Examination of lysine requirement of healthy young male adults on a Chinese habitual diet by the modified indicator amino acid oxidation method

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Abstract

There is currently no reference for intake of lysine for Chinese people; therefore, the present study was conducted to determine the lysine requirement of Chinese young male adults on a habitual Chinese mixed diet based on the modified indicator amino acid oxidation method. Seven young men with a mean age of 23.7 ± 2.2 years that were healthy based on questionnaire, physical examinations and screening tests were evaluated. Subjects were evaluated over five consecutive 7 day periods, during which time they were administered decreasing amounts of lysine via the diet (65, 55, 45, 35, 25 mg·kg^{-1}·d^{-1}). Subjects were allowed to adapt from day 1 to 6 and the isotopes were measured on day 7 in each period. The subjects' body weights, body compositions and plasma proteins were also examined during the study. Amino acid kinetics were measured based on the indicator amino acid oxidation technique using the $^{13}CO_2$ release rate and phenylalanine oxidation rate to estimate lysine requirements. Body weights, body compositions, and plasma proteins of subjects did not change significantly relative to those at baseline. The mean and the upper 95% CI of lysine requirements of Chinese habitual diets were determined to be 58.41 and 70.09 mg·kg^{-1}·d^{-1}, respectively, based on the $^{13}CO_2$ release rate and 54.28 and 65.14 mg·kg^{-1}·d^{-1}, respectively, based on the phenylalanine oxidation rate.

Key Words: Lysine, dietary requirement, men, stable isotope

Introduction

Lysine is an indispensible amino acid in humans that cannot be synthesized by the body and must instead be acquired from the diet. Animal foods and bean products are rich in lysine, but cereals are not. Indeed, lysine is the first limiting amino acid in cereals such as wheat and rice, which are staple foods in China. More than 50% of dietary protein consumed in China is cereal protein, especially in undeveloped regions, where cereal protein accounts for 87% of the daily dietary protein intake [1]. The reference intake of protein for Chinese adults is 1.0-1.2 g·kg^{-1}·d^{-1} [2]. Although most people consume sufficient amounts of protein, the consumption of animal and soy protein is low, especially in rural areas [2]. This likely results in inadequate dietary lysine intake by some Chinese residents. However, no reference for lysine intake by Chinese residents has been developed to date, and few related studies have been conducted; therefore, it has not been possible to evaluate lysine intake by Chinese residents. In the present study, the lysine requirement of a small number of healthy young Chinese male adults was examined to determine the best method for investigation of the requirements of necessary amino acids of Chinese populations.

The current requirement for lysine (30 mg·kg^{-1}·d^{-1}) is derived from tracer studies conducted using the 24 h indicator amino acid balance method (IAAB) [3,4], during which the first 12 hours are fasting (overnight) and the second 12 hours are fed (small meals at half-hour intervals). This method is considered to provide the best stable isotope data currently available [5]. However, there are several drawbacks to this method. For example, the sophisticated facilities and staff required to perform the accurate balance studies make it difficult to ensure that sufficiently different intake levels are provided around the requirement level, which may yield inaccurate results [5]. When compared with the IAAB method, the indicator amino acid oxidation (IAAO) method is simpler and not reliant on high levels of precision during measurement of amino acid oxidation; thus, this method can include more than six intake levels around the requirement level [5]. This technique has been extensively deployed for determination of requirements of several indispensible amino acids [6]. However, there are some problems associated with this method. Specifically, there are no prior periods of adaptation to experimental diets, even though some studies have shown that adaptation influences the overall need for amino acids [7-9]. Additionally, the experimental diets in all studies are purified amino acids mixtures, which are absorbed more rapidly and completely than amino acids provided in

habitual mixed diets. Accordingly, the results cannot reflect the actual requirements of amino acids in the normal daily diet. In the present study, we employed a modified experimental design based on the IAAO method with 6 days of prior adaptation and Chinese habitual mixed diets as the experimental diets.

Subjects and Methods

Subjects

Seven adult male volunteers from Yangzhou University (age 23.7 ± 2.2 years, weight 62.7 ± 4.2 kg, height 1.73 ± 0.03 m) were recruited for participation in this study. Volunteers were considered to be eligible if they were found to be healthy on the basis of clinical history, which was determined using questionnaire of their activities of daily living, physical examination, and screening tests, including complete blood count, blood chemistry workup and hepatic and renal functions. Exclusion criteria were as follows: recent weight loss, unusual dietary practices, nutritional supplements, smoking or alcohol consumption, chronic disease, endocrine disorder, atypical sleeping or exercise schedules. The subjects' habitual intake of lysine was estimated to be 48 $mg \cdot kg^{-1} \cdot d^{-1}$ based on a continuous 24h-dietary recall for 3 days.

The purpose of the study and the potential risks involved were explained fully to each subject and written consent was obtained. Approval from the Ethical Review Committee, Tianjin Medical University was also obtained (20090309). The study protocol was approved by the Committee of the National Natural Science Foundation of China (NO. 30901191).

Study protocol

The study was carried out for five consecutive periods of 7 days each. There were five different lysine intakes (65, 55, 45, 35, 25 $mg \cdot kg^{-1} \cdot d^{-1}$) in all. Each subject received one intake of lysine every period with the intake level provided in descending order. Subjects were instructed to maintain a light physical activity level and were weighed and examined for body composition on the morning of the sixth day of each period to confirm weight maintenance and observe the effects of different lysine intake on body composition. The first six days of each period were adaptation days, and the seventh day was an isotope day, on which stable isotopes were administered to subjects and breath and blood samples were collected.

Diets

Diets during the adaptation periods were provided in the form of Chinese habitual mixed food with a three-day rotation of menus (including meat, eggs, fish, milk, soy products, vegetables, fruits, rice and wheat flour) (Table 1). The everyday lysine intake of each subject was calculated according to body weight and designated lysine level and was distributed into three meals with a ratio of $3:4:3$ throughout the day. The estimated energy requirement of each subject was calculated using equations for prediction of the basal metabolic rate of Chinese adults [10] based on the individual's age, sex, weight and height. Basal metabolic rate values were multiplied by an activity coefficient of 1.7 to obtain an appropriate energy intake [11]. The contents of lysine and other nutrients in foods were based on the China Food Composition for food quantity [12]. The dietary protein level was 1.16 $g \cdot kg^{-1} \cdot d^{-1}$ and protein from animal and soy products was controlled at 40% a day. A crystalline amino acid mixture that simulated the composition of egg essential amino acids (with L-alanine replacing the N content of lysine) was provided to subjects when dietary protein was insufficient. Crystalline L-Phenylalanine and L-Tyrosine were provided to subjects if

Table 1. Menu of the 5th week (25 $mg \cdot kg^{-1} \cdot d^{-1}$ of lysine intake) for subject No.1[1]

	Day 1	Day 2	Day 3
Breakfast (g)	Apple (161)	Pear (243)	Apple (265)
	Fried bread (179)	Fried bread (175)	Fried bread (181)
	Lotus root starch (40)	Lotus root starch (24)	Lotus root starch (24)
Lunch (g)	Fried pumpkin (151)	Fried lotus root (212)	Fried rapeseed (137)
	Braised tofu (79)	Fried spiced tofu (41)	Steamed egg custard (20)
	Steamed rice (352)	Steamed rice (352)	Steamed rice (353)
	Seaweed soup (unlimited)	Seaweed soup (unlimited)	Seaweed soup (unlimited)
Supper (g)	Fried lettuce (181)	Fried cucurbita maxima (155)	Fried potatoes (241)
	Roast pork balls (12)	Fried spiced tofu (18)	
	Steamed rice (352)	Steamed rice (353)	Steamed rice (352)
	White radish soup (unlimited)	White radish soup (unlimited)	White radish soup (unlimited)
Snacks	Potato starchy noodles (unlimited)	Potato starchy noodles (unlimited)	Potato starchy noodles (unlimited)
	Fruit drops (unlimited)	Fruit drops (unlimited)	Fruit drops (unlimited)
Phenylalanine (mg)	0	0	0
Tyrosine (mg)	1174.42	1149.00	1175.35
Crystalline mixed amino acids (g)	27.00	26.00	26.40

[1] The menu was a three-day rotation, so the menu of the 4th to 6th day was the same.

their diet did not contain adequate amounts of phenylalanine (15 mg·kg^{-1}·d^{-1}) and tyrosine (40 mg·kg^{-1}·d^{-1}) every day [13]. Subjects were allowed to eat starchy noodles and fruit drops if the meal did not meet their energy requirements. No other foods or beverages were consumed except low-protein fruits (apple and pear) and water.

Diets of the five isotopic days were the same (Chinese cabbage, pork and rice). The foods on the isotopic day were divided into seven isocaloric, isonitrogenous meals and consumed hourly to ensure a metabolic steady state during the fed condition.

All foods in each meal were weighed accurately and recorded both before and after consumption by each subject to determine the actual intake of each food. Samples of each food were analyzed for lysine, phenylalanine, tyrosine, total nitrogen, fat, carbohydrate, water and ash.

Isotope studies

The isotopic labeled tracers used in this study were NaH^{13}CO$_3$ with 99% enrichment and L-[1-^{13}C]-Phenylalanine with 99% enrichment (Cambridge Isotope Laboratories). Stock solutions of NaH^{13}CO$_3$ (2.0 g·L^{-1}) and L-[1-^{13}C]-Phenylalanine (5.0 g·L^{-1}) were prepared in purified water by passage through a 0.22 μm filter (Carriglwohill, Ireland) and then dispensed into disinfectant bottles and stored at 4℃ until use.

Isotope studies were carried out on day 7 of each period in a temperature-controlled room at Yangzhou University. The entire day's meal was divided into seven equal parts, which the subjects consumed hourly from 10:00 to 16:00. The constant intakes of L-[1-^{13}C]-Phenylalanine (8.0μmol·kg^{-1}·h^{-1}) were administered every 20 min and lasted for 4h (from 13:00 to 17:00) immediately after the intake of NaH^{13}CO$_3$ (2.1μmol·kg^{-1}) and priming L-[1-^{13}C]-Phenylalanine (4.0μmol·kg^{-1}) which were provided at 13:00. The isotopic steady state in the metabolic pool was represented by plateaus in ^{13}CO$_2$ enrichments in breath. A plateau was defined as a CV < 5% and the absence of a significant slope. The difference between mean breath ^{13}CO$_2$ enrichments of the baseline and plateau samples was used to determine atom percent excess (APE) above baseline in the isotopic steady state [14].

One baseline breath and blood samples were collected immediately before the first intake of isotopes. To determine the exact metabolic trend of phenylalanine in the body, nine breath samples were collected at 14:00, 15:00, 16:00, 16:15, 16:30, 16:45, 17:00, 17:15, and 17:30. Breath samples were stored at room temperature pending analysis. Immediately after the isotope intakes ended, 3 min breath samples were collected into a 100 L Douglas Bag (Harvard Apparatus). Following sample collection, the bag was connected to an infrared monitor (GXH-3010E, Hua Yun, Beijing) to measure the percentage of CO$_2$ in the sample, after which a vacuum extraction system (Schlumberger, Netherland) was used to determine total sample volumes and the sample was evacuated at 10 L·min^{-1}. Barometric pressure and sample temperature were recorded to obtain volumes at standard temperature and pressure. The CO$_2$ analyzer was calibrated with a 2.9% CO$_2$ reference standard (China National Institute of Standardization). To reduce harm to subjects, only one blood sample (3 ml) was withdrawn into heparinized syringes at 16:45. Blood was sampled from a catheter placed in the antecubital fossa of the right arm. The catheter tubing was flushed with heparin after a blood sample was taken to prevent clotting. Blood samples were kept on ice until centrifugation at 4℃ for plasma separation and the plasma was stored at -20℃ until further analysis. All subjects were required to rest throughout the study.

Analytical methods

Body compositions were examined using a body composition analyzer (EX/COM DF850, Japan). Amino acids in experimental foods were examined using an amino acid autoanalyzer (Hitachi 853-50), protein content was determined by Kjeldahl analysis, fat content was measured by Soxhelt extraction and acid solution, the contents of water and ash were detected by the weight method and carbohydrate content was determined by the subtraction method. Expired ^{13}CO$_2$ enrichment was measured using a ^{13}C breath analyzer (ZHP-2001, KYKY Technology Co., Beijing) and expressed as APE relative to a reference standard of compressed CO$_2$ gas. Plasma prealbumin (PA) and retinol-binding protein (RBP) levels were determined by ELISA (ELISA kit, RB Co., USA). Plasma L-[1-^{13}C]-Phenylalanine enrichment was measured using a triple quadrupole LC/MS system (Agilent 6460, USA). The individual components of plasma were separated on an Eclipse Plus C18 column (4.6×150 mm, 5μm, Agilent, USA) and eluted with a binary LC gradient (10-50% aqueous acetonitrile containing 0.1% formic acid). The retention time was 6 min. Selected-ion chromatograms were obtained by monitoring the fragmentation of the protonated [M+H$^+$] molecule at m/z = 166 (L-phenylalanine) and 167 (L-[1-^{13}C]-Phenylalanine) for precursor (parent) ion and at m/z = 130 for the product (daughter) ion.

Calculations

The ^{13}C enrichment in expired air was expressed through δ[15]:

$$\delta = (^{13}C \text{ enrichment}_{sample} - {}^{13}C \text{ enrichment}_{PDB}) / {}^{13}C \text{ enrichment}_{PDB} \times 1000,$$

where δ is the relative enrichment of ^{13}C in breath. PDB indicates pee deebeleminite, which contains a ^{13}C/^{12}C of 0.0112372.

Whole body phenylalanine flux was calculated from the dilution of isotopes in the body amino acid pool at isotopic steady state [16]:

$$Q = i[E_i/E_p - 1],$$

where Q is the rate of phenylalanine flux (μmol·kg^{-1}·h^{-1}), i is the isotope infusion rate (μmol·kg^{-1}·h^{-1}), E_i is the increase over naturally present in enrichment of the infused isotope (APE), E_p is the increase over baseline in enrichment of plasma

phenylalanine at the isotopic plateau (APE).

The rate of phenylalanine oxidation was calculated using the following equation [16]:

$$O = F^{13}CO_2(1/E_p\text{-}1/E_i) \times 100,$$

where O represents the phenylalanine oxidation ($\mu mol \cdot kg^{-1} \cdot h^{-1}$) and $F^{13}CO_2$ represents the rate of $^{13}CO_2$ released by phenylalanine tracer oxidation ($\mu mol \cdot kg^{-1} \cdot h^{-1}$) calculated by the following equation:

$$F^{13}CO_2 = (FCO_2)(ECO_2)(44.6)(60) / (W)(0.82)(100),$$

where FCO_2 is the CO_2 production rate ($mL \cdot min^{-1}$), ECO_2 is the increase over baseline in enrichment of expired breath at isotopic steady state (APE), the constants 44.6 $\mu mol \cdot mL^{-1}$ and 60 $min \cdot h^{-1}$ converted FCO_2 to $\mu mol \cdot h^{-1}$, W is the weight (kg) of the subject, 0.82 is the correction factor for CO_2 retained in the body due to the bicarbonate fixation [17], and the factor 100 converts APE to a fraction.

Statistical analysis

Results are expressed as the means ± SD. Paired-t tests were performed to compare the body weight, body composition and levels of plasma PA and RBP during the study periods with those at baseline. Estimates of the mean protein requirement intake were derived by breakpoint analysis of the rate of release of $^{13}CO_2$ and rate of phenylalanine oxidation using the mixed procedure of SAS (version 8.2, SAS Institute) [18] followed by a 2-phase linear regression crossover model as described by Zello *et al.* [19].

Results

Dietary structures

Actual lysine intakes were somewhat higher than designated levels because lysine in some experimental foods was higher than that in the reference of the China Food Composition. The intakes of phenylalanine and tyrosine both met the requirements. The intakes of protein, fat, carbohydrate and energy were also sufficient. The ratio of animal and soy protein in experimental diets was 45%-48% and the energy proportions from protein, fat and carbohydrates were 10%-12%, 26%-34% and 55%-59%, respectively, which were all reasonable according to Dietary Guidelines for Chinese Residents [20] (Table 2).

Subject physical features

As shown in Table 3, the weights and body compositions did not change significantly over the five 7 d study periods relative to the baseline ($P > 0.05$), demonstrating that the subjects were in energy balance during the study and that different lysine intakes did not affect body compositions during the experiment.

Levels of plasma PA and RBP

Subject optical density of plasma PA and RBP did not change significantly over the five 7 d study periods relative to the baseline ($P > 0.05$) (Table 4), suggesting that different lysine intakes did not affect plasma proteins during the experiment.

Table 2. Intakes of main amino acids, macronutrients and energy (n = 7)

Designated lysine intake ($mg \cdot kg^{-1} \cdot d^{-1}$)	Actual lysine intake ($mg \cdot kg^{-1} \cdot d^{-1}$)	Phenylalanine intake ($mg \cdot kg^{-1} \cdot d^{-1}$)	Tyrosine intake ($mg \cdot kg^{-1} \cdot d^{-1}$)	Protein intake ($g \cdot kg^{-1} \cdot d^{-1}$)	Fat intake ($g \cdot kg^{-1} \cdot d^{-1}$)	Carbohydrate intake ($g \cdot kg^{-1} \cdot d^{-1}$)	Energy intake ($kcal \cdot kg^{-1} \cdot d^{-1}$)
25	26.07 ± 0.26 [1]	47.55 ± 0.85	39.37 ± 0.32	1.25 ± 0.01	0.97 ± 0.03	6.25 ± 0.25	40.92 ± 1.22
35	41.37 ± 0.67	45.67 ± 0.98	37.92 ± 0.30	1.14 ± 0.01	1.20 ± 0.04	5.96 ± 0.25	42.10 ± 1.37
45	49.01 ± 0.33	48.20 ± 0.73	39.41 ± 0.14	1.24 ± 0.01	1.10 ± 0.03	5.59 ± 0.20	39.90 ± 0.99
55	63.36 ± 0.99	48.84 ± 0.83	41.68 ± 0.42	1.13 ± 0.01	1.03 ± 0.03	5.52 ± 0.23	38.97 ± 1.21
65	76.51 ± 0.70	49.17 ± 1.03	43.04 ± 0.34	1.28 ± 0.02	0.88 ± 0.03	5.87 ± 0.25	40.28 ± 0.98

[1] Mean ± SD.

Table 3. Weight and body compositions of subjects before and during the experiment (n = 7)

Index	Baseline	1st week	2nd week	3rd week	4th week	5th week
Weight (kg)	62.66 ± 4.15 [1]	62.54 ± 4.10	62.45 ± 4.22	62.49 ± 4.13	62.56 ± 4.11	62.69 ± 4.18
P-value [2]		0.09	0.06	0.23	0.60	0.89
BMI (kg/m^2)	20.96 ± 1.18	20.92 ± 1.17	20.89 ± 1.19	20.91 ± 1.20	20.93 ± 1.19	20.98 ± 1.25
P-value [2]		0.10	0.06	0.23	0.61	0.86
Percentage body fat (%)	16.20 ± 3.1	16.20 ± 3.5	16.50 ± 3.5	16.80 ± 3.4	16.50 ± 3.7	16.30 ± 3.3
P-value [2]		0.91	0.35	0.05	0.44	0.82
Muscle mass (kg)	23.64 ± 2.53	23.48 ± 2.60	23.33 ± 2.69	23.26 ± 2.47	23.25 ± 2.47	23.24 ± 2.44
P-value [2]		0.20	0.12	0.07	0.06	0.05
Percentage muscle (%)	37.70 ± 2.1	37.90 ± 2.4	37.60 ± 2.6	37.40 ± 2.2	37.30 ± 2.4	37.90 ± 2.1
P-value [2]		0.28	0.66	0.10	0.07	0.29

[1] Mean ± SD.
[2] Paired-t tests were performed to compare body weight, BMI, and body compositions during the study periods with those at baseline.

Table 4. Optical density of plasma PA and RBP before and during the experiment (n = 7)

Time	PA (OD value)	*P*-value[1]	RBP (OD value)	*P*-value[1]
Baseline	0.54 ± 0.73[2]		0.78 ± 1.11	
1st week	0.54 ± 0.68	0.13	0.75 ± 1.05	0.25
2nd week	0.58 ± 0.74	0.11	0.79 ± 1.14	0.81
3rd week	0.56 ± 0.73	0.76	0.74 ± 1.07	0.19
4th week	0.62 ± 0.81	0.86	0.79 ± 1.13	0.82
5th week	0.56 ± 0.71	0.39	0.76 ± 1.07	0.48

PA = prealbumin; RBP = retinol-binding protein
[1] Paired-t tests were performed to compare PA and RBP in study periods with those at baseline.
[2] Mean ± SD.

Phenylalanine kinetics

As shown in Fig. 1, steady state of phenylalanine enrichment was obtained during 210 to 270 min after the first intake of isotope. As shown in Table 5, phenylalanine flux decreased with increasing intake of lysine and with phenylalanine oxidation. These findings indicate that increasing phenylalanine participates in protein synthesis with the increasing intake of lysine until the intake of lysine meets the requirements of the body.

Lysine requirement derived from phenylalanine oxidation

As shown in Fig. 2 and Fig. 3, all subjects showed a similar pattern in which the rate of release of $^{13}CO_2(F^{13}CO_2)$ and rate of phenylalanine oxidation (O) decreased with increasing lysine intake to a point that was close to the requirements, after which
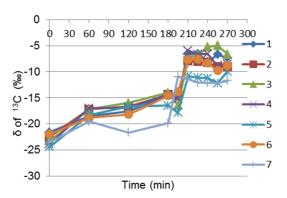

Fig. 1. δ of $^{13}CO_2$ in expired air

Table 5. Phenylalanine flux, rate of oxidation and rate of $^{13}CO_2$ production at different dietary lysine levels (n = 7)

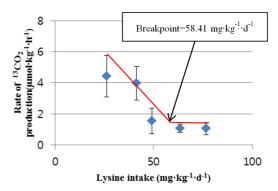| Lysine level, mg·kg⁻¹·d⁻¹	Phenylalanine flux (Q), μmol·kg⁻¹·h⁻¹	Rate of phenylalanine oxidation (O), μmol·kg⁻¹·h⁻¹	Rate of $^{13}CO_2$ released by [1-^{13}C]-Phenylalanine ($F^{13}CO2$), μmol·kg⁻¹·h⁻¹
26.07	147.35 ± 60.71[1]	78.72 ± 28.81	4.43 ± 1.34
41.37	87.63 ± 25.99	43.08 ± 16.59	3.97 ± 1.09
49.01	68.02 ± 11.79	12.75 ± 6.02	1.56 ± 0.82
63.36	54.63 ± 5.89	7.29 ± 2.21	1.06 ± 0.28
76.51	61.11 ± 14.92	9.67 ± 4.00	1.05 ± 0.39

[1] Mean ± SD.

Fig. 2. Effect of lysine intake on oxidation of L-[1-^{13}C]-Phenylalanine determined from the rate of release of $^{13}CO_2$ ($F^{13}CO_2$). The breakpoint estimates the lysine requirement. The breakpoint was determined by using 2-phase linear regression crossover analysis to minimize the total sum of squares in error for the combine line.
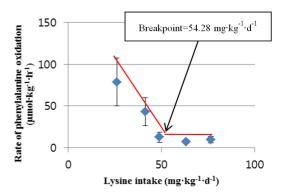

Fig. 3. Effect of lysine intake on oxidation of L-[1-^{13}C]-Phenylalanine determined from the rate of L-[1-^{13}C]-Phenylalanine oxidation (O). The breakpoint estimates the lysine requirement. The breakpoint was determined by using 2-phase linear regression crossover analysis to minimize the total sum of squares in error for the combine line.

it remained relatively stable. By using a two-phase linear regression crossover model, a breakpoint in the $F^{13}CO_2$ response curve was identified at a dietary lysine intake of 58.41 mg·kg⁻¹·d⁻¹, and the upper 95% CI was 70.09 mg·kg⁻¹·d⁻¹. Similarly, a breakpoint in the O response curve was identified at a dietary lysine intake of 54.28 mg·kg⁻¹·d⁻¹, and the upper 95% CI was 65.14 mg·kg⁻¹·d⁻¹.

Discussion

The IAAO method has been widely used to measure the requirements of necessary amino acids of adults and children [19,21]. However, these studies used purified amino acid mixtures other than habitual diets as experimental food. Since the digestibility and absorptivity of purified amino acids are higher than those of normal mixed diets, the results of those studies did not reflect the real amino acids requirement of daily habitual diets. To avoid this problem, the present study used Chinese habitual diets as the experimental diets with accurately

controlled lysine and protein intake. On the isotope day, the indicator amino acid L-[1-^{13}C]-Phenylalanine was provided with food to obtain similar digestion and absorption of amino acids in the diet. The adaptation period of each lysine intake level was 6 d, which was longer than that of other IAAO studies in which it was only 2 d [14,21,22], and similar to the adaptation periods of IAAB studies, which were 6 to 8 days [3,4]. The five dietary lysine intake levels contained the recommended requirement of FAO/WHO/UNU in 2007 (30 mg·kg^{-1}·d^{-1}) [4], that of the USA in 2005 (31 mg·kg^{-1}·d^{-1}) [23], the results of a 24 h dietary recall survey conducted over 3 continuous days of subjects before the study (48 mg·kg^{-1}·d^{-1}), and the results of a previous study of protein requirements of Chinese male adults (not published, 64 mg·kg^{-1}·d^{-1}). The experimental dietary lysine levels were administered from high to low because if the opposite administration was employed, subjects with low lysine levels would require a long adaptation time.

The results showed that the intakes of protein, energy, phenylalanine and tyrosine were adequate, the proportion of dietary animal and soybean protein and the energy proportions from protein, fat and carbohydrate were all in accordance with Chinese customary dietary structure. Throughout the experiment, the body weights of all subjects did not change significantly relative to those at baseline, indicating that the subjects were in energy balance during the study. To determine if different lysine intakes had effects on the plasma proteins, we measured two sensitive indicators plasma PA and RBP, during the study. The two plasma proteins were both hepatic proteins with half-lives of 1.9 d and 3-12 h respectively. The very short half-lives make them sensitive and specific to changes in indispensible amino acids in the body over the short term [24-26]. The results showed that there were no significant differences between the five experimental plasma protein levels and the baseline levels. Moreover, there were no significant differences in body compositions. Therefore, the results suggest that the lysine intake levels (26-77 mg·kg^{-1}·d^{-1}) had no effect on the plasma proteins and anthropometry among subjects during the 35 d study. This may have been because the subjects were healthy young men aged 22 to 26 years old who had a strong capacity for self-regulation. When the lysine intake was below the physiological requirements, body protein would be degraded to amino acids in the blood to meet the need for synthesis of plasma proteins, while above the physiological requirement, spare lysine is oxidized. Accordingly, even if lysine intake was low or high, the plasma proteins will remain unchanged. In theory, when lysine intake is under physiological requirements, body protein, especially muscle protein, will be degraded, resulting in decreased lean body mass; however, this was not observed in the present study. The main reason for this may be that the 6 d adaptation was not long enough for muscles to decrease significantly, although there was a slight decreasing trend in muscle mass (Table 3).

The lysine requirement with the IAAO method in the present study was 58.41 mg·kg^{-1}·d^{-1} based on the rate of ^{13}CO$_2$ production

and 54.28 mg·kg^{-1}·d^{-1} based on the rate of L-[1-^{13}C]-Phenylalanine oxidation. Because previous studies have suggested that the rate of ^{13}CO$_2$ production is a more sensitive endpoint measurement for tracer oxidation in adults and in children [8,21,27], we selected 58.41 mg·kg^{-1}·d^{-1} as the lysine requirement of the subjects. These results were much higher than those observed in other studies [4,19,28,29]. This was likely for the following reasons. Allowing for the anti-nutritional factors in Chinese habitual mixed diets, such as dietary fiber, the availability of protein in Chinese mixed diet is about 70% [10], which is much lower than the amount of purified amino acids mixture, which will be absorbed very rapidly and completely. Therefore, the lysine requirement in mixed diet is higher than that in crystalline amino acid mixture. Additionally, the IAAO method is not a direct measure of the amino acids maintenance requirement, but is primarily a measure of the intake of the test amino acid as a proportion of its content in the amino acid mixture required for postprandial protein deposition [5]. The extent of protein deposition after meals relates to the overall requirement and must balance fasting losses; therefore, the IAAO result determines the amino acid requirement for the maximum rate of postprandial protein anabolism [5]. This may be higher than the IAAB study results. Moreover, the present study providedthe daily intake in 7 hourly meals rather than 10 hourly meals in the IAAB method [3], which results in a 43% higher hourly intake than the IAAB method, and may result in a higher potential net protein deposition rate and a higher amino acid requirement than the IAAB method.

In summary, the lysine requirement of the Chinese habitual mixed diet of healthy young male adults is 58 mg·kg^{-1}·d^{-1}. No method is entirely reliable for determining the dietary requirements for indispensable amino acids at present [5]; however, the method described herein provides improvements to the experimental diets and extends the adaptation time, making the results more in line with the actual requirements of the Chinese population.

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