



## Protein quality assessment: Impact of expanding understanding of protein and amino acid needs for optimal health

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### Abstract

Protein quality describes characteristics of a protein in relation to its ability to achieve defined metabolic actions. Traditionally, this has been discussed solely in the context of a protein's ability to provide specific patterns of amino acids to satisfy the demands for synthesis of protein as measured by animal growth or, in humans, nitrogen balance. As understanding of protein's actions expands beyond its role in maintaining body protein mass, the concept of protein quality must expand to incorporate these newly emerging actions of protein into the protein quality concept. New research reveals increasingly complex roles for protein and amino acids in regulation of body composition and bone health, gastrointestinal function and bacterial flora, glucose homeostasis, cell signaling, and satiety. The evidence available to date suggests that quality is important not only at the minimum Recommended Dietary Allowance level but also at higher intakes. Currently accepted methods for measuring protein quality do not consider the diverse roles of indispensable amino acids beyond the first limiting amino acid for growth or nitrogen balance. As research continues to evolve in assessing protein's role in optimal health at higher intakes, there is also need to continue to explore implications for protein quality assessment.

### Topic

- amino acids
- satiation
- nitrogen balance
- glucose homeostasis
- healthcare quality assessment

### Issue Section

Supplement: Protein Summit 2007: Exploring the Impact of High-Quality Protein on Optimal Health

**Keywords:** substituted Li ferrite, magnetostatic and spin waves, microstrip array antenna, X-band frequency range

### Introduction

As addressed in earlier papers in this supplement and at the Summit, there is strong evidence emerging of a positive role for protein in promoting optimal health at intakes beyond the Recommended Dietary Allowance. There is new focus on the roles of protein related to lean body mass retention during calorie restriction and aging, weight control, insulin secretion and action, and bone and cardiovascular health. To date most studies have focused on the quantity of protein and its relative proportion to carbohydrate and fat needed to achieve any benefit. Much of this work builds on a long-established concept that protein intakes above the minimum for nitrogen balance can have important anabolic influences on muscle and bone through an anabolic drive of amino acids <sup>[1]</sup> as well as considerable experimental support for specific influence of indispensable amino acids such as the branched-chain amino acids, especially leucine, on regulation of muscle protein synthesis <sup>[2]</sup>. Additionally, interest in dietary protein has grown to include actions within the gastrointestinal tract that may reflect protein structure and composition independently from its post absorptive amino acid profile <sup>[3]</sup>. Protein quality describes characteristics of a protein in relation to its ability to achieve defined metabolic actions. Traditionally, this has been discussed solely in the context of a food protein's ability to provide specific patterns of amino acids to satisfy the

demands for synthesis of protein and other specific metabolites. As understanding of protein's actions expands beyond its role in maintaining body protein mass and satisfying metabolic demands for biosynthetic pathways, it is clear that the concept of protein quality must expand to incorporate these newly emerging actions of protein.

Which protein characteristics are important for which processes or functions? In the context of a brief review of the strengths and weaknesses of current methods for assessing protein quality, this paper will explore particular characteristics of the protein consumed that could impact optimal health and would need to be considered in an expanded protein quality concept. Clearly, for this newly emerging area, our main objective will be to define research questions for future exploration.

### Defining protein quality

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. For this reason, in the present context of optimal protein intakes, discussion of "what sort" is equally relevant as the question of "how much." There are 2 important aspects of protein quality: 1) the characteristics of the protein and the food matrix in which it is consumed, and 2) the demands of the individual

consuming the food, as influenced by age, health status, physiologic status, and energy balance. Multiple factors influence protein quality, and these issues have been debated extensively for decades. In light of increasingly diverse functions of protein in human health, the appropriate endpoints by which the “how much” question is investigated become equally important for the assessment of protein quality. With respect to dietary protein's ability to satisfy metabolic demands in relation to maintaining muscle and bone, significant data have emerged to suggest that protein's role in health may be based on factors that are not captured by current protein quality estimates. The current aim of protein quality evaluation is to determine the ability of a protein to meet maintenance needs plus special needs for growth, pregnancy, or lactation: “The lowest level of dietary protein intake that will balance the losses of nitrogen from the body, and thus maintain the body protein mass, in persons at energy balance with modest levels of physical activity, plus, in children or pregnant/lactating women, the needs associated with the deposition of tissues or the secretion of milk at rates consistent with good health.”<sup>[4]</sup>

Current protein quality methods assess animal growth (protein efficiency ratio) or, in humans, nitrogen balance, where both digestibility and the suitability of the amino acid pattern of absorbed amino acids (biological value) determines net protein utilization. The practical difficulties and poor sensitivity of the nitrogen balance method has led to the adoption of the protein digestibility-corrected amino acid score (PDCAAS) approach.

The PDCAAS, which was introduced by the Food and Agriculture Organization of the World Health Organization (FAO/WHO) in 1991<sup>[5]</sup>, is the current internationally approved method for protein quality assessment<sup>[4]</sup>. Briefly, PDCAAS is based on the combination of an age-related amino acid reference pattern that is representative of human requirements plus estimates of the digestibility of the protein. The amount of potentially limiting amino acids in the test protein is compared with their respective content in the appropriate reference pattern, identifying the single most limiting amino acid that determines the amino acid score. The current consensus is that meeting the minimum requirements for lysine, methionine, and tryptophan, the most limiting amino acids in poor quality proteins, determines the amino acid score and will lead to a plateau of nitrogen retention<sup>[4]</sup>. At the plateau of nitrogen balance, any further increase in plasma amino acids would stimulate increased oxidation and elimination of the excess amino acids, implying that protein quality above requirements does not matter. This score is assumed to predict biological value, or the anticipated ability of the absorbed test protein to fulfill human amino acid requirements. The score is then corrected for digestibility giving the PDCAAS value, which is assumed to predict net protein utilization.

Inherent in PDCAAS or nitrogen balance is that provision of substrate for protein synthesis and other pathways is limited by available (digested and absorbed) indispensable amino acids. Thus, protein utilization is predicted from expected digestibility and the amino acid composition of the protein. These 2 characteristics of the protein determine the ability of a dietary protein to meet minimum human amino acid requirements for nitrogen balance and, hence, its nutritional quality. For protein mixtures in a meal, the score is calculated from the amino acid pattern of the digested protein mixture. Because available protein in food will be

first limited by digestibility, which cannot exceed 100%, PDCAAS cannot exceed 100%. Thus, in calculating PDCAAS values, amino acid score values >100% are truncated. Whereas PDCAAS values of diets based on mixtures of proteins will reflect the complementation of proteins that might be deficient in one or more indispensable amino acids (IAA), this is also the foundation of one criticism of the PDCAAS approach for those with higher IAA levels. Specifically, the truncation of the PDCAAS value and the calculation of the amino acid score based on only the first limiting amino acid arguably underestimate the power of a high-quality protein to balance the IAA composition of inferior proteins.

### Body Protein Metabolism

Assessing protein quality with respect to its efficiency in supporting body protein metabolism should include consideration of the capacity of the diet to provide substrate needs for protein synthesis and any other biosynthetic pathways, ie, a suitable source of nitrogen and IAA (lysine, threonine, valine, isoleucine, leucine, methionine, phenylalanine, tryptophan, and histidine). However, to this assessment method should be added provision of sufficient signal amino acids, (eg, leucine), required for those regulatory steps whereby metabolism is optimized and anabolism is stimulated<sup>[2,7]</sup>. It is arguable that current methods used for assessing protein quality have only evaluated substrate needs rather than any provision of regulatory amino acids.

Evaluation of protein quality with the PDCAAS approach measures the protein's metabolic effectiveness at a dietary intake that meets minimum requirements. By this measure, protein requirements are low compared with most nutritionally complete habitual diets. Indeed, applying an adaptive metabolic demand model of protein homeostasis<sup>[8]</sup>, protein requirements may be even lower after complete adaptation to the extent that a dietary recommendation based on the true minimum intake for nitrogen equilibrium would become of questionable nutritional significance.

Furthermore, in the context of an adaptive model and the higher habitual protein intakes in subjects consuming the currently recommended healthy diet, it has been suggested that the assessment of protein quality by amino acid scoring becomes problematic, with the metabolic demand for amino acids reflecting a complex adaptive response to varying intakes of protein and amino acids<sup>[9,10]</sup>. This means that as protein intake increases, for example toward the upper half of the current acceptable macronutrient density range<sup>[11]</sup>, both the metabolic demands for amino acids and the consequent fate of the dietary amino acids will become increasingly difficult to predict in terms of generating a single reference amino acid pattern against which to judge protein quality, especially across the entire life span and in all physiologic conditions. For example, leucine regulation of muscle protein synthesis via the mammalian target of rapamycin signal cascade requires increases in intracellular leucine concentration, which also increases amino acid oxidation<sup>[12]</sup>. 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, within the context of potential benefits associated with higher protein intakes, it is important to consider to what extent the quality of the protein (eg, amino acid profile) influences its anabolic

signaling. Although concern has always been expressed about the importance of dietary protein for the elderly, especially in the context of the 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 [13] or the nitrogen-balance data which form the basis of the current PDCAAS reference pattern [4]. However, emerging experimental evidence suggests that there is an age-related change in the regulatory influence of IAA on muscle protein synthesis that will reduce the effectiveness of dietary protein to maintain muscle mass [14, 15].

Muscle growth and maintenance occurs in response to a complex interplay of stimuli, including physical activity, hormonal signaling, and substrate supply. However, amino acids are a prerequisite for muscle protein synthesis, and a dietary supplement of IAA is a potent stimulus [16]. There is, in fact, a dose-response relation between IAA concentrations in the blood and muscle protein synthesis [14, 15, 17, 18]. In the elderly, there is, at the same time, decreased sensitivity and responsiveness of muscle protein synthesis to IAA [19, 20]. Currently, human studies have not identified the mechanisms of these effects. Although intervention studies point to the need for a combination of both nutritional support and resistance exercise, the ideal amino acid pattern of the extra protein involved is unknown. There is limited evidence to date on the relative influence of different protein sources on increasing muscle mass in human trials. Studies measuring the effects of meat-containing and lactoovovegetarian diets, coupled with resistance training protocols, on muscle mass have been mixed [21, 22], although methodology varied and the research is only beginning to emerge. According to Wilkinson *et al* [23], fluid skim milk promoted greater muscle protein accretion than a soy protein beverage when consumed after resistance exercise. Phillips *et al* [24] have suggested that any improved nitrogen retention observed with milk compared with 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 2 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 protein synthesis through synergistic effects with insulin in signal transduction pathways and in the presence of adequate dietary energy. As proposed by Garlick [7], there are worthwhile research opportunities 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. A review of the leucine literature by Layman [25] estimated that stimulation of muscle protein synthesis would be optimized with 18 g IAA, including 2.5 g leucine, at each of 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 [26], or perhaps even the conditionally indispensable amino acid [6] for determination of protein quality. Although leucine is abundant in a variety of protein

sources, confirmation of the need for particularly high intakes of leucine at each meal, particularly within a calorie-restricted diet, could have implications for choosing a protein source.

**Table 1:** Leucine and BCAA content of foods

	<b>Leucine</b>	<b>BCAA</b>
Whey protein isolate	14%	26%
Milk protein	10%	21%
Egg protein	8.5%	20%
Muscle protein	8%	18%
Soy protein isolate	8%	18%
Wheat protein	7%	15%

1. Values reflect g amino acids/100 g protein. BCAA, branched-chain amino acid.
2. Adapted from Layman and Baum (12).
3. **Source:** USDA Food Composition Tables.

In addition to muscle, bone is also an important target for anabolic influences of dietary protein. In a rat model there is a clear dose response of bone length growth to protein intakes in excess of those associated with maximal muscle growth [27, 28], with dietary protein-induced changes in proteoglycan synthesis rates in rat skeletal muscle and bone linked to changes in plasma and tissue insulin-like growth factor-I levels [29]. This is consistent with more rapid catch-up growth in height in children with increased dietary protein intake (15% compared with 7.5% energy) [30], which was associated with higher serum concentrations of IGF-I. Also in relation to the adult bone, it has been argued that dietary proteins enhance IGF-1, a factor that exerts positive activity on skeletal development and bone formation, and are as essential as calcium and vitamin D for bone health and osteoporosis prevention [32]. Although there is no consistent evidence to suggest differences in dietary protein sources on such influences [32], it is of obvious importance to establish how the amino acid pattern of the protein supply influences such responses not only at the level of the osteocyte but also in terms of IGF-1 production.

### **Dietary Protein and AMINO Acid Bioavailability**

A second important issue in quality evaluation relates to the bioavailability or digestibility of a protein or the capacity 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 antinutritional factors in foods. These issues have been addressed with particular attention to animal compared with plant proteins.

As mentioned previously, the PDCAAS value is calculated by first scoring the test protein against an appropriate reference amino acid pattern, then correcting for digestibility. The currently accepted method for assessing digestibility is based on measures of fecal nitrogen in a rat assay. Fecal 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 [33], although other monogastric species, such as the pig, have been considered [34]. It has also been noted that research is needed to assess the impact of kinetic differences between

proteins in the intestinal lumen when measuring ileal digestibility<sup>[35]</sup>.

Sarwar<sup>[36]</sup> and Schaafsma<sup>[6]</sup> have argued that digestibility factors developed from the rat bioassay may not appropriately correct for the range of antinutrient effects in the food matrix, both naturally occurring and formed through processing methods. Although heat, oxidation, and other treatments are carried out for consumer protection and benefit, they can lead to formation of Maillard compounds, oxidized sulfur amino acid, D-amino acids, or cross-linked peptide chains, which limit amino acid bioavailability. The multiple antinutritional factors present in foods have led Sarwar and Schaafsma to also question the biological efficiency of complementation of low-quality with high-quality proteins.

Also, as stated above, the truncation procedure and the restriction to only 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 inferior proteins<sup>[6]</sup>.

A high 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.

### **Protein quality in relation to energy turnover and 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, the impact of protein quality in populations with varying levels of energy turnover has not been considered in the past. However, it is logical to question the influence of energy turnover on amino acid needs and the consequent reference amino acid pattern for assessing protein quality in any target population. 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 activity<sup>[4]</sup>. 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<sup>[37]</sup>. In subjects who are otherwise in energy balance, the protein utilization effects of varying levels of physical activity are very poorly understood.

In the context of the obesity epidemic, there is an important potential role for protein as a part of diets aiming to limit weight gain or help with weight loss. Several mechanisms have been proposed to explain the well-documented influence of dietary protein's role in body weight regulation, such as thermogenesis<sup>[38]</sup>, improved body composition<sup>[39,40]</sup>, improved glycemic control<sup>[41]</sup>, and, as discussed below, appetite regulation<sup>[42-44]</sup>. These effects have been assumed to relate to the quantity of dietary protein and its relative proportion compared with the other macronutrients. However, there is evidence to suggest mechanisms that would have implications for protein quality assessment<sup>[12, 25, 45-48]</sup>. Improved glycemic control is important in the context of management of type 2 diabetes and also in relation to body-weight 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 carbohydrate (with low biologically available glucose), and 50% from fat is effective in improving glycemic control in people with type 2 diabetes without an adverse effect on serum lipids or renal function<sup>[49,50]</sup>. 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 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 branched-chain amino acids 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<sup>[12]</sup>. The overall significance of protein or the amino acid pattern on glucose homeostasis through insulin secretion, de novo glucose production, or alanine recycling has not been investigated.

### **Satiety Induction**

As indicated above, 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<sup>[51]</sup>, it is difficult to predict how quality will modulate protein's influence within the satiety cascade given the likelihood of both pre- and postabsorptive signaling. Proteins that are more rapidly digested (fast proteins), such as whey, appear to have greater influence on satiety than casein (a slow protein), which clots in the stomach and induces a slower hyperaminoacidemia<sup>[46]</sup>. In part, the difference in rate of digestion alters levels of the gut hormones glucagon-like peptide-1 and cholecystokinin<sup>[46]</sup>. Hence, another feature of protein that influences its effectiveness (ie, "quality") in terms of appetite regulation relates to its tertiary structure and consequent behavior in the gastrointestinal tract. Protein structure is a characteristic not currently addressed in quality evaluation.

Another potential mechanism in satiety induction involves the presence of bioactive amino acid sequences, which may be absorbed and have metabolic effects that increase satiety<sup>[42]</sup>. Casomorphins, casein-derived peptides, slow gastrointestinal motility via gastric opioid receptors which mediate lower postprandial plasma amino acid concentrations, thereby preventing the satiating effect of higher plasma amino acid levels. Caseinomacropptide, a glycosylated peptide comprising 15% to 20% of whey products, stimulates cholecystokinin production, which leads to greater satiety<sup>[52]</sup>. Finally, it is well known that proteins increase diet-induced thermogenesis, and it has been shown that this effect is closely associated with satiation/satiety.

### **Summary**

It is clear that protein plays a role in promoting optimal health. Many avenues are emerging for exploring protein's potential and elucidating the mechanisms at play in lean

body mass retention, weight control, reduced inflammation, insulin sensitivity, and bone and cardiovascular health. The evidence available to date suggests that quality is important not only at the Recommended Daily Allowance but also at higher intakes. It is also evident that quality at higher compared with lower intakes is important for different reasons. Examination of the increasingly complex roles emerging for protein reveals these differences. The roles for IAAs in lean body mass retention, cell signaling, bone health, glucose homeostasis, and satiety induction are particularly intriguing and worthy of further study. Noting that currently accepted methods for protein quality evaluation do not capture the importance of IAAs beyond the first limiting amino acid, and given the long-standing debate regarding assessment of bioavailability, research assessing protein's role in optimal health at higher intakes should also explore implications for protein quality assessment. The authors' responsibilities were as follows—DJM, DKL, DT, and GS: contributed to the conception, drafting, and revision of this manuscript. DT sits on the Nutrition Research Scientific Advisory Committee of the National Dairy Council. The Summit sponsors provided an honorarium (DKL) for efforts on the Steering Committee for organization of the meeting and preparation of manuscripts and an honorarium (DJM, DT, GS) for participation in a working group that reviewed and compiled the relevant published literature on this topic. The Beef Check off through the National Cattlemen's Beef Association and National Dairy Council provided research grants (DKL). The Beef Check off through the National Cattlemen's Beef Association, the National Dairy Council, and the Egg Nutrition Center also provided compensation for speaking/consulting (DKL). Reimbursements of travel costs and lodging were provided to DJM, DKL, DT and GS by the Protein Summit sponsors.

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