Evolving trends in protein quality assessment

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ABSTRACT

There is strong evidence emerging of the positive role for protein in promoting optimal health at intakes beyond the Recommended Dietary Allowance (RDA). There is new focus on the roles of protein related to lean body mass retention during calorie restriction and ageing, weight control, insulin secretion & action, and bone & cardiovascular health. To date, most studies have focused on the quantity of protein and its relative proportion to carbohydrate and fat required for the provision of structural units in the production of new tissues and cells as well as the repair of worn-out/dead tissues. In light of the increasingly diverse functional roles of protein in human health, the appropriate end-points by which protein composition is being investigated becomes equally important for the assessment of protein quality. With respect to dietary proteins’ abilities to satisfy metabolic demands in relation to maintaining the muscles and bones, significant data have emerged to suggest that proteins’ roles in health may be based on factors that are not captured by the current protein quality estimates. As the understanding of proteins’ activities expands beyond its roles in maintaining body protein mass and satisfying the different metabolic demands for biosynthetic pathways, it is clear that the concept of protein quality must expand to incorporate these newly emerging activities of proteins.

Introduction

Proteins are linear polymer of amino acids linked by amide bonds where the biological function is dictated by the sequence of amino acid residues within the complex which adopts a well-defined three dimensional conformation. Thus, the knowledge of protein structure is central to understanding the molecular basis of life. Protein quality, therefore describe characteristics of a protein in relation to its ability to achieve defined metabolic actions. New researches reveal increasingly complex roles for proteins and amino acids in regulation of body composition & bone health, gastrointestinal function & bacteria flora, glucose homeostasis, cell signaling and satiety 1. The evidence obtainable to date suggests that quality is not only important at the minimum Recommended Dietary Allowance (RDA) level, but also at higher intakes 2. As research continues to evolve in assessing proteins’ roles in optimal health at higher intakes, there is also need to spur and explore implications for protein quality assessment.

Several protein quality studies builds on a long established concept that protein intakes above the minimum for nitrogen balance can have important anabolic influences on the muscles and bones through an anabolic drive of amino acids as well as exerting considerable experimental support for specific influence of indispensable amino acids such as the...
unbranched-chain amino acids, especially leucine, on the regulation of muscle protein synthesis 3.

Additionally, interests in diary proteins have grown to include activities within the gastrointestinal tract that may reflect protein structure and composition independently from its post-absorbptive amino acid profile 4. The current challenge to understand which protein characteristics are essential for those metabolic processes and functions remains a mystery. In the present context of assessing protein quality, this review will explore particular characteristics of the protein consumed that could influence optimal health and would need to be considered in an expanded protein quality concept. Clearly, for this newly emerging area, the main objective will be to define research questions for future explorations.

Features of quality proteins

Protein quality is defined as a measure of the essentiality of a dietary protein for growth and maintenance of body tissues, and in animals; production of meat, eggs, wool and milk. It is a long accepted paradigm that protein quality is an important aspect of any consideration of human protein needs, as evidenced by extensive efforts to measure quality and standardize those measurements 5. In the perspective of optimal protein intakes, the discussion of ‘what sort’ is equally relevant as the question of ‘how much’. High quality proteins provide the exact amount of essential amino acids or ‘module’ the body needs to develop, build and maintain the muscles to function properly.

There are two (2) important aspects of protein quality; I. The characteristics of the protein and the food matrix in which it is consumed. II. The demands of the individual consuming the food, as influenced by age, health & physiological status and energy balance.

Nutritional values

Functionally, dietary proteins can be classified as either;

- Complete/high quality proteins
- Incomplete/low quality proteins

The quality of dietary proteins depends on the level at which it provides nutritional amounts of essential amino acids need for the overall body health, maintenance and growth. Animal proteins such as eggs, cheese, milk, meat and fish are considered high quality/complete proteins due to their inherent ability to provide sufficient concentrates of essential amino acids. However, proteins of plant origins such as grains, nuts, vegetables and fruits are usually termed low quality/Incomplete proteins because the lack one or more of the essential amino acids and/or has an improper balance of amino acid concentrates. Incomplete proteins can be complemented and consumed at the same time or within a short period of time to provide all the essential amino acids and obtain the maximum nutritional value from the amino acids. Such dietary combinations usually yields a high quality protein meal, providing sufficient amounts of proper balance of the essential amino acids required by the human system to function.

Not all proteins are equally beneficial and nutritious; the factors that determine whether a protein source is of high or low qualities include:

a) Digestibility (bioavailability): This describes how efficient enough a protein source can be digested and absorbed by the gastrointestinal system

b) Essential amino acid concentrates: A food source that contains all the essential amino acids in adequate amounts and at a similar level.

Based on the characteristics of individual dietary proteins, complete proteins comprises all the essential amino acids in the correct proportions based on the human dietary requirements which is in contrast to incomplete proteins 6. Efficient protein utilization
would readily be attained if a balanced variety of protein sources are ingested concurrently. Table 1 enlists the different limiting amino acid(s) in six dietary sources.

Table 1 Limiting Amino Acids in Diets

<table>
<thead>
<tr>
<th>Dietary Sources</th>
<th>Limiting Amino Acid(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>Lysine</td>
</tr>
<tr>
<td>Rice</td>
<td>Lysine</td>
</tr>
<tr>
<td>Maize</td>
<td>Lysine &amp; Tryptophan</td>
</tr>
<tr>
<td>Legumes</td>
<td>Tryptophan/ Cysteine/ Methionine</td>
</tr>
<tr>
<td>Pulses</td>
<td>Methionine/Cysteine</td>
</tr>
<tr>
<td>Eggs</td>
<td>None; reference for absorbable protein</td>
</tr>
</tbody>
</table>

Pulses are edible seeds of different forms of pod-bearing plants [For example, Beans]. Because proteins are crucial to physical and mental health, the list of health advantages it confers is almost endless. Feasting high quality protein food at each meal helps reduce appetite and energy requirements for converting proteins to carbohydrates, a process known as Gluconeogenesis. Proteins can also be converted to fat (indirectly) and stored in the adipocytes; however, the presence of nitrogenous moieties, an important signaling molecule essentially channeled for the synthesis of antibodies configures the human systems to preferentially hold onto nitrogen, which indicates the likelihood of proteins to be converted to carbohydrates rather than fats. These complex chemical reactions utilize extra energy (ATP) or its reduced equivalents (NADH+/FADH2) to burn more calories at rest, thus, weight loss progress is often enhanced due to increased metabolism.

Egg proteins

Eggs provide an affordable source of complete protein for sustained energy. Recent studies affirms that the high quality protein character of eggs makes a valuable contribution to muscle strength providing a source of sustained energy and promotes satiety; noting that quality protein remains an important nutrient factor for active individuals in all stages of life. The protein concentrate in raw eggs which is approximately 51% bioavailable as compared to its 91% protein bioavailability when cooked indicates that the protein of cooked eggs is nearly twice as absorbable as the protein from raw eggs.

Despite the cholesterol content of the egg yolk (vitellus), biochemical observations have reported that a moderate consumption of eggs [up to two (2) per day] does not seem to increase the risk associated with the coronary heart diseases, type II diabetes and/or cardiovascular diseases in healthy individuals.

Egg proteins: experimental findings

All natural, high quality proteins in eggs contribute to strength, power and energy in the ensuing ways:

a) Sustained energy: The proteins in eggs provides steady and sustained energy because it does not cause a surge in blood sugar or insulin levels, which can lead to a rebound effect or energy ‘crash’ as the sugar levels drop. Eggs are inexpensive nutrient-rich source of complete proteins and provide adequate concentrates of B vitamins and minerals required for the production of energy metabolites such as thiamin (B1), riboflavin (B2), vitamin B6, folic acid, vitamin B12, choline, iron, calcium, phosphorus and potassium.

b) Muscle strength: Dietary protein intake directly influences muscle mass, strength and function in individuals of all ages. The nutrient content of one (1) egg provides more than 6g of high quality proteins which can help in building and preserving muscle
mass which especially prevents muscle loss in older adults. Eggs are also rich in leucine, an essential amino acid that contributes to the muscles ability to utilize energy and assists in post-exercise muscle recovery.

c) **Gold-Standard protein:** The high quality protein constituent in eggs provides all the essential amino acids required to maintain muscle mass. In fact, the quality of egg protein is so high that scientist frequently uses eggs as the standard to evaluate the protein quality of other dietary foods.

d) **Satiety & weight management:** Studies have shown that administering two (2) eggs for breakfast on a daily basis have helped overweight dieters lose 65% more weight and feel more energetic than those who ate bagel breakfast of equal calorie and volume. Further observations revealed that individuals who ate high quality protein diets for breakfast, including eggs had greater sense of fullness throughout the day as compared to eating the same combinations at lunch or dinner.

Many people dieting do not seem to realize the importance of eating high quality protein when attempting to lose weight. At low protein concentrates, the human system actually breaks down faster than it repairs itself, eventually resulting in a lowered metabolism and of course, this may even be intense if frequent exercises are employed in order to lose weight. This does not mean that it is necessary to ingest higher concentrates of dietary proteins, in fact eating little is often the best rule. The importance of quality proteins when placed on a weight loss diet includes:

- **Reduction in the body fat concentrate:** There are two (2) essential amino acids which enable the body to burn body fat, these are lysine and methionine. These amino acids are metabolized via complex processes to produce a modified amino acid, carnitine which acts to channel fatty acids into the cells for catabolic energy generation. Another essential amino acid, phenylalanine is employed by the metabolic system to build insulin which helps to regulate sugar levels. A vast number of scientific evidences suggest that weight management is achieved much more quickly when there is a consistent control of blood sugar and insulin release. These factors account for the need to establish a standardized glucose intake dietary plan. It is significant to note that most processed sources of dietary protein such as meat and meat products contain high amounts of sodium derivatives in the form of salts mainly used for preservation and taste enhancers which causes water retention and contribute partly to weight gain.

**Protein quality evaluation**

The aim of evaluating protein quality is to determine the ability of a protein to meet maintenance needs plus special needs for growth, pregnancy/lactation. These needs are known as the lowest level of dietary protein intake that will balance the losses of nitrogen from the body to maintain the body protein mass, in persons at energy balance with the modest levels of physical activities plus, in children or pregnant/lactating women, the needs associated with the deposition of tissues or secretion of milk at rates consistent with good health.

**Methods of protein quality**

The quality of a dietary protein is predominantly assayed and estimated using five (5) different techniques; this includes:
a) **Protein efficiency ratio (PER):** It measures the ability of a protein to support the physiological growth. It represents the ratio of weight gain to the amount of protein consumed. It is significant to note that this technique suffers a major concern at which it may not be experimentally applicable to infants and children as it measures growth but not maintenance. Therefore, it may be of limited use in determining the protein needs of adults. Mathematically, the PER can be expressed in terms of the weight gain per gram of dietary protein administered.\(^5\)

\[
\text{PER} = \frac{\text{Physiological weight gain}}{\text{Dietary protein concentrate}}
\]

b) **Biological value (BV):** these measures the amount of bound-nitrogen retained relative to the nitrogen concentrates absorbed. The BV method reflects bioavailability and gives an accurate appraisal of maintenance needs. BV, being the proportion of absorbed protein retained in the body does not take account of its digestibility. It can be used to assess the requirements of protein derived from foods with known quality differences and measure the proportion of absorbed nitrogen which is retained and presumably utilized for metabolic protein synthesis as an accurate indicator for protein measurements. The chart below illustrates the biological value of six (6) dietary proteins from both animal and plant origins.\(^16\)

<table>
<thead>
<tr>
<th>Dietary Protein(s)</th>
<th>Biological Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg &amp; Human Milk</td>
<td>0.90 - 1.00</td>
</tr>
<tr>
<td>Meat &amp; Fish</td>
<td>0.75 - 0.80</td>
</tr>
<tr>
<td>Wheat Protein</td>
<td>0.50</td>
</tr>
<tr>
<td>Gelatine</td>
<td>0</td>
</tr>
</tbody>
</table>

c) **Nitrogen protein utilization (NPU):** Current methods of evaluating protein quality that analyze physiological growth (Protein Efficiency Ratio - PER) and nitrogen balance where both digestibility and bioavailability of the absorbed amino acids (Biological Value - BV) determines the Net Protein Utilization. This is described as the ratio of nitrogen utilization for tissue formation to the concentrates of nitrogen digested. The NPU is the proportion of dietary proteins that is retained in the body under specified experimental conditions taking account of the digestibility and bioavailability. By convention, NPU is measured at 10% dietary protein (NPU10) at which the protein synthesis mechanism can metabolize all the bioavailable proteins to assume a balance in the essential amino acids concentrates. However, feasting of dietary proteins as it is normally eaten can be referred to as NPU{\textunderscore}operative\(^5\).

d) **Amino acid score (AAS):** This is a chemical technique considered fast, consistent and inexpensive. It is a basic measure of protein quality which has not been adjusted for the digestibility of the dietary protein, yet it measures the indispensable amino acids present in a protein and compares the values with a reference protein. The protein is rated based on the most limiting indispensable amino acid and values greater than 1.0 are considered to indicate that the dietary protein contains essential amino acids in excess of the human requirements. In 1990, FAO/WHO reinstates that proteins having values higher than 1.0 be truncated as 1.0; thus, the truncation of AAS value for certain dietary proteins fails to reflect the ability of the protein to complement the nutritional value of a lower quality protein.\(^28\)

e) **Protein digestibility-corrected amino acid score (PDCAAS):** This is a form of biochemical assay that
employs the same technicality for the Amino Acid Score (AAS) with an additional digestibility component. The PDCAAS is the currently accepted measure of protein quality as it closely provides significant estimations as compared to determinations done in experimental studies. The practical difficulties and poor sensitivities of the NPU have led to the adoption of the PDCAAS approach. However, a number of nutritional experts seem to denote that this method requires further refinement and additional modifications might be seen in the future \cite{16, 18}.

**Quality evaluation: PDCAAS approach**

The Protein Digestibility-Corrected Amino Acid Score (PDCAAS) is a method of evaluating the protein quality based on the amino acid requirements of humans. The PDCAAS rating is a fairly recent evaluation method, it was adopted by the US Food and Drug Administration (FDA) and the Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO) in 1993 as the most preferred method of determining dietary protein quality. Briefly, PDCAAS is based on the combination of age-related amino acid reference pattern that is representative of human requirements plus estimates of the digestibility of the dietary protein. The amount of potentially limiting amino acids in the test protein is relatively compared with their respective composition in the standardized reference pattern, identifying the single most limiting amino acid that determines the amino acid score. The current consensus is that attaining the minimum requirements for lysine, methionine and tryptophan, the most limiting amino acids in low quality dietary proteins, determines the amino acid score and will result in optimal nitrogen retention. At the optimized nitrogen balance, any further increase in the plasma amino acids would stimulate increased oxidation and elimination of excess amino acids, implying that protein quality above human requirements is physiologically insignificant\cite{18}.

The amino acids score is assumed to predict the biological value as well as the anticipated ability of the absorbed test proteins to fulfill human amino acid requirements. The score is then corrected for digestibility giving the PDCAAS value, which is assumed to predict the net protein utilization of the dietary intakes. Inherent in the PDCAAS and/or Nitrogen balance is that provision of substrates for metabolic protein synthesis and other pathways is limited by the digested and absorbed indispensable amino acids (IAA). Thus, protein utilization is predicted from the expected digestibility and amino acid composition of the dietary proteins. These two features of dietary protein determine the ability of a dietary protein to meet the minimum human demands for nitrogen balance and nutritional quality \cite{16}.

For protein combinations in a meal, the PDCAAS score is calculated from the amino acid pattern of the digested protein constituents because the bioavailable proteins in food are first limited by digestibility which cannot exceed 100\% and the AAS having evaluated values greater than 100\% are truncated; thus, PDCAAS cannot exceed 100\%. The PDCAAS of diets based of protein mixtures reflects the complementation of proteins that might be limited in one or more indispensable amino acids (IAA); this is one of the inefficiency of the PDCAAS approach especially for dietary proteins with higher IAA levels. More specifically, the truncation of their PDCAAS value and the calculation of the AAS based on only the first limiting amino acid arguably underestimate the efficiency of a high quality dietary protein to balance the IAA composition of inferior proteins with a PDCAAS ranging from 1.00 - 0.00.

**PDCAAS: Methodology**
The PDCAAS approach substantially differs from the PER and BV techniques in evaluating the quality of a dietary protein. It allows evaluation based on the requirements of the human biological system as it measures the protein quality in accordance to the amino acid constituents (adjusted for digestibility) for two (2) to five (5) years old children which are considered as the most nutritionally-demanding age group. The BV method uses nitrogen absorption as its basis; however, it does not take into account certain factors influencing the digestion of the protein and remains of limited application in humans because it actually measures the maximal potential of quality which differs from the true estimate of quality at standard requirement levels.

Using the PDCAAS method, the protein quality profiling are determined by relatively comparing the amino acid profile of the dietary protein with established standards to generate a highest potential score of 1.00. This score indicates that the aftermath of protein digestion is the 100% provision of the required IAA per unit of protein. The PDCAAS is mathematically expressed as $\text{PDCAAS} = \text{AAS} \times \text{digestibility}$.

<table>
<thead>
<tr>
<th>Dietary Protein(s)</th>
<th>BV</th>
<th>AAS</th>
<th>Digestibility</th>
<th>PDCAAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whey</td>
<td>100%</td>
<td>1.14</td>
<td>99%</td>
<td>1.00</td>
</tr>
<tr>
<td>Egg</td>
<td>94%</td>
<td>1.21</td>
<td>98%</td>
<td>1.00</td>
</tr>
<tr>
<td>Casein</td>
<td>80%</td>
<td>1.00</td>
<td>99%</td>
<td>1.00</td>
</tr>
<tr>
<td>Soy</td>
<td>74%</td>
<td>0.99</td>
<td>95%</td>
<td>1.00</td>
</tr>
<tr>
<td>Beef</td>
<td>80%</td>
<td>0.94</td>
<td>98%</td>
<td>0.92</td>
</tr>
<tr>
<td>Gluten</td>
<td>54%</td>
<td>0.47</td>
<td>91%</td>
<td>0.25</td>
</tr>
<tr>
<td>Chickpea</td>
<td></td>
<td>1.00</td>
<td></td>
<td>0.78</td>
</tr>
<tr>
<td>Tubercles</td>
<td></td>
<td>0.90</td>
<td></td>
<td>0.74</td>
</tr>
<tr>
<td>Vegetable</td>
<td></td>
<td>0.89</td>
<td></td>
<td>0.73</td>
</tr>
<tr>
<td>Legumes</td>
<td></td>
<td>0.89</td>
<td></td>
<td>0.70</td>
</tr>
<tr>
<td>Cereals</td>
<td></td>
<td>0.69</td>
<td></td>
<td>0.59</td>
</tr>
<tr>
<td>Fruits</td>
<td></td>
<td>0.66</td>
<td></td>
<td>0.48</td>
</tr>
</tbody>
</table>

Sources:
- Protein Quality Evaluation, Report of the Joint FAO/WHO Consultation
- Reference Manual for U.S Whey products; 2nd Ed.

**PDCAAS: Limitations**

The PDCAAS takes no account of the location of protein digestion and as such, amino acids which are channeled beyond the terminal ileum of the human digestive system are less likely to be absorbed for metabolic activities. However, they may be absorbed by the inherent microbes of the intestines, making the absent in the faeces and appearing to have been digestible and absorbable. Similarly, amino acids that losses its activity as a result of anti-nutritional factors...
present in many diets are assumed to be digested by the PDCAAS. The PDCAAS method can also be considered incomplete, since human diets, except in times of famine, almost never contains only one kind of protein however, calculating the PDCAAS of a diet solely based on the PDCAAS of the individual amino acid constituents is inaccurate due to their complementation function. For instance, the PDCAAS evaluation of a combined diet with relatively equal quantities of cereals (0.59) which is rich in methionine and limited by lysine & chickpea (0.78) which is rich in lysine and limited by methionine would generate an overall PDCAAS of 1.00 because each constituent protein is complemented by the other.

Additionally, the fact that four (4) proteins, each with distinctively different amino acid profiles, produces an identical PDCAAS of 1.00 (Table 3) limits the applicability of the PDCAAS approach as a comparative tool. This difference in their amino acid profile specifies that they assume varying functionalities in the human physiology and should exhibit different scores. As a consequence, the PDCAAS approach provides no distinction of their functional performance relative to each other as they are all capped at 1.00 and identically rated. These anomalies can also be attributed to the ‘truncation effect’ which considers certain dietary proteins to be composed of essential amino acids in excess of the human dietary requirements.

Remodeling the PDCAAS Approach

- **Body Protein Metabolism Insights**

Protein quality assessment with respect to its efficiency in supporting body protein metabolism includes consideration of the dietary functionality to provide substrate needs for protein synthesis and other biosynthetic pathways that require a sustainable source of nitrogen and IAA (lysine, threonine, valine, isoleucine, leucine, methionine, phenylalanine, tryptophan and histidine). Additionally, the provision of sufficient signal amino acids (e.g. leucine) required for different regulatory mechanisms when metabolism is optimized and anabolism becomes activated. In contrast, the current PDCAAS methods have only evaluated substrate needs rather than any provision of regulatory amino acids.

The protein metabolic effectiveness at a dietary intake that meets minimum requirements indicates that as protein intake increases towards the upper half of the currently acceptable macronutrient density range, both the metabolic demands for amino acids and the consequent fate of the dietary amino acids will become increasingly difficult to predict by generating a single reference amino acid profile against which serves as the protein quality standards, especially across the life span and in all physiological conditions. As an instance, leucine regulation of muscle protein synthesis via the mammalian target of rapamycin signal cascade requires an increase in the intracellular leucine concentration, which also increases amino acid oxidation. The PDCAAS approach argues that increased amino acid oxidation reflects inefficient use of amino acids, but this ignores any transient signaling influence of specific amino acids before their oxidation; thus, it is important to consider to what extent the quality of the dietary protein (amino acid profiles) influences its anabolic signaling.

Although, concerns have also been expressed about the importance of dietary protein for the elderly, especially in the context of age-related loss of skeletal muscle mass (sarcopenia), there has not been a firm consensus that the published evidence indicates any measurable age-related change in the minimum protein requirement or the nitrogen-balance data which form the basis of the current PDCAAS.
reference pattern; however, emerging experimental results suggest that there is an age-related change in the regulatory influence of IAA on the muscle protein synthesis that will reduce the effectiveness of dietary proteins to maintain muscle mass. Muscle growth and maintenance occurs in response to a complex interplay of stimuli including physical activity, hormone signaling and substrate metabolism. However, amino acids are a pre-requisite for muscle protein synthesis and a dietary supplement of IAA is a potent stimulus; there is, in fact, a dose response relation between IAA concentrations in the blood and muscle protein synthesis. In the elderly, there is at the same time, a decreased sensitivity and responsiveness of muscle protein synthesis to IAA even though human studies have not identified the mechanism of these effects and the ideal amino acids pattern of the extra proteins involved in the complementation of dietary proteins for both nutritional support and resistance exercise remains unknown. There are limited evidences on the relative influence of different protein sources on increasing muscle mass in human trials. Studies measuring the effects of meat-containing and lacto-ovo-vegetarian diets, coupled with resistance training protocols on muscle mass have been mixed, although methodology varied and the research is only beginning to emerge. According to Wilkson, fluid-skim milk promoted greater muscle protein accretion than a soy protein beverage when consumed after resistance exercise. Philip, B. et al have suggested that any improved nitrogen retention observed in milk compared to soy consumption during a resistance training protocol may reflect differences in the amino acid profile during delivery to peripheral tissues. However, it is not known whether this is a function of different rates of digestion, peak postprandial amino acid flow through the splanchnic bed and consequent rates of amino acid oxidation and deamination (higher for soy than milk protein), or the different amino acid profiles of the two protein sources.

Although human evidence is beginning to emerge, there is abundant evidence from animal studies that sufficiently high doses of leucine may be particularly important in muscle synthesis through synergistic effects with insulin in signal transduction pathways and in the presence of adequate dietary energy. There are worthwhile research areas regarding the promising potential role for leucine in protein metabolism as well as the possibility of an intake threshold at which overstimulation by leucine could negatively impact glucose metabolism. It is estimated that stimulation of muscle protein synthesis would be optimized with 18g IAA including 2.5g leucine at each of the three (3) meals per day. A clear research goal is to identify the optimal dietary amino acid pattern in terms of specific amino acids, the total IAA content, or perhaps, even the conditionally IAA for the determination of protein quality. Even though leucine is abundant in a variety of dietary protein sources, confirmation of the need for high intakes of leucine at each meal, particularly within a calorie-restricted diet could have implications for choosing a protein source (Table 4).

### Table 4 Leucine & BCAA concentrates of proteins

<table>
<thead>
<tr>
<th>Dietary Sources</th>
<th>Leucine</th>
<th>BCAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whey protein isolate</td>
<td>14%</td>
<td>26%</td>
</tr>
<tr>
<td>Milk protein</td>
<td>10%</td>
<td>21%</td>
</tr>
<tr>
<td>Egg protein</td>
<td>8.5%</td>
<td>20%</td>
</tr>
<tr>
<td>Muscle protein</td>
<td>8%</td>
<td>18%</td>
</tr>
<tr>
<td>Soy protein isolate</td>
<td>8%</td>
<td>18%</td>
</tr>
</tbody>
</table>
### Amino Acid Bioavailability

A second significant function in quality evaluation relates to the bioavailability or digestibility of a protein and its capability to provide metabolically available nitrogen and amino acid to tissues and organs. The food matrix in which a protein is consumed can have significant impact on the bioavailability of amino acid for metabolic needs. Digestive losses and structural changes of amino acids are caused by numerous anti-nutritional factors in foods. These issues have been addressed with particular attention to animal compared with plant proteins 24.

As mentioned earlier, the PDCAAS value is calculated by first scoring the test protein against an appropriate reference amino acid pattern and correcting for digestibility. The currently accepted method for accessing digestibility is based on measures of faecal nitrogen in a rat assay. Faecal measures in this assay appear to appropriately assess human nitrogen digestibility. It has been noted, however, that ileal measures may better assess amino acid digestibility. Both cost and time involved in measuring true ileal digestibility in human subjects are intensive, although other monogastric species such as pigs have been considered 25. It has also been observed that research is needed to assess the impact of kinetic differences between proteins in the intestinal lumen when measuring ileal digestibility 26. Schaafsma and Sarwar 18, 24 have argued that digestibility factors developed from the rat bioassay may not be appropriately correct for the range of anti-nutritional effects in the food matrix, both naturally occurring and those formed via different processing techniques. Although heat, oxidation and other treatment options are carried out for consumer protection and benefit, this can lead to formation of maillard compounds, oxidized sulfur amino acids, D-amino acids or cross-linked peptide chains which limit amino acid bioavailability. The multiple anti-nutritional factors present in foods have led to also question the biological efficiency of complementation of low-quality with high-quality dietary proteins.

As stated above, the truncation procedure and calculation restriction to the first limiting amino acid are subject to criticism because these latter issues do not allow expression of the power of a high-quality protein to balance the IAA composition of the inferior proteins 18.

An ileal digestibility of proteins is also relevant for reducing the amount of dietary nitrogen entering the colon. Protein fermentation by the intestinal flora may result in the formation of toxic compounds, including ammonia, dihydrogen sulfide, indoles and phenols that could irritate the colonic epithelial cells and increase the risk of colon cancer 4.

### Energy Turnover & Glucose Homeostasis

Dietary protein function is not usually considered in relation to energy status and glucose homeostasis. Although energy intake and expenditure, either above or below metabolic needs influences protein utilization. Current evaluation of dietary protein utilization especially in relation to its quality assumes subjects are in energy balance, consuming nutritionally adequate diets and engaging in moderate rates of physical activities 27. Departure from energy balance markedly changes protein utilization and has been suggested as an important factor in the lack of reproducibility of the nitrogen balance studies 28.

In the context of obesity epidemic, there is an important potential role for protein as a part of diets aiming to limit weight gain or help in weight loss.

<table>
<thead>
<tr>
<th>Wheat protein</th>
<th>7%</th>
<th>15%</th>
</tr>
</thead>
</table>

Values reflects g amino acids/100g protein

BCAA = Branched-Chain Amino Acids
Several mechanisms have been proposed to explain the well-documented influence of dietary proteins’ roles in body weight regulation, such as thermogenesis $^{14}$, improved body composition $^{29}$, improved glycemic control and appetite regulation $^{30}$. These effects have been assumed to relate to the quantity of dietary protein and its relative proportion compared with other macronutrients; however, there is evidence to suggest mechanisms that would have implications for protein quality assessment.

Improved glycemic control is important in the context of the management of type II diabetes and also in relation to bodyweight regulation. Studies that have increased protein intakes at the expense of carbohydrates have shown that a diet with 30% of energy derived from protein, 20% from carbohydrates (with low biologically available glucose) and 50% from fat is effective at improving glycemic control in people with type II diabetes without an adverse effect on the serum lipids or renal function $^{18}$. There are several potential mechanisms of these influences of protein which might be responsive to the protein structure or amino acid profile. One is the influence of variation in amino acid composition on the magnitude and duration of postprandial insulin secretion, an important but relatively unexplored question in this context. Another factor is gluconeogenesis rates in relation to both the pattern of amino acids as substrates as well as their influence as regulators of the metabolic pathway. Individual amino acids differ as substrates for gluconeogenesis and the BCAA have a unique role in providing amino groups for production of alanine (from pyruvate) and recycling of glucose carbon from skeletal muscle to liver for gluconeogenesis. The overall significance of protein or the amino acid pattern on glucose homeostasis through insulin secretion, de novo glucose production and/or alanine recycling has not been investigated $^{31}$.

**Satiety Induction**

In the context of weight and energy balance regulation, dietary protein is now known to play an important role in appetite regulation. Thus, the effect of protein on satiety becomes a potential endpoint for quality assessment. Given the complexity of the neuroendocrine and metabolic mechanisms of appetite regulation, it is difficult to predict how quality modulates protein’s influence within the satiety cascade and the likelihood of both pre- and post-absorptive signaling $^{32}$. Proteins that are more rapidly digested (fast proteins), such as whey appears to have greater influence on satiety than casein (a slow protein), which clots in the stomach and induces a slower hyperaminoacidemia. In part, the difference in the rate of digestion alters levels of the gut hormone, glucagon-like peptide -1 and cholescystokinin (hormone that stimulates the gallbladder, making it contract and release bile) $^{33}$. Another feature of protein that influences its effectiveness (quality) in terms of appetite regulation relates to its tertiary structure and its consequent behavior in the gastrointestinal tract. Protein structure is a characteristic not currently addressed in quality evaluation. The potential mechanism in satiety induction involves the presence of bioactive amino acid sequences which may be absorbed and have metabolic effects that increase satiety. Casein-macropeptide, a glycosylated peptide comprising 15% - 20% whey products, stimulates cholescystokinin production, which leads to greater satiety. It is also well characterized that proteins increase diet-induced thermogenesis which have been shown to be closely associated with satiatiion $^{34}$.
Conclusions

It is clear that protein plays a role in promoting optimal health. Many avenues are emerging for exploring proteins’ potentials and elucidating the mechanisms at play in lean body mass retention, weight control, reduced inflammation, insulin sensitivity and bone & cardiovascular health. The evidence available to date suggests that quality is not only important at the recommended dietary allowance (RDA) but also at higher intakes.

The roles of IAAs in lean body mass retention, cell signaling, bone health, glucose homeostasis and satiety induction are particularly intriguing and worthy of further studies. Noting that the currently accepted methods for protein quality evaluation do not capture the importance of IAAs beyond the first limiting amino acids and given the long-standing debate regarding assessment of bioavailability, research assessing proteins’ roles in optimal health at higher intakes should also be explore implications for protein quality assessment.

References


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