supplement use: NR	protein to 3.0 g of carbohydrate Intended amino acid intake: NR Intended carbohydrate intake: 1.0 g protein to 3.0 g of carbohydrate Intended fat intake: NR Actual protein intake: 56 g Actual amino acid intake: NR Actual carbohydrate intake: 171 g Actual fat intake: 53 g Study duration: 12 wk Crossover details: Number of intakes per participant: NA Total intake observations: NA Wash out period: NA		
Martens, 2013[41] (23221572) Netherlands Very High HDI Outpatient RCT, cross over design Government, food products provided by Kellogg's Nederland, FrieslandCampina, and Solae, LCC. High ROB	Population: Adults (19-50 yr and 51->70 yr) Total sample N: 79 **Whey Protein:** N: 39 % Female: 59% Mean Age/Range/Age at Baseline: 34.5; SD 16.8 yr Race: NR Mean BMI at baseline: 23.1;	**Whey Protein and Soy Protein:** Composition of whey and soy protein were combined Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR	**Whey Protein:** Diet type: Mixed Protein source: 5% diet: 5% energy from wheat protein 15% diet: 5% energy from wheat protein and 10% energy from whey protein with a-lactalbumin 30% diet: 5% energy from wheat protein and 25%	Outcome measure: Nitrogen balance Measure/Method of Assessment: Nitrogen balance calculated as the difference between nitrogen excretion and nitrogen intake. Nitrogen excretion was

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	SD 3.5 kg/m^2 Health status/comorbidities: Healthy Obesity status: Normal weight to obese; BMI range: 18.2-33.9 kg/m^2 Pubertal status: NA Pregnant or lactating: NR Gestation stage: NR Lactation stage: NR Menopausal status: NR Income level: NR Education level: NR Physical activity level: Based on the Baecke Activity Questionnaire: 1.76; SD 0.15 PAL Medication use: None used aside from oral contraceptives in women. Supplement use: None **Soy Protein:** N: 40 % Female: 40% Mean Age/Range/Age at Baseline: 33.6; SD 18.6 yr Race: NR Mean BMI at baseline: 24.3; SD 3.2 kg/m^2 Health status/comorbidities: Healthy Obesity status: Normal weight to obese; BMI range: 18.1-33.4 kg/m^2 Pubertal status: NA Pregnant or lactating: NR Gestation stage: NR	Intended protein intake: 5% of energy from protein, 15% of energy from protein, 30% of energy from protein Intended amino acid intake: NR Intended carbohydrate intake: Adjusted accordingly with protein amounts (60%, 50%, 35%) Intended fat intake: Maintained constant at 35% of energy from fat. **5% of energy from protein** Actual protein intake: 0.3; SD 0.1 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 3.7; SD 1.2 g/kg/d Actual fat intake: 1.0; SD 0.3 g/kg/d **15% of energy from protein** Actual protein intake: 0.9; SD 0.3 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 3.1; SD 1.1 g/kg/d Actual fat intake: 1.0; SD 0.3 g/kg/d **30% of energy from protein** Actual protein intake: 1.6; SD 0.5 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake:	energy from whey protein with a-lactalbumin Energy status: Eucaloric Dietary method of assessment: The energy content of meals was calculated from the nutrition information on the food items or from the standard Dutch food-composition table. Each meal was weighed before eating. Leftovers for meals and snacks were weighed after eating. Energy and macronutrient intakes were calculated per subject. How protein was administered: Food was served as ready-to-eat meals of three different variants for breakfast, lunch, and dinner. Meals were provided ad libitum for 30 min. and subjects were instructed to eat until they felt comfortably full. After each meal, snack items were provided in individual boxes for ad libitum consumption at home. Protein assessment method: Nitrogen excretion, measured from 24-h urine collections at baseline (day 0) and at days 5 and 11, was used as a biomarker for protein intake. Nitrogen concentrations were measured with a nitrogen	measured from 24-h urine collection at baseline, d 5, and d 11 with an elemental analyser. Total nitrogen output was calculated as 24-h urinary nitrogen plus 10% to account for normal losses via feces and other losses. Isotope used: NA

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Lactation stage: NR Menopausal status: NR Income level: NR Education level: NR Physical activity level: Based on the Baecke Activity Questionnaire: 1.75; SD 0.14 PAL Medication use: None used aside from oral contraceptives in women. Supplement use: None	1.8; SD 0 g/kg/d Actual fat intake: 0.8; SD 0.3 g/kg/d Study duration: 12 d per diet Crossover details: Number of intakes per participant: 3 Total intake observations: 237 Wash out period: ~6 wk	analyzer and total nitrogen output was calculated as 24-h urinary nitrogen plus 10% to account for normal losses via feces and other losses. **Soy Protein:** Diet type: Mixed Protein source: 5% diet: 5% energy from wheat protein 15% diet: 5% energy from wheat protein and 10% energy from soy protein 30% diet: 5% energy from wheat protein and 25% soy Energy status: Eucaloric Dietary method of assessment: The energy content of meals was calculated from the nutrition information on the food items or from the standard Dutch food-composition table. Each meal was weighed before eating. Leftovers for meals and snacks were weighed after eating. Energy and macronutrient intakes were calculated per subject. How protein was administered: Food was served as ready-to-eat meals of three different variants for breakfast, lunch, and dinner. Meals were provided ad libitum for 30 min. and subjects were instructed to	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
			eat until they felt comfortably full. After each meal, snack items were provided in individual boxes for ad libitum consumption at home. Protein assessment method: Nitrogen excretion, measured from 24-h urine collections at baseline (day 0) and at days 5 and 11, was used as a biomarker for protein intake. Nitrogen concentrations were measured with a nitrogen analyzer and total nitrogen output was calculated as 24-h urinary nitrogen plus 10% to account for normal losses via feces and other losses.	
Martens, 2014[42] (24760974) Netherlands and United States Very High HDI Outpatient RCT, cross over design Government, food products provided by Kellogg's Nederland, FrieslandCampina, and Solae, LCC. High ROB	Population: Adults (19-50 yr and 51->70 yr) Total sample N: 58 **Beef Protein:** N: Total: 58, Maastricht 28, Purdue 30 % Female: Total: 48.3%, Maastricht: 50%, Purdue: 46.7% Mean Age/Range/Age at Baseline: Total: 33; SD 16 yr, Maastricht: 38; SD 19 yr, Purdue: 29; SD 11 yr Race: NR Mean BMI at baseline: Total: 24.4; SD 4.0 kg/m^2, Maastricht: 24.2; SD 2.4 kg/m^2, Purdue: 24.7; SD 5.1	**Beef Protein:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 5% of energy from protein, 15% of energy from protein, 30% of energy from protein Intended amino acid intake: NR Intended carbohydrate intake: Adjusted accordingly with protein amounts (60%, 50%, 35%) Intended fat intake:	**Beef Protein:** Diet type: Mixed Protein source: 5% diet: 5% energy from wheat protein 15% diet: 5% energy from wheat protein and 10% energy from beef protein 30% diet: 5% energy from wheat protein and 25% energy from beef protein Energy status: Eucaloric Dietary method of assessment: Calculated from nutrition information on the food items and from standard food-composition tables and software (Nutrition Data System for Research). Each	Outcome measure: Nitrogen balance Measure/Method of Assessment: Nitrogen balance calculated as the difference between nitrogen excretion and nitrogen intake Nitrogen excretion was measured from 24-h urine collection at baseline, d 5, and d 11 with an elemental analyser. Total nitrogen output was calculated as 24-h urinary nitrogen plus 10% to account for normal losses via feces and other losses.

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	kg/m^2 Health status/comorbidities: Healthy Obesity status: Normal weight to obese; BMI range: Total: 18.7-38.7 kg/m^2, Maastricht: 20.0-28.9 kg/m^2, Purdue: 18.7-38.7 kg/m^2 Pubertal status: NA Pregnant or lactating: NR Gestation stage: NR Lactation stage: NR Menopausal status: NR Income level: NR Education level: NR Physical activity level: Based on the Baecke Activity Questionnaire: Total: 1.73; SD 0.16 PAL, Maastricht: 1.76; SD 0.17 PAL, Purdue: 1.70; SD 0.14 PAL Medication use: None used aside from oral contraceptives in women. Supplement use: None	Maintained constant at 35% of energy from fat. **5% of energy from protein:** Actual protein intake: Total: 0.4; SD 0.1 g/kg/d Actual amino acid intake: Total: NR Actual carbohydrate intake: Total: 5.0; SD 1.1 g/kg/d Actual fat intake: Total: 1.2; SD 0.2 g/kg/d Actual protein intake: Maastricht: 0.4; SD 0.1 g/kg/d Actual amino acid intake: Maastricht: NR Actual carbohydrate intake: Maastricht: 4.4; SD 0.9 g/kg/d Actual fat intake: Maastricht: 1.2; SD 0.2 g/kg/d Actual protein intake: Purdue: 0.5; SD 0.1 g/kg/d Actual amino acid intake: Purdue: NR Actual carbohydrate intake: Purdue: 5.5; SD 1.0 g/kg/d Actual fat intake: Purdue: 1.2; SD 0.2 g/kg/d **15% of energy from protein:** Actual protein intake: Total: 1.2; SD 0.3 g/kg/d Actual amino acid intake: Total: NR Actual carbohydrate intake:	meal was weighed before it was provided to subjects. Leftovers for meals and snacks were weighed, after which energy and macronutrient intakes were calculated per subject. How protein was administered: Food was served as ready-to-eat meals of three different variants for breakfast, lunch, and dinner. Meals were provided ad libitum for 30 min. and subjects were instructed to eat until they felt comfortably full. After each meal, snack items were provided in individual boxes for ad libitum consumption at home. Protein method of assessment: Nitrogen excretion, measured from 24-h urine collections at baseline (day 0) and at days 5 and 11, was used as a biomarker for protein intake. Nitrogen concentrations were measured with an elemental analyzer and Integra COBAS 400 plus. Total Nitrogen output was calculated as 24-h urinary nitrogen plus 10% to account for normal losses via feces and other losses.	 Isotope used: NA

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Total: 4.2; SD 0.9 g/kg/d Actual fat intake: Total: 1.2; SD 0.3 g/kg/d Actual protein intake: Maastricht: 1.1; SD 0.2 g/kg/d Actual amino acid intake: Maastricht: NR Actual carbohydrate intake: Maastricht: 3.7; SD 0.7 g/kg/d Actual fat intake: Maastricht: 1.2; SD 0.2 g/kg/d Actual protein intake: Purdue: 1.3; SD 0.3 g/kg/d Actual amino acid intake: Purdue: NR Actual carbohydrate intake: Purdue: 4.6; SD 0.9 g/kg/d Actual fat intake: Purdue: 1.2; SD 0.3 g/kg/d **30% of energy from protein:** Actual protein intake: Total: 2.0; SD 0.5 g/kg/d Actual amino acid intake: Total: NR Actual carbohydrate intake: Total: 3.2; SD 0.9 g/kg/d Actual fat intake: Total: 1.1; SD 0.3 g/kg/d Actual protein intake: Maastricht: 1.8; SD 0.5 g/kg/d Actual amino acid intake: Maastricht: NR		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Actual carbohydrate intake: Maastricht: 2.5; SD 0.6 g/kg/d Actual fat intake: Maastricht: 1.0; SD 0.3 g/kg/d Actual protein intake: Purdue: 2.1; SD 0.5 g/kg/d Actual amino acid intake: Purdue: NR Actual carbohydrate intake: Purdue: 3.8; SD 0.7 g/kg/d Actual fat intake: Purdue: 1.2; SD 0.2 g/kg/d Study duration:12 d per diet Crossover details: Number of intakes per participant: 3 Total intake observations: 174 Wash out period: ~6 wk		

Abbreviations: AA = amino acid; BMI = body mass index; CVD = cardiovascular disease; d = day; F_n = daily fecal nitrogen excretion; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation; g = gram; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; HPro = higher protein; I_n = daily dietary protein intake; IAAO = indicator amino acid oxidation; kg/m^2 = kilogram per square meter; LPro = lower protein; M_n = daily miscellaneous nitrogen excretions; MPro = medium protein; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; OM = older men; OW = older women; PAL = physical activity level; PMID = PubMed Identification Number; RCT = randomized controlled trial; RDA = recommended dietary allowances; SD = standard deviation; SE = standard error; U_n = daily urinary nitrogen excretion; wk = week; YW = younger women; YM = younger men; yr = year.

Table E.5. Protein non-RCTs infants

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Sims, 2020[67] (32401302) United States Very High HDI Outpatient Non-RCT, longitudinal study Government High ROB	Population: Infants Total sample N: 174 **Infants (born to women who are NW):** N: 88 % Female: 39.8% Mean Age/Range/Age at Baseline: Start: 2 wk End:9 mo; gestational age: 39.14; SEM 0.11 wk Race: 85.2% Caucasian; 14.8% non-Caucasian Mean BMI at baseline: NR Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NA Medication use: NR Supplement use: NR **Infants (born to women who are OW)** N: 86 % Female: 43% Mean Age/Range/Age at Baseline: Start: 2 wk End: 9 mo; gestational age: 39.37; SEM 0.09 wk	**Infants (born to women who are NW):** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR End of study protein intake: Protein intake reported for 2 wk-9 mo in figure of original paper End of study amino acid intake: NR End of study carbohydrate intake: Carbohydrate intake reported for 2 wk-9 mo in figure of original paper End of study fat intake: Fat intake reported for 2 wk-9 mo in figure of original paper Duration/Follow up: ~8.5 mo **Infants (born to women who are OW)** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR End of study protein intake:	**Infants (born to women who are NW and born to women who are OW)** Diet type: Breastfed and/or mixed feed from formula Protein source: Human milk and/or formula Energy status: NR Dietary assessment method: Daily human milk intake was assessed by measuring the infant's weight before and after a nursing session combined with 3-d weighed food records. How protein was administered: Received protein from human milk and/or formula Protein assessment method: Macronutrients were measured using a Miris Human Milk Analyzer	Outcome measure: Length-for-age z score; Association of length-for-age z score and daily protein intake Measure/Method of Assessment: Infant weight and length were measured using a tared scale and a length board with a sliding foot piece. All z scores were calculated based on the WHO Child Growth Standards Isotope used: NA

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Race: 87.2% Caucasian; 12.8% non-Caucasian Mean BMI at baseline: NR Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NA Medication use: NR Supplement use: NR	Protein intake reported for 2 wk-9 mo in figure of original paper End of study amino acid intake: NR End of study carbohydrate intake: Carbohydrate intake reported for 2 wk-9 mo in figure of original paper End of study fat intake: Fat intake reported for 2 wk-9 mo in figure of original paper Duration/Follow up: ~8.5 mo		
Olga, 2023[66] (36259139) United Kingdom Very High HDI Outpatient Non-RCT, longitudinal study Government, academic, industry, nonprofit Very High ROB	Population: Infants Total sample N: 70 **Infants:** N: 70 % Female: 41.4% Mean Age/Range/Age at Baseline: Start: Birth End:12 mo; gestational age: 40.36; SD 1.08 wk Race: 94.3% White/European, remaining % NR Mean BMI at baseline: 13.78; SD 1.16 kg/m^2 Health status/comorbidities: NR Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA	**Infants:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA End of study protein intake: 6 wk: 9.1; SD 2.1 g/d End of study amino acid intake: 6 wk: NR End of study carbohydrate intake: 6 wk: 50.8; SD 11.7 g/d End of study fat intake: 6 wk: 29.6; SD 17.4 g/d Duration/Follow up: 12 mo Crossover details:	**Infants:** Diet type: Exclusive breastfeeding for 6 wk followed by mixed feeding (breastfeeding and formula) Protein source: Human milk and/or formula Energy status: NR Dietary assessment method: The volume of breastmilk consumed by each infant at 4-6 wk of age was measured using the dose-to-the-mother deuterium-oxide turnover technique. Mothers were asked to hand or pump express their BM samples after feeding their infants at each visit from birth until 12 mo of age, if still BF. Expression was done from the same breast last used to	Outcome measure: Length gain; Length-SDS; Association between BM protein intake and length gain Measure/Method of Assessment: Measured infant weight, and length. Weight, length, and BMI values were converted to sex- and age-adjusted standard deviation scores using the British 1990 growth reference at birth and the WHO International Growth Standard. Isotope used: NA

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Menopausal status: NA Income level: NR Education level: NR Physical activity level: NA Medication use: NR Supplement use: NR	Number of intakes per participant: NA Total intake observations: NA Wash out period: NA	feed the infants. BM were measured for lactose, and fat by ^{1}H-NMR. How protein was administered: Received protein from human milk and/or formula Protein assessment method: Total nitrogen was measured by the Dumas method to calculate BM protein concentration	

Abbreviations: BM = breast milk; g/d = gram per day; HDI = human development index; mo = month; kg/m^2 = kilogram per square meter; N = number; NA = not applicable; NR = not reported; NW = normal weight; OW = overweight/obese; PMID = PubMed Identification Number; RCT = randomized controlled trial; SDS = standard deviation score; SEM = standard error of the mean; WHO = World Health Organization; wk = week

Table E.6. Protein non-RCTs adults (19-50 years)

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Li, 2013[63] (23981551) China High HDI Outpatient Non-RCT, cross over design Government High ROB	Population: Adults (19-50 yr) Total sample N: 19 **Males:** N: 10 % Female: 0% Mean Age/Range/Age at Baseline: 21.1; SD 1.1 yr Race: NR Mean BMI at baseline: 22.4; SD 2.1 kg/m^2 Health status/comorbidities: Healthy	**Males:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 0.75, 0.82, 0.89, 0.97, 1.05 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake:	**Males and Females:** Diet type: Standard Chinese diet Protein source: Mixed Energy status: Eucaloric Dietary assessment method: Before and after taking the meal, each food was weighed and recorded to determine actual intake. Diet samples were analyzed for contents of protein, fat, ash and moisture. Total	Outcome measures: Protein requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]-leucine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: Light physical activity Medication use: NR Supplement use: NR **Females (Arm 2):** N: 9 % Female: 100% Mean Age/Range/Age at Baseline: 21.3; SD 1.1 yr Race: NR Mean BMI at baseline: 21.0; SD 2.0 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NR Gestation stage: NR Lactation stage: NR Menopausal status: NR Income level: NR Education level: NR Physical activity level: Light physical activity Medication use: NR Supplement use: NR	NR Intended fat intake: NR **0.75 g/kg/d protein** Actual protein intake: 0.76; SD 0.02 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 6.1; SD 0.9 g/kg/d Actual fat intake: 1.3; SD 0.2 g/kg/d **0.82 g/kg/d protein:** Actual protein intake: 0.83; SD 0.01 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 5.6; SD 0.8 g/kg/d Actual fat intake: 1.1; SD 0.2 g/kg/d **0.89 g/kg/d protein:** Actual protein intake: 0.91; SD 0.02 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 5.7; SD 0.8 g/kg/d Actual fat intake: 1.5; SD 0.3 g/kg/d **0.97 g/kg/d protein:** Actual protein intake: 0.97; SD 0.01 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 5.9; SD 0.5 g/kg/d Actual fat intake: 1.2; SD 0.2 g/kg/d	carbohydrate and energy was calculated. How protein was administered: The daily protein intake of each subject was calculated according to his/her body weight and distributed into three meals with a ratio of 3:4:3. Each meal was provided to participants and contained one kind of staple food (rice or steamed roll or steamed bread), one kind of high-quality protein food (pork, chicken, shrimp, egg, tofu), one kind of vegetable, and one kind of fruit (except breakfast). Protein assessment method: For the staple, protein was calculated according to measured protein values of rice and wheat flour and water content. For meat and vegetables, protein contents were calculated based on the China food composition for food quantity. Before and after taking the meal, each food was weighed and recorded to determine actual intake. Diet samples were analyzed for contents of protein.	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		1.05 g/kg/d protein: Actual protein intake: 1.02; SD 0.02 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 5.7; SD 0.7 g/kg/d Actual fat intake: 1.1; SD 0.1 g/kg/d Study duration: 6 d (5 d adaptation followed by 1 study d) **Females:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 0.75, 0.82, 0.89, 0.97, 1.05 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR **0.75 g/kg/d protein** Actual protein intake: 0.74; SD 0.02 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 5.2; SD 0.4 g/kg/d Actual fat intake: 1.1; SD 0.1 g/kg/d		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		0.82 g/kg/d protein: Actual protein intake: 0.79; SD 0.02 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 5.0; SD 0.5 g/kg/d Actual fat intake: 1.1; SD 0.3 g/kg/d **0.89 g/kg/d protein:** Actual protein intake: 0.87; SD 0.07 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 5.3; SD 0.5 g/kg/d Actual fat intake: 1.4; SD 0.3 g/kg/d **0.97 g/kg/d protein:** Actual protein intake: 0.95; SD 0.01 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 5.6; SD 0.2 g/kg/d Actual fat intake: 1.3; SD 0.2 g/kg/d **1.05 g/kg/d protein:** Actual protein intake: 0.99; SD 0.02 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 4.5; SD 0.2 g/kg/d Actual fat intake: 0.9; SD 0.0 g/kg/d Study duration: 6 d (5 d		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		adaptation followed by 1 study d) Crossover details: Number of intakes per participant: 5 Total intake observations: 95 Wash out period: 3 d		

Abbreviations: BMI = body mass index; d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; leucine]; g/kg/d = grams per kilogram per day; HDI = human development index; IAAO = indicator amino acid oxidation; kg/m^2 = kilogram per square meter; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification number; RCT = randomized controlled trial; SD = standard deviation; yr = year.

Lysine

Table E.7. Lysine RCTs adults (19-50 years)

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and methods of assessment)
Paoletti, 2022[45] (34871427) Canada Very High HDI Outpatient RCT, cross over design Government, Pfizer Consumer Healthcare donated the multivitamins, protein-free powder for experimental diets provided by Mead Johnson Nutritionals High ROB	Population: Adults (19-50 yr) Total sample N: 5 **Intervention: Varied Lysine intakes** N: 5 % Female: 0% Mean Age/Range/Age at Baseline: 28.3; SD 3.4 yr Race: NR Mean BMI at baseline: 23.4; SD 1.9 kg/m^2 Health status/comorbidities: Healthy	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d Intended amino acid intake: free crystalline AA diet: 5, 8, 12 and 15 mg/kg/d; cooked sorghum diet: 8.2,	**Adaptation Period:** Diet type: Meals provided as either 1) free crystalline amino acid 2) cooked sorghum 3) sorghum and lentil in a mixed meal, depending on whether the reference, sorghum or sorghum and lentil diet was under investigation. Nonprotein energy provided as protein-free powder, flavored with Tang and Fresh Plus crystals, grapeseed oil,	Outcome measure: $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1–13C] phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and methods of assessment)
	Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: Multivitamin tablet	12.5, and 15.7 mg/kg/d; sorghum and lentil in a mixed meal: 17 mg/kg/d; phenylalanine and tyrosine intake kept constant; alanine adjusted to maintain a constant nitrogen intake. See Table 1 in the original paper for more information Intended carbohydrate intake: ~53% of energy from carbohydrate Intended fat intake: ~37% of energy from fat Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.0 g/kg/d Intended amino acid intake: free crystalline AA diet: 5, 8, 12 and 15 mg/kg/d; cooked sorghum diet: 8.2, 12.5, and 15.7 mg/kg/d;	and protein-free cookies Protein source: 1) free crystalline amino acid 2) cooked sorghum 3) sorghum and lentil in a mixed meal depending on whether the reference, sorghum or sorghum and lentil diet was under investigation Energy status: Eucaloric Dietary assessment method: The calorie, carbohydrate, fat, and tyrosine contents were based on the composition of sorghum taken from the USDA database and of lentils from the Canadian Nutrient database. How protein was administered: Provided as 4 equal meals per day Protein assessment method: The amino acid and protein content of the sorghum was analyzed by ion-exchange chromatography with postcolumn derivation with ninhydrin; The AA and protein compositions of the lentils were analyzed by Evonik Degussa Canada **Study Day:** Diet type: Meals provided as either 1) free crystalline amino acid 2) cooked sorghum 3) sorghum and	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and methods of assessment)
		sorghum and lentil in a mixed meal: 17 mg/kg/d; phenylalanine and tyrosine intake kept constant; alanine adjusted to maintain a constant nitrogen intake. See Table 1 in the original paper for more information Intended carbohydrate intake: ~53% of energy from carbohydrate Intended fat intake: ~37% of energy from fat Actual protein intake: 1.0 g/kg/d Actual amino acid intake: free crystalline AA diet: 5, 8, 12 and 15 mg/kg/d; cooked sorghum diet: 8.2, 12.5, and 15.7 mg/kg/d; sorghum and lentil in a mixed meal: 17 mg/kg/d; phenylalanine and tyrosine intake kept constant; alanine adjusted to maintain a constant nitrogen intake. See Table 1 in the original paper for more information Actual carbohydrate intake: ~53% of energy from carbohydrate Actual fat intake: ~37% of energy from fat Study duration: 1 d (d 3) Crossover details:	lentil in a mixed meal, depending on whether the reference, sorghum or sorghum and lentil diet was under investigation. Nonprotein energy provided as protein-free powder, flavored with Tang and Fresh Plus crystals, grapeseed oil, and protein-free cookies Protein source: 1) free crystalline amino acid 2) cooked sorghum 3) sorghum and lentil in a mixed meal depending on whether the reference, sorghum or sorghum and lentil diet was under investigation Energy status: Eucaloric Dietary assessment method: The calorie, carbohydrate, fat, and tyrosine contents were based on the composition of sorghum taken from the USDA database and of lentils from the Canadian Nutrient database How protein was administered: Provided as 9 hourly isonitrogenous, isocaloric meals Protein assessment method: The amino acid and protein content of the sorghum was analyzed by ion-exchange chromatography with postcolumn derivation with	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and methods of assessment)
		Number of intakes per participant: 8 Total intake observations: 36 Wash out period: ≥1 wk	ninjydrin; The amino acid and protein compositions of the lentils were analyzed by Evonik Degussa Canada	

Abbreviations: AA = amino acid; BMI = body mass index; 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; HDI = human development index; IAAO = indicator amino acid oxidation; N = number; NA = not applicable; NR = not reported; PMID = PubMed identification Number; RCT = randomized controlled trial; REE = resting energy expenditure; RoB = risk of bias; SD = standard deviation; USDA = United States Department of Agriculture; wk = week; yr = year.

Table E.8. Lysine non-RCTs adults (19-50 years)

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Millward, 2000[65] (10871569) United Kingdom Very high HDI Outpatient Non-randomized intervention, cross-over design Industry, nonprofit High ROB	Population: Adults (19-50 yr) Total sample N: 6 **Intervention: Milk and Wheat Meals** N: 6 % Female: 33% Mean Age/Range/Age at Baseline: 32.0; SD 11.4 yr Race: NR Mean BMI at baseline: 21.5; SD 2.2 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NR Gestation stage: NR Lactation stage: NR Menopausal status: NR	**Intervention 1 (Arm 1): Milk; Low Protein** Baseline protein intake: 1.19; SD 0.07 g/kg/d for all subjects Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 2-3 % of energy from protein Intended amino acid intake: NR Intended carbohydrate intake: 60% of energy from carbohydrate Intended fat intake: Adjusted with protein to maintain an isoenergetic diet	**Intervention 1 (Arm 1): Milk; Low Protein** Diet type: Milk protein meal (potato dextrose, double cream, full-cream milk) Protein source: Animal Energy status: Eucaloric Dietary assessment method: Meals individually formulated for each subject How protein was administered: Each test meal was designed and provided to the participant at 3 h into the study protocol for low protein and 6 h for high protein. Participants were fed every 30 min over the 3 h of each meal. Protein assessment method:	Outcome measures: Lysine requirement estimate calculated from the lysine content of the EAR for wheat protein; leucine oxidation and balance; nitrogen and lysine balance Measure/Method of Assessment: [1-13C] leucine balance protocol Isotope used: L-[1-13C] leucine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: NR	Actual protein intake: 2.2; SD 0.1% of energy from protein Actual amino acid intake: 10.4; SD 0.0 µmol/kg/h lysine; 20.1; SD 0.1 µmol/kg/h leucine Actual carbohydrate intake: 60.2; SD 4.8 % of energy from carbohydrate Actual fat intake: 37.6; SD 2.9 % of energy from fat Study duration: 9-h infusion which consisted of 0-3 h postabsorptive state, 3-6 h milk low protein, 6-9 h milk high protein **Comparator (Arm 2): Milk; High Protein** Baseline protein intake: 1.19; SD 0.07 g/kg/d for all subjects Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 12-14 % of energy from protein Intended amino acid intake: NR Intended carbohydrate intake: 60% of energy from carbohydrate Intended fat intake: Adjusted with protein to maintain an	Kjeldahl analysis **Comparator (Arm 2): Milk; High Protein** Diet type: milk protein meal (potato dextrose, double cream, skim milk) Protein source: Animal Energy status: Eucaloric Dietary assessment method: Meals individually formulated for each subject How protein was administered: Each test meal was designed and provided to the participant at 3 h into the study protocol for low protein and 6 h for high protein. Participants were fed every 30 min over the 3 h of each meal. Protein assessment method: Kjeldahl analysis **Comparator (Arm 3): Wheat; Low Protein** Diet type: wheat protein meal (stone-ground, soy-free, whole-meal wheat bread, margarine, and potato dextrose served as crust-free bread slices with margarine and a drink of the potato dextrose dissolved in water flavored with some sugar-free orange soda) Protein source: Plant Energy status: Eucaloric	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		isoenergetic diet Actual protein intake: 13.2; SD 0.6 of energy from protein Actual amino acid intake: 60.7; SD 3.9 μmol/kg/h lysine; 83.0; SD 4.8 μmol/kg/h leucine Actual carbohydrate intake: 60.1; SD 3.9 % of energy from carbohydrate Actual fat intake: 26.7; SD 1.8 % of energy from fat Study duration: 9-h infusion which consisted of 0-3 h postabsorptive state, 3-6 h milk low protein, 6-9 h milk high protein **Comparator (Arm 3): Wheat; Low protein** Baseline protein intake: 1.19; SD 0.07 g/kg/d for all subjects Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 2-3 % of energy from protein Intended amino acid intake: NR Intended carbohydrate intake: 60% of energy from carbohydrate Intended fat intake: Adjusted	Dietary assessment method: Meals individually formulated for each subject How protein was administered: Each test meal was designed and provided to the participant at 3 h into the study protocol for low protein and 6 h for high protein. Participants were fed every 30 min over the 3 h of each meal. Protein assessment method: Kjeldahl analysis **Comparator (Arm 4): Wheat; High protein** Diet type: wheat protein meal (stone-ground, soy-free, whole-meal wheat bread, margarine, and potato dextrose served as crust-free bread slices with margarine and a drink of the potato dextrose dissolved in water flavored with some sugar-free orange soda) Protein source: Plant Energy status: Eucaloric Dietary assessment method: Meals individually formulated for each subject How protein was administered: Each test meal was designed and provided to the participant at 3 h into the study protocol for low protein and 6 h for high	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		with protein to maintain an isoenergetic diet Actual protein intake: 2.1; SD 0.1 % of energy from protein Actual amino acid intake: 2.1; SD 0.2 μmol/kg/h lysine; 10.3; SD 0.5 μmol/kg/h leucine Actual carbohydrate intake: 61.6; SD 3.9 % of energy from carbohydrate Actual fat intake: 36.3; SD 2.7 % of energy from fat Study duration: 9-h infusion which consisted of 0-3 h postabsorptive state, 3-6 h wheat low protein, 6-9 h wheat high protein **Comparator (Arm 4): Wheat; High Protein** Baseline protein intake: 1.19; SD 0.07 g/kg/d for all subjects Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 12-14 % of energy from protein Intended amino acid intake: NR Intended carbohydrate intake: 60% of energy from carbohydrate Intended fat intake: Adjusted	protein. Participants were fed every 30 min over the 3 h of each meal. Protein assessment method: Kjeldahl analysis	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		with protein to maintain an isoenergetic diet Actual protein intake: 11.9; SD 0.4 % of energy from protein Actual amino acid intake: 12.5; SD 1.2 µmol/kg/h lysine; 42.8; SD 3.7 µmol/kg/h leucine Actual carbohydrate intake: 61.5; SD 4.2 % of energy from carbohydrate Actual fat intake: 26.6; SD 2.9 % of energy from fat Study duration: 9-h infusion which consisted of 0-3h postabsorptive state, 3-6 h wheat low protein, 6-9 h wheat high protein Crossover details: Number of intakes per participant: 2 (milk Low protein/High protein, and wheat Low protein/High protein) Total intake observations: 12 Wash out period: 3-12 mo		
Millward, 2002[64] (12450900) United Kingdom Very high HDI Outpatient Non-randomized intervention, cross-over design	Population: Adults (19-50 yr) Total sample N: 5 **Intervention: Milk and Wheat Meals** N: 5 % Female: 20% Mean Age/Range/Age at Baseline: 33.2; SD 12.8 yr	**Milk Meal** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 50%	**Milk Meal** Diet type: Milk protein meal (fresh skim milk and dissolved potato dextrose) Protein source: Animal Energy status: Eucaloric Dietary assessment method: Samples of the meals were taken and stored frozen	Outcome measures Lysine requirement estimate calculated from the lysine content of the EAR for wheat protein; leucine oxidation and balance; nitrogen and lysine balance Measure/Method of

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Industry, nonprofit High ROB	Race: NR Mean BMI at baseline: 22.6; SD 2.4 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NR Gestation stage: NR Lactation stage: NR Menopausal status: NR Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: NR	of the UK average daily protein intake (protein-energy ratio of 30%) Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: Kept as low as possible to maximize gastric emptying Actual protein intake: 32.3; SD 4.5% of energy from protein Actual amino acid intake: 64.2; SD 3.0 mg/kg leucine, 55.8; SD 2.6 lysine mg/kg Actual carbohydrate intake: 65.7; SD 8.7% of energy from carbohydrate Actual fat intake: 2.0; SD 0.3% of energy from fat Study duration: 9-h infusion which consisted of 0-3 h postabsorptive state and 3-9 h postprandial state **Wheat Meal** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 50% of the UK average daily protein intake (protein-energy	before measurement of leucine and nitrogen contents How protein was administered: Each test meal was designed and provided to the participant at 3 h into the study protocol Protein assessment method: Kjeldahl analysis **Wheat Meal** Diet type: Wheat protein meal (wheat gluten, plain flour, and potato dextrose) Protein source: Plant Energy status: Eucaloric Dietary assessment method: Samples of the meals were taken and stored frozen before measurement of leucine and nitrogen contents How protein was administered: Each test meal was designed and provided to the participant at 3 h into the study protocol Protein assessment method: Kjeldahl analysis	Assessment: [1-13C] leucine balance protocol Isotope used: L-[1-13C]leucine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		ratio of 30%) Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: Kept as low as possible to maximize gastric emptying Actual protein intake: 26.7; SD 4.1% of energy from protein Actual amino acid intake: 39.1; SD 4.8 mg/kg leucine; 9.5; SD 1.2 mg/kg lysine Actual carbohydrate intake: 72.3; SD 6.4% of energy from carbohydrate Actual fat intake: 1.0; SD 0.1% of energy from fat Study duration: 9-h infusion which consisted of 0-3 h postabsorptive state and 3-9 h postprandial state Crossover details: Number of intakes per participant: 2 Total intake observations: 10 Wash out period: mean 6 mo		

Abbreviations: BMI = body mass index; EAR = Estimated Average Requirement; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; kg/m^2 = kilogram per square meter; mg/kg/d = milligrams per kilogram per day; min = minute; mo = month; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; SD = standard deviation; yr = year

Methionine

Table E.9. Methionine RCTs adults (19-50 years)

Study (PMID) **Location (country)** **HDI** **Setting** **Study Design** **Funding Source** **ROB Score**	**Participants**	**Interventions/Exposure and Comparator (Content)**	**Intervention/Exposure and Comparator (Methods of Assessment)**	**Outcome (Measures and Methods of Assessment)**
Raguso, 2000[51] (10648263) United States Very High HDI Outpatient RCT, cross over design Government, nonprofit High risk ROB	Population: Adults (19-50 yr) *Total sample N: 6 **Intervention: Varied Methionine Intakes** N: 6 % Female: 0% Mean Age/Range/Age at Baseline: 22.8; SD 2.2 yr Race: NR Mean BMI at baseline: NR, Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: Multivitamin-multimineral capsule (1 per d), potassium tablet (3 per d), calcium tablet (4 per d), sodium chloride tablet (2 per d). Choline supplements (2 per d supplying 500 mg/d)	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d (160 mg/kg/d nitrogen) Intended amino acid intake: High methionine: 13 mg/kg/d methionine, 0 mg/kg/d cysteine; low methionine: 5 mg/kg/d methionine, 0 mg/kg/d cysteine; methionine plus cysteine 5 mg/kg/d methionine, 6.5 mg/kg/d cysteine; dispensable amino acids were adjusted to maintain a constant total nitrogen content for all diets. See Table 2 in the original paper for more information Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR	**Adaptation Period:** Diet type: Protein-free wheat-starch, butter cookies and a sherbet-based drink along with an L-amino acid mixture Protein source: L-amino acid mixture Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Provided as 3 isoenergetic, isonitrogenous meals Protein assessment method: NR **Study Day:** Diet type: Protein-free wheat-starch, butter cookies and a sherbet-based drink along with an L-amino acid mixture Protein source: L-amino acid mixture Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Provided after 3-h after the start of the tracer study and received small isoenergetic meals every 30 min	Outcome measure: Sulfur amino acid kinetic balance Measure/Method used: 24-h amino acid balance method; taken as the sum of the 12-h fasting and 12-h fed sulfur amino acid balance Isotope used: [1-13C]cysteine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Study duration: 6 d (d 1-6) **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.0 g/kg/d (160 mg/kg/d nitrogen) Intended amino acid intake: High methionine:13 mg/kg/d methionine, 0 mg/kg/d cysteine; low methionine: 5 mg/kg/d methionine, 0 mg/kg/d cysteine; methionine plus cysteine 5 mg/kg/d methionine, 6.5 mg/kg/d cysteine; dispensable amino acids were adjusted to maintain a constant total nitrogen content for all diets. See Table 2 in the original paper for more information Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: 1.0 g/kg/d (160 mg/kg/d nitrogen) Actual amino acid intake: High methionine:13 mg/kg/d methionine, 0 mg/kg/d cysteine; low methionine: 5 mg/kg/d methionine, 0 mg/kg/d cysteine; methionine	Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		plus cysteine 5 mg/kg/d methionine, 6.5 mg/kg/d cysteine; dispensable amino acids were adjusted to maintain a constant total nitrogen content for all diets. See Table 2 in the original paper for more information Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 1 d (d 7) Crossover details: Number of intakes per participant: 3 Total intake observations: 16 Wash out period: 10-20 d		

Abbreviations: BMI = body mass index; d = day; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; mg/d = milligrams per day; mg/kg/d = milligrams per kilogram per day; min = minute; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; SD = standard deviation; yr = year.

*Participant characteristics reported for n=6 participants. For sulfur amino acid balance data was available for n=6 for the high methionine diet, n=5 for the low methionine diet, and n=5 for the methionine plus cysteine diet.

Table E.10. Methionine RCTs adults (51->70 years)

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and methods of assessment)
Paoletti, 2023[46] (37356549) Canada Very high HDI Outpatient	Population: Adults (51->70 yr) Total sample N: 15	**Adaptation Period (Same for Men and Women):** Baseline protein intake: Men: 1.11; SEM 0.10 g/kg/d,	**Adaptation Period (Same for Men and Women):** Diet type: Lactose-free milkshake maintenance diet	Outcome measure: Total sulfur AA requirement estimate calculated from $F^{13}CO_2$

Study (PMID) **Location (country)** **HDI** **Setting** **Study Design** **Funding Source** **ROB Score**	**Participants**	**Interventions/Exposure and Comparator (Content)**	**Intervention/Exposure and Comparator (Methods of Assessment)**	**Outcome (Measures and methods of assessment)**
RCT, cross over design Government, Mead Johnson Nutritionals donated the protein-free powder High ROB	**Intervention: Varied Methionine Intakes (Men)** N: 7 % Female: 0% Mean Age/Range/Age at Baseline: 67.3; SEM 3.10 yr Race: NR Mean BMI at baseline: 28.0; SEM 1.08 kg/m^2 Health status/comorbidities: In good health; those with hypertension were included if their blood pressure was well controlled and their antihypertensive medications were consumed as prescribed by their physician Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: Daily 50+ multivitamin-mineral supplement and 500 mg/kg/d choline **Intervention: Varied Methionine Intakes (Women)** N: 8 % Female: 100% Mean Age/Range/Age at	Women: 1.10; SEM 0.10 g/kg/d Baseline amino acid intake: Men: 15.9; SEM 5.59 g/kg/d total sulfur AA, Women: 19.1; SEM 4.52 g/kg/d total sulfur AA Baseline carbohydrate intake: NR Baseline fat intake: Men: 83.7; SEM 10.9 g/d, Women: 81.7; SEM 8.37 g/d Intended protein intake: 1.0 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day (Same for Men and Women):** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA	Protein source: Animal Energy status: Eucaloric Dietary assessment method: NR; Habitual dietary intake assessed by 3-d dietary food record How protein was administered: Provided as 4 equal meals per day Protein assessment method: NR; Habitual dietary intake assessed by 3-d dietary food record **Study Day (Same for Men and Women):** Diet type: Liquid formula composed of protein-free powder, orange-flavored drink crystals, grape seed oil, a crystalline AA mixture, and protein-free cookies Protein source: Crystalline AA mixture (patterned after egg protein) Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Consumed as 8 hourly isocaloric meals Protein assessment method: NR	Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and methods of assessment)
	Baseline: 69.1; SEM 2.55 yr Race: NR Mean BMI at baseline: 25.5; SEM 1.27 kg/m^2 Health status/comorbidities: In good health; those with hypertension were included if their blood pressure was well controlled and their antihypertensive medications were consumed as prescribed by their physician Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NR Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: Daily 50+ multivitamin-mineral supplement and 500 mg/kg/d choline	Intended protein intake: 1.0 g/kg/d Intended amino acid intake: 5, 10, 15, 19, 25, 35, or 40 mg/kg/d methionine, 0 mg/kg/d cysteine, 25 mg/kg/d phenylalanine, 40 mg/kg/d tyrosine, L-alanine adjusted to keep diets isonitrogenous Intended carbohydrate intake: 50% energy from carbohydrate Intended fat intake: 34% energy from fat Actual protein intake: 1.0 g/kg/d Actual amino acid intake: 5, 10, 15, 19, 25, 35, or 40 mg/kg/d methionine, 0 mg/kg/d cysteine, 25 mg/kg/d phenylalanine, 40 mg/kg/d tyrosine, L-alanine adjusted to keep diets isonitrogenous Actual carbohydrate intake: 50% energy from carbohydrate Actual fat intake: 34% energy from fat Study duration: 1 d (d 3) Crossover details: Number of intakes per participant: 2-7 Total intake observations: 83 Wash out period: 1-2 wk		

Abbreviations: AA = amino acid; BMI = body mass index; 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; HDI = human development index; IAAO = indicator amino acid oxidation; kg = kilogram; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RoB = risk of bias; SD = standard deviation; wk = week; yr = year

Threonine

Table E.11. Threonine RCTs adults (19-50 years)

Study (PMID) **Location (country)** **HDI** **Setting** **Study Design** **Funding Source** **ROB Score**	**Participants**	**Interventions/Exposure and Comparator (Content)**	**Intervention/Exposure and Comparator (Methods of Assessment)**	**Outcome (Measures and Methods of Assessment)**
Borgonha, 2002[1] (11916756) United States Very high HDI Outpatient RCT Government; academic High ROB	Population: Adults (19-50 yr) Total sample N: 15 **Intervention/Arm 1: 7 mg/kg/d Threonine** N: 5 % Female: 60% Mean Age/Range/Age at Baseline: 23.4; SD 2.6 yr Race: NR Mean BMI at baseline: NR Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NR Gestation stage: NR Lactation stage: NR Menopausal status: NR Income level: NR Education level: NR Physical activity level: Encouraged to maintain their customary levels of physical activity during the dietary period but were asked to refrain from excessive or competitive exercise.	**Intervention/Arm 1: 7 mg/kg/d Threonine** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Intended amino acid intake: 7 mg/kg/d threonine; ~40 mg/kg/d leucine. See Table 1 in the original paper for more information Intended carbohydrate intake: Non-protein energy: ~60% Intended fat intake: Non-protein energy: ~40% Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR	**Intervention/Arm 1: 7 mg/kg/d Threonine** Diet type: Weight-maintaining diet based on an L-amino acid mixture with a sugar-oil formula and protein-free wheat-starch cookies Protein source: L-amino acid mixture Energy status: Eucaloric except for tracer infusion d 7 and 14 in which energy was reduced to 70% of usual intake Dietary assessment method: daily energy requirements determined with the use of diet histories and estimates of basal metabolic rates How protein was administered: Provided as 3 isoenergetic, isonitrogenous meals Protein assessment method: NR **Comparator/Arm 2: 15 mg/kg/d Threonine**	Outcome measure: Leucine balance and leucine oxidation Measure/Method of Assessment: 24-h IAAB/24-h IAAO method Isotope used: L-[1-13C] leucine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Medication use: NR Supplement use: daily multivitamin/mineral, choline (500 mg/d), dietary fiber (20g microcrystalline cellulose/d) **Comparator/Arm 2: 15 mg/kg/d Threonine** N: 5 % Female: 20% Mean Age/Range/Age at Baseline: 23.4; SD 2.6 yr Race: NR Mean BMI at baseline: NR Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NR Gestation stage: NR Lactation stage: NR Menopausal status: NR Income level: NR Education level: NR Physical activity level: Encouraged to maintain their customary levels of physical activity during the dietary period but were asked to refrain from excessive or competitive exercise. Medication use: NR Supplement use: daily multivitamin/mineral, choline (500mg/d), dietary fiber (20g microcrystalline cellulose/d) **Comparator/Arm 3: 46**	Study duration: 14 d (study d on d 7 and 14) **Comparator/Arm 2: 15 mg/kg/d Threonine** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Intended amino acid intake: 15 mg/kg/d threonine; ~40 mg/kg/d leucine. See Table 1 in the original paper for more information Intended carbohydrate intake: Non-protein energy: ~60% Intended fat intake: Non-protein energy: ~40% Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 14 d (study d on d 7 and 14) **Comparator/Arm 3: 46 mg/kg/d Threonine** Baseline protein intake: NR Baseline amino acid intake:	Diet type: weight-maintaining diet based on an L-amino acid mixture with a sugar-oil formula and protein-free wheat-starch cookies Protein source: L-amino acid mixture Energy status: Eucaloric except for tracer infusion d 7 and 14 in which energy was reduced to 70% of usual intake Dietary assessment method: daily energy requirements determined with the use of diet histories and estimates of basal metabolic rates How protein was administered: Provided as 3 isoenergetic, isonitrogenous meals Protein assessment method: NR **Comparator/Arm 3: 46 mg/kg/d Threonine** Diet type: weight-maintaining diet based on an L-amino acid mixture with a sugar-oil formula and protein-free wheat-starch cookies Protein source: L-amino acid mixture Energy status: Eucaloric except for tracer infusion d 7 and 14 in which energy was reduced to 70% of usual intake	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	mg/kg/d Threonine N: 5 % Female: 40% Mean Age/Range/Age at Baseline: 23.4; SD 2.6 yr Race: NR Mean BMI at baseline: NR Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NR Gestation stage: NR Lactation stage: NR Menopausal status: NR Income level: NR Education level: NR Physical activity level: Encouraged to maintain their customary levels of physical activity during the dietary period but were asked to refrain from excessive or competitive exercise. Medication use: NR Supplement use: daily multivitamin/mineral, choline (500mg/d), dietary fiber (20g microcrystalline cellulose/d)	NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Intended amino acid intake: 46 mg/kg/d threonine; ~40 mg/kg/d leucine. See Table 1 in the original paper for more information Intended carbohydrate intake: Non-protein energy: ~60% Intended fat intake: Non-protein energy: ~40% Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 14 d (study d on d 7 and 14) Crossover details: Number of intakes per participant: NA Total intake observations: NA Wash out period: NA	Dietary assessment method: daily energy requirements determined with the use of diet histories and estimates of basal metabolic rates How protein was administered: Provided as 3 isoenergetic, isonitrogenous meals Protein assessment method: NR	

Abbreviations: BMI = body mass index; d = day; g = gram; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; IAAB = indicator amino acid balance; mg/d = milligrams per day; mg/kg/d = milligrams per kilogram per day; N = number, NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; SD = standard deviation; yr = year.

Total Branched Chain Amino Acids

Table E.12. Total branched chain amino acids RCTs adults (19-50 years)

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Riazi, 2003[52] (12730426) 2003 Canada Very high HDI Outpatient RCT, cross over design Government High ROB	Population: Adults (19-50 yr) Total sample N: 7 **Intervention: Varied Total BCAA Intakes** N: 7 % Female: 0% Mean Age/Range/Age at Baseline: 26.1; SD 6.6 yr Race: NR Mean BMI at baseline: 24.1; SD 2.3 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: None used Supplement use: Multivitamin supplement	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.0 g/kg/d	**Adaptation Period:** Diet type: Milkshake diet Protein source: Animal Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Provided a milkshake diet supplemented with additional protein and calories to meet each participant's requirements Protein assessment method: NR **Study Day:** Diet type: Flavored liquid protein-free formula along with an L-amino acid mixture based on the amino acid profile of egg protein, plus two protein-free cookies. Protein source: L-amino acid mixture based on the amino acid profile of egg protein Energy status: Eucaloric Dietary assessment method: NR How protein was administered: All diets prepared and weighed in the research kitchen and were provided as 9 hourly isonitrogenous, isocaloric	Outcome measure: Total BCAA requirement estimate calculated from $F^{13}CO_2$, phenylalanine oxidation, and 9-h phenylalanine balance Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Intended amino acid intake: initial design: 26, 34, 50, 66, 80, 100 and 120 mg/kg/d BCAA mixture. adjusted design: 34, 50, 66, 80, 100, 120, 140, 160, and 180 mg/kg/d BCAA mixture; BCAA proportions: 38.5% leucine, 32.5% valine, and 29% isoleucine; 15 mg/kg/d phenylalanine; 40 mg/kg/d tyrosine. See Table 2 in the original paper for more information Intended carbohydrate 53% of energy from carbohydrate Intended fat intake: 37% of energy from fat Actual protein intake: 1.0 g/kg/d Actual amino acid intake: initial design: 26, 34, 50, 66, 80, 100 and 120 mg/kg/d BCAA mixture. adjusted design: 34, 50, 66, 80, 100, 120, 140, 160, and 180 mg/kg/d BCAA mixture; BCAA proportions: 38.5% leucine, 32.5% valine, and 29% isoleucine; 15 mg/kg/d phenylalanine; 40 mg/kg/d tyrosine. See Table 2 in the original paper for more information Actual carbohydrate 53% of energy from carbohydrate Actual fat intake: 37% of	meals Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		energy from fat Study duration: 1 d (d 3) Crossover details: Adaptation period: Number of intakes per participant: 4-9 Total intake observations: 53 Wash out period: NR		

Abbreviations: BCAA = branched-chain amino acids; BMI = body mass index; 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; HDI = human development index; IAAO = indicator amino acid oxidation; kg/m^2 = kilogram per square meter; mg/kg/d = milligrams per kilogram per day; N = number, NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; SD = standard deviation; yr = year.

Tryptophan

Table E.13. Tryptophan RCTs children and adolescents

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Al-Mokbel, 2019[13] (30753549) Canada Very High HDI Outpatient RCT, cross over design Government High ROB	Population: Children and Adolescents Total sample N: 7 **Intervention: Varied Tryptophan Intake** N: 7 % Female: 57.1% Mean Age/Range/Age at Baseline: 10.2; SD 1.77 yr Race: NR Mean BMI at baseline: 16.7;	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.5 g/kg/d Intended amino acid intake: NR	**Adaptation Period:** Diet type: Standardized diet (based on participant's typical diet) Protein source: NR Energy status: Eucaloric Dietary assessment method: A 3-d food record was collected prior to the adaptation period to develop a standardized diet based on each participant's typical diet.	Outcome measure: Tryptophan requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	SD 2.97 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NR Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: None Supplement use: a daily multivitamin supplement	Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.5 g/kg/d Intended amino acid intake: 0.5–10.0 mg/kg/d tryptophan; 25 mg/kg/d phenylalanine; 61 mg/kg/d tyrosine; alanine adjusted to keep diet isonitrogenous. See Table 2 in original paper for more information Intended carbohydrate intake: 53% of energy from carbohydrate Intended fat intake: 37% of energy from fat Actual protein intake: 1.5 g/kg/d	Additionally, a food record was collected before each study to ensure adherence to the prescribed diet How protein was administered: Prescribed a diet based on each participant's typical diet Protein assessment method: NR **Study Day:** Diet type: Protein-free liquid formula made with protein-free powder, flavored with Tang and Fresh Plus crystals (Lynch Foods), grapeseed oil, crystalline amino acid mixture, patterned after egg protein, and protein-free cookies Protein source: Amino acid mixture on the basis of the egg protein pattern Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Each participant was provided with 8 hourly isocaloric isonitrogenous meals Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Actual amino acid intake: 0.5–10.0 mg/kg/d tryptophan; 25 mg/kg/d phenylalanine; 61 mg/kg/d tyrosine; alanine adjusted to keep diet isonitrogenous. See Table 2 in original paper for more information Actual carbohydrate intake: 53% of energy from carbohydrate Actual fat intake: 37% of energy from fat Study duration: 1 d (d 3) Crossover details: Number of intakes per participant: 2-7 Total intake observations: 36 Wash out period: 1–2 wk		

Abbreviations: BMI = body mass index; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation; g/kg/d = grams per kilogram per day, h = hour; HDI = human development index; IAAO = indicator amino acid oxidation; kg/m^2 = kilogram per square meter; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; SD = standard deviation; wk = week; yr = year.

Appendix F. Study Characteristics (Low or Moderate RoB)

Protein

Table F.1. Protein RCTs children and adolescents

Study (PMID) **Location (country)** **HDI** **Setting** **Study Design** **Funding Source** **ROB Score**	**Participants**	**Interventions/Exposure and Comparator (Content)**	**Intervention/Exposure and Comparator (Methods of Assessment)**	**Outcome (Measures and Methods of Assessment)**
Elango, 2011[20] (22049165) Canada Very High HDI Outpatient RCT, cross over design Government, protein-free powder for experimental diets provided by Mead Johnson Nutritionals Moderate risk ROB	Population: Children and Adolescents Total sample N: 7 **Intervention: Varied Protein Intakes** N: 7 % Female: 28.6% Mean Age/Range/Age at Baseline: 8.4; SD 1.4 yr Race: NR Mean BMI at baseline: NR Health status/comorbidities: Considered healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: None Supplement use: Daily multivitamin supplement	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.5 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA	**Adaptation Period:** Diet type: Standardized diet (typical foods consumed by participants) Protein source: NR Energy status: Eucaloric Dietary assessment method: Created the standardized diet for the adaptation d based on a 3-d food record; collected a 2-d food record before each study to ensure consistency of dietary intakes How protein was administered: Created the standardized diet for the adaptation d based on a 3-d food record; collected a 2-d food record before each study to ensure consistency of dietary intakes Protein assessment method: 2-d food record **Study Day** Diet type: Protein-free liquid formula made with protein-free powder, flavored drink crystals, corn oil, and protein-	Outcome measure: Protein requirement estimate calculated from phenylalanine oxidation and $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Baseline fat intake: NA Intended protein intake: 0.1-2.56 g/kg/d Intended amino acid intake: Based on egg protein pattern; L-Phenylalanine intake kept constant (30.5 mg/kg/d); Tyrosine intake kept constant (60.1 mg/kg/d); See table 2 in original paper for more information Intended carbohydrate intake: 48-66% of energy from carbohydrates Intended fat intake: 33% of energy from fat Actual protein intake: 0.1-2.56 g/kg/d Actual amino acid intake: Based on egg protein pattern; L-Phenylalanine intake kept constant (30.5 mg/kg/d); Tyrosine intake kept constant (60.1 mg/kg/d); See table 2 in original paper for more information Actual carbohydrate intake: 48-66% of energy from carbohydrates Actual fat intake: 33% of energy from fat Study duration: 1 d (d 3) Crossover details: Number of intakes per	free cookies and a crystalline L-amino acid mixture provided Protein source: crystalline L-amino acid mixture on the basis of the egg protein pattern Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Each subject provided with 8 hourly isocaloric meals Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		participant: 7-9 Total intake observations: 56 Wash out period: ≥1 wk		

Abbreviations: BMI = body mass index; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; g = gram; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; h/wk = hours per week; IAAO = indicator amino acid oxidation; kg/m^2 = kilogram per square meter; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; SD = standard deviation; wk = week; yr = year

Table F.2. Protein RCTs pregnant people

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Stephens, 2015[54] (25527661) Canada Very High HDI Outpatient RCT, cross over design Government, protein-free powder for experimental diets provided by Mead Johnson Nutritionals, Ajinomoto Inc. donated the L-amino acids. Moderate ROB	Population: Pregnant People Total sample N: 29; 7 studied in both early and late gestation **Intervention: Varied Protein Intakes (early gestation)** N: 17 % Female: 100% Mean Age/Range/Age at Baseline: 30.6: SD 3.9 yr Race: NR Mean BMI at baseline: 22.1; SD 2.9 kg/m^2 Health status/comorbidities: Considered healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: Pregnant Gestation stage: Early	**Adaptation Period (early and late gestation)** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.5 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: 1.44; SD 0.30 g/kg/d (early gestation); 1.47; SD 0.53 g/kg/d (late gestation) Actual amino acid intake: NR	**Adaptation Period (early and late gestation)** Diet type: Maintenance diet (food sources favored by participants) Protein source: NR Energy status: Eucaloric Dietary assessment method: Created diet protein recommendations for the adaptation d based on a 2-d food record; collected a 2-d food record before each study to ensure consistency of dietary intakes. How protein was administered: Participants provided with maintenance diet protein recommendations Protein assessment method: collected a 2-d food record	Outcome measure: Protein requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	gestation (11-20 wk), 16.5: SD 2.6 wk Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: Two women reported using an antidepressant during their pregnancy, 1 woman reported using a steroid inhaler, and 1 woman reported using an over-the-counter stool softener. No medications were taken on the study d. Supplement use: Daily Prenatal Multivitamin supplements **Intervention: Varied Protein Intakes (late gestation)** N: 19 % Female: 100% Mean Age/Range/Age at Baseline: 30.3: SD 2.8 yr Race: NR Mean BMI at baseline: 21.8; SD 2.9 kg/m^2 Health status/comorbidities: Considered healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: Pregnant Gestation stage: Late	Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day (early and late gestation)** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 0.22-2.56 g/kg/d Intended amino acid intake: Based on egg protein pattern; L-Phenylalanine intake kept constant (30.5 mg/kg/d); Tyrosine intake kept constant (60.1 mg/kg/d); see supplemental Table 1 in original paper for more information Intended carbohydrate intake: 42-60% of energy from carbohydrates Intended fat intake: 37% of energy from fat Actual protein intake: 0.22-2.56 g/kg/d Actual amino acid intake: Based on egg protein pattern; L-Phenylalanine intake kept constant (30.5 mg/kg/d);	before each study to ensure consistency of dietary intakes **Study Day (early and late gestation)** Diet type: Small protein shake and protein-free cookies (Shakes consisted of protein-free liquid formula made with protein-free powder, flavored drink crystals, and corn oil. Additionally, test protein was provided as a crystalline L-amino acid mixture based on egg-protein composition) Protein source: L-amino acid mixture based on egg-protein composition Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants received 8-hourly isocaloric and isonitrogenous meals Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	gestation (32-38 wk), 35.4: SD 1.8 wk Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: Two women reported using an antidepressant during their pregnancy, 1 woman reported using a steroid inhaler, and 1 woman reported using an over-the-counter stool softener. No medications were taken on the study d. Supplement use: Daily Prenatal Multivitamin supplements	Tyrosine intake kept constant (60.1 mg/kg/d); see supplemental Table 1 in original paper for more information Actual carbohydrate intake: 42-60% of energy from carbohydrates Actual fat intake: 37% of energy from fat Study duration: 1 d (d 3) Crossover details: Number of intakes per participant: 1-4 Total intake observations: 78 (35 early gestation, 43 late gestation) Wash out period: ≥ 5 d		

Abbreviations: BMI = body mass index; 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; HDI = human development index; IAAO = indicator amino acid oxidation; kg/m^2 = kilogram per square meter; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; SD = standard deviation; wk = week; yr = year

Table F.3. Protein RCTs adults (19-50 years)

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Humayun, 2007[27] (17921376) Canada Very High HDI Outpatient	Population: Adults (19-50 yr) Total sample N: 8 **Intervention: Varied Protein Intakes**	**Adaptation Period** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake:	**Adaptation Period** Diet type: Maintenance milkshake diet Protein source: Animal Energy status: Eucaloric	Outcome measure: Protein requirement estimate calculated from $F^{13}CO_2$ Measure/Method of

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
RCT, cross over design Government, protein-free powder for experimental diets provided by Mead Johnson Nutritionals Moderate ROB	N: 8 % Female: 0% Mean Age/Range/Age at Baseline: 26.8; SE 2.0 yr Race: 25% South Asian, 37.5% East Asian, 12.5% African, 25% White Mean BMI at baseline: 23.3; SE 1.0 kg/m^2 Health status/comorbidities: Considered healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: None Supplement use: NR	NR Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: 3.25% fat added to daily portion of milk shakes Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 0.10, 0.30, 0.60, 0.90, 1.2, 1.5, and 1.8 g/kg/d Intended amino acid intake: Amount representative of egg protein composition; L-phenylalanine intake was kept constant at 30.5 mg/kg/d and L-tyrosine intake was kept	Dietary assessment method: NR How protein was administered: Participants received their milkshake diet in weighed daily portions and supplemented with additional protein and energy to meet each participant's requirements Protein assessment method: NR **Study Day:** Diet type: Protein-free liquid formula containing protein-free powder, flavoring crystals, corn oil, the crystalline AA mixture, and protein free cookies Protein source: Crystalline AA mixture Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants were provided with the study diet as 8 isocaloric hourly meals Protein assessment method: NR	Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		constant at 40.7 mg/kg/d; See Table 2 in original paper for more information Intended carbohydrate intake: 48-66% of energy from carbohydrate Intended fat intake: 33% of energy from fat Actual protein intake: 0.10, 0.30, 0.60, 0.90, 1.2, 1.5, and 1.8 g/kg/d Actual amino acid intake: Amount representative of egg protein composition; L-phenylalanine intake was kept constant at 30.5 mg/kg/d and L-tyrosine intake was kept constant at 40.7 mg/kg/d; See Table 2 in original paper for more information Actual carbohydrate intake: 48-66% of energy from carbohydrate Actual fat intake: 33% of energy from fat Study duration: 1 d (d 3) Crossover details: Number of intakes per participant: 7 Total intake observations: 56 Wash out period: ≥1 wk		

Abbreviations: AA = amino acid; BMI = body mass index; d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; HDI = human development Index; IAAO = indicator amino acid oxidation; kg/m^2 = kilogram per square meter; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; SD = standard deviation; SE = standard error; wk = week; yr = year

Table F.4. Protein RCTs adults (51->70 years)

Study (PMID) **Location (country)** **HDI** **Setting** **Study Design** **Funding Source** **ROB Score**	**Participants**	**Interventions/Exposure and Comparator (Content)**	**Intervention/Exposure and Comparator (Methods of Assessment)**	**Outcome (Measures and Methods of Assessment)**
Mao, 2020[40] (32140711) China High HDI Outpatient RCT, cross over design Nonprofit Moderate ROB	Population: Adults (51->70 yr) Total sample N: 14 **Intervention: Varied Protein Intakes (female)** N: 7 % Female: 100% Mean Age/Range/Age at Baseline: 73.1: SD 4.95 yr Race: NR Mean BMI at baseline: 25.2; SD 2.85 kg/m^2 Health status/comorbidities: Considered healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NR Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: NR **Intervention: Varied protein intakes (male)** N: 7 % Female: 0% Mean Age/Range/Age at Baseline: 70.9: SD 5.76 yr Race: NR Mean BMI at baseline: 24.7; SD 3.87 kg/m^2	**Adaptation Period (same for female and male)** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day (same for female and male)** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 0.3, 0.6, 0.9, 1.2, 1.5, and 1.8	**Adaptation Period (same for female and male)** Diet type: Standard Chinese diet. Each meal contained 1 staple food (e.g., rice, steamed roll, or steamed bread), 1 high-quality protein food (such as pork, chicken, egg, or tofu), 1 vegetable, and 1 fruit (except at breakfast). Protein source: Mixed; (high-quality protein food (such as pork, chicken, egg, or tofu)) Energy status: Eucaloric Dietary assessment method: Before and after taking the meal, each food was weighed and recorded in order to determine intake per food. The types and amounts of consumed foods were collected for all subjects and tested for the concentration of major macronutrients. How protein was administered: Each subject received prepared standard Chinese diets Protein assessment method: Same as above. **Study day (same for female and male)** Diet type: Lactalbumin powder, protein-free biscuits,	Outcome measure: Protein requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Health status/comorbidities: Considered healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: NR	g/kg/d Intended amino acid intake: Represents amino acid composition of lactalbumin; 62.8 mg/kg/d phenylalanine and 61.4 mg/kg/d tyrosine, both kept constant; See Table 1 in original paper for more information Intended carbohydrate intake: 38.5%–62.9% energy from carbohydrates Intended fat intake: 33% energy from fat Actual protein intake: 0.3, 0.6, 0.9, 1.2, 1.5, and 1.8 g/kg/d Actual amino acid intake: Represents amino acid composition of lactalbumin; 62.8 mg/kg/d phenylalanine and 61.4 mg/kg/d tyrosine, both kept constant; See Table 1 in original paper for more information Actual carbohydrate intake: 38.5%–62.9% energy from carbohydrates Actual fat intake: 33% energy from fat Study duration: 1 d (d 3) Crossover details: Number of intakes per participant: 6, except for 2 who received 4 (2 subjects missing 0.9 and 1.5 g/kg	protein-free and fried starch slices, and protein-free lotus root starch. Protein source: Lactalbumin powder Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Each subject received 8 hourly isocaloric meals Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		dose) Total intake observations: 80 Wash out period: 1 wk		
Morse, 2001[44] (11682582) United States Very High HDI Outpatient RCT, cross over design Government, academic Moderate risk ROB	Population: Adults (51->70 yr) Total sample N: 11 **Intervention: Varied Protein Intakes (low, medium, high)** N: 11 % Female: 100% Mean Age/Range/Age at Baseline: 75; SEM 4.0 yr Race: NR Mean BMI at baseline: 27.3; SEM 3.8 kg/m^2 Health status/comorbidities: Considered healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NR Income level: NR Education level: NR Physical activity level: NR Medication use: Women on estrogen replacement therapy (N=9). Supplement use: One multivitamin taken daily.	**Low Protein (LPro) (Arm 1):** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 0.50 g/kg/d protein Intended amino acid intake: NR Intended carbohydrate intake: Nonprotein energy 65% Intended fat intake: Nonprotein energy 35% Actual protein intake: Day 1: 0.18; SEM 0.01 g/kg/d; Day 2-18: 0.53; SEM 0.02 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 330; SEM 9.5 g/d Actual fat intake: 77; SEM 2.0 g/d Study duration: 18 d **Medium Protein (MPro) (Arm 2):** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR	**Intervention: Varied Protein Intakes (low, medium, high)** Diet type: Mixed (menu with solid foods and a protein supplement mixture) Protein source: Mixed, excluded meats (High-quality animal-based proteins were included). Energy status: Eucaloric Dietary method of assessment: The energy, protein, carbohydrate, and fat contents of each cycle menu were calculated by Nutritionist V computer software. How protein was administered: Each participant provided all meals as a 3-d rotating menu. All women agreed to scrape and rinse all dishes, glassware, and utensils with water and to consume the rinsing. Protein assessment method: Food homogenates were made of each woman's three daily menus during the second week of the study. These samples were measured for total nitrogen content via the Elementar Macro N Nitrogen analyzer.	Outcome measure: Protein requirement estimate calculated from nitrogen balance Measure/Method of Assessment: Nitrogen balance measured during week 2 (d 7-10) and week 3 (d 14-17) of each trial and calculated as I_n - (U_n+F_n+M_n), where M_n, assumed to be 8 mg of nitrogen/kg/d. Isotope used: NA

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Baseline fat intake: NR Intended protein intake: 0.75 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: Nonprotein energy 65% Intended fat intake: Nonprotein energy 35% Actual protein intake: Day 1: 0.18; SEM 0.01 g/kg/d; Day 2-18: 0.76; SEM 0.02 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 313; SEM 8.8 g/d Actual fat intake: 78; SEM 2.0 g/d Study duration: 18 d **High Protein (HPro) (Arm 3):** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.00 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: Nonprotein energy 65% Intended fat intake: Nonprotein energy 35%	Dietary protein intake (g/kg/d) was calculated by assuming that each gram of nitrogen was equivalent to 6.25 g of protein.	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Actual protein intake: Day 1: 0.18; SEM 0.01 g/kg/d; Day 2-18: 1.06; SEM 0.05 g/kg/d Actual amino acid intake: NR Actual carbohydrate intake: 302; SEM 8.6 g/d Actual fat intake: 74; SEM 2.0 g/d Study duration: 18 d Crossover details: Number of intakes per participant: 3 Total intake observations: 33 Wash out period: minimum of 7 d		
Rafii, 2015[49] (25320185) Canada Very High HDI Outpatient RCT, cross over design Government, multivitamins donated by Pfizer Consumer Healthcare, protein-free powder for experimental diets provided by Mead Johnson Nutritionals Moderate ROB	Population: Adults (51->70 yr) Total sample N: 12 **Intervention: Varied Protein Intakes** N: 12 % Female: 100% Mean Age/Range/Age at Baseline: 74.3; SD 7.4 yr Race: 91.67% Caucasian, 8.33% Asian Mean BMI at baseline: 24.8; SD 2.3 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: 3.25% fat per daily portion of milkshake Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake:	**Adaptation Period:** Diet type: Lactose-free milkshake maintenance diet Protein source: Animal Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Diet was weighed in daily portions for each subject and supplemented with additional protein and carbohydrate depending on each subject's requirement and consumed as 4 equal meals Protein assessment method: NR **Study Day:**	Outcome measure: Protein requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Lactation stage: NA Menopausal status: NR Income level: NR Education level: NR Physical activity level: All subjects engaged in some level of physical activity. Medication use: None used that could affect protein or AA metabolism. Subjects with hypertension could take blood pressure medication as prescribed Supplement use: Daily multivitamin supplement	NR Actual fat intake: NR Study duration: 2 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 0.2-2.0 g/kg/d Intended amino acid intake: Amount similar to that of egg-protein composition; L-phenylalanine intake kept constant at 30.0 mg/kg/d and L-tyrosine intake kept constant at 40.0 mg/kg/d; See Table 1 in original paper for more information Intended carbohydrate intake: 39-58% of energy from carbohydrate Intended fat intake: 35% of energy from fat Actual protein intake: 0.2-2.0 g/kg/d Actual amino acid intake: Amount similar to that of egg-protein composition; L-phenylalanine intake kept constant at 30.0 mg/kg/d and L-tyrosine intake kept	Diet type: Protein-free liquid formula made with protein-free powder; flavored drink crystals; grape seed oil; a crystalline AA mixture, patterned after egg protein (representing various protein intake amounts); and protein-free cookies. Protein source: Crystalline AA mixture, patterned after egg protein (representing various protein intake amounts) Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Each subject received 8 hourly isocaloric meals Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		constant at 40.0 mg/kg/d; See Table 1 in original paper for more information Actual carbohydrate intake: 39-58% of energy from carbohydrate Actual fat intake: 35% of energy from fat Study duration: 1 d (d 3) Crossover details: Number of intakes per participant: 2-11 Total intake observations: 83 Wash out period: 1 to 2 wk		
Rafii, 2015[50] (26962173) Canada Very high HDI Outpatient RCT, cross over design Government, Pfizer Consumer Healthcare donated the multivitamins and Mead Johnson Nutritionals donated the protein-free powder Moderate ROB	Population: Adults 51->70 yr Total sample N: 6 **Intervention: Varied Protein Intake** N: 6 % Female: 0% Mean Age/Range/Age at Baseline: 71.3; SD 4.50 yr Race: 83% White; 17% African Canadian Mean BMI at baseline: 27.7; SD 3.47 kg/m^2 Health status/comorbidities: Healthy; Participants with hypertension were not excluded if their blood pressure was well controlled, and their antihypertensive medications were taken as prescribed. Obesity status: NR	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR	**Adaptation Period:** Diet type: Lactose-free milkshake maintenance diet Protein source: Animal Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Provided to participants to be consumed as 4 equal meals. Protein assessment method: NR **Study Day:** Diet type: Protein-free liquid formula made with protein-free powder, flavored drink crystals, grape seed oil, and a crystalline AA mixture and protein-free cookies. Protein source: Crystalline	Outcome measure: Protein requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: Daily multivitamin	Study duration: 2 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 0.2-2.0 g/kg/d Intended amino acid intake: 30 mg/kg/d phenylalanine; 40 mg/kg/d tyrosine; See table 1 in original paper for more information Intended carbohydrate intake: 37-57% of energy from carbohydrate Intended fat intake: Up to 40% of energy from fat Actual protein intake: 0.2-2.0 g/kg/d Actual amino acid intake: 30 mg/kg/d phenylalanine; 40 mg/kg/d tyrosine; See table 1 in original paper for more information Actual carbohydrate intake: 37-57% of energy from carbohydrate Actual fat intake: Up to 40% of energy from fat Study duration: 1 d (d 3)	AA mixture patterned after egg protein Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Each participant received 8 hourly isocaloric meals. Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Crossover details: Number of intakes per participant: 7 Total intake observations: 42 Wash out period: 1-2 wk		

Abbreviations: AA = amino acid; BMI = body mass index; 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; HDI = human development index; IAAO = indicator amino acid oxidation; kg/m^2 = kilogram per square meter; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; SD = standard deviation; wk = week; yr = year

Table F.5. Protein RCTs adults (19-50 years and 51->70 years)

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Walrand, 2008[58] (18697911) United States Very High HDI Outpatient RCT, cross over design Government Moderate ROB	Population: Adults (19-50 and 51->70 yr) Total sample N: 19 **Younger (usual and high protein)** N: 10 % Female: 50% Mean Age/Range/Age at Baseline: 24.3; SE 1.2 yr Race: NR Mean BMI at baseline: 23.3; SE 1.0 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA	**Younger (usual protein; Arm 1):** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.5 g/kg FFM/d Intended amino acid intake: NR Intended carbohydrate intake: 50% of energy from carbohydrate Intended fat intake: Adjusted to keep the diets isocaloric Actual protein intake: protein	**Younger and Older (usual and high protein)** Diet type: NR Protein source: NR Energy status: Eucaloric Dietary assessment method: All food was prepared in the Metabolic Kitchen at the Clinical Research Unit of the Mayo Clinic Center for Translational Science Activities. Compliance to the diet was checked by measuring 24-h urinary nitrogen and protein oxidation rate at the end of each 10 d dietary period How protein was administered: Prepared foods provided to participants	Outcome measure: Nitrogen balance and leucine oxidation Measure/Method of Assessment: AA kinetics (calculated using steady-state Tracer dilution technique and the reciprocal pool model) Nitrogen balance method (Urinary nitrogen was measured and nitrogen balance calculated by subtracting 24-h urinary nitrogen excretion from nitrogen intakes.) Isotope used: L-[15N]lysine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Menopausal status: NR Income level: NR Education level: NR Physical activity level: NR Medication use: Excluded if taking medications such as B-blockers, steroids, and any medication affecting metabolism or muscle, endocrine, cardiovascular or digestive function Supplement use: NR **Older (usual and high protein)** N: 9 % Female: 44% Mean Age/Range/Age at Baseline: 70.0; SE 1.8 yr Race: NR Mean BMI at baseline: 27.2; SE 0.9 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NR Income level: NR Education level: NR Physical activity level: NR Medication use: Excluded if taking medications such as B-blockers, steroids, and any medication affecting metabolism or muscle,	intake g/kg FFM/d: 1.5 SE 0.0, Protein intake g/kg BW/d: 1.04; SE 0.03, Protein intake g/d: 72.7 SE 6.1 Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 10 d **Younger (high protein; Arm 2):** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 3.0 g/kg FFM/d Intended amino acid intake: NR Intended carbohydrate intake: 50% of energy from carbohydrate Intended fat intake: Adjusted to keep the diets isocaloric Actual protein intake: Protein intake g/kg FFM/d: 3.0; SE 0.0, Protein intake g/kg BW/d: 2.08; SE 0.07, Protein intake g/d: 146.5; SE 12.9 Actual amino acid intake: NR Actual carbohydrate intake: NR	during the intervention period Protein assessment method: Compliance to the diet was checked by measuring 24-h urinary nitrogen and protein oxidation rate at the end of each 10 d dietary period	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	endocrine, cardiovascular or digestive function Supplement use: NR	Actual fat intake: NR Study duration: 10 d **Older (usual protein; Arm 3):** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.5 g/kg FFM/d Intended amino acid intake: NR Intended carbohydrate intake: 50% of energy from carbohydrate Intended fat intake: Adjusted to keep the diets isocaloric Actual protein intake: Protein intake g/kg FFM/d: 1.5; SE 0.0, Protein intake g/kg BW/d: 0.89; SE 0.05, Protein intake g/d: 68.6; SE 4.6 Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 10 d **Older (high protein; Arm 4):** Baseline protein intake: NR Baseline amino acid intake:		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 3.0 g/kg FFM/d Intended amino acid intake: NR Intended carbohydrate intake: 50% of energy from carbohydrate Intended fat intake: Adjusted to keep the diets isocaloric Actual protein intake: Protein intake g/kg FFM/d: 3.0; SE 0.0, Protein intake g/kg BW/d: 1.79; SE 0.10, Protein intake g/d: 137.1; SE 9.5 Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 10 d Crossover details: Number of intakes per participant: 2 Total intake observations: 38 Wash out period: 2-8 wk		

Abbreviations: AA = amino acid; BMI = body mass index; d = day; g/d = grams per day; g/kg FFM/d = grams per kilogram fat-free mass per day; h = hour; HDI = human development index; kg/m^2 = kilogram per square meter; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; SD = standard deviation; SE = standard error; wk = week; yr = year

Table F.6. Protein non-RCTs infants

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Kittisakmontri, 2022[68] (36235599) Thailand Very High HDI Outpatient Non-RCT; Multicenter, prospective cohort study Government, academic Moderate ROB	Population: Infants Total sample N: 145 **Infants:** N: 145 % Female: 49.7% Mean Age/Range/Age at Baseline: Start: 6 mo End:12 mo; gestational age: 38.8; SD 1.0 wk Race: NR Mean BMI at baseline: NR Health status/comorbidities: Healthy term infants Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: Family income per month: <10,000: 7.6%, 10,000-29,999: 44.8%, 30,000-49,999: 35.2%, ≥ 50,000: 12.4% Education level: Maternal educational attainment: Did not receive formal education: 1.4%, below bachelor's degree: 51%, bachelor's degree and above: 47.6% Physical activity level: NA Medication use: Excluded those who regularly received medication Supplement use: NR	**Infants:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR End of study protein intake: Average % of energy from protein: 6 mo: 7.8%, 9 mo: 12.6%, 12 mo: 15.6% of energy from protein End of study amino acid intake: NR End of study carbohydrate intake: NR End of study fat intake: NR Duration/Follow up: 6 mo	**Infants:** Diet type: Breastfeeding and complementary food Protein source: Mixed Energy status: NR Dietary assessment method: Dietary intake was estimated using a FFQ for the semi-quantitative estimation of habitual intake alongside a 24-h recall interview and a 3-d FR was also collected at 9 and 12 months for quantitative estimation. Protein assessment method: Dietary intakes were converted to energy and nutrients using the Institute of Nutrition Mahidol University Calculation (INMUCAL)-Nutrient program version 4.0 (2018).	Outcome measure: Length-for-age z score; conditional growth length-for-age z score Measure/Method of Assessment: Body weight and the recumbent length of infants were measured using an electronic scale and a standard wooden measuring board. All outcomes were calculated using WHO Anthro version 3.2.2 Isotope used: NA

Abbreviations: BMI = body mass index; d = day; FFQ = food frequency questionnaire; FR = food record; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation; HDI = human development index; kg/m^2 = kilogram per square meter; N = number; NR = not reported; PMID = PubMed Identification Number; wk = week; yr = year

Table F.7. Protein non-RCTs adults (19-50 years)

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Atinmo, 2010[61] (NA) Nigeria Low HDI Outpatient Non-RCT, cross over design Government Low ROB	Population: Adults (19-50 yr) Total sample N: 18 **Northern Nigeria (Arm 1):** N: 7 % Female: 0% Mean Age/Range/Age at Baseline: Mean age of all arms combined: 23.3; SD 1.5 yr Race: NR Mean BMI at baseline: NR Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: University students Physical activity level: NR Medication use: NR Supplement use: Vitamin and mineral supplementation **Southern Nigeria:** N: 11 % Female: 0% Mean Age/Range/Age at Baseline: Mean age of all	**Northern Nigeria (Arm 1):** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 0.4, 0.7, 0.8 and 0.9 g/kg/d; 0.1 g/kg/d consumed one d prior to study Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: 74.85; SD 5.71; 112.84; SD 9.14; 130.03; SD 9.66; 156.23; SD 12.17 mg/kg/d nitrogen Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 1 d adapted to very low protein diet, 10 d on study diet **Southern Nigeria (Arm 2):** Baseline protein intake: NR	**Northern Nigeria (Arm 1) and Southern Nigeria (Arm 2):** Diet type: Ordinary Nigerian mixed diets (including root tubers, cereals, vegetables, and animal products) Protein source: Mixed Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Prepared meals were provided to subjects in patterns the subjects were accustomed to, i.e. 7-8 a.m., 1-2 p.m., and 7-8 p.m. Protein assessment method: Modified micro-kjeldahl method of Munro and Fleck	Outcome measure: Protein requirement estimate calculated from nitrogen balance Measure/Method of Assessment: Nitrogen balance calculated from intake, fecal, urine, and miscellaneous losses. Skin nitrogen loss was taken as 10.14 mg/kg/d nitrogen. Isotope used: NA

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	arms combined: 23.3; SD 1.5 yr Race: NR Mean BMI at baseline: NR Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: University students Physical activity level: NR Medication use: NR Supplement use: Vitamin and mineral supplementation	Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 0.4, 0.7, 0.8 and 0.9 g/kg/d; 0.1 g/kg/d consumed one d prior to study Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: 63.92; SD 5.39; 92.71; SD 7.90; 116.79; SD 9.78; 147.69; SD 12.07 mg/kg/d nitrogen Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 1 d adapted to very low protein diet, 10 d on study diet Crossover details: Number of intakes per participant: 4 Total intake observations: 72 Wash out period: 3 d		

Abbreviations: BMI = body mass index; d = day; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; kg/m^2 = kilogram per square meter; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year

Isoleucine

Table F.8. Isoleucine RCTs infants

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
de Groof, 2014[2] (24284437) China Medium HDI Admitted to the Neonatology Department of the Fudan Children's Hospital, Shanghai, China. RCT Nonprofit, Study formulas were manufactured by SHS United Kingdom Low ROB	Population: Infants Total sample N: 22 **Intervention: Varied Isoleucine Intakes** N: 22 % Female: 59% Mean Age/Range/Age at Baseline: 12; SD 5 d; gestational age: 39.5; SD 1.2 wk Race: 100% Asian Mean BMI at baseline: NR Health status/comorbidities: clinically stable condition and considered healthy; initially admitted to the hospital for the following reasons: N=8 unconjugated hyperbilirubinemia, N=7 pneumonia with negative blood cultures, N=3 suspicion of infection with negative blood cultures, N=2 cardiac arrhythmia, N=1 asphyxia, N=1 pneumothorax Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR	**Adaptation Period:** Baseline protein intake: 2.53; SD 0.25 g/kg/d Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: NR Intended amino acid intake: 5-216 mg/kg/d isoleucine; 166 mg/kg/d phenylalanine and tyrosine; L-alanine added separately to make the formula isonitrogenous; See Table 1 in original paper for more information Intended carbohydrate intake: 54 g/100g formula Intended fat intake: 23 g/100g formula Actual protein intake: 2.96; SD 0.15 g/kg/d Actual amino acid intake: 5-216 mg/kg/d isoleucine; 166 mg/kg/d phenylalanine and tyrosine; L-alanine added separately to make the formula isonitrogenous; See Table 1 in original paper for more information Actual carbohydrate intake: 54	**Adaptation Period:** Diet type: Study formula Protein source: AA-based formula Energy status: 475 kcal/100g formula Dietary assessment method: NR How protein was administered: Infants received the study formula every 3 h Protein assessment method: NR **Study Day:** Diet type: Study formula Protein source: AA-based formula Energy status: 475 kcal/100g formula Dietary assessment method: NR How protein was administered: Infants received the study formula by a continuous drip feeding. Protein assessment method: NR	Outcome: Isoleucine requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: [1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Physical activity level: NA Medication use: NR Supplement use: NR	g/100g formula Actual fat intake: 23 g/100g formula Study duration: 1 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: NR Intended amino acid intake: 5-216 mg/kg/d isoleucine; 166 mg/kg/d phenylalanine and tyrosine; L-alanine added separately to make the formula isonitrogenous; See Table 1 in original paper for more information Intended carbohydrate intake: 54 g/100g formula Intended fat intake: 23 g/100g formula Actual protein intake: 2.96; SD 0.15 g/kg/d Actual amino acid intake: 5-216 mg/kg/d isoleucine; 166 mg/kg/d phenylalanine and tyrosine; L-alanine added separately to make the formula isonitrogenous; See Table 1 in original paper for more information		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Actual carbohydrate intake: 54 g/100g formula Actual fat intake: 23 g/100g formula Study duration: 1 d (d 2) Crossover details: Number of intakes per participant: NA Total intake observations: NA Wash out period: NA		

Abbreviations: AA = amino acid; BMI = body mass index; d = day; $F^{13}CO_2$ = the fraction of 13CO2 recovery from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; IAAO = indicator amino acid oxidation; kg = kilogram; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RoB = risk of bias; SD = standard deviation; wk = week

Leucine

Table F.9. Leucine RCTs infants

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
de Groof, 2014[2] (24284437) China Medium HDI Admitted to the Neonatology Department of the Fudan Children's Hospital, Shanghai, China. RCT Nonprofit, Study formulas were manufactured by SHS	Population: Infants Total sample N: 33 **Intervention: Varied Leucine Intakes:** N: 33 % Female: 51.5% Mean Age/Range/Age at Baseline: 11; SD 4 d; gestational age: 39.4; SD 1.4	**Adaptation Period:** Baseline protein intake: 2.78; SD 0.38 g/kg/d Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: NR	**Adaptation Period:** Diet type: Study formula Protein source: AA based formula Energy status: 475 kcal/100g formula Dietary assessment method: NR How protein was administered: Infants	Outcome: Leucine requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: [1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
United Kingdom Low ROB	wk Race: 100% Asian Mean BMI at baseline: NR Health status/comorbidities: clinically stable condition and considered healthy; initially admitted to the hospital for the following reasons: N=21 unconjugated hyperbilirubinemia, N=6 pneumonia with negative blood cultures, N=2 asphyxia, N=1 bloody stool, N=1 wet lung, N=1 constipation, N=1 urine tract infection Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NA Medication use: NR Supplement use: NR	Intended amino acid intake: 5-370 mg/kg/d leucine; 166 mg/kg/d phenylalanine and tyrosine; L-alanine added separately to make the formula isonitrogenous; See Table 1 in original paper for more information Intended carbohydrate intake: 54 g/100g formula Intended fat intake: 23 g/100g formula Actual protein intake: 2.98; SD 0.2 g/kg/d Actual amino acid intake: 5-370 mg/kg/d leucine; 166 mg/kg/d phenylalanine and tyrosine; L-alanine added separately to make the formula isonitrogenous; See Table 1 in original paper for more information Actual carbohydrate intake: 54 g/100g formula Actual fat intake: 23 g/100g formula Study duration: 1 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA	received the study formula every 3 h Protein assessment method: NR **Study Day:** Diet type: Study formula Protein source: AA based formula Energy status: 475 kcal/100g formula Dietary assessment method: NR How protein was administered: Infants received the study formula by a continuous drip feeding. Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Intended protein intake: NR Intended amino acid intake: 5-370 mg/kg/d leucine; 166 mg/kg/d phenylalanine and tyrosine; L-alanine added separately to make the formula isonitrogenous; See Table 1 in original paper for more information Intended carbohydrate intake: 54 g/100g formula Intended fat intake: 23g/100g formula Actual protein intake: 2.98; SD 0.2 g/kg/d Actual amino acid intake: 5-370 mg/kg/d leucine; 166 mg/kg/d phenylalanine and tyrosine; L-alanine added separately to make the formula isonitrogenous; See Table 1 in original paper for more information Actual carbohydrate intake: 54 g/100g formula Actual fat intake: 23g/100g formula Study duration: 1 d (d 2) Crossover details: Number of intakes per participant: NA Total intake observations: NA Wash out period: NA		

Abbreviations: AA = amino acid; BMI = body mass index; d = day; $F^{13}CO_2$ = the fraction of 13CO2 recovery from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; IAAO = indicator amino acid oxidation; kg = kilogram; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RoB = risk of bias; SD = standard deviation; wk = Week

Table F.10. Leucine RCTs adults (19-50 years)

Study (PMID) **Location (country)** **HDI** **Setting** **Study Design** **Funding Source** **ROB Score**	**Participants**	**Interventions/Exposure and Comparator (Content)**	**Intervention/Exposure and Comparator (Methods of Assessment)**	**Outcome (Measures and Methods of Assessment)**
Kurpad, 2001[30] (11722955) 2001 India Low HDI Outpatient RCT, cross over design Government, industry Moderate ROB	Population: Adults (19-50 yr) Total sample N: 20 **Intervention: Varied Leucine Intakes:** N: 20 % Female: 0% Mean Age/Range/Age at Baseline: 21.35; SD 1.11 yr Race: NR Mean BMI at baseline: 20.05; SD 1.5 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: Maintain their customary physical activity levels Medication use: NR Supplement use: Multivitamin-multimineral capsule, 500 mg choline and dietary fiber (20g ispagul)	**Adaptation Period:** Baseline protein intake: 0.9; SD 0.1 g/kg/d Baseline amino acid intake: <70 mg/kg/d leucine Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Intended amino acid intake: 14, 22, 30, and 40 mg/kg/d leucine; total amino acids: 1000 mg/g mixture. See Table 2 in original paper for more information Intended carbohydrate intake: 56% of energy from carbohydrate Intended fat intake: 43% of energy from fat Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 6 d (d 1-6)	**Adaptation Period:** Diet type: Weight maintaining diet based on an L-amino acid mixture Protein source: L-amino acid mixture Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants received their daily intake as 3 isoenergetic isonitrogenous meals except on d 6 where participants received 10 isoenergetic, isonitrogenous small meals Protein assessment method: NR **Study Day** Diet type: Weight maintaining diet based on an L-amino acid mixture Protein source: L-amino acid mixture Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants received 10 isoenergetic,	Outcome measure: Leucine requirement estimate calculated from 24-h IAAB and nitrogen balance Measure/Method of Assessment: 24-h IAAB method and nitrogen balance method Isotope used: [13C]leucine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Study Day: Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Intended amino acid intake: 14, 22, 30, and 40 mg/kg/d leucine; total amino acids: 1000 mg/g mixture. See Table 2 in original paper for more information Intended carbohydrate intake: 56% of energy from carbohydrate Intended fat intake: 43% of energy from fat Actual protein intake: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Actual amino acid intake: 14, 22, 30, and 40 mg/kg/d leucine; total amino acids: 1000 mg/g mixture. See Table 2 in original paper for more information Actual carbohydrate intake: 56% of energy from carbohydrate Actual fat intake: 43% of energy from fat	isonitrogenous small meals Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Study duration: 1 d (d 7) Crossover details: Number of intakes per participant: 2 Total intake observations: 40 (24-h IAAB method), 36 (nitrogen balance) Wash out period: 1-4 wk		

Abbreviations: AA = amino acid; BMI = body mass index; d = day; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; IAAO = indicator amino acid oxidation; kg = kilogram; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RoB = risk of bias; SD = standard deviation; wk = Week; yr = year

Table F.11. Leucine RCTs adults (51->70 years)

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Szwiega, 2021[55] (33330915) Canada Very High HDI Outpatient RCT, cross over design Government, protein-free powder for experimental diets provided by Mead Johnson Nutritionals Moderate ROB	Population: Adults (51->70 yr) Total sample N: 16 **Interventions: Varied Leucine Intakes (Males)** N: 7 % Female: 0% Mean Age/Range/Age at Baseline: 70.4; SEMs 2.2 yr Race: NR Mean BMI at baseline: 27.2, SEMs 0.6 kg/m^2 Health status/comorbidities: Healthy; Subjects with hypertension were not	**Adaptation Period: (Same for Males and Females)** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR	**Adaptation Period: (Same for Males and Females)** Diet type: Lactose-free milkshake maintenance diet Protein source: Animal Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants consumed each milkshake as 4 equal meals. Protein assessment method: NR **Study Day (Same for Males**	Outcome measure: Leucine requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	excluded if their blood pressure was well controlled for at least 2 mo Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: daily 50+ multivitamin-mineral **Interventions: Varied Leucine Intakes (Females)** N: 9 % Female: 100% Mean Age/Range/Age at Baseline: 70.7; SEMs 1.7 yr Race: NR Mean BMI at baseline: 29.2, SEMs 1.3 kg/m^2 Health status/comorbidities: Healthy; Subjects with hypertension were not excluded if their blood pressure was well controlled for at least 2 mo. Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NR Income level: NR	Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day: (Males)** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.0 g/kg/d Intended amino acid intake: 20, 30, 45, 55, 70, 80, 90, 105 and 120 mg/kg/d leucine; 25 mg/kg/d phenylalanine; 40 mg/kg/d tyrosine. See Table 2 in original paper for more information Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: 1.0 g/kg/d Actual amino acid intake: 20, 30, 45, 55, 70, 80, 90, 105 and 120 mg/kg/d leucine; 25 mg/kg/d phenylalanine; 40 mg/kg/d tyrosine. See Table 2 in original paper	**and Females)** Diet type: Liquid formula made with protein-free powder, orange-flavored drink crystals, grape seed oil, a crystalline amino acid mixture patterned after egg protein, and protein free cookies. Protein source: Crystalline amino acid mixture patterned after egg protein. Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants consumed the liquid formula diet as 8 hourly isocaloric meals. Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Education level: NR Physical activity level: NR Medication use: NR Supplement use: daily 50+ multivitamin-mineral	for more information Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 1 d (d 3) **Study Day: (Females)** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.0 g/kg/d Intended amino acid intake: 20, 45, 55, 70, 80, 90, and 120 mg/kg/d leucine; 25 mg/kg/d phenylalanine; 40 mg/kg/d tyrosine. See Table 2 in original paper for more information Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: 1.0 g/kg/d Intended amino acid intake: 20, 45, 55, 70, 80, 90, and 120 mg/kg/d leucine; 25 mg/kg/d phenylalanine; 40 mg/kg/d tyrosine. See Table 2 in original paper for more information. Actual carbohydrate intake:		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		NR Actual fat intake: NR Study duration: 1 d (d 3) Crossover details: (Males) Number of intakes per participant: 5-7 Total intake observations: 45 Wash out period: 1 to 2 wk Crossover details: (Females) Number of intakes per participant: 3-7 Total intake observations: 48 Wash out period: 1 to 2 wk		

Abbreviations: AA = amino acid; BMI = body mass index; 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; HDI = human development index; IAAO = indicator amino acid oxidation; kg = kilogram; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RoB = risk of bias; SD = standard deviation; SEM = standard error of the mean; wk = week, yr = year

Lysine

Table F.12. Lysine RCTs infants

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and methods of assessment)
Huang, 2011[5] (22049162) China High HDI Admitted to the Neonatal Ward in the Children's	Population: Infants Total sample N: 21 **Intervention: Varied Lysine Intakes**	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake:	**Adaptation Period:** Diet type: Elemental Formula Protein source: Free AA-based formula Energy status: 108 kcal/kg/d	Outcome measure: Lysine requirement estimate calculated from $F^{13}CO_2$ Measure/Method of

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and methods of assessment)
Hospital of Fudan University RCT Industry Low ROB	N: 21 % Female: 43% Mean Age/Range/Age at Baseline: 12; SD 6 d; gestational age: 39; SD 1 wk Race: NR Mean BMI at baseline: NR Health status/comorbidities: clinically stable condition and considered healthy; initially admitted to the hospital for the following reasons: N=15 unconjugated hyperbilirubinemia, N=3 pneumonia, N=2 infection suspicion, N=1 skin infection Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NA Medication use: Intravenous antibiotics (peniciliins and/or cephalosporins) given to 15 infants. Supplement use: NA	NR Baseline fat intake: NR Intended protein intake: ~2.96 g/kg/d Intended amino acid intake: 15-240 mg/kg/d lysine; 166 mg/kg/d phenylalanine; 166 mg/kg/d tyrosine; Nitrogen intake kept constant by the substitution of L-alanine. Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: 2.99; SD 0.02 g/kg/d Actual amino acid intake: 15-240 mg/kg/d lysine; 166 mg/kg/d phenylalanine; 166 mg/kg/d tyrosine; Nitrogen intake kept constant by the substitution of L-alanine. Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 24 h (d 1) **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: ~2.96	Dietary assessment method: NR How protein was administered: Infants received the study formula every 3 h Protein assessment method: NR **Study Day:** Diet type: Elemental Formula Protein source: Free AA-based formula Energy status: 108 kcal/kg/d Dietary assessment method: NR How protein was administered: Infants received the study formula hourly Protein assessment method: NR	Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and methods of assessment)
		g/kg/d Intended amino acid intake: 15-240 mg/kg/d lysine; 166 mg/kg/d phenylalanine; 166 mg/kg/d tyrosine; Nitrogen intake kept constant by the substitution of L-alanine. Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: 2.99; SD 0.02 g/kg/d Actual amino acid intake: 15-240 mg/kg/d lysine; 166 mg/kg/d phenylalanine; 166 mg/kg/d tyrosine; Nitrogen intake kept constant by the substitution of L-alanine. Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 15 h (d 2) Crossover details: Number of intakes per participant: NA Total intake observations: NA Wash out period: NA		

Abbreviations: AA = amino acid; BMI = body mass index; $F^{13}CO_2$ = the fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; IAAO = indicator amino acid oxidation; kg = kilogram; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RoB = risk of bias; SD = standard deviation; wk = week

Table F.13. Lysine RCTs children and adolescents

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Elango, 2007[19] (17684206) Canada Very high HDI Outpatient RCT, cross over design Government, protein-free powder for experimental diets provided by Mead Johnson Nutritionals Moderate ROB	Population: Children and Adolescents Total sample N: 5 **Intervention: Varied Lysine Intakes:** N: 5 % Female: 20% Mean Age/Range/Age at Baseline: 8.4; SD 0.9 yr Race: NR Mean BMI at baseline: NR Health status/comorbidities: Healthy Obesity status: NR Pubertal status: Tanner stage I and II Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: None used Supplement use: Daily multivitamin supplement	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.5 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.5 g/kg/d Intended amino acid intake: 5, 15, 25, 35, 50, 65, and 80	**Adaptation Period:** Diet type: Maintenance diet (based on 3-d food records) Protein source: NR Energy status: Eucaloric Dietary assessment method: 3-d food record used to determine maintenance diet; food recorded collected to ensure consistency of intake How protein was administered: Prescribed to a maintenance diet with typical foods consumed by the participants Protein assessment method: NR **Study Day:** Diet type: Protein-free powder flavored with Tang and Kool-Aid, corn oil, a crystalline L-amino acid mixture (based on egg protein composition) and protein-free cookies Protein source: Crystalline L-amino acid mixture based on egg protein composition Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Consumed experimental diet as 8 hourly	Outcome measure: Lysine requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		mg/kg/d lysine, 25 mg/kg/d phenylalanine, 61 mg/kg/d tyrosine, alanine adjusted to maintain a constant nitrogen intake. See Table 2 in original paper for more information Intended carbohydrate intake: 53% of energy from carbohydrate Intended fat intake: 37% of energy from fat Actual protein intake: 1.5 g/kg/d Actual amino acid intake: 5, 15, 25, 35, 50, 65, and 80 mg/kg/d lysine, 25 mg/kg/d phenylalanine, 61 mg/kg/d tyrosine, alanine adjusted to maintain a constant nitrogen intake. See Table 2 in original paper for more information Actual carbohydrate intake: 53% of energy from carbohydrate Actual fat intake: 37% of energy from fat Study duration: 1 d (d 3) Crossover details: Number of intakes per participant: 7 Total intake observations: 35 Wash out period: ≥ 1 wk	isonitrogenous, isocaloric meals Protein assessment method: NR	
Pillai, 2010[48] (19923398) India Low HDI	Population: Children and Adolescents Total sample N: 6	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake:	**Adaptation Period:** Diet type: Standardized diet Protein source: NR	Outcome measure: Lysine requirement estimate calculated from $F^{13}CO_2$

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Outpatient RCT, cross over design Government, protein-free powder for experimental diets provided by Mead Johnson Nutritionals Moderate ROB	**Intervention: Varied Lysine Intakes:** N: 6 % Female: 50% Mean Age/Range/Age at Baseline: 8.4; SD 0.8 yr Race: NR Mean BMI at baseline: 15.3; SD 0.9 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NR Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: Encouraged to maintain customary physical activity levels Medication use: NR Supplement use: Daily multivitamin supplement during the study period except on the experimental study d	NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.5 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.5 g/kg/d Intended amino acid intake: 5, 15, 25, 35, 50, 65, and 80 mg/kg/d lysine, 25 mg/kg/d phenylalanine, 61 mg/kg/d tyrosine, alanine adjusted to maintain a constant nitrogen intake. See Table 2 in original	Energy status: Provided at an amount to ensure age-appropriate growth Dietary assessment method: 3-d food records used to determine standardized diet and used to ensure consistency of dietary intakes during the adaptation period How protein was administered: Food supplied that was typically consumed by the participants Protein assessment method: NR **Study Day:** Diet type: Protein-free powder flavored with Tang and Kool-Aid, corn oil, amino acid mixture, and protein-free wheat starch cookies. Protein source: Amino acid mixture Energy status: Provided at an amount to ensure age-appropriate growth Dietary assessment method: NR How protein was administered: Consumed experimental diet as 8 hourly isonitrogenous, isocaloric meals Protein assessment method: NR	Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		paper for more information Intended carbohydrate intake: ~53% of energy from carbohydrate Intended fat intake: ~37% of energy from fat Actual protein intake: 1.5 g/kg/d Actual amino acid intake: 5, 15, 25, 35, 50, 65, and 80 mg/kg/d lysine, 25 mg/kg/d phenylalanine, 61 mg/kg/d tyrosine, alanine adjusted to maintain a constant nitrogen intake. See Table 2 in original paper for more information Actual carbohydrate intake: ~53% of energy from carbohydrate Actual fat intake: ~37% of energy from fat Study duration: 1 d (d 3) Crossover details: Number of intakes per participant: 7 Total intake observations: 42 Wash out period: ≥ 1 wk		

Abbreviations: BMI = body mass index; d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; g= grams; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; IAAO = indicator amino acid oxidation; kcal/kg/d = kilocalorie per kilogram per day; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RoB = risk of bias; SD = standard deviation; wk = week; yr = year

Table F.14. Lysine RCTs pregnant people

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Payne, 2018[47] (29378056) Canada Very High HDI Outpatient RCT, cross over design Government, nonprofit Moderate ROB	Population: Pregnant People Total sample N: 33 **Intervention: Varied Lysine Intake (Early Gestation):** N: 14 % Female: 100% Mean Age/Range/Age at Baseline: 29.5; SD 3.2 yr Race: NR Mean BMI at baseline: 23.7; SD 3.8 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: Pregnant Gestation stage: 16.3; SD 2.1wk (12-19 wk) Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: None used Supplement use: NR **Intervention: Varied Lysine Intake (Late Gestation):** N: 19 % Female: 100% Mean Age/Range/Age at Baseline: 30.5; SD 3.87 yr Race: NR Mean BMI at baseline: 21.5;	**Adaptation Period: (Same for Early and Late Gestation):** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.5 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day: (Same for Early and Late Gestation)** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.5	**Adaptation Period: (Same for Early and Late Gestation):** Diet type: Prescribed maintenance diet Protein source: NR Energy status: NR Dietary assessment method: Two-day detailed dietary records were collected to estimate baseline protein and calorie intake and to determine food choices. Participants kept a 2-d food record during the maintenance diet to ensure adequate dietary protein intake How protein was administered: NR Protein assessment method: NR **Study Day: (Same for Early and Late Gestation)** Diet type: liquid formula composed of protein-free powder, flavored drink crystals, and corn oil. Along with a crystalline L-amino acid mixture based on egg-protein composition. Protein source: crystalline L-amino acid mixture based on egg-protein composition. Energy status: Eucaloric	Outcome measure: Lysine requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	SD 2.4 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: Pregnant Gestation stage: 34.9; SD 1.6 wk (33-39 wk) Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: None used Supplement use: NR	g/kg/d Intended amino acid intake: 6–84 mg/kg/d lysine; 30.5 mg/kg/d phenylalanine, 61 mg/kg/d tyrosine, L-alanine content was altered to ensure all meals were isonitrogenous. Intended carbohydrate intake: ~50% of energy from carbohydrate Intended fat intake: 35% of energy from fat Actual protein intake: 1.5 g/kg/d Actual amino acid intake: 6–84 mg/kg/d lysine; 30.5 mg/kg/d phenylalanine, 61 mg/kg/d tyrosine, L-alanine content was altered to ensure all meals were isonitrogenous. Actual carbohydrate intake: ~50% of energy from carbohydrate Actual fat intake: 35% of energy from fat Study duration: 1 d (d 3) Crossover details: (Early Gestation) Number of intakes per participant: 1-5 Total intake observations: 27 Wash out period: ≥5 d Crossover details: (Late Gestation)	Dietary assessment method: NR How protein was administered: Participants received 8 hourly meals Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Number of intakes per participant: 1-4 Total intake observations: 36 Wash out period: ≥5 d		

Abbreviations: BMI = body mass index; d = day; g= grams; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; IAAO = indicator amino acid oxidation; kcal/kg/d = kilocalorie per kilogram per day; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RoB = risk of bias; SD = standard deviation; wk = week; yr = year

Table F.15. Lysine RCTs adults (19-50 years)

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Kriengsinyos, 2004[29] (15308475) Canada Very High HDI Outpatient RCT, cross over design Government, academic Moderate ROB	Population: Adults (19-50 yr) Total sample N: 5 **Intervention: Varied Lysine Intakes:** N: 5 % Female: 100% Mean Age/Range/Age at Baseline: 33.6; SD 5.9 yr Race: NR Mean BMI at baseline: 22.9; SD 3.4 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NR Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status:	**Adaptation Period: (Same for Follicular and Luteal Phases):** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: 3.25% per milkshake Actual protein intake: NR	**Adaptation Period: (Same for Follicular and Luteal Phases):** Diet type: Milkshake diet Protein source: Animal Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants received the milkshake diet as three meals and snacks spread throughout the d. Protein assessment method: NR **Study Day: (Same for Follicular and Luteal Phases):** Diet type: Flavored,	Outcome measure: Lysine requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Conducted during the follicular and luteal phases of the menstrual cycle. The follicular phase was conducted on d 3-7 immediately after the 1st d of menstrual bleeding and the luteal phase was conducted on 4-7 d before the onset of next menstrual bleeding Income level: NR Education level: NR Physical activity level: NR Medication use: None used Supplement use: daily multivitamin supplement	Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day: (Same for Follicular and Luteal Phases):** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.0 g/kg/d Intended amino acid intake: 10, 25, 30, 35, 40, 45, and 60 mg/kg/d lysine; 15 mg/kg/d phenylalanine, 40 mg/kg/d tyrosine; alanine intake adjusted to maintain a constant nitrogen intake. See Table 2 in the original paper for more information Intended carbohydrate intake: 53% of energy from carbohydrate Intended fat intake: 37% of energy from fat Actual protein intake: 1.0 g/kg/d Actual amino acid intake: 10, 25, 30, 35, 40, 45, and 60	nonprotein liquid formula diet with protein free cookies along with a crystalline L-amino acid mixture based on the amino acid composition of egg protein Protein source: Crystalline L-amino acid mixture based on the amino acid composition of egg protein Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants received the diet as 8 isocaloric, isonitrogenous meals. Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		mg/kg/d lysine; 15 mg/kg/d phenylalanine, 40 mg/kg/d tyrosine; alanine intake adjusted to maintain a constant nitrogen intake. See Table 2 in the original paper for more information Actual carbohydrate intake: 53% of energy from carbohydrate Actual fat intake: 37% of energy from fat Study duration: 1 d (d 3) Crossover details: Adaptation Period: Number of intakes per participant: 7 per phase (14 total) Total intake observations: 35 observations per phase (70 total) Wash out period: 1 or 2 IAAO studies conducted during each phase for each menstrual cycle.		
Kurpad, 2001[31] (11333843) India Low HDI Outpatient RCT, cross over design Government Moderate ROB	Population: Adults (19-50 yr) Total sample N: 16 **Intervention: Varied Lysine Intakes:** N: 16 % Female: 0% Mean Age/Range/Age at Baseline: 19.78; SD 2.26 yr Race: NR Mean BMI at baseline: 19.97;	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: <60 mg/kg/d lysine Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 160 mg/kg/d nitrogen (1.0 g/kg/d protein)	**Adaptation Period:** Diet type: Weight-maintaining diet Protein source: L-amino acid mixture Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants were provided with 3	Outcome measure: Lysine requirement estimate calculated from 24-h IAAO, 12-h fed IAAO, and 24-h IAAB Measure/Method of Assessment: 24-h IAAO and 24-h IAAB methods Isotope used: [13C]leucine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	SD 1.22 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: Encouraged to maintain customary levels of physical activity Medication use: NR Supplement use: Multivitamin-multimineral capsule, 500 mg choline, and dietary fiber	Intended amino acid intake: 12, 20, 28, and 36 mg/kg/d lysine; ~93 mg/kg/d leucine; 999.99 total amino acids mg/g mixture. See Table 2 in the original paper for more information Intended carbohydrate intake: ~56% of energy from carbohydrate Intended fat intake: ~43% of energy from fat Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 8 d (d 1-8) **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Intended amino acid intake: 12, 20, 28, and 36 mg/kg/d lysine; ~93 mg/kg/d leucine; 999.99 total amino acids mg/g mixture. See Table 2 in the original paper for more	isoenergetic isonitrogenous meals Protein assessment method: NR **Study Day:** Diet type: Weight-maintaining diet Protein source: L-amino acid mixture Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants were provided with 10 small isoenergetic isonitrogenous meals Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		information Intended carbohydrate intake: ~56% of energy from carbohydrate Intended fat intake: ~43% of energy from fat Actual protein intake: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Actual amino acid intake: 12, 20, 28, and 36 mg/kg/d lysine; ~93 mg/kg/d leucine; 999.99 total amino acids mg/g mixture. See Table 2 in the original paper for more information Actual carbohydrate intake: ~56% of energy from carbohydrate Actual fat intake: ~43% of energy from fat Study duration: 1 d (d 9) Crossover details: Number of intakes per participant: 2 Total intake observations: 32 Wash out period: 1-4 wk		
Kurpad, 2002[33] (12145014) India Low HDI Outpatient RCT, cross over design Government, nonprofit Low ROB	Population: Adults (19-50 yr) Total sample N: 18 **Intervention: Varied Lysine Intakes:** N: 18 % Female: 0% Mean Age/Range/Age at	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: < 60 mg/kg/d lysine Baseline carbohydrate intake: NR Baseline fat intake: NR	**Adaptation Period:** Diet type: Weight-maintaining diet Protein source: L-amino acid mixture Energy status: Eucaloric Dietary assessment method: Habitual intakes	Outcome measure: Lysine requirement estimate calculated from 24-h IAAO, 12-h fed IAAO, and 24-h IAAB Measure/Method of Assessment: 24-h IAAO and

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Baseline: 19.12, SD 1.11 yr Race: NR Mean BMI at baseline: 20.77; SD 1.64 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NR Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: Encouraged to maintain customary levels of physical activity Medication use: NR Supplement use: Multivitamin-multimineral capsule, 500 mg choline and dietary fiber	Intended protein intake: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Intended amino acid intake: 12, 20, 28, and 36 mg/kg/d lysine; 50 mg/kg/d leucine; 1000 mg/kg/d total amino acids Intended carbohydrate intake: 56% of energy from carbohydrate Intended fat intake: 43% of energy from fat Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 6 d (d 1-6, first adaptation period); 13 d (d 8-20, second adaptation period) **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Intended amino acid intake: 12, 20, 28, and 36 mg/kg/d lysine; 50 mg/kg/d leucine;	determined from 3-day weighed dietary intake record; specifics for adaptation period NR How protein was administered: Participants were provided with 3 isoenergetic isonitrogenous meals Protein assessment method: NR **Study Day:** Diet type: Weight-maintaining diet Protein source: L-amino acid mixture Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants were provided with 10 small isoenergetic isonitrogenous meals Protein assessment method: NR	24-h IAAB methods Isotope used: [13C]leucine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		1000 mg/kg/d total amino acids. See Table 2 in the original paper for more information Intended carbohydrate intake: 56% of energy from carbohydrate Intended fat intake: 43% of energy from fat Actual protein intake: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Actual amino acid intake: 12, 20, 28, and 36 mg/kg/d lysine; 50 mg/kg/d leucine; 1000 mg/kg/d total amino acids. See Table 2 in the original paper for more information Actual carbohydrate intake: 56% of energy from carbohydrate Actual fat intake: 43% of energy from fat Study duration: 1 d (d 7, first study d); 1 d (d 21, second study d) Crossover details: Number of intakes per participant: 2 Total intake observations: 36 Wash out period: 4-6 wk		
Elango, 2009[18] (19369367) Canada Very high HDI Outpatient	Population: Adults (19-50 yr) Total sample N: 5 **Intervention: Varied Lysine**	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR	**Adaptation Period:** Diet type: Milkshake-based maintenance diet Protein source: Animal	Outcome measure: $F^{13}CO_2$ Measure/Method of Assessment: IAAO method

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
RCT, cross over design Government Moderate ROB	**Intakes:** N: 5 % Female: 0% Mean Age/Range/Age at Baseline: 23.6; SD 2.7 yr Race: NR Mean BMI at baseline: 25.1; SD 1.6 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: None used Supplement use: daily multivitamin/mineral supplement	Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d (prior to 7-d study) **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.0 g/kg/d Intended amino acid intake: 5, 20, 35, 70 mg/kg/d lysine, 15 mg/kg/d phenylalanine, 40 mg/kg/d tyrosine; alanine adjusted with varying lysine intakes to maintain a constant nitrogen intake. See Table 2	Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants were instructed to add a predetermined volume of homogenized milk to their daily portion of milkshakes and to drink the shakes at regular times throughout the d. Protein assessment method: NR **Study Day:** Diet type: Protein-free formula, corn oil, crystalline L-amino acid mixture, and protein-free cookies. Protein source: a crystalline amino acid mixture patterned after egg protein. Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants received 8 hourly isonitrogenous and isocaloric meals Protein assessment method: NR	 Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		in original paper for more information Intended carbohydrate intake: 53% of energy from carbohydrate Intended fat intake: 37% of energy from fat Actual protein intake: 1.0 g/kg/d Actual amino acid intake: 5, 20, 35, 70 mg/kg/d lysine, 15 mg/kg/d phenylalanine, 40 mg/kg/d tyrosine; alanine adjusted with varying lysine intakes to maintain a constant nitrogen intake. See Table 2 in original paper for more information Actual carbohydrate intake: 53% of energy from carbohydrate Actual fat intake: 37% of energy from fat Study duration: 7 d (IAAO method conducted on day 1, 3 and 7) Crossover details: Number of intakes per participant: 12 Total intake observations: 60 Wash out period: >1 wk		

Abbreviations: BMI = body mass index; CI = confidence interval; d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; g= grams; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; IAAO = indicator amino acid oxidation; IAAB = indicator amino acid balance; kcal/kg/d = kilocalorie per kilogram per day; mg/kg/d = milligrams per kilogram per day; mo = month; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RoB = risk of bias; SD = standard deviation; wk = week; yr = year

Table F.16. Lysine non-RCTs adults (19-50 years)

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
El-Khoury, 2000[62] (10871570) United States Very high HDI Outpatient Non-randomized intervention trial Government, nonprofit Low risk ROB	Population: Adults (19-50 yr) Total sample N: 11 **Intervention Arm 1: Low Lysine Intake:** N: 5 % Female: 60% Mean Age/Range/Age at Baseline: 21 SD 1 yr Race: NR Mean BMI at baseline: NR Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: None of the participants were pregnant (a negative pregnancy was required 2-3 d before the study started) Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: Maintained usual physical activity level and avoided excessive or competitive exercise Medication use: NR Supplement use: Dietary fiber 20 g microcrystalline cellulose and a choline supplement of 500 mg/d	**Intervention Arm 1: Low Lysine Intake:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d (160 mg/kg/d nitrogen) Intended amino acid intake: 14-15 mg/kg/d lysine Intended carbohydrate intake: ~60% of energy from carbohydrate Intended fat intake: ~40% of energy from fat Actual protein intake: NR Actual amino acid intake: 15.53; SD 0.53 mg/kg/d lysine Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 6 d adaptation (d 1-6); 1 d (d 7) study d **Comparator Arm 2: Intermediate Lysine Intake:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR	**Intervention Arm 1: Low Lysine Intake:** Diet type: Protein-free wheat starch cookies and flavored drinks with an L-amino acid mixture with a profile close to that of chicken egg protein Protein source: L-amino acid mixture based on chicken egg protein Energy status: Eucaloric until d 6 and then energy was reduced by 20% to account for the reduced physical activity on the d 7 Dietary assessment method: A dietary history and estimation of basal metabolic rates were used to determine the mean daily energy intake requirements to maintain body weight. How protein was administered: During the 6-d adaptation period, the diet was provided as 2 isoenergetic, isonitrogenous meals. Then, 10 isoenergetic, isonitrogenous meals, one meal every hour was given on the study d (d 7) Protein assessment method: NR **Comparator Arm 2:**	Outcome measure: Lysine balance and lysine oxidation Measure/Method of Assessment: 24-h whole body lysine [1-13C] oxidation and 24-h whole body lysine balance Isotope used: L-[1-13C]lysine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Comparator Arm 2: Intermediate Lysine Intake: N: 6 % Female: 0% Mean Age/Range/Age at Baseline: 23 SD 5 yr Race: NR Mean BMI at baseline: NR Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: Maintained usual physical activity level and avoided excessive or competitive exercise Medication use: NR Supplement use: Dietary fiber 20 g microcrystalline cellulose and a choline supplement of 500 mg/d	Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d (160 mg/kg/d nitrogen) Intended amino acid intake: 28-29 mg/kg/d lysine Intended carbohydrate intake: ~60% of energy from carbohydrate Intended fat intake: ~40% of energy from fat Actual protein intake: NR Actual amino acid intake: 29.1; SD 0.24 mg/kg/d lysine Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 6 d adaptation (d 1-6); 1 d (d 7) study d Crossover details: Number of intakes per participant: NA Total intake observations: NA Wash out period: NA	**Intermediate Lysine Intake:** Diet type: Protein-free wheat starch cookies and flavored drinks with an L-amino acid mixture with a profile close to that of chicken egg protein Protein source: L-amino acid mixture based on chicken egg protein Energy status: Eucaloric until d 6 and then energy was reduced by 20% to account for the reduced physical activity on the d 7 Dietary assessment method: A dietary history and estimation of basal metabolic rates were used to determine the mean daily energy intake requirements to maintain body weight. How protein was administered: During the 6-d adaptation period, the diet was provided as 2 isoenergetic, isonitrogenous meals. Then, 10 isoenergetic, isonitrogenous meals, one meal every hour was given on the study d (d 7) Protein assessment method: NR	

Abbreviations: BMI = body mass index; d = day; g= grams; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; IAAO = indicator amino acid oxidation; IAAB = indicator amino acid balance; kcal/kg/d = kilocalorie per kilogram per day; mg/kg/d = milligrams per kilogram per day; mo = months; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RoB = risk of bias; SD = standard deviation; wk = Week

Methionine

Table F.17. Methionine RCTs infants

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Huang, 2012[6] (22492372) China High HDI Admitted to the Neonatal Ward in the Children's Hospital of Fudan University RCT Nonprofit, study formulas were manufactured by SHS UK Moderate ROB	Population: Infants Total sample N: 33 **Intervention: Varied Methionine Intakes:** N: 33 % Female: 27% Mean Age/Range/Age at Baseline: 13; SD 6 d; gestational age: 39; SD 1 wk Race: NR Mean BMI at baseline: NR Health status/comorbidities: Clinically stable and considered healthy; initially admitted to the hospital for the following reasons: N=15 unconjugated hyperbilirubinemia, N=6 pneumonia with a negative blood culture, N=4 asphyxia, N=5 infection suspicion with a negative blood culture, N=1 wet lung, N=1 observation that was due to uterine bleeding, N=1 pending results of toxoplasmosis, other agents, rubella, cytomegalovirus, and herpes simplex virus, which were negative Obesity status: NR Pubertal status: NA Pregnant or lactating: NA	**Adaptation Period:** Baseline protein intake: 2.5; SD 0.4 g/kg/d Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: ~2.96 g/kg/d Intended amino acid intake: 3-59 mg/kg/d methionine; 91 mg/kg/d cysteine; 166 mg/kg/d phenylalanine and tyrosine; 13 g/100g formula total amino acids. See Table 1 in original paper for more information Intended carbohydrate intake: 54 g/100 g formula Intended fat intake: 23 g/100 g formula Actual protein intake: 3.0; SD 0.1 g/kg/d Actual amino acid intake: 3-59 mg/kg/d methionine; 91 mg/kg/d cysteine; 166 mg/kg/d phenylalanine and tyrosine; 13 g/100g formula total amino acids. See Table 1 in original paper for more information	**Adaptation Period:** Diet type: Study formula Protein source: Elemental formula that was based on free amino acids Energy status: 108; SD 14 kcal/kg/d Dietary assessment method: NR How protein was administered: Infants were fed the study formula every 3 h Protein assessment method: NR **Study Day:** Diet type: Study formula Protein source: Elemental formula that was based on free amino acids Energy status: 475 kcal/100g formula Dietary assessment method: NR How protein was administered: Infants were fed the study formula every h Protein assessment method: NR	Outcome measure: Methionine requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NA Medication use: N=28 were on intravenous antibiotics (penicillins and/or cephalosporins) Supplement use: NR	Actual carbohydrate intake: 54 g/100g formula Actual fat intake: 23 g/100g formula Study duration: 1 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: ~2.96 g/kg/d Intended amino acid intake: 3-59 mg/kg/d methionine; 91 mg/kg/d cysteine; 166 mg/kg/d phenylalanine and tyrosine; 13 g/100g formula total amino acids. See Table 1 in original paper for more information Intended carbohydrate intake: 54 g/100 g formula Intended fat intake: 23 g/100 g formula Actual protein intake: 3.0; SD 0.1 g/kg/d Actual amino acid intake: 3-59 mg/kg/d methionine; 91 mg/kg/d cysteine; 166 mg/kg/d phenylalanine and tyrosine; 13 g/100g formula total amino acids. See Table 1		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		in original paper for more information Actual carbohydrate intake: 54 g/100 g formula Actual fat intake: 23 g/100 g formula Study duration: 1 d (d 2) Crossover details: Number of intakes per participant: NA Total intake observations: NA Wash out period: NA		

Abbreviations: BMI = body mass index; d = day; g= grams; $F^{13}CO_2$ = the fraction of 13CO2 recovery from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; IAAO = indicator amino acid oxidation; IAAB = indicator amino acid balance; kcal/kg/d = kilocalorie per kilogram per day; mg/kg/d = milligrams per kilogram per day; mo = months; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RoB = risk of bias; SD = standard deviation; wk = week

Table F.18. Methionine RCTs children and adolescents

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Humayun, 2006[28] (17093160) Canada Very High HDI Outpatient RCT, cross over design Government, protein-free powder for experimental diets provided by Mead	Population: Children and Adolescents Total sample N: 6 **Intervention: Varied Methionine Intakes:** N: 6 % Female: 16.6% Mean Age/Range/Age at Baseline: 9.4; SD 2.3 yr	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.5 g/kg/d	**Adaptation Period:** Diet type: Maintenance diet Protein source: NR Energy status: Eucaloric Dietary assessment method: Study dietitian used 3-d food record to create adaptation diet How protein was administered: The	Outcome measure: Methionine requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Johnson Nutritionals Moderate ROB	Race: NR Mean BMI at baseline: NR Health status/comorbidities: Healthy Obesity status: NR Pubertal status: Early puberty except 1 male in mid puberty Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: None used Supplement use: Daily supplement of vitamin B complex with vitamin C	Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.5 g/kg/d Intended amino acid intake: 0, 2.5, 5, 7.5, 10, or 15 mg/kg/d methionine; 12 mg/kg/d cysteine. See Table 2 in original paper for more information Intended carbohydrate intake: 53% of energy from carbohydrate Intended fat intake: 37% of energy from fat Actual protein intake: 1.5 g/kg/d	maintenance diet was prescribed by the study dietitian according to the participant's 3-d food record Protein assessment method: NR **Study Day:** Diet type: Protein-free liquid formula (flavored with soft drink crystals), corn oil, the crystalline amino acid mixture (based on the amino acid composition of egg protein), and protein-free cookies. Protein source: Crystalline amino acid mixture (based on the amino acid composition of egg protein). Energy status: Eucaloric Dietary assessment method: NR How protein was administered: NR Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Actual amino acid intake: 0, 2.5, 5, 7.5, 10, or 15 mg/kg/d methionine; 12 mg/kg/d cysteine. See Table 2 in original paper for more information Actual carbohydrate intake: 53% of energy from carbohydrate Actual fat intake: 37% of energy from fat Study duration: 1 d (d 3) Crossover details: Number of intakes per participant: 6 Total intake observations: 36 Wash out period: ≥1 wk		
Turner, 2006[57] (16522909) Canada Very high HDI Outpatient RCT, cross over design Government, Mead Johnson Nutritionals (Canada) donated the protein-free powder Moderate ROB	Population: Children and Adolescents Total sample N: 6 **Intervention: Varied Methionine Intakes** N: 6 % Female: 17% Mean Age/Range/Age at Baseline: 9.1; SD 2.2 yr Race: NR Mean BMI at baseline: NR Health status/comorbidities: Healthy Obesity status: NR Pubertal status: N=5 early puberty, N=1 midpuberty Pregnant or lactating: NA Gestation stage: NA	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.5 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: 53% of energy from carbohydrate Intended fat intake: 37% of energy from fat Actual protein intake: NR	**Adaptation Period:** Diet type: Maintenance diet Protein source: NR Energy status: Eucaloric Dietary assessment method: 3-d food records used to determine maintenance diet. How protein was administered: NR Protein assessment method: NR **Study Day:** Diet type: Protein-free liquid formula, corn oil, the crystalline AA mixture, and protein-free cookies. Protein source: Crystalline AA mixture (composition	Outcome measure: Total sulfur AA requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: None used Supplement use: Daily B vitamins	Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.5 g/kg/d Intended amino acid intake: 0, 5, 10, 15, 25, and 35 mg/kg/d methionine, 0 mg/kg/d cysteine, 25.13 mg/kg/d phenylalanine, 61.10 mg/kg/d tyrosine Intended carbohydrate intake: 53% of energy from carbohydrate Intended fat intake: 37% of energy from fat Actual protein intake: 1.5 g/kg/d Actual amino acid intake: 0, 5, 10, 15, 25, and 35 mg/kg/d methionine, 0 mg/kg/d cysteine, 25.13 mg/kg/d phenylalanine, 61.10 mg/kg/d tyrosine Actual carbohydrate intake:	based on egg protein) Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Consumed study diet as 8 isonitrogenous and isocaloric hourly meals Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		53% of energy from carbohydrate Actual fat intake: 37% of energy from fat Study duration: 1 d (d 3) Crossover details: Number of intakes per participant: 6 Total intake observations: 36 Wash out period: ≥ 1 wk		

Abbreviations: AA= amino acid; BMI = body mass index; d = day; g= grams; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; IAAO = indicator amino acid oxidation; kcal/kg/d = kilocalorie per kilogram per day; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RoB = risk of bias; SD = standard deviation; wk = week; yr = year

Table F.19. Methionine RCTs adults (19-50 years)

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Di Buono, 2001[16] (11722957) Canada Very High HDI Outpatient RCT, cross over design Government, protein-free powder for experimental diets provided by Mead Johnson Nutritionals, multivitamin supplements provided by Whitehall Robins	Population: Adults (19-50 yr) Total sample N: 6 **Intervention: Varied Methionine Intakes:** N: 6 % Female: 0% Mean Age/Range/Age at Baseline: 26.3; SD 3.6 yr Race: NR Mean BMI at baseline: 26.4; SD 2.9 kg/m^2	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d Intended amino acid intake: NR	**Adaptation Period:** Diet type: Milkshake diet. Protein source: Animal Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Provided in the form of milkshakes, which were weighed in daily portions for each participant and supplemented with	Outcome measure: Methionine requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C] phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Inc Moderate ROB	Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: None used Supplement use: Daily multivitamin supplement	Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.0 g/kg/d Intended amino acid intake: 0, 2.5, 5.0, 7.5, 10.0, and 13.0 mg/kg/d methionine; 21 mg/kg/d cysteine, 14 mg/kg/d phenylalanine, 40 mg/kg/d tyrosine. See Table 2 in original paper for more information Intended carbohydrate intake: 55% of energy from carbohydrate Intended fat intake: 35% of energy from fat Actual protein intake: 1.0 g/kg/d	additional protein and energy, depending on each participant's requirements Protein assessment method: NR **Study Day:** Diet type: Liquid formula (protein-free powder flavored with orange fruit crystals) and protein-free cookies along with a crystalline amino acid mixture based on the AA composition of egg protein Protein source: crystalline amino acid mixture based on the AA composition of egg protein Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants were provided with diets portioned into isoenergetic, isonitrogenous meals and consumed them hourly Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Actual amino acid intake: 0, 2.5, 5.0, 7.5, 10.0, and 13.0 mg/kg/d methionine; 21 mg/kg/d cysteine, 14 mg/kg/d phenylalanine, 40 mg/kg/d tyrosine. See Table 2 in original paper for more information Actual carbohydrate intake: 55% of energy from carbohydrate Actual fat intake: 35% of energy from fat Study duration: 1 d (d 3) Crossover details: Number of intakes per participant: 6 Total intake observations: 36 Wash out period: ≥1 wk		
Di Buono, 2001[17] (11722956) Canada Very High HDI Outpatient RCT, cross over design Government, protein-free powder for experimental diets provided by Mead Johnson Nutritionals, multivitamin supplements provided by Whitehall Robins Inc Moderate ROB	Population: Adults (19-50 yr) Total sample N: 6 **Intervention: Varied Methionine Intakes:** N: 6 % Female: 0% Mean Age/Range/Age at Baseline: 29; SD 6 yr Race: NR Mean BMI at baseline: 26.78; SD 2.47 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR	**Adaptation Period:** Diet type: Milkshake diet Protein source: Animal Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Provided in the form of milkshakes, which were weighed in daily portions for each participant and supplemented with additional protein and energy, depending on each participant's requirements Protein assessment method: NR	Outcome measure: Total sulfur amino acid requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: None used Supplement use: Daily multivitamin supplement	Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.0 g/kg/d Intended amino acid intake: initial design: 0, 6.5, 13.0, 19.5, 26.0, and 32.0 mg/kg/d methionine; 0 mg/kg/d cysteine; 14 mg/kg/d phenylalanine; 40 mg/kg/d tyrosine. See Table 2 in original paper for more information Intended carbohydrate intake: 55% of energy from carbohydrate Intended fat intake: 35% of energy from fat Actual protein intake: 1.0 g/kg/d Actual amino acid intake: initial design: 0, 6.5, 13.0, 19.5, 26.0, and 32.0 mg/kg/d methionine; 0 mg/kg/d	**Study Day:** Diet type: Liquid formula (protein-free powder, flavored with orange and fruit crystals) and protein-free cookies along with a crystalline amino acid mixture based on the amino acid composition of egg protein Protein source: Crystalline amino acid mixture, based on the amino acid composition of egg protein. Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants were provided with diets portioned into isoenergetic, isonitrogenous meals and consumed them hourly Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		cysteine; 14 mg/kg/d phenylalanine; 40 mg/kg/d tyrosine. See Table 2 in original paper for more information Actual carbohydrate intake: 55% of energy from carbohydrate Actual fat intake: 35% of energy from fat Study duration: 1 d (d 3) Crossover details: Adaptation Period: Number of intakes per participant: 6 Total intake observations: 36 Wash out period: ≥1 wk		
Humayun, 2007[26] (17634258) Canada Very high HDI Outpatient RCT, cross over design Government, Mead Johnson Nutritionals (Canada) donated the protein-free powder Moderate ROB	Population: Adults (19-50 yr) Total sample N: 7 **Intervention: Varied Methionine Intakes and Sources** N: 7 % Female: 0% Mean Age/Range/Age at Baseline: 26.5; SEM 2.2 yr Race: NR Mean BMI at baseline: 23.6; SEM 0.7 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake:	**Adaptation Period** Diet type: Milkshake diet Protein source: Animal Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants were provided the milkshake diet. Protein assessment method: NR **Study Day:** Diet type: Protein-free liquid formula, corn oil and crystalline AA mix or crystalline AA mix plus casein or crystalline AA mix	Outcome measure: Phenylalanine oxidation (% dose) Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: None used Supplement use: Daily multivitamin	NR Actual fat intake: NR Study duration: 2 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA **AA mix** Intended protein intake: 1.0 g/kg/d Intended amino acid intake: Total sulfur AA provided at 20, 40, 50, 60, and 70% of the mean total sulfur AA requirement (13 mg/kg/d), 30.5 mg/kg/d phenylalanine, 40 mg/kg/d tyrosine, see Table 2 in original paper for more information Intended carbohydrate intake: 52% of energy from carbohydrate Intended fat intake: 36% of energy from fat Actual protein intake: 1.0 g/kg/d Actual amino acid intake: Total sulfur AA provided at 20, 40, 50, 60, and 70% of the mean total sulfur AA requirement (13 mg/kg/d),	plus SPI and protein-free cookies Protein source: Crystalline AA mix, crystalline AA mix plus casein, crystalline AA mix plus SPI Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Consumed study day diet as 8 hourly isonitrogenous, isocaloric meals Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		30.5 mg/kg/d phenylalanine, 40 mg/kg/d tyrosine, see Table 2 in original paper for more information Actual carbohydrate intake: 52% of energy from carbohydrate Actual fat intake: 36% of energy from fat **Casein:** Intended protein intake: 1.0 g/kg/d Intended amino acid intake: Total sulfur AA provided at 40, 50, 60 and 70% of the meal total sulfur AA requirement (13 mg/kg/d), 30.5 mg/kg/d phenylalanine, 40 mg/kg/d tyrosine, see Table 2 in original paper for more information Intended carbohydrate intake: 52% of energy from carbohydrate Intended fat intake: 36% of energy from fat Actual protein intake: 1.0 g/kg/d Actual amino acid intake: Total sulfur AA provided at 40, 50, 60 and 70% of the meal total sulfur AA requirement (13 mg/kg/d), 30.5 mg/kg/d phenylalanine, 40 mg/kg/d tyrosine, see Table 2 in original paper for more		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		information Actual carbohydrate intake: 52% of energy from carbohydrate Actual fat intake: 36% of energy from fat **SPI** Intended protein intake: 1.0 g/kg/d Intended amino acid intake: Total sulfur AA provided at 40, 50, 60 and 70% of the meal total sulfur AA requirement (13 mg/kg/d), 30.5 mg/kg/d phenylalanine, 40 mg/kg/d tyrosine, see Table 2 in original paper for more information Intended carbohydrate intake: 52% of energy from carbohydrate Intended fat intake: 36% of energy from fat Actual protein intake: 1.0 g/kg/d Actual amino acid intake: Total sulfur AA provided at 40, 50, 60 and 70% of the meal total sulfur AA requirement (13 mg/kg/d), 30.5 mg/kg/d phenylalanine, 40 mg/kg/d tyrosine, see Table 2 in original paper for more information Actual carbohydrate intake: 52% of energy from		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		carbohydrate Actual fat intake: 36% of energy from fat Study duration: 1 d (d 3) Crossover details: Number of intakes per participant: 13 Total intake observations: 91 Wash out period: ≥ 1 wk		
Kurpad, 2003[36] (12716672) India Low HDI Outpatient RCT, cross over design Government, industry Moderate ROB	Population: Adults (19-50 yr) Total sample N: 21 **Intervention: Varied Methionine Intakes:** N: 21 % Female: 0% Mean Age/Range/Age at Baseline: 20.6; SD 1.5 yr Race: NR Mean BMI at baseline: 21.4; SD 1.3 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: 0.5 g/d choline	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d Intended amino acid intake: 3, 6, 9, 13, 18, 21, and 24 mg/kg/d methionine, 0 mg/kg/d cysteine. See Table 2 in original paper for more information Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 6 d (d 1-6)	**Adaptation Period:** Diet type: Weight-maintaining diet based on an L-amino acid mixture. Protein source: L-amino acid mixture. Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants received their daily intake as 3 isoenergetic isonitrogenous meals except on d 6 where participants received 10 isoenergetic, isonitrogenous small meals Protein assessment method: NR **Study Day:** Diet type: Weight-maintaining diet based on an L-amino acid mixture. Protein source: L-amino acid mixture. Energy status: Eucaloric	Outcome measure: Total sulfur amino acid requirement estimate calculated from 24-h IAAO and 24-h IAAB Measure/Method of Assessment: 24-h IAAO and 24-h IAAB method Isotope used: [13C]leucine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Study Day: Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.0 g/kg/d Intended amino acid intake: 3, 6, 9, 13, 18, 21, and 24 mg/kg/d methionine, devoid of cysteine. See Table 2 in original paper for more information Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: 1.0 g/kg/d Actual amino acid intake: 3, 6, 9, 13, 18, 21, and 24 mg/kg/d methionine, devoid of cysteine. See Table 2 in original paper for more information Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 1 d (d 7) Crossover details: Number of intakes per participant: 3	Dietary assessment method: NR How protein was administered: Participants received 10 isoenergetic, isonitrogenous small meals Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Total intake observations: 63 Wash out period: NR		
Kurpad, 2004[37] (15585764) India Low HDI Outpatient RCT, cross over design Government, industry Moderate risk	Population: Adults (19-50 yr) Total sample N: 42 **Intervention: Varied Methionine Intakes with 5 mg/kg/d Cysteine:** N: 21 % Female: 0% Mean Age/Range/Age at Baseline: 22.0; SD 1.8 yr Race: NR Mean BMI at baseline: 21.6; SD 1.6 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: NR **Comparator: Varied Methionine Intakes with 12 mg/kg/d Cysteine:** N: 21 % Female: 0% Mean Age/Range/Age at Baseline: 21.7; SD 2.8 yr Race: NR Mean BMI at baseline: 21.6;	**Adaptation Period: (Varied Methionine Intakes with 5 mg/kg/d Cysteine):** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Intended amino acid intake: 3, 6, 9, 13, 18, 21, and 24 mg/kg/d methionine, 5 mg/kg/d cysteine. See Table 2 in original paper for more information Intended carbohydrate intake: 56% of energy from carbohydrate Intended fat intake: 43% of energy from fat Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 6 d (d 1-6) **Study Day: (Varied Methionine Intakes with 5 mg/kg/d Cysteine):**	**Adaptation Period: (Same for Varied Methionine Intakes with 5 mg/kg/d Cysteine and with 12 mg/kg/d Cysteine):** Diet type: weight-maintaining diet based on an L-amino acid mixture Protein source: L-amino acid mixture Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants received their daily intake as 3 isoenergetic isonitrogenous meals except on d 6 where participants received 10 isoenergetic, isonitrogenous small meals Protein assessment method: NR **Study Day: (Same for Varied Methionine Intakes with 5 mg/kg/d Cysteine and with 12 mg/kg/d Cysteine):** Diet type: weight-maintaining diet based on an L-amino acid mixture Protein source: L-amino acid mixture Energy status: Eucaloric Dietary assessment	Outcome measure: Methionine requirement estimate calculated from 24-h IAAO and 24-h IAAB Measure/Method of Assessment: 24-h IAAO and 24-h IAAB method Isotope used: [13C] leucine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	SD 2.0 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: NR	Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Intended amino acid intake: 3, 6, 9, 13, 18, 21, and 24 mg/kg/d methionine, 5 mg/kg/d cysteine. See Table 2 in original paper for more information Intended carbohydrate intake: 56% of energy from carbohydrate Intended fat intake: 43% of energy from fat Actual protein intake: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Actual amino acid intake: 3, 6, 9, 13, 18, 21, and 24 mg/kg/d methionine, 5 mg/kg/d cysteine. See Table 2 in original paper for more information Actual carbohydrate intake: 56% of energy from carbohydrate Actual fat intake: 43% of energy from fat Study duration: 1 d (d 7)	method: NR How protein was administered: Participants received 10 isoenergetic, isonitrogenous small meals Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Crossover details: Number of intakes per participant: 3 Total intake observations: 63 Wash out period: NR **Adaptation Period: (Varied Methionine Intakes with 12 mg/kg/d Cysteine):** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Intended amino acid intake: 3, 6, 9, 13, 18, 21, and 24 mg/kg/d methionine, 12 mg/kg/d cysteine. See Table 3 in original paper for more information Intended carbohydrate intake: 56% of energy from carbohydrate Intended fat intake: 43% of energy from fat Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 6 d (d 1-6)		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Study Day: (Varied Methionine Intakes with 12 mg/kg/d Cysteine): Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Intended amino acid intake: 3, 6, 9, 13, 18, 21, and 24 mg/kg/d methionine, 12 mg/kg/d cysteine. See Table 3 in original paper for more information Intended carbohydrate intake: 56% of energy from carbohydrate Intended fat intake: 43% of energy from fat Actual protein intake: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Actual amino acid intake: 3, 6, 9, 13, 18, 21, and 24 mg/kg/d methionine, 12 mg/kg/d cysteine. See Table 3 in original paper for more information Actual carbohydrate intake: 56% of energy from carbohydrate		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Actual fat intake: 43% of energy from fat Study duration: 1 d (d 7) Crossover details: Number of intakes per participant: 3 Total intake observations: 63 Wash out period: NR		

Abbreviations: AA = amino acid; BMI = body mass index; d = day; g= grams; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; IAAO = indicator amino acid oxidation; IAAB = indicator amino acid balance; kcal/kg/d = kilocalorie per kilogram per day; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RoB = risk of bias; SD = standard deviation; SPI = soy protein isolate; wk = week; yr = year

Table F.20. Phenylalanine RCTs infants

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Hogewind-Schoonenboom, 2015[4] (25926506) China High HDI Admitted to the Neonatal Department of the Fudan Children's Hospital, Shanghai, China RCT Nonprofit, Study formulas were manufactured by SHS UK Moderate ROB	Population: Infants Total sample N: 20 **Intervention: Varied Phenylalanine Intakes:** N: 20 % Female: 55% Mean Age/Range/Age at Baseline: 13; SD 6 d; Gestational age: 38.9; SD 1 wk Race: NR Mean BMI at baseline: NR Health status/comorbidities:	**Adaptation Period:** Baseline protein intake: 2.0; SD 0.1 g/kg/d Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 2.96 g/kg/d Intended amino acid intake: 5-166 mg/kg/d phenylalanine; 166 mg/kg/d	**Adaptation Period:** Diet type: Study formula Protein source: AA–based formula Energy status: 103; SD 7 kcal/kg/d Dietary assessment method: NR How protein was administered: Infants were fed the study formula every 2-3 h Protein assessment method: NR	Outcome measure: Phenylalanine requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: [1-13C] lysine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Clinically stable and considered healthy; Initially admitted to the hospital for the following reasons: N=16 unconjugated hyperbilirubinemia, N=3 pneumonia with negative blood cultures, N=1 wet lung Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NA Medication use: NR Supplement use: NR	tyrosine, 252 mg/kg/d lysine, L-alanine was added to keep the amount of nitrogen constant. See Table 1 in original paper for more information Intended carbohydrate intake: 54 g per 100 g formula Intended fat intake: 23 g per 100 g formula Actual protein intake: 2.96 g/kg/d Actual amino acid intake: 5-166 mg/kg/d phenylalanine; 166 mg/kg/d tyrosine, 252 mg/kg/d lysine, L-alanine was added to keep the amount of nitrogen constant. See Table 1 in original paper for more information Actual carbohydrate intake: 54 g per 100 g formula Actual fat intake: 23 g per 100 g formula Study duration: 1 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 2.96 g/kg/d	**Study Day:** Diet type: Study formula Protein source: AA–based formula Energy status: 108 kcal/kg/d Dietary assessment method: NR How protein was administered: Infants were fed the study formula hourly Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Intended amino acid intake: 5-166 mg/kg/d phenylalanine; 166 mg/kg/d tyrosine, 252 mg/kg/d lysine, L-alanine was added to keep the amount of nitrogen constant. Total amino acids: 13 g/100g formula; See Table 1 in original paper for more information Intended carbohydrate intake: 54 g per 100 g formula Intended fat intake: 23 g per 100 g formula Actual protein intake: 2.96 g/kg/d Actual amino acid intake: 5-166 mg/kg/d phenylalanine; 166 mg/kg/d tyrosine, 252 mg/kg/d lysine, L-alanine was added to keep the amount of nitrogen constant. Total amino acids: 13 g/100g formula; See Table 1 in original paper for more information Actual carbohydrate intake: 54 g per 100 g formula Actual fat intake: 23 g per 100 g formula Study duration: 1 d (d 2) Crossover details: Number of intakes per participant: NA Total intake observations: NA Wash out period: NA		

Abbreviations: BMI = body mass index; d = day; g= grams; $F^{13}CO_2$ = the fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; lysine]; g/kg/d = grams per kilogram per day; h = hour; HDI = human development Index; IAAO = indicator amino acid oxidation; kcal/kg/d = kilocalorie per kilogram per day; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RoB = risk of bias; SD = standard deviation; wk = week

Table F.21. Phenylalanine RCTs children and adolescents

Study (PMID) **Location (country)** **HDI** **Setting** **Study Design** **Funding Source** **ROB Score**	**Participants**	**Interventions/Exposure and Comparator (Content)**	**Intervention/Exposure and Comparator (Methods of Assessment)**	**Outcome (Measures and Methods of Assessment)**
Hsu, 2007[23] (17314698) Canada Very high HDI Outpatient RCT, cross over design Government Moderate ROB	Population: Children and Adolescents Total sample N: 5 **Intervention: Varied Phenylalanine Intakes:** N: 5 % Female: NR Mean Age/Range/Age at Baseline: 9.1; SD 1.4 yr Race: NR Mean BMI at baseline: NR Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NR Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: NR	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.5 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA	**Adaptation Period:** Diet type: Standard diet (menu plans provided according to typical foods consumed by participant) Protein source: NR Energy status: Amount provided to ensure age-appropriate growth Dietary assessment method: Food records were collected to ensure consistency of dietary intake before the study d How protein was administered: Menu plans were provided according to typical food consumed by the subjects Protein assessment method: NR **Study Day:** Diet type: Liquid formula with protein-free cookies along with a crystalline L-amino acid mixture based on the amino acid profile of egg protein Protein source: Crystalline L-amino acid mixture based on	Outcome measure: Total aromatic amino acid requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]lysine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Intended protein intake: 1.5 g/kg/d Intended amino acid intake: 5, 10, 20, 30, 40, 50, 60, and 70 mg/kg/d phenylalanine; 0 mg/kg/d tyrosine; 64 mg/kg/d lysine; Alanine was in various amounts to keep the meals isonitrogenous. See Table 2 in original paper for more information Intended carbohydrate intake: 52% of energy from carbohydrate Intended fat intake: 37% of energy from fat Actual protein intake: 1.5 g/kg/d Actual amino acid intake: 5, 10, 20, 30, 40, 50, 60, and 70 mg/kg/d phenylalanine; 0 mg/kg/d tyrosine; 64 mg/kg/d lysine; Alanine was in various amounts to keep the meals isonitrogenous. See Table 2 in original paper for more information Actual carbohydrate intake: 52% of energy from carbohydrate Actual fat intake: 37% of energy from fat Study duration: 1 d (d 3) Crossover details:	the amino acid profile of egg protein Energy status: Amount provided to ensure age-appropriate growth Dietary assessment method: NR How protein was administered: Participants received 8 hourly isocaloric, isonitrogenous meals in the liquid formula Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Number of intakes per participant: 8 Total intake observations: 40 Wash out period: NR		

Abbreviations: BMI = body mass index; d = day; g= grams; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; lysine]; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; IAAO = indicator amino acid oxidation; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RoB = risk of bias; SD = standard deviation; yr = year

Table F.22. Phenylalanine RCTs pregnant people

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Ennis, 2020[22] (31758682) Canada Very high HDI Outpatient RCT, cross over design Government Low ROB	Population: Pregnant People Total sample N: 23* **Intervention: Varied Phenylalanine Intakes (Early Gestation DAAO Method):** N: 9 % Female: 100% Mean Age/Range/Age at Baseline: 29.3; SD 2.6 yr Race: NR Mean BMI at baseline: 23.5; SD 2.9 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: Pregnant Gestation stage: 17.5; SD	**Adaptation Period: (Same for Early and Late Gestation DAAO Method and Late Gestation IAAO Method):** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.5 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR Actual amino acid intake: NR	**Adaptation Period: (Same for Early and Late Gestation DAAO Method and Late Gestation IAAO Method):** Diet type: Standardized diet Protein source: NR Energy status: Eucaloric Dietary assessment method: 2-day diet records were obtained to create the adaptation period diet How protein was administered: Personalized diet recommendations were created and prescribed to each participant. Protein assessment method: NR **Study Day: (Same for Early**	Outcome measure: Phenylalanine requirement estimates calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method and DAAO method Isotope used: IAAO method: L-[1-13]leucine DAAO method: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	1.9 wk(13-19 wk) Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: N=2 used Diclectin (doxylamine succinate-pyridoxine) for morning sickness, N=4 used Synthroid (levothyroxine) for mild hypothyroidism, gestational stage this occurred in NR, No prescription medications were taken on the study d Supplement use: Prenatal vitamins **Intervention: Varied Phenylalanine Intakes (Late Gestation DAAO Method):** N: 9 % Female: 100% Mean Age/Range/Age at Baseline: 29.5; SD 2.4 yr Race: NR Mean BMI at baseline: 22.2; SD 2.5 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: Pregnant Gestation stage: 36.1; SD 1.9 wk (33-39 wk)	Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day: (Same for Early and Late Gestation DAAO Method):** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.5 g/kg/d Intended amino acid intake: 5.5-30.5 mg/kg/d phenylalanine; 61 mg/kg/d tyrosine. See Table 2 in original paper for more information. Intended carbohydrate intake: ~53% of energy from carbohydrate Intended fat intake: 37% of energy from fat Actual protein intake: 1.5 g/kg/d Actual amino acid intake: 5.5-30.5 mg/kg/d phenylalanine; 61 mg/kg/d tyrosine. See Table 2 in original paper for more information. Actual carbohydrate intake:	**and Late Gestation DAAO Method and Late Gestation IAAO Method):** Diet type: Liquid formula (protein-free powder, orange-flavored drink crystals, corn oil, and a crystalline L-amino acid mixture based on egg-protein composition) and protein-free cookies. Protein source: Crystalline L-amino acid mixture based on egg-protein composition Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Provided the liquid formula as 8 hourly meals Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: N=2 used Diclectin (doxylamine succinate-pyridoxine) for morning sickness, N=4 used Synthroid (levothyroxine) for mild hypothyroidism, gestational stage this occurred in NR, No prescription medications were taken on the study d Supplement use: Prenatal vitamins **Intervention: Varied Phenylalanine Intakes (Late Gestation IAAO Method):** N: 13 % Female: 100% Mean Age/Range/Age at Baseline: 30.9; SD 3.8 yr Race: NR Mean BMI at baseline: 22.0; SD 3.0 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: Pregnant Gestation stage: 35.9; SD 2.0 wk (33-39 wk) Lactation stage: NA	~53% of energy from carbohydrate Actual fat intake: 37% of energy from fat Study duration: 1 d (d 3) **Study Day: (Late Gestation IAAO Method):** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.5 g/kg/d Intended amino acid intake: 2.5-30.5 mg/kg/d phenylalanine; 61 mg/kg/d tyrosine. See Table 2 in original paper for more information. Intended carbohydrate intake: ~53% of energy from carbohydrate Intended fat intake: 37% of energy from fat Actual protein intake: 1.5 g/kg/d Actual amino acid intake: 2.5-30.5 mg/kg/d phenylalanine; 61 mg/kg/d tyrosine. See Table 2 in original paper for more information. Actual carbohydrate intake:		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: N=2 used Diclectin (doxylamine succinate-pyridoxine) for morning sickness, N=4 used Synthroid (levothyroxine) for mild hypothyroidism, gestational stage this occurred in NR, No prescription medications were taken on the study d Supplement use: Prenatal vitamins	~53% of energy from carbohydrate Actual fat intake: 37% of energy from fat Study duration: 1 d (d 3) Crossover details: (early and late gestation DAAO method and late gestation IAAO method) Number of intakes per participant: ≤ 6 Total intake observations: 76 (26 early gestation DAAO method, 25 late gestation DAAO method, 25 late gestation IAAO method) Wash out period: ≥5 d		
Ennis, 2020[21] (33188409) Canada Very high HDI Outpatient RCT, cross over design Government Low ROB	Population: Pregnant people Total sample N: 19[**] **Intervention: Varied Phenylalanine Intakes (Early Gestation):** N: 10 % Female: 100% Mean Age/Range/Age at Baseline: 32.3; SD 3.0 yr Race: NR Mean BMI at baseline: 25.0; SD 3.0 kg/m^2 (pre-pregnancy) Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating:	**Adaptation Period (Same for Early and Late Gestation):** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.5 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR Actual amino acid intake: NR	**Adaptation Period (Same for Early and Late Gestation):** Diet type: Standardized diet Protein source: NR Energy status: Eucaloric Dietary assessment method: Detailed 2-d diet records were obtained to create a personalized diet recommendation. Analysis of the diet records using the Food Processor Nutrition Analysis Software was used to measure adherence How protein was administered: Personalized diet recommendations were created and prescribed to	Outcome measure: Total aromatic amino acid requirement estimates calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]leucine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Pregnant Gestation stage: 17.2; SD 2.4 wk (13-19 wk) Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: None used on the study d; N=2 reported recent use of pyridoxine/doxylamine, N=1 reported use of levothyroxine, and N=1 reported use of fluoxetine, gestational stage this occurred in NR Supplement use: Prenatal vitamins **Intervention: Varied Phenylalanine Intakes (Late Gestation):** N: 10 % Female: 100% Mean Age/Range/Age at Baseline: 30.0; SD 5.0 yr Race: NR Mean BMI at baseline: 23.5; SD 3.8 kg/m^2 (pre-pregnancy) Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: Pregnant Gestation stage: 34.1; SD	Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day: (Same for Early and Late Gestation):** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.5 g/kg/d Intended amino acid intake: 5, 25, 40, 50, 60, 70, 85, 100 mg/kg/d phenylalanine; 0 mg/kg/d tyrosine; serine and glutamine content were altered depending on the phenylalanine intake to ensure all meals were isonitrogenous. See Table 2 in original paper for more information. Intended carbohydrate intake: 53% of energy from carbohydrate Intended fat intake: 37% of energy from fat Actual protein intake: 1.5 g/kg/d Actual amino acid intake: 5, 25, 40, 50, 60, 70, 85, 100	each participant. Protein assessment method: Analysis of the diet records using the Food Processor Nutrition Analysis Software **Study Day: (Same for Early and Late Gestation):** Diet type: Liquid formula (protein-free powder, orange flavored drink powder, corn oil, and protein as a crystalline L-amino acid mixture modeled after egg-protein composition) and protein-free cookies Protein source: Crystalline L-amino acid mixture modeled after egg-protein composition Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Provided the liquid formula as 8 hourly meals Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	2.5 wk (33-39 wk) Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: None used on the study d; N=2 reported recent use of pyridoxine/doxylamine, N=1 reported use of levothyroxine, and N=1 reported use of fluoxetine, gestational stage this occurred in NR Supplement use: Prenatal vitamins	mg/kg/d phenylalanine; 0 mg/kg/d tyrosine; serine and glutamine content were altered depending on the phenylalanine intake to ensure all meals were isonitrogenous. See Table 2 in original paper for more information. Actual carbohydrate: 53% of energy from carbohydrate Actual fat intake: 37% of energy from fat Study duration: 1 d (d 3) Crossover details: (early gestation) Number of intakes per participant: 1-6 Total observations: 24 Wash out period: ≥5 d Crossover details: (late gestation) Number of intakes per participant: 1-5 Total observations: 27 Wash out period: ≥5 d		

Abbreviations: BMI = body mass index; d = day; g= grams; DAAO = direct amino acid oxidation; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; leucine and phenylalanine]; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; IAAO = indicator amino acid oxidation; kcal/kg/d = kilocalorie per kilogram per day; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; rob = risk of bias; SD = standard deviation; wk = week; yr = year

*Five participants were studied in both early and late gestation; Three participants were studied using both the IAAO method and DAAO methods

**One participant was studied at both early and late gestation

Table F.23. Phenylalanine RCTs adults (19-50 years)

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Hsu, 2006[24] (16400054) Canada Very high HDI Outpatient RCT, cross over design Government Moderate ROB	Population: Adults (19-50 yr) Total sample N: 5 **Intervention: Varied Phenylalanine Intakes:** N: 5 % Female: 0% Mean Age/Range/Age at Baseline: 29.4; SD 4.7 yr Race: NR Mean BMI at baseline: 23.9; SD 3.2 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: No use of pharmacologic therapy or hormonal treatment Supplement use: NR	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.0 g/kg/d Intended amino acid intake: 5, 10, 15, 25, 35, 45,	**Adaptation Period:** Diet type: Milkshake diet Protein source: Animal Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Provided participants with the milkshake diet Protein assessment method: NR **Study Day:** Diet type: Liquid formula and protein-free cookies Protein source: Crystalline L-amino acid mixture based on the amino acid profile of egg protein Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants received 8 hourly isocaloric, isonitrogenous meals Protein assessment method: NR	Outcome measure: Aromatic amino acid requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]lysine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		60, and 70 mg/kg/d phenylalanine; 0 mg/kg/d tyrosine; 45 mg/kg/d lysine; alanine provided in varying amounts to keep the meals isonitrogenous. See Table 2 in original paper for more information. Intended carbohydrate intake: 52% of energy from carbohydrate Intended fat intake: 37% of energy from fat Actual protein intake: 1.0 g/kg/d Actual amino acid intake: 5, 10, 15, 25, 35, 45, 60, and 70 mg/kg/d phenylalanine; 0 mg/kg/d tyrosine; 45 mg/kg/d lysine; alanine provided in varying amounts to keep the meals isonitrogenous. See Table 2 in original paper for more information. Actual carbohydrate intake: 52% of energy from carbohydrate Actual fat intake: 37% of energy from fat Study duration: 1 d (d 3) Crossover details: Number of intakes per participant: 8; except for N=1 who received 7 Total intake observations: 39 Wash out period: NR		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Hsu, 2006[25] (16549457) Canada Very high HDI Outpatient RCT, cross over design Government Moderate ROB	Population: Adults (19-50 yr) Total sample N: 7* **Intervention: Varied Phenylalanine Intakes (Part A):** N: 5 % Female: 0% Mean Age/Range/Age at Baseline: 29.4; SD 4.7 yr Race: NR Mean BMI at baseline: 23.9; SD 3.2 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: NR **Intervention: Varied Phenylalanine Intakes (Part B):** N: 5 % Female: 0% Mean Age/Range/Age at Baseline: 30.4; SD 5.4 yr Race: NR Mean BMI at baseline: 23.9; SD 3.2 kg/m^2 Health	**Adaptation Period: (Same for Part A and B)** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day: (Part A):** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.0 g/kg/d Intended amino acid intake: 5, 10,15, 25, 35, 45, 60 mg/kg/d	**Adaptation Period: (Same for Part A and B)** Diet type: Milkshake diet Protein source: Animal Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Provided participants with the milkshake diet Protein assessment method: NR **Study Day: (Same for Part A and B):** Diet type: Liquid formula and protein free cookies along with a crystalline L-amino acid mixture based on the amino acid profile of egg protein Protein source: Crystalline L-amino acid mixture based on the amino acid profile of egg protein Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants received 8 hourly isocaloric, isonitrogenous meals Protein assessment method: NR	Outcome measure: Aromatic amino acid requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]leucine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: NR	phenylalanine; 0 mg/kg/d tyrosine; total BCAA 170 mg/kg/d (65.5 mg/kg/d leucine, 49.3 mg/kg/d isoleucine, 55.3 mg/kg/d valine); glycine was given in various amounts to keep the meals isonitrogenous. See Table 2 in original paper for more information. Intended carbohydrate intake: 52% of energy from carbohydrate Intended fat intake: 37% of energy from fat Actual protein intake: 1.0 g/kg/d Actual amino acid intake: 5, 10,15, 25, 35, 45, 60 mg/kg/d phenylalanine; 0 mg/kg/d tyrosine; total BCAA 170 mg/kg/d (65.5 mg/kg/d leucine, 49.3 mg/kg/d isoleucine, 55.3 mg/kg/d valine); glycine was given in various amounts to keep the meals isonitrogenous. See Table 2 in original paper for more information. Actual carbohydrate intake: 52% of energy from carbohydrate Actual fat intake: 37% of energy from fat Study duration: 1 d (d 3)		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Study Day: (Part B): Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.0 g/kg/d Intended amino acid intake: 5, 15, 25, 35, 45, 55, 65 mg/kg/d phenylalanine; 0 mg/kg/d tyrosine; total BCAA 177.1 mg/kg/d (45.0 mg/kg/d leucine, 62.4 mg/kg/d isoleucine, 69.7 mg/kg/d valine); glycine was given in various amounts to keep the meals isonitrogenous. See Table 2 in original paper for more information. Intended carbohydrate intake: 52% of energy from carbohydrate Intended fat intake: 37% of energy from fat Actual protein intake: 1.0 g/kg/d Actual amino acid intake: 5, 15, 25, 35, 45, 55, 65 mg/kg/d phenylalanine; 0 mg/kg/d tyrosine; total BCAA 177.1 mg/kg/d (45.0 mg/kg/d leucine, 62.4 mg/kg/d isoleucine, 69.7 mg/kg/d valine); glycine was given in		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		various amounts to keep the meals isonitrogenous. See Table 2 in original paper for more information. Actual carbohydrate intake: 52% of energy from carbohydrate Actual fat intake: 37% of energy from fat Study duration: 1 d (d 3) Crossover details: (Part A) Number of intakes per participant: 7 Total intake observations: 35 Wash out period: NR Crossover details: (Part B) Number of intakes per participant: 7 Total intake observations: 35 Wash out period: NR		
Kurpad, 2006[35] (16762944) India Low HDI Outpatient RCT, cross over design Government Moderate ROB	Population: Adults (19-50 yr) Total sample N: 32 **Intervention: Varied Phenylalanine Intakes:** N: 32 % Female: 0% Mean Age/Range/Age at Baseline: 21.8; SD 2.7 yr Race: NR Mean BMI at baseline: 21.5; SD 1.6 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d (160 mg/kg/d nitrogen Intended amino acid intake: 19, 23, 27, 31, 35, 38, 43, and 47 mg/kg/d phenylalanine; 0 mg/kg/d tyrosine. See Table 2 in original paper for more	**Adaptation Period:** Diet type: Weight-maintaining diet based on an L- amino acid mixture Protein source: L- amino acid mixture Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Daily dietary intake was provided in 3 isoenergetic, isonitrogenous meals Protein assessment method:	Outcome measure: Aromatic amino acid requirement calculated from 24-h IAAO, 12-h fed IAAO, and 24-h IAAB Measure/Method of Assessment: 24-h IAAO and 24-h IAAB method Isotope used: [13C]leucine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: Encouraged to maintain their customary levels of physical activity but were asked to refrain from excessive or competitive exercise Medication use: NR Supplement use: NR	information. Intended carbohydrate intake: 56% of energy from carbohydrate Intended fat intake: 43% of energy from fat Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 6 d (d 1-6) **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.0 g/kg/d (160 mg/kg/d nitrogen) Intended amino acid intake: 19, 23, 27, 31, 35, 38, 43, and 47 mg/kg/d phenylalanine; 0 mg/kg/d tyrosine. See Table 2 in the original paper for more information Intended carbohydrate intake: 56% of energy from fat Intended fat intake:43% of energy from fat Actual protein intake: 1.0 g/kg/d (160 mg/kg/d nitrogen)	NR **Study Day:** Diet type: Weight-maintaining diet based on an L- amino acid mixture Protein source: L- amino acid mixture Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants were provided with 10 small isoenergetic isonitrogenous meals Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Actual amino acid intake: 19, 23, 27, 31, 35, 38, 43, and 47 mg/kg/d phenylalanine; 0 mg/kg/d tyrosine. See Table 2 in the original paper for more information Actual carbohydrate intake: 56% of energy from carbohydrate Actual fat intake: 43% of energy from fat Study duration: 1 d (d 7) Crossover details: Number of intakes per participant: 2 Total intake observations: 64 Wash out period: NR		

Abbreviations: BCCA = branched chain amino acid; BMI = body mass index; d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; leucine or lysine]; g= grams; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; IAAB = indicator amino acid balance; IAAO = indicator amino acid oxidation; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RoB = risk of bias; SD = standard deviation; yr = year
*Three subjects completed both Part A and B

Table F.24. Phenylalanine RCTs adults (51->70 years)

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Martin, 2019[43] (31271193) Canada Very high HDI Outpatient	Population: Adults (51->70 yr) Total sample N: 12 **Intervention: Varied**	**Adaptation Period: (Same for Men and Women):** Baseline protein intake: NR Baseline amino acid intake:	**Adaptation Period: (Same for Men and Women):** Diet type: Lactose-free milkshake maintenance diet	Outcome measure: Phenylalanine requirement estimate calculated from $F^{13}CO_2$

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
RCT, cross over design Government, seniors' multivitamins provided by Pfizer Consumer Healthcare, protein-free powder for experimental diets provided by Mead Johnson Nutritionals Moderate ROB	**Phenylalanine Intake (Men):** N: 6 % Female: 0% Mean Age/Range/Age at Baseline: 70.8; SD 5.4 yr Race: NR Mean BMI at baseline: 26.4; SD 4.9 kg/m^2 Health status/comorbidities: Excluded if they had a recent history of weight loss, diabetes, kidney disease or other illness known to affect protein metabolism Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: N= 2 subjects were on anti-hypertensive medications; N=2 were on cholesterol-lowering medications, N=1 was taking a blood thinner, a medication to help with smoking cessation and hypothyroidism, N=1 was taking strontium for improvement of bone density does not specify if this is in men or women Supplement use: N=8 subjects were on daily multivitamin and/or vitamin D,	NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day: (Same for Men and Women):** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.0 g/kg/d Intended amino acid intake: 7.2-40 mg/kg/d phenylalanine; 40 mg/kg/d tyrosine; and alanine was supplied to balance the nitrogen with varying phenylalanine intake	Protein source: Animal Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Study diets prepared and provided to participants in which they were instructed to consume them in 4 meals 3 h apart. Protein assessment method: NR **Study Day: (Same for Men and Women):** Diet type: Protein-free liquid formula made with protein-free powder, flavored drink crystals, grape seed oil, and protein-free cookies along with the crystalline amino acid mixture (patterned after egg protein) Protein source: Crystalline amino acid mixture Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Provided 8 hourly isocaloric, isonitrogenous meals Protein assessment method: NR	Measure/Method of Assessment: DAAO method Isotope used: L-[1–13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	N=3 were taking fish oil, N=2 were taking calcium and magnesium supplements, does not specify if this is in men or women **Intervention: Varied Phenylalanine Intake (Women):** N: 6 % Female: 100% Mean Age/Range/Age at Baseline: 76.7; SD 7.0 yr Race: NR Mean BMI at baseline: 25.3; SD 4.4 kg/m^2 Health status/comorbidities: Excluded if they had a recent history of weight loss, diabetes, kidney disease or other illness known to affect protein metabolism Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NR Income level: NR Education level: NR Physical activity level: NR Medication use: N= 2 women were on low-dose Premarin for management of postmenopausal symptoms; N= 2 subjects were on anti-hypertensive medications; N=2 were on cholesterol-	Intended carbohydrate intake: 51% of energy from carbohydrate Intended fat intake: 36% of energy from fat Actual protein intake: 1.0 g/kg/d Actual amino acid intake: 7.2-40 mg/kg/d phenylalanine; 40 mg/kg/d tyrosine; and alanine was supplied to balance the nitrogen with varying phenylalanine intake Actual carbohydrate intake: 51% of energy from carbohydrate Actual fat intake: 36% of energy from fat Study duration: 1 d (d 3) Crossover details: (all) Number of intakes per participant: 3-7 Total intake observations: 66 (31 in men, 35 in women) Wash out period: 1–2 wk		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	lowering medications, N=1 was taking a blood thinner, a medication to help with smoking cessation and hypothyroidism, N=1 was taking strontium for improvement of bone density does not specify if this is in men or women Supplement use: N=8 subjects were on daily multivitamin and/or vitamin D, N=3 were taking fish oil, N=2 were taking calcium and magnesium supplements, does not specify if this is in men or women			

Abbreviations: BMI = body mass index; d = day; DAAO = direct amino acid oxidation; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; g= grams; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RoB = risk of bias; SD = standard deviation; wk = week

Threonine

Table F.25. Threonine RCTs infants

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Hogewind-Schoonenboom, 2015[3] (25844708) China Medium HDI Admitted to the Neonatal	Population: Infants Total sample N: 35; 32 analyzed **Intervention: Varied**	**Adaptation Period:** Baseline protein intake: 3.0; SD 0.6 g/kg/d Baseline amino acid intake: NR	**Adaptation Period:** Diet type: Study formula Protein source: Amino acid-based formula with an identical composition to	Outcome: Threonine requirement estimate calculated from $F^{13}CO_2$

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Department of the Fudan Children's Hospital, Shanghai, China RCT Nonprofit, study formulas were manufactured by SHS UK Moderate ROB	**Threonine Intakes:** N: 32 % Female: 40.6% Mean Age/Range/Age at Baseline: 10; SD 4 d; gestational age: 39; SD 1 wk Race: NR Mean BMI at baseline: NR Health status/comorbidities: Clinically stable condition and considered healthy; initially admitted to the hospital for the following reasons: N=20 unconjugated hyperbilirubinemia, N=4 suspected infection with negative blood culture, N=3 pneumonia with negative blood culture, N=2 wet lung, N=1 asphyxia, N=1 bronchiolitis, N=1 polycythemia Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NA Medication use: 69% received intravenous antibiotics Supplement use: NR	Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: ~2.96 g/kg/d Intended amino acid intake: 5-182 mg/kg/d threonine; 166 mg/kg/d phenylalanine and tyrosine; total amino acid amount 13g/100g formula Intended carbohydrate intake: 54g/100g formula Intended fat intake: 23g/100g formula Actual protein intake: ~2.96 g/kg/d Actual amino acid intake: 5-182 mg/kg/d threonine; 166 mg/kg/d phenylalanine and tyrosine; total amino acid amount 13g/100g formula Actual carbohydrate intake: 54g/100g formula Actual fat intake: 23g/100g formula Study duration: 1 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA	regular Neocate (SHS International) Energy status: 113; SD 17 kcal/kg/d Dietary assessment method: NR How protein was administered: Infants received the study formula every 3 h Protein assessment method: NR **Study Day:** Diet type: Study formula Protein source: Amino acid-based formula with an identical composition to regular Neocate (SHS International) Energy status: 108 kcal/kg/d Dietary assessment method: NR How protein was administered: Infants received the study formula by hourly bolus feeding Protein assessment method: NR	Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Intended protein intake: ~2.96 g/kg/d Intended amino acid intake: 5-182 mg/kg/d threonine; 166 mg/kg/d phenylalanine and tyrosine; total amino acid amount 13g/100g formula Intended carbohydrate intake: 54g/100g formula Intended fat intake: 23g/100g formula Actual protein intake: ~2.96 g/kg/d Actual amino acid intake: 5-182 mg/kg/d threonine; 166 mg/kg/d phenylalanine and tyrosine; total amino acid amount 13g/100g formula Actual carbohydrate intake: 54g/100g formula Actual fat intake: 23g/100g formula Study duration: 1 d (d 2) Crossover details: Number of intakes per participant: NA Total intake observations: NA Wash out period: NA		

Abbreviations: BMI = body mass index; d = day; g= grams; $F^{13}CO_2$ = the fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; IAAO = indicator amino acid oxidation; IAAB = indicator amino acid balance; kcal/kg/d = kilocalorie per kilogram per day; mg/kg/d = milligrams per kilogram per day; mo = months; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RoB = risk of bias; SD = standard deviation; wk = week

Table F.26. Threonine RCTs adults (19-50 years)

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Kurpad, 2002[32] (12324292) India Low HDI Outpatient RCT, cross over design Government, nonprofit Moderate ROB	Population: Adults (19-50 yr) Total sample N: 16 **Intervention: Varied Threonine Intakes:** N: 16 % Female: 0% Mean Age/Range/Age at Baseline: 19.6; SD 1.2 yr Race: NR Mean BMI at baseline: 21.0; SD 1.6 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: Encouraged to maintain their customary physical activity levels but were asked to refrain from excessive or competitive exercise. Medication use: NR Supplement use: A multivitamin-multimineral supplement, a choline supplement of 500 mg was given daily and dietary fiber was provided as 20 g ispagul	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d (160 mg/kg/d nitrogen) Intended amino acid intake: 7, 11, 15, 19, 22, and 27 mg/kg/d threonine; 40 mg/kg/d leucine; Total amino acids: 1000 mg/kg/d Intended carbohydrate intake: 56% energy from carbohydrate Intended fat intake: 43% energy from fat Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 6 d (d 1-6) **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA	**Adaptation Period:** Diet type: Weight-maintaining diet based on an L-amino acid mixture Protein source: L-amino acid mixture Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants received their daily intake as 3 isoenergetic isonitrogenous meals Protein assessment method: NR **Study Day:** Diet type: Weight-maintaining diet based on an L-amino acid mixture Protein source: L-amino acid mixture Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants received their daily intake as 10 hourly isoenergetic, isonitrogenous small meals Protein assessment method: NR	Outcome measure: Threonine requirement estimate calculated from fasted and fed plasma amino acid response, 24-h IAAO , 12-h fed IAAO and 24-h IAAB Measure/Method of Assessment: Plasma amino acid response, 24-h IAAO, and 24-h IAAB method Isotope used: [1-13C]leucine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	when requested by the subject	Intended protein intake: 1.0 g/kg/d (160 mg/kg/d nitrogen) Intended amino acid intake: 7, 11, 15, 19, 22, and 27 mg/kg/d threonine; 40 mg/kg/d leucine; Total amino acids: 1000 mg/kg/d. See Table 2 in original paper for more information Intended carbohydrate intake: 56% energy from carbohydrate Intended fat intake: 43% energy from fat Actual protein intake: 1.0 g/kg/d (160 mg/kg/d nitrogen) Actual amino acid intake: 7, 11, 15, 19, 22, and 27 mg/kg/d threonine; 40 mg/kg/d leucine; Total amino acids: 1000 mg/kg/d. See Table 2 in original paper for more information Actual carbohydrate intake: 56% energy from carbohydrate Actual fat intake: 43% energy from fat Study duration: 1 d (d 7) Crossover details: Number of intakes per participant: 3 Total intake observations: 48		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		total observations Wash out period: 2-4 wk		
Wilson, 2000[59] (10702170) Canada Very high HDI Outpatient RCT, cross over design Government, academic, protein-free powder for experimental diets provided by Mead Johnson Nutritionals Moderate ROB	Population: Adults (19-50 yr) Total sample N: 6 **Intervention: Varied Threonine Intakes:** N: 6 % Female: 0% Mean Age/Range/Age at Baseline: 26.5; SD 6.8 yr Race: NR Mean BMI at baseline: 25.7; SD 4.0 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: Subjects were encouraged to maintain their normal levels of physical activity and were provided with preprinted forms on which to record all activities throughout the study Medication use: NR Supplement use: NR	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.0 g/kg/d (10% of energy from protein) Intended amino acid intake: 47 mg/kg/d threonine; 14 mg/kg/d phenylalanine; 40 mg/kg/d tyrosine Intended carbohydrate intake: 53% of energy from carbohydrate Intended fat intake: 37% of energy from fat Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: Two 9 d periods; consumed the adaptation diet on d 1-2, 4-5, and 7-8. **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA	**Adaptation Period:** Diet type: Flavored liquid-formula diet, a crystalline amino acid mixture and protein-free cookies Protein source: Crystalline AA mixture based on egg protein Energy status: Eucaloric Dietary assessment method: Diets were prepared and weighed in the research kitchen, specifics NR How protein was administered: Participants provided with 4 isoenergetic, isonitrogenous meals Protein assessment method: NR **Study Day:** Diet type: Flavored liquid-formula diet, a crystalline amino acid mixture and protein-free cookies Protein source: Crystalline amino acid mixture based on egg protein Energy status: Eucaloric Dietary assessment method: Diets were prepared and weighed in the research kitchen, specifics NR How protein was administered: The meals at 0800 and 1200 were divided	Outcome measure: Threonine requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.0 g/kg/d (10% of energy from protein) Intended amino acid intake: 5, 10, 15, 20, 25, 30, or 35 mg/kg/d threonine; 14 mg/kg/d phenylalanine; 40 mg/kg/d tyrosine. See Table 2 in original paper for more information Intended carbohydrate intake: 53% of energy from carbohydrate Intended fat intake: 37% of energy from fat Actual protein intake: 1.0 g/kg/d (10% of energy from protein) Actual amino acid intake: 5, 10, 15, 20, 25, 30, or 35 mg/kg/d threonine; 14 mg/kg/d phenylalanine; 40 mg/kg/d tyrosine. See Table 2 in original paper for more information Actual carbohydrate intake: 53% of energy from carbohydrate Actual fat intake: 37% of energy from fat Study duration: Two 9 d periods; received the test	into 6 equal parts, which the subjects consumed hourly beginning 2 h before infusion of the Tracer Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		intake and tracer infusion on d 3, 6 and 9 Crossover details: Number of intakes per participant: 6 Total intake observations: 36 Wash out period: ≥2 wk but < 1 mo		

Abbreviations: BMI = body mass index; d = day; g= grams; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; h = hour; IAAO = indicator amino acid oxidation; IAAB = indicator amino acid balance; kcal/kg/d = kilocalorie per kilogram per day; mg/kg/d = milligrams per kilogram per day; mo = months; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RoB = risk of bias; SD = standard deviation; wk = week; yr = year

Total Branched Chain Amino Acids

Table F.27. Total branched chain amino acids RCTs children and adolescents

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Mager, 2003[39] (14608071) Canada Very high HDI Outpatient RCT, cross over design Government Moderate ROB	Population: Children and Adolescents Total sample N: 5 **Intervention: Varied BCAA Intakes:** N: 5 % Female: 80% Mean Age/Range/Age at Baseline: 8.5; SD 1.2 yr Race: NR Mean BMI at baseline: NR	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 1.5 g/kg/d Intended amino acid intake: NR	**Adaptation Period:** Diet type: Standardized diet Protein source: NR Energy status: Provided at an amount to ensure age-appropriate growth Dietary assessment method: Food records were collected to ensure consistency of dietary intake before each study d How protein was	Outcome measure: BCAA requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	Health status/comorbidities: Healthy Obesity status: NR Pubertal status: Tanner stage 1.2; SD 0.4 Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: Excluded if taking medication that alter protein or energy metabolism Supplement use: Multivitamin supplements	Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: ~10% of energy from protein Intended amino acid intake: 75, 85, 100, 125, 150, 200 and 225 mg/kg/d BCAA providing 29% isoleucine, 38.5% leucine, and 32.5% valine. See Table 2 in original paper for more information Intended carbohydrate: 53% of energy from carbohydrate Intended fat intake: 37% of energy from fat Actual protein intake: ~10% of energy from protein Actual amino acid intake: 75, 85, 100, 125, 150, 200 and	administered: NR Protein assessment method: NR **Study Day:** Diet type: Flavored protein-free liquid formula, crystalline L-amino acid study mixture, and protein free cookies Protein source: L-amino acid mixture based on the amino acid composition of egg protein Energy status: 75% of daily energy and protein needs Dietary assessment method: NR How protein was administered: Consumed liquid formula diet as 9 isonitrogenous, isoenergetic hourly meals Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		225 mg/kg/d BCAA providing 29% isoleucine, 38.5% leucine, and 32.5% valine. See Table 2 in original paper for more information Actual carbohydrate: 53% of energy from carbohydrate Actual fat intake: 37% of energy from fat Study duration: 1 d (d 3) Crossover details: Number of intakes per participant: 7 Total intake observations: 35 Wash out period: ≥ 1 wk		

Abbreviations: BMI = body mass index; d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; g= grams; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; IAAO = indicator amino acid oxidation; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RoB = risk of bias; SD = standard deviation; wk = week; yr = year

Table F.28. Total branched chain amino acids RCTs adults (19-50 years)

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and methods of assessment)
Riazi, 2003[53] (14608070) Canada Very High Outpatient RCT, cross over design Government, protein-free powder for experimental diets provided by Mead	Population: Adults (19-50 yr) Total sample N: 5 Intervention: Varied Branched Chain Amino Acid Intakes N: 5 % Female: 0%	Adaptation Period: Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR	Adaptation Period: Diet type: Milkshake diet Protein source: Animal Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Provided a	Outcome measure: Phenylalanine oxidation and $F^{13}CO_2$ Measure/Method of Assessment: IAAO method

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and methods of assessment)
Johnson Nutritionals, multivitamin supplements provided by Whitehall Robins Moderate ROB	Mean Age/Range/Age at Baseline: 26.8; SD 6.7 yr Race: NR Mean BMI at baseline: 24.8; SD 2.4 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: Maintained ordinary levels of activity Medication use: None used Supplement use: Multivitamin supplement	Intended protein intake: NR Intended amino acid intake: NR Intended carbohydrate intake: NR Intended fat intake: NR Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 2 d Study Day: Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 1.0 g/kg/d Intended amino acid intake: Isoleucine provided at requirement amount and leucine and valine provided at 10% and 20% less than the requirement; Leucine provided at requirement amount 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	milkshake diet supplemented with additional protein and energy to meet each subject's requirements Protein assessment method: NR Study Day: Diet type: Flavored liquid protein-free formula along with an L-amino acid mixture based on the amino acid profile of egg protein, plus two protein-free cookies. Protein source: L-amino acid mixture based on the amino acid profile of egg protein Energy status: Eucaloric Dietary assessment method: NR How protein was administered: All diets were prepared and weighed in the research kitchen and were provided in 9 hourly isonitrogenous, isocaloric meals Protein assessment method: NR	Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and methods of assessment)
		requirement, 15 mg/kg/d phenylalanine Intended carbohydrate intake: 53% of energy from carbohydrate Intended fat intake: 37% of energy from fat Actual protein intake: 1.0 g/kg/d Actual amino acid intake: Isoleucine provided at requirement amount and leucine and valine provided at 10% and 20% less than the requirement; Leucine provided at requirement amount 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, 15 mg/kg/d phenylalanine Actual carbohydrate intake: 53% of energy from carbohydrate Actual fat intake: 37% of energy from fat Study duration: 1 d (d 3) Crossover details: Number of intakes per participant: 6 Total intake observations: 35; 30 from this study and 5		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and methods of assessment)
		brought from the previous study[52] Wash out period: NR		

Abbreviations: BMI = body mass index; 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; kg/m^2 = kilogram per meter squared; mg/kg/d = milligram per kilogram per day; N = number; NA = not applicable; NR = not reported; RCT = randomized controlled trial; SD = standard deviation; yr = year

Tryptophan

Table F.29. Tryptophan RCTs infants

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content, administrator, and duration)	Outcome (Measures and Methods of Assessment)	Risk of Bias Score
Huang, 2014[7] (24824360) China High HDI Admitted to the Children's Hospital of Fudan University RCT Nonprofit, Study formulas were manufactured by SHS UK Moderate ROB	Population: Infants Total sample N: 30 **Intervention: Varied Tryptophan Intakes:** N: 30 % Female: 43% Mean Age/Range/Age at Baseline: 9; SD 4 d; gestational age: 39; SD 1 wk Race: NR Mean BMI at baseline: NR Health status/comorbidities: Healthy; initially admitted to the hospital for the following reasons: N=12 unconjugated hyperbilirubinemia, N=7 pneumonia, N=4 fetal distress, N=3 infection	**Adaptation Period:** Baseline protein intake: 2.2; SD 0.4 g/kg/d (range: 1.7-3.4 g/kg/d) Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 2.96 g/kg/d Intended amino acid intake: 0.5-73 mg/kg/d tryptophan; 166 mg/kg/d phenylalanine and tyrosine; Total amino acids: 13g/100g formula; L-alanine content varied depending on the tryptophan	**Adaptation Period:** Diet type: Study formula Protein source: Elemental formula based on free amino acids Energy status: 105; SD 17 kcal/kg/d Dietary assessment method: NR How protein was administered: Infants received the study formula every 3 h Protein assessment method: NR **Study Day:** Diet type: Study formula Protein source: Elemental	Outcome: Tryptophan requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Isotope used: L-[1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content, administrator, and duration)	Outcome (Measures and Methods of Assessment)	Risk of Bias Score
	suspicion with a negative blood culture, N=2 wet lung, N=1 meconium-stained amniotic fluid, N=1 peripheral cyanosis Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NA Medication use: N=29 received intravenous antibiotics Supplement use: NR	level to maintain an isonitrogenous diet. See Table 1 in original paper for more information Intended carbohydrate intake: 54 g/100 g formula Intended fat intake: 23 g/100 g formula Actual protein intake: 2.98; SD 0.01 g/kg/d Actual amino acid intake: 0.5-73 mg/kg/d tryptophan; 166 mg/kg/d phenylalanine and tyrosine; Total amino acids: 13g/100g formula; L-alanine content varied depending on the tryptophan level to maintain an isonitrogenous diet. See Table 1 in original paper for more information Actual carbohydrate intake: 54 g/100g formula Actual fat intake: 23 g/100g formula Study duration: 1 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 2.96 g/kg/d	formula based on free amino acids Energy status: 108 kcal/kg/d Dietary assessment method: NR How protein was administered: Infants received the study formula every hour Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content, administrator, and duration)	Outcome (Measures and Methods of Assessment)	Risk of Bias Score
		Intended amino acid intake: 0.5-73 mg/kg/d tryptophan; 166 mg/kg/d phenylalanine and tyrosine; Total amino acids: 13g/100g formula; L-alanine content varied depending on the tryptophan level to maintain an isonitrogenous diet. See Table 1 in original paper for more information Intended carbohydrate intake: 54 g/100 g formula Intended fat intake: 23 g/100 g formula Actual protein intake: 2.98; SD 0.01 g/kg/d Actual amino acid intake: 0.5-73 mg/kg/d tryptophan; 166 mg/kg/d phenylalanine and tyrosine; Total amino acids: 13g/100g formula; L-alanine content varied depending on the tryptophan level to maintain an isonitrogenous diet. See Table 1 in original paper for more information Actual carbohydrate intake: 54 g/100 g formula Actual fat intake: 23 g/100 g formula Study duration: 1 d (d 2) Crossover details: Number of intakes per participant: NA		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content, administrator, and duration)	Outcome (Measures and Methods of Assessment)	Risk of Bias Score
		Total intake observations: NA Wash out period: NA		

Abbreviations: BMI = body mass index; d = day; g= grams; $F^{13}CO_2$ = the fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; IAAO = indicator amino acid oxidation; kcal/kg/d = kilocalorie per kilogram per day; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RoB = risk of bias; SD = standard deviation; wk = week

Valine

Table F.30. Valine RCTs infants

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
de Groof, 2014[2] (24284437) China Medium HDI Admitted to the Neonatology Department of the Fudan Children's Hospital, Shanghai, China. RCT Nonprofit, Study formulas were manufactured by SHS United Kingdom Low ROB	Population: Infants Total sample N: 28 **Intervention: Varied Valine Intakes:** N: 28 % Female: 0% Mean Age/Range/Age at Baseline: 15; SD 7d; gestational age: 39.5; SD 1.2 wk Race: 100% Asian Mean BMI at baseline: NR Health status/comorbidities: clinically stable condition and considered healthy; initially admitted to the hospital for the following reasons: N=13 pneumonia with negative blood cultures, N=5	**Adaptation Period:** Baseline protein intake: 2.43; SD 0.27 g/kg/d Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: NR Intended amino acid intake: 5-236 mg/kg/d valine; 166 mg/kg/d phenylalanine and tyrosine; L-alanine added separately to make the formula isonitrogenous. See Table 1 in original paper for more information Intended carbohydrate intake: 54 g/100g formula	**Adaptation Period:** Diet type: Study formula Protein source: AA-based formula Energy status: 475 kcal/100g formula Dietary assessment method: NR How protein was administered: Infants received the study formula every 3 h Protein assessment method: NR **Study Day:** Diet type: Study formula Protein source: AA-based formula Energy status: 475 kcal/100g	Outcome: Valine requirement estimate calculated from $F^{13}CO_2$ Measure/Method of Assessment: IAAO method Tracer Isotope used: [1-13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
	hyperbilirubinemia, N=4 asphyxia, N=2 pneumothorax, N=2 suspicion of infection with negative blood cultures, N=1 respiratory syncytial virus bronchiolitis, N=1 humerus fracture Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NA Medication use: NR Supplement use: NR	Intended fat intake: 23 g/100g formula Actual protein intake: 2.96; SD 0.15 g/kg/d Actual amino acid intake: 5-236 mg/kg/d valine; 166 mg/kg/d phenylalanine and tyrosine; L-alanine added separately to make the formula isonitrogenous. See Table 1 in original paper for more information Actual carbohydrate intake: 54 g/100g formula Actual fat intake: 23 g/100g formula Study duration: 1 d **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: NR Intended amino acid intake: 5-236 mg/kg/d valine; 166 mg/kg/d phenylalanine and tyrosine; L-alanine added separately to make the formula isonitrogenous. See Table 1 in original paper for more information Intended carbohydrate intake:	formula Dietary assessment method: NR How protein was administered: Infants received the study formula by a continuous drip feeding. Protein assessment method: NR	

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		54 g/100g formula Intended fat intake: 23g/100g formula Actual protein intake: 2.96; SD 0.15 g/kg/d Actual amino acid intake: 5-236 mg/kg/d valine; 166 mg/kg/d phenylalanine and tyrosine; L-alanine added separately to make the formula isonitrogenous. See Table 1 in original paper for more information Actual carbohydrate intake: 54 g/100g formula Actual fat intake: 23g/100g formula Study duration: 1 d (d 2) *Crossover details: Number of intakes per participant: NA Total intake observations: NA Wash out period: NA		

Abbreviations: BMI = body mass index; d = day; $F^{13}CO_2$ = the fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; g= grams; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; IAAO = indicator amino acid oxidation; IAAB = indicator amino acid balance; kcal = kilocalorie; mg/kg/d = milligrams per kilogram per day; mo = months; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RoB = risk of bias; SD = standard deviation; wk = week

*One subject was studied at 2 different intakes and all others were studied at one intake.

Table F.31. Valine RCTs adults (19-50 years)

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
Kurpad, 2005[34] (16087981) India Low HDI Outpatient RCT, cross over design Government Moderate ROB	Population: Adults (19-50 yr) Total sample N: 18 **Intervention: Varied Valine Intakes:** N: 18 % Female: 0% Mean Age/Range/Age at Baseline: 21.5 SD 2.5 yr Race: NR Mean BMI at baseline: 21.4; SD 1.6 kg/m^2 Health status/comorbidities: Healthy Obesity status: NR Pubertal status: NA Pregnant or lactating: NA Gestation stage: NA Lactation stage: NA Menopausal status: NA Income level: NR Education level: NR Physical activity level: NR Medication use: NR Supplement use: NR	**Adaptation Period:** Baseline protein intake: NR Baseline amino acid intake: NR Baseline carbohydrate intake: NR Baseline fat intake: NR Intended protein intake: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Intended amino acid intake: 5, 10, 15, 20, 25, 30, and 35mg/kg/d valine; 40 mg/kg/d leucine; Total amino acids: 1000 mg/g mixture. See Table 2 in original paper for more information Intended carbohydrate intake: 56% of energy from carbohydrate Intended fat intake: 43% of energy from fat Actual protein intake: NR Actual amino acid intake: NR Actual carbohydrate intake: NR Actual fat intake: NR Study duration: 6 d (d 1-6) **Study Day:** Baseline protein intake: NA Baseline amino acid intake: NA	**Adaptation Period:** Diet type: Weight maintaining diet based on an L-amino acid mixture Protein source: L-amino acid mixture Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants received their daily intake as 3 isoenergetic isonitrogenous meals except on d 6 where participants received 10 isoenergetic, isonitrogenous small meals Protein assessment method: NR **Study Day:** Diet type: Weight maintaining diet based on an L-amino acid mixture Protein source: L-amino acid mixture Energy status: Eucaloric Dietary assessment method: NR How protein was administered: Participants received 10 isoenergetic, isonitrogenous small meals Protein assessment method: NR	Outcome measure: Valine requirement estimate calculated from 24-h IAAO, 12-h fed IAAO, 24-h IAAB, and $F^{13}CO_2$ Measure/Method of Assessment: 24-h IAAO and 24-h IAAB method Isotope used: [13C]phenylalanine

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Baseline carbohydrate intake: NA Baseline fat intake: NA Intended protein intake: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) mg nitrogen/kg/d) Intended amino acid intake: 5, 10, 15, 20, 25, 30, and 35mg/kg/d valine; 40 mg/kg/d leucine; Total amino acids: 1000 mg/g mixture. See Table 2 in original paper for more information Intended carbohydrate intake: 56% of energy from carbohydrate Intended fat intake: 43% of energy from fat Actual protein intake: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Actual amino acid intake: 5, 10, 15, 20, 25, 30, and 35mg/kg/d valine; 40 mg/kg/d leucine; See Table 2 in original paper for more information Actual carbohydrate intake: 56% of energy from carbohydrate Actual fat intake: 43% of energy from fat Study duration: 1 d (d 7)		

Study (PMID) Location (country) HDI Setting Study Design Funding Source ROB Score	Participants	Interventions/Exposure and Comparator (Content)	Intervention/Exposure and Comparator (Methods of Assessment)	Outcome (Measures and Methods of Assessment)
		Crossover details: Number of intakes per participant: 3 Total intake observations: 54 Wash out period: NR		

Abbreviations: BMI = body mass index; d = day; $F^{13}CO_2$ = proportion of tracer oxidized [tracer; phenylalanine]; g = grams; g/kg/d = grams per kilogram per day; h = hour; HDI = human development index; IAAB = indicator amino acid balance; IAAO = indicator amino acid oxidation; kcal/kg/d = kilocalorie per kilogram per day; kg/m^2 = kilogram per meter squared; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RoB = risk of bias; SD = standard deviation; yr = year

Appendix G. Protein and Amino Acid Requirement Estimates of Studies Not in the Analytic Set

Summary of Findings

Of those studies not included in the analytic set, 10 reported protein and indispensable amino acid requirement estimates. Protein requirements were calculated in one RCT and one non-RCT for adults 19-50 years, two RCTs for adults 51->70 years, and 1 RCT for adults 19-50 and 51->70 years. For indispensable amino acid requirements, two non-RCTs calculated lysine requirements for adults 19-50 years, one RCT calculated methionine requirements for adults 51->70 years, one RCT calculated total branched chain amino acids requirements for adults 19-50 years, and one RCT calculated tryptophan requirements for children and adolescents. A summary of findings is presented in Tables G.1-G.8 and detailed findings for each study can be found in Tables G.9-G.16.

Protein

Adults (19-50 years)

One RCT[38] and one non-RCT[63] addressed the question of the average daily protein requirements for adults 19-50 years.

In the RCT, [38] participants underwent 6 adaptation days and 1 study day in which they consumed test protein intakes ranging from 0.7-1.10 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 leucine oxidation data. Table G.1 provides a summary of findings.

In the non-RCT, [63] participants underwent 5 adaptation days and 1 study day in which they consumed test protein intakes ranging from 0.75-1.05 g/kg/d. The IAAO method was performed, and the protein requirement estimate was calculated using a biphasic linear regression crossover model on the $F^{13}CO_2$ data. Table G.2 provides a summary of findings.

Table G.1. Protein requirement estimates high RoB RCTs adults (19-50 years)

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
Protein requirement estimate **1. $F^{13}CO_2$** **2. Leucine oxidation**	Tian, 2011[38] (21859657) RCT See note Data obtained following each study d (d 7)	China NR 21.6 yr 100%	I: 0.70-1.10 g/kg/d *A: 0.79-1.17 g/kg/d	1. Breakpoint: 0.91 g/kg/d; upper 95% CI: 1.09 g/kg/d 2. Breakpoint: 0.92 g/kg/d; upper 95% CI: 1.10 g/kg/d

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

Note: N values for each outcome not provided. Initially 20 subjects were recruited and received 3 intakes each (60 total observations). However, it is unclear if this is the number of total participants and observations analyzed.

*Actual intake is shown as the average range

Table G.2. Protein requirement estimates high RoB non-RCTs adults (19-50 years)

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
***Protein requirement estimate** **1. $F^{13}CO_2$ (M)** **2. $F^{13}CO_2$ (F)** **3. $F^{13}CO_2$ (combined)**	Li, 2013[63] (23981551) non-RCT (19; 95) Data obtained following each study d (d 6)	China NR M: 21.1 yr F: 21.3 yr 47.4%	I: 0.75-1.05 g/kg/d **A: M: 0.76-1.02 g/kg/d, F: 0.74-0.99 g/kg/d	1. Breakpoint: 0.88 g/kg/d; Safe intake level: 0.98 g/kg/d 2. Breakpoint: 0.85 g/kg/d; Safe intake level: 0.97 g/kg/d 3. Breakpoint: 0.87 g/kg/d; Safe intake level: 0.98 g/kg/d

Abbreviations: A = actual; d = day; F = female; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; leucine]; 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; yr = year

*No significant difference in requirement estimates was observed between males and females.

**Actual intake is shown as the average range

Adults (51->70 years)

Two RCTs addressed the question of the average daily protein requirements for adults 51->70 years. [56, 60] In both studies, participants underwent 2 adaptation days (1.0 g/kg/d protein) and 1 study day in which they consumed test protein intakes ranging from 0.1-1.8 g/kg/d. [56, 60] Additionally, in both studies the IAAO method was used. The protein requirement estimate was calculated using a 4-parameter nonlinear mixed model in one study[56] and a non-linear mixed effects model in the other. [60] Table G.3 provides a summary of findings.

Table G.3. Protein requirement estimates high RoB RCTs adults (51->70 years)

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
Protein requirement estimate **1. $F^{13}CO_2$ (assuming normal distribution)** **1. $F^{13}CO_2$ (assuming log normal)**	Tang, 2014[56] (24429540) RCT (6; 42) Data obtained following each study d (d 3)	United States NR 82 yr 100%	0.1-1.8 g/kg/d	1. Breakpoint: 0.85 g/kg/d; Adequate allowance: 1.15 g/kg/d 2. Breakpoint: 0.96 g/kg/d; adequate allowance: 1.39 g/kg/d; ~RDA (EAR x 1.24): 1.05 g/kg/d
Protein requirement estimate **1. $F^{13}CO_2$ (M)** **2. $F^{13}CO_2$ (F)** **3. $F^{13}CO_2$ (combined)**	Wu, 2023[60] (38073288) RCT (16; 106) Data obtained following each study d (d 3)	China NR M: 70.6 yr F: 71 yr 43.8%	0.1-1.8 g/kg/d	1. Breakpoint: 0.93 g/kg/d; upper 95 CI: NR 2. Breakpoint: 0.94 g/kg/d; upper 95% CI: NR 3. Breakpoint: 0.94 g/kg/d; upper 95% CI: 1.13; RNI (EAR + SD (1.96)): 1.36 g/kg/d; RNI (CV (1.25) x EAR): 1.18 g/kg/d

Abbreviations: CI = confidence interval; CV = coefficient of variation; d = day; EAR = estimated average requirement; 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; M = male; N = number; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; RDA = recommended dietary allowance; RNI = recommended nutrient intake; SD = standard deviation; yr = year

Adults (19-50 and 51->70 years)

One RCT addressed the question of the average daily protein requirements for adults 19-50 and 51->70 years. [14] In this study, participants underwent 1 adaptation day (<0.2 g/kg/d) 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 3 (day 14-17) and the protein requirement estimate was calculated using linear regression and inverse prediction of protein intake and nitrogen balance. Notably, the publication by Campbell et al. [14] and Morse et al. [44] (included in the analytic set) are from the study. The publication by Morse et al. [44] reports findings for older women, which is a subset of the entire population. The findings of the entire population are reported by Campbell et al. [14] and include younger men, younger women, older men, and older women. The Morse et al. [44] publication reports findings for protein requirement estimates from nitrogen balance data both at week 2 and week 3. The Campbell et al. [14] publication reports findings for protein requirement estimates from nitrogen balance data at week 3. The publication by Morse et al. [44] received a moderate risk of bias and the publication by Campbell et al. [14] received high risk of bias because of the attrition rate of the population being reported on (i.e., Morse et al. [44] subset of population [older women] vs Campbell et al. [14] entire population [younger men, younger women, older men, and older women]).The data presented here includes the findings for the entire population, including the older women previously reported in the Morse et al. [44] publication and included in the main report. Table G.4 provides a summary of findings.

Table G.4. Protein requirement estimates high RoB RCTs adults (19-50 years and 51->70 years)

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
¥Protein requirement estimate **1. Nitrogen balance (YM)** **2. Nitrogen balance (YW)** **3. Nitrogen balance (OM)** **4. Nitrogen balance (OW)** **5. Nitrogen balance (combined)**	Campbell, 2008[14] (18996869) RCT *(40; 120) Data obtained from day 14-17 of each 18-d trial	United States YM: 90.9% White, 9.1% Asian YW: 83.3% White, 16.7% African American OM: 100% White OW: 100% White YM: 29 yr YW: 30 yr OM: 72 yr OW: 75 yr	I: LPro:0.5 MPro: 0.75 HPro: 1.0 g/kg/d **A: YM: 0.51, 0.77, 1.02 g/kg/d YW: 0.50, 0.74, 0.98 g/kg/d OM: 0.51, 0.77, 1.01 g/kg/d OW: 0.50, 0.76, 1.01 g/kg/d	1. BW basis: Mean: 0.54 g/kg/d; 97.5th percentile: 0.83 g/kg/d 1. FFM basis: Mean: 0.73 g/kg FFM/d; 97.5th percentile: 1.21 g/kg FFM/d 2. BW basis: Mean: 0.67 g/kg/d; 97.5th percentile: 0.89 g/kg/d 2. FFM basis: Mean: 0.92 g/kg FFM/d; 97.5th percentile: 1.22 g/kg FFM/d

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
		54.8%		3. BW basis: Mean: 0.65 g/kg/d; 97.5th percentile: 0.83 g/kg/d 3. FFM basis: Mean: 0.92 g/kg FFM/d; 97.5th percentile: 1.20 g/kg FFM/d 4. BW basis: Mean: 0.53 g/kg/d; 97.5th percentile: 0.75 g/kg/d 4. FFM basis: Mean: 0.96 g/kg FFM/d; 97.5th percentile: 1.36 g/kg FFM/d 5. BW basis Mean: 0.59 g/kg/d; 97.5th percentile: 0.85 g/kg/d 5. FFM basis: Mean: 0.88 g/kg/d; 97.5th percentile: 1.28 g/kg FFM/d

Abbreviations: A = actual; BW = body weight; d = day; FFM = fat free mass; g/kg/d = grams per kilogram per day; HPro = higher protein; I = intended; OM = older men; OW = older women; LPro = lower protein; MPro = medium protein; n = number; RCT = randomized controlled trial; PMID = PubMed Identification Number; YM = younger men; yr = year; YW = younger women

*n=42 participants completed the study and data for these participants are reported for study characteristics and test protein intakes, shown here. However, the findings data is from n=40 participants (10 YM, 11 YW, 8 OM, 11 OW)

**Actual intake is shown as the average for each protein level and each population group (LPro, MPro, HPro)

¥No significant difference was found for younger and older subjects and men and women on a body weight basis or for younger and older subjects, men and women, and age-by-sex interaction on a fat free mass basis. A significant age-by-sex interaction was observed, on a body weight basis, in which older women had a significantly lower mean protein requirement than older men.

Lysine

Adults (19-50 years)

Two non-RCTs addressed the question of the average daily lysine requirements for adults 19-50 years. [64, 65] In one study, [64] participants received both a milk and wheat-based diet on two separate occasions in which they were provided a low protein milk or wheat diet (2-3% of energy from protein) and a high protein milk or wheat diet (12-14% of energy from protein). In the other study, [65] participants received a milk-based (average 32.3% of energy from protein) and wheat-based meal (average 26.7% of energy from protein) on two separate occasions. In both studies, participants were studied over a 9-hour period using a [1-13C] leucine balance protocol and the lysine requirement estimate was calculated based on the lysine content of the estimated average requirement of wheat protein. Table G.5 provides a summary of findings.

Table G.5. Lysine requirement estimates high RoB non-RCTs adults (19-50 years)

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
Lysine requirement estimate **1. EAR of wheat protein**	Millward, 2000[65] (10871569) RCT (6; 12) Data obtained following each 9-h infusion	United Kingdom NR 32 yr 33%	I (% energy from protein): Milk LPro: 2-3% Milk HPro: 12-14% Wheat LPro: 2-3% Wheat HPro: 12-14% A: Milk LPro: 2.2 ± 0.1% Milk HPro: 13.2 ± 0.6% Wheat LPro: 2.1 ± 0.1% Wheat HPro: 11.9 ± 0.4%	1. EAR for lysine: 23.2 ± 2.0 mg/kg/d
Lysine requirement estimate **1. EAR of wheat protein**	Millward, 2002[64] (12450900) RCT (5, 10) Data obtained following each 9-h infusion	United Kingdom NR 33.2 yr 20%	I (% energy from protein): Milk meal: 50% of UK average daily intake Wheat meal: 50% of UK average daily intake A: Milk meal: 32.3 ± 4.5% Wheat meal: 26.7 ± 4.1%	1. EAR for lysine: 18.3 ± 1.0 g/kg/d

Abbreviations: EAR = estimated average requirement; g/kg/d = grams per kilogram per day; h = hour; N = number; NR = not reported; PMID = PubMed Identification Number; yr = year
Note: Values shown are mean ± standard deviation

Methionine

Adults (51->70 years)

One RCT addressed the question of the average daily methionine requirements for adults (51->70 years).[46] In this study, participants underwent 2 adaptation days and 1 study day (1.0 g/kg/d protein). Participants received 2-7 methionine intakes ranging from 5-40 mg/kg/d methionine and 0 mg/kg/d cysteine. The IAAO method was performed and the total sulfur amino acid requirement estimate was calculated using a mixed-effect change point regression model on the $F^{13}CO_2$ data. Table G.6 provides a summary of findings.

Table G.6. Methionine requirement estimates high RoB RCTs adults (51->70 years)

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
***Total sulfur amino acid requirement estimate** **1. $F^{13}CO_2$ (M)** **2. $F^{13}CO_2$ (W)**	Paoletti, 2023[46] (37356549) RCT (15; 83) Data obtained following each study d (d 3)	Canada NR M: 67.3 yr W: 69.1 yr 53.3%	1.0 g/kg/d 5-40 mg/kg/d methionine, 0 mg/kg/d cysteine	1. BW basis: Breakpoint: 26.2 mg/kg/d; upper 95% CI: 32.1 mg/kg/d 1. FFM basis: Mean requirement: 36.9 ± 1.42 mg/kg FFM/d; upper 95% CI: 39.7 mg/kg/d 2. BW basis: Breakpoint: 17.1 mg/kg/d; upper 95% CI: 23.7 mg/kg/d 2. FFM basis: Mean requirement: 25.6 ± 0.90 mg/kg FFM/d; upper 95% CI 27.4 mg/kg/d

Abbreviations: BW = body weight; 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; M = men; mg/kg/d = milligram per kilogram per day; n = number; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; W = women; yr = year

*Men had a significantly higher mean total sulfur amino acid requirement estimate than women on a body weight and fat free mass basis. Mean requirement ± standard error of the mean.

Total Branched Chain Amino Acids

Adults (19-50 years)

One RCT addressed the question of the average daily total branched chain amino acids requirements for adults (19-50 years).[52] Participants underwent 2 adaptation days and 1 study day (1.0 g/kg/d protein). Participants received 4-9 total branched chain amino acid intakes ranging from 34-180 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 $F^{13}CO_2$, phenylalanine oxidation, and 9-h phenylalanine balance data. Table G.7 provides a summary of findings.

Table G.7. Total branched chain amino acids requirement estimates high RoB RCTs adults (19-50 years)

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
Total branched chain amino acids requirements **1. $F^{13}CO_2$ (all intake levels)** **2. $F^{13}CO_2$ (180 mg/kg/d level removed)** **3. Phenylalanine oxidation (all intake levels)** **4. Phenylalanine oxidation (all intake levels)** **5. 9-h Phenylalanine balance (all intake levels)** **6. 9-h Phenylalanine balance (180 mg/kg/d intake level removed)**	Riazi, 2003[52] (12730426) RCT (7; 53) Data obtained following each study d (d 3)	Canada NR 26.1 yr 0%	1.0 g/kg/d 34-180 mg/kg/d total BCAA	1. Breakpoint: 144 mg/kg/d; upper 95% CI: 209.9 mg/kg/d 2. Breakpoint: 136.6 mg/kg/d; upper 95% CI: 201.5 mg/kg/d 3. Breakpoint: 125.7 mg/kg/d; upper 95% CI: 170.7 mg/kg/d 4. Breakpoint: 121.7 mg/kg/d; upper 95% CI: 168.5 mg/kg/d 5. Breakpoint: 138 mg/kg/d; upper 95% CI: 183.5 mg/kg/d 6. Breakpoint: 135.8 mg/kg/d; upper 95% CI: 182.7 mg/kg/d

Abbreviations: BCAA = branched chain amino acid; 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; mg/kg/d = milligram per kilogram per day; n = number; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year

Tryptophan

Children and Adolescents

One RCT addressed the question of the average daily tryptophan requirements for children and adolescents. [13] In this study, participants underwent 2 adaptation days and 1 study day (1.5 g/kg/d protein). Participants received 2-7 tryptophan intakes ranging from 0.5-10 mg/kg/d. The IAAO method was performed, and the tryptophan requirement estimate was calculated using a nonlinear mixed-effects model on the $F^{13}CO_2$ data. Table G.8 provides a summary of findings.

Table G.8. Tryptophan requirement estimates high RoB RCTs 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
Tryptophan requirement estimate **1. $F^{13}CO_2$**	Al-Mokbel, 2019[13] (30753549) RCT (7; 36) Data obtained following each study d (d 3)	Canada NR 10.2 yr 57.1%	1.5 g/kg/d 0.5-10 mg/kg/d tryptophan	1. Breakpoint: 4.7 mg/kg/d; upper 95% CI: 6.1 mg/kg/d

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

Detailed Findings Tables

Protein

Table G.9. Protein requirement estimates high RoB RCTs adults (19-50 years)

Study (PMID)	Statistics/Confounders	Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate
Tian, 2011[38] (21859657)	Statistics: 2-phase linear regression crossover model Confounders adjusted for: None	**Outcome: Protein requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 7) *Total (N analyzed): see note Protein amount: Intended: 0.70-1.10 g/kg/d; Actual: 0.79-1.17 g/kg/d	**Outcome: $F^{13}CO_2$** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (d 7) *Total (N analyzed): see note Protein amount: Intended: 0.70-1.10 g/kg/d; Actual: 0.79-1.17 g/kg/d 0.79 g/kg/d protein: 0.64; SD 0.25 umol/kg/h	**Outcome: Leucine oxidation** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (d 7) *Total (N analyzed): see note Protein amount: Intended: 0.70-1.10 g/kg/d; Actual: 0.79-1.17 g/kg/d 0.79 g/kg/d protein: 9.2; SD

Study (PMID)	Statistics/Confounders	Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate
		Breakpoint: 0.91 g/kg/d Upper 95% CI: 1.09 g/kg/d Lower 95% CI: NR **Outcome: Protein requirement estimate calculated from leucine oxidation** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 7) *Total (N analyzed): see note Protein amount: Intended: 0.70-1.10 g/kg/d; Actual: 0.79-1.17 g/kg/d Breakpoint: 0.92 g/kg/d Upper 95% CI: 1.10 g/kg/d Lower 95% CI: NR	0.91 g/kg/d protein: 0.77; SD 0.11 umol/kg/h 0.92 g/kg/d protein: 0.70; SD 0.24 umol/kg/h 0.99 g/kg/d protein: 0.72; SD 0.16 umol/kg/h 1.07 g/kg/d protein: 1.02; SD 0.27 umol/kg/h 1.17 g/kg/d protein: 1.03; SD 0.42 umol/kg/h	2.7 umol/kg/h 0.91 g/kg/d protein: 10.1; SD 4.9 umol/kg/h 0.92 g/kg/d protein: 11.6; SD 5.0 umol/kg/h 0.99 g/kg/d protein: 10.4; SD 4.8 umol/kg/h 1.07 g/kg/d protein: 13.1; SD 6.2 umol/kg/h 1.17 g/kg/d protein: 16.6; SD 6.3 umol/kg/h Comparisons and p-values Leucine oxidation was significantly affected by protein intake (p-value NR)

Abbreviations: CI = confidence interval; d = day; F13CO2 = rate of 13CO2 released from tracer oxidation [tracer; leucine]; g/kg/d = grams per kilogram per day; N = number; NR = not reported; PMID = PubMed Identification Number; SD = standard deviation; umol/kg/h = micromole per kilogram per hour
*N values for each outcome not provided. Initially 20 subjects were recruited and received 3 intakes each (60 total observations). However, it is unclear if this is the number of total participants and observations analyzed.

Table G.10. Protein requirement estimates high RoB non-RCTs adults (19-50 years)

Study (PMID)	Statistics/Confounders	Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate
Li, 2013[63] (23981551)	Statistics: Biphasic linear regression crossover model Confounders adjusted for: None	**Outcome: Protein requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint; SD, Safe intake level Timepoint: Data obtained following	**Outcome: $F^{13}CO_2$** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (d 6)

Study (PMID)	Statistics/Confounders	Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate
		each study d (d 6) Total (N analyzed): Male: 10 Female: 9; 95 total observations Protein amount: Intended: 0.75-1.05 g/kg/d Actual: Male: 0.76-1.02 g/kg/d; Female: 0.74-0.99 g/kg/d Male: Breakpoint: 0.88; SD 0.05 g/kg/d Safe intake level: 0.98 g/kg/d Female: Breakpoint: 0.85; SD 0.06 g/kg/d Safe intake level: 0.97 g/kg/d All participants: Breakpoint: 0.87 g/kg/d Safe intake level: 0.98 g/kg/d Comparisons and p-values There was no significant difference for the breakpoint and safe intake level for males and females.	Total (N analyzed): Male: 10 Female: 9; 95 total observations Protein amount: Intended: 0.75-1.05 g/kg/d Actual: Male: 0.76-1.02 g/kg/d; Female: 0.74-0.99 g/kg/d Male: 0.76 g/kg/d protein: 0.66; SD 0.15 umol/kg/h 0.83 g/kg/d protein: 0.64; SD 0.15 umol/kg/h 0.91 g/kg/d protein: 0.67; SD 0.22 umol/kg/h 0.97 g/kg/d protein: 1.10; SD 0.35 umol/kg/h 1.02 g/kg/d protein: 1.10; SD 0.25 umol/kg/h Female: 0.74 g/kg/d protein: 0.74; SD 0.15 umol/kg/h 0.79 g/kg/d protein: 0.71; SD 0.16 umol/kg/h 0.87 g/kg/d protein: 0.74; SD 0.15 umol/kg/h 0.95 g/kg/d protein: 1.04; SD 0.10 umol/kg/h 0.99 g/kg/d protein: 1.03; SD 0.29 umol/kg/h Comparisons and p-values No difference in breath $^{13}CO_2$ excretion was observed among subjects when 0.75-0.89 g/kg/d protein was consumed ($p>0.05$) but was significantly different among subjects when 0.89-1.05 g/kg/d protein was consumed ($p<0.05$).

Abbreviations: d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; leucine]; g/kg/d = grams per kilogram per day; N = number, PMID = PubMed Identification Number; SD = standard deviation

Table G.11. Protein requirement estimates high RoB RCTs adults (51->70 years)

Study (PMID)	Statistics/Confounders	Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate
Tang, 2014[56] (24429540)	Statistics: 4-parameter nonlinear mixed model Confounders adjusted for: None	**Outcome: Protein requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint (Lower 95%CI, Upper 95% CI), Adequate protein allowance (Lower 95%CI, Upper 95% CI) Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 6; 42 total observations Protein amount: 0.1-1.8 g/kg/d Assuming the data are normally distributed: Breakpoint: 0.85 (0.60, 1.09) g/kg/d Adequate protein allowance: 1.15 (0.77, 1.54) g/kg/d Log normal assumption model: Breakpoint: 0.96 (0.71, 1.22) g/kg/d Adequate protein allowance: 1.39 (0.84-1.93) g/kg/d Approximation RDA (1.24 times the EAR): 1.05 (0.75, 1.35) g/kg/d	**Outcome: $F^{13}CO_2$** Outcome parameter: NA Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 6; 42 total observations Protein amount: 0.1-1.8 g/kg/d Data reported in figures of original paper

Study (PMID)	Statistics/Confounders	Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate
Wu, 2023[60] (38073288)	Statistics: Nonlinear mixed-effects model Confounders: None	**Outcome: Protein requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI, RNI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): Male: 9 Female: 7; 106 total observations Protein amount: 0.1-1.8 g/kg/d Breakpoint: Male: 0.93 g/kg/d; 1.31 g/kg FFM/d; Female: 0.94 g/kg/d; 1.47 g/kg FFM/d; All participants: 0.94 g/kg/d Upper 95% CI: All participants: 1.13 g/kg/d Lower 95% CI: All participants: 0.75 g/kg/d RNI (EAR plus 1.96 SD): All participants: 1.36 g/kg/d RNI (CV of the protein EAR, 1.25 times EAR): All participants: 1.18 g/kg/d	**Outcome: $F^{13}CO_2$** Outcome parameter: NA Timepoint: Data obtained following each study d (d 3) Total (N analyzed): Male: 9 Female: 7; 106 total observations Protein amount: 0.1-1.8 g/kg/d Data reported in figures of original paper

Abbreviations: CI = confidence interval; CV = coefficient of variation; d = day; EAR = estimated average requirement; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation[tracer; phenylalanine]; FFM = fat free mass; g/kg/d = grams per kilogram per day; N = number; NA = not applicable; PMID = PubMed Identification Number; RDA = recommended dietary allowance; RNI = recommended nutrient intake; SD = standard deviation

Table G.12. Protein requirement estimates high RoB RCTs adults (19-50 years and 51->70 years)

Study (PMID)	Statistics/Confounders	Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate
Campbell, 2008[14] (18996869)	Statistics: Linear regression and inverse predication; Two-factor ANOVA and Student's t test; Three-factor, repeated measures ANOVA and Student's t test; one-sample t test Confounders adjusted for: None	**Outcome: Protein requirement estimate calculated from nitrogen balance** Outcome parameter: Mean; SD (Lower 95% CI, Upper 95% CI for the mean), 97.5th percentile	**Outcome: Nitrogen balance** Outcome parameter: Mean; SD Timepoint: Data obtained from d 14-17 of the 18-d trials

Study (PMID)	Statistics/Confounders	Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate
		Timepoint: Data obtained from d 14-17 of the 18-d trials Total (N analyzed): 40; 120 total observations Protein amount: Intended LPro: 0.50 g/kg/d, MPro: 0.75 g/kg/d, HPro: 1.0 g/kg/d YM: LPro: 0.51; SD 0.01 g/kg/d, MPro: 0.77; SD 0.02 g/kg/d, HPro: 1.02; SD 0.02 g/kg/d YW: LPro: 0.50; SD 0.04 g/kg/d, MPro: 0.74; SD 0.02 g/kg/d, HPro: 0.98; SD 0.02 g/kg/d OM: LPro: 0.51; SD 0.01 g/kg/d, MPro: 0.77; SD 0.02 g/kg/d, HPro: 1.01; SD 0.02 g/kg/d OW: LPro: 0.50; SD 0.02 g/kg/d, MPro: 0.76; SD 0.03 g/kg/d, HPro: 1.01; SD 0.03 g/kg/d YM: Protein requirement estimate: (N=10) Mean: 0.54; SD 0.15 (0.43, 0.64), 97.5th percentile: 0.83 g/kg/d; (N=11) Mean 0.73; SD 0.24 (0.57, 0.90), 97.5th percentile: 1.21 g/kg FFM/d YW: Protein requirement estimate: (N=11) Mean: 0.67; SD 0.12 (0.59, 0.74), 97.5th percentile: 0.89 g/kg/d (N=10) Mean: 0.92; SD 0.15 (0.81, 1.03), 97.5th percentile: 1.22 g/kg FFM/d	Total (N analyzed): YM: 11, YW: 12, OM: 8, OW: 11, All participants: 40; 126 total observations Protein amount: Intended LPro: 0.50 g/kg/d, MPro: 0.75 g/kg/d, HPro: 1.0 g/kg/d YM: LPro: 0.51; SD 0.01 g/kg/d, MPro: 0.77; SD 0.02 g/kg/d, HPro: 1.02; SD 0.02 g/kg/d YW: LPro: 0.50; SD 0.04 g/kg/d, MPro: 0.74; SD 0.02 g/kg/d, HPro: 0.98; SD 0.02 g/kg/d OM: LPro: 0.51; SD 0.01 g/kg/d, MPro: 0.77; SD 0.02 g/kg/d, HPro: 1.01; SD 0.02 g/kg/d OW: LPro: 0.50; SD 0.02 g/kg/d, MPro: 0.76; SD 0.03 g/kg/d, HPro: 1.01; SD 0.03 g/kg/d YM: LPro: -4; SD 13 mg/kg/d nitrogen MPro: 12; SD 18 mg/kg/d nitrogen HPro: 29; SD 19 mg/kg/d nitrogen YW: LPro: -8; SD 11 mg/kg/d nitrogen MPro: 11; SD 18 mg/kg/d nitrogen HPro: 30; SD 19 mg/kg/d nitrogen OM: LPro: -11; SD 9 mg/kg/d nitrogen MPro: 16; SD 10 mg/kg/d nitrogen HPro: 24; SD 18 mg/kg/d nitrogen OW: LPro: 3; SD 9 mg/kg/d nitrogen MPro: 9; SD 12 mg/kg/d nitrogen

Study (PMID)	Statistics/Confounders	Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate
		OM: Protein requirement estimate: (N=8) Mean: 0.65; SD 0.09 (0.57, 0.73), 97.5th percentile: 0.83 g/kg/d (N=8) Mean: 0.92; SD 0.14 (0.80, 1.04), 97.5th percentile: 1.20 g/kg FFM/d OW: Protein requirement estimate: (N=11) Mean: 0.53; SD 0.11 (0.45, 0.60), 97.5th percentile: 0.75 g/kg/d (N=11) Mean: 0.96; SD 0.20 (0.82, 1.09), 97.5th percentile: 1.36 g/kg FFM/d All younger participants: Protein requirement estimate: (N=21) Mean: 0.61; SD 0.14, 97.5th percentile 0.89 g/kg/d (N=21) Mean: 0.82; SD 0.22, 97.5th percentile 1.25 g/kg FFM/d All older participants: Protein requirement estimate: (N=19) Mean: 0.58; SD 0.12, 97.5th percentile 0.81 g/kg/d (N=19) Mean: 0.94; SD 0.18, 97.5th percentile 1.29 g/kg FFM/d All men participants: Protein requirement estimate: (N=18) Mean: 0.59; SD 0.14, 97.5th percentile 0.85 g/kg/d (N=19) Mean: 0.81; SD 0.22, 97.5th percentile 1.24 g/kg FFM/d All women participants: Protein requirement estimate: (N=22) Mean: 0.60; SD 0.13, 97.5th percentile 0.85 g/kg/d	HPro: 45; SD 10 mg/kg/d nitrogen Comparisons and p-values Nitrogen balance was significantly different from LPro to MPro to HPro for all groups (YM, YW, OM, OW) ($p<0.05$).

Study (PMID)	Statistics/Confounders	Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate
		(N=21) Mean: 0.94; SD 0.18, 97.5th percentile 1.29 g/kg FFM/d All participants: Protein requirement estimate: (N=40) Mean: 0.59; SD 0.13, 97.5th percentile: 0.85 g/kg/d (N=40) Mean: 0.88; SD 0.21, 97.5th percentile: 1.28 g/kg FFM/d Comparisons and p-values No significant difference in the protein requirement were observed between younger and older subjects (p = 0.565, 95% CI mean difference: -0.11, 0.06) or between men and women (p = 0.849, 95% CI mean difference: -0.08,0.09) when expressed on a body weight basis. There was a significant age-by-sex interaction (p=0.002) in which the mean protein requirement was lower for OW than OM but was no different between YW and YM. No significant differences in the protein requirement were observed when expressed on a fat-free mass basis for younger and older subjects (p=0.070), men and women (p=0.053), or age-by-sex interaction (p=0.253). There was no significant difference between the 97.5th percentile (adequate protein allowance) for all subjects compared to the current RDA.	

Abbreviations: ANOVA = analysis of variance; CI = confidence interval; d = day; FFM = fat free mass; g/kg/d = grams per kilogram per day; HPro = higher protein; LPro = lower protein; MPro = medium protein; N = number); OM = older men; OW = older women; RDA = recommended dietary allowance; SD = standard deviation; YM = younger men; YW = younger women

Lysine

Table G.13. Lysine requirement estimates high RoB RCTs adults (19-50 years)

Study (PMID)	Statistics/Confounders	Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate
Millward, 2000[65] (10871569)	Statistics: Two-way fixed effects MANOVA analyzed as within-subject, repeated measures; paired t-test Confounders adjusted for: None	**Outcome: Lysine requirement estimate calculated from the EAR of wheat protein** Outcome parameter: Mean; SD Timepoint: Data obtained following each 9 h infusion Total (N analyzed): 4 M, 2 F; 12 total observations Protein amount: Milk LPro: 2.2; SD 0.1% of energy from protein Milk HPro: 13.2; SD 0.6% of energy from protein Wheat LPro: 2.1; SD 0.1% of energy from protein Wheat HPro: 11.9; SD 0.4% of energy from protein Amino acid amount: Milk LPro: 10.4; SD 0.0 µmol/kg/h lysine Milk HPro: 60.7; SD 3.9 µmol/kg/h lysine Wheat LPro: 2.1; SD 0.2 µmol/kg/h lysine Wheat HPro: 12.5; SD 1.2 µmol/kg/h lysine *EAR for wheat protein: 0.89; SD 0.08 g/kg/d	**Outcome: $PPU_{nitrogen}$** Outcome parameter: Mean; SD Timepoint: Data obtained following each 9 h infusion Total (N analyzed): 6; 12 total observations Protein amount: Milk LPro: 2.2; SD 0.1% of energy from protein Milk HPro: 13.2; SD 0.6% of energy from protein Wheat LPro: 2.1; SD 0.1% of energy from protein Wheat HPro: 11.9; SD 0.4% of energy from protein Amino acid amount: Milk LPro: 10.4; SD 0.0 µmol/kg/h lysine Milk HPro: 60.7; SD 3.9 µmol/kg/h lysine Wheat LPro: 2.1; SD 0.2 µmol/kg/h lysine Wheat HPro: 12.5; SD 1.2 µmol/kg/h lysine $PPU_{nitrogen}$: Milk: 1.00; SD 0.09 fractional efficiency of nitrogen utilization

Study (PMID)	Statistics/Confounders	Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate
		*EAR for lysine: 23.2; SD 2.0 mg/kg/d	Wheat: 0.68; SD 0.06 fractional efficiency of nitrogen utilization Comparisons and p-values: The $PPU_{nitrogen}$ was significantly lower for wheat than for milk ($p \leq 0.0001$).
Millward, 2002[64] (12450900)	Statistics: ANOVA with repeated measures; simple t tests Confounders adjusted for: None	**Outcome: Lysine requirement estimate calculated from the EAR of wheat protein** Outcome parameter: Mean; SD Timepoint: Data obtained following each 9 h infusion (3 h premeal, 6 h postmeal) Total (N analyzed): 4 M, 1 F; 10 total observations Protein amount: Milk: 32.3; SD 4.5% of energy from protein Wheat: 26.7; SD 4.1% of energy from protein Amino acid amount: Milk: 55.8; SD 2.6 mg/kg lysine Wheat: 9.5; SD 1.2 mg/kg lysine *EAR for wheat protein: 0.98; SD 0.05 g/kg/d *EAR for lysine: 18.3; SD 1.0 g/kg/d	**Outcome: $PPU_{nitrogen}$** Outcome parameter: Mean; SD Timepoint: Data obtained following each 9 h infusion Total (N analyzed): 5; 10 total observations Protein amount: Milk: 32.3; SD 4.5% of energy from protein Wheat: 26.7; SD 4.1% of energy from protein Amino acid amount: Milk: 55.8; SD 2.6 mg/kg lysine Wheat: 9.5; SD 1.2 mg/kg lysine $PPU_{nitrogen}$: Milk: 0.93; SD 0.02 fractional efficiency of nitrogen utilization Wheat: 0.61; SD 0.03 fractional efficiency of nitrogen utilization Comparisons and p-values: The $PPU_{nitrogen}$ was significantly greater with the milk meal compared with the wheat meal ($p \leq 0.0001$).

Abbreviations: ANOVA = analysis of variance; EAR = estimated average requirement; F = female; g/kg/d = grams per kilogram per day; h = hour; HPro = higher protein; LPro = low protein M = male; MANOVA = multivariate analysis of variance; mg/kg/d = milligram per kilogram per day; N = number; PPU = postprandial protein utilization; SD = standard deviation

*Note: EAR for wheat (g/kg/d) = 0.6 x $PPU_{nitrogen}$ of wheat. 0.6 represents the current EAR for protein. EAR for lysine (mg/kg/d) = EAR for wheat x lysine content of wheat protein.

Methionine

Table G.14. Methionine requirement estimates high RoB RCTs adults (51->70 years)

Study (PMID)	Statistics/Confounders	Total Sulfur Amino Acid Requirement Estimate	Data Used to Calculate Total Sulfur Amino Acid Requirement Estimate
Paoletti, 2023[46] (37356549)	Statistics: Mixed-effect change-point regression model with subject as a random effect; overlap in 95% CI was calculated to determine sex differences. Confounders adjusted for: Methionine intake treated as a covariate	**Outcome: Total sulfur amino acid requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): Men: 7; Women 8; Total 15; 83 total observations Protein amount: 1.0 g/kg/d Amino acid amount: 5-40 mg/kg/d methionine, 0 mg/kg/d cysteine Men: Breakpoint: 26.2 mg/kg/d Upper 95% CI: 32.1 mg/kg/d Lower 95% CI: 20.3 mg/kg/d R^2_m = 0.39, R^2_c = 0.89, p<0.001 Mean requirement: 36.9; SEM 1.42 mg/kg FFM/d Upper 95% CI: 39.7 mg/kg FFM/d Lower 95% CI: 34.1 mg/kg FFM/d Women: Breakpoint: 17.1 mg/kg/d Upper 95% CI: 23.7 mg/kg/d Lower 95% CI: 10.5 mg/kg/d	**Outcome: $F^{13}CO_2$** Outcome parameter: Mean; SEM Timepoint: Data obtained following each study d (d 3) Total (N analyzed): Men: 7; Women 8; Total 15; 83 total observations Protein amount: 1.0 g/kg/d Amino acid amount: 5-40 mg/kg/d methionine, 0 mg/kg/d cysteine Men: 5 mg/kg/d methionine: 0.95; SEM 0.06 mmol/kg/h 10 mg/kg/d methionine: 0.86; SEM 0.05 mmol/kg/h 15 mg/kg/d methionine: 0.82; SEM 0.05 mmol/kg/h 19 mg/kg/d methionine: 0.73; SEM 0.07 mmol/kg/h 25 mg/kg/d methionine: 0.64; SEM 0.10 mmol/kg/h 35 mg/kg/d methionine: 0.63; SEM 0.06 mmol/kg/h 40 mg/kg/d methionine: 0.61; SEM 0.05 mmol/kg/h Women: 5 mg/kg/d methionine: 0.81; SEM 0.07 mmol/kg/h 10 mg/kg/d methionine: 0.77; SEM

Study (PMID)	Statistics/Confounders	Total Sulfur Amino Acid Requirement Estimate	Data Used to Calculate Total Sulfur Amino Acid Requirement Estimate
		R^2_m = 0.22, R^2_c = 0.79, p<0.001 Mean requirement: 25.6; SEM 0.90 mg/kg FFM/d Upper 95% CI: 27.4 mg/kg FFM/d Lower 95% CI: 23.8 mg/kg FFM/d Comparisons and p-values Men had a significantly higher mean total sulfur amino acid requirement estimate than women on a body weight (difference in CI: 9.1; SEM 8.85, p value NR) and FFM basis (p=0.0005).	0.07 mmol/kg/h 15 mg/kg/d methionine: 0.70; SEM 0.06 mmol/kg/h 19 mg/kg/d methionine: 0.65; SEM 0.03 mmol/kg/h 25 mg/kg/d methionine: 0.63; SEM 0.05 mmol/kg/h 35 mg/kg/d methionine: 0.60; SEM 0.08 mmol/kg/h 40 mg/kg/d methionine: 0.66; SEM 0.04 mmol/kg/h

Abbreviations: CI = confidence interval; d = day; FFM = fat free mass; F13CO2 = rate of 13CO2 released 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; SEM = standard error of the mean

Total Branched Chain Amino Acids

Table G.15. Total branched chain amino acids requirement estimates high RoB RCTs adults (19-50 years)

Study (PMID)	Statistics/Confounders	Total Branched Chain Amino Acids Requirement Estimate	Data Used to Calculate Total Branched Chain Amino Acids Requirement Estimate	Data Used to Calculate Total Branched Chain Amino Acids Requirement Estimate	Data Used to Calculate Total Branched Chain Amino Acids Requirement Estimate
Riazi, 2003[52] (12730426)	Statistics: Two-phase linear regression crossover model; Three-factor general linear model; ANOVA with repeated measures followed by Student-Newman-Keuls post hoc test Confounders adjusted for: None	**Outcome: Total branched chain amino acid requirement estimate calculated from $F^{13}CO_2$ (with all intake levels)** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3)	**Outcome: $F^{13}CO_2$** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 7; 53 total observations Protein amount: 1.0 g/kg/d	**Outcome: Phenylalanine oxidation** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 7; 52 total observations Protein amount: 1.0	**Outcome: 9-h Phenylalanine balance** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 7; 52 total observations Protein amount: 1.0 g/kg/d

Study (PMID)	Statistics/Confounders	Total Branched Chain Amino Acids Requirement Estimate	Data Used to Calculate Total Branched Chain Amino Acids Requirement Estimate	Data Used to Calculate Total Branched Chain Amino Acids Requirement Estimate	Data Used to Calculate Total Branched Chain Amino Acids Requirement Estimate
		Total (N analyzed): 7; 53 total observations Protein amount: 1.0 g/kg/d Amino acid amount: 34-180 mg/kg/d total BCAA Breakpoint: 144; SE 32.6 mg/kg/d Upper 95% CI: 209.9 mg/kg/d Lower 95% CI: 78.6 mg/kg/d $r^2 = 0.267$, $p = 0.0004$ **Outcome: Total branched chain amino acid requirement estimate calculated from $F^{13}CO_2$ (without 180 mg/kg/d level)** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 7; 50 total observations Protein amount: 1.0 g/kg/d	Amino acid amount: 34-180 mg/kg/d total BCAA 34 mg/kg/d total BCAA: 0.694; SD 0.14 umol/kg/h 50 mg/kg/d total BCAA: 0.631; SD 0.14 umol/kg/h 66 mg/kg/d total BCAA: 0.616; SD 0.16 umol/kg/h 80 mg/kg/d total BCAA: 0.620; SD 0.15 umol/kg/h 100 mg/kg/d total BCAA: 0.589; SD 0.15 umol/kg/h 120 mg/kg/d total BCAA: 0.492; SD 0.17 umol/kg/h 140 mg/kg/d total BCAA: 0.489; SD 0.05 umol/kg/h 160 mg/kg/d total BCAA: 0.485; SD 0.06 umol/kg/h 180 mg/kg/d total BCAA: 0.424; SD 0.15 umol/kg/h Comparisons and p-values Rate of $^{13}CO_2$ release varied significantly by subject ($P<0.05$) but was not affected by order of intake.	g/kg/d Amino acid amount: 34-180 mg/kg/d total BCAA 34 mg/kg/d total BCAA: 5.9; SD 1.1 umol/kg/h 50 mg/kg/d total BCAA: 5.05; SD 1.6 umol/kg/h 66 mg/kg/d total BCAA: 4.9; SD 1.7 umol/kg/h 80 mg/kg/d total BCAA: 4.1; SD 1.5 umol/kg/h 100 mg/kg/d total BCAA: 3.9; SD 1.5 umol/kg/h 120 mg/kg/d total BCAA: 3.7; SD 1.8 umol/kg/h 140 mg/kg/d total BCAA: 3.1; SD 0.4 umol/kg/h 160 mg/kg/d total BCAA: 3.5; SD 1.3 umol/kg/h 180 mg/kg/d total BCAA: 2.9; SD 0.6 umol/kg/h Comparisons and p-values Phenylalanine oxidation was not different among subjects ($p = 0.059$) but was significantly affected by total BCAA intake ($p=0.01$).	Amino acid amount: 34-180 mg/kg/d total BCAA 34 mg/kg/d total BCAA: 12.2; SD 10.2 umol/kg/h 50 mg/kg/d total BCAA: 19.5; SD 14.2 umol/kg/h 66 mg/kg/d total BCAA: 21.1; SD 14.9 umol/kg/h 80 mg/kg/d total BCAA: 28.0; SD 12.4 umol/kg/h 100 mg/kg/d total BCAA: 27.8; SD 10.8 umol/kg/h 120 mg/kg/d total BCAA: 32.1; SD 15.9 umol/kg/h 140 mg/kg/d total BCAA: 37.2; SD 3.9 umol/kg/h 160 mg/kg/d total BCAA: 34.6; SD 12.3 umol/kg/h 180 mg/kg/d total BCAA: 38.8; SD 5.1 umol/kg/h Comparisons and p-values Phenylalanine balance was significantly affected by total BCAA intake (p-value NR).

Study (PMID)	Statistics/Confounders	Total Branched Chain Amino Acids Requirement Estimate	Data Used to Calculate Total Branched Chain Amino Acids Requirement Estimate	Data Used to Calculate Total Branched Chain Amino Acids Requirement Estimate	Data Used to Calculate Total Branched Chain Amino Acids Requirement Estimate
		Amino acid amount: 34-180 mg/kg/d total BCAA Breakpoint: 136.6; SE 32.3 mg/kg/d Upper 95% CI: 201.5 mg/kg/d Lower 95% CI: 71.7 mg/kg/d $r^2 = 0.237$, $p = 0.0017$ **Outcome: Total branched chain amino acid requirement estimate calculated from phenylalanine oxidation (with all intake levels)** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 7; 52 total observations Protein amount: 1.0 g/kg/d Amino acid amount: 34-180 mg/kg/d total BCAA Breakpoint: 125.7; SE 22.4 mg/kg/d			

Study (PMID)	Statistics/Confounders	Total Branched Chain Amino Acids Requirement Estimate	Data Used to Calculate Total Branched Chain Amino Acids Requirement Estimate	Data Used to Calculate Total Branched Chain Amino Acids Requirement Estimate	Data Used to Calculate Total Branched Chain Amino Acids Requirement Estimate
		Upper 95% CI: 170.7 mg/kg/d Lower 95% CI: 80.6 mg/kg/d $r^2 = 0.299$, $p = 0.0002$ **Outcome: Total branched chain amino acid requirement estimate calculated from phenylalanine oxidation (without 180 mg/kg/d level)** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 7; 49 total observations Protein amount: 1.0 g/kg/d Amino acid amount: 34-180 mg/kg/d total BCAA Breakpoint: 121.7; SE 23.3 mg/kg/d Upper 95% CI: 168.5 mg/kg/d Lower 95% CI: 78.8 mg/kg/d			

Study (PMID)	Statistics/Confounders	Total Branched Chain Amino Acids Requirement Estimate	Data Used to Calculate Total Branched Chain Amino Acids Requirement Estimate	Data Used to Calculate Total Branched Chain Amino Acids Requirement Estimate	Data Used to Calculate Total Branched Chain Amino Acids Requirement Estimate
		r^2 = 0.275, p = 0.0007 **Outcome: Total branched chain amino acid requirement estimate calculated from phenylalanine balance (with all intake levels)** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 7; 52 total observations Protein amount: 1.0 g/kg/d Amino acid amount: 34-180 mg/kg/d total BCAA Breakpoint: 138; SE 22.7 mg/kg/d Upper 95% CI: 183.5 mg/kg/d Lower 95% CI: 92.5 mg/kg/d r^2 = 0.401, p = <0.0001 **Outcome: Total branched chain amino acid requirement**			

Study (PMID)	Statistics/Confounders	Total Branched Chain Amino Acids Requirement Estimate	Data Used to Calculate Total Branched Chain Amino Acids Requirement Estimate	Data Used to Calculate Total Branched Chain Amino Acids Requirement Estimate	Data Used to Calculate Total Branched Chain Amino Acids Requirement Estimate
		estimate calculated from phenylalanine balance (without 180 mg/kg/d level) Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 7; 49 total observations Protein amount: 1.0 g/kg/d Amino acid amount: 34-180 mg/kg/d total BCAA Breakpoint: 135.8; SE 23.4 mg/kg/d Upper 95% CI: 182.7 mg/kg/d Lower 95% CI: 88.9 mg/kg/d $r^2 = 0.373$, $p = <0.0001$			

Abbreviations: ANOVA = analysis of variance; BCAA = branched chain amino acids; CI = confidence interval; d = day; F13CO2 = rate of 13CO2 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; SD = standard deviation; SE = standard error; umol/kg/h = micromole per kilogram per hour

Tryptophan

Table G.16. Tryptophan requirement estimates high RoB RCTs children and adolescents

Study (PMID)	Statistics/Confounders	Tryptophan Requirement Estimate	Data Used to Calculate Tryptophan Requirement Estimate
Al-Mokbel, 2019[13] (30753549)	Statistics: Nonlinear mixed-effects model with observations within subjects regarded as statistically dependent; Mixed linear model with the subject as a random variable Confounders adjusted for: None	**Outcome: Tryptophan requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 7; 36 total observations Protein amount: 1.5 g/kg/d Amino acid amount: 0.5-10 mg/kg/d tryptophan Breakpoint: 4.7 mg/kg/d Upper 95% CI: 6.1 mg/kg/d Lower 95% CI: 3.3 mg/kg/d $r^2 = 0.42$	**Outcome: $F^{13}CO_2$** Outcome parameter: NA Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 7; 36 total observations Protein amount: 1.5 g/kg/d Amino acid amount: 0.5-10 mg/kg/d tryptophan Data reported in figures of original paper

Abbreviations: CI = confidence interval; d = day; F13CO2 = rate of 13CO2 released from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilograms per day; mg/kg/d = milligram per kilogram per day; N = number

Appendix H. Findings of Studies in the Analytic Set

Protein

Table H.1. Protein requirement estimates RCTs children and adolescents

Study (PMID)	Statistics/Confounders	Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate
Elango, 201120 (22049165)	Statistics: 2-phase linear regression crossover model; Mixed linear model with the subject as a random variable. Confounders adjusted for: None	Outcome: Protein requirement estimate calculated from phenylalanine oxidation Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 7; 56 total observations Protein amount: 0.1-2.56 g/kg/d Breakpoint: 1.25 g/kg/d Upper 95% CI: 1.5 g/kg/d Lower 95% CI: NR r2 = 0.75 Outcome: Protein requirement estimate calculated from F13CO2 Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3)	Outcome: Phenylalanine oxidation Outcome parameter: NA Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 7; 56 total observations Protein amount: 0.1-2.56 g/kg/d Data reported in figures of original paper	Outcome: F13CO2 Outcome parameter: NA Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 7; 56 total observations Protein amount: 0.1-2.56 g/kg/d Data reported in figures of original paper

Study (PMID)	Statistics/Confounders	Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate
		Total (N analyzed): 7; 56 total observations Protein amount: 0.1-2.56 g/kg/d Breakpoint: 1.3 g/kg/d Upper 95% CI: 1.55 g/kg/d Lower 95% CI: NR r2 = 0.85		

Abbreviations: BMI = body mass index; 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; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; SE = standard error

Table H.2. Protein requirement estimates RCTs pregnant people

Study (PMID)	Statistics/Confounders	Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate
Stephens, 2015[54] (25527661)	Statistics: 2-phase linear regression crossover model with the use of mixed models to account for repeated measures within subject, as well as missing observations Confounders adjusted for: None	**Outcome: Protein requirement estimate calculated from $F^{13}CO_2$ (Early Gestation)** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 17; 35 total observations Protein amount: 0.22-2.56 g/kg/d Breakpoint: 1.22 g/kg/d Upper 95% CI: 1.66 g/kg/d Lower 95% CI: 0.79 g/kg/d r^2 = 0.60 **Outcome: Protein requirement estimate calculated from $F^{13}CO_2$ (Late Gestation)**	**Outcome: $F^{13}CO_2$ (Early Gestation)** Outcome parameter: NA Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 17; 35 total observations Protein amount: 0.22-2.56 g/kg/d Data reported in figures of original paper **Outcome: $F^{13}CO_2$ (Late Gestation)** Outcome parameter: NA Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 19; 43 total observations

Study (PMID)	Statistics/Confounders	Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate
		Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 19; 43 total observations Protein amount: 0.22-2.56 g/kg/d Breakpoint: 1.52 g/kg/d Upper 95% CI: 1.77 g/kg/d Lower 95% CI: 1.28 g/kg/d $r^2 = 0.63$	Protein amount: 0.22-2.56 g/kg/d Data reported in figures of original paper

Abbreviations: BMI = body mass index; 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; N = number; NA = not applicable; PMID = PubMed Identification Number

Table H.3. Protein requirement estimates RCTs adults (19-50 years)

Study (PMID)	Statistics/Confounders	Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate
Humayun, 2007[27] (17921376)	Statistics: Bi-phase linear regression cross over model; repeated measures ANOVA Confounders adjusted for: None	**Outcome: Protein requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 8; 56 total observations Protein amount: 0.1-1.8 g/kg/d Breakpoint: 0.93 g/kg/d Upper 95% CI: 1.24 g/kg/d Lower 95% CI: NR	**Outcome: $F^{13}CO_2$** Outcome parameter: NA Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 8; 56 total observations Protein amount: 0.1-1.8 g/kg/d Data reported in figures of original paper

Abbreviations: ANOVA = analysis of variance; 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; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number

Table H.4. Protein requirement estimates RCTs adults (51->70 years)

Study (PMID)	Statistics/Confounders	Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate
Mao, 2020[40] (32140711)	Statistics: Nonlinear mixed-effects model Confounders adjusted for: None	**Outcome: Protein requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 14; 80 total observations Protein amount: 0.3-1.8 g/kg/d Breakpoint: 0.91 g/kg/d (95% CI: 0.76, 1.06 g/kg/d) Upper 95% CI: 1.17 g/kg/d (95% CI: 0.89, 1.45 g/kg/d) Lower 95% CI: NR	**Outcome: $F^{13}CO_2$** Outcome parameter: NA Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 14; 80 total observations Protein amount: 0.3-1.8 g/kg/d Data reported in figures of original paper
Morse, 2001[44] (11682582)	Statistics: Linear regression; paired t tests (two sided): Within-trial comparisons; Repeated measures ANOVA: among-trial comparisons Confounders adjusted for: None	**Outcome: Protein requirement estimate calculated from nitrogen balance** Outcome parameter: Mean protein requirement; SD, protein allowance Timepoint: 2 wk Total (N analyzed): 11; 33 total observations Protein amount: I: LPro: 0.5 g/kg/d, MPro: 0.75 g/kg/d, HPro: 1.0 g/kg/d A: LPro: 0.53; SE 0.02 g/kg/d, MPro: 0.76; SE 0.02 g/kg/d, HPro: 1.06; SE 0.05 g/kg/d Mean protein requirement: 0.7; SD 0.1 g/kg/d	**Outcome: Nitrogen balance** Outcome parameter: Mean; SE Timepoint: 2 wk Total (N analyzed): 11 Protein amount: I: LPro: 0.5 g/kg/d, MPro: 0.75 g/kg/d, HPro: 1.0 g/kg/d A: LPro: 0.53; SE 0.02 g/kg/d, MPro: 0.76; SE 0.02 g/kg/d, HPro: 1.06; SE 0.05 g/kg/d LPro: -14.5; SE 3.1 mg/kg/d nitrogen MPro: 3.8; SE 2.5 mg/kg/d nitrogen HPro: 23.4; SE 3.3 mg/kg/d nitrogen Timepoint: 3 wk

Study (PMID)	Statistics/Confounders	Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate
		Protein allowance: 0.90 g/kg/d Timepoint: 3 wk Total (N analyzed): 11; 33 total observations Protein amount: LPro: 0.53; SE 0.02 g/kg/d, MPro: 0.76; SE 0.02 g/kg/d, HPro: 1.06; SE 0.05 g/kg/d Mean protein requirement: 0.56; SD 0.10 g/kg/d Protein allowance: 0.76 g/kg/d Comparisons and p-values The mean protein requirement at wk 2 was significantly different than wk 3 ($p<0.0004$). The mean protein requirement at wk 2 was also significantly different than the current assumed mean protein requirement of 0.6g/kg/d ($p<0.005$).	Total (N analyzed): 11 Protein amount: LPro: 0.53; SE 0.02 g/kg/d, MPro: 0.76; SE 0.02 g/kg/d, HPro: 1.06; SE 0.05 g/kg/d LPro: 0.1; SE 2.7 mg/kg/d nitrogen MPro: 8.5; SE 3.6 mg/kg/d nitrogen HPro: 42; SE 3 mg/kg/d nitrogen Comparisons and p-values Net nitrogen balance became more positive with increased protein intake. Shift toward a more positive nitrogen balance from week 2 and week 3 apparent within trials and due to difference in urinary nitrogen excretion which was statistically different for LPro ($p<0.002$) and HPro ($p<0.002$).
Rafii, 2015[49] (25320185)	Statistics: Nonlinear mixed model; observations within subjects were regarded as statistically dependent; Mixed linear model with subject as a random variable Confounders adjusted for: None	**Outcome: Protein requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (n analyzed): 12; 83 total observations Protein amount: 0.2-2.0 g/kg/d Breakpoint: 0.96 g/kg/d Upper 95% CI: 1.29 g/kg/d Lower 95% CI: 0.65 g/kg/d	**Outcome: $F^{13}CO_2$** Outcome parameter: NA Timepoint: Data obtained following each study d (d 3) Total (n analyzed): 12; 83 total observations Protein amount: 0.2-2.0 g/kg/d Data reported in figures of original paper

Study (PMID)	Statistics/Confounders	Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate
		r^2 = 0.58	
Rafii, 2015[50] (26962173)	Statistics: Nonlinear mixed-effects model; mixed linear model with subject as a random variable Confounders adjusted for: None	**Outcome: Protein requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 6; 42 total observations Protein amount: 0.2-2.0 g/kg/d Breakpoint: 0.94 g/kg/d Upper 95% CI: 1.24 g/kg/d Lower 95% CI: 0.64 g/kg/d r^2 = 0.64, SE = 0.11	**Outcome: $F^{13}CO_2$** Outcome parameter: NA Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 6; 42 total observations Protein amount: 0.2-2.0 g/kg/d Data reported in figures of original paper

Abbreviations: A= actual intake; ANOVA = analysis of variance; CI = confidence interval; d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; F_n = daily fecal nitrogen excretions; g/kg/d = grams per kilogram per day; HPro = high protein; I = intended intake; LPro = low protein; M_n = daily miscellaneous nitrogen excretions; MPro = medium protein; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; SE = standard Error; U_n is daily urinary nitrogen excretion; wk = week

Table H.5. Protein requirement estimates non-RCTs adults (19-50 years)

Study (PMID)	Statistics/Confounders	Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate
Atinmo, 2010[61] (NA)	Statistics: Linear regression; Two-way ANOVA with subjects as blocks; student t-test Confounders adjusted for: None	**Outcome: Protein requirement estimate calculated from nitrogen balance (Northern Nigeria Arm)** Outcome parameter: Mean maintenance requirement; SD Timepoint: Days 5-10 Total (N analyzed): 7; 28 total observations Protein amount: 0.4-0.9 g/kg/d	**Outcome: Nitrogen balance (Northern Nigeria Arm)** Outcome parameter: Mean; SD Timepoint: Days 5-10 Total (N analyzed): 7 per intake level Protein amount: 0.4-0.9 g/kg/d 0.4 g/kg/d protein: -21.04; SD 7.07 mg/kg/d nitrogen

Study (PMID)	Statistics/Confounders	Protein Requirement Estimate	Data Used to Calculate Protein Requirement Estimate
		Mean maintenance requirement: 108.01 mg/kg/d nitrogen SD: 9.45 mg/kg/d nitrogen **Outcome: Protein requirement estimate calculated from nitrogen balance (South Eastern Nigeria Arm)** Outcome parameter: Mean maintenance requirement; SD Timepoint: Days 5-10 Total (N analyzed): 11; 44 total observations Protein amount: 0.4-0.9 g/kg/d Mean maintenance requirement: 110.82 mg/kg/d nitrogen SD: 14.53 mg/kg/d nitrogen Comparisons and p-values The overall (both arms combined) mean maintenance requirement was 109.725 mg/kg/d nitrogen (0.686 g/kg/d protein) with an SD of 12.56 mg/kg/d and a protein allowance of 0.843 g/kg/d.	0.7 g/kg/d protein: 2.85; SD 8.54 mg/kg/d nitrogen 0.8 g/kg/d: 14.90; SD 10.22 mg/kg/d nitrogen 0.9 g/kg/d: 30.57; SD 14.02 mg/kg/d nitrogen Comparisons and p-values 0.9 g/kg/d protein intake had significantly different nitrogen balance from all other levels of protein intake ($p<0.05$) **Outcome: Nitrogen balance (South Eastern Nigeria Arm)** Outcome parameter: Mean; SD Timepoint: Days 5-10 Total (N analyzed): 11 per intake level Protein amount: 0.4-0.9 g/kg/d 0.4 g/kg/d protein: -28.33; SD 11.02 mg/kg/d nitrogen 0.7 g/kg/d protein: -11.64; SD 9.86 mg/kg/d nitrogen 0.8 g/kg/d: 7.04; SD 11.34 mg/kg/d nitrogen 0.9 g/kg/d: 22.20; SD 13.01 mg/kg/d nitrogen Comparisons and p-values 0.9 g/kg/d protein intake had significantly different nitrogen balance from all other levels of protein intake ($p<0.05$)

Abbreviations: ANOVA = analysis of variance; g/kg/d = grams per kilogram per day; mg nitrogen/kg/d = milligrams nitrogen per kilogram per day; N = number; PMID = PubMed Identification Number; SD = standard deviation

Table H.6. Protein requirement not calculated; nitrogen balance and leucine oxidation RCTs adults (19-50 and 51->70 years)

Study (PMID)	Statistics/Confounders	Nitrogen Balance	Leucine Oxidation
Walrand, 2008[58] (18697911)	Statistics: Repeated-measures ANOVA Confounders adjusted for: None	**Outcome: Nitrogen balance** Outcome parameter: Mean; SE Timepoint: Days 9-11 Total (N analyzed): 10 (younger); 9 (older) Protein amount: 1.5 g/kg FFM/d (usual protein); 3.0 g/kg FFM/d Younger (usual protein): 2.77; SE 0.11 g/kg FFM/d Younger (high protein): 5.42; SE 0.45 g/kg FFM/d Older (usual protein): 2.48; SE 0.12 g/kg FFM/d Older (high protein): 5.32; SE 0.18 g/kg FFM/d Comparisons and p-values Nitrogen balance was significantly higher on the high protein compared to usual protein diet in younger adults ($p<0.05$). Nitrogen balance was significantly higher on the high protein compared to usual protein diet in older adults ($p<0.05$) There was no difference in nitrogen balance between younger and older adults regardless of diet.	**Outcome: Leucine oxidation** Outcome parameter: Mean; SE Timepoint: Day 11 Total (N analyzed): 10 (younger); 9 (older) Protein amount: 1.5 g/kg FFM/d (usual protein); 3.0 g/kg FFM/d Younger (usual protein): 27.4; SE 1.8 μmol/kg FFM/min Younger (high protein): 38.2; SE 2.1 μmol/kg FFM/min Older (usual protein): 31.2; SE 1.5 μmol/kg FFM/min Older (high protein): 37.3; SE 1.3 μmol/kg FFM/min Comparisons and p-values Leucine oxidation was significantly higher on the high protein compared to usual protein diet in younger adults ($p<0.05$) Leucine oxidation was significantly higher on the high protein compared to usual protein diet in younger adults ($p<0.05$) Younger adults showed a greater increase from usual protein to high protein (39%) than older (20%) adults.

Abbreviations: FFM/d = fat-free mass per day; g/kg = grams per kilogram; N = number; NR = not reported; PMID = PubMed Identification Number; SE = standard error; wk = week

Table H.7. Protein requirement not calculated; growth outcomes non-RCTs infants

Study (PMID)	Statistics/Confounders	Length-for-age z score	Conditional Length-for-age z score
Kittisakmontri, 2022[68] (36235599)	Statistics: Simple linear regression; bivariate correlation and general linear models Confounders adjusted for: Conditional growth was also adjusted for baseline size at 6 mo; Linear growth: prenatal diagnosis, paternal height, non-protein energy, calcium intake, zinc intake, type of milk, maternal smoking, maternal education, family income, working mom, protein, duration of breastfeeding, infection, birth length, sex, maternal height;	Outcome: Length-for-age z score Outcome parameter: LAZ Timepoint: 6, 9 and 12 mo Total (N analyzed): 36 (HPro), 73 (MPro), 36 (LPro) Protein amount: High Pro: ≥12.9% of energy from protein; Median Pro: 11-12.8% of energy from protein; Low Pro: ≤10.9% of energy from protein HPro: 6 mo: -0.15 LAZ 9 mo: -0.17 LAZ 12 mo: -0.19 LAZ MPro 6 mo: -0.55 LAZ 9 mo: -0.48 LAZ 12 mo: -0.55 LAZ LPro: 6 mo: -0.66 LAZ 9 mo: -0.69 LAZ 12 mo: -0.64 LAZ Comparisons and p-values Mean difference (lower and upper 95% CI) is shown HPro vs MPro: 6 mo: -0.4 (95% CI: -0.05, 0.84) 9 mo: 0.32 (95% CI: -0.14, 0.77) 12 mo: 0.35 (95% CI: -0.09, 0.8) No difference between groups at any timepoint.	Outcome: Conditional length-for-age z score Outcome parameter: conditional LAZ mean; SD (lower and upper 95% CI) Timepoint: 6-12 mo Total (N analyzed): 145 Protein amount: HPro: ≥12.9% of energy from protein; MPro: 11-12.8% of energy from protein; LPro: ≤10.9% of energy from protein Conditional LAZ at 12 mo: HPro: 0.07; SD 1.18 (95% CI: -0.33, 0.47) MPro: -0.01; SD 0.82 (95% CI: -0.20, 0.18) LPro: -0.04; SD 1.14 (95% CI: -0.42, 0.35) Comparisons and p-values No significant difference in conditional growth status at 12 mo Pearson's correlation between protein intake at 6-9 mo (12.6% average protein intake) and 9-12 mo (15.6% average protein intake) and conditional LAZ do not show a significant difference (6-9 mo: r = 0.09, p = 0.26; 9-12 mo: r = 0.07, p = 0.39)

Study (PMID)	Statistics/Confounders	Length-for-age z score	Conditional Length-for-age z score
		HPro vs LPro: 6 mo: 0.5 (95% CI: -0.01, 1.01) 9 mo: 0.52 (95% CI: -0.01, 1.05) 12 mo: 0.45 (95% CI: -0.07, 0.96) No difference between groups at any timepoint. MPro vs LPro: 6 mo: 0.11 (95% CI: -0.34, 0.55) 9 mo: 0.2 (95% CI: -0.24, 0.65) 12 mo: 0.1 (95% CI: -0.35, 0.54) No difference between groups at any timepoint.	

Abbreviations: CI = confidence interval; g/kg/d = grams per kilogram per day; HPro = high protein; LAZ = length-for-age z score; LPro = low protein; mo = month; MPro = median protein; N = number; PMID = PubMed Identification Number; vs = versus

Isoleucine

Table H.8. Isoleucine requirement estimates RCTs infants

Study (PMID)	Statistics/Confounders	Isoleucine Requirement Estimate	Data Used to Calculate Isoleucine Requirement Estimate
de Groof, 2014[2] (24284437)	Statistics: 2-phase regression model Confounders adjusted for: None	**Outcome: Isoleucine requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 2) Total (N analyzed): 22 Protein amount: average 2.96; SD 0.15 g/kg/d Amino Acid amount: 5-216 mg/kg/d isoleucine Breakpoint: 105 mg/kg/d Upper 95% CI: 150 mg/kg/d Lower 95% CI: 60 mg/kg/d $r^2 = 0.61$, $p<0.001$	**Outcome: $F^{13}CO_2$** Outcome parameter: NA Timepoint: Data obtained following each study d (d 2) Total (N analyzed): 22 Protein amount: 2.96; SD 0.15 g/kg/d Amino Acid amount: 5-216 mg/kg/d isoleucine Data reported in figures of original paper

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 = milligrams per kilogram per day; N = number; NA = not applicable; PMID = PubMed Identification; SD = standard deviation

Leucine

Table H.9. Leucine requirement estimates RCTs infants

Study (PMID)	Statistics/Confounders	Leucine Requirement Estimate	Data Used to Calculate Leucine Requirement Estimate
de Groof, 2014[2] (24284437)	Statistics: 2-phase regression model Confounders adjusted for: None	**Outcome: Leucine requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 2) Total (N analyzed): 33 Protein amount: 2.98; SD 0.2 g/kg/d Amino acid amount: 5-370 mg/kg/d leucine Breakpoint: 140 mg/kg/d Upper 95% CI: 245 mg/kg/d Lower 95% CI: 35 mg/kg/d r^2 = 0.26, p<0.01	**Outcome: $F^{13}CO_2$** Outcome parameter: NA Timepoint: Data obtained following each study d (d 2) Total (N analyzed): 33 Protein amount: 2.96; SD 0.2 g/kg/d Amino acid amount: 5-370 mg/kg/d leucine Data reported in figures of original paper

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 = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification; SD = standard deviation

Table H.10. Leucine requirement estimates RCTs adults (19-50 years)

Study (PMID)	Statistics/Confounders	Leucine Requirement Estimate	Data Used to Calculate Leucine Requirement Estimate	Data Used to Calculate Leucine Requirement Estimate
Kurpad, 2001[30] (11722955)	Statistics: Mixed-models linear regression model; Mixed-models ANOVA Confounders: Leucine intake as a continuous covariate in the regression model	**Outcome: Leucine requirement estimate calculated from 24-h leucine balance** Outcome parameter: Zero balance intercept, Upper 95% CI, Lower 95% CI Timepoint: Data obtained	**Outcome: 24-h Leucine balance** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (d 7) Total (N analyzed) 10 per	**Outcome 24-h Nitrogen balance** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (d 7) Total (N analyzed) 9 per

Study (PMID)	Statistics/Confounders	Leucine Requirement Estimate	Data Used to Calculate Leucine Requirement Estimate	Data Used to Calculate Leucine Requirement Estimate
		following each study d (d 7) Total (N analyzed): 20; 40 total observations Protein amount: 1.0 g/kg/d protein (160 mg/kg/d nitrogen) Amino Acid amount: 14-40 mg/kg/d leucine Zero balance intercept: 37.3 mg/kg/d Upper 95% CI: 50 mg/kg/d Lower 95% CI: 32 mg/kg/d p<0.0001 for the intercept **Outcome: Leucine requirement estimate calculated from 24-h nitrogen balance** Outcome parameter: Zero balance intercept, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 7) Total (N analyzed): 18; 36 total observations Protein amount: 1.0 g protein/kg/d (160 mg nitrogen/kg/d) Amino Acid amount: 14-40 mg/kg/d leucine Zero balance intercept: 37.6 mg/kg/d	intake level Protein amount: 1.0 g protein/kg/d (160 mg nitrogen/kg/d) Amino Acid amount: 14-40 mg/kg/d leucine 14 mg/kg/d leucine: -16.5; SD 10 mg/kg/d 22 mg/kg/d leucine: -9; SD 9.4 mg/kg/d 30 mg/kg/d leucine: -3.3; SD 9.8 mg/kg/d 40 mg/kg/d leucine: 0.5; SD 10.2 mg/kg/d Comparisons and p-values Overall, daily leucine balance differed significantly between the 4 leucine intakes (p=0.002). Leucine balance at 14 and 22 mg/kg/d were significantly different from zero (p<0.05 for both). Leucine balance at 30 and 40 mg/kg/d were significantly different from 14 mg/kg/d (p<0.05 for both).	intake level Protein amount: 1.0 g protein/kg/d (160 mg nitrogen/kg/d) Amino Acid amount: 14-40 mg/kg/d leucine 14 mg/kg/d leucine: -12.7; SD 14.8 mg/kg/d 22 mg/kg/d leucine: -17.9; SD 18.9 mg/kg/d 30 mg/kg/d leucine: -3.9; SD 26.3 mg/kg/d 40 mg/kg/d leucine: 1; SD 22.3 mg/kg/d Comparisons and p-values Overall, there was a tendency of an effect of leucine intake on nitrogen balance (p=0.060). Nitrogen balance at 14 mg/kg/d tended to be different than zero (p=0.080) Nitrogen balance at 22 mg/kg/d was significantly different than zero (p=0.017)

Study (PMID)	Statistics/Confounders	Leucine Requirement Estimate	Data Used to Calculate Leucine Requirement Estimate	Data Used to Calculate Leucine Requirement Estimate
		Upper 95% CI: ND Lower 95% CI: ND p=0.002 for the intercept		

Abbreviations: CI = confidence interval; d = day; g/kg/d = grams per kilogram per day; mg/kg/d = milligrams per kilogram per day; N = number; ND = not determined; NR = not reported; PMID = PubMed Identification; SD = standard deviation

Table H.11. Leucine requirement estimate RCTs adults (51->70 years)

Study (PMID)	Statistics/Confounders	Leucine Requirement Estimate	Data Used to Calculate Leucine Requirement Estimate
Szwiega, 2021[55] (33330915)	Statistics: Biphasic linear mixed-effects model; t-test; with subject as a random effect Confounders adjusted for: None	**Outcome: Leucine requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (1 d each) Total (N analyzed): 16 (7 males, 9 females); total observations 93 (45 males, 48 females) Protein amount: 1.0 g/kg/d Amino Acid amount: 20-120 mg/kg/d leucine Males: Breakpoint: 77.8 mg/kg/d Upper 95% CI: 81 mg/kg/d Lower 95% CI: 74.7 mg/kg/d r^2= 0.449 and p<0.0001 Mean requirement: 115; SEM 3.2 mg/kg FFM/d Upper 95% CI: 125.4 mg/kg FFM/d Lower 95% CI: 109.4 mg/kg FFM/d Females:	**Outcome: $F^{13}CO_2$** Outcome parameter: NA Timepoint: Data obtained following each study d (1 d each) Total (N analyzed): Total (N analyzed): 16 (7 males, 9 females); total observations 93 (45 males, 48 females) Protein amount: 1.0 g/kg/d Amino Acid amount: 20-120 mg/kg/d leucine Data reported in figures of original paper

Study (PMID)	Statistics/Confounders	Leucine Requirement Estimate	Data Used to Calculate Leucine Requirement Estimate
		Breakpoint: 78.2 mg/kg/d Upper 95% CI: 82 mg/kg/d Lower 95% CI: 74.5 mg/kg/d r^2=0.468 and p<0.0001 Mean requirement: 127.6; SEM 2.4 mg/kg FFM/d Upper 95% CI: 133.7 mg/kg FFM/d Lower 95% CI: 120.4 mg/kg FFM/d Combined: Breakpoint: 78.5 mg/kg/d Upper 95% CI: 81 mg/kg/d Lower 95% CI: 76.1 mg/kg/d r^2=0.456 and p<0.0001 NR on a FFM basis Comparisons and p-values No significant difference in the breakpoint was found between males and females on a body weight basis but a significant difference in the mean requirement for males and females was observed on a FFM basis (p=0.005).	

Abbreviations: CI = confidence interval; d = day; EAR = estimated average requirement; FFM/d = fat free mass per 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 = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification; SD = standard deviation; SEM = standard error of the mean

Lysine

Table H.12. Lysine requirement estimates RCT infants

Study (PMID)	Statistics/Confounders	Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate
Huang, 2011[5] (22049162)	Statistics: Biphasic linear regression crossover model	**Outcome: Lysine requirement estimate calculated from $F^{13}CO_2$ (first isotopic plateau)**	**Outcome: $F^{13}CO_2$** Outcome parameter: NA

Study (PMID)	Statistics/Confounders	Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate
	Confounders adjusted for: None	Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 2) Total (N analyzed): 21 Protein amount: 2.99; SD 0.02 g/kg/d Amino acid amount: 15-240 mg/kg/d lysine Breakpoint: 130 mg/kg/d Upper 95% CI: 188.4 mg/kg/d Lower 95% CI: 71.6 mg/kg/d $r^2 = 0.46$ $p<0.0001$ **Outcome: Lysine requirement estimate calculated from $F^{13}CO_2$ (second isotopic plateau)** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 2) Total (N analyzed): 21 Protein amount: 2.99; SD 0.02 g/kg/d Amino acid amount: 15-240 mg/kg/d lysine Breakpoint: 130 mg/kg/d Upper 95% CI: 183.7 mg/kg/d Lower 95% CI: 76.3 mg/kg/d $r^2 = 0.51$ $p<0.0001$	Timepoint: Data obtained following each study d (d 2) Total (N analyzed): 21 Protein amount: 2.99; SD 0.02 g/kg/d Amino acid amount: 15-240 mg/kg/d lysine Data reported in figures of original paper

Study (PMID)	Statistics/Confounders	Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate
		Outcome: Lysine requirement estimate calculated from phenylalanine oxidation (urinary enrichment) Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 2) Total (N analyzed): 21 Protein amount: 2.99; SD 0.02 g/kg/d Amino acid amount: 15-240 mg/kg/d lysine Breakpoint: 130 mg/kg/d Upper 95% CI: 183.2 mg/kg/d Lower 95% CI: 76.8 mg/kg/d $r^2 = 0.51$ $p<0.0001$ **Outcome: Lysine requirement estimate calculated from phenylalanine oxidation (plasma enrichment)** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 2) Total (N analyzed): 20 Protein amount: 2.99; SD 0.02 g/kg/d Amino acid amount: 15-240 mg/kg/d lysine Breakpoint: 130 mg/kg/d	

Study (PMID)	Statistics/Confounders	Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate
		Upper 95% CI: 185.6 mg/kg/d Lower 95% CI: 74.4 mg/kg/d r^2 = 0.49 p<0.0001	

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 = milligrams per kilogram per day; N = number; NA = not applicable; PMID = PubMed Identification; SD = standard deviation

Table H.13. Lysine requirement estimates RCTs children and adolescents

Study (PMID)	Statistics/Confounders	Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate
Elango, 2007[19] (17684206)	Statistics: 2-phase linear regression crossover model; Mixed linear model with subject as a random variable. Confounders adjusted for: None	**Outcome: Lysine requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 5; 35 total observations Protein amount: 1.5 g/kg/d protein Amino Acid amount: 5-80 mg/kg/d lysine Breakpoint: 35 mg/kg/d Upper 95% CI: 58 mg/kg/d Lower 95% CI: NR	**Outcome: $F^{13}CO_2$** Outcome parameter: NA Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 5; 35 total observations Protein amount: 1.5 g/kg/d protein Amino Acid amount: 5-80 mg/kg/d lysine Data reported in figures of original paper

Study (PMID)	Statistics/Confounders	Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate
Pillai, 2010[48] (19923398)	Statistics: 2-phase linear regression crossover model; Mixed linear model with participant as a random variable. Confounders adjusted for: None	**Outcome: Lysine requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 6; 42 total observations Protein amount: 1.5 g/kg/d protein Amino Acid amount: 5-80 mg/kg/d lysine Breakpoint: 33.5 mg/kg/d Upper 95% CI: 46.6 mg/kg/d Lower 95% CI: NR	**Outcome: $F^{13}CO_2$** Outcome parameter: NA Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 6; 42 total observations Protein amount: 1.5 g/kg/d protein Amino Acid amount: 5-80 mg/kg/d lysine Data reported in figures of original paper

Abbreviations: 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; mg/kg/d = milligrams per kilogram per day; NR = not reported; N = number; NA = not applicable; PMID = PubMed Identification Number

Table H.14. Lysine requirement estimates RCTs pregnant people

Study (PMID)	Statistics/Confounders	Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate
Payne, 2018[47] (29378056)	Statistics: Bi-phase linear regression crossover model; Mixed linear regression model with subject as a random variable Confounders adjusted for: None	**Outcome: Lysine requirement estimate calculated from $F^{13}CO_2$ (Early Gestation)** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 14, 27 total observations Protein amount: 1.5 g/kg/d protein Amino Acid amount: 6–84 mg/kg/d	**Outcome: $F^{13}CO_2$** Outcome parameter: NA Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 6; 42 total observations Protein amount: 1.5 g/kg/d protein Amino Acid amount: 5-80 mg/kg/d lysine

Study (PMID)	Statistics/Confounders	Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate
		lysine Breakpoint: 36.6 mg/kg/d Upper 95% CI: 46.2 mg/kg/d Lower 95% CI: NR $r^2 = 0.484$ **Outcome: Lysine requirement estimate calculated from $F^{13}CO_2$ (Late Gestation)** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 19; 36 total observations Protein amount: 1.5 g/kg/d protein Amino Acid amount: 6–84 mg/kg/d lysine Breakpoint: 50.3 mg/kg/d Upper 95% CI: 60.4 mg/kg/d Lower 95% CI: NR $r^2 = 0.664$	Data reported in figures of original paper

Abbreviations: 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; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; SD = standard deviation

Table H.15. Lysine requirement estimates RCTs adults (19-50 years)

Study (PMID)	Statistics/Confounders	Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate
Kriengsinyos, 2004[29] (15308475)	Statistics: Two-phase linear regression model; two-sample t-test; Mixed-model ANOVA	**Outcome: Lysine requirement estimate calculated from $F^{13}CO_2$ (Follicular Phase)**	**Outcome: $F^{13}CO_2$** Outcome parameter: NA	NA	NA

Study (PMID)	Statistics/Confounders	Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate
	Confounders adjusted for: Subject and sex hormones	Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 5, 35 total observations Protein amount: 1.0 g/kg/d protein Amino Acid amount: 10-60 mg/kg/d lysine Breakpoint: 35 mg/kg/d Upper 95% CI: 47.9 mg/kg/d Lower 95% CI: 22.1 mg/kg/d **Outcome: Lysine requirement estimate calculated from $F^{13}CO_2$ (Luteal Phase)** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 5, 35 total observations Protein amount: 1.0 g/kg/d protein	Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 5, 35 total observations Protein amount: 1.0 g/kg/d protein Amino Acid amount: 10-60 mg/kg/d lysine Data reported in figures of original paper Comparisons and p-values Mean $F^{13}CO_2$ at each level of lysine intake was 13-24% higher during the luteal phase compared with the follicular phase (P=0.02)		

Study (PMID)	Statistics/Confounders	Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate
		Amino Acid amount: 10-60 mg/kg/d lysine Breakpoint: 37.7 mg/kg/d Upper 95% CI: 43.6 mg/kg/d Lower 95% CI: 31.8 mg/kg/d Comparisons and p-values The breakpoint estimate obtained in the luteal phase was significantly higher than that obtained in the follicular phase (P=0.025)			
Kurpad, 2001[31] (11333843)	Statistics: Two-phase linear regression models; Mixed-models ANOVA Confounders adjusted for: None	**Outcome: Lysine requirement estimate calculated from 24-h IAAO** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (24-h, d 7) Total (N analyzed): 16, 32 total observations Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 12-36 mg/kg/d lysine	**Outcome: 24-h Leucine oxidation** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (24-h, d 7) Total (N analyzed): 8 per intake level Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 12-36 mg/kg/d lysine 12 mg/kg/d lysine: 104.1; SD 11 mg/kg/d	**Outcome: 12-h fed Leucine oxidation** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (12-h fed, d 7) Total (N analyzed): 8 per intake level Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 12-36 mg/kg/d lysine 12 mg/kg/d lysine: 70.4; SD 8.4 mg/kg/d	**Outcome: 24-h Leucine balance** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (24-h, d 7) Total (N analyzed): 8 per intake level Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 12-36 mg/kg/d lysine 12 mg/kg/d lysine: 3.3; SD 11 mg/kg/d

Study (PMID)	Statistics/Confounders	Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate
		Breakpoint: 28.7 mg/kg/d Upper 95% CI: 48 mg/kg/d Lower 95% CI: 21 mg/kg/d **Outcome: Lysine requirement estimate calculated from 12-h fed IAAO** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (12-h fed, d 7) Total (N analyzed): 16, 32 total observations Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 12-36 mg/kg/d lysine Breakpoint: 28.2 mg/kg/d Upper 95% CI: 48 mg/kg/d Lower 95% CI: 20 mg/kg/d **Outcome: Lysine requirement estimate calculated from 24-h IAAB**	20 mg/kg/d lysine: 97.8; SD 11.9 mg/kg/d 28 mg/kg/d lysine: 87.3; SD 11.7 mg/kg/d 36 mg/kg/d lysine: 87.3; SD 7.8 mg/kg/d Comparisons and p-values 28 and 26 mg/kg/d lysine 24-h IAAO significantly different from 12 mg/kg/d lysine intake ($p<0.05$ for both)	20 mg/kg/d lysine: 64.3; SD 10.4 mg/kg/d 28 mg/kg/d lysine: 58.1; SD 5.8 mg/kg/d 36 mg/kg/d lysine: 58.0; SD 7.3 mg/kg/d Comparisons and p-values Overall significant effect of lysine intake and of metabolic phase ($p<0.05$)	20 mg/kg/d lysine: 9.1; SD 10.4 mg/kg/d 28 mg/kg/d lysine: 19.7; SD 11.4 mg/kg/d 36 mg/kg/d lysine: 20.7; SD 7.6 mg/kg/d Comparisons and p-values Overall, there was a significant effect of lysine intake on 24-h IAAB method ($p<0.05$). 20, 28, and 36 mg/kg/d lysine 24-h IAAB were all significantly different from zero ($p<0.05$ for all). 28 and 36 mg/kg/d lysine 24-h IAAB method was significantly different than 12 mg/kg/d lysine intake ($p<0.05$ for both).

Study (PMID)	Statistics/Confounders	Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate
		Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (12-h fed, d 7) Total (N analyzed): 16, 32 total observations Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 12-36 mg/kg/d lysine Breakpoint: 29.7 mg/kg/d Upper 95% CI: 49 mg/kg/d Lower 95% CI: 22 mg/kg/d			
Kurpad, 2002[33] (12145014)	Statistics: Two-phase linear regression model; Mixed models ANOVA Confounders adjusted for: Mean and SD for the combined day 7 and day 21 data were adjusted for the correlation between observations on days 7 and 21	**Outcome: Lysine requirement estimate calculated from 24-h IAAO** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (24-h, d 7) Total (N analyzed): 18, 36 total observations Protein amount: 160	**Outcome: 24-h Leucine oxidation** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (24-h, d 7) Total (N analyzed): 9 per intake level Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein)	**Outcome: 12-h fed Leucine oxidation** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (12-h fed, d 7) Total (N analyzed): 9 per intake level Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein)	**Outcome: 24-h Leucine balance** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (24-h, d 7) Total (N analyzed): 9 per intake level Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein)

Study (PMID)	Statistics/Confounders	Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate
		mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 12-36 mg/kg/d lysine Breakpoint: 31 mg/kg/d Upper 95% CI: 40 mg/kg/d Lower 95% CI: 26 mg/kg/d Timepoint: Data obtained following each study d (24-h, d 21) Total (N analyzed): 16, 36 total observations Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 12-36 mg/kg/d lysine Breakpoint: 31 mg/kg/d Upper 95% CI: 48 mg/kg/d Lower 95% CI: 23 mg/kg/d Timepoint: Data obtained following each study d (24-h, d 7 and 21) Total (N analyzed): 16, 36 total observations per d 7 and d 21; 72 total observations	Amino Acid amount: 12-36 mg/kg/d lysine 12 mg/kg/d lysine: 62.3; SD 5.2 mg/kg/d 20 mg/kg/d lysine: 56.7; SD 6 mg/kg/d 28 mg/kg/d lysine: 51.2; SD 1 mg/kg/d 36 mg/kg/d lysine: 47.9; SD 5.7 mg/kg/d Timepoint: Data obtained following each study d (24-h, d 21) Total (N analyzed): 8 per intake level Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 12-36 mg/kg/d lysine 12 mg/kg/d lysine: 60.1; SD 5.5 mg/kg/d 20 mg/kg/d lysine: 56.9; SD 5.9 mg/kg/d 28 mg/kg/d lysine: 52.6; SD 3.3 mg/kg/d 36 mg/kg/d lysine: 51.5; SD 4.7 mg/kg/d Timepoint: Data obtained following each study d (24-h, d 7 and 21) Total (N analyzed): 18 per intake level	Amino Acid amount: 12-36 mg/kg/d lysine 12 mg/kg/d lysine: 35.0; SD 3.3 mg/kg/d 20 mg/kg/d lysine: 30.3; SD 4.2 mg/kg/d 28 mg/kg/d lysine: 25.9; SD 3.4 mg/kg/d 36 mg/kg/d lysine: 26.6; SD 6 mg/kg/d Timepoint: Data obtained following each study d (12-h fed, d 21) Total (N analyzed): 9 per intake level Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 12-36 mg/kg/d lysine 12 mg/kg/d lysine: 33.8; SD 7.1 mg/kg/d 20 mg/kg/d lysine: 30.5; SD 6.8 mg/kg/d 28 mg/kg/d lysine: 29.1; SD 5.4 mg/kg/d 36 mg/kg/d lysine: 28.0; SD 3.5 mg/kg/d Timepoint: Data obtained following each study d (12-h fed, d 7 and 21) Total (N analyzed): 18 per intake level	Amino Acid amount: 12-36 mg/kg/d lysine 12 mg/kg/d lysine: -11.3; SD 5.5 mg/kg/d 20 mg/kg/d lysine: -5.6; SD 5.7 mg/kg/d 28 mg/kg/d lysine: 1.2; SD 4.9 mg/kg/d 36 mg/kg/d lysine: 3.3; SD 5.4 mg/kg/d Timepoint: Data obtained following each study d (24-h, d 21) Total (N analyzed): 9 per intake level Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 12-36 mg/kg/d lysine 12 mg/kg/d lysine: -8.4; SD 5.8 mg/kg/d 20 mg/kg/d lysine: -5.6; SD 5.8 mg/kg/d 28 mg/kg/d lysine: -1.1; SD 3.1 mg/kg/d 36 mg/kg/d lysine: -0.2; SD 4.4 mg/kg/d Timepoint: Data obtained following each study d (24-h, d 7 and 21) Total (N analyzed): 18 per intake level

Study (PMID)	Statistics/Confounders	Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate
		Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 12-36 mg/kg/d lysine Breakpoint: 31 mg/kg/d Upper 95% CI: 38 mg/kg/d Lower 95% CI: 26 mg/kg/d **Outcome: Lysine requirement estimate calculated from 12-h fed IAAO** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (24-h, d 7 and 21) Total (N analyzed): 16, 36 total observations per d 7 and d 21; 72 total observations Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 12-36 mg/kg/d lysine Breakpoint: 26 mg/kg/d Upper 95% CI: 72 mg/kg/d	Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 12-36 mg/kg/d lysine 12 mg/kg/d lysine: 61.5; SD 6 mg/kg/d 20 mg/kg/d lysine: 56.6; SD 7.7 mg/kg/d 28 mg/kg/d lysine: 51.4; SD 3.3 mg/kg/d 36 mg/kg/d lysine: 50.3; SD 5.5 mg/kg/d Comparisons and p-values There was a significant interaction of lysine intake ($p<0.05$) The 24-h leucine oxidation rate was lower with the 28 and 36 mg intakes than with the 12 and 20 mg intakes ($p<0.05$) and daily leucine oxidation was not significantly different between the 12 and 20 mg intakes or between the 28 and 36 mg intakes	Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 12-36 mg/kg/d lysine 12 mg/kg/d lysine: 35.8; SD 4 mg/kg/d 20 mg/kg/d lysine: 29.9; SD 4.9 mg/kg/d 28 mg/kg/d lysine: 26.5; SD 4.5 mg/kg/d 36 mg/kg/d lysine: 28.0; SD 4.9 mg/kg/d Comparisons and p-values There was a significant interaction of lysine intake and of metabolic period ($p<0.05$) The 12-h fed leucine oxidation rate was lower with the 28 and 36 mg intakes than with the 12 mg/kg/d intake ($p<0.05$)	Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 12-36 mg/kg/d lysine 12 mg/kg/d lysine: -10.1; SD 6.4 mg/kg/d 20 mg/kg/d lysine: -5.3; SD 6.9 mg/kg/d 28 mg/kg/d lysine: 0; SD 3.4 mg/kg/d 36 mg/kg/d lysine: 0.7; SD 5.2 mg/kg/d Comparisons and p-values Without regard to infusion d (data from d 7 and 21 combined) 24-h IAAB method did not differ significantly between 12 and 20 mg/k/g lysine intakes or 28 and 36 mg/kg/d intakes. 24-h IAAB at 12 and 20 mg/kg/d lysine each differed from intake of 28 and 36 mg/kg/d ($p<0.02$). Without regard to infusion d (data from d 7 and 21 combined) 24-h IAAB method significantly differed from zero at 12 and 20 mg/kg/d lysine intake ($p<0.01$ for both).

Study (PMID)	Statistics/Confounders	Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate
		Lower 95% CI: 20 mg/kg/d **Outcome: Lysine requirement estimate calculated from 24-h IAAB** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (24-h, d 7) Total (N analyzed): 18, 36 total observations Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 12-36 mg/kg/d lysine Breakpoint: 31 mg/kg/d Upper 95% CI: 40 mg/kg/d Lower 95% CI: 26 mg/kg/d Timepoint: Data obtained following each study d (24-h, d 21) Total (N analyzed): 16, 36 total observations Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein)			

Study (PMID)	Statistics/Confounders	Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate	Data Used to Calculate Lysine Requirement Estimate
		Amino Acid amount: 12-36 mg/kg/d lysine Breakpoint: 31 mg/kg/d Upper 95% CI: 47 mg/kg/d Lower 95% CI: 23 mg/kg/d Timepoint: Data obtained following each study d (24-h, d 7 and 21) Total (N analyzed): 16, 36 total observations per d 7 and d 21; 72 total observations Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 12-36 mg/kg/d lysine Breakpoint: 31 mg/kg/d Upper 95% CI: 38 mg/kg/d Lower 95% CI: 26 mg/kg/d			

Abbreviations: ANOVA = analysis of variance; 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 = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; SD = standard deviation; µmol/L = micromoles per liter

Table H.16. Lysine requirement not calculated; $F^{13}CO_2$ RCTs adults (19-50 years)

Study (PMID)	Statistics/Confounders	$F^{13}CO_2$
Elango, 2009[18] (19369367)	Statistics: Repeated-measures ANOVA with 2 fixed effects (lysine intake and days of adaptation) and 1 random effect (participant)	**Outcome: $F^{13}CO_2$** Outcome parameter: Mean; SD (lower and upper

Study (PMID)	Statistics/Confounders	$F^{13}CO_2$
	Confounders adjusted for: None	95% CI) Timepoint: Data obtained following each study d (8-h, 3-d, 7-d) Total (N analyzed): 5 per intake level Protein amount: 1.0 g/kg/d Amino Acid amount: 5-70 mg/kg/d lysine 8h: 5 mg/kg/d lysine: 0.465; SD 0.093 (95% CI: 0.377-0.553) µmol/kg/h 20 mg/kg/d lysine: 0.365; SD 0.129 (95% CI: 0.270-0.460) µmol/kg/h 35 mg/kg/d lysine: 0.412; SD 0.097 (95% CI: 0.311-0.520) µmol/kg/h 70 mg/kg/d lysine: 0.316; SD 0.086 (95% CI: 0.210-0.423) µmol/kg/h 3-d: 5 mg/kg/d lysine: 0.495; SD 0.096 (95% CI: 0.407-0.583) µmol/kg/h 20 mg/kg/d lysine: 0.338; SD 0.030 (95% CI: 0.243-0.434) µmol/kg/h 35 mg/kg/d lysine: 0.346; SD 0.084 (95% CI: 0.243-0.446) µmol/kg/h 70 mg/kg/d lysine: 0.305; SD 0.123 (95% CI: 0.199-0.412) µmol/kg/h 7-d: 5 mg/kg/d lysine: 0.511; SD 0.039 (95% CI: 0.423-0.599) µmol/kg/h 20 mg/kg/d lysine: 0.317; SD 0.096 (95% CI: 0.221-0.413) µmol/kg/h 35 mg/kg/d lysine: 0.358; SD 0.086 (95% CI: 0.258-0.458) µmol/kg/h 70 mg/kg/d lysine: 0.280; SD 0.086 (95% CI: 0.173-0.386) µmol/kg/h Total: 8-h to 7-d combined: 5 mg/kg/d lysine: 0.490; SD 0.077 µmol/kg/h 20 mg/kg/d lysine: 0.340; SD 0.090 µmol/kg/h

Study (PMID)	Statistics/Confounders	$F^{13}CO_2$
		35 mg/kg/d lysine: 0.372; SD 0.088 μmol/kg/h 70 mg/kg/d lysine: 0.300; SD 0.094 μmol/kg/h 95% CI NR Comparisons and p-values There were no significant differences in $F^{13}CO_2$ due to adaptation periods (p=0.614) and no significant interactions between lysine intake and adaptation period (p=0.665). The simultaneous 95% CI for the difference of means between 8 h and 3 d: -0.0276–0.0641 (p= 0.604), 8 h and 7 d: -0.0229–0.0688 (p= 0.453), and 3 and 7 d: -0.0411–0.0505 (p= 0.967). Significantly higher oxidation was observed when lysine intake was 5 mg/kg/d compared to 20, 35, and 70 mg/kg/d (p=0.0001).

Abbreviations: ANOVA = analysis of variance; 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; mg/kg/d = milligrams per kilogram per day; N = number; NR = not reported; PMID = PubMed Identification Number; SD = standard deviation; μmol/kg/h = micromoles per kilogram per hour;

Table H.17. Lysine requirement not calculated; 24-hour lysine balance and whole-body lysine oxidation non-RCTs adults (19-50 years)

Study (PMID)	Statistics/Confounders	24-h Lysine Balance	24-h Whole body lysine oxidation
El-Khoury, 2000[62] (10871570)	Statistics: Paired t tests; Group t test; One sample, one tailed t test Confounders adjusted for: None	**Outcome: 24-h Lysine balance** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (24-h, d 7) Total (N analyzed): 11 Protein amount: 1.0 g/kg/d (160 mg nitrogen/kg/d) Amino Acid amount: LL:14-15 mg/kg/d; average lysine intake: 15.53; SD 0.53 mg/kg/d IL: 28-29 mg/kg/d; average lysine intake: 29.1; SD 0.24 mg/kg/d LL: -12.4; SD 9.2 mg/kg/d IL: 1.8; SD 17.7 mg/kg/d Comparisons and p-values The low lysine group had a significantly different 24-h lysine balance than the intermediate lysine group (p<0.025). The low lysine group had a 24-h lysine balance that was significantly different from zero (p<0.001).	**Outcome: 24-h Whole body lysine oxidation** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (12-h fasted, d 7; 12-h fed, d 7; 24-h, day 7) Total (N analyzed): 11 Protein amount: 1.0 g/kg/d (160 mg nitrogen/kg/d) Amino Acid amount: LL:14-15 mg/kg/d; average lysine intake: 15.53; SD 0.53 mg/kg/d IL: 28-29 mg/kg/d; average lysine intake: 29.1; SD 0.24 mg/kg/d 12-h fasted, d 7: LL: 14.7; SD 4.6 mg/kg/d IL: 15.1; SD 7 mg/kg/d 12-h fed, d 7: LL: 13.2; SD 5.4 mg/kg/d IL: 12.2; SD 5 mg/kg/d 24-h, d 7: LL: 27.9; SD 8.8 mg/kg/d IL: 27.3; SD 17.6 mg/kg/d Comparisons and p-values No difference between groups at 12-h fasted, 12-h fed or 24-h timepoints.

Abbreviations: CI = confidence interval; g/kg/d = grams per kilogram per day; h = hour; IL = intermediate lysine; LL = low lysine; mg/kg/d = milligrams per kilogram per day; NR = not reported; N = number; PMID = PubMed Identification Number; SD = standard deviation

Methionine

Table H.18. Methionine requirement estimates RCTs infants

Study (PMID)	Statistics/Confounders	Methionine Requirement Estimate	Data Used to Calculate Methionine Requirement Estimate
Huang, 2012[6] (22492372)	Statistics: Bi-phasic linear regression crossover model Confounders adjusted for: None	**Outcome: Methionine requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI. Timepoint: Data obtained following each study d (d 2) Total (N analyzed): 33 Protein amount: 3.0; SD 0.1 g/kg/d Amino Acid amount: 3-59 mg/kg/d methionine and 91 mg/kg/d cysteine Breakpoint: 38 mg/kg/d Upper 95% CI: 48 mg/kg/d Lower 95% CI: 27 mg/kg/d $r^2 = 0.59$, $p<0.0001$	**Outcome: $F^{13}CO_2$** Outcome parameter: NA Timepoint: Data obtained following each study d (d 2) Total (N analyzed): 33 Protein amount: 2.96 g/kg/d Amino Acid amount: 3-59 mg/kg/d methionine and 91 mg/kg/d cysteine Data reported in figures of original paper

Abbreviations: 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; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number

Table H.19. Methionine requirement estimates RCTs children and adolescents

Study (PMID)	Statistics/Confounders	Methionine Requirement Estimate	Data Used to Calculate Methionine Requirement Estimate
Humayun, 2006[28] (17093160)	Statistics: Biphasic linear regression crossover model; Repeated-measures ANOVA Confounders adjusted for: None	**Outcome: Methionine requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 6; 36 total observations	**Outcome: $F^{13}CO_2$** Outcome parameter: NA Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 6; 36 total observations Protein amount: 1.0 g/kg/d

Study (PMID)	Statistics/Confounders	Methionine Requirement Estimate	Data Used to Calculate Methionine Requirement Estimate
		Protein amount: 1.0 g/kg/d Amino Acid amount: 0-15 mg/kg/d methionine and 21 mg/kg/d cysteine Breakpoint: 5.8 mg/kg/d Upper 95% CI: 7.3 mg/kg/d Lower 95% CI: NR	Amino Acid amount: 0-15 mg/kg/d methionine and 21 mg/kg/d cysteine Data reported in figures of original paper Comparisons and p-values: $F^{13}CO_2$ at 0 mg/kg/d methionine differed significantly from all other intake levels ($p<0.05$). $F^{13}CO_2$ at 2.5 mg/kg/d methionine intake differed significantly from those at 7.5, 10, and 15 mg/kg/d methionine intakes ($p<0.05$).
Turner, 2006[57] (16522909)	Statistics: Biphase linear regression crossover model; Repeated measures analysis of variance Confounders adjusted for: None	**Outcome: Total sulfur amino acid requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 6; 36 total observations Protein amount: 1.5 g/kg/d Amino Acid amount: 0-35 mg/kg/d methionine; 0 mg/kg/d cysteine Breakpoint: 12.9 mg/kg/d Upper 95% CI: 17.2 mg/kg/d Lower 95% CI: NR	**Outcome: $F^{13}CO_2$** Outcome parameter: NA Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 6; 36 total observations Protein amount: 1.5 g/kg/d Amino Acid amount: 0-35 mg/kg/d methionine; 0 mg/kg/d cysteine Data reported in figures of original paper

Abbreviations: 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; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; PMID = PubMed Identification Number; SD = standard deviation

Table H.20. Methionine requirement estimates RCTs adults (19-50 years)

Study (PMID)	Statistics/Confounders	Methionine Requirement Estimate	Data Used to Calculate Methionine Requirement Estimate	Data Used to Calculate Methionine Requirement Estimate
Di Buono, 2001[16] (11722957)	Statistics: 2-phase linear regression crossover model; Repeated-measures ANOVA Confounders adjusted for: None	**Outcome: Methionine requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 6; 36 total observations Protein amount: 1.0 g/kg/d Amino Acid amount: 0-13 mg/kg/d methionine; 21 mg/kg/d cysteine Breakpoint: 4.5 mg/kg/d Upper 95% CI: 10.1 mg/kg/d Lower 95% CI: NR	**Outcome: $F^{13}CO_2$** Outcome parameter: NA Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 6; 36 total observations Protein amount: 1.0 g/kg/d Amino Acid amount: 0-13 mg/kg/d methionine; 21 mg/kg/d cysteine Data reported in figures of original paper	NA
Di Buono, 2001[17] (11722956)	Statistics: 2-phase linear regression crossover model; Repeated-measures ANOVA Confounders: None; To identify the possible sources of variation in individual $F^{13}CO_2$ responses, an analysis of covariance was carried out between $F^{13}CO_2$ and methionine intake and lean body mass. Inclusion of lean body mass in this analysis was found was not significant.	**Outcome: Total sulfur amino acid requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 6; 36 total observations Protein amount: 1.0 g/kg/d Amino Acid amount: 0-13 mg/kg/d methionine; 0 mg/kg/d cysteine	**Outcome: $F^{13}CO_2$** Outcome parameter: NA Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 6; 36 total observations Protein amount: 1.0 g/kg/d Amino Acid amount: 0-13 mg/kg/d methionine; 0 mg/kg/d cysteine Data reported in figures of original paper	NA

Study (PMID)	Statistics/Confounders	Methionine Requirement Estimate	Data Used to Calculate Methionine Requirement Estimate	Data Used to Calculate Methionine Requirement Estimate
		Breakpoint: 12.6 mg/kg/d Upper 95% CI: 21 mg/kg/d Lower 95% CI: NR		
Kurpad, 2003[36] (12716672)	Statistics: Two-phase linear regression model which considered multiple measures; Mixed-models ANOVA Confounders adjusted for: None	**Outcome: Total sulfur amino acid requirement estimate calculated from 24-h IAAO** Outcome parameter: Breakpoint, Upper 95% CI Timepoint: Data obtained following each study d (d 7) Total (N analyzed): 21; 63 total observations Protein amount: 1.0 g/kg/d Amino Acid amount: 3-24 mg/kg/d methionine; 0 mg/kg/d cysteine Breakpoint: 14 mg/kg/d Upper 95% CI: 23 mg/kg/d Lower 95% CI: 11 mg/kg/d **Outcome: Total sulfur amino acid requirement estimate calculated from 24-h IAAB** Outcome parameter: Breakpoint, Upper 95% CI Timepoint: Data obtained following each study d (d 7) Total (N analyzed): 21; 63 total observations Protein amount: 1.0 g/kg/d	**Outcome: 24-h Leucine oxidation** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (d 7) Total (N analyzed): 9 per intake level Protein amount: 1.0 g/kg/d Amino Acid amount: 3-24 mg/kg/d methionine; 0 mg/kg/d cysteine 3 mg/kg/d methionine: 50.8; SD 7.4 mg/kg/d 6 mg/kg/d methionine: 47.6; SD 4.8 mg/kg/d 9 mg/kg/d methionine: 49.6; SD 3.6 mg/kg/d 13 mg/kg/d methionine: 41.8; SD 3.5 mg/kg/d 18 mg/kg/d methionine: 41.4; SD 6.3 mg/kg/d 21 mg/kg/d methionine: 43.9; SD 3.6 mg/kg/d 24 mg/kg/d methionine: 41.7; SD 1.8 mg/kg/d Comparisons and p-values Overall, there was a significant effect of methionine intake on oxidation (P<0.0001).	**Outcome: 24-h Leucine balance** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (d 7) Total (N analyzed): 9 per intake level Protein amount: 1.0 g/kg/d Amino Acid amount: 3-24 mg/kg/d methionine; 0 mg/kg/d cysteine 3 mg/kg/d methionine: -11; SD 7.4 mg/kg/d 6 mg/kg/d methionine: -8; SD 4.9 mg/kg/d 9 mg/kg/d methionine: -10.3; SD 3.6 mg/kg/d 13 mg/kg/d methionine: -2.7; SD 3.7 mg/kg/d 18 mg/kg/d methionine: -2.1; SD 6.5 mg/kg/d 21 mg/kg/d methionine: -4.3; SD 3.6 mg/kg/d 24 mg/kg/d methionine: -2; SD 2.3 mg/kg/d Comparisons and p-values Overall, there was a significant effect of methionine intake on 24-h IAAB (p<0.0001).

Study (PMID)	Statistics/Confounders	Methionine Requirement Estimate	Data Used to Calculate Methionine Requirement Estimate	Data Used to Calculate Methionine Requirement Estimate
		Amino Acid amount: 3-24 mg/kg/d methionine; 0 mg/kg/d cysteine Breakpoint: 15 mg/kg/d Upper 95% CI: 27 mg/kg/d Lower 95% CI: 11 mg/kg/d	13, 18, and 24 mg/kg/d methionine intakes were significantly different from 3, 6, and 9 mg/kg/d methionine intakes ($p<0.05$ for all).	24 mg/kg/d methionine intake was significantly different from 3, 6, and 9 mg/kg/d methionine intakes ($p<0.05$). 13, 18, and 21 mg/kg/d methionine intake was significantly different from 3 and 9 mg/kg/d methionine intakes ($p<0.05$ for all). 3, 6, 9, and 21 mg/kg/d methionine intakes were all significantly different from zero balance ($p<0.001$ for all).
Kurpad, 2004[37] (15585764)	Statistics: Two-phase linear regression model which took into account multiple measures; Mixed-models ANOVA Confounders adjusted for: None	**Outcome: Methionine requirement estimate calculated from 24-h IAAO** Outcome parameter: Breakpoint, Upper 95% CI Timepoint: Data obtained following each study d (d 7) Total (N analyzed): 21; 63 total observations per methionine and cysteine group Protein amount: 1.0 g/kg/d Amino Acid amount: 3-24 mg/kg/d methionine; 5 mg/kg/d cysteine Breakpoint: 20 mg/kg/d Upper 95% CI: 26 mg/kg/d Lower 95% CI: 17 mg/kg/d	**Outcome: 24-h Leucine oxidation** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (d 7) Total (N analyzed): 9 per intake level Protein amount: 1.0 g/kg/d Amino Acid amount: 3-24 mg/kg/d methionine; 5 mg/kg/d cysteine 3 mg/kg/d methionine: 47.3; SD 4 mg/kg/d 6 mg/kg/d methionine: 48.1; SD 2.6 mg/kg/d 9 mg/kg/d methionine: 44.9; SD 4.2 mg/kg/d 13 mg/kg/d methionine: 42.2;	**Outcome: 24-h Leucine balance** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (d 7) Total (N analyzed): 9 per intake level Protein amount: 1.0 g/kg/d Amino Acid amount: 3-24 mg/kg/d methionine; 5 mg/kg/d cysteine 3 mg/kg/d methionine: -7.5; SD 3.9 mg/kg/d 6 mg/kg/d methionine: -8.5; SD 2.7 mg/kg/d 9 mg/kg/d methionine: -5.4; SD 4.3 mg/kg/d 13 mg/kg/d methionine: -2.5;

Study (PMID)	Statistics/Confounders	Methionine Requirement Estimate	Data Used to Calculate Methionine Requirement Estimate	Data Used to Calculate Methionine Requirement Estimate
		Amino Acid amount: 3-24 mg/kg/d methionine; 12 mg/kg/d cysteine Breakpoint: 10 mg/kg/d Upper 95% CI: 16 mg/kg/d Lower 95% CI: 8 mg/kg/d **Outcome: Methionine requirement estimate calculated from 24-h IAAB** Outcome parameter: Breakpoint, Upper 95% CI Timepoint: Data obtained following each study d (d 7) Total (N analyzed): 21; 63 total observations per methionine and cysteine group Protein amount: 1.0 g/kg/d Amino Acid amount: 3-24 mg/kg/d methionine; 5 mg/kg/d cysteine Breakpoint: 20 mg/kg/d Upper 95% CI: 25 mg/kg/d Lower 95% CI: 17 mg/kg/d Amino Acid amount: 3-24 mg/kg/d methionine; 12 mg/kg/d cysteine Breakpoint: 10 mg/kg/d Upper 95% CI: 16 mg/kg/d Lower 95% CI: 8 mg/kg/d Comparisons and p-values	SD 3.9 mg/kg/d 18 mg/kg/d methionine: 40.9; SD 3.4 mg/kg/d 21 mg/kg/d methionine: 38.1; SD 3 mg/kg/d 24 mg/kg/d methionine: 39.6; SD 1.6 mg/kg/d Comparisons and p-values Significant effect of methionine intake (p<0.001) Amino Acid amount: 3-24 mg/kg/d methionine; 12 mg/kg/d cysteine 3 mg/kg/d methionine: 48.1; SD 4.3 mg/kg/d 6 mg/kg/d methionine: 43.6; SD 4.8 mg/kg/d 9 mg/kg/d methionine: 42.7; SD 3.0 mg/kg/d 13 mg/kg/d methionine: 40.3; SD 3 mg/kg/d 18 mg/kg/d methionine: 40.6; SD 4 mg/kg/d 21 mg/kg/d methionine: 40.2; SD 4.2 mg/kg/d 24 mg/kg/d methionine: 40.3; SD 2.7 mg/kg/d Comparisons and p-values Significant effect of methionine intake (p<0.001) 24-h leucine oxidation was significantly higher at 3 mg/kg/d methionine intake than any other intake level (P<0.05)	SD 4.1 mg/kg/d 18 mg/kg/d methionine: -1.5; SD 3.3 mg/kg/d 21 mg/kg/d methionine: 1.2; SD 3 mg/kg/d 24 mg/kg/d methionine: 0.1; SD 1.7 mg/kg/d Comparisons and p-values 24-h leucine balance was affected by methionine intake (P<0.0001). 24-h leucine balance was significantly different from zero balance at the 3, 6, 9 and 13 mg/kg/d methionine intakes (P<0.01); 24-h leucine balance was significantly lower at the 3 mg/kg/d intake than at the 9, 13, 18, 21, and 24 mg/kg/d intakes, at the 6 mg/kg/d intake than at the 13, 18, 21, and 24 mg/kg/d intakes, at the 9 mg/kg/d intake than at the 18, 21, and 24 mg/kg/d intakes and at the 13 mg/kg/d intake than at the 21 and 24 mg/kg/d intakes (P<0.05). Leucine balance was not significantly different among the 18, 21, and 24 mg/kg/d intakes. Amino Acid amount: 3-24 mg/kg/d methionine; 12

Study (PMID)	Statistics/Confounders	Methionine Requirement Estimate	Data Used to Calculate Methionine Requirement Estimate	Data Used to Calculate Methionine Requirement Estimate
		Estimated requirements significantly differed when 5 mg/kg/d cysteine compared with 12 mg/kg/d cysteine was provided but both requirement estimates were not different from when 0 mg/kg/d was provided in the study by Kurpad et al. above[36] (p values noted provided)		mg/kg/d cysteine 3 mg/kg/d methionine: -8.9; SD 4.6 mg/kg/d 6 mg/kg/d methionine: -4; SD 4.8 mg/kg/d 9 mg/kg/d methionine: -3.4; SD 3.4 mg/kg/d 13 mg/kg/d methionine: -1.1; SD 3.2 mg/kg/d 18 mg/kg/d methionine: -1.2; SD 4.3 mg/kg/d 21 mg/kg/d methionine: -0.7; SD 4 mg/kg/d 24 mg/kg/d methionine: -0.4; SD 3 mg/kg/d Comparisons and p-values 24-h leucine balance was affected by methionine intake (P<0.0001) 24-h leucine balance was significantly different from zero balance at 3, 6, and 9 mg/kg/d intakes (P<0.01). 24-h Leucine balance was significantly lower at the 3 mg/kg/d intake than at each other intake (P<0.05) and was significantly lower at the 6 mg/kg/d intake than at the 24 mg/kg/d intake (P<0.05) but otherwise was not significantly different among intakes.

Abbreviations: ANOVA = analysis of variance; 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; IAAB = indicator amino acid balance; IAAO = indicator amino acid oxidation; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; SD = standard deviation

Table H.21. Methionine requirement not calculated; phenylalanine oxidation RCTs adults (19-50 years)

Study (PMID)	Statistics/Confounders	Total Sulfur Amino Acid Oxidation
Humayun, 2007[26] (17634258)	Statistics: "MIXED" procedure with "subject" as a random variable; paired t test Confounders adjusted for: Age, weight, and VCO_2 and their interactions were tested but they were found to be non-significant.	**Outcome: Phenylalanine oxidation (% administered dose)** Outcome parameter: NA Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 7; 91 total observations Protein amount: 1.0 g/kg/d Amino acid amount: Crystalline AA mix: 20-70% of TSAA requirement (13 mg/kg/d); Casein: 40-70% of TSAA requirement (13 mg/kg/d); SPI: 40-70% of TSAA requirement (13 mg/kg/d) Data reported in figures of original paper Comparisons and p-values Crystalline AA mix: An increase in TSAA intake resulted in a linear decrease in L[1-13C] phenylalanine oxidation. Slope = -0.055 + 0.01 (r^2 = 0.91, p = 0.011). Casein: An increase in TSAA intake resulted in no change in L[1-13C] phenylalanine oxidation. Slope = -0.017 + 0.02 (r^2 = 0.24, p = 0.50). SPI: An increase in TSAA intake resulted in no change in L[1-13C] phenylalanine oxidation. Slope = 0.006 + 0.02 (r^2 = 0.02, p = 0.86).

Abbreviations: AA = amino acid; d = day; g/kg/d = grams per kilogram per day; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; PMID = PubMed Identification; SPI = soy protein isolate; TSAA = total sulfur amino acid; VCO_2 = rate of carbon dioxide production

Phenylalanine

Table H.22. Phenylalanine requirement estimates RCTs infants

Study (PMID)	Statistics/Confounders	Phenylalanine Requirement Estimate	Data Used to Calculate Phenylalanine Requirement Estimate
Hogewind-Schoonenboom, 2015[4] (25926506)	Statistics: Nonlinear regression model Confounders adjusted for: None	**Outcome: Phenylalanine requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint; Lower 95% CI, Upper 95% CI Timepoint: data obtained following each study d (d 2) Total (N analyzed): 20 Protein amount: 2.96 g/kg/d Amino Acid amount: 5-166 mg/kg/d phenylalanine and 166 mg/kg/d tyrosine Breakpoint; 58 mg/kg/d Upper 95% CI: 78 mg/kg/d Lower 95% CI: 38 mg/kg/d $r^2 = 0.62$, $p<0.001$	**Outcome: $F^{13}CO_2$** Outcome parameter: NA Timepoint: data obtained following each study d (d 2) Total (N analyzed): 20 Protein amount: 2.96 g/kg/d Amino Acid amount: 5-166 mg/kg/d phenylalanine and 166 mg/kg/d tyrosine Data reported in figures of original paper

Abbreviations: CI = confidence interval; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; lysine]; g/kg/d = grams per kilogram per day; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; PMID = PubMed Identification Number

Table H.23. Phenylalanine requirement estimates RCTs children and adolescents

Study (PMID)	**Statistics/Confounders**	**Phenylalanine Requirement Estimate**	**Data Used to Calculate Phenylalanine Requirement Estimate**
Hsu, 2007[23] (17314698)	Statistics: Two-phase linear regression crossover model with subject as a random effect Confounders adjusted for: None	**Outcome: Total aromatic amino acid requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: data obtained following each study d (d 3) Total (N analyzed): 5; 40 total observations Protein amount:1.5 g/kg/d Amino Acid amount: 5-70 mg/kg/d phenylalanine, 0 mg/kg/d tyrosine Breakpoint: 28 mg/kg/d Upper 95% CI: NR Lower 95% CI: NR Comparisons and p-values Hsu et al. note in this study that this is a biologically implausible value.	**Outcome: $F^{13}CO_2$** Outcome parameter: Mean; SD Timepoint: data obtained following each study d (d 3) Total (N analyzed): 5 per intake level Protein amount:1.5 g/kg/d Amino Acid amount: 5-70 mg/kg/d phenylalanine, 0 mg/kg/d tyrosine 5 mg/kg/d phenylalanine: 1.84; SD 0.32 µmol/kg/h 10 mg/kg/d phenylalanine: 1.55; SD 0.26 µmol/kg/h 20 mg/kg/d phenylalanine: 1.48; SD 0.32 µmol/kg/h 30 mg/kg/d phenylalanine: 1.35; SD 0.29 µmol/kg/h 40 mg/kg/d phenylalanine: 1.43; SD 0.23 µmol/kg/h 50 mg/kg/d phenylalanine: 1.46; SD 0.22 µmol/kg/h 60 mg/kg/d phenylalanine: 1.4; SD 0.32 µmol/kg/h 70 mg/kg/d phenylalanine: 1.52; SD 0.18 µmol/kg/h Overall phenylalanine intake significantly affected $F^{13}CO_2$

Study (PMID)	Statistics/Confounders	Phenylalanine Requirement Estimate	Data Used to Calculate Phenylalanine Requirement Estimate
			(p=0.01). There was no difference between 10, 20, 30, 40, 50, 60 or 70 mg/kg/d phenylalanine intakes but 5 mg/kg/d was significantly different than 30, 40, and 60 mg/kg/d phenylalanine intakes (P<0.05).

Abbreviations: CI = confidence interval; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; lysine]; g/kg/d = grams per kilogram per day; mg/kg/d = milligrams per kilogram per day; N = number; NR = not reported; PMID = PubMed Identification Number; SD = standard deviation; µmol/kg/h = micromoles per kilogram per hour

Table H.24. Phenylalanine requirement estimates RCTs pregnant people

Study (PMID)	Statistics/Confounders	Phenylalanine Requirement Estimate	Data Used to Calculate Phenylalanine Requirement Estimate
Ennis, 2020[22] (31758682)	Statistics: Biphase linear regression crossover analysis, with subject as a random variable; Pooled 2-sample t test Confounders adjusted for: None	**Outcome: Phenylalanine requirement estimate calculated from $F^{13}CO_2$ (Early Gestation DAAO Method)** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 9, 26 total observations Protein amount: 1.5 g/kg/d protein Amino Acid amount: 5-30.5 mg/kg/d phenylalanine, 61 mg/kg/d tyrosine Breakpoint: 15.14 mg/kg/d Upper 95% CI: 19.9 mg/kg/d Lower 95% CI: 10.4 mg/kg/d r^2 = 0.87 **Outcome: Phenylalanine**	**Outcome: $F^{13}CO_2$ (Early Gestation DAAO Method)** Outcome parameter: NA Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 9, 26 total observations Protein amount: 1.5 g/kg/d protein Amino Acid amount: 5-30.5 mg/kg/d phenylalanine, 61 mg/kg/d tyrosine Data reported in figures of original paper **Outcome: $F^{13}CO_2$ (Late Gestation DAAO Method)** Outcome parameter: NA Timepoint: Data obtained following each study d (d 3)

Study (PMID)	Statistics/Confounders	Phenylalanine Requirement Estimate	Data Used to Calculate Phenylalanine Requirement Estimate
		requirement estimate calculated from $F^{13}CO_2$ (Late Gestation DAAO Method) Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 9, 25 total observations Protein amount: 1.5 g/kg/d protein Amino Acid amount: 5-30.5 mg/kg/d phenylalanine, 61 mg/kg/d tyrosine Breakpoint: 21.05 mg/kg/d Upper 95% CI: 24.7 mg/kg/d Lower 95% CI: 17.4 mg/kg/d $r^2 = 0.79$ **Outcome: Phenylalanine requirement estimate calculated from $F^{13}CO_2$ (late gestation IAAO method)** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 13, 25 total observations Protein amount: 1.5 g/kg/d protein Amino Acid amount: 2.5-30.5 mg/kg/d phenylalanine, 61 mg/kg/d tyrosine	Total (N analyzed): 9, 25 total observations Protein amount: 1.5 g/kg/d protein Amino Acid amount: 5-30.5 mg/kg/d phenylalanine, 61 mg/kg/d tyrosine Data reported in figures of original paper **Outcome: $F^{13}CO_2$ (Late Gestation IAAO Method)** Outcome parameter: NA Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 13, 25 total observations Protein amount: 1.5 g/kg/d protein Amino Acid amount: 2.5-30.5 mg/kg/d phenylalanine, 61 mg/kg/d tyrosine Data reported in figures of original paper

Study (PMID)	Statistics/Confounders	Phenylalanine Requirement Estimate	Data Used to Calculate Phenylalanine Requirement Estimate
		Breakpoint: 21.36 mg/kg/d Upper 95% CI: 32.2 mg/kg/d Lower 95% CI: 10.5 mg/kg/d $r^2 = 0.37$ Comparisons and p-values Comparison of the mean requirement estimates obtained by the DAAO method found a significant difference ($p<0.0001$) between gestation stages	
Ennis, 2020[21] (33188409)	Statistics: Biphase linear regression crossover analysis with subject as a random variable. Confounders adjusted for: None	**Outcome: Total aromatic amino acid requirement estimate calculated from $F^{13}CO_2$ (Early Gestation)** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 10, 24 total observations Protein amount: 1.5 g/kg/d protein Amino Acid amount: 5-100 mg/kg/d phenylalanine, 0 mg/kg/d tyrosine Breakpoint: 43.57 mg/kg/d Upper 95% CI: 58.8 mg/kg/d Lower 95% CI: 28.3 mg/kg/d $r^2 = 0.56$ **Outcome: Total aromatic amino acid requirement estimate calculated from $F^{13}CO_2$ (Late Gestation)**	**Outcome: $F^{13}CO_2$ (Early Gestation)** Outcome parameter: NA Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 10, 24 total observations Protein amount: 1.5 g/kg/d protein Amino Acid amount: 5-100 mg/kg/d phenylalanine, 0 mg/kg/d tyrosine Data reported in figures of original paper **Outcome: $F^{13}CO_2$ (Late Gestation)** Outcome parameter: NA Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 10, 27 total observations

Study (PMID)	Statistics/Confounders	Phenylalanine Requirement Estimate	Data Used to Calculate Phenylalanine Requirement Estimate
		Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 10, 27 total observations Protein amount: 1.5 g/kg/d protein Amino Acid amount: 5-100 mg/kg/d phenylalanine, 0 mg/kg/d tyrosine Breakpoint: 49.56 mg/kg/d Upper 95% CI: 63.1 mg/kg/d Lower 95% CI: 36.1 mg/kg/d $r^2 = 0.67$ Comparisons and p-values Comparison of early and late stage mean requirements showed a significant difference ($p<0.01$)	Protein amount: 1.5 g/kg/d protein Amino Acid amount: 5-100 mg/kg/d phenylalanine, 0 mg/kg/d tyrosine Data reported in figures of original paper

Abbreviations: CI = confidence interval; DAAO = direct amino acid oxidation; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; leucine or phenylalanine]; g/kg/d = grams per kilogram per day; mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; SD = standard deviation; TAA = total amino acid

Table H.25. Phenylalanine requirement estimates RCTs adults (19-50 years)

Study (PMID)	Statistics/Confounders	Phenylalanine Requirement Estimate	Data Used to Calculate Phenylalanine Requirement Estimate	Data Used to Calculate Phenylalanine Requirement Estimate
Hsu, 2006[24] (16400054)	Statistics: Two-phase linear regression crossover model; ANOVA with subject as a random effect Confounders adjusted for: None	**Outcome: Aromatic amino acid requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, upper 95% CI, lower 95% CI	**Outcome: $F^{13}CO_2$** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (d 3)	NA

Study (PMID)	Statistics/Confounders	Phenylalanine Requirement Estimate	Data Used to Calculate Phenylalanine Requirement Estimate	Data Used to Calculate Phenylalanine Requirement Estimate
		Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 5; 39 total observations Protein amount: 1.0 g/kg/d Amino acid amount: 5-70 mg/kg/d phenylalanine; 0 mg/kg/d tyrosine Model 5 vs 3: Breakpoint: 43.73 mg/kg/d Upper 95% CI: NR Lower 95% CI: NR Model 5 vs 3 indicates that 5 phenylalanine intakes were on one line and 3 phenylalanine intakes were on the other. r^2 = 0.290, p=0.002; The mean standard error was 0.052, the standard error of choose was 0.180 and the CV was 17.298 Model 6 vs 2: Breakpoint: 51.71 mg/kg/d Upper 95% CI: NR Lower 95% CI: NR Model 6 vs 2 indicates that 6 phenylalanine intakes were on one line and 2 phenylalanine intakes were on the other. r^2 = 0.295, p=0.002; The mean standard error was 0.052, the standard error of choose was 0.109 and the SV was 17.240 Comparisons and p-values	Total (N analyzed): 5; per intake except 4 for 70 mg/kg/d phenylalanine Protein amount: 1.0 g/kg/d Amino acid amount: 5-70 mg/kg/d phenylalanine; 0 mg/kg/d tyrosine 5 mg/kg/d phenylalanine: 1.566; SD 0.302 µmol/kg/h 10 mg/kg/d phenylalanine: 1.443; SD 0.255 µmol/kg/h 15 mg/kg/d phenylalanine: 1.399; SD 0.308 µmol/kg/h 25 mg/kg/d phenylalanine: 1.365; SD 0.224 µmol/kg/h 35 mg/kg/d phenylalanine: 1.245; SD 0.269 µmol/kg/h 45 mg/kg/d phenylalanine: 1.216; SD 0.222 µmol/kg/h 60 mg/kg/d phenylalanine: 1.121; SD 0.127 µmol/kg/h 70 mg/kg/d phenylalanine: 1.154; SD 0.151 µmol/kg/h Comparisons and p-values Phenylalanine intake significantly affected $F^{13}CO_2$ (P=0.02). There was a significant difference between $F^{13}CO_2$ for 5 mg/kg/d phenylalanine and 60 mg/kg/d phenylalanine (P<0.05). There was no difference between 5, 10, 15, 25, 35, 45 or 70 mg/kg/d phenylalanine intakes. Additionally, there	

Study (PMID)	Statistics/Confounders	Phenylalanine Requirement Estimate	Data Used to Calculate Phenylalanine Requirement Estimate	Data Used to Calculate Phenylalanine Requirement Estimate
		The average aromatic amino acid requirement based on the average of model 5 vs 3 and 6 vs 2 = 48 mg/kg/d.	was no difference between 10, 15, 25, 35, 45, 60, and 70 mg/kg/d phenylalanine intakes.	
Hsu, 2006[25] (16549457)	Statistics: Mixed procedure of SAS (version 8.2, SAS institute) followed by a 2-phase linear regression crossover model; Proc mixed with subject included as a random effect Confounders adjusted for: None	**Outcome: Aromatic amino acid requirement estimate calculated from $F^{13}CO_2$ (Part A)** Outcome parameter: Breakpoint, upper 95% CI, lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 5, 35 total observations Protein amount: 1.0 g/kg/d Amino acid amount: 5-60 mg/kg/d phenylalanine; 0 mg/kg/d tyrosine Breakpoint analysis and aromatic amino acid requirement estimate unable to be calculated r^2=0.29; the ideal IAAO method pattern of the partition of any indispensable amino acid between oxidation and protein synthesis did not occur with the higher daily leucine intake in Part A. The $F^{13}CO_2$ remained steady and was reduced only when the phenylalanine intake was >35 mg/kg/d.	**Outcome: $F^{13}CO_2$ (Part A)** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 5 per intake level Protein amount: 1.0 g/kg/d Amino acid amount: 5-60 mg/kg/d phenylalanine; 0 mg/kg/d tyrosine 5 mg/kg/d phenylalanine: 2.13; SD 0.24 µmol/kg/h 10 mg/kg/d phenylalanine: 2.22; SD 0.25 µmol/kg/h 15 mg/kg/d phenylalanine: 2.18; SD 0.51 µmol/kg/h 25 mg/kg/d phenylalanine: 2.2; SD 0.44 µmol/kg/h 35 mg/kg/d phenylalanine: 1.99; SD 0.18 µmol/kg/h 45 mg/kg/d phenylalanine: 1.96; SD 0.19 µmol/kg/h 60 mg/kg/d phenylalanine: 1.88; SD 0.31 µmol/kg/h Comparisons and p-values Overall, for Part A phenylalanine intake did not affect enrichment of expired	NA

Study (PMID)	Statistics/Confounders	Phenylalanine Requirement Estimate	Data Used to Calculate Phenylalanine Requirement Estimate	Data Used to Calculate Phenylalanine Requirement Estimate
		Outcome: Aromatic amino acid requirement estimate calculated from $F^{13}CO_2$ (Part B) Outcome parameter: Breakpoint; SE, upper 95% CI, lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 5 per intake level Protein amount: 1.0 g/kg/d Amino acid amount: 5-65 mg/kg/d phenylalanine; 0 mg/kg/d tyrosine Breakpoint: 41.9; SE 16 mg/kg/d Upper 95% CI: NR Lower 95% CI: NR r^2=0.17, p=0.048	$^{13}CO_2$ ($F^{13}CO_2$) (P=0.23) **Outcome: $F^{13}CO_2$ (Part B)** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 5 per intake level Protein amount: 1.0 g/kg/d Amino acid amount: 5-65 mg/kg/d phenylalanine; 0 mg/kg/d tyrosine 5 mg/kg/d phenylalanine: 1.62; SD 0.18 µmol/kg/h 15 mg/kg/d phenylalanine: 1.75; SD 0.21 µmol/kg/h 25 mg/kg/d phenylalanine: 1.66; SD 0.44 µmol/kg/h 35 mg/kg/d phenylalanine: 1.53; SD 0.43 µmol/kg/h 45 mg/kg/d phenylalanine: 1.36; SD 0.21 µmol/kg/h 55 mg/kg/d phenylalanine: 1.49; SD 0.33 µmol/kg/h 65 mg/kg/d phenylalanine: 1.41; SD 0.19 µmol/kg/h Comparisons and p-values Overall, for Part B, Phenylalanine intake did not affect enrichment of expired $^{13}CO_2$ ($F^{13}CO_2$) (P=0.06)	
Kurpad, 2006[35] (16762944)	Statistics: 2-phase linear random-effects regression	**Outcome: Aromatic amino acid requirement estimate**	**Outcome: Leucine oxidation (12-h fed and 24-**	**Outcome: 24-h Leucine balance**

Study (PMID)	Statistics/Confounders	Phenylalanine Requirement Estimate	Data Used to Calculate Phenylalanine Requirement Estimate	Data Used to Calculate Phenylalanine Requirement Estimate
	models Confounders adjusted for: None	**calculated from 24-h IAAO** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (24-h, d 7) Total (N analyzed): 32, 64 total observations Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 19-47 mg/kg/d phenylalanine, 0 mg/kg/d tyrosine Breakpoint: 37 mg/kg/d Upper 95% CI: >47 mg/kg/d Lower 95% CI: 31 mg/kg/d **Outcome: Aromatic amino acid requirement estimate calculated from 12-h fed IAAO** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Total (N analyzed): 32, 64 total observations Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 19-47 mg/kg/d phenylalanine, 0 mg/kg/d tyrosine	**h)** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (12-h fed, d 7) Total (N analyzed): 8 per intake level Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 19-47 mg/kg/d phenylalanine, 0 mg/kg/d tyrosine 19 mg/kg/d phenylalanine: 26; SD 4.5 mg/kg/d 23 mg/kg/d phenylalanine: 23.4; SD 3.3 mg/kg/d 27 mg/kg/d phenylalanine: 22.8; SD 4.4 mg/kg/d 31 mg/kg/d phenylalanine: 23.3; SD 4.1 mg/kg/d 35 mg/kg/d phenylalanine: 20.9; SD 5.4 mg/kg/d 38 mg/kg/d phenylalanine: 20; SD 4.1 mg/kg/d 43 mg/kg/d phenylalanine: 23; SD 1.9 mg/kg/d 47 mg/kg/d phenylalanine: 20; SD 2.1 mg/kg/d Comparisons and p-values 12-h fed leucine oxidation at phenylalanine intakes of 19, 23, and 27 mg/kg/d was significantly greater than	Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (12-h, d 7) Total (N analyzed): 8 per intake level Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 19-47 mg/kg/d phenylalanine, 0 mg/kg/d tyrosine 19 mg/kg/d phenylalanine: -9.3; SD 8.6 mg/kg/d 23 mg/kg/d phenylalanine: -4.9; SD 4.9 mg/kg/d 27 mg/kg/d phenylalanine: -3.8; SD 5 mg/kg/d 31 mg/kg/d phenylalanine: -2.9; SD 8.7 mg/kg/d 35 mg/kg/d phenylalanine: -2; SD 5 mg/kg/d 38 mg/kg/d phenylalanine: 1.2; SD 6.1 mg/kg/d 43 mg/kg/d phenylalanine: -1.5; SD 1.9 mg/kg/d 47 mg/kg/d phenylalanine: 0.8; SD 3.9 mg/kg/d Comparisons and p-values 24-h leucine balance was significantly lower than zero balance at phenylalanine intakes of 19, 23, 27 and 31 mg/kg/d ($p \le 0.01$), but was

Study (PMID)	Statistics/Confounders	Phenylalanine Requirement Estimate	Data Used to Calculate Phenylalanine Requirement Estimate	Data Used to Calculate Phenylalanine Requirement Estimate
		Breakpoint: 36 mg/kg/d Upper 95% CI: >47 mg/kg/d Lower 95% CI: 28 mg/kg/d **Outcome: Aromatic amino acid requirement estimate calculated from 24-h IAAB** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (12-h fed, d 7) Total (N analyzed): 32, 64 total observations Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 19-47 mg/kg/d phenylalanine, 0 mg/kg/d tyrosine Breakpoint: 38 mg/kg/d Upper 95% CI: > 47 mg/kg/d Lower 95% CI: 31 mg/kg/d	oxidation above the breakpoint (37 mg/kg/d) (P<0.01) but not at phenylalanine intakes of 31 and 35 mg/kg/d. Timepoint: Data obtained following each study d (24-h, d 7) Total (N analyzed): 8 per intake level Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino Acid amount: 19-47 mg/kg/d phenylalanine, 0 mg/kg/d tyrosine 19 mg/kg/d phenylalanine: 49.5; SD 8.5 mg/kg/d 23 mg/kg/d phenylalanine: 44.8; SD 5.7 mg/kg/d 27 mg/kg/d phenylalanine: 43.5; SD 4.7 mg/kg/d 31 mg/kg/d phenylalanine: 43; SD 8.2 mg/kg/d 35 mg/kg/d phenylalanine: 42.1; SD 5.1 mg/kg/d 38 mg/kg/d phenylalanine: 38.7; SD 5.8 mg/kg/d 43 mg/kg/d phenylalanine: 42; SD 2.3 mg/kg/d 47 mg/kg/d phenylalanine: 39.6; SD 4.1 mg/kg/d Comparisons and p-values 24-h leucine oxidation at phenylalanine intakes of 19, 23, and 27 mg/kg/d was	not significantly different from zero balance at phenylalanine intakes of 35 mg/kg/d. Leucine balance at intakes of 19, 23, 27, and 31 mg/kg/d were significantly lower than balance above the breakpoint ($p \leq 0.05$) but not at phenylalanine intakes of 35 mg/kg/d.

Study (PMID)	Statistics/Confounders	Phenylalanine Requirement Estimate	Data Used to Calculate Phenylalanine Requirement Estimate	Data Used to Calculate Phenylalanine Requirement Estimate
			significantly greater than oxidation above the breakpoint (37 mg/kg/d) ($P<0.05$) but not at phenylalanine intakes of 31 and 35 mg/kg/d.	

Abbreviations: CI = confidence interval; $F^{13}CO_2$ = 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 = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; SD = standard deviation; SE = standard error; µmol/kg/h = micromoles per kilogram per hour; vs = versus

Table H.26. Phenylalanine requirement estimates RCTs adults (51->70 years)

Study (PMID)	Statistics/Confounders	Phenylalanine Requirement Estimate	Data Used to Calculate Phenylalanine Requirement Estimate
Martin, 2019[43] (31271193)	**Statistics**: Mixed model 2-phase linear regression crossover analysis; 2 sample t test Confounders adjusted for: None	**Outcome: Phenylalanine requirement estimate calculated from $F^{13}CO_2$** Outcome parameters: breakpoint, upper 95% CI, lower 95% CI and mean requirement; SD Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 12 (6 men; 6 women) 66 total observations Protein amount: 1.0 g/kg/d Amino acid amount: 7.2-40 mg/kg/d phenylalanine, 40 mg/kg/d tyrosine Men: Breakpoint: 9.3 mg/kg/d Upper 95% CI: NR Lower 95% CI: NR Mean requirement: 11.9; SD 0.94 mg/kg FFM/d	**Outcome: $F^{13}CO_2$** Outcome parameters: NA Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 12 (6 men; 6 women) 66 total observations Protein amount: 1.0 g/kg/d Amino acid amount: 7.2-40 mg/kg/d phenylalanine, 40 mg/kg/d tyrosine Data reported in figures of original paper

Study (PMID)	Statistics/Confounders	Phenylalanine Requirement Estimate	Data Used to Calculate Phenylalanine Requirement Estimate
		Women: Breakpoint: 8.4 mg/kg/d Upper 95% CI: NR Lower 95% CI: NR Mean requirement: 12.8; SD 0.94 mg/kg FFM/d Combined: Breakpoint: 9.03 mg/kg/d Upper 95% CI: 15.9 mg/kg/d Lower 95% CI: NR $r^2 = 0.79$, $p<0.01$ Comparisons and p-values No difference in requirement estimates between men and women on a body weight basis (p=0.98) or on a FFM basis (p-0.11).	

Abbreviations: CI = confidence interval; d = day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; FFM/d = fat-free mass per day; g/kg/d = grams per kilogram per day; mg/kg/d = milligrams per Kilogram per Day; N = number; NR = not reported; PMID = PubMed Identification Number; SD = standard deviation

Threonine

Table H.27. Threonine requirement estimates RCTs infants

Study	Statistics/Confounders	Threonine Requirement Estimate	Data Used to Calculate Threonine Requirement Estimate
Hogewind-Schoonenboom, 2015[3] (25844708)	Statistics: Two-phase linear regression crossover model; two-factor general linear model Confounders adjusted for: None	**Outcome: Threonine requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: data obtained following each study d (d 2) Total (N analyzed): 32 Protein amount: 2.96 g/kg/d Amino Acid amount: 5-182 mg/kg/d threonine Breakpoint: 68 mg/kg/d Upper 95% CI: 104 mg/kg/d Lower 95% CI: 32 mg/kg/d $r^2 = 0.37$	**Outcome: $F^{13}CO_2$** Outcome parameter: NA Timepoint: data obtained following each study d (d 2) Total (N analyzed): 32 Protein amount: 2.96 g/kg/d Amino Acid amount: 5-182 mg/kg/d threonine Data reported in figures of original paper Comparisons and p-values Threonine intake had a significant effect on $F^{13}CO_2$ (p=0.012).

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 = milligrams per kilogram per day; N = number; NA = not applicable; PMID = PubMed Identification Number

Table H.28. Threonine requirement estimates RCTs adults (19-50 years)

Study	Statistics/Confounders	Threonine Requirement Estimate	Data Used to Calculate Threonine Requirement Estimate	Data Used to Calculate Threonine Requirement Estimate	Data Used to Calculate Threonine Requirement Estimate
Kurpad, 2002[32] (12324292)	Statistics: Two-phase linear regression model; Mixed-models ANOVA Confounders adjusted for: None	**Outcome: Threonine requirement estimate calculated from 24-h IAAO** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each	**Outcome: Leucine oxidation (24-h and 12-h fed)** Outcome parameter: Mean; SD Timepoint: data obtained following each study d (24-h, d 7)	**Outcome: 24-h Leucine balance** Outcome parameter: Mean; SD Timepoint: data obtained following each study d (24-h, d 7) Total (N analyzed): 16	**Outcome: Plasma amino acid response (fasted, 3-h fed, and 6-h fed)** Outcome parameter: Mean; SD Timepoint: data obtained following each study d (fasted, d 7)

Study	Statistics/Confounders	Threonine Requirement Estimate	Data Used to Calculate Threonine Requirement Estimate	Data Used to Calculate Threonine Requirement Estimate	Data Used to Calculate Threonine Requirement Estimate
		study d (24-h, d 7) Total (N analyzed): 6; 48 total observations Protein amount: 1.0 g/kg/d (160 mg /kg/d Nitrogen) Amino Acid amount: 7-27 mg/kg/d threonine Breakpoint: 15 mg/kg/d Upper 95% CI: 25 mg/kg/d Lower 95% CI: 11 mg/kg/d **Outcome: Threonine requirement estimate calculated from 12-h fed IAAO** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (24-h, d 7) Total (N analyzed): 16; 48 total observations Protein amount: 1.0 g/kg/d (160 mg nitrogen/kg/d) Amino Acid amount: 7-27 mg/kg/d threonine Breakpoint: 15 mg/kg/d Upper 95% CI: ND Lower 95% CI: ND	Total (N analyzed): 16 (4-12 per intake level) Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino acid amount: 7-27 mg/kg/d threonine 7 mg/kg/d threonine: 49.7; SD 5.8 mg/kg/d 11 mg/kg/d threonine: 46.5; SD 3.7 mg/kg/d 15 mg/kg/d threonine: 42.8; SD 5 mg/kg/d 19 mg/kg/d threonine: 43.4; SD 6.7 mg/kg/d 22 mg/kg/d threonine: 44.4; SD 3.8 mg/kg/d 27 mg/kg/d threonine: 41.7; SD 3 mg/kg/d Comparisons and p-values 15, 19, and 22 mg/kg/d threonine 24-h IAAO were significantly different from 7 and 11 mg/kg/d threonine intakes ($p<0.05$ for all). There was a significant interaction between metabolic period and threonine intake	(4-12 per intake level) Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino acid amount: 7-27 mg/kg/d threonine 7 mg/kg/d threonine: -9.3; SD 5.8 mg/kg/d 11 mg/kg/d threonine: -6.3; SD 3.8 mg/kg/d 15 mg/kg/d threonine: -1.4; SD 5.7 mg/kg/d 19 mg/kg/d threonine: -2.5; SD 7.3 mg/kg/d 22 mg/kg/d threonine: -3.8; SD 4.2 mg/kg/d 27 mg/kg/d threonine: -0.7; SD 3.2 mg/kg/d Comparisons and p-values 15, 19, and 27 mg/kg/d threonine 24-h IAAB was significantly different from 7 mg/kg/d ($p<0.05$ for all) 24-h IAAB was significantly different from 0 at 7 and 11 mg/kg/d threonine intakes ($p<0.01$ for both)	 Total (N analyzed): 16 (4-12 per intake level) Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino acid amount: 7-27 mg/kg/d threonine 7 mg/kg/d threonine: 48; SD 10 μmol/L 11 mg/kg/d threonine: 72; SD 12 μmol/L 15 mg/kg/d threonine: 58; SD 11 μmol/L 19 mg/kg/d threonine: 85; SD 41 μmol/L 22 mg/kg/d threonine: 74; SD 27 μmol/L 27 mg/kg/d threonine: 98; SD 34 μmol/L Outcome parameter: Mean; SD Timepoint: data obtained following each study d (3-h fed d 7) Total (N analyzed): 16 (4-12 per intake level) Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein)

Study	Statistics/Confounders	Threonine Requirement Estimate	Data Used to Calculate Threonine Requirement Estimate	Data Used to Calculate Threonine Requirement Estimate	Data Used to Calculate Threonine Requirement Estimate
		Outcome: Threonine requirement estimate calculated from 24-h IAAB Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (24-h, d 7) Total (N analyzed): 16; 48 total observations Protein amount: 1.0 g/kg/d (160 mg/kg/d Nitrogen) Amino Acid amount: 7-27 mg/kg/d threonine Breakpoint: 15 mg/kg/d Upper 95% CI: 27 mg/kg/d Lower 95% CI: 11 mg/kg/d **Outcome: Threonine requirement estimate calculated from fasted plasma amino acid response** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (24-h, d 7)	(p=0.03). Outcome parameter: Mean; SD Timepoint: data obtained following each study d (12-h fed, d 7) Total (N analyzed): 16 (4-12 per intake level) Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino acid amount: 7-27 mg/kg/d threonine 7 mg/kg/d threonine: 24.3; SD 3.5 mg/kg/d 11 mg/kg/d threonine: 23.3; SD 2.6 mg/kg/d 15 mg/kg/d threonine: 21.1; SD 3.2 mg/kg/d 19 mg/kg/d threonine: 22.4; SD 4.1 mg/kg/d 22 mg/kg/d threonine: 22.5; SD 4.6 mg/kg/d 27 mg/kg/d threonine: 20.2; SD 2.1 mg/kg/d Comparisons and p-values 15 and 22 mg/kg/d threonine 12-h fed	Daily leucine balance was significantly affected by threonine intake (p=0.02)	Amino acid amount: 7-27 mg/kg/d threonine 7 mg/kg/d threonine: 64; SD 29 µmol/L 11 mg/kg/d threonine: 66; SD 10 µmol/L 15 mg/kg/d threonine: 72; SD 9 µmol/L 19 mg/kg/d threonine: 90; SD 39 µmol/L 22 mg/kg/d threonine: 90; SD 24 µmol/L 27 mg/kg/d threonine: 120; SD 25 µmol/L Outcome parameter: Mean; SD Timepoint: data obtained following each study d (6-h fed d 7) Total (N analyzed): 16 (4-12 per intake level) Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino acid amount: 7-27 mg/kg/d threonine 7 mg/kg/d threonine: 71; SD 27 µmol/L

Study	Statistics/Confounders	Threonine Requirement Estimate	Data Used to Calculate Threonine Requirement Estimate	Data Used to Calculate Threonine Requirement Estimate	Data Used to Calculate Threonine Requirement Estimate
		Total (N analyzed): 6; 48 total observations Protein amount: 1.0 g/kg/d (160 mg/kg/d Nitrogen) Amino Acid amount: 7-27 mg/kg/d threonine Breakpoint: 15 mg/kg/d Upper 95% CI: ND Lower 95% CI: ND **Outcome: Threonine requirement estimate calculated from 3-h fed plasma amino acid response** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (24-h, d 7) Total (N analyzed): 16; 48 total observations Protein amount: 1.0 g/kg/d (160 mg/kg/d Nitrogen) Amino Acid amount: 7-27 mg/kg/d threonine Breakpoint: 13 mg/kg/d Upper 95% CI: 18 mg/kg/d Lower 95% CI: 3 mg/kg/d	IAAO was significantly different from 7 mg/kg/d ($p<0.05$ for both)		11 mg/kg/d threonine: 72; SD 15 µmol/L 15 mg/kg/d threonine: 72; SD 22 µmol/L 19 mg/kg/d threonine: 103; SD 43 µmol/L 22 mg/kg/d threonine: 96; SD 17 µmol/L 27 mg/kg/d threonine: 120; SD 11 µmol/L

Study	Statistics/Confounders	Threonine Requirement Estimate	Data Used to Calculate Threonine Requirement Estimate	Data Used to Calculate Threonine Requirement Estimate	Data Used to Calculate Threonine Requirement Estimate
		Outcome: Threonine requirement estimate calculated from 6-h fed plasma amino acid response Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (24-h, d 7) Total (N analyzed): 6; 48 total observations Protein amount: 1.0 g/kg/d (160 mg/kg/d Nitrogen) Amino Acid amount: 7-27 mg/kg/d threonine Breakpoint: 13 mg/kg/d Upper 95% CI: 19 mg/kg/d Lower 95% CI: 1 mg/kg/d			
Wilson, 2000[50] (10702170)	Statistics: Two-phase linear regression crossover model; Two-factor general linear model Confounders adjusted for: None	**Outcome: Threonine requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: data obtained following each study d (d 3) Total (N analyzed): 6, 36	**Outcome: $F^{13}CO_2$** Outcome parameter: NA Timepoint: data obtained following each study d (d 3) Total (N analyzed): 6, 36 total observations Protein amount: 1.0	NA	NA

Study	Statistics/Confounders	Threonine Requirement Estimate	Data Used to Calculate Threonine Requirement Estimate	Data Used to Calculate Threonine Requirement Estimate	Data Used to Calculate Threonine Requirement Estimate
		total observations Protein amount: 1.0 g/kg/d protein Amino Acid amount: 5-35 mg/kg/d threonine Breakpoint: 19 mg/kg/d Upper 95% CI: 26.2 mg/kg/d Lower 95% CI: NR	g/kg/d protein Amino Acid amount: 5-35 mg/kg/d threonine Data reported in figures of original paper Comparisons and p-values Threonine intake had a significant effect on $F^{13}CO_2$ ($p=0.002$).		

Abbreviations: 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; mg/kg/d = milligrams per kilogram per day; N = number; ND = not determined; NR = not reported; PMID = PubMed Identification Number; SD = standard deviation; μmol/L = micromoles per liter

Total Branched Chain Amino Acids

Table H.29. Total branched chain amino acids requirement estimates RCTs children and adolescents

Study (PMID)	Statistics/Confounders	Total Branched Chain Amino Acid Requirement Estimate	Data Used to Calculate Total Branched Chain Amino Acid Requirement Estimate
Mager, 2003[39] (14608071)	Statistics: Two-phase linear regression crossover model; Three-factor general linear model ANOVA Confounders adjusted for: None	**Outcome: Total BCAA requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 5; 35 total observations Protein amount: ~10% of energy from protein Amino Acid amount: 75-225 mg/kg/d	**Outcome: $F^{13}CO_2$** Outcome parameter: Mean; SD Timepoint: Data obtained following each study d (d 3) Total (N analyzed) 5 per intake level Protein amount: ~10% of protein as energy Amino Acid amount: 75-225 mg/kg/d BCAA 75 mg/kg/d BCAA: 1.02; SD 0.16

Study (PMID)	Statistics/Confounders	Total Branched Chain Amino Acid Requirement Estimate	Data Used to Calculate Total Branched Chain Amino Acid Requirement Estimate
		BCAA Breakpoint: 147.3 mg/kg/d Upper 95% CI: 191.5 mg/kg/d Lower 95% CI: 103.5 mg/kg/d $r^2 = 0.25$, $p<0.01$	µmol/kg/h 85 mg/kg/d BCAA: 1.01; SD 0.25 µmol/kg/h 100 mg/kg/d BCAA: 1.0; SD 0.24 µmol/kg/h 125 mg/kg/d BCAA: 0.93; SD 0.24 µmol/kg/h 150 mg/kg/d BCAA: 0.77; SD 0.12 µmol/kg/h 200 mg/kg/d BCAA: 0.84; SD 0.04 µmol/kg/h 225 mg/kg/d BCAA: 0.77; SD 0.27 µmol/kg/h Comparisons and p-values $F^{13}CO_2$ production was related to total BCAA intake (P=0.0137) and affected by individual study subject (P=0.012).

Abbreviations: ANOVA = analysis of variance; BCAA = branched chain amino acid; CI = confidence interval; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; mg/kg/d = milligrams per kilogram per day; N = number; NR = not reported; PMID = PubMed Identification Number; SD = standard deviation; µmol/kg/h = micromoles kilograms per hour

Table H.30. Total branched chain amino acids requirement not calculated; phenylalanine oxidation and $F^{13}CO_2$ RCTs adults (19-50 years)

Study (PMID)	Statistics/Confounders	$F^{13}CO_2$	Phenylalanine Oxidation
Riazi, 2003[53] (14608070)	Statistics: Repeated-measures ANOVA; Student-Newman-Keuls; Orthogonal contrast Confounders adjusted for: None	**Outcome: $F^{13}CO_2$** Outcome parameter: Mean; SD, Mean difference from the breakpoint Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 5; 35 total observations; 30 from this study and 5 brought from the previous study[52] Protein amount: 1.0 g/kg/d Amino Acid amount:	**Outcome: Phenylalanine oxidation** Outcome parameter: Mean; SD, Mean difference from the breakpoint Timepoint: Data obtained following each study d (d 3) Total (N analyzed): 5; 35 total observations; 30 from this study and 5 brought from the previous study[52] Protein amount: 1.0 g/kg/d Amino Acid amount:

Study (PMID)	Statistics/Confounders	$F^{13}CO_2$	Phenylalanine Oxidation
		Isoleucine provided at requirement amount and leucine and valine provided at 10 and 20% less than the requirement (Ile constant). Leucine provided at requirement amount and isoleucine and valine provided at 10 and 20% less than the requirement (Leu constant). Valine provided at requirement amount and isoleucine and leucine provided at 10 and 20% less than the requirement (Val constant). Visual breakpoint: 0.45; SD 0.099 umol/kg/h Ile constant –10%: 0.58; SD 0.10 umol/kg/h 27.3% mean difference Ile constant –20%: 0.65; SD 0.08 umol/kg/h 42.7% mean difference Leu constant-10%: 0.60; SD 0.09 umol/kg/h 31.3% mean difference Leu constant-20%: 0.61; SD 0.13 umol/kg/h 35.2% mean difference Val constant –10%: 0.58; SD 0.16 umol/kg/h 27.3% mean difference Val constant –20%: 0.59; SD 0.21 umol/kg/h 30.4% mean difference Comparison and p-values	Isoleucine provided at requirement amount and leucine and valine provided at 10 and 20% less than the requirement. Leucine provided at requirement amount and isoleucine and valine provided at 10 and 20% less than the requirement. Valine provided at requirement amount and isoleucine and leucine provided at 10 and 20% less than the requirement. Visual breakpoint: 2.89; SD 1.14 umol/kg/h Ile constant –10%: 3.98; SD 0.94 umol/kg/h 37.7% mean difference Ile constant –20%: 4.93; SD 1.11 umol/kg/h 70.5% mean difference Leu constant-10%: 4.08; SD 1.70 umol/kg/h 41.2% mean difference Leu constant-20%: 4.79; SD 1.23 umol/kg/h 65.7% mean difference Val constant –10%: 3.94; SD 1.53 umol/kg/h 36.3% mean difference Val constant –20%: 4.02; SD 0.70 umol/kg/h 39% mean difference Comparison and p-values

Study (PMID)	Statistics/Confounders	$F^{13}CO_2$	Phenylalanine Oxidation
		Ile constant: Significant difference in $F^{13}CO_2$ compared to the visual breakpoint after a 20% reduction ($p=0.007$) but not 10% ($p>0.058$) and no difference between 10% and 20% was observed ($p=0.27$) Leu constant: Significant difference in $F^{13}CO_2$ compared to the visual breakpoint after a 20% reduction ($p=0.038$) but not 10% ($p=0.059$) and no difference between 10% and 20% was observed ($p=0.81$). Val constant: No significant difference in $F^{13}CO_2$ compared to the visual breakpoint after 10 and 20% reduction ($p=0.025$, $p=0.199$) and no difference between 10% and 20% was observed ($p=0.88$).	Ile constant: Significant difference in phenylalanine oxidation compared to the visual breakpoint after a 20% reduction ($p=0.012$) but not 10% ($p=0.158$) and no difference between 10% and 20% was observed ($p=0.223$) Leu constant: Significant difference in phenylalanine oxidation compared to the visual breakpoint after a 20% reduction ($p=0.018$) but not 10% ($p=0.125$) and no difference between 10% and 20% was observed ($p=0.358$). Val constant: No significant difference in $F^{13}CO_2$ compared to the visual breakpoint after 10 and 20% reduction ($p=0.176$, $p=0.144$) and no difference between 10% and 20% was observed ($p=0.909$).

Abbreviations: ANOVA = analysis of variance; d = day; g/kg/d = grams per kilogram per day; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; Ile = isoleucine; Leu = leucine PMID = PubMed Identification Number; SD = standard deviation; umol/kg/hr = micromole per kilogram per hour; Val = valine

Tryptophan

Table H.31. Tryptophan requirement estimates RCTs infants

Study (PMID)	Statistics/Confounders	Tryptophan Requirement Estimate	Data Used to Calculate Tryptophan Requirement Estimate
Huang, 2014[7] (24824360)	Statistics: Biphasic linear regression crossover model Confounders adjusted for: None	**Outcome: Tryptophan requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 2) Total (N analyzed): 30 Protein amount: 2.98; SD 0.01 g/kg/d Amino Acid amount: 5-73 mg/kg/d tryptophan Breakpoint: 15 mg/kg/d Upper 95% CI: 31 mg/kg/d Lower 95% CI: NR r^2=0.17	**Outcome: $F^{13}CO_2$** Outcome parameter: NA Timepoint: Data obtained following each study d (d 2) Total (N analyzed): 30 Protein amount: 2.96 g/kg/d Amino Acid amount: 5-73 mg/kg/d tryptophan Data reported in figures of original paper

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 = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number

Valine

Table H.32. Valine requirement estimates RCTs infants

Study (PMID)	Statistics/Confounders	Valine Requirement Estimate	Data Used to Calculate Valine Requirement Estimate
de Groof, 2014[2] (24284437)	Statistics: 2-phase regression model adjusted to account for repeated measure Confounders adjusted for: None	**Outcome: Valine requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: Data obtained following each study d (d 2) *Total (N analyzed): 28 subjects and 29 observations Protein amount: average 2.96; SD 0.15 g/kg/d Amino Acid amount: 5-236 mg/kg/d valine Breakpoint: 110 mg/kg/d Upper 95% CI: 164 mg/kg/d Lower 95% CI: 56 mg/kg/d $r^2 = 0.35$, $p=0.001$	**Outcome: $F^{13}CO_2$** Outcome parameter: NA Timepoint: Data obtained following each study d (d 2) *Total (N analyzed): 28 subjects and 29 observations Protein amount: 2.96; SD 0.15 g/kg/d Amino Acid amount: 5-236 mg/kg/d valine Data reported in figures of original paper

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 = milligrams per kilogram per day; N = number; NA = not applicable; NR = not reported; PMID = PubMed Identification Number
*One subject was studied at 2 different intakes and all others were studied at one intake.

Table H.33. Valine requirement estimate RCTs adults (19-50 years)

Study (PMID)	Statistics/Confounders	Valine Requirement Estimate	Data Used to Calculate Valine Requirement Estimate	Data Used to Calculate Valine Requirement Estimate	Data Used to Calculate Valine Requirement Estimate	Data Used to Calculate Valine Requirement Estimate
Kurpad, 2005[34] (16087981)	Statistics: Two-phase linear random effects regression models	**Outcome: Valine requirement estimate calculated from 24-h IAAO**	**Outcome: 24-h phenylalanine oxidation** Outcome parameter: Mean;	**Outcome: 12-h fed phenylalanine oxidation** Outcome parameter: Mean;	**Outcome: 24-h phenylalanine balance** Outcome parameter: Mean;	**Outcome: $F^{13}CO_2$ (rate of phenylalanine oxidation in the fed state)**

Study (PMID)	Statistics/Confounders	Valine Requirement Estimate	Data Used to Calculate Valine Requirement Estimate	Data Used to Calculate Valine Requirement Estimate	Data Used to Calculate Valine Requirement Estimate	Data Used to Calculate Valine Requirement Estimate
	Confounders adjusted for: None	Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: data obtained following each study d (d 7) Total (N analyzed): 18; 54 total observations Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino acid amount: 5-35 mg/kg/d valine Breakpoint: 17 mg/kg/d Upper 95% CI: ≥ 35 mg/kg/d Lower 95% CI: 11 mg/kg/d **Outcome: Valine requirement estimate calculated from 12-h fed IAAO** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: data	SD Timepoint: data obtained following each study d (24-h, d 7) Total (N analyzed): 18 (7-9 per intake level) Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino acid amount: 5-35 mg/kg/d valine 5 mg/kg/d valine: 35.1; SD 5.9 mg/kg/d 10 mg/kg/d valine: 31.8; SD 5.5 mg/kg/d 15 mg/kg/d valine: 29.7; SD 8.1 mg/kg/d 20 mg/kg/d valine: 26.7; SD 6.7 mg/kg/d 25 mg/kg/d valine: 28.3; SD 2.7 mg/kg/d 30 mg/kg/d valine: 31.5; SD 6.7 mg/kg/d	SD Timepoint: data obtained following each study d (12-h, d 7) Total (N analyzed): 18 (7-9 per intake level) Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino acid amount: 5-35 mg/kg/d valine 5 mg/kg/d valine: 19.2; SD 3.4 mg/kg/d 10 mg/kg/d valine: 17.1; SD 2.8 mg/kg/d 15 mg/kg/d valine: 15; SD 4.6 mg/kg/d 20 mg/kg/d valine: 13.2; SD 3.7 mg/kg/d 25 mg/kg/d valine: 13.8; SD 2 mg/kg/d 30 mg/kg/d valine: 15.2; SD 3.6 mg/kg/d 35 mg/kg/d valine:	SD Timepoint: data obtained following each study d (24-h, d 7) Total (N analyzed): 18 (7-9 per intake level) Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino acid amount: 5-35 mg/kg/d valine 5 mg/kg/d valine: 2.6; SD 5.4 mg/kg/d 10 mg/kg/d valine: 6.5; SD 6 mg/kg/d 15 mg/kg/d valine: 7.9; SD 7.8 mg/kg/d 20 mg/kg/d valine: 11.3; SD 7.7 mg/kg/d 25 mg/kg/d valine: 9.9; SD 2.5 mg/kg/d 30 mg/kg/d valine: 6.5; SD 7.5 mg/kg/d 35 mg/kg/d valine: 10.9; SD 5 mg/kg/d Comparisons and p-	Outcome parameter: Mean; SD Timepoint: data obtained following each study d (24-h, d 7) Total (N analyzed): 18 (7-9 per intake level) Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino acid amount: 5-35 mg/kg/d valine 5 mg/kg/d valine: 0.14; SD 0.03 10 mg/kg/d valine: 0.13; SD 0.03 15 mg/kg/d valine: 0.12; SD 0.02 20 mg/kg/d valine: 0.11; SD 0.04 25 mg/kg/d valine: 0.11; SD 0.03 30 mg/kg/d valine: 0.12; SD 0.04 35 mg/kg/d valine: 0.11; SD 0.03

Study (PMID)	Statistics/Confounders	Valine Requirement Estimate	Data Used to Calculate Valine Requirement Estimate	Data Used to Calculate Valine Requirement Estimate	Data Used to Calculate Valine Requirement Estimate	Data Used to Calculate Valine Requirement Estimate
		obtained following each study d (d 7) Total (N analyzed): 18, 54 observations Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino acid amount: 5-35 mg/kg/d valine Breakpoint: 18 mg/kg/d Upper 95% CI: ≥ 35 mg/kg/d Lower 95% CI: 13 mg/kg/d **Outcome: Valine requirement estimate calculated from 24-h IAAB** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: data obtained following each study d (d 7) Total (N analyzed): 18, 54 observations Protein amount: 160 mg/kg/d nitrogen	35 mg/kg/d valine: 26.4; SD 5.1 mg/kg/d Comparisons and p-values: 24-h phenylalanine oxidation at the 5 and 10 mg/kg/d valine intakes was significantly higher than oxidation above the breakpoint (17 mg/kg/d) ($p<0.05$) but the 15 mg/kg/d valine intake was not significantly different from oxidation above the breakpoint.	12.7; SD 2.2 mg/kg/d Comparisons and p-values: 12-h fed phenylalanine oxidation at the 5 and 10 mg/kg/d valine intakes was significantly higher than oxidation above the breakpoint (18 mg/kg/d) ($p<0.01$) but the 15 mg/kg/d valine intake was not significantly different from oxidation above the breakpoint.	values: Phenylalanine balance was not significantly different from zero balance at an intake of 5 mg/kg/d valine ($p=0.06$) but was significantly different from zero balance at the 10 and 15 mg/kg/d valine intakes and intakes above the breakpoint (17 mg/kg/d) ($p<0.01$). Phenylalanine balance at the 5 and 10 mg/kg/d valine intakes was significantly lower than balance above the breakpoint (17 mg/kg/d) ($p<0.05$) but at the 15 mg/kg/d valine intake was not significantly different from balance above the breakpoint.	Comparisons and p-values: $F^{13}CO_2$ ratio at the 5 and 10 mg/kg/d valine intakes was significantly lower than the $F^{13}CO_2$ ratio above the breakpoint (20 mg/kg/d) ($p<0.05$) but the 15 mg/kg/d valine intake was not significantly different from the $F^{13}CO_2$ ratio above the breakpoint (20 mg/kg/d).

Study (PMID)	Statistics/Confounders	Valine Requirement Estimate	Data Used to Calculate Valine Requirement Estimate	Data Used to Calculate Valine Requirement Estimate	Data Used to Calculate Valine Requirement Estimate	Data Used to Calculate Valine Requirement Estimate
		(1.0 g/kg/d protein) Amino acid amount: 5-35 mg/kg/d valine Breakpoint: 17 mg/kg/d Upper 95% CI: 28 mg/kg/d Lower 95% CI: 11 mg/kg/d **Outcome: Valine requirement estimate calculated from $F^{13}CO_2$** Outcome parameter: Breakpoint, Upper 95% CI, Lower 95% CI Timepoint: data obtained following each study d (d 7) Total (N analyzed): 18, 54 observations Protein amount: 160 mg/kg/d nitrogen (1.0 g/kg/d protein) Amino acid amount: 5-35 mg/kg/d valine Breakpoint: 20 mg/kg/d Upper 95% CI: ≥ 35				

Study (PMID)	Statistics/Confounders	Valine Requirement Estimate	Data Used to Calculate Valine Requirement Estimate	Data Used to Calculate Valine Requirement Estimate	Data Used to Calculate Valine Requirement Estimate	Data Used to Calculate Valine Requirement Estimate
		mg/kg/d Lower 95% CI: 12 mg/kg/d				

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; IAAB= indicator amino acid balance; IAAO = indicator amino acid oxidation; mg/kg/d = milligrams per kilogram per day; N = number; NR = not reported; PMID = PubMed Identification Number; RoB = risk of bias; SD = standard deviation

Appendix I. Strength of Evidence

Protein

Table I.1. Strength of evidence protein requirement estimates RCTs children and adolescents

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Elango, 2011[20] (22049165)	Protein requirement estimate 1. Phenylalanine oxidation 2. $F^{13}CO_2$	Children and Adolescents	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 (N=7; 56)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: 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; NA = not applicable; PMID = PubMed Identification Number; RCT = randomized controlled trial
*Includes study design and ROB Score

Table I.2. Strength of evidence protein requirement estimates RCTs pregnant people

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Stephens, 2015[54] (25527661)	Protein requirement estimate 1. $F^{13}CO_2$ (early gestation) 2. $F^{13}CO_2$ (late gestation)	Pregnant People	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 (N=17; 35 early gestation) (N=19; 43 late gestation)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: CI = confidence interval; $F^{13}CO_2$ = rate of tracer oxidation to $^{13}CO_2$ [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; N = number; NA = not applicable; PMID = PubMed Identification Number; RCT = randomized controlled trial
*Includes study design and ROB Score

Table I.3. Strength of evidence protein requirement estimates RCTs adults (19-50 years)

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Humayun, 2007[27] (17921376)	Protein requirement estimate 1. $F^{13}CO_2$	Adults (19-50 yr)	1. Breakpoint: 0.93 g/kg/d, upper 95% CI: 1.24 g/kg/d (N=8; 56)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: CI = confidence interval; $F^{13}CO_2$ = rate $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; g/kg/d = grams per kilogram per day; N = number; NA = not applicable; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year
*Includes study design and ROB Score

Table I.4. Strength of evidence protein requirement estimates RCTs adults (51->70 years)

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Mao, 2020[40] (32140711)	Protein requirement estimate 1. $F^{13}CO_2$	Adults (51->70 yr)	1. Breakpoint: 0.91 g/kg/d; upper 95% CI: 1.17 g/kg/d (N=14; 80)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion
Rafii, 2015[49] (25320185)	Protein requirement estimate 1. $F^{13}CO_2$	Adults (51->70 yr)	1. Breakpoint: 0.96 g/kg/d; upper 95% CI: 1.29 g/kg/d (N=12; 83)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion
Rafii, 2015[50] (26962173)	Protein requirement estimate 1. $F^{13}CO_2$	Adults (51->70 yr)	1. Breakpoint: 0.94 g/kg/d, upper 95% CI: 1.24 g/kg/d (N=6; 42)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion
Morse, 2001[44] (11682582)	Protein requirement estimate 1. Nitrogen balance (week 2) 2. Nitrogen balance (week 3)	Adults (51->70 yr)	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 (N=11; 33)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: 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; NA = not applicable; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year
*Includes study design and ROB Score

Table I.5. Strength of evidence protein requirement estimates non-RCTs adults (19-50 years)

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Atinmo, 2010[61] (NA)	Protein requirement estimate 1. Nitrogen balance (Northern Nigeria arm) 2. Nitrogen balance (South Eastern Nigeria arm)	Adults (19-50 yr)	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: 12.56 mg/kg/d nitrogen (N=7; 28 Northern Nigeria arm (N=11; 44 South Eastern Nigeria arm)	1 Non-RCT Low risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: mg/kg/d = milligrams per kilogram per day; N = number; NA = not applicable; PMID = PubMed Identification Number; RCT = randomized control trial; SD = standard deviation; yr = year

*Includes study design and ROB Score

Table I.6. Strength of evidence protein requirement not calculated; nitrogen balance and leucine oxidation RCTs adults (19-50 and 51->70 years)

Study (PMID)	Outcome Comparison	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Walrand, 2008[58] (18697911)	Nitrogen balance 1. Younger	Adults (19-50 and 51->70 yr)	1. No difference 2. Significantly	1 RCT Moderate risk: 1	Direct	Unknown	Imprecise	NA	Insufficient	Insufficient evidence on which to

Study (PMID)	Outcome Comparison	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
	adult vs older adult 2. Usual protein younger adults vs High protein younger adults 3. Usual protein older adults vs high protein older adults		increased on the high protein diet 3. Significantly increased on the high protein diet (N=19; 38)							draw a conclusion
Walrand, 2008[58] (18697911)	Leucine oxidation 1. Younger adult vs older adult 2. Usual protein younger adults vs High protein younger adults 3. Usual protein older adults vs high protein older adults	Adults (19-50 and 51->70 yr)	1. No difference 2. Significantly increased on the high protein diet 3. Significantly increased on the high protein diet (N=19; 38)	1 RCT Moderate risk: 1	Direct	Unknown	Imprecise	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: N = number; NA = not applicable; PMID = PubMed Identification Number; RCT = randomized controlled trial; vs = versus; yr = year
*Includes study design and ROB Score

Table I.7. Strength of evidence protein requirement not calculated; growth outcomes non-RCTs infants

Study (PMID)	Outcome Comparison	Population	Findings (N analyzed)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Kittisakmontri, 2022[68] (36235599)	LAZ 6 months: 1. HPro vs LPro 2. HPro vs MPro 3. MPro vs LPro LAZ 9 months 1. HPro vs LPro 2. HPro vs MPro 3. MPro vs LPro LAZ 12 months: 1. HPro vs LPro 2. HPro vs MPro 3. MPro vs LPro	Infants	6 months: 1. no difference 2. no difference 3. no difference 9 months: 1. no difference 2. no difference 3. no difference 12 months: 1. no difference 2. no difference 3. no difference N=145 (N=36 HPro, N=73 MPro, N=36 LPro)	1 non-RCT Moderate risk: 1	Direct	Unknown	Precise	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Study (PMID)	Outcome Comparison	Population	Findings (N analyzed)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Kittisakmontri, 2022[68] (36235599)	Conditional LAZ 12 months: 1. HPro vs LPro 2. HPro vs MPro 3. MPro vs LPro	Infants	12 months: 1. no difference 2. no difference 3. no difference N=145 (N=36 HPro, N=73 MPro, N=36 LPro)	1 non-RCT Moderate risk: 1: 1	Direct	Unknown	Precise	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: HPro = high protein; LAZ = length-for-age z score; LPro = low protein; MPro = median protein; N = number; NA = not applicable; PMID = PubMed Identification Number; RCT = randomized controlled trial; vs = versus*Includes study design and ROB Score

Isoleucine

Table I.8. Strength of evidence isoleucine requirement estimates RCTs infants

Study (PMID)	Outcome Data used to calculate requiremen t	Populatio n	Findings (N analyzed; total observations)	Limitations *	Directnes s	Study Consistenc y	Precision Reportin g	Reportin g Bias	Grade	Conclusio n
de Groof, 2014[2] (24284437)	Isoleucine requirement estimate 1. $F^{13}CO_2$	Infants	1. Breakpoint: 105 mg/kg/d, upper 95% CI 150 mg/kg/d (N=22; 22)	1 RCT Low risk: 1	Direct	Unknown	Unable to be determine d	NA	Insufficien t	Insufficient evidence on which to draw a conclusion

Abbreviations: $F^{13}CO_2$ = The fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial
*Includes study design and ROB Score

Leucine

Table I.9. Strength of evidence leucine requirement estimates RCTs infants

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
de Groof, 2014[2] (24284437)	Leucine requirement estimate 1. $F^{13}CO_2$	Infants	1. Breakpoint: 140 mg/kg/d, upper 95% CI 245 mg/kg/d (N=33; 33)	1 RCT Low risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: CI = confidence interval; $F^{13}CO_2$ = The fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; mg/kg/d = milligram per kilogram per day; N = number; NA = not applicable; PMID = PubMed Identification Number; RCT = randomized controlled trial
*Includes study design and ROB Score

Table I.10. Strength of evidence leucine requirement estimates RCTs adults (19-50 years)

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Kurpad, 2001[30] (11722955)	Leucine requirement estimate 1. 24-h IAAB 2. Nitrogen balance	Adults (19-50 yr)	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 (N=20; 40 leucine balance; 36 nitrogen balance)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: CI = confidence interval; h = hour; IAAB = indicator amino acid balance; mg/kg/d = milligram per kilogram per day; N = number; NA = Not applicable; ND = not determined; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year
*Includes study design and ROB Score

Table I.11. Strength of evidence leucine requirement estimate RCTs adults (51->70 years)

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Szwiega, 2021[55] (33330915)	Leucine requirement estimate 1. $F^{13}CO_2$ (men) 2. $F^{13}CO_2$ (women) 3. $F^{13}CO_2$ (combined)	Adults (51->70 yr)	1. Breakpoint: 77.8 mg/kg/d, upper 95% CI: 81 mg/kg/d 2. Breakpoint: 78.2 mg/kg/d, upper 95% CI: 82 mg/kg/d 3. Breakpoint: 78.5 mg/kg/d, upper 95% CI: 81 mg/kg/d (N=16; 93)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: CI = confidence interval; $F^{13}CO_2$ = rate of $^{13}CO_2$ release from tracer oxidation [tracer; phenylalanine]; mg/kg/d = milligram per kilogram per day; N = number; NA = not applicable; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year
*Includes study design and ROB Score

Lysine

Table I.12. Strength of evidence lysine requirement estimates RCTs infants

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Huang, 2011[5] (22049162)	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)	Infants	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 (N=21; 21)	1 RCT Low risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: CI = confidence interval; $F^{13}CO_2$ = The fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; mg/kg/d = milligrams per kilogram per day; N = number; PMID = PubMed Identification; RCT = randomized controlled trial
*Includes study design and ROB Score

Table I.13. Strength of evidence lysine requirement estimates RCTs children and adolescents

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Elango, 2007[19] (17684206)	Lysine requirement estimate 1. $F^{13}CO_2$	Children and Adolescents	1. Breakpoint: 35 mg/kg/d, upper 95% CI: 58 mg/kg/d (N= 5; 35)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion
Pillai, 2010[48] (19923398)	Lysine requirement estimate 1. $F^{13}CO_2$	Children and Adolescents	1. Breakpoint: 33.5 mg/kg/d, upper 95% CI: 46.6 mg/kg/d (N= 6; 42)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: CI = confidence interval; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; mg/kg/d = milligram per kilogram per day; N = number; NA = not applicable; PMID = PubMed Identification Number; RCT = randomized controlled trial
*Includes study design and ROB Score

Table I.14. Strength of evidence lysine requirement estimates RCTs pregnant people

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Payne, 2018[47] (29378056)	Lysine requirement estimate Lysine intake: 6-84 mg/kg/d 1. $F^{13}CO_2$ (early gestation) 2. $F^{13}CO_2$ (late gestation)	Pregnant People	1. Breakpoint: 36.6 mg/kg/d, upper 95% CI: 46.2 mg/kg/d 2. Breakpoint: 50.3 mg/kg/d, upper 95% CI: 60.4 mg/kg/d (N= 14; 27 early gestation) (N= 19; 36 late gestation)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: CI = confidence interval; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; mg/kg/d = milligram per kilogram per day; N = number; NA = not applicable; PMID = PubMed Identification Number; RCT = randomized controlled trial
*Includes study design and ROB Score

Table I.15. Strength of evidence lysine requirement estimates RCTs adults (19-50 years)

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Kurpad, 2001[31] (11333843)	Lysine requirement estimate 1. 24-h IAAO 2. 12-h fed IAAO 3. 24-h IAAB	Adults (19-50 yr)	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:	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
			29.7 mg/kg/d, upper 95% CI: 49 mg/kg/d (N= 16; 32)							
Kurpad, 2002[33] (12145014)	Lysine requirement estimate 1. 24-h IAAO (day 7) 2. 24-h IAAB (day 7) 3. 24-h IAAO (day 21) 4. 24-h IAAB (day 21) 5. 24-h IAAO (day 7 and 21) 6. 12-h fed IAAO (day 7 and 21) 7. 24-h IAAB (day 7 and 21)	Adults (19-50 yr)	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, 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%	1 RCT Low risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
			CI: 72 mg/kg/d 7. Breakpoint: 31 mg/kg/d, upper 95% CI: 38 mg/kg/d (N= 18; 36)							
Kriengsinyos, 2004[29] (15308475)	Lysine requirement estimate 1. $F^{13}CO_2$ (follicular) 2. $F^{13}CO_2$ (luteal)	Adults (19-50 yr)	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 (N= 5; 35 follicular phase) (N=5; 35 luteal phase)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

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

Table I.16. Strength of evidence lysine requirement not calculated; $F^{13}CO_2$ RCTs adults (19-50 years)

Study (PMID)	Outcome Comparison	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Elango, 2009[18] (19369367)	$F^{13}CO_2$ 1. 8-h adaptation vs 3-day adaptation vs 7-day 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	Adults (19-50 yr)	1. No difference 2. Higher oxidation at lower lysine intake (N= 5; 60)	1 RCT Moderate risk: 1	Direct	Unknown	Imprecise	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; mg/kg/d = milligram per kilogram per day; N = number; NA = not applicable; PMID = PubMed Identification Number; RCT = randomized controlled trial; vs = versus; yr = year
*Includes study design and ROB Score

Table I.17. Strength of evidence lysine requirement not calculated; 24-hour lysine balance and whole-body lysine oxidation non-RCTs adults (19-50 years)

Study (PMID)	Outcome Comparison	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
El-Khoury, 2000[62] (10871570)	Lysine balance 1. Low lysine (14-15 mg/kg/d) vs intermediate lysine (28-29 mg/kg/d)	Adults (19-50 yr)	1. Significant difference between groups (N= 11 (N=5 low lysine, N=6 intermediate lysine)	1 Non-RCT Low risk: 1	Direct	Unknown	Precise	NA	Insufficient	Insufficient evidence on which to draw a conclusion
El-Khoury, 2000[62] (10871570)	Lysine oxidation 1. Low lysine (14-15 mg/kg/d) vs intermediate lysine (28-29 mg/kg/d)	Adults (19-50 yr)	1. No difference (N= 11 (N=5 low lysine, N=6 intermediate lysine)	1 Non-RCT Low risk: 1	Direct	Unknown	Imprecise	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: mg/kg/d = milligram per kilogram per day; N = number; NA = not applicable; PMID = PubMed Identification Number; RCT = randomized controlled trial; vs = versus; yr = year
*Includes study design and ROB Score

Methionine

Table I.18. Strength of evidence methionine requirement estimates RCTs infants

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Huang, 2012[6] (22492372)	Methionine requirement estimate 1. $F^{13}CO_2$	Infants	1. Breakpoint: 38 mg/kg/d, upper 95% CI 48 mg/kg/d (N=33; 33)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: CI = confidence interval; $F^{13}CO_2$ = the fraction of 13CO2 recovery from tracer oxidation [tracer; phenylalanine]; mg/kg/d = milligram per kilogram per day; N = number; NA = not applicable; PMID = PubMed Identification Number; RCT = randomized controlled trial
*Includes study design and ROB Score

Table I.19. Strength of evidence methionine requirement estimates RCTs children and adolescents

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Humayun, 2006[28] (17093160)	Methionine requirement estimate 1. $F^{13}CO_2$	Children and Adolescents	1. Breakpoint: 5.8 mg/kg/d, upper 95% CI 7.3 mg/kg/d (N=6; 36)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion
Turner, 2006[57] (16522909)	Total sulfur amino acid requirement estimate 1. $F^{13}CO_2$	Children and Adolescents	1. Breakpoint: 12.9 mg/kg/d, upper 95% CI: 17.2 mg/kg/d (N=6; 36)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; NA = not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial
*Includes study design and ROB Score

Table I.20. Strength of evidence methionine requirement estimates RCTs adults (19-50 years)

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Di Buono, 2001[16] (11722957)	Methionine requirement estimate 1. $F^{13}CO_2$	Adults (19-50 yr)	1. Breakpoint: 4.5 mg/kg/d, upper 95% CI: 10.1 mg/kg/d (N=6; 36)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion
Di Buono, 2001[17] (11722956)	Total sulfur amino acid requirement estimate 1. $F^{13}CO_2$	Adults (19-50 yr)	1. Breakpoint: 12.6 mg/kg/d, upper 95% CI: 21 mg/kg/d (N=6; 36)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion
Kurpad, 2004[37] (15585764)	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)	Adults (19-50 yr)	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	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
			(N=21; 63, 5 mg/kg/d cysteine) (N=21; 63, 12 mg/kg/d cysteine)							
Kurpad, 2003[36] (12716672)	Total sulfur amino acid requirement estimate 1. 24-h IAAO 2. 24-h IAAB	Adults (19-50 yr)	1. Breakpoint: 14 mg/kg/d, upper 95% CI: 23 mg/kg/d 2. Breakpoint: 15 mg/kg/d, upper 95% CI 27 mg/kg/d (N=21; 63)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

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

Table I.21. Strength of evidence methionine requirement not calculated; phenylalanine oxidation RCTs adults (19-50 years)

Study (PMID)	Outcome Comparison	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Humayun, 2007[26] (17634258)	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	Adults (19-50 yr)	1. Linear decrease 2. No change 3. No change (N=7; 91)	1 RCT Moderate risk: 1	Direct	Unknown	Imprecise	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: AA = amino acid; N = number; PMID = PubMed Identification; RCT = randomized controlled trial; SPI = soy protein isolate; TSAA = total sulfur amino acid; yr = year
*Includes study design and ROB Score

Phenylalanine

Table I.22. Strength of evidence phenylalanine requirement estimates RCTs infants

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Hogewind-Schoonenboom, 2015[4] (25926506)	Phenylalanine requirement estimate 1. $F^{13}CO_2$	Infants	1. Breakpoint: 58 mg/kg/d, upper 95% CI 78 mg/kg/d (N=20; 20)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: CI = confidence interval; $F^{13}CO_2$ = fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; lysine]; mg/kg/d = milligram per kilogram per day; N = number; NA = not applicable; PMID = PubMed Identification Number; RCT = randomized controlled trial
*Includes study design and ROB Score

Table I.23. Strength of evidence phenylalanine requirement estimates RCTs children and adolescents

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Hsu, 2007[23] (17314698)	Aromatic amino acid requirement estimate 1. $F^{13}CO_2$	Children and Adolescents	1. Breakpoint: 28 mg/kg/d, upper 95% CI: NR (N=5; 40)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: CI = confidence interval; $F^{13}CO_2$=rate of $^{13}CO_2$ released from tracer oxidation [tracer; lysine]; mg/kg/d = milligram per kilogram per day; N = number; NA = Not applicable; NR = not reported; PMID = PubMed Identification Number; RCT = randomized controlled trial
*Includes study design and ROB Score

Table I.24. Strength of evidence phenylalanine requirement estimates RCTs pregnant people

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Ennis, 2020[22] (31758682)	Phenylalanine requirement estimate 1. $F^{13}CO_2$ (early gestation; DAAO) 2. $F^{13}CO_2$ (late gestation; DAAO) 3. $F^{13}CO_2$ (late	Pregnant People	1. Breakpoint: 15.14 mg/kg/d, 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,	1 RCT Low risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
	gestation; IAAO)		upper 95% CI: 32.2 mg/kg/d (N= 9; 26, early gestation; DAAO) (N= 9; 25, late gestation; DAAO) (N= 13; 25, late gestation; IAAO)							
Ennis, 2020[21] (33188409)	Aromatic amino acid requirement estimate 1. $F^{13}CO_2$ (early gestation) 2. $F^{13}CO_2$ (late gestation)	Pregnant People	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 (N= 10; 24, early gestation) (N= 10; 27, late gestation)	1 RCT Low risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: CI = confidence interval; DAAO = direct amino acid oxidation; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; leucine or phenylalanine]; IAAO = indicator amino acid oxidation; mg/kg/d = milligram per kilogram per day; N = number; NA = not applicable; PMID = PubMed Identification Number; RCT = randomized

controlled trial
*Includes study design and ROB Score

Table I.25. Strength of evidence phenylalanine requirement estimates RCTs adults (19-50 years)

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Hsu, 2006[24] (16400054)	Aromatic amino acid requirement estimate 1. $F^{13}CO_2$ (model 5vs 3)** 2. $F^{13}CO_2$ (model 6vs 2)**	Adults (19-50 yr)	1. Breakpoint: 47.73 mg/kg/d, upper 95% CI: NR 2. Breakpoint: 51.71 mg/kg/d, upper 95% CI: NR (N=5; 39)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion
Hsu, 2006[25] (16549457)	Aromatic amino acid requirement estimate 1. $F^{13}CO_2$ (part A) 2. $F^{13}CO_2$ (part B)	Adults (19-50 yr)	1. Breakpoint: ND mg/kg/d, upper 95% CI: ND 2. Breakpoint: 41.9 mg/kg/d, upper 95% CI: NR (N=5; 35, Part A) (N=5; 35, Part B)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Kurpad, 2006[35] (16762944)	Aromatic amino acid requirement estimate 1. 24-h IAAO 2. 12-h fed IAAO 3. 24-h IAAB	Adults (19-50 yr)	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 (N=32; 64)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: CI = confidence interval; $F^{13}CO_2$ = 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; mg/kg/d = milligram per kilogram per day; N = number; NA = not applicable; NR = not reported; ND = unable to be determined; PMID = PubMed Identification Number; RCT = randomized controlled trial; vs = versus; yr = year

*Includes study design and ROB Score

**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

Table I.26. Strength of evidence phenylalanine requirement estimates RCTs adults (51->70 years)

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Martin, 2019[43] (31271193)	Phenylalanine requirement estimates 1. $F^{13}CO_2$ (men) 2. $F^{13}CO_2$ (women) 3. $F^{13}CO_2$ (combined)	Adults (51->70 yr)	1. Breakpoint: 9.3 mg/kg/d, upper 95% CI: NR 2. Breakpoint: 8.4 mg/kg/d, upper 95% CI: NR 3. Breakpoint: 9.03 mg/kg/d, upper 95% CI: 15.9 mg/kg/d (N= 12; 66)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

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

Threonine

Table I.27. Strength of evidence threonine requirement estimates RCTs infants

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Hogewind-Schoonenboom, 2015[3] (25844708)	Threonine requirement estimate 1. $F^{13}CO_2$	Infants	1. Breakpoint: 68 mg/kg/d, upper 95% CI 104 mg/kg/d (N=32; 32)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: CI = confidence interval; $F^{13}CO_2$ = the fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; mg/kg/d = milligram per kilogram per day; N = number; NA = not applicable; PMID = PubMed Identification Number; RCT = randomized controlled trial
*Includes study design and ROB Score

Table I.28. Strength of evidence threonine requirement estimates RCTs adults (19-50 years)

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Kurpad, 2002[32] (12324292)	Threonine requirement estimate 1. fasted plasma amino acid response 2. 3-h fed plasma amino acid response 3. 6-h fed plasma amino acid response 4. 24-h IAAO 5. 12-h fed IAAO 6. 24-h IAAB	Adults (19-50 yr)	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: 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 (N=16; 48)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion
Wilson, 2000[50] (10702170)	Threonine requirement estimate 1. $F^{13}CO_2$	Adults (19-50 yr)	1. Breakpoint: 19 mg/kg/d, upper 95% CI: 26.2 mg/kg/d (N= 6; 36)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: CI = confidence interval; $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; h = hour; IAAB = indicator amino acid balance; IAAO = indicator amino acid oxidation; mg/kg/d = milligram per kilogram per day; N = number; NA = not applicable; ND = unable to be reliably determined; PMID = PubMed Identification Number; RCT = randomized controlled trial; yr = year
*Includes study design and ROB Score

Total Branched Chain Amino Acids

Table I.29. Strength of evidence total branched chain amino acids requirement estimates RCTs children and adolescents

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Mager, 2003[39] (14608071)	Total branched chain amino acid requirement estimate 1. $F^{13}CO_2$	Children and Adolescents	1. Breakpoint: 147.3 mg/kg/d, upper 95% CI 191.5 mg/kg/d (N=5; 35)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: CI = confidence interval; $F^{13}CO_2$=rate of release of $^{13}CO_2$ from tracer oxidation [tracer; phenylalanine]; mg/kg/d = milligram per kilogram per day; N = number; NA = not applicable; PMID = PubMed Identification Number; RCT = randomized controlled trial
*Includes study design and ROB Score

Table I.30. Strength of evidence total branched chain amino acids requirement not calculated; $F^{13}CO_2$ and phenylalanine oxidation RCTs adults (19-50 years)

Study (PMID)	Outcome Comparison	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Riazi, 2003[53] (14608070)	$F^{13}CO_2$ 1. Ile constant-10% vs visual breakpoint 2. Ile constant-20% vs	Adults (19-50 yr)	1. No difference 2. Significant increase 3. No difference 4. No difference	1 RCT Moderate risk: 1	Direct	Unknown	Imprecise	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Study (PMID)	Outcome Comparison	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
	visual breakpoint 3. Ile constant-10% vs Ile constant-20% 4. Lue constant-10% vs visual breakpoint 5. Leu constant-20% vs visual breakpoint 6. Leu constant-10% vs Leu constant-20% 7. Val constant-10% vs visual breakpoint 8. Val constant-20% vs visual breakpoint 9. Val		5. Significant increase 6. No difference 7. No difference 8. No difference 9. No difference (5; 35 total observations; 30 from this study and 5 brought from the previous study[52])							

Study (PMID)	Outcome Comparison	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
	constant-10% vs Val constant-20%									
Riazi, 2003[53] (14608070)	Phenylalanine oxidation 1. Ile constant-10% vs visual breakpoint 2. Ile constant-20% vs visual breakpoint 3. Ile constant-10% vs Ile constant-20% 4. Lue constant-10% vs visual breakpoint 5. Leu constant-20% vs visual breakpoint 6. Leu constant-10% vs Leu	Adults (19-50 yr)	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 (5; 35 total observations; 30 from this study and 5 brought from the previous study[52])	1 RCT Moderate risk: 1	Direct	Unknown	Imprecise	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Study (PMID)	Outcome Comparison	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
	constant-20% 7. Val constant-10% vs visual breakpoint 8. Val constant-20% vs visual breakpoint 9. Val constant-10% vs Val constant-20%									

Abbreviations: $F^{13}CO_2$ = rate of $^{13}CO_2$ released from tracer oxidation [tracer; phenylalanine]; Ile = isoleucine; Leu = leucine; N = number; PMID = PubMed Identification; RCT = randomized controlled trial; Val = valine; yr = year
*Includes study design and ROB Score

Tryptophan

Table I.31. Strength of evidence tryptophan requirement estimates RCTs infants

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations*	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Huang, 2014[7] (24824360)	Tryptophan requirement estimate 1. $F^{13}CO_2$	Infants	1. Breakpoint: 15 mg/kg/d, upper 95% CI 31 mg/kg/d (N=30; 30)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: CI = confidence interval; $F^{13}CO_2$ = the fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; mg/kg/d = milligram per kilogram per day; N = number; NA = not applicable; PMID = PubMed Identification Number; RCT = randomized controlled trial
*Includes study design and ROB Score

Valine

Table I.32. Strength of evidence valine requirement estimates RCTs infants

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations *	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
de Groof, 2014[2] (24284437)	Valine requirement estimate 1. $F^{13}CO_2$	Infants	1. Breakpoint: 110 mg/kg/d, upper 95% CI 164 mg/kg/d (N=28; 29)	1 RCT Low risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

Abbreviations: CI = confidence interval; $F^{13}CO_2$= The fraction of $^{13}CO_2$ recovery from tracer oxidation [tracer; phenylalanine]; mg/kg/d = milligram per kilogram per day; N = number; NA = not applicable; PMID = PubMed Identification Number; RCT = randomized controlled trial
*Includes study design and ROB Score

Table I.33. Strength of evidence valine requirement estimate RCTs adults (19-50 years)

Study (PMID)	Outcome Data used to calculate requirement	Population	Findings (N analyzed; total observations)	Limitations *	Directness	Study Consistency	Precision Reporting	Reporting Bias	Grade	Conclusion
Kurpad, 2005[34] (16087981)	Valine requirement estimate 1. 24-h IAAO 2. 12-h fed IAAO 3. 24-h IAAB 4. $F^{13}CO_2$	Adults (19-50 yr)	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 (N=18; 54)	1 RCT Moderate risk: 1	Direct	Unknown	Unable to be determined	NA	Insufficient	Insufficient evidence on which to draw a conclusion

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

*Includes study design and ROB Score

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