Lysine from Cooked White Rice Consumed by Healthy Young Men Is Highly Metabolically Available When Assessed Using the Indicator Amino Acid Oxidation Technique

Ivo R. D. Prolla, M. Rafii, G. Courtney-Martin, Rajavel Elango, Leila P. da Silva, Ronald O. Ball, and Paul B. Pencharz

Abstract

Cooked white rice (CWR) provides up to 71% of the dietary protein for many people worldwide. The protein digestibility-corrected amino acid (AA) score is the method adopted by FAO/WHO to evaluate protein quality. Our group has proposed the metabolic availability (MA) of AAs as another determinant of protein quality. It measures the percentage of an indispensable AA that is incorporated during protein synthesis. This study is the first to our knowledge to assess the MA of L-lysine (L-Lys) from CWR in humans using the indicator AA oxidation (IAAO) technique. Three amounts of L-Lys, 10, 15, and 19 mg·kg⁻¹·d⁻¹ (28.5, 42.8, and 54.3% of the mean L-Lys requirement of 35 mg·kg⁻¹·d⁻¹), were studied in 5 healthy young men in a repeated-measures design. To test the principle that the Maillard reaction has an effect on the MA of L-Lys, we also assessed the MA of L-Lys in oven-browned, cooked rice (n = 3) in the amount of 19 mg·kg⁻¹·d⁻¹ L-Lys. The MA of L-Lys was estimated by comparing the IAAO response with varying L-Lys intakes in rice compared with the IAAO response to varying L-Lys intakes in the reference protein (crystalline AA mixture patterned after egg protein) using the slope ratio method. The MA of L-Lys from CWR was high (97%), but the effect of the Maillard reaction reduced it to 70%. The results show that despite its relatively low content in rice, L-Lys has a high MA when the rice is cooked without being browned. J. Nutr. 143: 302–306, 2013.

Introduction

For many people worldwide, cooked, white rice (CWR)12 serves as the most important protein source in the diet. In some rice-eating countries, the mean rice intake is ~187 kg⁻¹ · person⁻¹ · y⁻¹ and contributes up to 71% of dietary protein (1). Therefore, for many countries, rice grains with a high protein content are of great interest.

The nutritional value of dietary proteins is related to the indispensable amino acid (IAA) pattern and, more importantly, the "proportion of dietary amino acid (AA) that is digested and absorbed in a form suitable for protein synthesis; this is called AA availability" (2) or bioavailability or metabolic availability (MA). The IAA of L-lysine (L-Lys) is the first limiting AA in cereal grains, which in rice has an AA score of 62% (3). This fact restricts the use of all other AAs present in the rice for protein synthesis, causing them to be considered “in excess” and, consequently, oxidized (4). In addition, cooking rice has been shown to decrease the digestibility of nitrogen by laboratory rats (5) and L-Lys can become unavailable due to the Maillard reaction with carbohydrates (6). Thus, this reaction further decreases the MA of L-Lys and consequently impairs the nutritional value of rice protein. Ultimately, the MA of L-Lys is an important factor regarding the amount of rice to be consumed by populations to match daily protein requirements, particularly when this cereal is the major dietary protein source (1).
The quality of dietary proteins in foodstuffs for human consumption has been assessed by balance studies in animals (7,8) or by the protein digestibility-corrected AA score (PDCAAS) (3). For rice, the PDCAAS is ~56% (3), which is considered low. Although the PDCAAS is currently the most used and recommended method, there have been many criticisms concerning its value (9).

Besides PDCAAS, different methods for assessing AA availability have been proposed (10,11). The indicator AA oxidation (IAAO) technique is currently a well-accepted method for the determination of protein and AA requirements in animals (12) and humans (13–15). The IAAO technique has been used to assess the MA of different AAs in pigs (16,17) and humans (18) and its benefits in determining the MA of IAs were recently discussed (19–21). Briefly, this method is based on the observation that, if one AA is limiting for protein synthesis, all other AAs are in excess (including the indicator AA, such as L-[1-13C] phenylalanine) and must be oxidized (22). Consequently, changes in the oxidation of the indicator AA following the intake of the test or reference protein will reflect the whole-body MA of the limiting AA at the site of protein synthesis, accounting for all losses of dietary AA during digestion, absorption, and cellular metabolism. In other words, the higher the oxidation of the indicator AA, the lower the MA of the test AA for protein synthesis and vice versa (18). The aim of this study was to assess the MA of L-Lys from CWR protein in young adult men by using the IAAO technique.

Materials and Methods

Subjects. Five young, healthy, adult males completed the experiment (on an outpatient basis) in the Clinical Investigation Unit at the Hospital for Sick Children, Toronto, ON, Canada. Subject characteristics, body composition, and energy requirements were assessed at entrance and prior to each individual experiment (Table 1). There was no history of recent weight loss, illness, or medication use at the time of entry or during the study, as determined by medical history. The study protocol and goal were explained to each subject. Informed written consent was obtained from each participating subject after the protocol was fully explained to them. Subjects received financial compensation for their inconvenience. The study was approved by the Research Ethics Board of the Hospital for Sick Children, Toronto, ON, Canada.

Study design and dietary intervention. This study was conducted in 2 main parts. The objective of the first part was to assess the MA of L-Lys from CWR protein by comparing the slopes of the IAAO response following the graded intakes of L-Lys in CWR compared with the reference protein, using the slope ratio method.

A reference slope was constructed from the IAAO response measured following the feeding of graded intakes of L-Lys from a reference protein (crystalline AA mixture patterned after egg protein) (Table 2). The slope was constructed from 3 graded intakes of L-Lys studied in random order. The amounts of L-Lys studied were 10, 15, and 19 mg·kg⁻¹·day⁻¹, representing 28.5, 42.8, and 54.3%, respectively, of the mean L-Lys requirement (35 mg·kg⁻¹·day⁻¹) (23).

The MA of L-Lys in rice was determined by substituting a portion of the AA-based diet with CWR (polished long grain white rice, Selection). We chose this rice because of its similar nutrient content to rice commonly eaten in East Asia, where a number of countries are among the highest rice consumers in the world. It also has a similar nutrient profile to rice commonly eaten in the Middle East and Brazil. Two amounts of L-Lys intake were studied in the rice in random order: 15 and 19 mg·kg⁻¹·day⁻¹, with 10 mg·kg⁻¹·day⁻¹ serving as the base L-Lys intake provided by the AA mixture. The carbohydrate, protein, and L-Lys contents of the rice studied were 78.2, 6.75, and 0.24 g·100 g⁻¹ of raw rice, respectively. These values were based on the mean among long, medium, and short raw, white rice grains and were taken from the USDA table (24) (Table 2). We performed our own analysis (macronutrient and AA content) on the actual rice used in the experiment and the results were very similar to those obtained by the USDA (Supplemental Table 1). The AA composition of the rice was matched to that of the reference protein by adding individual crystalline AA to the cooked rice.

For the second part of the experiment, the effect of the Maillard reaction on the MA of L-Lys from rice was determined as a proof of principle. For testing the effect of the Maillard reaction, 3 subjects consumed oven-browned cooked rice (OBCR) at the highest L-Lys intake (19 mg·kg⁻¹·day⁻¹). The same white rice was first browned in the oven, after which it was cooked.

Study protocol. Oxidation studies were performed on d 3 after 2 d of adaptation to the test diet. During the 2 adaptation days, subjects received an AA-based liquid diet (25), providing 1 of the 3 randomly assigned L-Lys amounts, energy; resting energy expenditure measured by open-circuit indirect calorimetry (Vmax Encore, Viasys Healthcare) × 1.7 and protein (1.0 g·kg⁻¹·day⁻¹). The nonprotein energy in the diet was provided as a protein-free powder (Product 80056, Mead Johnson) flavored with Tang and KoolAid crystals (Kraft Foods, Don Mills), corn oil, and protein-free cookies (26). The adaptation diet was consumed as 4 equal meals with 52, 36, and 12% of energy from carbohydrates, fat, and protein, respectively. On d 3, following a 10-h overnight fast, subjects came to the Investigation Unit at The Hospital for Sick Children, Toronto, ON for a period of 10 h. The oxidation study day diet content was similar to that of the adaptation diet. It was consumed as 9 isonitrogenous and isoenergetic hourly meals, with each meal representing 1/9 level of intake of the OBCR.

TABLE 1 Characteristics of healthy young men who participated in the IAAO studies for the determination of MA of L-Lys in CWR

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>33.2 ± 7.3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>76.5 ± 8.1</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.73 ± 0.08</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.4 ± 1.7</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>56.6 ± 7.6</td>
</tr>
<tr>
<td>Resting metabolic rate, MJ·d⁻¹</td>
<td>6.7 ± 0.83</td>
</tr>
</tbody>
</table>

1 Mean ± SEM, n = 5. CWR, cooked white rice; IAAO, indicator amino acid oxidation; MA, metabolic availability.

2 Determined by bioelectrical impedance analysis.

3 Determined by open-circuit indirect calorimetry.

Table 1

Oxidation studies were performed on d 3 after 2 d of adaptation to the test diet. During the 2 adaptation days, subjects received an AA-based liquid diet (25), providing 1 of the 3 randomly assigned L-Lys amounts, energy; resting energy expenditure measured by open-circuit indirect calorimetry (Vmax Encore, Viasys Healthcare) × 1.7 and protein (1.0 g·kg⁻¹·day⁻¹). The nonprotein energy in the diet was provided as a protein-free powder (Product 80056, Mead Johnson) flavored with Tang and KoolAid crystals (Kraft Foods, Don Mills), corn oil, and protein-free cookies (26). The adaptation diet was consumed as 4 equal meals with 52, 36, and 12% of energy from carbohydrates, fat, and protein, respectively. On d 3, following a 10-h overnight fast, subjects came to the Investigation Unit at The Hospital for Sick Children, Toronto, ON for a period of 10 h. The oxidation study day diet content was similar to that of the adaptation diet. It was consumed as 9 isonitrogenous and isoenergetic hourly meals, with each meal representing 1/9 level of intake of the OBCR.

Tracer protocol. The tracer protocol started orally on d 3 of each experiment (study day) with the fifth meal, using NaH¹³CO₃ (0.176 mg·kg⁻¹·day⁻¹) and L-[¹³C] phenylalanine (99 atom % excess, Cambridge Isotope Laboratories; 40 µmol·kg⁻¹·h⁻¹) as prime and 15 µmol·kg⁻¹·h⁻¹.
TABLE 2 AA composition of reference and test protein consumed by healthy young men who participated in the IAAO study on MA of l-Lys in CWR.1,2

<table>
<thead>
<tr>
<th>AA</th>
<th>AA mixture</th>
<th>Rice3</th>
</tr>
</thead>
<tbody>
<tr>
<td>l-Arginine</td>
<td>74.5 g·g−1</td>
<td>100</td>
</tr>
<tr>
<td>l-Asparagine</td>
<td>33.1</td>
<td>N.I.</td>
</tr>
<tr>
<td>l-Aspartic acid</td>
<td>33.1</td>
<td>93.9</td>
</tr>
<tr>
<td>l-Lysine</td>
<td>75.1</td>
<td>44.8</td>
</tr>
<tr>
<td>l-Cysteine</td>
<td>21.9</td>
<td>20.5</td>
</tr>
<tr>
<td>l-Glutamine</td>
<td>56.2</td>
<td>N.I.</td>
</tr>
<tr>
<td>l-Glutamic acid</td>
<td>56.2</td>
<td>194.9</td>
</tr>
<tr>
<td>l-Glycine</td>
<td>33.0</td>
<td>45.6</td>
</tr>
<tr>
<td>l-Histidine</td>
<td>22.5</td>
<td>23.5</td>
</tr>
<tr>
<td>l-Isoleucine</td>
<td>62.4</td>
<td>43.2</td>
</tr>
<tr>
<td>l-Leucine</td>
<td>82.6</td>
<td>82.7</td>
</tr>
<tr>
<td>l-Methionine</td>
<td>29.5</td>
<td>23.5</td>
</tr>
<tr>
<td>l-Phenylalanine</td>
<td>54.2</td>
<td>53.5</td>
</tr>
<tr>
<td>l-Proline</td>
<td>41.6</td>
<td>47.0</td>
</tr>
<tr>
<td>l-Serine</td>
<td>93.2</td>
<td>52.6</td>
</tr>
<tr>
<td>l-Threonine</td>
<td>46.7</td>
<td>35.8</td>
</tr>
<tr>
<td>l-Tryptophan</td>
<td>15.5</td>
<td>11.6</td>
</tr>
<tr>
<td>l-Tyrosine</td>
<td>40.4</td>
<td>33.4</td>
</tr>
<tr>
<td>l-Valine</td>
<td>69.7</td>
<td>61.0</td>
</tr>
<tr>
<td>l-Alanine</td>
<td>61.5</td>
<td>57.9</td>
</tr>
<tr>
<td>Total</td>
<td>983</td>
<td>1025</td>
</tr>
</tbody>
</table>

1 The reference protein was a crystalline AA mixture patterned after the AA composition of egg protein. AA, amino acid; CWR, cooked white rice; IAAO, indicator amino acid oxidation; MA, metabolic availability; N.I., no information (from USDA tables).
2 The test protein was a combination of crystalline AA mixture [providing 10 mg·kg−1·d−1 of l-Lys (base)] plus CWR (2.05 or 3.68 g·kg−1·d−1) of raw rice, providing 5 or 9 mg·kg−1·d−1 of l-Lys, to meet 42.9 or 54.3% of the daily l-Lys requirement of 35 mg·kg−1·d−1, respectively).
3 Polished white rice AA profile, USDA National Nutrient Database for Standard Reference; values represent the mean among long, medium, and short grain values (24).
4 Actual concentrations of AAs in HCl form: in AA mixture: arginine, 62.1 mg·g−1; l-Lys, 60.6 mg·g−1; in raw rice: arginine, 83.4 mg·g−1; l-Lys, 36.2 mg·g−1.

**Results**

**Linearity of response to Lys intake from free AA diet.** As l-Lys intake from the AA mixture increased from 10 to 15 to 19 mg·kg−1·d−1 (28.6–54.3% of l-Lys requirement of 35 mg·kg−1·d−1), the l-[1-13C]phenylalanine oxidation (OXPhe) decreased linearly. Application of linear regression determined a negative slope of the best-fit line of −0.00899 (SEM = 0.0023; P < 0.05).

**MA of Lys in CWR.** l-Lys intake (5–9 mg·kg−1·d−1 above base) from CWR had a significant effect on OXPhe (slope = −0.00872; SEM = 0.0023; P < 0.05). However, the replacement of l-Lys from the AA mixture with l-Lys from CWR did not produce a change in OXPhe from that observed with the AA mixture (P = 0.85). The ratio of the response to additional l-Lys intake from the CWR compared with that of l-Lys from the AA mixture was 0.9699. Thus, the MA of l-Lys from CWR was 97% and did not significantly differ from the AA mixture (Table 3).

**MA of Lys in OBCR.** There was an increase in OXPhe when OBCR was consumed by men at a l-Lys intake of 9 mg·kg−1·d−1 above base. Browing the rice decreased the MA of l-Lys from 97 to 70% (P = 0.02).

**Discussion**

The FAO/WHO considers PDCAAS as the method of choice for the measurement of protein quality in human nutrition (28). However, many criticisms about this method have risen in the literature (9). Among them, the use of fecal instead of ileal digestibility is of main concern. In this sense, the true ileal AA digestibility coefficients, as performed in human ileostomates, may provide more accurate results (21). However, methodological difficulties in testing different protein sources in these subjects limit the broad use of this technique. In addition, the PDCAAS is dependent on the concentration of the AAs

**TABLE 3** MA of L-Lys in CWR and OBCR based on IAAO of l-[1-13C]phenylalanine

<table>
<thead>
<tr>
<th>Lysine source</th>
<th>n</th>
<th>Slope equation</th>
<th>MA</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA mixture</td>
<td>5</td>
<td>−0.00892 × 1.11</td>
<td>100</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CWR</td>
<td>5</td>
<td>−0.00872 × 1.11</td>
<td>97</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>OBCR</td>
<td>3</td>
<td>−0.00630 × 1.11</td>
<td>70</td>
<td>0.02</td>
</tr>
</tbody>
</table>

1 CWR, cooked white rice; IAAO, indicator amino acid oxidation; MA, metabolic availability; OBCR, oven-browned cooked rice.
2 MA from AA mixture was assumed to be 100%.
Based on our data, the MA of L-Lys from cooked rice is 97%, as a limiting AA, in cereal-based diets consumed by humans. However, rice was shown to have a high TD of the limiting AA. For instance, in black beans, the TD for total nitrogen is 72%, whereas the TD of essential AAs such as methionine and arginine is 51% and 77%, respectively. The correction of AA scores for crude protein digestibility (as is usually done) is not adequate to accurately reflect the digestibility of individual AAs in proteins (29).

Our group has used the IAAO technique, a noninvasive method, to assess AA requirements in adults and children (30,31). Recently, we applied the same technique to determine the MA of different AAs from various food protein sources, such as casein and soy protein isolate in humans (18) and peas, corn, and barley in animals (16,17). In the present study, the IAAO technique was used to assess the MA of L-Lys from the protein in a whole food: cooked rice. As far as we know, this is the first study to determine the MA of an AA from a common supermarket-purchased grain using the IAAO technique in humans. For this, we studied different amounts of L-Lys intake provided by a mix of free L-AAs plus CWR (test protein). Based on apparent and true ideal AA digestibility of a crystalline AA mixture in animals (pigs and cockerels), we assumed that the MA of all AAs provided in the mix was 100% (32).

In countries where cereals and legumes provide the main protein source in the diet, both quality and amount of the protein consumed should be taken into account. For malnourished children, e.g., it is suggested that 24–26 g protein · 1000 kcal−1 with a PDCAAS of at least 70% would be the preferable profile of the diet (33). However, there is no statement about the lowest digestibility and MA values of the IAAs of the protein source. Consequently, any isolated or combined vegetable protein source might fulfill the requirements related to quantity and PDCAAS mentioned above. CWR presents a low protein: energy ratio (18.5–20.8 g protein · 1000 kcal−1) (24) and a low PDCAAS (36%) (3). This suggests that rice is not an adequate protein source. However, rice was shown to have a high TD value for both the protein (90%) and Lys (100%) (3). Nevertheless, information is lacking on the bioavailability of the L-Lys, as a limiting AA, in cereal-based diets consumed by humans. Based on our data, the MA of L-Lys from cooked rice is 97%, meaning that almost all L-Lys provided by cooked rice is effectively digested, absorbed, and incorporated into proteins. These results are critical for any populations where rice is the main protein source in the diet.

In rats, the digestibility of protein and L-Lys from cooked, milled rice was 90 and 100%, respectively; because the AA score for the rice used was 62.2%, the PDCAAS was 56% (3). In our study, the MA of L-Lys was 97%. It means that 97% of the total amount of the L-Lys present in the diet was actually digested, absorbed, and used for protein synthesis. Because the mean daily amount of L-Lys requirement was previously determined (23) to be 35 mg · kg−1 · d−1, the AA score for our rice was calculated to be 1.0 or 100% [(35.5 g · L-Lys · g−1 of rice protein/35 mg · L-Lys · g−1 protein) × 100 = 101.4%]. Considering 90% (3) as the TD value for the rice protein and the new AA score (1.0 or 100%) to recalculate the PDCAAS, it was found to be 90% (90% × 1.0, or 100%), much higher than the 56% previously assumed (3). The suggestion is that rice protein is of a much higher quality than that stated in past reports.

Comparisons between TD of protein (used in PDCAAS calculation) and limiting AAs in vegetable protein fed to rats showed that the crude protein digestibility may not be a good predictor of the bioavailability of limiting AAs (29). The corrections for TD of individual AAs for all the foods assessed lowered the scores by 11–47% (29). For instance, in black beans the AA score and TD of protein were 89 and 72%, respectively. Then, the PDCAAS for black beans was considered low: 64%. Despite the high TD for black bean protein, the TD for its limiting AAs, methionine and cystine, were very low (51 and 46%, respectively). According to this study (29), the TD of each of the 9 IAAs should be taken into account and provide a more reliable value for the AA ratio called the available AA score. This new score was used to reanalyze the PDCAAS. For black beans, it was found to be 42%, which is even lower than the previous value of 64% (29). Based on our L-Lys values, the available AA score is significantly higher (97%) (3.0 × 97%) than the previously assumed 62%. However, the available AA score for the remaining IAAs in rice still needs to be assessed. In this sense, the MA using the IAAO technique is a better option, because it is able to assess the actual amount of each AA provided in the diet that is effectively digested and used for protein synthesis.

The higher MA obtained in this study might be related to the low amount of antinutritional factors (e.g., fiber and phytate) that have a negative impact on the bioavailability of nutrients in foods. We used polished white rice in our study. Although the rice used in the current study has a similar nutrient profile to rice consumed in many rice-eating countries around the world, it is possible that other kinds of rice with higher fiber contents might have a lower MA. The most important antinutritional food constituent in diets in low-income countries is phytate. It is present in cereal products and forms insoluble complexes with a range of nutrients, inhibiting the absorption of proteins (34). In CWR, the phytate content is remarkably low (1.2–3.7 mg · g−1 dry matter) compared with other cereals and legumes (35). This is due to the fact that the germ and pericarp, which contain 87.6% of the phytate, are removed during industry processing of the rice grains. Besides that, thermal and soaking methods can reduce the phytate content in cereals (34). These facts may explain the higher MA of Lys observed in our study.

The Maillard reaction can decrease the availability of some essential AAs (33,34). In cereal-based foodstuffs L-Lys is the most reactive and thus most affected (36). In our study, we also aimed at demonstrating this effect on L-Lys in rice by first browning it in an oven before cooking. For this part of the experiment, only 3 of the original 5 subjects were available. When they were fed the OBCR, we observed an upward shift in slope (namely, increment in the rate of OXPhe). It suggests that L-lys incorporation into proteins was decreased after the rice was browned (MA = 70%). This result is important for populations where rice is considered the primary protein source, because excessive heat exposure resulting in burning of the rice would result in a decrease in its nutritional value. Because the Maillard reaction can decrease the availability of some essential AAs other than L-Lys (37), it is not known from this study whether the change in the oxidation slope happened exclusively due to the impairment in L-Lys availability. This further illustrates the sensitivity of the IAAO technique to changes in AA content in the diets.

In summary, this was the first study, to our knowledge, to determine the MA of L-Lys in a store-bought, whole-food item, CWR, using the IAAO technique. The MA of L-Lys in men (97%) was similar to that derived for its TD in animals (100%) and much higher than that suggested by the PDCAAS value for rice. The methodology applied in this study is suitable for assessing the MA of any IAAs in foods and consequently can be used to update the nutritional value of different dietary AAs and protein sources.
Acknowledgments
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Literature Cited