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REVIEW ARTICLE

Amino acid requirements in humans: with a special emphasis on the metabolic availability of amino acids

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Abstract Due to advances made in the development of stable isotope based carbon oxidation methods, the determination of amino acid requirements in humans has been an active area of research for the past 2 decades. The indicator amino acid oxidation (IAAO) method developed in our laboratory for humans has been systematically applied to determine almost all indispensable amino acid requirements in adult humans. Nutritional application of experimentally derived amino acid requirement estimates depends upon the capacity of food proteins to meet the amino acid requirements in humans. Therefore, there is a need to know the proportion of dietary amino acids which are bioavailable, or metabolically available to the body for protein synthesis following digestion and absorption. Although this concept is widely applied in animal nutrition, it has not been applied to human nutrition due to lack of data. We developed a new in vivo method in growing pigs to identify the metabolic availability of amino acids in foods using the IAAO concept. This metabolic availability method has recently been adapted for use in humans. As this newly developed IAAO based method to determine metabolic availability of amino acids in foods is suitable for rapid and routine analysis in humans, it is a major step forward in defining the protein quality of food sources and integrating amino acid requirement data with dietary amino acid availability of foods.

Keywords Amino acid requirements · Humans · Indicator amino acid oxidation · Bioavailability · Metabolic availability

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Abbreviations

BCAA	Branched chain amino acids
DAAO	Direct amino acid oxidation
DRI	Dietary reference intakes
EAR	Estimated average requirement
IAAO	Indicator amino acid oxidation
IDAA	Indispensable amino acids
MA	Metabolic availability
NPU	Net protein utilization
NPPU	Net postprandial protein utilization
PDCAAS	Protein digestibility-corrected amino acid score
PER	Protein efficiency ratio
RDA	Recommended dietary allowance
SAA	Sulfur amino acids
TD	True digestibility

Introduction

Due to advances made in the development and application of stable isotope based carbon oxidation methods, the determination of amino acid requirements in humans has been an active area of research for the past 2 decades (Pencharz and Ball 2003). The indicator amino acid oxidation (IAAO) method independently developed to determine amino acid requirements for humans in our laboratory has been applied to study virtually all the indispensable amino acid (IDAA) requirements in humans (Elango et al. 2008a). The IAAO and the IAAO based method, 24 h IAAO/indicator amino acid balance (IAAB; Kurpad et al. 2002, 2003), were recently accepted as the most appropriate to determine amino acid requirements of humans (DRI 2005; FAO 2007). For detailed discussions about the various methods, their advantages and disadvantages, and requirement estimates, the reader is referred to earlier publications (DRI 2005; Pencharz and Ball 2003; Young and El-Khoury 1996; Zello et al. 1995).

Nutritional application of the experimentally derived amino acid requirement estimates must be integrated with our knowledge of the capacity of food proteins to efficiently meet the amino acid requirements of humans. Normal growth and maintenance of health in humans requires all amino acids (IDAA, conditionally IDAA and dispensable amino acids) to be provided in appropriate quantity and form that is biologically utilizable (Pencharz and Young 2006). This aspect commonly referred to as availability or bioavailability, is very important to know because food proteins vary greatly in both the concentration and bioavailability of the IDAA and conditionally IDAA. Although this concept is widely and routinely applied in animal nutrition (e.g. NRC 1998), it has not been applied to human nutrition due to lack of data. Using the IAAO concept, we have recently developed a new method to determine the whole body bioavailability, termed as "metabolic availability", of IDAA for protein synthesis from dietary protein sources (Moehn et al. 2005; Humayun et al. 2007).

This review will briefly describe the IAAO method, summarize human amino acid requirements determined using the IAAO method and outline the recent application of the IAAO concept for the determination of "metabolic availability" of amino acids from food sources.

Indicator amino acid oxidation

The IAAO technique is based on the concept that when one IDAA is deficient for protein synthesis, then all other amino acids including the indicator amino acid (another IDAA, usually L-[1-^{13}C]phenylalanine) are in excess and therefore will be oxidized (Elango et al. 2008b). This is primarily because, except for the developing conceptus (Wu et al. 2008a, b), excess amino acids cannot be stored and therefore must be partitioned between incorporation into protein or oxidation. With increasing intake of the limiting amino acid, oxidation of the indicator amino acid will decrease, reflecting increasing incorporation into protein. Once the requirement is met for the limiting amino acid, there will be no further change in the oxidation of the indicator amino acid with increasing intake of the test amino acid (Fig. 1). The inflection point where the oxidation of the indicator amino acid stops decreasing and reaches a plateau is referred to as the 'breakpoint' (Fig. 1). The breakpoint, identified with the use of bi-phase linear regression analysis, indicates the EAR of the limiting (test) amino acid (Elango et al. 2008a, b; Pencharz and Ball 2003).

Advantages of the indicator amino acid oxidation technique

The IAAO method has several advantages in determining amino acid requirements when compared to the other available methods. The minimally invasive IAAO protocol involves 2-day adaptation to a fixed protein intake and study day adaptation to the test amino acid intake (Thorpe et al. 1999; Bross et al. 1998). Each study day involves 8 hourly meals, with sampling of breath for measurement of $^{13}CO_2$ enrichment ($F^{13}CO_2$), and urine for phenylalanine kinetics. This protocol has been validated for oral delivery of isotope (Kriengsinyos et al. 2002) and measurement of urinary isotopic enrichment to calculate amino acid kinetics (Wykes et al. 1990). The short-term adapted fed state IAAO model allows each subject to participate in multiple studies over a range of intakes (deficient to excess), and

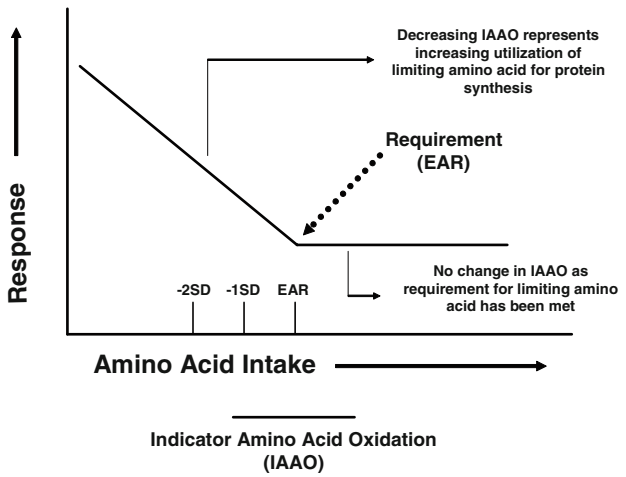

Fig. 1 Pattern of response observed in indicator amino acid oxidation with increasing intake of limiting amino acid. *EAR* Estimated average requirement, *SD* standard deviation

amino acid requirements in each individual can be determined.

The breakpoint estimate of requirement is usually determined from the rate of oxidation ($F^{13}CO_2$) of the labeled tracer (usually 1-^{13}C-phenylalnine). This is one of the key strengths of the IAAO method; this variable represents an end point measurement which takes into account all losses and uses by the body. Recently, we showed in healthy young adults that the breakpoint for indicator amino acid oxidation measured using $F^{13}CO_2$ was very similar to the breakpoint for phenylalanine hydroxylation measured using apo B-100, a hepatic export protein which is synthesized from intrahepatocyte amino acids (Rafii et al. 2008); and thus represents the intracellular enrichment of phenylalanine at the site of protein synthesis in the liver. These data are strong support for the initial observation by Ball and Bayley (1986) that phenylalanine oxidation was inversely related to change in liver protein synthesis. Amino acid oxidation measured using plasma enrichments may be in error, because plasma is not the true precursor pool from which protein synthesis takes place. Therefore, the use of $F^{13}CO_2$ circumvents the complex issue of measuring the true precursor pool enrichment in humans during routine studies where relative rates are being compared.

Amino acid requirements determined using the IAAO technique

This minimally invasive IAAO method has been systematically applied to determine IDAA requirements in adult humans (Table 1) (Elango et al. 2008a, b; Pencharz and Ball 2003). In the recent DRI for macronutrients report (DRI 2005), and the report of a joint WHO/FAO/UNU expert consultation on protein and amino acid requirements in human nutrition (FAO 2007), IDAA requirement values derived using the IAAO method were the main data utilized to make recommendations for adult humans. The minimally invasive IAAO, which is well suited for application in vulnerable populations, has been applied to determine amino acid requirements in school-age children, parenterally fed neonates, metabolic disorders and in diseased populations (Elango et al. 2008a, b; Brunton et al. 1998). The reader is referred to recent reviews and publications for requirement estimates and related discussions (Elango et al. 2008a, b; FAO 2007; DRI 2005; Pencharz and Ball 2003).

Table 1 Mean (average) indispensable amino acid requirements in adult humans

Amino acid	IAAO[1] (mean requirement) (mg kg^{-1} d^{-1})	DRI[2] (EAR) (mg kg^{-1} d^{-1})	FAO/WHO/UNU[3] (average requirement) (mg kg^{-1} d^{-1})
Histidine	–	11	10
Isoleucine	42	15	20
Leucine	55	34	39
Lysine	35	31	30
Methionine (with no cysteine)	12.6	15	15
Phenylalanine (with no tyrosine)	42	27	25
Threonine	19	16	15
Tryptophan	4	4	4
Valine	47	19	26
Total BCAA[4]	144	–	–

[1] Indicator amino acid oxidation (Pencharz and Ball 2003; Elango et al. 2008a)

[2] Dietary reference intakes (DRI 2005)

[3] Food and Agriculture Organization/World Health Organization/United Nations University (FAO/WHO/UNU 2007)

[4] IAAO based requirements for isoleucine, leucine and valine in adults are derived from total BCAA requirements and the BCAA proportion in egg protein

Bioavailability of amino acids for body needs

The practical application of amino acid requirement estimates is to provide adequate amino acid nutrition (Baker 2008). Optimal dietary protein intake will provide all the 20 amino acids (indispensable, conditionally indispensable and dispensable amino acids) in the correct proportions to meet the body's needs for metabolic functions including intestinal integrity (Wang et al. 2008), modulation of gene expression (Palii et al. 2008), protein synthesis (Suryawan et al. 2008; Lewis and Bayley 1995), and regulation of cellular signaling pathways (Flynn et al. 2008; Rhoads and Wu 2008). This requires knowledge of the capacity of food proteins to efficiently meet the nitrogen and amino acid requirements in humans.

Amino acid composition of foods varies greatly (Table 2). The concentrations of lysine, sulfur amino acids (methionine and cystine) and threonine are especially important (Baker 2008; Stipanuk et al. 2008). In cereals, such as rice and wheat, lysine concentrations are significantly lower compared to foods of animal origin. In legumes, such as chickpeas and soybeans, methionine concentrations are significantly lower compared to animal foods (Table 2). Therefore, the nutritional quality of food proteins varies widely, with the animal foods being labeled as "high quality" protein sources when compared against plant proteins (Young and Pellett 1994; Singh 2002). The nutritional quality of food proteins are also influenced by *digestibility*, a

Table 2 Selected amino acid composition of some common foods

Food	Lysine (g/100 g[a])	Methionine (g/100 g[a])	Cystine (g/100 g[a])	Threonine (g/100 g[a])	Total protein (g/100 g[a])
Rice, white	0.258 (36)[b]	0.168 (24)	0.146 (21)	0.255 (36)	7.1
Wheat, flour	0.378 (28)	0.212 (16)	0.317 (23)	0.395 (29)	13.7
Peas	0.317 (59)	0.082 (15)	0.032 (6)	0.203 (38)	5.4
Chickpea	1.291 (67)	0.253 (13)	0.259 (13)	0.716 (37)	19.3
Soybean, cooked	1.108 (67)	0.224 (14)	0.268 (16)	0.723 (44)	16.6
Egg	0.914 (72)	0.380 (30)	0.272 (22)	0.556 (44)	12.6
Chicken	1.818 (85)	0.592 (28)	0.274 (13)	0.904 (42)	21.4
Beef	1.785 (83)	0.565 (26)	0.227 (11)	0.846 (40)	21.4

Data from (USDA/ARS 2006): composition of foods: raw, processed, prepared, USDA National Nutrient Database for Standard Reference, Release 19, August 2006. US Department of Agriculture, Agricultural Research Service

[a] Values are per 100 g of food

[b] Values in parenthesis are mg/g protein

measure of the dietary intake which is made available to the body after digestion and absorption (Moughan 2005). The study of "protein quality evaluation" of foods aims to determine the capacity of food protein sources to satisfy the metabolic demand for both amino acids and nitrogen.

Protein quality evaluation

One of the earliest approaches to assess the nutritional quality of proteins for humans was the use of bioassays using growing rats. The protein efficiency ratio (PER) method which determines the ability of a protein to support growth in young rapidly growing rats has been applied since 1919 for the routine assessment of protein quality of foods (Young and Pellett 1994). The PER is calculated as the body weight gain in gram per gram of test protein consumed. However, the PER over estimates the value of animal proteins and under estimates the value of vegetable proteins. This is primarily because the rapid growth rate of rats increases the proportion of the protein which needs to be IDAA, in comparison to the slow growth rates in humans (FAO 1991).

Protein digestibility-corrected amino acid score (PDCAAS)

Due to the disadvantages of the PER method, an alternative method, the amino acid score method was proposed for the routine assessment of protein foods during a joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert consultation on protein quality evaluation in 1989 (FAO 1991). The amino acid score is defined as the concentration of the limiting amino acid in the food protein and is expressed as a percentage of the concentration of the same limiting amino acid in a reference amino acid pattern, which is the essential amino acid requirements of preschool-age children, as recommended by FAO (1985) (Young and Pellett 1994). The amino acid score for each protein derived by the above described procedure is subsequently corrected for true fecal protein digestibility (TD) of the test protein, as determined by using a rat model. TD is defined as the difference between the dietary intake of protein nitrogen and fecal nitrogen excretion, expressed as a % of the dietary nitrogen intake. Endogenous fecal nitrogen is taken into account by feeding rats a protein free diet (Schaafsma 2005). This modified amino acid score method is referred to as the protein digestibility-corrected amino acid score (PDCAAS), and is currently recommended as the method of choice for routine assessment of protein quality of foods (Schaafsma 2005; FAO 1991).

PDCAAS is therefore defined as:

PDCAAS (%) = (mg of first limiting amino acid in 1 g of test protein/mg of the same amino acid in 1 g reference protein) × TD (%)

Concerns relating to PDCAAS

Since its introduction in 1991, PDCAAS has been extensively studied and various limitations have been reported (Schaafsma 2005; Reeds et al. 2000). Specific concerns regarding the PDCAAS method include the adoption of the usage of mean requirement (EAR) estimates as reference, rather than the safe or RDA estimates. Furthermore, the current reference pattern is restricted to the IDAA and does not take into account the conditionally IDAA (Schaafsma 2005). The PDCAAS method also does not recognize nutritional value of high quality proteins, because values higher than 100% are truncated to 100%. Therefore,

differences between two proteins such as milk and soy proteins are not distinguished, although the actual concentrations of some IDAA and the capacity to complement other food sources are higher in milk than in soy protein (FAO 1991).

The PDCAAS method also utilizes true digestibility (TD) values derived from fecal digestibility coefficients in a rat model. This has many problems at several levels, including the usage of rat digestibility values and the usage of fecal versus ileal digestibility values. Nearly all of the absorption of amino acids occurs in the small intestine, prior to the end of the ileum, which means that fecal digestibility is a poor estimate of actual amino acid availability. There is extensive evidence demonstrating microbial degradation of protein and amino acids in the hindgut (Moughan 2003; Darragh and Hodgkinson 2000). Therefore, fecal digestibility values tend to overestimate the availability of many amino acids in dietary protein (Darragh and Hodgkinson 2000). Conversely, microbes in the hindgut also synthesize many amino acids, which can lead to underestimation of the ileal availability. Extensive research in pigs (NRC 1998) clearly demonstrates that ileal digestibility coefficients, which are determined by measurements made from the quantity of amino acids remaining at the end of the small intestine, or ileum, provide a more accurate estimate of protein and amino acid availability. Ileal digestibility values for humans have been attempted in studies conducted on healthy adult ileostomates (Moughan 2005), but the method is not suited for routine application. Despite being a major improvement compared to fecal digestibility, ileal digestibility of amino acids is not equal to the true amino acid availability for two reasons: not all absorbed amino acids are in a form that is biologically available (see discussion below), and the quantity of amino acids excreted into the gut, called endogenous protein loss (NRC 1998), varies with different foods (Myrie et al. 2008) and must be accurately accounted for.

The PDCAAS method also does not take into account the large variations which exist between digestibility values for entire proteins and individual amino acids (Fuller and Tomé 2005). For example, in human milk, indispensable amino acid digestibility ranged from 86% for threonine to 100% for methionine and tyrosine (Fuller and Tomé 2005).

Another key concern with the PDCAAS method is with respect to application of the method in foods which have been heat processed. Protein foods subjected to heat/alkaline processing to improve food flavour and texture, or sterilization/pasteurization may cause the formation of compounds that render the amino acids unavailable for protein synthesis, for example: Maillard compounds, oxidized forms of sulfur amino acids, D-amino acids, and cross-linked peptide chains, such as lysinolalanine (Gilani et al. 2005). Furthermore, some foods naturally contain antinutritional factors, such as trypsin inhibitors in soy protein, tannins in legumes and cereals, and phytates in cereals. These decrease the bioavailability of amino acids from the food sources (Myrie et al. 2008); the PDCAAS method does not take into account these factors and tends to overestimate the protein quality of such products (Gilani et al. 2005; Sarwar 1997).

Newer stable isotope based methods for in vivo estimation of amino acid bioavailability of foods

Due to the several disadvantages of the PDCAAS method, as outlined above, there is a need to develop newer stable isotope based methods, which will provide a more accurate estimate of the nutritional value of food protein sources. We (Moehn et al. 2005, 2007; Humayun et al. 2007) have recently applied the IAAO method using carbon (^{14}C or ^{13}C-labeled amino acid) oxidation as an indicator of amino acid availability, while others (Tomé and Bos 2000; Mariotti et al. 1999; Bos et al. 1999; Gausserès et al. 1997) have applied the use of [^{15}N]-labeled proteins to determine net postprandial protein utilization (NPPU), or [^{13}C]-leucine balance to predict PPU (Millward et al. 2000, 2002).

Net postprandial protein utilization (NPPU)

[^{15}N]-Labeled proteins (milk, soy protein isolate and wheat) have been used to measure the metabolic fate of dietary nitrogen after its consumption. NPPU is calculated using true ileal digestibility and ^{15}N-labeled protein deamination parameters. The dietary nitrogen collected in urine, and that retained in the body in the form of urea is added (Tomé and Bos 2000). NPPU values determined for milk, soy protein isolate and wheat are 81, 78 and 66%, respectively (Bos et al. 1999, 2005; Tomé and Bos 2000; Mariotti et al. 1999). This method is a major advancement in the evaluation of protein quality; however, it is restricted to foods which can be intrinsically labeled with ^{15}N and during the study, ileal digesta is collected via a naso-intestinal intubation technique. Therefore, this method is unlikely to be widely adopted for routine application.

Postprandial protein utilization (PPU)

Millward et al. (2002) used a [1-^{13}C]-leucine balance protocol and a single meal of wheat or milk protein to predict postprandial protein utilization (PPU). From the measurement of ^{13}C-leucine oxidation, leucine and nitrogen balances are predicted using the cumulative difference between the pre-meal and post-meal leucine oxidation rate,

which is converted to a nitrogen retention value based on an assumed body tissue protein leucine:nitrogen ratio, and the meal nitrogen intake. Nitrogen utilization is assumed to be equivalent to "leucine intake less the meal-dependent leucine oxidation", and utilization of wheat protein is assumed to be limited by the lysine content (Millward et al. 2002). Using this method the authors predicted wheat PPU to be 0.61 using the single meal pattern (Millward et al. 2002) or 0.68 using frequent small meals (Millward et al. 2000). The method as described above involves several assumptions which have not been validated in subsequent studies, and has been severely criticized by others (Kurpad and Young 2003). Furthermore, it is not possible to accurately determine the PPU for nitrogen present in the test meal by the present method because it is impossible to estimate how much of leucine oxidized in the 6 h postprandial period is due to specifically exogenous (dietary) and endogenous sources of leucine. It is unclear, whether these issues related to the assumptions in the PPU method can be resolved or not.

Application of the IAAO method to determine the metabolic availability (MA) of amino acids

The IAAO method can be applied to determine the bioavailability or metabolic availability (MA) of amino acids (Ball et al. 2005; Moehn et al. 2005, 2007). IAAO is inversely proportional to the rate of protein synthesis (Ball and Bayley 1986; Rafii et al. 2008). Therefore, at a given amino acid intake, relative difference in the IAAO rate between test and reference proteins will be proportional to the whole body MA of the test amino acid for protein synthesis, and thus account for all losses of dietary amino acids during digestion, absorption, and cellular metabolism (Figs. 1, 2). The total losses taken into account by the IAAO method include: incomplete digestion and absorption, gut endogenous amino acid losses and the unavailability of absorbed amino acids from foods due to the presence of anti-nutritional factors, Maillard compounds formed due to processing of foods, D-amino acids, and cross-linked proteins such as lysinoalanine. In simple terms, the higher the oxidation of the indicator amino acid, the lower is the metabolic availability of the test amino acid for protein synthesis and vice versa.

Metabolic availability of lysine in pigs

The initial development of the IAAO method to measure MA was conducted in growing pigs. The MA of lysine from peas in growing pigs (15–18 kg) was determined by comparing the oxidation of the indicator amino acid (intravenously delivered [1-^{14}C]phenylalanine) to that of pigs fed free lysine (crystalline form) (Moehn et al. 2005). It has been shown previously that the true digestibility of crystalline amino acids in pigs is essentially 100% (Baker 1992; Chung and Baker 1992). To determine metabolic availability using this method, key criteria must be fulfilled: (1) the test amino acid must be first limiting to ensure that it is the dietary intake of this amino acid that drives the change in indicator oxidation rates. (2) The response of the oxidation rate to increments of the test amino acid must be predictable to allow calculation of availability. For this criterion to be met, the intake of the limiting amino acid must be below the lower confidence interval (CI) of the requirement in every individual (Fig. 2).

The test diets, raw peas and heated peas (to render some of the lysine unavailable by Mallaird reaction), were fed at the 80% of lysine requirement level and IAAO measured (Moehn et al. 2005). Replacing the free lysine with equal amounts of protein bound lysine from both samples of raw pea's increased IAAO oxidation, demonstrating a lower availability of lysine for protein synthesis (Table 3). Heated peas increased IAAO greater than raw peas, demonstrating that lysine availability was decreased due to the heating process. MA was calculated from the ratio of the response to the addition of lysine intake from peas/heated peas to that of free lysine (Table 3). The MA of lysine from raw peas was determined to be 88%, compared to 55% from heated peas. These values were comparable to earlier published estimates of 85 and 48% for raw peas and similarly heated peas, respectively; determined using slope-ratio growth assays (van Barneveld et al. 1994).

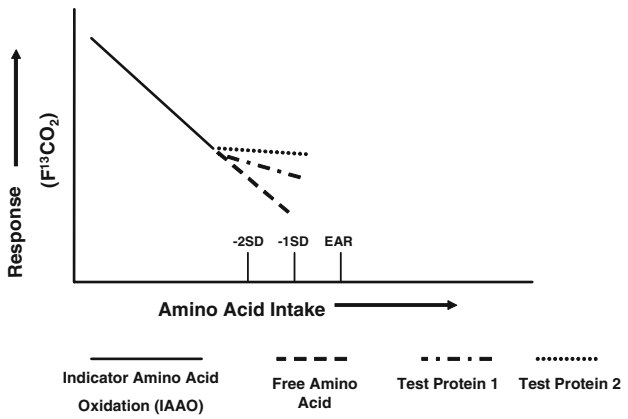

Fig. 2 Pattern of response observed in indicator amino acid oxidation due to intake of free (crystalline) amino acids and intact proteins. *EAR* Estimated average requirement; *SD* standard deviation. Metabolic availability (MA) is calculated from the ratio of the response to the addition of amino acid intake from test proteins to that of free (crystalline) amino acids. To ensure that the test amino acid is first limiting, the intake of the test amino acid must be below the lower confidence interval (CI), i.e. 1 or 2SD below the EAR in every individual

Table 3 Metabolic availability of lysine in peas fed to Swine

Amino Acid Intake	IAAO response (% of dose/g lysine intake)	Metabolic availability (%)
Moehn et al. (2005)[a]		
Free lysine (crystalline form)	−3.16 ± 0.39	100
Raw peas	−2.81 ± 0.44	88.8
Heated peas	−1.73 ± 0.41	54.8
Moehn et al. (2007)[b]		
Free lysine (crystalline form)	−3.63 ± 0.43	100
Soybean meal	−3.18 ± 0.32	87.5
Canola meal	−2.59 ± 0.31	71.4
Cottonseed meal	−2.73 ± 0.38	75.1
Raw peas	−2.75 ± 0.29	75.8
Heated peas	−2.48 ± 0.30	68.3
Heated peas plus free lysine	−2.78 ± 0.27	76.5

[a] Values are mean ± SEM, $n = 4$ growing pigs (Moehn et al. 2005)

[b] Values are mean ± SEM, $n = 8$ growing pigs (Moehn et al. 2007)

The method was adapted for the use of oral isotope delivery (Moehn et al. 2007) to make the method more applicable for routine use. The MA of lysine from soybean meal (87.5%, Table 3) correlated well with reported values of 88% for soybean meal based on standardized ileal digestibility (Moehn et al. 2007). The MA of raw and heated peas was 76 and 68.3%, respectively. Different feed stuffs and less severe heating conditions were applied in the latter study, which rendered less lysine unavailable. When heated peas were supplemented with free lysine, to the amount which was calculated to be lost during heating, the availability of lysine was determined to be 76.5%, similar to the raw peas (Table 3). Therefore, the MA method is sensitive to changes in lysine bioavailability.

Metabolic availability of sulfur amino acids in humans

We (Humayun et al. 2007) recently adapted the method in humans to determine the MA of SAA from casein versus soy protein isolate (SPI) using L-[1-^{13}C]phenylalanine as an indicator amino acid. Healthy young men received free methionine (crystalline form) at 20, 40, 50 and 70% of the TSAA requirement (13 mg/kg/d) previously determined in our laboratory (Di Buono et al. 2001). With increasing intake of free methionine, a linear decrease in indicator oxidation rate was observed. SPI was also tested to be first limiting for protein synthesis in these subjects, by the addition of free methionine to a test diet containing 40% of the TSAA requirement. It was observed that indicator oxidation decreased significantly due to the addition of free methionine to SPI test diet when compared to the unsupplemented group (Humayun et al. 2007). The test proteins, casein and SPI were tested at 60% of the TSAA requirement in the same subjects and the IAAO response compared against the IAAO response observed with the addition of free methionine (Table 4). All other amino acids, except the SAA, were present in excess and identical in content among the reference and test proteins. Therefore, changes in the IAAO between diets with free methionine versus SAA from casein or SPI reflected the metabolic availability of the SAA. The MA of SAA in casein and SPI were determined to be 87 and 72%, respectively, which are comparable to earlier published net protein utilization (NPU) values of 80–85% for milk proteins and 71–78% for soy proteins (Table 4).

In the human study, we also studied IAAO responses with the provision of graded increases in TSAA (40, 50, 60 and 70%) from casein and SPI, while supplementing all other amino acids to ensure that only the SAA from test proteins were limiting. We did not observe a linear decrease in IAAO with increasing SAA from test proteins, as we had observed for the graded increases in free methionine (Humayun et al. 2007). We reasoned that this was possibly due to the provision of a mixture of free and protein-bound amino acids in the minimally adapted IAAO protocol. To ensure that reliable estimates for TSAA availability can be obtained, we repeated the study in the same human subjects at the 60% TSAA intake level and virtually similar metabolic availability for both casein (90.5 vs. 90.6%) and soy protein (70.6 vs. 69.9%) were obtained in the second experiment, compared to the first experiment (Humayun et al. 2007). The 60% TSAA intake level was chosen to determine the availability because it is 1SD below the EAR for methionine in each individual (Di Buono et al. 2001). Future studies in humans need to be conducted to further validate this new concept to determine "metabolic availability". This application to determine amino acid availability is more practical and suitable for routine application in various food sources than the PDCAAS method and the other stable isotope based method in humans (using ^{15}N-labeled proteins, Tomé and Bos 2000). Compared to the ^{15}N-labeled protein method

Table 4 Metabolic availability of sulfur amino acids in casein and soy protein fed to humans

Amino acid intake	Breath $^{13}CO_2$ (% of dose)	Metabolic availability (%)
Free methionine (crystalline form)	10.3 ± 0.5	100
Casein	11.6 ± 0.6	87.4
Soy protein isolate (SPI)	13.2 ± 0.5	71.8

Values are mean ± SEM, $n = 7$ healthy young men (Humayun et al. 2007)

the IAAO method is easier to conduct, less expensive, and less invasive because it does not involve collection of ileal digesta via naso-intestinal intubation.

Conclusions

Amino acid requirements in human adults, neonates, children, and in diseased individuals have been systematically studied using the IAAO method. This concept has also been applied to determine the in vivo availability, termed "metabolic availability", of amino acids from foods for protein synthesis in humans. This method is suitable for rapid and routine application due to the minimal invasiveness involved and is a major step forward in defining the protein quality of food sources. Future studies need to be conducted to further validate the method for routine application and we believe that this method may have the potential to revolutionize the field of protein quality evaluation in humans.

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