Indicator Amino Acid Oxidation: Concept and Application1–3

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3Abbreviations used: AAA, aromatic amino acid; BCAA, branched chain amino acid; DRI, dietary recommended intake; IAAO, indicator amino acid oxidation; IDAA, indispensable amino acid; MA, metabolic availability; PKU, phenylketonuria; SAA, sulfur amino acid; SPI, soy protein isolate; TPN, total parenteral nutrition.
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Abstract
The indicator amino acid oxidation (IAAO) method is based on the concept that when 1 indispensable amino acid (IDAA) is deficient for protein synthesis, then all other IDAA, including the indicator amino acid, will be oxidized. With increasing intakes of the limiting amino acid, IAAO will decrease, reflecting increasing incorporation into protein. Once the requirement for the limiting amino acid is met, there will be no further change in the indicator oxidation. Originally, the IAAO method was designed to determine amino acid requirements in growing pigs. The minimally invasive IAAO method developed in humans has been systematically applied to determine IDAA requirements in adults. Due to its noninvasive nature, the IAAO method has also been used to determine requirements for amino acids in neonates and children, and in disease. The IAAO model has recently been applied to determine the metabolic availability (MA) of amino acids from dietary proteins and to determine total protein requirements. The IAAO method is robust, rapid, and reliable; it has been used to determine amino acid requirements in different species, across the life cycle, and in diseased populations. The recent application of IAAO to determine MA of amino acids and protein requirements is also very novel.

Introduction
The indicator amino acid oxidation (IAAO)8 technique is based on the concept that when 1 indispensable amino acid (IDAA) is deficient for protein synthesis, then all other amino acids, including the indicator amino acid (another IDAA, usually L-[1-13C]phenylalanine), will be oxidized (1). Fundamentally, this is because 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 (Fig. 1).

The IAAO method was initially developed by Bayley et al. (2–5) for the determination of amino acid requirements in young growing pigs in a series of elegant experiments. Ball and Bayley (6) validated the concept that the IAAO is inversely related to protein synthesis by showing that the recovery of 14C-phenylalanine radioactivity in piglet liver protein increased with increasing intake of dietary protein and was greatest with the dietary level of protein that minimized phenylalanine oxidation. 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 mean or estimated average requirement of the limiting (test) amino acid (1). Currently, IAAO and the IAAO-based method, 24-h indicator amino acid balance, are accepted as appropriate for the determination of amino acid requirements (7). For discussions about the various methods to determine amino acid requirements, their advantages and disadvantages, and requirement values, the reader is referred to earlier comprehensive reviews (1,8–10). The current article will briefly review developments in the application of the IAAO method to determine amino acid requirements, and introduce the recent adaptation of the IAAO model to determine total protein requirements and the metabolic availability (MA) of amino acids from foods.

Amino acid requirements in adult humans. The initial application of the IAAO method in adult humans was accomplished by Zello et al. (11) to determine the lysine requirement using intravenous L-[1-13C]phenylalanine as the indicator amino acid. Breath and blood were collected to measure 13CO2 and plasma phenylalanine enrichment, respectively. Biphase linear regression analysis identified the lysine requirement as 36.9 mg kg⁻¹ d⁻¹, which was considerably higher than the 1985 FAO/WHO/UNU recommendations of 12 mg kg⁻¹ d⁻¹ (11). To make the IAAO protocol less invasive, Bross et al. (12) validated the IAAO method with hourly oral isotope doses and sampling of urine to measure isotopic enrichment. To test whether the route of isotope infusion has an impact on the determination of the breakpoint, or requirement estimate, Kriengsinyos et al. (13) determined the lysine requirement in subjects infused i.v. or orally with L-[1-13C]phenylalanine (Fig. 1). Identical requirement estimates of 36.6 mg kg⁻¹ d⁻¹ for lysine were determined with both routes of isotope infusion. This minimally invasive IAAO method has...
have been systematically applied to determine IDAA requirements in adult humans (10), except histidine (14), for which no requirement could be determined (Table 1). These requirement values obtained using the IAAO method were used to derive amino acid intakes in the recent dietary recommended intakes (DRI) for macronutrients (7).

One of the criticisms of the IAAO model has been that subjects are only adapted to the test amino acid intake on the study day. To examine whether prolonged days of adaptation are necessary to determine requirements using the IAAO method, Moehn et al. (15) tested phenylalanine oxidation following various periods of adaptation to different lysine and protein intakes in growing and adult pigs. Phenylalanine oxidation did not differ following 2 or 10 d of adaptation to lysine or protein intake. Furthermore, we recently demonstrated that L-[1-13C] phenylalanine oxidation, measured as \( F^{13} \text{CO}_2 \) which is the primary variable used for determination of the breakpoint, did not differ following 8 h, 3 d, or 7 d of adaptation to a wide range of lysine intakes in young men (16). Therefore, the minimally invasive model provides valid estimates of amino acid requirements to be determined in short periods of time and thus is highly suitable for application in vulnerable populations.

**Amino acid requirements in children.** Determination of amino acid requirements in children has traditionally been difficult, because it is impractical and unethical to feed deficient amino acid intakes for prolonged periods of time. Therefore, current recommendations for amino acids in children are based on a factorial method. Development of the minimally invasive IAAO model enabled the direct determination of requirements for: total branched chain amino acids (BCAA) (17), total sulfur amino acids (SAA) (18), methionine (with cysteine) (19), and lysine (20) in healthy school-age children (6–11 y) (Table 1). Requirement estimates in children were similar to the estimates in adult humans, which suggests that the experimentally derived values predominantly reflect maintenance requirements and do not take into account all the growth needs (10). To ensure proper growth in children of this age group, we recommend addition of the calculated growth component to the requirement estimate, which has been discussed in detail recently by Elango et al. (10). These recent IAAO studies are the first to our knowledge to be conducted in children using stable isotopes and have clearly established that the factorial method of calculating requirements is indeed valid in healthy children.

**Amino acid requirements in disease.** Dietary management of specific diseases requires knowledge of nutrient requirements to have a successful clinical outcome. Metabolic disorders such as phenylketonuria (PKU) require tyrosine supplementation with phenylalanine restriction and maple syrup urine disease requires BCAA restriction. The minimally invasive IAAO model was used to determine tyrosine (21) and phenylalanine (22) requirements in children with classical PKU and the requirements were 19 and 14 mg kg\(^{-1}\)d\(^{-1}\), respectively. These values suggest that the ratio of aromatic amino acids (AAA) is 60% of tyrosine and 40% of phenylalanine, which is considerably different from the current recommendation of 80 and 20%, respectively, for the management of patients with PKU (9). Similarly, the mean total BCAA requirements in maple syrup urine disease patients was determined to be 45 mg kg\(^{-1}\)d\(^{-1}\) compared with the requirements of 144 mg kg\(^{-1}\)d\(^{-1}\) in healthy people (23).

Children with liver disease are hypothesized to have increased BCAA requirements based on measurements of plasma amino acid concentrations. We therefore applied the IAAO method using L-[1,13C]phenylalanine to determine total BCAA needs in children with cholestatic liver disease (24). The mean requirement was 209 mg kg\(^{-1}\)d\(^{-1}\), which is 30% higher than the mean requirement estimate of 147 mg kg\(^{-1}\)d\(^{-1}\) determined earlier in healthy children (17). Using a similar protocol, the mean total BCAA requirements in children after liver transplantation was determined to be 172 mg kg\(^{-1}\)d\(^{-1}\) (25). Therefore, post liver transplantation BCAA requirements are lower compared with children with liver disease but remain higher compared with the requirements for healthy children. These IAAO-derived requirement values are the first direct estimates, to our knowledge, in various diseases and disorders in children.

**Amino acid requirements in neonates.** Amino acid requirement studies in preterm and term neonates are extremely difficult to conduct due to ethical and practical concerns. During the adaptation of the IAAO method from growing pigs to adult humans, simultaneous work was conducted to adapt the IAAO protocol in...
lysine from peas and heated peas in 15-kg pigs. The MA of lysine determine the MA of free lysine compared with protein-bound Vaminolact was deficient in AAA (phenylalanine and 3.1% of total amino acids, respectively. Currently available (using the IAAO method in piglets (27) and human neonates (33) increased protein synthesis. Similarly, the new parenteral profile supplementation with AAA reduced lysine oxidation and hence increased intakes of the limiting amino acid, oxidation of the in- the IAAO model using14C-phenylalanine to et al. (34) applied the IAAO model using14C-lysine as the indicator amino acid, it was observed that Vaminolact was deficient in AAA (phenylalanine + tyrosine) and supplementation with AAA reduced lysine oxidation and hence increased protein synthesis. Similarly, the new parenteral profile was deficient in SAA (methionine + cysteine). Evidence that results from animal studies are readily applicable in human neonates is provided by comparing earlier tyrosine requirements determined using the IAAO method in piglets (27) and human neonates (33) (Table 2). At a constant phenylalanine intake, the mean tyrosine requirements in piglets and neonates were determined to be 2.7 and 3.1% of total amino acids, respectively. Currently available TPN solutions provide 0.9% of the total amino acids as tyrosine and clearly are not sufficient to promote normal growth and protein accretion in neonates.

Application of IAAO to determine MA of amino acids. The nutritional value of amino acids is directly related to the availability of amino acids metabolically at the site of protein synthesis. MA is dependent on digestibility, subsequent absorption, and the fraction of absorbed amino acids that is utilized for protein synthesis. Currently, the nutritional value of proteins is determined by cumbersome long-term growth/balance studies, or by using the protein digestibility-corrected amino acid score method. The IAAO method, which is based on the concept that with increasing intakes of the limiting amino acid, oxidation of the indicator amino acid will decrease and therefore reflect increasing whole body protein synthesis, can be applied to determine the bioavailability or MA of amino acids. At a given amino acid intake, differences in the IAAO rate between test and reference proteins will be proportional to the whole body MA of the test amino acid and thus account for all losses of dietary amino acids during digestion, absorption, and cellular metabolism. Moehn et al. (34) applied the IAAO model using 14C-phenylalanine to determine the MA of free lysine compared with protein-bound lysine from peas and heated peas in 15-kg pigs. The MA of lysine from peas was determined to be 88%, compared with 55% from heated peas. These values are comparable to earlier published estimates of 85% for peas and 48% for heated peas, determined using slope-ratio growth assays.

Humayun et al. (35) recently adapted the method in humans to determine the MA of SAA from casein vs. soy protein isolate (SPI) using t-[1-13C]phenylalanine as the indicator amino acid. All other amino acids except the SAA were present in excess and identical in content between the test proteins. Therefore, changes in the IAAO between free methionine vs. SAA from casein or SPI will reflect MA. The MA of SAA in casein and SPI were 87 and 72%, respectively, and are comparable to earlier published net protein utilization values of 80–85% for milk proteins and 71–78% for soy proteins. The IAAO method to determine MA has the potential to revolutionize the field of determination of protein quality of foods and is preferable to existing methods, because it can be conducted in a relatively short period of time in a minimally invasive way.

TABLE 2 Comparison of IAAO-derived requirements in piglets vs. human neonates fed parenterally

<table>
<thead>
<tr>
<th>Species</th>
<th>Reference</th>
<th>Phenylalanine intake</th>
<th>Mean tyrosine requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piglet</td>
<td>(27)</td>
<td>4.1</td>
<td>2.7</td>
</tr>
<tr>
<td>Neonate</td>
<td>(33)</td>
<td>4.0</td>
<td>3.1</td>
</tr>
</tbody>
</table>

% of total amino acids

1 Currently available TPN formulas provide 0.9% of total amino acids as tyrosine.

Application of IAAO to determine protein requirements. Traditionally, total protein requirements for humans have been determined using nitrogen balance. The recent DRI recommendations for mean and population safe intakes of 0.66 and 0.8 g·kg⁻¹·d⁻¹, respectively, of high quality protein in adult humans are based on meta-analysis of nitrogen balance studies using linear regression analysis (7). Considering the inherent problems associated with the nitrogen balance method (1), we hypothesized that the protein requirements are underestimated. Therefore, we examined the total protein requirement in adult humans using the IAAO method (36), as previously applied in young pigs (6). Graded intakes of a mixture of amino acids in the pattern present in egg protein, except phenylalanine, ranging from 0.1 to 1.8 g·kg⁻¹·d⁻¹, were fed and indicator amino acid (t-[1-13C]phenylalanine) oxidation was measured. The mean protein requirement was 0.93 g·kg⁻¹·d⁻¹ and is 41% higher than the current DRI recommendation. This value is also in agreement with our reanalysis of previous nitrogen balance studies using bi-phase linear regression analysis, which identified a mean protein requirement of 0.91 g·kg⁻¹·d⁻¹ (36). The IAAO-derived protein requirements for adult humans are significantly higher than current recommendations and suggest an urgent need to reassess protein intake recommendations.

In conclusion, the IAAO method is a robust, rapid, and reliable method to determine amino acid requirements in different species, across the life cycle, and in diseased populations. The novel application of the IAAO to determine MA is a major step forward in the determination of protein quality of various foods. The recent adaptation of the IAAO method to determine protein requirements in humans suggest that reassessment of protein intake recommendations for adult humans is necessary. Due to the minimally invasive nature of the IAAO method, it is now possible to determine amino acid and protein requirements in other vulnerable populations, including pregnant and lactating women and the elderly.

Literature Cited

Indicator amino acid oxidation technique
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