The Journal of Nutrition xxx (xxxx) xxx

American Society for Nutrition
Excellence in Nutrition Research and Practice

JN THE JOURNAL OF NUTRITION

journal homepage: https://jn.nutrition.org/

Perspectives

Perspective: Developing a Nutrient-Based Framework for Protein Quality

Shavawn M. Forester [1,*], Emily M. Jennings-Dobbs [1], Shazia A. Sathar [2], Donald K. Layman [3]

[1] *Nutrient Institute LLC, Reno, NV, United States;* [2] *WISEcode, Reno, NV, United States;* [3] *Department of Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL*

A B S T R A C T

The future of precision nutrition requires treating amino acids as essential nutrients. Currently, recognition of essential amino acid requirements is embedded within a generalized measure of protein quality known as the PDCAAS (Protein Digestibility-Corrected Amino Acid Score). Calculating the PDCAAS includes the FAO/WHO/UNU amino acid score, which is based on the limiting amino acid in a food, that is, the single amino acid with the lowest concentration compared to the reference standard. That "limiting" amino acid score is then multiplied by a bioavailability factor to obtain the PDCAAS, which ranks proteins from 0.0 (poor quality) to 1.0 (high quality). However, the PDCAAS has multiple limitations: it only allows for direct protein quality comparison between 2 proteins, and it is not scalable, transparent, or additive. We therefore propose that shifting the protein quality evaluation paradigm from the current generalized perspective to a precision nutrition focus treating amino acids as unique, metabolically active nutrients will be valuable for multiple areas of science and public health. We report the development and validation of the Essential Amino Acid 9 (EAA-9) score, an innovative, nutrient-based protein quality scoring framework. EAA-9 scores can be used to ensure that dietary recommendations for each essential amino acid are met. The EAA-9 scoring framework also offers the advantages of being additive and, perhaps most importantly, allows for personalization of essential amino acid needs based on age or metabolic conditions. Comparisons of the EAA-9 score with PDCAAS demonstrated the validity of the EAA-9 framework, and practical applications demonstrated that the EAA-9 framework is a powerful tool for precision nutrition applications.

Keywords: amino acids, protein quality, DIAAS, lysine, leucine, PDCAAS, dietary recommendations, precision nutrition

Introduction

Amino acids are unique nutrients with individual dietary requirements and distinct, noninterchangeable metabolic functions [1]. Just as vitamins A, B_6, C, and D have distinct functions and metabolic requirements despite being grouped as vitamins, amino acids are equally distinct despite being grouped as protein. Nevertheless, an understanding of amino acids as unique nutrients has not yet been clearly incorporated into nutrition recommendations or protein quality scores.

Most consumer-facing dietary guidelines still treat amino acids as interchangeable equivalents by generically representing them as "protein." This generalization is built into nutrition recommendations such as the Dietary Guidelines for Americans (DGA) and the Nutrition Facts Panel, which both use protein as a surrogate for amino acid requirements [2,3]. The DGA and Nutrition Facts Panel are informed by DRI reports, which specify the amounts of the 9 essential amino acids (EAAs) that must be consumed in the diet. However, DRI data is not intended for consumers and is not built in an easily accessible, user-friendly format for professionals' use [4].

Currently, there is no available dietary guideline framework that evaluates food or meal protein quality based on the distinct metabolic roles and requirements of EAAs or that allows professionals or consumers to customize a diet based on specific individual requirements for one or more EAAs.

Understanding amino acids as individual nutrients requires understanding their unique metabolic roles beyond the fundamental role for protein synthesis (Table 1) [5–12]. For example, leucine (Leu) is a dietary signal that activates the mTOR

Abbreviations: AAS, amino acid score; DGA, Dietary Guidelines for Americans; DIAAS, Digestible Indispensable Amino Acid Score; EAA, essential amino acid; EAA-9, Essential Amino Acid 9; PDCAAS, Protein Digestibility-Corrected Amino Acid Score; SR Legacy, USDA National Nutrient Database for Standard Reference Legacy Release.

* Corresponding author. *E-mail address:* shavawn@nutrientinstitute.org (S.M. Forester).

https://doi.org/10.1016/j.tjnut.2023.06.004
Received 21 February 2023; Received in revised form 9 May 2023; Accepted 6 June 2023; Available online xxxx

Table 1
Metabolic roles of essential amino acids in optimal human health

Essential amino acid	Metabolic roles in addition to protein synthesis
Histidine	Histamine and carnosine synthesis [5]
Isoleucine	Anaphoretic role in citric acid cycle [6]
Leucine	Activation of the mTORC1 pathway; alanine and glutamine synthesis [7,8]
Lysine	Carnitine synthesis (fatty acid oxidation) [4]
Methionine	One-carbon metabolism for RNA and DNA; precursor for cysteine, glutathione, and taurine [9,10]
Phenylalanine	Dopamine synthesis (neurotransmitter) [11]
Threonine	Mucin production within gastrointestinal tract [12]
Tryptophan	Serotonin and nicotinic acid synthesis [4]
Valine	Anaphoretic role in citric acid cycle [6]

Abbreviation: mTORC1, mammalian target of rapamycin complex 1.

(mammalian target of rapamycin) complex to initiate protein synthesis [13], which is an important component of maintaining muscle protein turnover. Optimizing Leu signaling for muscle health requires 2.5 to 3.0 g of Leu per meal or approximately 7.5 g/d [14]. In contrast, the Leu recommendation embedded within the current protein requirements is only 2.9 g/d for a 70 kg adult. That large discrepancy (2.9 g/d compared with 7.5 g/d) exists because current protein recommendations are based on nitrogen balance, which estimates the role of Leu as a building block for protein but does not account for Leu's other metabolic roles [15,16]. Consequently, if a dietitian were preparing a diet to boost a patient's muscle health, the current generic protein recommendations are unlikely to provide sufficient Leu to meet that dietary need.

Methionine (Met) is another example of an EAA with unique and important metabolic roles. Dietary Met intake impacts synthesis of the amino acid cysteine (Cys) and of the antioxidants glutathione and taurine [9]. Met also plays a critical role in 1-carbon metabolism, driving choline metabolism, and as a methyl donor in DNA methylation [17]. Threonine (Thr) offers yet another example of an EAA with a specific metabolic role: nearly 75% of dietary intake of Thr is used to produce the mucin lining the inner surface of the gastrointestinal tract. The thickness of the protective mucin layer is proportional to dietary Thr intake [12].

Developing dietary recommendations that account for each of these distinct, noninterchangeable EAA roles for every individual is an essential component for achieving personalized precision nutrition. Thus, a nutrient-based protein quality scoring framework is required to improve on the existing models of generalized protein recommendations.

Limitations of current approaches to evaluating protein quality

The current standard for evaluating protein quality, on an amino acid level, is the Protein Digestibility-Corrected Amino Acid Score (PDCAAS). PDCAAS is calculated by multiplying the FAO/WHO/UNU amino acid score (AAS) by protein digestibility [18] (Figure 1). The FAO/WHO/UNU AAS is calculated as the proportion of the limiting amino acid in 1 g of protein compared to the same amino acid in a reference pattern. This reference pattern is derived by dividing daily amino acid recommendations by the mean protein recommendation. The digestibility factor used for PDCAAS is determined from measurements of

$$\text{FAO/WHO/UNU amino acid score} = \frac{\text{mg of amino acid in 1 g test protein}}{\text{mg of amino acid in reference pattern}}$$

$$\text{PDCAAS} = \text{digestibility} \times \text{amino acid score}$$

Figure 1. Calculation of FAO/WHO/UNU amino acid score and PDCAAS.
FAO/WHO/UNU amino acid score calculated using limiting amino acid and truncated at 100%; equations are further defined in the 2007 FAO/WHO/UNU report [18]. Abbreviation: PDCAAS, Protein Digestibility-Corrected Amino Acid Score.

fecal nitrogen losses, assuming any fecal nitrogen represents nondigested dietary protein. PDCAAS may be calculated for a multi-ingredient food by using the weighted average digestible amino acid content. However, such a calculation is only possible if the digestibility of each component that contributes to overall protein content of the mixture is known [18].

PDCAAS is currently being replaced by DIAAS (Digestible Indispensable Amino Acid Score) as stated in the 2013 FAO protein quality report [19]. DIAAS uses the same FAO/WHO/UNU AAS but shifts from digestibility factors determined by fecal nitrogen to nitrogen measurements at the end of the ileum, in part because colonic bacteria have been shown to alter or contribute to fecal nitrogen [20]. Additionally, DIAAS values are not truncated at 1.0 (ie, 100%) because truncating AAS removes nutritional differences between higher quality protein foods such as eggs, milk, and soy [19].

PDCAAS and DIAAS measurements allow for comparison of protein quality between single foods or ingredients. However, the practical applications of PDCAAS and DIAAS are constrained by analytical complexity. Digestibility scores are only available for a limited number of foods, and the respective abilities of PDCAAS and DIAAS to evaluate complex food products and meals are limited because neither score is scalable or additive. Furthermore, PDCAAS and DIAAS are of limited utility for professionals and consumers because both methods are intended to compare proteins, but neither can be personalized to meet specific amino acid requirements aimed at optimizing health.

Optimizing health by personalizing amino acid requirements is an important application of precision nutrition. One person's dietary needs may be different from another based on multiple factors, including age, health and disease status, physical fitness, genetic background, location, or dietary preferences. For instance, a person over the age of 70 may need more dietary Leu to build muscle than would someone younger. A person committed to an exclusively plant-based diet may need to consciously choose combinations of foods that will provide sufficient amounts of lysine (Lys) or Met. A person undergoing cancer treatment may need to adjust the proportions of certain dietary EAAs [21]. All of these are examples of precision nutrition, tailoring an individual's nutrition to meet specific needs. Such personalization is only possible if values for individual nutrients within a food are known and are incorporated into a protein quality scoring framework. We therefore propose that a nutrient-based analytical framework, designed to account for each EAA within each food, represents a timely and optimizable approach to protein quality and amino acid requirements.

In this prospectus, we describe, validate, and propose a new nutrient-based protein quality score, EAA-9 (Essential Amino Acid 9). The EAA-9 framework is a robust, transparent, additive, and scalable protein quality score for use in scoring multi-ingredient foods and meals with the aim of tracking progress toward meeting amino acid requirements. We show that the EAA-9 framework can be validated against the existing international measurement standards represented by PDCAAS and DIAAS and demonstrate its utility as both a scientific and consumer-facing tool.

Introducing the Essential Amino Acid Score 9 (EAA-9)

Current methods of scoring protein quality, such as PDCAAS, compare the quality of proteins from different food sources but are not built to assess dietary requirements for individual EAAs or evaluate complex meals. To advance understanding of protein quality, a score must facilitate the evaluation of protein intake and determine the extent to which amino acid needs are satisfied in an applied context.

We identified 5 properties deemed necessary for a complete protein quality scoring framework: *1*) it can be used to compare protein quality across foods; *2*) it allows for representation of selected amino acid nutrients; *3*) it can evaluate personalized amino acid requirements; *4*) it can be used additively across meals; and *5*) it yields a score that represents meeting EAA RDAs. As depicted in Table 2, the proposed EAA-9 score incorporates all 5 of those properties.

Development of the EAA-9 scoring framework

Just as dietary recommendations should account for each of the 14 essential vitamins, a nutrient-based model for protein quality must represent all 9 EAAs. RDAs for each of the 9 EAAs have been established by the National Academies of Sciences, Engineering, and Medicine (formerly the Institute of Medicine) [4].Therefore, a nutrient-based protein quality model must incorporate those 9 RDAs.

Once the 9 EAAs and corresponding RDAs were identified, the calculation strategy required 2 initial decisions: *1*) whether to use truncated or nontruncated scores, and *2*) whether to use average scores or the limiting amino acid score. Consistent with the FAO/WHO/UNU amino acid scoring decision for DIAAS, we adopted a nontruncated score that can exceed the RDA [19]. This decision made sense biologically because scoring with a nontruncated calculation strategy gives credit to food (ie, proteins) that provides EAAs in excess of daily recommendations.

Next, we considered 2 scoring approaches: one using the average percentage of the EAA RDAs met and one using the

$$\text{EAA-9} = \min\left(\frac{\text{His (mg/svg)}}{\text{His RDA}}, \frac{\text{Ile (mg/svg)}}{\text{Ile RDA}}, \frac{\text{Leu (mg/svg)}}{\text{Leu RDA}}, \frac{\text{Lys (mg/svg)}}{\text{Lys RDA}}, \frac{\text{Met + Cys (mg/svg)}}{\text{Met + Cys RDA}}, \frac{\text{Phe + Tyr (mg/svg)}}{\text{Phe + Tyr RDA}}, \frac{\text{Thr (mg/svg)}}{\text{Thr RDA}}, \frac{\text{Trp (mg/svg)}}{\text{Trp RDA}}, \frac{\text{Val (mg/svg)}}{\text{Val RDA}}\right) \times 100$$

Figure 2. EAA-9 framework.
EAA-9 mathematical framework. Calculation is based on the minimum percentage of the RDA met per serving(s) of food, where the minimum is the lowest percentage met by a single amino acid. EAA RDAs are satisfied when the EAA-9 score for foods consumed in a day reaches 100%. Abbreviations: EAA, essential amino acid; svg, serving.

minimum percentage met (ie, the percentage of the RDA met by the limiting amino acid). Although both the average and minimum scores can be useful, the average score could hide a limiting amino acid. For example, a standard 2 tbsp serving of peanut butter (FDC_ID 172469) meets an average of 16.0% of the EAA RDAs for a 70 kg man but only contains 8.1% of the RDA for the limiting EAA Lys [22]. To ensure that all 9 essential nutrients were accounted for, because they are not metabolically interchangeable, we chose to use the minimum percentage of RDA met. The EAA-9 score therefore reflects the lower bound of recommendations met rather than the average amount. Moreover, evaluating a protein based on the limiting amino acid aligns with the use of the limiting amino acid in the FAO/WHO/UNU AAS.

In summary, the proposed EAA-9 (equation in Figure 2) score represents the ability of a food to meet EAA RDAs and addresses the limitations of PDCAAS as shown in Table 2. The EAA-9 score can be used to compare protein quality for single ingredients, multi-ingredient foods, and meals. Most notably, it can be used additively over multiple meals, tracking progress toward meeting EAA recommendations. When the EAA-9 score for foods consumed in a day reaches 100%, all EAA requirements have been met.

Validation of EAA-9 Against FAO/WHO/UNU AAS Used in PDCAAS

Validation of the EAA-9 approach required comparison to the established protein quality standard, PDCAAS. This analysis was not straightforward because the proposed EAA-9 score and PDCAAS are calculated using different units and are created for different purposes. However, we achieved a robust evaluation in 2 steps. First, we directly compared the EAA-9 score to the FAO/WHO/UNU AAS, which is an independent component of the PDCAAS calculation. Second, we compared 2 versions of PDCAAS, one calculated using the EAA-9 score ($\text{PDCAAS}_{\text{EAA-9}}$ = EAA-9 × protein digestibility) and one calculated using the FAO/

Table 2
Features of protein quality scoring methods

	FAO/WHO/UNU amino acid score	PDCAAS	EAA-9
Compare protein quality between foods	✓	✓	✓
Allows for representation of selected amino acid nutrients			✓
Can be modified to reflect personalized amino acid recommendations			✓
Is additive to create amino acid complete meals			✓
Score of 100% guarantees all 9 EAA RDAs			✓
Protein quality score can incorporate protein digestibility factors when available	✓	✓	✓

Comparison of current protein scoring systems against proposed EAA-9 framework. Identifies notable functionality and application of amino acid scoring methods. Abbreviations: EAA, essential amino acid; EAA-9, Essential Amino Acid 9; PDCAAS, Protein Digestibility-Corrected Amino Acid Score

WHO/UNU AAS ($PDCAAS_{FAO/WHO/UNU}$ = FAO/WHO/UNU amino acid score × protein digestibility).

Comparison of EAA-9 to FAO/WHO/UNU AAS

The USDA National Nutrient Database for Standard Reference, Legacy Release (SR Legacy) was utilized in this analysis, as it is the most trusted and comprehensive source of food composition data and one of the only publicly available sources of amino acid composition in foods. Foods with nonzero values for all 9 EAAs and protein were included for calculation (n = 4734, Supplemental Table 1) [22]. EAA-9 was calculated using the RDAs for a 70 kg adult; the FAO/WHO/UNU AAS was calculated as defined in the Report of the Joint FAO/WHO/UNU Expert Consultation on Protein Quality Evaluation (2007) [18]. Table 3 shows the RDAs and FAO/WHO/UNU amino acid scoring pattern. EAA recommendations were converted to units equivalent to the respective RDA and FAO/WHO/UNU recommendations using organization-specific protein recommendations of 0.66 g/kg/d for FAO/WHO/UNU and 0.8 g/kg/d for RDA [4].

To facilitate a one-to-one comparison of EAA-9 and FAO/WHO/UNU AASs without altering the FAO/WHO/UNU framework, the EAA-9 scores for each food were transformed to mg of EAA per 56 g of protein (representing the RDA for protein, 0.8 g/kg), as shown in Figure 3.

The FAO/WHO/UNU AASs did not follow a normal distribution due to differences in the amino acid content of plant-based compared with animal-based foods and truncation at 100%. We therefore used Spearman's rank correlation coefficient to compare the nonlinear scores. Spearman rank correlation indicates the level of association between 2 nonlinearly distributed variables. A correlation coefficient of 1 (or 100%) would indicate that the scores for all foods were ranked in the same order, and a 0 would indicate no correlation between the ranks of the 2 scores. As shown in Figure 4A, the results of Spearman's rank correlation showed a very strong relationship with a correlation coefficient (ρ) of 0.8181 and P value $< 2.2e^{-16}$. Any scoring deviations that existed could be explained by differences between the FAO/WHO/UNU scoring pattern and the RDA for each amino acid. If the FAO/WHO/UNU scoring pattern of the limiting amino acid for a food is higher than the RDA, the food will fall to the left of the main line in Figure 4A; if the RDA is higher, the food will fall to the right of the line. Due to the truncation of the FAO/WHO/UNU AAS, the line flattens out at the top. The series of linear relationships in Figure 4A visually represented the natural clustering of scores based on the limiting amino acid of each food. Due to the strength of the relationship, we concluded the 2 scoring frameworks produced nearly identical results.

Comparison of PDCAAS calculated with $PDCAAS_{EAA-9}$ to $PDCAAS_{FAO/WHO/UNU}$

To calculate PDCAAS, protein digestibility scores for 220 foods were obtained from the Genesis R&D Food Manual [23]. For each food listed in Genesis, a Registered Dietitian Nutritionist identified an equivalent match in SR Legacy to obtain amino acid profiles. In total, 95 foods were included for comparison. As with the comparison of AASs, Spearman's rank correlation was chosen in assessment of PDCAAS due to nonlinearly distributed scores. When used to assess the association between $PDCAAS_{EAA-9}$ and $PDCAAS_{FAO/WHO/UNU}$, Spearman's rank correlation indicated that there was an extremely strong relationship with a correlation coefficient (ρ) of 0.9072 and P value $<$ $2.2e^{-16}$ (Figure 4B). Thus, the 2 scoring frameworks produced nearly identical results. All data required for calculations can be found inSupplemental Tables 1–3.

Applications of the EAA-9 Score

Although the EAA-9 and FAO/WHO/UNU scoring framework share many similarities, they differ largely in their application. The FAO/WHO/UNU AAS framework compares the relative quality of 2 proteins, whereas the EAA-9 can be used to compare proteins and to track progress toward meeting EAA requirements. In addition to scoring single foods, the EAA-9 framework simplifies the complex nature of complementing amino acids, thus allowing users to score a combination of foods or meals. The EAA-9 score can be used either additively or cumulatively. Both approaches are valuable.

With the additive method, each food is scored individually, and the resulting scores are added ($EAA\text{-}9_{Additive} = EAA\text{-}9_{Food\ A} + EAA\text{-}9_{Food\ B}$). This score tends to be conservative because it is calculated using the limiting amino acid of each food and does not consider the total amino acid profile. Consequently, it is possible to meet requirements for all EAAs with an additive score below 100%. The benefit of the additive framework lies in the

Table 3
Amino acid recommendations used in calculation of amino acid scores

Essential amino acid	RDA	FAO/WHO/UNU scoring pattern	RDA	FAO/WHO/UNU scoring pattern
	mg/70 kg/d		mg/g protein	
Histidine	980	693	17.5	15
Isoleucine	1330	1386	23.7	30
Leucine	2940	2726	52.5	59
Lysine	2660	2079	47.5	45
Methionine + Cysteine	1330	1016	23.7	22
Phenylalanine + Tyrosine	2310	1756	41.2	38
Threonine	1400	1063	25.0	23
Tryptophan	350	277	6.2	6
Valine	1680	1802	30.0	39

Two recommendations for intake of EAAs: RDAs and FAO/WHO/UNU scoring patterns. RDAs are defined in mg/70 kg/d, converted to mg/g of protein by dividing by RDA for protein (0.8 g/kg/d = 56 g/70 kg/d) [4]. FAO/WHO/UNU scoring pattern defined in mg/g protein converted into mg/70 kg/d by multiplying by the FAO mean protein recommendation (0.66 g/kg/d = 42.6 g/70 kg/d) [18].

$$\text{EAA-9} = \overbrace{\frac{\text{mg of limiting EAA}}{\text{RDA of limiting EAA}\left(\text{mg}\cdot 70\ \text{kg}^{-1}\cdot \text{d}^{-1}\right)}}^{\text{EAA-9}} \times \overbrace{0.8\ \text{g protein}\left(\text{kg}^{-1}\cdot \text{d}^{-1}\right)\times 70\ \text{kg}}^{\text{RDA for protein}}$$

$$= \frac{\text{mg of limiting EAA}}{\text{RDA of limiting EAA}\left(\text{mg}\cdot 70\ \text{kg}^{-1}\cdot \text{d}^{-1}\right)} \times 56\ \text{g protein}\left(\text{d}^{-1}\right)$$

$$= \frac{56\times\ (\text{mg of limiting EAA per g protein})}{\text{RDA of limiting EAA}\ (\text{mg}\cdot 70\ \text{kg}^{-1})}$$

Figure 3. Conversion of EAA-9 from mg/kg body weight/d to mg/g protein/d.
Calculation for conversion of EAA-9 from using RDAs in mg/70 kg/d to mg/g protein; intended for use in one-to-one comparison with FAO/WHO/UNU amino acid score. Abbreviation: EAA, essential amino acid.

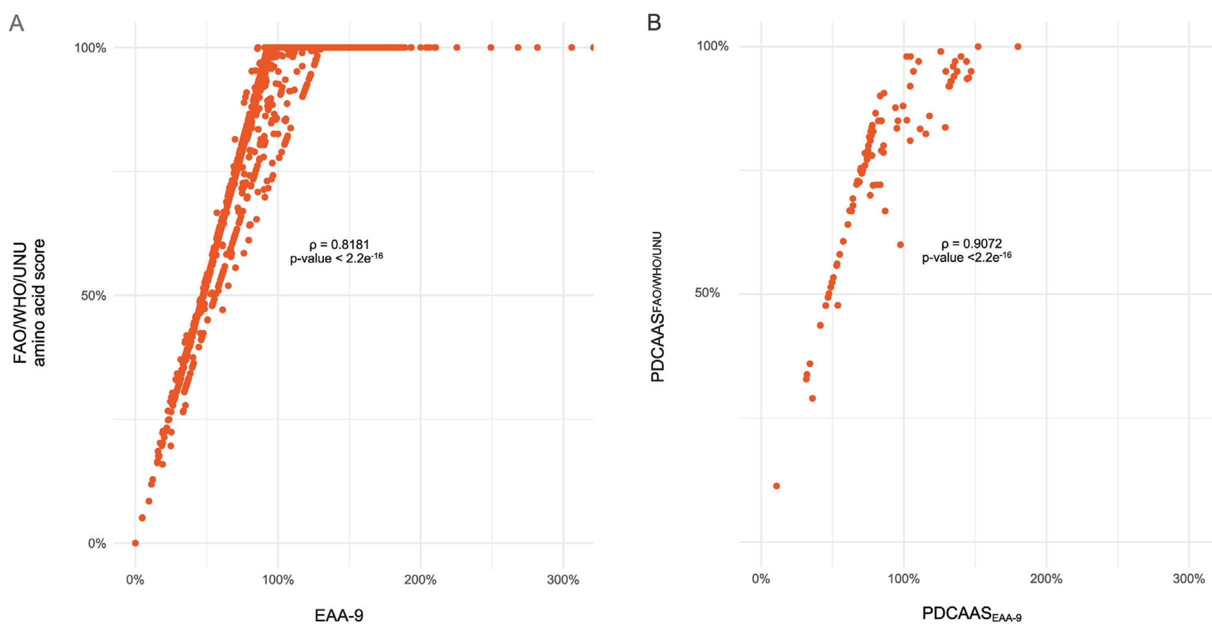

Figure 4. Comparison of scoring frameworks.
(A) Relationship between EAA-9 scoring framework to standard FAO/WHO/UNU amino acid score (n = 4734 foods); and (B) relationship of PDCAAS using EAA-9 and FAO/WHO/UNU amino acid score (n = 95 foods). Each dot represents one food. A correlation coefficient (ρ) above 0.6 indicates a strong correlation. P value of .05 or lower indicates strong statistical significance. $\text{PDCAAS}_{\text{EAA-9}}$ = EAA-9 × protein digestibility; $\text{PDCAAS}_{\text{FAO/WHO/UNU}}$ = FAO/WHO/UNU amino acid score × protein digestibility. Abbreviations: EAA-9, Essential Amino Acid 9; PDCAAS, Protein Digestibility-Corrected Amino Acid Score.

ability to build meals and diets using individual ingredient and food scores: higher scoring foods could be easily substituted while eliminating risks of suboptimal intake.

With the cumulative method, the EAA content of each food is summed prior to scoring ($\text{EAA-9}_{\text{Cumulative}} = \text{EAA-9}_{(\text{Food A} + \text{Food B})}$). Therefore, a cumulative score is accurate; a score of 100% meets exactly the RDA for the limiting EAA and meets or exceeds RDAs for all other EAAs. The cumulative approach may be best suited for research settings in which individual food scores are less important because they may not accurately reflect total consumption.

Considering its unique additive and cumulative capabilities, the EAA-9 score is applicable in a broad range of settings, from research and clinical use to personal consumer use. To demonstrate EAA-9 scoring in an applied example, recipes were identified from MyPlate Kitchen, an interactive site for consumers and professionals to find recipes that follow the DGA [24]. The additive and cumulative EAA-9 scores corresponding to the MyPlate recipe for 1 serving of the Rice Bowl Breakfast with Fruit and Nuts [25] were calculated (Figure 5), portions of each ingredient are shown in Table 4. As expected, the additive and cumulative scoring methods resulted in slightly different scores.

The additive and cumulative EAA-9 methods can also be applied to multiple meals consumed in a day. As an example, we calculated both the additive and cumulative scores for 3 MyPlate meals (Table 4): Rice Bowl Breakfast with Fruit and Nuts, Chicken Waldorf Salad, and Easy Stuffed Pasta Shells [25–27]. The score calculation is shown in Figure 6 (calculation details and SR Legacy foods provided in Supplemental Table 4).

The utility of the additive approach versus the cumulative approach to EAA-9 scoring can be of benefit beyond scientific study. For example, if EAA-9 were implemented in a consumer-facing application such as MyFitnessPal, a user may prefer to be conservative and overshoot amino acid targets. The

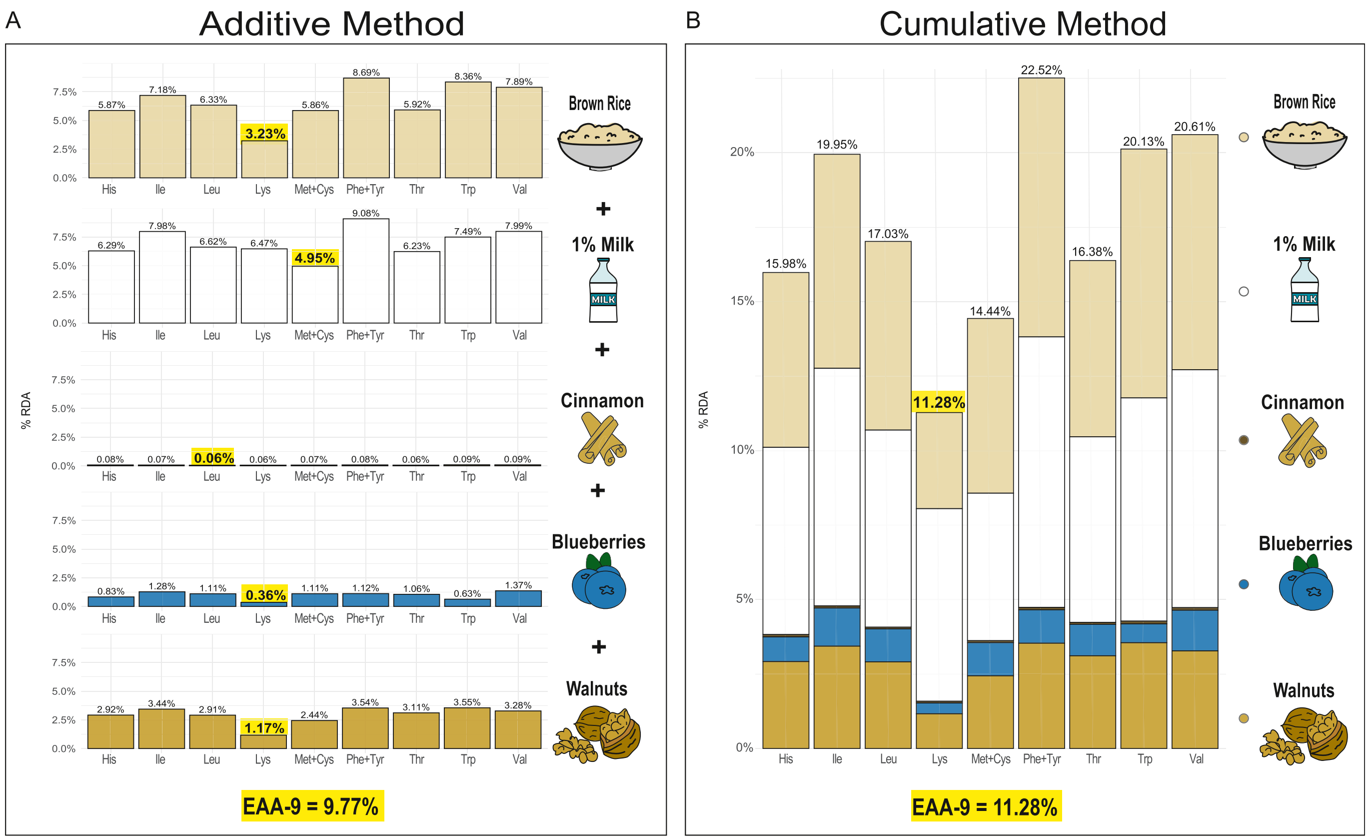

Figure 5. Two valid methods of calculating the EAA-9 score.
An example of an (A) additive and (B) cumulative approach to scoring multiple foods. Additive EAA-9 score calculated as the sum of ingredient EAA-9 scores. Cumulative EAA-9 score calculated by summing amino acid nutrients first then scoring. % RDA defined as the amount of each amino acid provided by the food divided by the RDA. Calculation details and SR Legacy foods provided in Supplemental Table 4. Abbreviations: EAA, essential amino acid; svg, serving.

application therefore could apply an additive model. This flexibility in use demonstrates the robustness of the EAA-9 score as a simple yet accurate approximation of amino acid contribution toward meeting daily requirements.

Furthermore, the additive EAA-9 approach could be of great value for individuals who wish to optimize the protein quality of their meals while conforming to a plant-based diet. Table 5 provides EAA-9 scores for each ingredient in a vegan meal adapted from the MyPlate Kitchen recipe for Bean and Rice Burritos [28]. The framework's flexibility allows for simple addition or subtraction of ingredients. In this case, adding tofu improved the protein quality score of the meal. As with all meal creation, individual EAA-9 food scores can be used to replace and complement lower scoring foods and assist in meeting amino acid needs.

In addition to the calculation examples in previous tables and figures, EAA-9 scores for a standard portion size of all applicable SR Legacy foods are provided in Excel format in Supplemental Table 5.

Precision EAA-9 scoring

The EAA-9 scoring framework was developed with flexibility, personalization, and precision in mind. With the advent of precision nutrition and the appreciation for the role of EAAs, future research may differentiate EAA recommendations based on characteristics such as sex or the usage of lean body mass instead of body weight. Although the RDAs for each EAA were used to calculate the examples above, the EAA-9 framework can also be used to implement alternative recommendations or requirements. For instance, although the current RDA of Leu is 2.9 g/d, research suggests that Leu intake at ~7.5 g/d can overcome anabolic resistance in older adults and optimize muscle health [14,15]. To reflect this discovery and optimize dietary Leu, an EAA-9 score was calculated with a requirement of 7500 mg/d of Leu (Figure 7). To use the previous example, a Rice Bowl Breakfast with Fruit and Nuts [25] had a personalized cumulative EAA-9 score of 6.67%, lower than the RDA-based cumulative EAA-9 score of 11.28% but accurately reflecting higher metabolic needs of Leu to maintain muscle mass in older adults.

Additional flexibility in the EAA-9 framework includes the ability to choose which amino acids are used in the calculation. For example, a subset of the amino acids, such as Leu, Lys, and Met, can be used in place of the full set of 9 EAAs. This feature may be of interest to research scientists and clinical professionals wanting to compare more metabolically active or more limiting amino acids rather than amino acids such as histidine, which does not have a clearly defined adult requirement [29]. For example, $^{1}/_{3}$ cup of plain low-fat yogurt (FDC_ID 170886) would have a score of 3.12% when calculated using only the requirements of Leu, Lys, and Met + Cys (formula depicted in Figure 8) compared to an EAA-9 score of 1.75% [22]. As with all scoring systems, opportunistic users could game the system. If

Table 4
MyPlate meals, ingredients, and recipe portions in 1 serving

Meal	Ingredient	Portion
Meal 1: Rice Bowl Breakfast with Fruit and Nuts	cooked brown rice	½ cup
	nonfat (skim) milk (or 1% milk)	¼ cup
	cinnamon	¼ tsp
	chopped fruit (try a mixture - apples, bananas, raisins, berries, peaches)	½ cup
	chopped nuts (try walnuts or almonds)	1 tbsp
Meal 2: Chicken Waldorf Salad	low-fat mayonnaise	4 teaspoons
	nonfat or low-fat plain yogurt	4 teaspoons
	lemon juice	½ teaspoon
	salt	1/16 teaspoon
	chopped cooked chicken breast	¾ cup
	red apple, diced	¼ medium
	halved red or green grapes	¼ cup
	sliced celery	¼ cup
	chopped walnuts, divided (toasted if desired)	2 tablespoons
Meal 3: Easy Stuffed Pasta Shells	frozen chopped spinach, thawed	1 ¼ ounces
	cottage cheese, low-fat	1 ½ ounces
	mozzarella, part skim shredded	3 tablespoons
	dried oregano	3/16 teaspoon
	black pepper	1/32 teaspoon
	light tomato basil pasta sauce, low-sodium	3 ¼ ounces
	water	⅛ cup
	pasta shells, uncooked (large)	¾ ounce

Meal ingredients and portion sizes for 1 serving of recipes identified from MyPlate Kitchen [25–27].

the food industry "spiked" a food with 1, 2, or all 3 of these EAAs, the result would be a high score, even though the overall protein quality would still be poor.

Discussion

The topic of protein quality is not new for food science or nutrition research. However, advances in the biochemical understanding of amino acids as metabolically unique nutrients and increasing consumer awareness regarding protein quality demonstrate a need to shift from the mindset and mathematics that treat protein and its component amino acids as generic and interchangeable. Moving forward, it is important to build on the foundation of past protein quality scoring systems to provide a more transparent, scalable, and personalizable framework that facilitates meeting essential nutrient recommendations.

The innovative EAA-9 model is a nutrient-focused approach that allows for flexible, additive, and scalable evaluations of protein quality. Current practice relies on nitrogen measurements to estimate the protein content of foods and protein requirements in the human body while using total protein consumption as an indicator of amino acid intake. However, the

A

$$\begin{aligned}
\text{EAA-9}_{\text{Additive}} &= \sum \text{EAA-9 ingredient scores for meal 1} + \sum \text{EAA-9 ingredient scores for meal 2} + \sum \text{EAA-9 ingredient scores for meal 3} \\
&= (3.23\% + 4.95\% + 0.06\% + 0.36\% + 1.17\%) + (0.18\% + 1.75\% + 92.37\% + 0.08\% + 0.28\% + 0.17\% + 2.33\%) + (1.78\% + 15.55\% + 8.63\% + 0.05\% + 1.07\% + 2.38\%) \\
&= 9.77\% + 97.16\% + 29.46\% \\
&= \mathbf{136.39\%}
\end{aligned}$$

B

$$\begin{aligned}
\text{EAA-9}_{\text{Cumulative}} &= \min\left(\frac{\text{Total His}}{\text{His RDA}}, \frac{\text{Total Ile}}{\text{Ile RDA}}, \frac{\text{Total Leu}}{\text{Leu RDA}}, \frac{\text{Total Lys}}{\text{Lys RDA}}, \frac{\text{Total Met + Cys}}{\text{Met + Cys RDA}}, \frac{\text{Total Phe + Tyr}}{\text{Phe + Tyr RDA}}, \frac{\text{Total Thr}}{\text{Thr RDA}}, \frac{\text{Total Trp}}{\text{Trp RDA}}, \frac{\text{Total Val}}{\text{Val RDA}}\right) \\
&= \min\left(\frac{1902.15\text{ mg}}{980\text{ mg}}, \frac{2770.19\text{ mg}}{1330\text{ mg}}, \frac{4829.08\text{ mg}}{2940\text{ mg}}, \frac{4653.36\text{ mg}}{2660\text{ mg}}, \frac{1997.47\text{ mg}}{1330\text{ mg}}, \frac{4762.24\text{ mg}}{2310\text{ mg}}, \frac{2507.04\text{ mg}}{1400\text{ mg}}, \frac{726.29\text{ mg}}{350\text{ mg}}, \frac{3109.78\text{ mg}}{1600\text{ mg}}\right) \\
&= \min(194.10\%, 208.28\%, 164.25\%, 174.94\%, 150.19\%, 206.16\%, 179.07\%, 207.51\%, 185.11\%) \\
&= \mathbf{150.19\%}
\end{aligned}$$

Figure 6. Additive and cumulative EAA-9 scores for MyPlate meals.
Comparison, providing the EAA-9 mathematical framework for 2 scoring options: (A) additive and (B) cumulative. Additive EAA-9 score calculated as the sum of ingredient EAA-9 scores. Cumulative EAA-9 score calculated by summing amino acid nutrients first, then scoring. Calculation details and SR Legacy foods provided in Supplemental Table 4. Abbreviations: EAA, Essential Amino Acid; SR Legacy, USDA National Nutrient Database for Standard Reference Legacy Release.

Table 5
Applying the EAA-9 framework for optimizing a plant-based diet

Ingredient	Serving size	EAA-9
Rice (cooked)	¼ cup	1.61%
Onion	$^1/_8$ small onion	0.04%
Kidney beans	¼ cup	6.45%
Flour tortillas	1 tortilla	2.71%
Salsa	1 tbsp	0.18%
Additive EAA-9 score =		10.99%
Firm tofu	$^1/_8$ block	8.16%
Additive EAA-9 score =		19.15%

Additive EAA-9 calculation for improving the protein quality of a vegan meal. Additive EAA-9 score of 10.99% is increased to 19.15% by adding tofu. Recipe for meal adapted from MyPlate recipe for "Bean and Rice Burritos," cheese was removed from this recipe [28]. Calculation details and SR Legacy foods provided in Supplemental Table 4. Abbreviation: EAA, essential amino acid.

9 EAAs—not nitrogen—are required for growth, development, metabolic functions, and healthy aging. We therefore propose that shifting the protein quality evaluation paradigm from the current generalized perspective to a precision nutrition focus that treats amino acids as unique, metabolically active nutrients will be valuable for multiple areas of science and public health. We further propose that the EAA-9 scoring framework provides the ideal tool for executing the paradigm shift toward precision nutrition for 3 reasons.

First, personalized nutrition and precision nutrition aim to "develop more comprehensive and dynamic nutritional recommendations based on shifting, interacting parameters in a person's internal and external environment throughout life" [30]. However, total-protein-based recommendations are designed for an average person who does not actually exist and are therefore not representative of the vast majority of the population. The current approach fails at the level of the individual. Future applications of precision nutrition depend on additional functionality that current dietary recommendations and protein quality models do not possess.

Second, food labeling profoundly obscures understanding about EAA needs. Protein, as listed on a food label, is a chemical estimate of total nitrogen multiplied by a factor of 6.25 to obtain total protein [3]. The factor of 6.25 approximates the average percentage of nitrogen (16%) in amino acids [31], but nitrogen composition varies widely from phenylalanine: 8.5% and Leu: 10.7% to glycine: 18.7% and arginine: 32.2% [32]. Thus, the true average differs across proteins, and therefore also differs across foods with plant proteins generally containing higher amounts of nonessential amino acids that have higher percentages of nitrogen. Furthermore, the current characterization of total protein on food labels suggests that all proteins (and therefore all their component amino acids) are interchangeable. The implication is that the proteins from different food products are additive to achieve a daily requirement. For example, if a cereal box label simply combines a serving of 4 g of wheat protein with 6 g of milk protein to total 10 g of protein, a consumer could well take that to mean the 2 protein sources are of equal value. Additionally, substituting 6 g of milk with 6 g of soy "milk" to reach the same total protein further extends this generalization. However, wheat, milk, and soy proteins do not have the same amounts of EAAs and are not nutritionally equal. For optimal health and precision nutrition, that estimate is simply not accurate. Although the food label provides an opportunity to incorporate protein quality, displayed as the Percent Daily Value (%DV) derived from PDCAAS, this calculation is severely limited and not scalable [3]. A nutrient-based score such as EAA-9 would provide a robust indication of protein quality, which is a vital component when considering the nutritional benefits that a protein source of food can provide.

Third, in dietary recommendations, nitrogen balance continues to be the gold standard for estimating the RDA for protein [4]. The nitrogen balance approach has been widely criticized for underestimating true protein requirements and does not take amino acids into account. In contrast, the EAA-9 approach allows researchers and practitioners to treat amino acids as individual nutrients to assess protein quality, nutrient density, and both metabolic and health outcomes. The analytical ability for measuring amino acids has been overcome, yet economic and logistical challenges still exist. Financial and scientific backing are needed to support updated dietary protein recommendations informed primarily by amino acid-based scientific understanding rather than a vague requirement estimated by nitrogen measurements.

Personalized requirement

$$\text{EAA-9} = \min\left(\frac{\text{His (mg/svg)}}{\text{His RDA}}, \frac{\text{Ile (mg/svg)}}{\text{Ile RDA}}, \frac{\text{Leu (mg/svg)}}{7500\ \text{(mg/d)}}, \frac{\text{Lys (mg/svg)}}{\text{Lys RDA}}, \frac{\text{Met + Cys (mg/svg)}}{\text{Met + Cys RDA}}, \frac{\text{Phe + Tyr (mg/svg)}}{\text{Phe + Tyr RDA}}, \frac{\text{Thr (mg/svg)}}{\text{Thr RDA}}, \frac{\text{Trp (mg/svg)}}{\text{Trp RDA}}, \frac{\text{Val (mg/svg)}}{\text{Val RDA}}\right) \times 100$$

Figure 7. Example of a personalized EAA-9 calculation.
EAA-9 modified to reflect leucine recommendation for older adults [15]. Abbreviations: EEA-9, Essential Amino Acid 9; svg, serving.

$$\underset{\text{(EAA-9 subset)}}{\text{EAA-3}} = \min\left(\frac{\text{Leu (mg/svg)}}{\text{Leu RDA}}, \frac{\text{Lys (mg/svg)}}{\text{Lys RDA}}, \frac{\text{Met + Cys (mg/svg)}}{\text{Met + Cys RDA}}\right) \times 100$$

Figure 8. Applying the EAA-9 framework using a subset of EAAs.
EAA-9 framework modified to include a subset of essential amino acids: Leu, Lys, Met+Cys. Abbreviations: EAA, essential amino acid; svg, serving.

The simple yet powerful EAA-9 framework provides a valuable, validated tool for measuring protein quality in a personalized way. It has utility for researchers, clinicians, public health officials, and consumers alike. However, a tool alone is not enough. The tool is limited by data availability; currently available protein data often do not include separate measurements for the EAAs. The future of precision nutrition requires treating amino acids as essential nutrients. Our understanding of amino acids as essential nutrients is scientifically established—now is the time to put that knowledge into practice.

Acknowledgments

We thank Olivia Comin, RDN, for assistance in data collection and data quality control, and Stephanie Westcot, PhD, for manuscript review and editing assistance. The authors' responsibilities were as follows—DKL, SMF: project conception; SMF, DKL: development of overall research plan and study oversight; EMJ-D, SAS: performed data analysis; EMJ-D: performed statistical analysis; SMF, EMJ-D, DKL: wrote the paper; SMF: had primary responsibility for final content; and all authors: read and approved the final manuscript.

Author disclosures

DKL serves on speaker bureaus for National Dairy Council and National Cattlemen's Beef Association (NCBA), as a research advisor for NCBA, and as a Scientific Advisory Board member for the Nutrient Institute and Herbalife Nutrition Foundation. SMF is Executive Director of and EMJ-D is a contractor for the Nutrient Institute 501(c)(3).

Funding

This work was supported by the Nutrient Institute, a 501(c)(3) not-for-profit organization.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tjnut.2023.06.004.

References

[1] A.J. Rose, Amino acid nutrition and metabolism in health and disease, Nutrients 11 (11) (2019) 2623, https://doi.org/10.3390/nu11112623.
[2] U.S. Department of Agriculture and U.S. Department of Health and Human Services, Dietary Guidelines for Americans, 2020-2025 [Internet], 9th Ed., U.S. Department of Agriculture, 2020. Available from: http://www.dietaryguidelines.gov/.
[3] 21 CFR 101.9 - Nutrition labeling of food, eCFR [Internet], National Archives, 2016 [cited 2023 Feb 2]. Available from: https://www.ecfr.gov/current/title-21/chapter-I/subchapter-B/part-101/subpart-A/section-101.9.
[4] Institute of Medicine, Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids [Internet], 2005th ed, National Academies Press, 2005, pp. 589–768, https://doi.org/10.17226/10490.
[5] M.E. Brosnan, J.T. Brosnan, Histidine metabolism and function, J. Nutr. 150 (suppl 1) (2020) 2570S–2575S, https://doi.org/10.1093/jn/nxaa079.
[6] S.M. Hutson, A.J. Sweatt, K.F. Lanoue, Branched-chain [corrected] amino acid metabolism: implications for establishing safe intakes, J Nutr 135 (6) (2005) 1557S–1564S, https://doi.org/10.1093/jn/135.6.1557S (suppl).
[7] C.J. Lynch, Role of leucine in the regulation of mTOR by amino acids: revelations from structure–activity studies, J. Nutr. 131 (3) (2001), https://doi.org/10.1093/jn/131.3.861S, 861S–865S.
[8] F. Li, Y. Yin, B. Tan, X. Kong, G. Wu, Leucine nutrition in animals and humans: mTOR signaling and beyond, Amino Acids 41 (5) (2011) 1185–1193, https://doi.org/10.1007/s00726-011-0983-2.
[9] M.F. McCarty, J.J. DiNicolantonio, An increased need for dietary cysteine in support of glutathione synthesis may underlie the increased risk for mortality associated with low protein intake in the elderly, Age (Dordr). 37 (5) (2015) 96, https://doi.org/10.1007/s11357-015-9823-8.
[10] G.S. Ducker, J.D. Rabinowitz, One-carbon metabolism in health and disease, Cell Metab 25 (1) (2017) 27–42, https://doi.org/10.1016/j.cmet.2016.08.009.
[11] J.D. Fernstrom, M.H. Fernstrom, Tyrosine, phenylalanine, and catecholamine synthesis and function in the brain, J. Nutr. 137 (6) (2007) 1539S–1547S, https://doi.org/10.1093/jn/137.6.1539S (supp 1).
[12] L.L. Munasinghe, J.L. Robinson, S.V. Harding, J.A. Brunton, R.F. Bertolo, Protein synthesis in mucin-producing tissues is conserved when dietary threonine is limiting in piglets, J. Nutr. 147 (2) (2017) 202–210, https://doi.org/10.3945/jn.116.236786.
[13] S. Zhang, X. Zeng, M. Ren, X. Mao, S. Qiao, Novel metabolic and physiological functions of branched chain amino acids: a review, J. Anim. Sci. Biotechnol. 8 (2017) 10, https://doi.org/10.1186/s40104-016-0139-z.
[14] D.K. Layman, T.G. Anthony, B.B. Rasmussen, S.H. Adams, C.J. Lynch, G.D. Brinkworth, et al., Defining meal requirements for protein to optimize metabolic roles of amino acids, Am. J. Clin. Nutr. 101 (6) (2015) 1330S–1338S, https://doi.org/10.3945/ajcn.114.084053.
[15] S.M. Phillips, D. Paddon-Jones, D.K. Layman, Optimizing adult protein intake during catabolic health conditions, Adv. Nutr. 11 (4) (2020) S1058–S1069, https://doi.org/10.1093/advances/nmaa047.
[16] M.C. Devries, C. McGlory, D.R. Bolster, A. Kamil, M. Rahn, L. Harkness, et al., Leucine, not total protein, content of a supplement is the primary determinant of muscle protein anabolic responses in healthy older women, J. Nutr. 148 (7) (2018) 1088–1095, https://doi.org/10.1093/jn/nxy091.
[17] D.S. Froese, B. Fowler, M.R. Baumgartner, Vitamin B12, folate, and the methionine remethylation cycle-biochemistry, pathways, and regulation, J. Inherit. Metab. Dis. 42 (4) (2019) 673–685, https://doi.org/10.1002/jimd.12009.
[18] Food and Agriculture Organization of the United Nations, World Health Organization & United Nations University, Protein and amino acid requirements in human nutrition: report of a joint FAO/WHO/UNU expert consultation [Internet], World Health Organization, 2007 [cited 2022 Dec 1]. Available from: https://apps.who.int/iris/handle/10665/43411.
[19] Food and Agriculture Organization of the United Nations, Dietary protein quality evaluation in human nutrition: report of an FAO Expert Consultation [Internet], 2013. Available from: https://www.fao.org/ag/humannutrition/35978-02317b979a686a57aa4593304ffc17f06.pdf.
[20] S.M. Rutherfurd, A.C. Fanning, B.J. Miller, P.J. Moughan, Protein digestibility-corrected amino acid scores and digestible indispensable amino acid scores differentially describe protein quality in growing male rats, J. Nutr. 145 (2) (2015) 372–379, https://doi.org/10.3945/jn.114.195438.
[21] J.S. Kang, Dietary restriction of amino acids for cancer therapy, Nutr. Metab. (Lond) 17 (2020) 20, https://doi.org/10.1186/s12986-020-00439-x.
[22] D.B. Haytowitz, J.K.C. Ahuja, X. Wu, M. Somanchi, M. Nickle, Q.A. Nguyen, et al., USDA national nutrient database for standard reference, legacy release [Internet], FoodData Central. Nutrient Data Laboratory, Beltsville Human Nutrition Research Center, ARS, USDA, 2019 [cited 2022 May 27]. Available from: https://fdc.nal.usda.gov/.
[23] J. Hohnstein, A. Triplett, Protein Digestibility - Genesis R&D Food Manual - Confluence [Internet], ESHA Docs. ESHA Research, 2019 [cited 2022 Jul 25]. Available from: https://esharesearch.atlassian.net/wiki/spaces/GENFOOD/pages/738820126/Protein+Digestibility.
[24] MyPlate Kitchen [Internet]. MyPlate. United States Department of Agriculture [cited 2022 Dec 2]. Available from: https://www.myplate.gov/myplate-kitchen/recipes.
[25] Rice Bowl Breakfast with Fruit and Nuts | MyPlate [Internet]. MyPlate. United States Department of Agriculture [cited 2022 Dec 2]. Available from: https://www.myplate.gov/recipes/supplemental-nutrition-assistance-program-snap/rice-bowl-breakfast-fruit-and-nuts.

[26] Chicken Waldorf Salad | MyPlate [Internet]. MyPlate. United States Department of Agriculture [cited 2022 Dec 2]. Available from: https://www.myplate.gov/recipes/myplate-cnpp/chicken-waldorf-salad.
[27] Easy Stuffed Pasta Shells | MyPlate [Internet]. MyPlate. United States Department of Agriculture [cited 2022 Dec 2]. Available from: https://www.myplate.gov/recipes/supplemental-nutrition-assistance-program-snap/easy-stuffed-pasta-shells.
[28] Bean and Rice Burritos | MyPlate [Internet]. MyPlate. United States Department of Agriculture [cited 2022 Dec 7]. Available from: https://www.myplate.gov/recipes/supplemental-nutrition-assistance-program-snap/bean-and-rice-burritos.
[29] W. Kriengsinyos, M. Rafii, L.J. Wykes, R.O. Ball, P.B. Pencharz, Long-term effects of histidine depletion on whole-body protein metabolism in healthy adults, J. Nutr. 132 (11) (2002) 3340–3348, https://doi.org/10.1093/jn/132.11.3340.
[30] J. de Toro-Martín, B.J. Arsenault, J.P. Després, M.C. Vohl, Precision nutrition: a review of personalized nutritional approaches for the prevention and management of metabolic syndrome, Nutrients 9 (8) (2017) 913, https://doi.org/10.3390/nu9080913.
[31] M. Hayes, Measuring protein content in food: an overview of methods, Foods 9 (10) (2020) 1340, https://doi.org/10.3390/foods9101340.
[32] [Internet], The Merck index: an encyclopedia of chemicals, drugs, and biologicals, The Merck Index Online. Royal Society of Chemistry, 2013 [cited 2023 Feb 2]. Available from: https://www.rsc.org/Merck-Index/.