The Validity of Estimated Dietary Amino Acid and Protein Values via the Amino Acid Composition Table 2015

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Summary The amino acid composition table (AACT) plays a pivotal role in examining the association between dietary amino acid intake and physical conditions. The updated version, AACT 2015, has been markedly expanded; however, most additions are not based on analytical values. The Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) recommend that protein contents be calculated as the sum of amino acid residues (PROTCAA). However, due to the lack of a validated AACT, protein content calculated as reference nitrogen multiplied by a nitrogen to protein conversion factor (PROTRN) is still commonly used. In this study, validity of the estimated dietary amino acid values via the AACT 2015 was examined by comparing differences between the estimated and analytical values, for 14 consecutive days' meals provided in an elder care facility. There were no major differences between the analytical and estimated values over the 14 d; however, noticeable daily differences sometimes emerged. These results indicate that the AACT 2015 may contain accidental errors, but allows the estimation of habitual amino acid intake. In the near future, PROTCAA will become the international standard. It will be necessary to convert PROTRN values to PROTCAA to refer to past reports and data; we have determined a correction factor (0.896) for this conversion.

Key Words amino acid composition table 2015, Kjeldahl analysis, amino acid analysis, protein-to-tryptophan conversion factor, protein contents calculated as the sum of amino acid residues

The amount of proteins in the body is maintained constant through continuous anabolism and catabolism. When degraded, their components return to the amino acid pool. Our bodies reuse these amino acids, but a small portion of them is lost, and needs to be supplemented, in one's diet. Protein requirements not only include this loss, but also the increased needs during growth, pregnancy, illness, injury, and recovery. The composition of the amino acid pool varies according to the characteristics of one's diet. For example, most plant proteins contain a lower ratio of essential amino acids, while most animal proteins contain the ideal ratios. Therefore, vegetarians who eat no animal products may suffer from subclinical protein malnutrition (1).

The protein digestibility-corrected amino acid score has been adopted by the Food and Agriculture Organization (FAO) of the United Nations and the World Health Organization (WHO) and is calculated according to the limiting amino acids, such as lysine, tryptophan, threonine, and the sulfur-containing amino acids (methionine and cystine) to determine the protein quality of human nutrition (2). It should be noted that this scoring pattern is derived from the essential amino acid requirements of 2- to 5-y-old children (3). Nutrient requirements can easily change, depending on age, gender, life-stage, life-style, and health state. For example, the utilization

and absorption of many nutrients is less efficient in the elderly, resulting in increased requirements (4). Therefore, there is a need for studies estimating the amino acid requirements during various individual conditions. A validated amino acid composition table (AACT) is necessary to estimate amino acid intake. Aging leads to significant loss in muscle mass, strength, and the ability to independently perform activities of daily living. The oral intake of essential amino acids, particularly leucine (5) and its metabolite β -hydroxy- β -methyl butyrate (HMB) (6), promotes skeletal muscle protein synthesis in the elderly. However, most functional studies on the use of amino acids, to prevent lifestyle-related diseases, are intervention studies, because amino acid content databases are insufficient to estimate dietary amino acid intake in Asian countries, including Japan (7). The Japanese Ministry of Education, Culture, Sports, Science, and Technology publishes the AACT, which was revised in 2010 after a gap of 24 y.

The Japanese food composition table 2010 included 1,878 food items; however, amino acid values were available for only 337. The updated AACT 2015 markedly increased the number of items listed with amino acid values (from 337 to 1,558); however, most of them (approximately 1,000) are not based on analytical values. Therefore, AACT 2015 contain substitution errors in matching nutritionally different foods when substituting for missing foods in the tables. The AACT 2015

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is still under development, and its validity has not yet been evaluated.

In general, protein content calculated as reference nitrogen multiplied by a nitrogen to protein conversion factor (PROTRN) has been used to assess the protein nutritional status for many years. Calculation of PROTRN is based on the assumption that almost all nitrogen exists in proteins. However, nitrogen is also present in variable quantities in other compounds, such as creatine, choline and nucleotides (8). This is one reason why the FAO and WHO recommended the use of protein contents calculated as the sum of amino acid residues (PROTCAA). However, PROTRN is still commonly used, because of the lack of a validated AACT. In addition, it has been observed that contents determined by PROTCAA tend to be lower than those calculated by PROTRN (9). However, few studies have examined the use of a correction factor to convert PROTRN results to PROTCAA.

The purpose of this study was to evaluate the validity of estimated dietary amino acid and protein values in the AACT 2015. We used 14 consecutive days' meals, provided in an elder care facility, as samples for amino acid analysis. We assessed the correlation between estimated values in the AACT 2015 and chemical analytical values. In addition, we examined the relationship between PROTRN and analytical value of PROTCAA (AVO-PROTCAA).

MATERIALS AND METHODS

Chemicals and reagents. Hexane and Type-H amino acid standard solution were purchased from Wako Pure Chemical Industries (Osaka, Japan). Mobile phase and reaction solution for HPLC were purchased from Shimadzu (Kyoto, Japan). Fat-free milk was purchased from Morinaga Milk Industry (Tokyo, Japan).

Pretreatment method for chemical analysis. We used the meals provided to one person for 14 consecutive days (including breakfast, lunch, dinner, and a snack), in an elder care facility, as samples for our chemical analysis. To prepare homogeneous samples with less water, the samples were homogenized, in a volume of water, equivalent to approximately 40% of the meal weight, and freeze-dried.

The dried samples were re-homogenized in a volume of hexane, equivalent to approximately 20% of the dried sample weight. They were filtered using Whatman No. 5 filter paper. The residues were dried for 5 h in a constant-temperature dryer at 50°C.

We evaluated the validity of the pretreatment method using spike recovery experiments. Fat-free milk containing known amounts of amino acids (equivalent to 25 g of PROTRN) were spiked into additive-free samples (meals, $n=3$). Recovered amounts were calculated as the average of spiked samples (meals, $n=3$) minus those of additive-free samples (meals, $n=3$).

Quantitative determination by PROTRN. Samples (approximately 0.75 g) were digested using a Kjeldahl System (Buchi Co., Flawil, Switzerland) that consisted of a digestion unit (Speed Digester K-425) with a scrub-

ber (Scrubber K-415), according to the manufacturer's instructions. Digestion was performed using concentrated sulfuric acid (15 mL) and a Kjeltab C mixed catalyst digestion tablet (Actac Inc, Tokyo, Japan). The digested samples were distilled after reaction with 75 mL of 33% (w/w) NaOH. The distillate was collected in 60 mL of 2% (w/v) boric acid and titrated using sulfuric acid (0.05 mol/L). The endpoint of the titration was determined with a pH indicator. The protein content of each sample was obtained by multiplying the total nitrogen content by 6.25 (the nitrogen-to-protein conversion factor). PROTCAA was calculated as the sum of the individual amino acid residues (the molecular weight of each amino acid minus the molecular weight of water) (8).

Hydrolysis for amino acid analysis. Proteins need to be completely hydrolyzed to single amino acids prior to amino acid analysis. However, there is no single hydrolysis method that completely cleaves all proteins to single amino acids without changing their chemical structures (10). Therefore, we hydrolyzed the protein using three different methods, according to the official compendium of methods described for the AACT 2015, with slight modifications. Asparagine and glutamine were not individually quantified, because they are converted to aspartic acid and glutamic acid, respectively, during hydrolysis. Hence, we assayed 18 amino acids using three different HPLC methods, depending on the hydrolysis conditions. Amino acids have been symbolized by three letter codes. In addition, we use the abbreviation (Cys)₂ for cystine. Asp, Thr, Ser, Glu, Pro, Gly, Ala, Val, Met, Ile, Leu, Tyr, Phe, His, Lys, and Arg were analyzed after acidic hydrolysis with 6 M HCl for 24 h at 110°C (11). (Cys)₂ was oxidized with performic acid before hydrolysis, as it is partially destroyed in acidic conditions (12). Trp was analyzed after alkali hydrolysis with 5 M barium hydroxide for 12 h at 110°C (13).

Chromatography. Amino acid analysis was performed using an HPLC system (Shimadzu) equipped with a quaternary low-pressure gradient unit (FCV-10AL), three pumps (LC-10AD vp), two degassers (DGU-14A), an autosampler (SIL-10AD vp), a column oven (CTO-10A vp), a fluorescence detector (RF-10A XL) and a system controller (SCL-10A vp). Amino acids in acidic hydrolysates with or without performic acid oxidation were measured by the post-column fluorometric detection of their *o*-phthalaldehyde derivatives with a Shimpack Amino-Na ion exchange column (Shimadzu), and SC-30/S0504Na ammonium trap column (Shimadzu) according to the manufacturer's instructions. Trp, in a basic hydrolysate, was measured by fluorometric detection on an Intersil ODS-2 250–4.6 5 μ m column (GL Science, Tokyo, Japan) according to the official compendium method described with the AACT 2015.

Nutritional value calculations. Recipes for each meal and Excel Eiyoukun version 8 nutrition calculation software (Kenpakusya, Tokyo, Japan) were used to calculate the amino acid and protein content in the meals. This software uses the AACT 2015.

Statistical analysis. All results are expressed as the

Fig. 1. Amino acid analysis of the hydrolysates of two reference samples, (A) bovine serum albumin (BSA) and (B) fat-free milk. The theoretical values were calculated using the amino acid sequence of BSA. The listed value of fat-free milk is from the amino acid composition table 2015. Bars represent the mean amino acid contents. Error bars represent the standard deviation among triplicate analytical values.

mean $\pm$ standard deviation. The Pearson correlation coefficient measures the strength and direction of the linear relationship between two variables (x and y). All statistical analyses were performed with SPSS for Windows, version 24 (Nippon IBM, Tokyo, Japan).

RESULTS AND DISCUSSION

Amino acid analysis validation

To confirm the accuracy of amino acid analysis, we compared the analytical values with theoretical and listed ones. The amount of amino acids in 100 g of BSA (amino acid sequence known) was calculated and shown as a theoretical value in Fig. 1A.

The listed value of fat-free milk was derived from the AACT 2015 (Fig. 1B). When these values were compared with the analytical ones, no remarkable difference was observed. In addition, the precision of our amino acid analysis was estimated by the coefficient of variation (COV). The COVs of analytical values were all $<12\%$.

Evaluation of the validity of pretreatment method for amino acid analysis

Pretreatment methods for the analysis of amino acid content are not provided in the official compendium methods with the AACT. Hence, delipidated and finely milled samples were prepared according to previously reported procedures (14) with minor modifications. To evaluate the validity of a pretreatment method for chemical analysis, fat-free milk containing a known amount of amino acids was spiked into additive-free samples (Fig. 2). The spiked- and recovered-amounts of amino acid were strongly correlated ($R=0.989$). These results suggest that our pretreatment method was appropriate for a meal to achieve the purpose of this study, although

Fig. 2. Spike and recovery experiment of amino acid analysis. Fat-free milk containing known amounts of amino acids was spiked into additive-free samples. Recovered amounts were calculated as the average of spiked samples (meals, $n=3$) minus those of additive-free samples (meals, $n=3$). Bars represent the mean amino acid amount. Error bars represent the standard deviation among triplicate analytical values of spiked samples.

Fig. 3. Amino acid analysis of 14 consecutive days' meals provided in an elder care facility. Percent estimated values were calculated using the amino acid composition table 2015. (A) Each value represents the amount of amino acids in 14 consecutive days' meals (average per day). Bars represent the mean amino acid contents. Error bars represent the standard deviation of daily variations ($n=14$). (B) Each datapoint represents the 14 consecutive days' meals (average per day). The graph of the direct variation equation is a straight line through the origin. (C) Each datapoint represents the amount of amino acids in each meal. The graph of the direct variation equation is a straight line through the origin.

the results did not indicate that it can be applied to all the foods listed in AACT 2015. In the case of amino acid analysis of walnut, which has a high fat content, 10 times as much hexane was added as the sample (15). Thus, the pretreatment method for amino acid analysis will vary depending on the characteristics of each food. *Evaluation of the validity of estimated dietary amino acid intake using the AACT 2015*

The main objective of this study was to examine the

Fig. 4. Relationship between protein content calculated as reference nitrogen multiplied by a nitrogen to protein conversion factor (PROTRN) and tryptophan (Trp) content. (A) Scatter diagram based on the listed values in the amino acid composition table 2015. (B and C) Scatter diagram based on the direct analytical values of 14 days' meals. The direct variation equation is a straight line through the origin. AVO-PROTRN: analytical value of PROTRN.

Fig. 5. Relationship between protein content calculated as reference nitrogen multiplied by a nitrogen to protein conversion factor (PROTRN) and protein content calculated as the sum of amino acid residues (PROTCAA). (A) Scatter diagram based on the listed values in the amino acid composition table 2015. (B and C) Scatter diagram based on the direct analytical values of 14 d' meals. The direct variation equation is a straight line through the origin. AVO-PROTRN: analytical value of PROTRN, AVO-PROTCAA: analytical value of PROTCAA.

differences between the estimated values in the AACT 2015 and directly analyzed values from amino acid analysis. Calculated values cannot evaluate the influence of factors such as cooking-mediated loss and substitution errors. In this study, we used 14 consecutive days' meals provided in an elder care facility as samples for amino acid analysis and compared the analytical values to the estimated values in the AACT 2015 (Fig. 3A). The REPs of each amino acid were within 15%, and the MAPE was 8.3% (Fig. 3A and 3B). These results indicate that the AACT 2015 allows the estimation of habitual amino acid intake. The relationship between analytical and estimated values was examined with a scatter plot (14 d, 18 amino acids/d; Fig. 3B and 3C). Scatter plots help visualize errors between analytical and estimated values.

The AACT is necessary to examine associations between dietary amino acid intake and physical conditions. Suga et al. established an amino acid database and estimated the amino acid intake of Japanese adults using a 16-d diet record. This database greatly contributed to the development of the AACT 2015. They suggested that the influence of excluding foods with missing amino acid values was relatively small; however, they were unable to compensate for losses during food preparation and cooking (16). Some cooked foods in the AACT 2015 were calculated using raw ingredient values, without consideration of cooking-mediated loss. On the other hand, the food composition table 2015 expresses the nutrient values of food components with adjustments for cooking-related loss. Ismail et al. reported that

the amino acid composition of eggs was changed after cooking (14). However, little is known about the general influence of cooking on the amino acid composition. In this study, there were no major differences between the analytical and estimated amino acid values over the 14 d. These results indicate that the influence of errors such as cooking-mediated loss, missing values, and substituted values will be relatively small. In contrast, the slope of the scatter plot was slightly lower than 1 (Fig. 3B and 3C). It is also suggested that these deviations (about 5%) could be errors due to the listed values of AACT 2015.

Trp is converted to niacin in the human body. Hence, to assess niacin nutritional status, it is important to know how much niacin is converted from our diets. The Dietary Reference of Intakes for Japanese (DRIs-J) is the dietary guidance which applied an evidence-based approach utilizing a systematic review process (17). In this report (DRI-J), it is estimated that 60 mg of Trp is converted to 1 mg of niacin.

The amount of Trp is estimated to be approximately 1% (w/w) of the PROTRN value in the DRIs-J, though this has not been proven because of the lack of a validated AACT. Hence, we examined the relationship between Trp content and PROTRN values using a scatter plot and a direct variation equation through the origin.

As shown in Fig. 4A, the correction factor to convert PROTRN values to Trp amounts was 0.0117 for all items listed in the AACT 2015. However, these results did not reflect weighting values such as general amounts and frequencies of foods in Japanese diets. Conversely,

we determined a correction factor (0.0102, Fig. 4B; 0.0099, Fig. 4C) which were calculated from the direct analysis values of 14 d of meals, reflecting not only the weighting value but also error factors such as cooking-mediated loss, missing values, and substitution values. This is the first study to elucidate the validity of the correction factor (0.01) in the DRIs-J 2015 to convert PROTRN to Trp by direct amino acid analysis.

Relationship between protein content calculated by PROTRN and PROTCAA

The FAO and WHO recommended the use of PROTCAA; however PROTRN is generally used because of the lack of a validated AACT. The correction factor to convert PROTRN to PROTCAA was 0.849, for all items listed in the AACT 2015 (Fig. 5A). However, this result did not reflect the general amounts and frequencies of foods in Japanese diets.

In this study, we examined the relationship between the analytical value of PROTRN (AVO-PROTRN) and AVO-PROTCAA through scatter plots and direct variation equations using Origin. We calculated a correction factor (0.920, Fig. 5B; 0.889, Fig. 5C) from the direct analysis values of 14 d meals, reflecting not only the weighted values, but also error factors such as cooking-mediated loss, missing values, and substitution values. Although we used analytical values as gold standards, the problem remains that it is not clear whether the pre-treatment method is appropriate for all foods. As these values were calculated based on meals fed in an elder care facility, sampling bias may occur when we apply this factor to the general Japanese population. To establish a more precise correction factor, further investigation, using a wide range of ages, would be needed. This will allow the adaptation of the PROTCAA method, and the conversion of PROTRN values to PROTCAA when referring to past reports and data.

CONCLUSION

This is the first study to show the validity of dietary amino acid intake estimation using the Japanese AACT 2015. We found that although the AACT 2015 may include relatively small errors such as cooking-mediated loss, missing values, and substituted values, it allows the estimation of habitual amino acid intake. In addition, we have determined a correction factor (0.896) to convert PROTRN values to PROTCAA values, and elucidate the validity of the correction factor (0.01) in the DRIs-J 2015 to convert PROTRN values to those corresponding to Trp. Our study lays a foundation for the examination of amino acid requirements under individual conditions, and the association between dietary amino acid intake and physical conditions.

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