The Journal of Nutrition
Nutrient Requirements and Optimal Nutrition

Protein Requirements of Elderly Chinese Adults Are Higher than Current Recommendations

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ABSTRACT

Background: Due to a lack of research data on the protein requirements of the elderly in China, the estimated average requirement (EAR) and the recommended nutrient intake (RNI) of protein in the elderly remain the same as those in young and middle-aged people at 0.98 g/(kg·d).

Objective: The objective of this study was to determine the protein requirements of healthy Chinese adults >65y old through use of the indicator amino acid oxidation (IAAO) method.

Methods: Seven healthy adult men and 7 healthy adult women participated in the study, with protein intakes ranging from 0.3 to 1.8 g/(kg·d). The diets were isocaloric and provided energy at a 1.5 resting energy expenditure. Protein was given based on the lactalbumin. Phenylalanine and tyrosine were added to protein doses of 0.3–1.5 g/kg according to the highest dose of protein content [1.8 g/(kg·d)]. Phenylalanine and tyrosine concentrations were kept constant at each protein dose. The mean protein requirement was determined by applying a nonlinear mixed-effects model analysis to the $F^{13}CO_2$, which identified a breakpoint in $F^{13}CO_2$ in response to graded amounts of protein. This trial was registered with the Chinese clinical trial registry as ChiCTR-BOC-17010930.

Results: Protein EAR and RNI for healthy elderly Chinese adults were determined to be 0.91 and 1.17 g/(kg·d), respectively, based on the indicator amino acid oxidation technique.

Conclusions: The estimates of protein requirements for Chinese adults >65 y in the present study are 3.4% and 19.4% higher than the current estimated requirements, 0.88 g/(kg·d) for EAR and 0.98 g/(kg·d) for RNI. *J Nutr* 2020;150:1208–1213.

Keywords: protein requirement, indicator amino acid oxidation, older adults, stable isotope, phenylalanine oxidation

Introduction

China has an ageing society. According to population data released by the National Bureau of Statistics, by the end of 2016, the number of elderly people aged 60 and over in China was 231 million, accounting for 16.7% of the total population. China is the only country in the world with an elderly population of over 200 million, which is expected to exceed 300 million in 2025 and 400 million in 2033. It will peak at 487 million in 2053, accounting for a quarter of the world's elderly population (1). At present, China is not only the country with the largest number of elderly people in the world, but also one of the countries with the most rapid increase in the number of elderly people. With increasing age, obvious changes occur in the bodies of most elderly people. Whether there are differences in protein requirements between elderly and young people has always been one of the important issues in the field of human nutrition. In 2010, the Chinese Nutrition Society began the revision of Dietary Reference Intakes (DRIs) for Chinese residents. Due to a lack of domestic research data on the protein requirements of the elderly and limited up-to-date research abroad, protein EARs and RNIs in elderly people remain the same as those in young and middle-aged people, i.e., 0.88 g/(kg·d) for EAR and 0.98 g/(kg·d) for RNI (2).

Indicator amino acid oxidation (IAAO), which was initially used to study amino acid requirements, is a robust method for calculating protein requirements by measuring changes in the oxidation of a labeled amino acid (3–5). In recent years, the use

Supported by the Danone Diet Nutrition Research & Communication Grant Evaluation of the Protein Requirement in Old Adults.
Author disclosures: The authors report no conflicts of interest.
DM and FC share first authorship.
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Abbreviations used: DRI, Dietary Reference Intake; EAR, estimated average requirement; IAAO, indicator amino acid oxidation; RNI, recommended nutrient intake; REE, resting energy expenditure.

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Manuscript received September 29, 2019. Initial review completed November 27, 2019. Revision accepted January 30, 2020.
First published online March 6, 2020; doi: https://doi.org/10.1093/jn/nxaa031.

of IAAO to study the protein requirements of adults, children, the elderly, and pregnant women has gradually increased (6–11). To determine the protein requirements of the elderly in China, the IAAO technique was applied to Chinese adults >65 y.

Materials and Methods

Subjects

Twenty elderly adult volunteers, 10 men and 10 women, were recruited for the present study. All subjects first underwent a routine medical examination. Eventually, 7 men and 7 women were selected who met the requirements for study participation. The inclusion criteria of subjects included were age 65–80 y; BMI (kg/m^2) 18.5–30; no weight loss in the previous 6 mo; no chronic disease or acute illness known to influence protein and amino acid metabolism such as diabetes, kidney disease, liver disease or cancer; no gastrointestinal diseases; and no recent infectious diseases, surgery, or antibiotic therapy within 8 wk of the commencement of this study. Participants were also required to have normal results in the routine medical examination, including complete blood count, blood chemistry, and hepatic and renal functions. The present study was conducted according to the guidelines in the Declaration of Helsinki and was approved by the Ethical Committee of the Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention. Each subject provided signed informed consent prior to the initiation of the study. This trial was registered at the Chinese clinical trial registry as ChiCTR-BOC-17010930.

Experimental design

The experimental design was based on the minimally invasive IAAO model used to determine protein requirements (7, 8). Each subject consumed 6 different protein intake amounts [0.3, 0.6, 0.9, 1.2, 1.5, and 1.8 g/(kg·d)], with each protein dose lasting 3 d, with a 1-wk period between doses. The first 2 d of the study were the adaptation days, during which the subjects received a daily 1.0 g/kg body weight protein intake as part of a standard Chinese diet, and 1.7 resting energy expenditure (REE). REE was measured by continuous, open-circuit indirect calorimetry (Cortex metamax3B). The daily 1.0 g/(kg·d) protein intake (35%, 35%, and 30% of protein consumed at breakfast, lunch, and dinner, respectively) was calculated for each subject according to body weight. Each meal contained 1 staple food (e.g., rice, steamed roll, or steamed bread), 1 high-quality protein food (such as pork, chicken, egg, or tofu), 1 vegetable, and 1 fruit (except at breakfast). Each component food of the meal was cooked individually. On the first 2 d of each protein dose, the dietary types were the same and subjects were required to consume all foods. Before and after taking the meal, each food was weighed and recorded in order to determine actual intake per food. The types and amounts of consumed foods were collected for all subjects and tested for the concentration of main macronutrients (including protein, fat, and carbohydrate) and energy, in order to ensure that the actual protein intakes of the subjects were close to 1.0 g/(kg·d).

Day 3 was the oxidation day of the study. All subjects consumed 8 hourly isocaloric meals, and the energy was provided at 1.5 × REE. The experimental diet consisted of the following: lactalbumin powder, protein-free biscuits, protein-free and fried starch slices, and protein-free lotus root starch. The study diet provided 33% energy as fat and variable energy from carbohydrate (38.5%–62.9%) and protein (3.0%–27.6%) according to the test protein intake. Each protein dose provided the same amounts of phenylalanine and tyrosine, which was ensured by determination of the amino acid composition of lactalbumin powder according to the Chinese standard GB 5009.124-2003. L-Phenylalanine (Brainstorm Nootropics) and L-tyrosine (Puritan's Pride) were added to protein doses of 0.3–1.5 g/kg according to the highest dose of daily protein content (1.8 g/kg). The quantity of phenylalanine supplied was 62.8 mg/(kg·d). Tyrosine was provided at 61.4 mg/(kg·d) to ensure an excess of tyrosine. Amino acid compositions of selected test protein intakes are provided in **Table 1**.

Tracer protocol

For each oxidation study day at each protein dose, the subjects consumed hourly meals for 4 h before the start of the oral tracer infusion protocol. Priming doses of 0.176 mg $NaH^{13}CO_3$/kg (99 atom percentage excess; Cambridge Isotope Laboratories) and 0.66 mg L-[1-^{13}C] phenylalanine/kg (99 atom percentage excess; Cambridge Isotope Laboratories) were ingested in the fifth hourly meal. An hourly oral dose of 1.2 mg/(kg·h) of L-[1-^{13}C] phenylalanine was commenced simultaneously (with the fifth meal) and continued for the remaining 3 h of the study.

Sample collection and analysis

Before the participants consumed the fifth hourly meal containing the indicator amino acid, 3 baseline breath samples (45, 30, and 15 min before) and 2 baseline urine samples (45 and 15 min before) were obtained. Four plateau breath and 4 plateau urine samples were collected every 30 min beginning 2.5 h after the fifth hourly meal.

Breath samples were collected in disposable expiratory bags (Beijing Famaruichi Technology Company) and stored at room temperature for further analysis. Urine samples were stored at −80°C until analysis. The enrichment of $^{13}CO_2$ in breath samples was analyzed using a ^{13}C breath analyzer (Heliview, Medichems). L-[1-^{13}C] phenylalanine enrichment in urine samples was measured using a TSQ triple quadrupole mass spectrometer (Thermo Fisher Scientific) in positive electrospray ionization mode, as described previously (12). Isotopic enrichment was expressed as mole percentage excess (MPE) and was calculated from peak area ratios at the isotopic steady state at plateau and at baseline.

Tracer kinetics

Isotope kinetics were estimated as previously described (6, 13, 14). The $^{13}CO_2$ rate in breath [$F^{13}CO_2$, μmol/(kg·h)] after the oxidation of ingested L-[1-^{13}C] phenylalanine was calculated by using the following equation:

$$F^{13}CO_2 = [(FCO_2)(ECO_2)(44.6)(60)]/[(W)(100)(0.82)] \quad (1)$$

where FCO_2 is the rate of carbon dioxide production (mL/min), ECO_2 is the $^{13}CO_2$ enrichment in expired breath, and W is body weight (kg). Factors of 44.6 (μmol/mL) and 60 (min/h) were used to convert FCO_2 to micromoles per hour; 0.82 is the factor to account for the fractional retention of $^{13}CO_2$ in the bicarbonate pool, and the factor 100 was used to convert the atom percentage excess to a fraction.Whole-body phenylalanine flux was calculated from the dilution of orally administered L-[1-^{13}C] phenylalanine into the plasma pool by using enrichment of L-[1-^{13}C] phenylalanine in urine, using the following equation:

$$Q = i\,[E_i/E_u - 1] \quad (2)$$

TABLE 1 Amino acid composition of reference protein and various test protein doses

	Reference protein,[1] mg/g	0.3 g protein/kg, mg/0.3 g	0.6 g protein/kg, mg/0.6 g	0.9 g protein/kg, mg/0.9 g	1.2 g protein/kg, mg/1.2 g	1.5 g protein/kg, mg/1.5 g	1.8 g protein/kg, mg/1.8 g
L-Alanine	59.7	17.9	35.8	53.8	71.7	89.6	108
L-Arginine	25.8	7.7	15.5	23.2	30.9	38.7	46.4
L-Aspartic acid	124	37.1	74.1	111	148	185	222
L-Cysteine	23.5	7.1	14.1	21.1	28.2	35.2	42.3
L-Glutamic acid	202	60.7	122	182	243	304	364
L-Glycine	20.3	6.1	12.2	18.2	24.3	30.4	36.5
L-Histidine	18.9	5.7	11.4	17.0	22.7	28.4	34.1
L-Isoleucine	66.3	19.9	39.8	59.7	79.6	99.5	119
L-Leucine	123	37.0	73.9	111	148	185	222
L-Lysine	101	30.3	60.6	90.9	121	151	182
L-Methionine	23.2	7.0	13.9	20.9	27.9	34.8	41.8
L-Valine	62.1	18.6	37.3	55.9	74.6	93.2	112
L-Proline	62.3	18.7	37.4	56.1	74.7	93.4	112
L-Serine	58.1	17.4	34.9	52.3	69.7	87.2	105
L-Threonine	83.6	25.1	50.2	75.3	100	125	151
L-Tryptophan	16.5	5.0	9.9	14.9	19.8	24.8	29.7
L-Phenylalanine[2]	34.9	10.5	20.9	31.4	41.9	52.4	62.8
Added L-phenylalanine[2]	—	52.4	41.9	31.4	20.9	10.5	0.0
L-Tyrosine[3]	34.1	10.2	20.5	30.7	40.9	51.1	61.4
Added L-tyrosine[3]	—	51.1	40.9	30.7	20.5	10.2	0.0

[1] Represents lactalbumin composition.
[2] L-Phenylalanine intake was kept constant at 62.8 mg/(kg·d).
[3] L-Tyrosine intake was kept constant at 61.4 mg/(kg·d).

Where Q is phenylalanine flux [μmol/(kg·h)]; i is the rate of L-[1-^{13}C] phenylalanine infused [μmol/(kg·h)]; and E_i and E_u denote the enrichment of L-[1-^{13}C] phenylalanine (MPE) in an orally administered state and in urine, respectively. Phenylalanine oxidation rate [*O*, μmol/(kg·h)] was calculated as follows:

$$O = F^{13}CO_2\,(1/E_u - 1/E_i) \times 100 \quad (3)$$

Statistical analysis

Study results are expressed as means ± SDs. ANOVA was used to test for differences in the body weight and phenylalanine flux and if there was a general difference, a post hoc analysis was performed by using the Student Newman–Keuls test for multiple comparisons. The oxidation rate of phenylalanine followed a nonnormal distribution, so a logarithm conversion was conducted before the ANOVA and Student Newman–Keuls analysis. The mean protein requirement was estimated by applying a nonlinear mixed-effects model (PROC NLMIXED, SAS Institute) to the $F^{13}CO_2$ data, described by Tang et al (6), as follows:

$$y_{ij} = b_0 + b_1\,(x_{ij} - b_{2ij}) + e_{ij},\ \text{when } x_{ij} < b_{2ij} \quad (4)$$

$$y_{ij} = b_0 + e_{ij},\ \text{when } x_{ij} \geq b_{2ij}. \quad (5)$$

Where x_{ij} is the protein intake for subject i at protein dose j; y_{ij} is the $F^{13}CO_2$ for subject i at protein dose j; b_1 is the slope of the linear part of the model; b_{2ij} is the breakpoint of the piecewise linear function; and b_0 is the intercept. The first-order method was used for approximating the integral of the likelihood over random effects.All statistical analyses were performed using the Statistical Analysis Systems 9.4 software. A value of $P < 0.05$ was considered statistically significant.

Results

Subject characteristics

The cohort characteristics are displayed in Table 2. Each subject was randomly assigned to receive a concentration of 0.3, 0.6, 0.9, 1.2, 1.5, and 1.8 g protein/kg body weight, for which 2 subjects were missing the 0.9 and 1.5 g/kg doses. At the different dietary protein doses, the subjects' body weights (Table 3) were not significantly different.

Dietary nutrient intakes

The diets in which the different protein doses were administered and were consistent during the 2 d before the tracer protocol was started, and the designed protein doses were maintained at 1.0 g/(kg·d). The actual main macronutrient and energy intakes of the subjects for the 6 dietary protein doses were 1.11 ± 0.112 g/(kg·d) for protein, 0.943 ± 0.167 g/(kg·d) for fat, 4.06 ± 0.450 g/(kg·d) for carbohydrate, and 29.4 ± 3.53 kcal/(kg·d) for energy.

Breath $^{13}CO_2$ excretion

The influence of protein intake on production of $^{13}CO_2$ from phenylalanine oxidation ($F^{13}CO_2$) in older adults is displayed in Figure 1. $F^{13}CO_2$ decreased as protein intake increased, consistent with an increase of L-[1-^{13}C] phenylalanine

TABLE 2 Characteristics of the elderly Chinese adults administered different protein doses[1]

	Subject sex	
Study cohort[1]	Male (*n* = 7)	Female (*n* = 7)
Age, y	70.9 ± 5.76	73.1 ± 4.95
Weight, kg	66.7 ± 12.1	57.8 ± 10.2
Height, cm	164.3 ± 6.58	151.1 ± 6.38
BMI, kg/m^2	24.7 ± 3.87	25.2 ± 2.85
REE, kcal/d	1400 ± 141	1164 ± 221

[1] Results are expressed as means ± SDs. REE, resting energy expenditure.

TABLE 3 Body weight of elderly Chinese adults administered different protein doses in 6 wk[1]

	Body weight at time of dose, kg					
	1 wk	2 wk	3 wk	4 wk	5 wk	6wk
Females	60.3 ± 11.1	59.4 ± 10.6	57.9 ± 10.8	57.7 ± 10.0	57.9 ± 10.3	57.9 ± 10.4
Males	68.8 ± 13.4	67.5 ± 12.9	66.3 ± 11.3	66.9 ± 11.8	66.6 ± 12.3	66.1 ± 12.5
All subjects	63.7 ± 12.5	62.6 ± 12.0	61.0 ± 11.2	61.1 ± 11.1	61.2 ± 11.6	61.0 ± 11.7

[1] Results are expressed as means ± SDs of body weights of 5 female and 7 male subjects for week 1 and week 2 and 7 female and 7 male subjects for weeks 3–6.There was no significant difference in subject body weights.

incorporation into the protein. This increase in incorporation of labeled phenylalanine continued until the protein intake reached the protein requirement and there was no further incorporation of labeled phenylalanine into the protein. The mean protein requirement (the upper limit of the 95% CI for the mean protein requirement) was 0.91 (95% CI: 0.76, 1.06) g/(kg·d), and the recommended intake was 1.17 (95% CI: 0.89, 1.45) g/(kg·d).

Phenylalanine Flux and Oxidation

Phenylalanine flux was not affected by differences in protein intake as required by the IAAO method (Table 4). The oxidation rate of phenylalanine decreased as the protein dose increased. The phenylalanine oxidation rate at the protein intake dose of 0.3 g/(kg·d) was significantly higher than that at other protein doses. The oxidation rate of phenylalanine at the protein intake dose of 0.6 g/(kg·d) was significantly higher than that at the protein intake doses of 1.5 g/(kg·d) and 1.8 g/(kg·d).

Discussion

With increasing age, the main changes that occur to the bodies of elderly people include the following: *1*) gradual decrease in lean body mass and relative increase in adipose tissue; *2*) declines in digestive organs and corresponding digestive functions, including chewing, digestion, absorption, and excretion; *3*) impairment of liver and kidney function, which is connected with the impairment of digestive organs; *4*) decrease in secretions from some endocrine glands and decreases in androgen and estrogen in male and female tissues, which affects catabolism and anabolism; and *5*) increased urinary calcium excretion in elderly women to a level significantly higher than that in middle-aged women. These changes are directly or indirectly related to the supply of protein.

Due to limited direct research on the protein requirements of elderly people, most countries have the same EAR and RNI for the elderly as for middle-aged and young people. In Australia and New Zealand, however, EAR and RNI are 25% higher for people >70 y old than for middle-aged and young people (15). Many scientific methods have been proposed to evaluate suitable protein requirements in humans, such as the nitrogen balance method, predictions from obligatory nitrogen loss, carbon balance, and the IAAO method. Of these, the nitrogen balance method is most commonly used to evaluate human protein requirements, and this approach

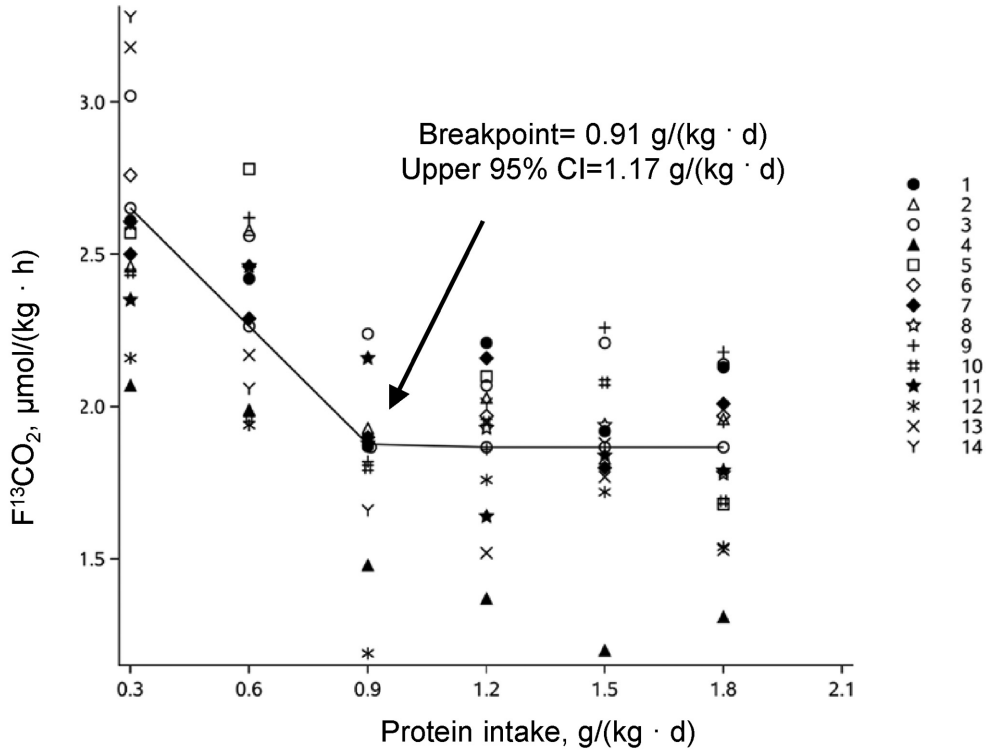

FIGURE 1 The relation between protein intake and breath $F^{13}CO_2$ production in elderly Chinese adults administered protein at 6 different doses. The data points represent the $^{13}CO_2$ excretion of individual subjects at each protein intake dose. The breakpoint represents the estimated mean protein requirement, which is 0.91 g/(kg·d), and the upper 95% CI is 1.17 g/(kg·d).

TABLE 4 Phenylalanine flux and oxidation in elderly Chinese adults administered different protein doses[1]

Protein doses, g/(kg·d)	*n*	Phenylalanine flux, μmol/(kg·h)	Phenylalanine oxidation, μmol/(kg·h)
0.3	14	20.9 ± 5.07	5.18 ± 1.53[a]
0.6	14	19.5 ± 8.24	4.12 ± 1.44[b]
0.9	12	20.9 ± 8.02	3.44 ± 1.08
1.2	14	19.0 ± 7.12	3.27 ± 0.918
1.5	12	17.1 ± 5.23	2.92 ± 0.768
1.8	14	17.7 ± 8.16	2.92 ± 0.934

[1]Results are expressed as means ± SDs. Phenylalanine flux was not affected by differences in protein intake. There were significant differences for the oxidation rates of phenylalanine with different protein doses. [a]The phenylalanine oxidation rate for the protein intake dose of 0.3 g/(kg·d) was significantly higher than that for other protein doses. [b]The phenylalanine oxidation rate at the protein intake dose of 0.6 g/(kg·d) was significantly higher than that at the protein intake doses of 1.5 and 1.8 g/(kg·d).

has been adopted in most countries. However, this method is cumbersome and reflects the balance between nitrogen intake and nitrogen excretion, making accurate assessment of human protein requirements difficult. Inadequacies result mainly from practical aspects related to the difficulties of making the appropriate measurements with sufficient accuracy and to the interpretation of the results. The IAAO method has been the most commonly used technique to study human protein requirements in recent years. In 2014, a study about the protein requirements of 6 elderly women aged 80–87 y showed that their EAR and RNI were 0.85 g/(kg·d) and 1.15 g/(kg·d), respectively (6). In 2015 and 2016, there were 2 further research papers on the protein requirements of 12 women and 6 men over 65 y of age (7, 8). For women over 65 y of age, protein EAR and RNI were 0.96 g/(kg·d) and 1.29 g/(kg·d) (7), respectively, while for men protein EAR and RNI were 0.94 g/(kg·d) and 1.24 g/(kg·d) (8), respectively. These studies suggest that the current recommendations may underestimate the protein requirements of elderly adults, based on nitrogen balance data (6–8). In the present study, the protein requirements of elderly Chinese citizens were lower than those of the studies mentioned above (7, 8), with an EAR and RNI of 0.91 g/(kg·d) and 1.17 g/(kg·d), respectively. In the IAAO method, egg protein is always used as the reference protein, whereas in the present study, we took market lactalbumin to be the main source of protein. The phenylalanine and tyrosine intake amounts were kept constant at 62.8 mg/(kg·d) and 61.4 mg/(kg·d), respectively, higher than in the studies previously mentioned (6–8). However, the breakpoint for elderly Chinese people was lower than for the elderly populations of other countries (6–8). Phenylalanine could be used as a suitable indicator at different protein doses when its intake exceeds the requirements of the body.

The current protein EAR and RNI values for elderly Chinese men are 0.88 g/(kg·d) and 0.98 g/(kg·d), respectively. Based on the weight representation of adult men and women aged 18–50 y, a daily protein RNI of 65 g for men and 55 g for women can be estimated. Based on the present study, protein RNI of 76 g and 64 g is recommended for men and women, respectively, which is almost 16% higher than current estimates. On the basis of the findings of the present study, we think that older people may need more protein to prevent ailments associated with declines in protein-related body funtions, such as sarcopenia. Sarcopenia is a common symptom in the elderly characterized by progressive muscle mass loss, reduced muscle strength, and reduced body activity. Such cases of sarcopenia are caused by multiple factors, of which diet and nutrition are a very important aspect, especially protein nutrition status. It has been reported that the overall incidence of sarcopenia in elderly Chinese people >60 y of age in urban and rural China is 9.8%, of which 6.7% are men, 12.0% are women, 13.1% represent rural older adults, and 7.0% represent urban adults (16). The decline of muscle function may begin at the age of 35 y (17), with an annual rate of decline of 1% to 2%. The rate of decline accelerates after the age of 50 y (18), and reaches a peak at the age of 75 y (19). Current studies suggest that an increase in protein intake, such as 25–30 g/d of high-quality protein per meal, and that protein intakes of 1.2 and 2.0 g/(kg·d) are conducive to muscle protein synthesis; improve muscle quality, quantity, and function (20–22); and actively prevent and improve sarcopenia in the elderly. In this regard, we suggest a higher protein intake for the elderly to reduce the occurrence of sarcopenia.

The IAAO method has many limitations, mainly concerning the dietary adaptation period. The IAAO method involves an 8-h amino acid oxidation metabolism experiment after the 2-d dietary maintenance period, although some researchers think that the adaptation period of 6–7 d is not needed before measuring the amino acid oxidation. That is, the IAAO method is not affected by the adaptation period (23, 24). However, other investigators think that during a stable period of intake, complicated changes occur in the rate of amino acid oxidation due to the amount of amino acid ingested, and that the absence of an adaptation period may lead to underestimation or overestimation of the minimum protein demand. However, the length of the adaptation period and whether or not a longer adaptation period will increase the difficulty of project implementation is still not known. Another limitation of the IAAO method is that the estimations of EAR and RNI have a large CI, with the CI of the estimated protein requirements overlapping with those of the current EAR and RNI. In our opinion individual variation is the main reason, especially with regard to elderly subjects.

Despite these disadvantages, the safety of the IAAO method enables study of the protein and amino acid requirements of different populations. Furthermore, changes in the amounts of $F^{13}CO_2$ can reflect the oxidation of labeled amino acids, and exhaled breath can easily be collected, which can greatly improve the subjects' compliance. Lastly, multilevel short-term tracer studies can be implemented in the same study subjects.

We used the term 'phenylalanine flux' for measurement by the administration of oral tracers, as many researchers have reported (6–11). There may be controversy over the appropriate utilization of this expression in the present study, because we caculated the phenylalanine flux according to the urine isotopic abundance. The tracer should be introduced in the compartment that you are going to sample when using the isotopic dilution approach. Regarding this issue, the use of this expression should be standardized.

A limitation of this study is that we didn't analyze the amount of D-^{13}C-phenylalanine in urine. The amino acids of the human body are almost all L-amino acids. and the L-^{13}C-phenylalanine (99%) was artificially added. Darling et al. reported that the presence of D-^{13}C-phenylalanine is ≥0.4% for infants (25). As a contaminant, D-^{13}C-phenylalanine could result in an overestimate for tracer enrichment. However, Tomlinson et al. reported that a developmental effect was found with the use of the same phenylalanine tracer, with a reduction

in the overestimate in children compared with infants and no effect on enrichment in adults (26). In our study, we did not analyze the amount of urine D-^{13}C-phenylalanine, which mignt be one reason that the flux data values were lower than those reported by Rafii et al (7, 8). Other reasons such as reference protein chosen and regional and ethnic differences might also explain the lower phenylalanine flux in our study.

This is the first study, to our knowledge, to evaluate the protein requirements of the elderly in China using the IAAO method. According to the production rate of $^{13}CO_2$ in breath exhaled at different protein doses, the mean EAR of protein in elderly Chinese adults is 0.91 (95% CI: 0.76, 1.06) g/(kg·d), and the RNI is 1.17 (95% CI: 0.89, 1.45) g/(kg·d), which are 3.4% and 19.4% higher than current estimates, respectively. More studies with larger sample sizes are needed to evaluate the protein requirements for elderly adults in different parts of China.

Acknowledgments

The authors' responsibilities were as follows—DM and ML: designed the research; FC, DM, RW, PB, YZ, WZ, JC, LY, XY, and ML: conducted the research; DM and ML: performed the statistical analysis; ML: wrote the paper; DM, FC, and ML: had primary responsibility for the final content; and all authors: read and approved the final manuscript.

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