Research Approaches and Methods for Evaluating the Protein Quality of Human Foods Proposed by an FAO Expert Working Group in 2014

Warren TK Lee, Robert Weisell, Janice Albert, Daniel Tomé, Anura V Kurpad, and Ricardo Uauy

Abstract
The Protein Digestibility Corrected Amino Acid Score (PDCAAS) has been adopted for assessing protein quality in human foods since 1991, and the shortcomings of using the PDCAAS have been recognized since its adoption. The 2011 FAO Expert Consultation recognized that the Digestible Indispensable Amino Acid Score (DIAAS) was superior to the PDCAAS for determining protein quality. However, there were insufficient human data on amino acid digestibility before adopting the DIAAS. More human data were needed before DIAAS could be implemented. In 2014, FAO convened an expert working group to propose and agree on research protocols using both human-based assays and animal models to study ileal amino acid digestibility (metabolic availability) of human foods. The working group identified 5 research protocols for further research and development. A robust database of protein digestibility of foods commonly consumed worldwide, including those consumed in low-income countries, is needed for an informed decision on adopting the DIAAS. A review on the impacts of using the DIAAS on public health policies is necessary. It would be advantageous to have a global coordinating effort to advance research and data collection. Collaboration with international and national agriculture institutes is desirable. Opportunities should be provided for young researchers, particularly those from developing countries, to engage in protein-quality research for sustainable implementation of DIAAS. To conclude, the DIAAS is a conceptually preferable method compared with the PDCAAS for protein and amino acid quality evaluation. However, the complete value of the DIAAS and its impact on public health nutrition cannot be realized until there are sufficient accumulated ileal amino acid digestibility data on human foods that are consumed in different nutritional and environmental conditions, measured by competent authorities. A future meeting may be needed to evaluate the size and quality of the data set and to determine the timeline for full adoption and implementation of the DIAAS.

Keywords: amino acids, protein, protein quality, digestibility, stable isotopes, DIAAS

Introduction
The FAO has a 60-y history in convening expert consultations for recommending protein requirements in humans (1). In 1989, the Protein Digestibility Correct Amino Acid Score (PDCAAS) was adopted by the Joint FAO/WHO Expert Consultation to determine protein quality in human foods (2). However, the PDCAAS method has received criticism since its adoption. In 2002, a Joint FAO/WHO/United Nations University Expert Consultation on Proteins and Amino Acids in Human Nutrition (3) reviewed the validity of these criticisms and recognized that the PDCAAS has a number of shortcomings: it does not credit additional nutritional value to high biological value proteins, it overestimates protein foods that contain antinutritional factors, and it overestimates protein foods of lower digestibility when supplemented with limiting amino acids. The 2002 FAO/WHO/United Nations University Expert Consultation recommended convening a further expert consultation to re-examine the validity of the PDCAAS for protein-quality evaluation and to suggest appropriate revisions or adoption of a better method to be applicable to a wider range of human diets (3). As a result, in 2011, the FAO convened an expert consultation in Auckland, New Zealand, to review methods for
determining dietary protein quality to reflect the current best practice (4). A newer protein-quality evaluation method, the Digestible Indispensable Amino Acid Score (DIAAS), was proposed. It was recognized that both ileal and fecal amino acid digestibility assays have limitations, but on balance of the evidence, ileal protein or amino acid digestibility determined at the terminal ileum, rather than the whole intestine, is considered better at reflecting the true quantity of amino acids absorbed. Therefore, the experts considered that the DIAAS should be based on the ileal digestibility of each amino acid studied in humans, or, if this is not possible, in growing pigs or growing rats in that order (4).

Although the 2011 Protein Quality Expert Consultation in principle recognized that the DIAAS is better than the PDCAAS in scoring protein quality, there was a lack of human data derived from the DIAAS (4). Virtually all of the existing DIAAS data came from the pig model, and there was also a lack of impact analysis on public health before the adoption of the DIAAS as the standard method for protein-quality evaluation. A shift toward the DIAAS could have implications on protein requirements in human nutrition and for scientific advice on protein nutrition. Therefore, the experts recommended that further research conducted in human subjects was needed before the DIAAS could be adopted (4). Against this background, in March 2014, the FAO convened an expert working group that brought together experts in protein and amino acids nutrition to update recent advances in protein-quality evaluation. The working group aimed to propose and agree on research protocols using both human-based assays and animal models to study ileal amino acid digestibility (protein quality) of human foods, especially foods and diets customarily consumed by persons in developing countries (5). In March 2015, the FAO released a new report to propose 5 research methods in the evaluation of protein quality of human foods (5), which is a follow-up of the 2011 Dietary Protein Quality Evaluation in Human Nutrition (4).

Protein-Quality Evaluation and Public Health Implications

Protein quality is related to true nitrogen and amino acid retention, currently measured as true net protein utilization, which is mainly related to the capacity of the protein to provide nitrogen and indispensable amino acids. This was the basis of the amino acid scoring approach developed in the 1980s, which is a method based on the comparison of the amino acid content of the protein under consideration with human amino acid requirements (an amino acid scoring system) and that was accepted as the most suitable approach. In addition, it was also recommended that amino acid score should be corrected for the potential incomplete digestibility of protein in the PDCAAS and DIAAS methods.

The experts recognized the relevance of protein quality in addressing the nutrition and public health agenda and its impacts on nutrition and health outcomes, in particular among vulnerable populations living in low-income countries in which people rely on plant-based diets. Even if plant foods are promoted in the agricultural systems, a clear idea of their digestibility must be developed, so that appropriate dietary recommendations can be made. Therefore, it is necessary to propose accurate methods to evaluate the quality of protein in human foods, especially those consumed by vulnerable populations from low-income countries. It is even more critical in developing countries in which environmental enteropathy could affect digestion and absorption (6).

Protein quality, not only quantity, exerts the true nutritional effects on body functions such as immunity, host defenses, linear growth, and the associated mental development and learning capacity (7). Long-term cohort studies from developing countries have indicated the need to improve food quality with consideration of linear growth rather than body weight as the relevant short-term outcome that will affect adult economic productivity and long-term health (8, 9).

The policy implications of these findings point to the first 1000 d as the critical time for long-term benefits for health, productivity, and well-being of populations. This period corresponds to the 9 mo of gestation starting from conception followed by the period from birth to 24 mo. This period is critical for lifelong health and economic productivity and is presently the focus of nutrition and health investment as part of global human and social development policies. Therefore, an improved understanding of protein quality in human foods would have a broad range of implications on public policies to reduce undernutrition, prevent childhood stunting, improve health and well-being, and improve rural livelihood and work productivity, thereby contributing to better economic development in the respective countries. Linear (height) growth is especially rapid during the first 4 y of life, and thus controlled field studies measuring length in a standardized manner can be used to evaluate the effect of protein quality on length gain over time (9–12 mo minimum in young infants; several years in children aged >24 mo given the slowdown of length gain after this age). Once the methods are in place, the possibilities can be explored to improve their cost-effectiveness.

Although there was a clear distinction between protein-quality evaluation and protein requirements in the working group, the experts recognized that protein quality and requirements are interlinked and this was taken into consideration when devising research protocols for human studies. The working group considered that both protein-quality evaluation and protein requirements need additional research, more particularly for vulnerable populations who consume predominantly plant-based diets.

Bridging Experimental Data between Animal and Human Studies

The working group was faced with the question, “What would legitimate the use of animal data for conclusions in humans?” Although recognizing that human data are preferable, the 2011 FAO Expert Consultation (4) believed that the fully validated pig-based assay would offer an opportunity to rapidly develop large data sets of ileal amino acid digestibility over a wide range of human foods at lower costs, because the pig’s digestive system and intestinal absorption processes are physiologically comparable to those of humans, although not totally comparable. In addition, the pig-based assay has already been standardized worldwide. However, the present working group stressed that validation studies are needed to compare the digestibility in the pig-based assay with that in human-based studies with the use of the same foods when consumed by humans. In addition, the experts pointed out that the data generated from these studies should apply to healthy adults but may not be applicable in groups with special protein requirements under specific conditions (e.g., old age, diseases, nutrient malabsorption, parasite burden, pregnancy or lactation, etc.).
The working group also agreed that appropriate in vivo isotopic methods for determining amino acid digestibility are urgently needed for direct comparison of amino acid digestibility in humans with that in a pig-based assay. Future research should also bridge the amino acid digestibility data between any newly developed in vivo isotopic methods and the pig-based assay.

Characteristics of an Ideal Method for Evaluating Dietary Protein Quality

Before reviewing currently available methods to propose research protocols, the challenges of characterizing an ideal method to determine the bioavailability of amino acids (protein quality) in human foods were also discussed. The key characteristics of an ideal method are summarized in Table 1.

The experts agreed on this framework as a starting point for evaluating and developing research protocols that could be used alone or in combination. In addition, the various research protocols could be part of a global coordinated effort to generate evidence to support the application of the DIAAS.

Proposed Research Protocols for Measuring Protein Digestibility in Human Foods

Although a qualitative scale is clearly an option for practical application purposes, there is also a need for quantitative reference values of amino acid availability and protein quality of the major protein sources in different populations, particularly in the current context of the needs for new alternative protein sources. The methods should be able to evaluate amino acid availability of different food sources in composite meals and diets in human populations.

The following methods that could serve as bases to develop standardized research protocols were reviewed and discussed by the working group:

- True ileal amino acid digestibility (4, 10, 11)
- Indicator amino acid oxidation (12–15)
- Postprandial protein utilization (16–18)
- Net postprandial protein utilization (19–21)
- A dual tracer approach to measuring the DIAAS (22–24)

The experts concluded that the DIAAS was a conceptually preferable method to the PDCAAS for the evaluation of protein quality research. In the long run, it is necessary to develop a method that can be used by governments to regulate claims on protein benefits from foods that contain protein.

The Way Forward: Addressing the Public Health Agenda and Knowledge Gap

In addition to proposing research protocols, the working group also suggested a link between protein quality and nutrition as well as between protein quality and the public health agenda. They also addressed the knowledge gap in protein-quality research.

A freely accessible, robust database of the amino acid digestibility of foods and diets commonly consumed in different parts of the world are needed for informed decisions regarding the DIAAS. Protein-quality research should also focus on diets of poor quality that are commonly consumed by vulnerable populations in different regions of the world. Before a final decision on the adoption of the DIAAS, there is a need to review the effects of using the DIAAS on public health policies and programs in comparison to those of the PDCAAS. In addition, research protocols to generate a reference database with escalated protein demands attributable to physiologic and pathologic conditions, hygiene, and different environmental conditions were also discussed.

To take the research agenda further, there is a need to identify research funds from both public and private sectors to carry out the research. It would be advantageous to have a global coordinated effort for funding research and data collection to generate a robust data set on the protein digestibility of foods and diets commonly consumed in different parts of the world. There is a need to foster collaboration with international and national agriculture research institutes to introduce protein-quality research with the use of stable isotopes. This would, however, require additional resources for human capacity development and the provision of research facilities. To ensure a wider and sustainable implementation of the DIAAS in dietary protein-quality evaluation at the country level, it is important to provide opportunities for young researchers, in particular those from developing countries, to participate in protein-quality research. In the long run, it is necessary to develop a method that can be used by governments to regulate claims on benefits from foods that contain protein.

Conclusions

The experts concluded that the DIAAS was a conceptually preferable method to the PDCAAS for the evaluation of protein

---

1 Codex Alimentarius Commission.
and amino acid quality. However, the complete value of the DIAAS cannot be realized until there are sufficient accumulated digestibility data on human foods determined by competent national and/or international authorities. Appropriate in vivo and noninvasive isotopic methods for determining amino acid digestibility in different populations, including those who are vulnerable, are needed. A future meeting may be needed to evaluate the size and quality of the data set and to determine the timeline for full adoption and implementation of the DIAAS.

Acknowledgments
WTKL drafted the original manuscript, refined the manuscript with comments provided by the coauthors, and has responsibility for final content; RW and JA provided intellectual inputs to improve the original draft of the manuscript; and RW, JA, DT, AVK, and RU provided comments and suggestions to improve the subsequent drafts of the manuscript before finalization. All authors read and approved the final manuscript.

References
1. FAO. Protein requirements: report of the FAO Committee. FAO Nutritional Series No. 16. Rome (Italy); FAO; 1957.