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Protein quality, nutrition and health

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Dietary proteins are energy macronutrients providing nitrogen, amino acids (AA), and energy. AAs are the main nitrogen-containing compounds in the body and are the precursors for the synthesis of body proteins and of several other AA-derived molecules. Among the 20 AAs included in protein sequence, 9 are classified as "nutritionally essential" or "indispensable" AA (IAA) because they cannot be synthesized in the body and must be provided by the diet. IAAs are limiting components for protein synthesis. An adequate intake of protein is required to support growth, maintenance, body functions, health and survival. Official definition of protein requirement is based on nitrogen balance. Protein quality is related to the capacity of protein to provide an adequate quantity of nitrogen and of each of the 9 IAAs for the different physiological situations in humans. Protein source is considered high quality for humans when the protein is readily digested, simultaneously providing an adequate quantity of nitrogen and of each of the 9 IAAs to maintain an adequate metabolic AA pool. The most accurate assessment of protein quality of foods for humans is through metabolic studies that measure nitrogen balance. The protein quality score is the ratio of the content of each IAA in the food and in a reference profile. This score corresponds to the calculated composition of a protein which, when meeting protein requirements, simultaneously meets the requirements of each of the 9 IAAs. AA scores as predictors of protein quality must be adjusted for protein and AA availability.

KEYWORDS

nutrition, protein for human health, protein quality, protein, amino acids

1 Introduction

Dietary proteins are macronutrients providing nitrogen, amino acids (AAs), and energy. In living organisms, nitrogen is mostly associated to AAs and AAs are mostly in the form of proteins. AAs are the main nitrogen-containing compounds in the body and are the precursors for the synthesis of body proteins and of several other AA-derived molecules, all involved in the structure of tissues and/or in all the functions of the organism.

There is a very large number of proteins in the body (~10,000 types) and each protein is characterized by a specific sequence of AAs encoded in the genetic code. Among the 20 AAs included in protein sequence, 9 are classified as "nutritionally essential" or "indispensable" AAs (IAAs) because they cannot be synthesized in the body and must be provided by the diet (1). The 11 other AAs are "dispensable" because they can be synthesized in the body from precursors available in the organism. In adult humans (female 57 kg, male 70 kg), the protein compartment is 8–12 kg (Figure 1). Despite the large number of body's proteins in the body, about half of these proteins are represented by four proteins—myosin, actin, collagen, and hemoglobin—and among them, about 25% is represented by collagen. Body protein have both

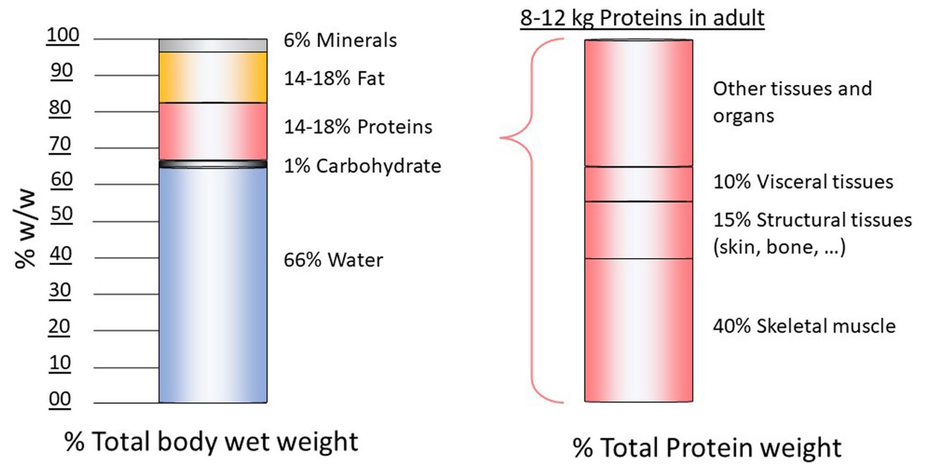

FIGURE 1
Body composition in healthy adult.

structural (muscle, skin, and bone) and physiological function (enzymes, hormones, receptors, antibodies, and cytokines).

Several health outcomes are associated with protein sufficiency such as body weight, body composition, muscle mass and strength, bone health, immune defenses, and most if not all physiological functions. An adequate intake of protein is required to support growth, maintenance, body functions, health, and survival. Protein quality is related to the capacity of protein to provide an adequate quantity of nitrogen and of each of the 9 IAAs for the different physiological situations in humans (1). The nutritive value of proteins from food and diet depends both on the amount of protein provided, but also on the AA composition and concentration, and on the bioavailability of protein-derived nitrogen and AAs. Protein quality matters because there are differences between the different food sources. Moreover, some forms of food storage and processing can affect protein quality (2).

Suitable markers for measuring the need for AAs and proteins and protein quality are derived from the different levels of AA metabolism and utilization in the body and from the functions of protein in the body (Figure 2; Table 1). Since the 1970/80s, the priority for international authorities of the United Nations (FAO/WHO/UNU) has been to define the requirement for nitrogen and IAA as criteria for protein quality to support body protein synthesis (1, 3, 4).

2 Protein and nitrogen requirements

Meeting protein nitrogen needs is required to maintain the body's protein pool that affect body composition and many if not all the functions in the body. Official definition of protein requirement is based on nitrogen balance – the usual protein intake that maintains a nitrogen balance in a person in good health, with normal body composition, normal energy balance and moderate physical activity. Determined by the nitrogen balance method in adult, the mean protein requirement is 0.66 g protein/kg/d (~40–50 g/d) and the recommended protein intake 0.83 g/kg/d (~50–60 g/d) (1). In different physiological situations such as infants, children, adolescents, pregnant women and lactating women, protein needs are derived from a factorial approach including nitrogen balance and additional protein deposition required for growth, pregnancy, or lactation.

Protein concentration or density (i.e., the amount of protein per unit of food) is a factor of food's protein quality (5, 6). Measuring nitrogen content with the Kjeldahl or Dumas methods and using a Nitrogen to Protein Conversion Factor remains the more frequently used approach for protein content in foods (7). The default conversion factor used for a mixture of protein sources is 6.25, corresponding to a nitrogen content of 16%. Specific protein conversion factors range from 5.7 (17.5% nitrogen) to 6.4 (15.6% nitrogen) for the major protein sources in the diet. The protein concentration in different food protein sources shows that animal product protein sources such as meat, milk, eggs, and some animal products are rich in protein with protein content of 30–70% (dry weight, dw). Among vegetables, pulses have the highest protein concentrations, ranging from 20–25% (dw) in most raw beans and peas to 35–38% in soybeans and lupines. Cereal seeds have a protein content of 15–20% (dw). Most nuts and edible seeds contain 8–18% protein (dw). Many oil seeds have 12–20% protein (dw), and the cake that remains after oil extrusion can have as much as 30–40% protein (dw).

3 Indispensable amino acid requirement and protein quality score

Maintaining optimal protein status required to provide in the diet a bioavailable form of an adequate quantity of protein with an adequate IAA profile.

The AA composition of proteins is usually calculated as milligrams AA per gram of protein. If they are reported as milligrams AA per gram of nitrogen, they are converted to the protein equivalents by multiplying by specific Nitrogen to Protein Conversion Factor. To calculate the AA content of a combination of food proteins, as in a food based on several protein sources or in a mixed diet, a weighted mean of the published or analytical results of each component should be used.

The protein required to achieve nitrogen balance must be of high quality. Protein quality is based on the capacity to provide an adequate

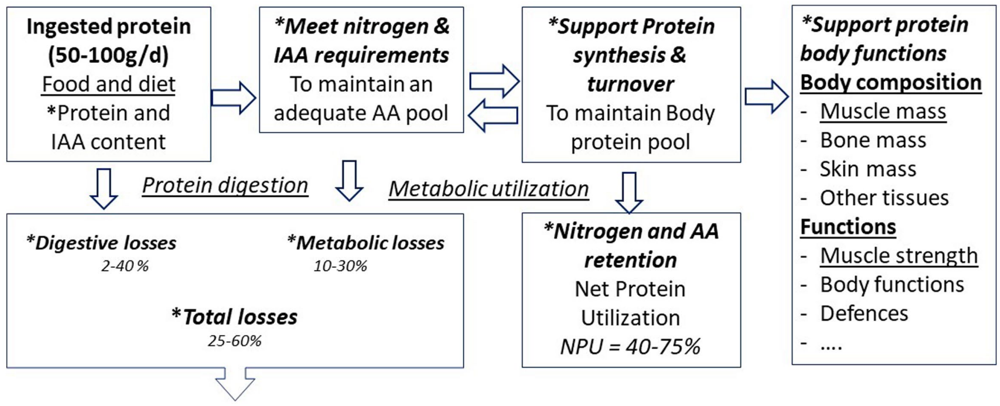

FIGURE 2
Proteinintake and utilization of nitrogen and amino acids (*markers of protein needs and protein quality) and markers and method for assessing protein quality.

TABLE 1 Markers and methods for assessing protein quality.

Protein, nitrogen & IAA requirements		
Protein requirement	Nitrogen balance	Digestive and metabolic nitrogen losses
Amino acid requirement	Amino acid oxidation	Stable isotopes amino acid balance
Protein, nitrogen & AA metabolic fate		
Protein, nitrogen & amino acid bioavailability	Faecal/ileal digestibility Amino acid availability	Faecal/ileal losses, dual isotope, Indicator AA oxidation, *In vitro*/*in silico* methods
Net protein utilization	Nitrogen/AA retention	Nitrogen/AA losses (stable isotopes)
Protein turnover	Whole body Protein synthesis	Stable isotopes amino acid balance and fluxes
	Muscle protein synthesis	Stable isotopes amino acid administration and muscle tissue sampling
Protein body functions		
Body composition	Lean mass, muscle mass, bone mass, …	
Body functions	Muscle strength, defenses, various functions, …	

quantity of nitrogen and of each of the 9 IAAs to achieve nitrogen balance and to support both protein turnover, and synthesis of the various AA derived component in the body. The 9 IAAs not synthesized in the body and limiting factors of AA utilization for protein synthesis must be provided at an adequate quantity and profile. IAA requirement for adult was initially determined by nitrogen balance in 1985 and re-evaluated in 2007 based on stable isotopes methods (1). IAA requirements were also determined for younger subjects by a factorial approach. The protein quality score is based on the ratio of the content of each IAA in the food and in a reference profile. The reference profile is the calculated composition of a protein which, when meeting protein requirements, simultaneously meets the requirements of each of the 9 IAAs. From the 2007 re-evaluation of IAA requirements, many foods such as cereals and legumes previously thought to be adequate in their IAA content, could be partially limited, particularly in lysine and Sulphur AA, respectively.

4 Correction of the score by digestibility

Protein sources are considered high quality for humans when the protein is readily digested, and nitrogen and AA readily absorbed and simultaneously providing an adequate quantity of nitrogen and of each of the 9 IAAs to maintain an adequate metabolic AA pool. A protein may have a good AA composition relative to the reference profile, but if it is not fully digested and its constituent AAs are not absorbed, its capacity to provide nitrogen and IAAs for human function will diminish.

Not all food proteins are digested, absorbed, and utilized to the same extent because of inherent differences in their source (e.g., inside vegetable cells with indigestible membranes), their physicochemical nature (e.g., protein configuration and AA binding), the presence of food constituents that modify digestion (e.g., dietary fiber, tannins, and other polyphenols), the presence of antinutritional factors that interfere with protein breakdown (e.g., trypsin inhibitors and lectins), and processing conditions that alter the nature or release of AAs (e.g., Maillard reaction and formation of polyAAs and methylmercaptan) (2, 8). Protein nitrogen digestibility values and more recently ileal AA digestibility values of specific foods and well-defined diets may be taken from reliable published data or must be determined, preferably in humans (3). When cost and practicality do not permit metabolic studies in humans to be performed, standardized methods in animal models are used (9). Nevertheless, animal data must be used with caution for foods and diets that are known or suspected of being handled differently by the human and animal intestines. When data are not available for a mixed diet, a weighted average can be calculated from the true digestibility of its constituent protein sources.

Consequently, AA scores as predictors of protein quality must be adjusted for protein digestibility and AA availability. The different scores are the “Chemical amino acid score,” the “Protein Digestibility-Corrected Amino acid Score” (PD-CAAS), and the “Digestible

Indispensable Amino Acid Score" (DIAAS) (1, 3, 8, 10). Stable isotope-based methods contribute to accumulate values for true protein and IAA digestibility from human food sources, including animal and plant protein sources. The True ileal digestibility assay is the best currently available approach to assess nitrogen and AA absorption. Digestibility measurements at the ileal level may provide a better measure of AA digestibility, however this may pose significant challenges (9). True ileal AA digestibility is assessed by different invasive or minimally invasive procedures in human, or alternatively in animal (pig or rat) models (9, 11–16).

For both IAA profile and bioavailability, plant protein are most often of lower quality than animal protein (6, 17). Digestibility of protein and IAA from plant protein sources are usually lower than for animal protein sources. The difference is more important when plant proteins are consumed in the form of complex flour or whole grains (treatment, matrix, and antinutritional factors) (2). This is particularly sensitive for younger subjects with higher protein and IAA requirements, i.e., a need for high protein quality. Protein quality also matters in the context of climate change (18). Reduction in diet-associated greenhouse gas emissions involves a shift toward plant-based diets that leads to reduce IAA content, particularly lysine and methionine and a risk to not meet IAA requirements.

TABLE 2 Protein digestibility, PD-CAAS and Net protein utilization of different protein sources.

	Protein digestibility %	PD-CAAS % (adult)	Limiting AA	Nitrogen/AA retention NPU %
Animal-source	75–99%	>100	–	–
Bovine Milk	94–99	>100	No	75
Meat (beef)	80–99	>100	No	75
Hen egg	80–97	>100	No	72
Plant sources	60–90%	70	–	–
Soy	75–90	86–100	Met+Cys	~70
Pea	70–90	71–78	Met+Cys	~70
Rice	65–85	50–58	Lys	–
Wheat	65–85	46–51	Lys	~60–65

Adapted from Fuller and Tomé (12), Gaudichon et al. (13), Tome (19), Gausseres et al. (20), Evenepoel et al. (21), Bos et al. (22–24), Gaudichon et al. (25), Tomé and Bos (26), Fromentin et al. (27), Oberli et al. (28), and Oberli et al. (29).

5 Protein quality and protein synthesis

As mentioned above, the most accurate assessment of protein quality of foods for humans is through clinical studies that measure nitrogen balance (1). Food proteins are fed to a group of individuals and nitrogen losses are determined. However, biological assays in laboratory animals have been used to assess food protein quality, based either on a protein's ability to support growth in young rats (protein efficiency ratio, PER) or on nitrogen retention (net protein utilization, NPU) (19). The PER and NPU remain useful indices for screening food protein quality and to validate theoretical models based on the AA composition of the target protein. PD-CAAS and DIAAS values in adults for animal and plant protein sources can be compared to the efficiency of nitrogen retention Net Protein Utilization (NPU) (Table 2).

AAs are the precursors of protein synthesis in the body. The body proteins and free AAs are in a continuous turnover through protein breakdown and synthesis at an overall rate of about 250–300 g/d (Figure 2). AAs in free form, circulating and present in tissues, are a small fraction of all body AAs (less than 100 g). The 9 IAAs are limiting factor of protein synthesis. The major anabolic factors that influence muscle protein synthesis are contractile activity and feeding. AAs, together with insulin, display an anabolic effect and stimulate muscle protein synthesis (10, 30–33). The ability of a protein source to stimulate protein synthesis have thus been used to assess protein quality. Moreover, among AA, the branched-chain AA (BCAA) have many important physiological roles and of the three BCAA, leucine is most notably a key regulator signaling molecules of muscle protein synthesis (MPS), exerting anabolic effects even in the presence of hyper-aminoacidemia (34).

Protein ingestion induces an increase in muscle protein synthesis (MPS, %/h) measured by stable isotopes method in young men (10, 33). For young adults at rest or with low body exercise 10 g or 20 g of high-quality whey protein result in a rise of MPS of 19 and 52%, respectively, from control 0 g while 40 g do not result in higher stimulation beyond consumption of 20 g. However, in young adults following whole-body exercise 40 g of protein did result in significantly higher MPS rate (35) and results in older adults also indicate a greater MPS response to 40 vs. 20 g whey protein (36). From different studies 40 g protein is consistently 10–20% higher compared to 20 g protein, albeit not always statistically significant (37). Lysine deficiency limits the capacity of wheat protein to induce an increase in MPS. Ingestion in older adult of 35 g wheat protein, deficient in lysine, does not induce an increase in MPS and an increase in MPS was induced by 60 g wheat protein, 35–40 g casein, chicken breast fillet, or lysine-enriched wheat and chickpea protein mixture (38). However, in younger adults an increase in MPS was observed in response to the ingestion of 30 wheat protein (39).

Interestingly, 8 weeks resistance training and intake of 46 g/day high-quality whey (WPC), beef (Beef), or hydrolyzed chicken (Chx) protein after workout improves body composition and muscle performance (38, 40). Lean body mass was significantly increased after 8-weeks resistance training with post workout consumption of a 46 g bolus of WPC, Beef or Chx protein, compared with a control (Maltodextrin) (41).

6 Conclusion

Protein requirements relate to the supply of metabolically available nitrogen and IAAs to balance nitrogen and AA losses, to support body protein turnover and synthesis and to maintain the body's protein pool. Several health outcomes are associated with protein and IAA sufficiency, including growth, body weight, muscle mass and strength, bone health, defenses, and most if not all physiological functions. AA scoring is the preferred approach to evaluate the protein quality. It correlates with other approaches of protein quality (nitrogen retention, protein synthesis, physiological functions). The lower IAA content of certain protein sources is at the origin of the risk of protein deficiency in certain diets. Reference values (data base) on IAA bioavailability of the different protein sources are required.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. WHO/FAO/UNU. *Protein and amino acid requirements in human nutrition* World Health Organization (2007).

2. Vissamsetti N, Simon-Collins M, Lin S, Bandyopadhyay S, Kuriyan R, Sybesma W, et al. Local sources of protein in low-and middle-income countries: how to improve the protein quality? *Curr Dev Nutr*. (2023) 8:102049. doi: 10.1016/j.cdnut.2023.102049

3. FAO. *Research approaches and methods for evaluating the protein quality of human foods*. Rome: Report of a FAO Expert Working Group (2014).

4. Tome D, Xipsiti M, Shertukde SP, Calvez J, Vasilopoulou D, Wijesinha-Bettoni R, et al. Context and perspectives for establishing a novel database for protein quality of human foods, as proposed by a joint food and agriculture Organization of the United Nations/International Atomic Energy Agency expert technical meeting in October 2022. *J Nutr*. (2023) 154:294–9. doi: 10.1016/j.tjnut.2023.12.050

5. Drewnowski A. Adjusting for protein quality by food source may affect nutrient density metrics. *Nutr Rev*. (2020) 79:1134–44. doi: 10.1093/nutrit/nuaa117

6. Pinckaers PJ, Trommelen J, Snijders T, Van Loon LJ. The anabolic response to plant-based protein ingestion. *Sports Med*. (2021) 51:59–74. doi: 10.1007/s40279-021-01540-8

7. Tome D, Cordella C, Dib O, Peron C. *Nitrogen and protein content measurement and nitrogen to protein conversion factors for dairy and soy protein-based foods: A systematic review and modelling analysis*. Geneva: World Health Organization (2019).

8. FAO. Expert consultation: dietary protein quality evaluation in human nutrition. *FAO Food Nutr Pap*. (2013) 92:1–66.

9. Shivakumar N, Jackson AA, Courtney-Martin G, Elango R, Ghosh S, Hodgkinson S, et al. Protein quality assessment of follow-up formula for young children and ready-to-use therapeutic foods: recommendations by the FAO expert working group in 2017. *J Nutr*. (2020) 150:195–201. doi: 10.1093/jn/nxz250

10. FAO. *Protein quality assessment in follow-up formula for young children and ready to use therapeutic foods*. Rome: (2018).

11. Devi S, Varkey A, Sheshshayee M, Preston T, Kurpad AV. Measurement of protein digestibility in humans by a dual-tracer method. *Am J Clin Nutr*. (2018) 107:984–91. doi: 10.1093/ajcn/nqy062

12. Fuller MF, Tomé D. In vivo determination of amino acid bioavailability in humans and model animals. *J AOAC Int*. (2005) 88:923–34. doi: 10.1093/jaoac/88.3.923

13. Gaudichon C, Bos C, Morens C, Petzke KJ, Mariotti F, Everwand J, et al. Ileal losses of nitrogen and amino acids in humans and their importance to the assessment of amino acid requirements. *Gastroenterology*. (2002) 123:50–9. doi: 10.1053/gast.2002.34233

14. Kashyap S, Shivakumar N, Varkey A, Duraisamy R, Thomas T, Preston T, et al. Ileal digestibility of intrinsically labeled hen's egg and meat protein determined with the dual stable isotope tracer method in Indian adults. *Am J Clin Nutr*. (2018) 108:980–7. doi: 10.1093/ajcn/nqy178

15. Lee WT, Weisell R, Albert J, Tomé D, Kurpad AV, Uauy R. Research approaches and methods for evaluating the protein quality of human foods proposed by an FAO expert working group in 2014. *J Nutr*. (2016) 146:929–32. doi: 10.3945/jn.115.222109

16. Moehn S, Bertolo RF, Pencharz PB, Ball RO. Development of the indicator amino acid oxidation technique to determine the availability of amino acids from dietary protein in pigs. *J Nutr*. (2005) 135:2866–70. doi: 10.1093/jn/135.12.2866

17. Van Vliet S, Kronberg SL, Provenza FD. Plant-based meats, human health, and climate change. *Front Sustain Food Syst*. (2020) 4:128.

18. Grasso AC, Olthof MR, Van Dooren C, Broekema R, Visser M, Brouwer IA. Protein for a healthy future: how to increase protein intake in an environmentally sustainable way in older adults in the Netherlands. *J Nutr*. (2021) 151:109–19. doi: 10.1093/jn/nxaa322

19. Tome D. Criteria and markers for protein quality assessment – a review. *Br J Nutr*. (2012) 108:S222–9. doi: 10.1017/S0007114512002565

20. Gausseres, N, Mahe, S, Benamouzig, R, Luengo, C, Ferriere, F, Rautureau, J, et al. [15N]- labeled pea flour protein nitrogen exhibits good ileal digestibility and postprandial retention in humans. *The Journal of nutrition*, (1997) 127:1160–1165.

21. Evenepoel, P, Geypens, B, Luypaerts, A, Hiele, M, Ghoos, Y, Rutgeerts, P, et al, Digestibility of cooked and raw egg protein in humans as assessed by stable isotope techniques. *The Journal of nutrition*, (1998) 128:1716–1722.

22. Bos, C, Mahé, S, Gaudichon, C, Benamouzig, R, Gausserès, N, Luengo, C, et al. Assessment of net postprandial protein utilization of 15N-labelled milk nitrogen in human subjects. *Br. J. Nutr.* (1999) 81:221–226.

23. Bos, C, Juillet, B, Fouillet, H, Turlan, L, Daré, S, Luengo, C, et al. Postprandial metabolic utilization of wheat protein in humans. *Am J Clin Nutr*, (2005) 81:87–94.

24. Bos, C, Airinei, G, Mariotti, F, Benamouzig, R, Bérot, S, Evrard, J, et al. The poor digestibility of rapeseed protein is balanced by its very high metabolic utilization in humans. *The Journal of nutrition.* (2007) 137:594–600.

25. Gaudichon, C, Mahé, S, Benamouzig, R, Luengo, C, Fouillet, H.l.n, Daré, S., et al. Net postprandial utilization of [15N]-labeled milk protein nitrogen is influenced by diet composition in humans. *The Journal of nutrition*, (1999) 129:890–895.

26. Tomé D, Bos C. c. Dietary protein and nitrogen utilization. *The Journal of nutrition* (2000) 130:1868S–1873S.

27. Fromentin, C, Tomé, D, Nau, F, Flet, L, Luengo, C, Azzout-Marniche, D, et al. Dietary proteins contribute little to glucose production, even under optimal gluconeogenic conditions in healthy humans. *Diabetes*, (2013) 62:1435–1442.

28. Oberli, M, Marsset-Baglieri, A, Airinei, G, Santé-Lhoutellier, V, Khodorova, N, Rémond, D, et al. High True Ileal Digestibility but Not Postprandial Utilization of Nitrogen from Bovine Meat Protein in Humans Is Moderately Decreased by High-Temperature, Long-Duration Cooking. *J Nutr*, (2015) 145:2221–8.

29. Oberli, M, Lan, A, Khodorova, N, Santé-Lhoutellier, V, Walker, F, Piedcoq, J, et al. Compared with raw bovine meat boiling but not grilling, barbecuing, or roasting decreases protein digestibility without any major consequences for intestinal mucosa in rats, although the daily ingestion of bovine meat induces histologic modifications in the colon. *The Journal of nutrition*, (2016) 146:1506–1513.

30. Dideriksen K, Reitelseder S, Holm L. Influence of amino acids, dietary protein, and physical activity on muscle mass development in humans. *Nutrients*. (2013) 5:852–76. doi: 10.3390/nu5030852

31. Paddon-Jones D, Campbell WW, Jacques PF, Kritchevsky SB, Moore LL, Rodriguez NR, et al. Protein and healthy aging. *Am J Clin Nutr*. (2015) 101:1339S–45S. doi: 10.3945/ajcn.114.084061

32. Volpi E, Mittendorfer B, Wolf SE, Wolfe RR. Oral amino acids stimulate muscle protein anabolism in the elderly despite higher first-pass splanchnic extraction. *Am J Physiol Endocrinol Metabol*. (1999) 277:E513–20. doi: 10.1152/ajpendo.1999.277.3.E513

33. Stokes T, Hector AJ, Morton RW, Mcglory C, Phillips SM. Recent perspectives regarding the role of dietary protein for the promotion of muscle hypertrophy with resistance exercise training. *Nutrients*. (2018) 10:180. doi: 10.3390/nu10020180

34. Plotkin DL, Delcastillo K, Van Every DW, Tipton KD, Aragon AA, Schoenfeld BJ. Isolated leucine and branched-chain amino acid supplementation for enhancing muscular strength and hypertrophy: a narrative review. *Int J Sport Nutr Exerc Metab*. (2021) 31:292–301. doi: 10.1123/ijsnem.2020-0356

35. Macnaughton LS, Wardle SL, Witard OC, Mcglory C, Hamilton DL, Jeromson S, et al. The response of muscle protein synthesis following whole-body resistance exercise is greater following 40 g than 20 g of ingested whey protein. *Physiol Rep*. (2016) 4:e12893. doi: 10.14814/phy2.12893

36. Yang Y, Breen L, Burd NA, Hector AJ, Churchward-Venne TA, Josse AR, et al. Resistance exercise enhances myofibrillar protein synthesis with graded intakes of whey protein in older men. *Br J Nutr*. (2012) 108:1780–8. doi: 10.1017/S0007114511007422

37. Trommelen J, Van Lieshout GA, Nyakayiru J, Holwerda AM, Smeets JS, Hendriks FK, et al. The anabolic response to protein ingestion during recovery from exercise has no upper limit in magnitude and duration in vivo in humans. *Cell Rep Med*. (2023) 4:101324. doi: 10.1016/j.xcrm.2023.101324

38. Gorissen SH, Witard OC. Characterising the muscle anabolic potential of dairy, meat and plant-based protein sources in older adults. *Proc Nutr Soc*. (2018) 77:20–31. doi: 10.1017/S002966511700194X

39. Pinckaers PJ, Kouw IW, Hendriks FK, Van Kranenburg JM, De Groot LC, Verdijk LB, et al. No differences in muscle protein synthesis rates following ingestion of wheat protein, milk protein, and their protein blend in healthy, young males. *Br J Nutr*. (2021) 126:1832–42. doi: 10.1017/S0007114521000635

40. Kouw IW, Pinckaers PJ, Le Bourgot C, Van Kranenburg JM, Zorenc AH, De Groot LC, et al. Ingestion of an ample amount of meat substitute based on a lysine-enriched, plant-based protein blend stimulates postprandial muscle protein synthesis to a similar extent as an isonitrogenous amount of chicken in healthy, young men. *Br J Nutr*. (2022) 128:1955–65. doi: 10.1017/S0007114521004906

41. Sharp MH, Lowery RP, Shields KA, Lane JR, Gray JL, Partl JM, et al. The effects of beef, chicken, or whey protein after workout on body composition and muscle performance. *J Strength Cond Res*. (2018) 32:2233–42. doi: 10.1519/JSC.0000000000001936