How an Isotope Technique Helps Determine Protein Quality

What should I know?

Proteins, alongside carbohydrates and fats, are referred to as macronutrients, as they must be consumed in large quantities to permit the human body to sustain its normal functions. Proteins are essential for body structure and growth, for regulating organ and tissue function and for protecting against infection. They are also an integral part of human genetic material. All enzymatic reactions and hormonal processes in the body depend on protein.

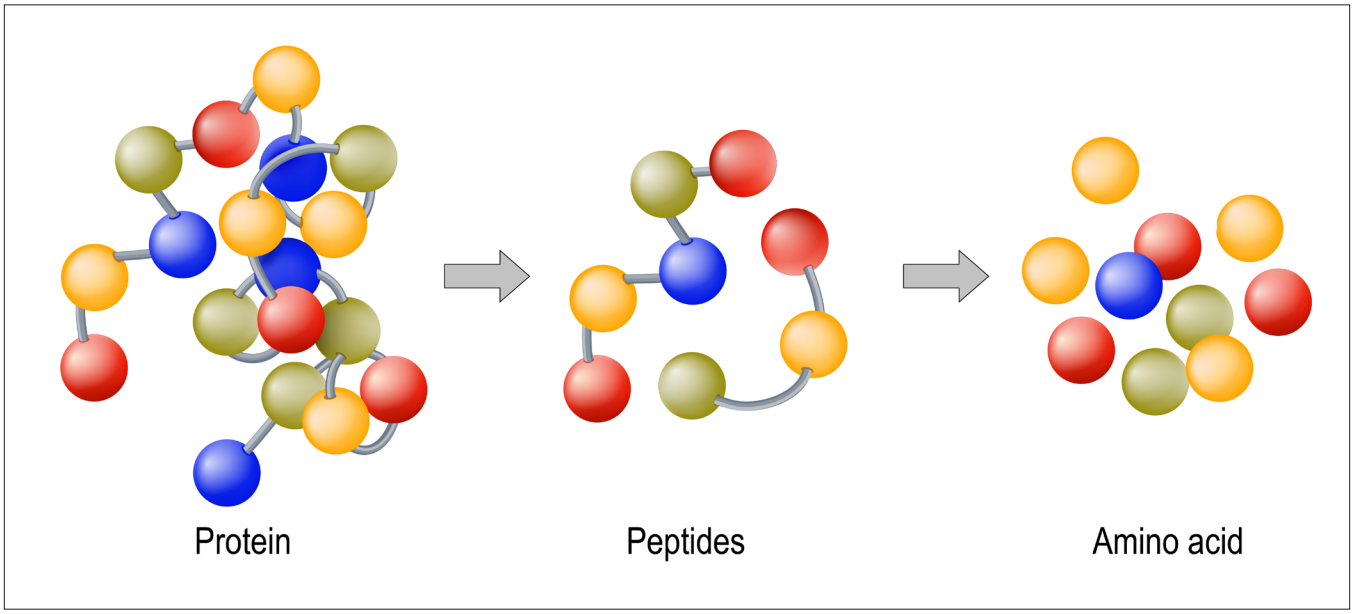
The supply of protein during the first two years of life not only determines growth, but also influences the risk of obesity and non-communicable diseases later in life, as well as the rate of recovery from acute undernutrition.

The IAEA supports Member States in the use of isotope techniques to provide vital data on the quality of protein, which, in turn, can contribute to improving nutritional programmes.

What is the composition of protein?

Proteins are made up of 20 amino acids, which are classified into two categories: non-essential (dispensable) and essential (indispensable).

Dispensable amino acids can be synthesized by the body, so they do not necessarily have to be present in the diet. They include alanine, aspartic acid, asparagine, glutamic acid and serine.



A combination of stable isotopes, deuterium and 13-carbon is used to track protein digestion. (Image: ©iStock.com/ttsz)

Phase 1: Bean amino acids are labelled with deuterium added to water during growth. (Photo: W. Kriengsinyos/Thailand)

However, indispensable amino acids cannot be produced by the body and therefore must be supplied through diet and/or supplements. Indispensable amino acids include histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. They are present in varying concentrations in different foods, such as meat, legumes, cereals, nuts and dairy products.

How is protein quality defined?

Protein quality is defined based on the capacity of a protein source to provide adequate amounts of indispensable amino acids. The combination of the presence of a given indispensable amino acid in a particular food and the proportion of the amino acid that is absorbed by the body after digestion is expressed as a score. Most cereal-based foods are deficient in the essential amino acids lysine, threonine and tryptophan, while legume-based foods are deficient in methionine. Proteins of animal origin such as eggs, milk, and meat tend to be better digestible and amino acids better absorbed compared with proteins derived from plant-based diets.

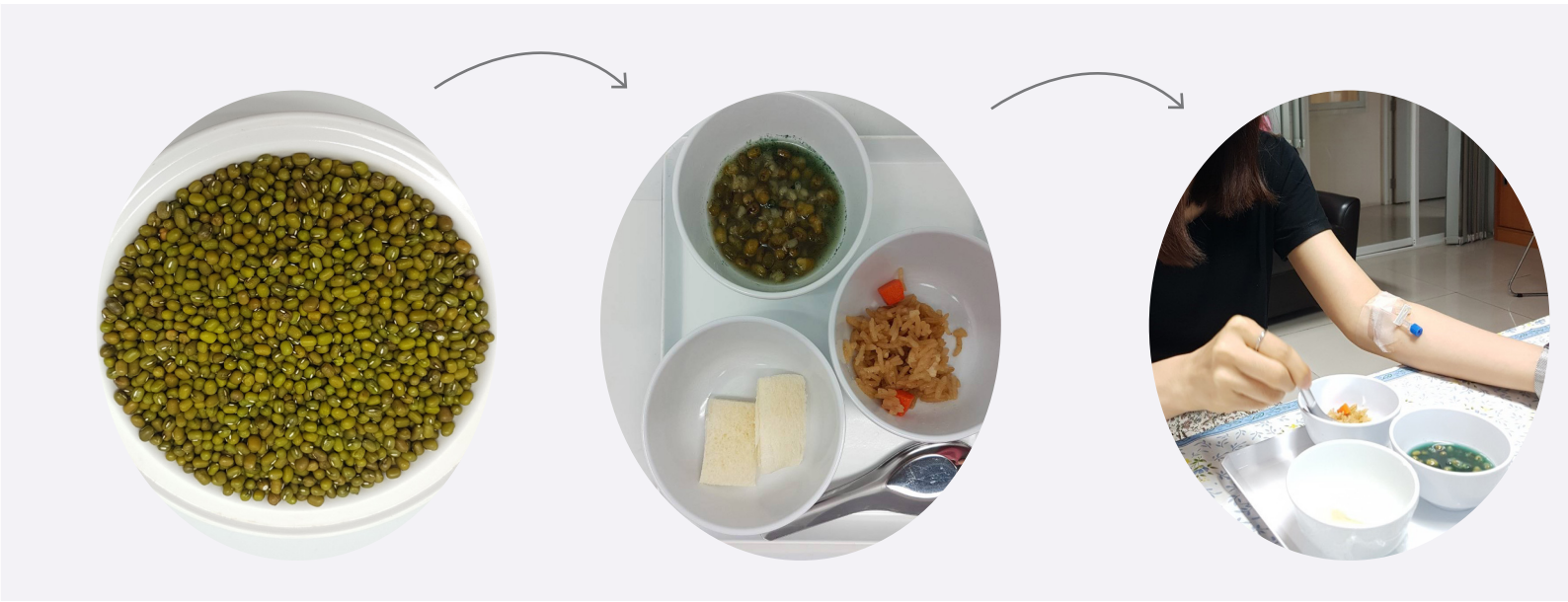
How does the dual stable isotope tracer technique help determine protein quality?

A new dual stable isotope tracer technique — developed as part of an IAEA coordinated research project (CRP) — compares the concentration of amino acids found in the blood after consuming a test meal to the concentration of a standard protein of known digestibility using isotopes, deuterium and carbon-13.

This method involves two steps:

1. Isotopic labelling of amino acids in a test food, such as milk or seeds, is achieved by adding deuterium oxide (D_2O) to drinking water for animals or to irrigation water for plants (Phase 1).

2. A test meal is prepared from the labelled edible portion of the test food, which is then consumed by the study participant along with another isotope (carbon-13)-labelled highly digestible protein source (Phase 2). Blood samples are collected before the test meal is consumed and again five, six, seven and eight hours after the test meal and the blood amino acid concentration is analysed. Additionally, breath samples are also collected. The ratio of the



Phase 2: Deuterium-labelled beans used to prepare test meal consumed by study participants to determine protein digestibility. (Photo: W. Kriengsinyos/Thailand)

differently labelled indispensable amino acids in the blood to those in the test meal consumed allows the true digestibility and absorption of the test protein to be determined[1]. The breath samples are analysed to assess the recovery of carbon-13 which is an additional indicator of protein digestion.

What are the benefits of using this technique?

Measuring the digestion of amino acids at the small-intestinal level, as recommended by the Food and Agriculture Organization of the United Nations (FAO), provides a more accurate picture than at the large-intestinal level. However, traditional methods for measuring amino acid digestibility in the small intestine are invasive. The dual isotope tracer method for measuring protein digestion is minimally invasive; only a few blood samples are required during an eight-hour feeding protocol.

What is the IAEA's role in the development of the dual isotope tracer method?

In 2015, the IAEA initiated, and has since supported, a four-year CRP entitled "Bioavailability of Proteins from Plant Based Diets". The CRP was implemented in seven low- and middle-income countries: Brazil, India, Jamaica, Mexico, Morocco, Pakistan and Thailand, with expert support from France and the United Kingdom. The IAEA provided financial support for field activities and purchased and supplied the necessary compounds containing the stable isotopes for all CRP sites. The IAEA also organized a training workshop to facilitate the standardized administration of the dual tracer protocol at all sites. Additionally, the IAEA convened three research coordination meetings in Vienna, where participants shared the lessons they had learnt, the challenges they had faced, and the results they had produced.

[1]The assumption is that the labelling process does not modify the functional behaviour of unlabelled amino acids.

What was the process for determining protein quality in the CRP?

The CRP participants established a method of growing legumes in water containing deuterium to intrinsically label the seed proteins. A standardized procedure was adopted and all seven countries participated in the labelling process: scientists in India and Thailand grew mung beans (Vigna radiata); in Brazil and Mexico, pinto beans (Phaseolus vulgaris); in Pakistan, chickpeas (Cicer arietinum); in Jamaica, kidney beans (Phaseolus vulgaris); and in Morocco, fava beans (Vicia faba).

Enough labelled beans were grown in all countries to conduct experiments among 7–10 adult volunteers. The isotope-labelled legume seeds were prepared as part of local meals and were consumed by volunteers. This knowledge enabled the protein quality score of the test meal to be calculated.

What success has been achieved in capacity building and knowledge sharing?

New data on the true protein digestibility of legumes in the seven countries are now available, and this will enable the FAO to develop protein quality recommendations. A network of mass spectrometry-based analytical capacity has been established for the analysis of the enrichment of deuterium and carbon-13 in legumes and blood samples from the experiments.

The CRP fostered the transfer of knowledge and skills, which resulted in a wider understanding of the use of stable isotope technology. This included researchers from Mahidol University, Thailand, receiving support from experts at the IAEA Collaborating Centre at St. John's Research Institute, Bangalore, India in order to build analytical capacity in protein digestion assessment. Similarly, scientists based at the Regional Designated Center for Nutrition, within the African Regional Co-operative Agreement for Research, Development and Training Related to Nuclear Science and Technology, in Rabat, Morocco, benefited from a fellowship and technical support from experts based at AgroParisTech, Paris, France. A detailed description of the dual isotope tracer method and its application has been published in the following three peer-reviewed articles:

1. Devi, S. et al. (2018). Measurement of protein digestibility in humans by a dual-tracer method, American Journal of Clinical Nutrition 107(6):984–991. https://pubmed.ncbi.nlm.nih.gov/29771297
2. Shivakumar, N. et al. (2019). Protein-quality evaluation of complementary foods in Indian children, American Journal of Clinical Nutrition 109(5): 1319–1327. https://pubmed.ncbi.nlm.nih.gov/30920607
3. Kashyap, S. et al. (2019). True ileal digestibility of legumes determined by dual-isotope tracer method in Indian adults, American Journal of Clinical Nutrition 110(4):873–882. https://pubmed.ncbi.nlm.nih.gov/31374575

IAEA Factsheets are produced by the Office of Public Information and Communication

Editor: Aabha Dixit • Design and layout: Ritu Kenn

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