Original Article

Examination of Chinese habitual dietary protein requirements of Chinese young female adults by an indicator amino acid method

Ying Tian PhD[1,2], Jianmin Liu PhD[3], Yuhui Zhang BS[4], Jianhua Piao BS[1], Lingyan Gou BS[4], Yuan Tian BS[1], Min Li PhD[1], Yibing Ji PhD[1], Xiaoguang Yang PhD[1]

[1]Laboratory of trace element nutrition MOH, Institute of nutrition and food safety, Chinese Center for Disease Control and Prevention, Beijing, China
[2]Department of nutrition, School of Tourism and Culinary Science, Yangzhou University, Yangzhou, China
[3]Hebei Medical University, Shijiazhuang, China
[4]Bethune Military Medical College, PLA Fourth Military Medical University, Shijiazhuang, China

Objective: To determine protein requirement of Chinese young female adults on habitual Chinese diet through indicator amino acid oxidation (IAAO) technique. Design: Twenty women with a mean (SD) age of 21.6 (0.9) years were healthy based on questionnaire, physical examinations and screening tests. There were three consecutive periods of 7 days each with six different intakes of protein (0.70, 0.78, 0.86, 0.94, 1.02 and 1.10 g/kg·d) within Chinese habitual diets (proportions of good-quality protein were 40 to 45%). Subjects were randomly allocated equally into two groups (1.10, 0.86, 0.78 g/kg·d for group 1 and 1.02, 0.94, 0.70 g/kg·d for group 2 from period 1 to period 3 in turn). Adaptation days were from day 1 to day 6 and the isotope study day was day 7 in each period. Amino acid kinetics was measured in non-menstrual periods, based on the IAAO technique. Two indicators (rate of release of $^{13}CO_2$ and rate of leucine oxidation) were used to estimate protein requirement by breakpoint analysis with a two-phase linear regression crossover model. Results: Mean and population safe protein requirements of Chinese habitual diets in non-menstrual periods from the rate of release of $^{13}CO_2$ were 0.91 and 1.09 g/kg·d, respectively. And from the rate of leucine oxidation were 0.92 and 1.10 g/kg·d, respectively. Conclusions: The protein requirement of young women on Chinese habitual diets in non-menstrual period was lower than the current protein reference intake for Chinese females. Further studies are necessary to explore female protein requirements during the whole menstrual cycle.

Key Words: protein requirement, indicator amino acid oxidation, mixed diet, stable isotope, women

INTRODUCTION

Although the nitrogen balance method is the gold standard in measuring protein requirement, it has many limitations.[1,2] Nitrogen intake tends to be overestimated and nitrogen excretion tends to be underestimated. Overestimation of nitrogen intake and underestimation of nitrogen excretion falsely results in positive nitrogen balance.[3] The indicator amino acid oxidation (IAAO) technique is an alternative approach relying on oxidation of stable isotopes to determine protein and amino acid requirements. It is a robust and reliable technique that has been successfully used in many studies on adults and children.[3,4] But the subjects in most studies consumed purified amino acids other than protein within mixed diets. The efficiency of absorption and utilization of purified amino acid mixtures are higher than those of mixed diet, so purified amino acids will be absorbed more rapidly and thoroughly than protein in mixed diets. Therefore the results can't reflect the real protein requirements of individuals consuming mixed diets.

The current protein reference intake for Chinese female adults was based on the experiment of protein requirement of Chinese male adults by the nitrogen balance method in the 1980's.[5] It was based on the assumption that there were no significant difference in the protein requirement between sexes and so the protein requirement per kilogram bodyweight per day of female adults equaled to that of the males, which is 1.16 g/kg·d. But the sex differences in body composition, generally higher fat and lower lean content of women compared with men, might be expected to result in a lower requirement per kilogram in women in line with their lower basal metabolic rate.[2] The result of Egun et al showed that there was a significant difference in the protein requirement between sexes.[6] However, data on protein requirements of female adults are still limited, especially with data

collected with the IAAO method in young females. So it is necessary to explore female protein requirement with the IAAO method with mixed diets to offer more information on the study of female protein requirement. In the present study, we determined the female protein requirement by feeding graded protein levels in habitual Chinese mixed diet and measuring changes in oxidation of orally administered L-[1-^{13}C]-Leucine.

MATERIALS AND METHODS
Subjects
Twenty adult female volunteers from Bethune Military Medical College (age 21.6±0.9 years, weight 55.9±4.7 kg, height 1.61±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, including a questionnaire on the activities of daily living, physical examination, and screening tests, including complete blood count, blood chemistry workup and hepatic and renal functions. Exclusion criteria were the following: recent weight loss, unusual dietary practices, nutritional supplement use, irregular menstrual cycle, chronic disease, endocrine disorder, atypical sleeping or exercise schedules.

The purpose of the study and the potential risks involved were explained fully to each subject and written consent was obtained. Approvals from the Ethical Review Committee, Institute for Nutrition and Food Safety, Chinese Center for Disease Control and Prevention were obtained.

Study protocol
The study was carried out for three consecutive periods, each period consisted of 7 days. There were six different protein intakes (0.70, 0.78, 0.86, 0.94, 1.02 and 1.10 g/kg·d) in all. Twenty subjects were divided equally into two groups and each group received three different protein intakes respectively. The intake level of protein for both groups was given from high to low in the three periods (i.e. 1.10, 0.86, 0.78 g/kg/day for group 1 and 1.02, 0.94, 0.70 g/kg/day for group 2 from period 1 to period 3 in turn).

Subjects were instructed to maintain light physical activity level and were weighed on the sixth day of each period to confirm weight maintenance throughout the study. All subjects were not in menstrual period during the study.

Diets
Diets during the adaptation period were provided in the form of a Chinese habitual diet with a three-day rotation of menus (including meat, egg, fish, milk, vegetables, rice and wheat flour). The daily protein intake of each subject was calculated according to her body weight and designed protein level, and was distributed into three meals with the ratio of 3:4:3 throughout the day. Protein contents in foods were calculated based on the China Food Composition for food quantity.[7] The level of dietary good-quality protein was controlled at 40% to 45% a day. Subjects were allowed to eat fried non-nitrogenous starchy cookies or starchy noodles if the meal couldn't meet her energy requirement. No other food or beverages

were consumed except low-protein fruits (apple, pear and grapes) and water.

Diets on the three isotope study days were the same and were similar to the recipes of adaptation days. Lunch was divided into four isocaloric, isonitrogenous meals and was consumed hourly to ensure metabolic steady state in the fed condition.

All foods in each meal were weighed and recorded both before and after consumption by each subject to get the actual intake of each food. Samples of each food were analyzed for total nitrogen, fat, carbohydrate, water and ash. Protein content was detected by Kjeldahl analysis, fat content was measured by Soxhelt extraction and acid solution, the contents of water and ash were detected by weight method and carbohydrate content was determined by subtraction method.

Isotope studies
The isotopic labeled tracers used in the study were NaH^{13}CO$_3$ with 99% enrichment and L-[1-^{13}C]-Leucine with 99% enrichment (Cambridge Isotope Laboratories). Stock solutions of NaH^{13}CO$_3$ (2.0 g/L) and L-[1-^{13}C]-Leucine (10.0 g/L) were prepared in purified water by passage through a 0.22 μm filter (Carriglwohill, Ireland) and were then dispensed into disinfectant bottles and stored at 4°C until used.

Isotope studies were carried out on day 7 of each period in a temperature-controlled room at Bethune Military Medical College. The constant intakes of L-[1-^{13}C]-Leucine (0.56 mg/kg·h) were given every 20 min and lasted for 4hours following the intake of NaH^{13}CO$_3$ (0.11 mg/kg) and priming L-[1-^{13}C]-Leucine (0.56 mg/kg). Lunch was divided into four equal parts, which the subjects consumed hourly immediately after the first intake of constant L-[1-^{13}C]-Leucine. The isotopic steady state in the metabolic pool was represented by plateaus in ^{13}CO$_2$ enrichments in the 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 above baseline at isotopic steady state.[8]

One baseline breath and blood samples were collected 30 min before the isotope protocol began. To determined the exact metabolic trend of leucine in the body, eleven breath samples were collected at 60, 120, 180, 195, 210, 225, 240, 255, 270 and 300 min. Breath samples were stored at room temperature pending analysis. Just after the isotope intakes ended, 3 min breath sample was collected into a 100 L Douglas Bag (Harvard Apparatus). After sample collection, the bag was connected to an infrared monitoring device (GXH-3010E, Hua Yun, Beijing) to measure the percentage of CO$_2$ in the sample, then the 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 three blood samples (3 ml) were withdrawn into heparinized syringes at 210, 225 and 240 min. 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°C for plasma separation and the plasma was stored at -20°C until further analysis. All subjects were required to rest throughout the study.

Analytical methods

Expired $^{13}CO_2$ enrichment was measured by ^{13}C breath analyzer (Helivew, Medichems, Korea) and was expressed as APE relative to a reference standard of compressed CO_2 gas. Plasma L-[1-^{13}C]-Leucine enrichment was measured by a triple quadrupole mass spectrometry MS API 4000 (ABI, USA) operated in positive ionization mode with a TurbolonSpray ionization probe source (operated at 5800V and 600°C), which was coupled to a 20A HPLC system (Shimadzu, Japan). The individual components were separated from Atlantics column (4.6×100 mm, 3.5 μm, Waters, USA) and eluted with a binary LC gradient (10-50% aqueous acetonitrile containing 0.1% formic acid). The retention time was 3 min. Selected-ion chromatograms were obtained by monitoring the fragmentation of the protonated $[M+H^+]$ molecule at m/z=132 (leucine) and 133 ($[^{13}C]$-leucine) for precursor (parent) ion and at m/z 86 at product (daughter) ion.

Calculations

The ^{13}C enrichment in expired air was expressed through δ‰: δ‰ = (^{13}C enrichment (sample)-^{13}C enrichment (PDB))/^{13}C enrichment (PDB)×1000, where δ‰ is the relative enrichment of ^{13}C in breath. PDB is the mineral pee dee beleminite, ^{13}C/^{12}C in PDB is 0.0112372.

Whole body leucine flux was calculated from the dilution of isotope in the body's amino acid pool at isotopic steady state:[9] $Q=i[E_i/E_p-1]$, where Q is the rate of leucine flux (μmol/kg·h), i is the isotope infusion rate (μmol/kg·h), and E_i and E_p are the enrichments as mole fractions of the infused isotope (APE) and the increase over baseline in enrichment of plasma leucine at isotopic plateau (APE).

The rate of leucine oxidation was calculated with the following equation:[9] $O=F^{13}CO_2(1/E_p-1/E_i)×100$, where O represents leucine oxidation (μmol/kg·h) and $F^{13}CO_2$ represents the rate of $^{13}CO_2$ released by leucine tracer oxidation (μmol/kg·h), calculated by the following eqution:
$F^{13}CO_2=(FCO_2)(ECO_2)(44.6)(60)/(W)(0.82)(100)$. Where FCO_2 is the CO_2 production rate (mL/min), ECO_2 is the enrichment in expired breath at isotopic steady state (APE), the constants 44.6 μmol/mL and 60 min/h converted FCO_2 to μmol/h, W is the weight (kg) of the subject, the factor 0.82 is the correction for CO_2 retained in the body due to the bicarbonate fixation,[10] and the factor 100 changes APE to a fraction.

Statistical analysis

Results are expressed as means (SD). Dunnett's tests were performed for the comparisons of body weight in study periods with that at baseline. Estimates of the mean protein requirement intake were derived by breakpoint analysis of $F^{13}CO_2$ and O data using the mixed procedure of SAS (version 8.2, SAS Institute)[11] followed by a 2-phase linear regression crossover model as described previously.[12]

RESULTS
Dietary Structures

Actual protein intakes were higher than designed levels because protein in staple food made from wheat in experimental diets was higher than that in the Chinese Food

Table 1. Intakes of main macronutrients and energy of six dietary protein levels (Mean ±SD)

Designed protein intake (g/kg·d)	Actual protein intake (g/kg·d)	Good quality protein (%)	Energy from protein (%)	Fat intake (g/kg·d)	Energy from fat (%)	Carbohydrate intake (g/kg·d)	Energy from carbohydrate (%)	Energy intake (kcal/kg·d)
0.70	0.79±0.04	40.6±3.6	9.6±0.9	1.2±0.3	30.4±4.9	5.0±0.5	60.0±4.5	33.6±3.9
0.78	0.91±0.07	40.5±4.3	10.0±1.3	1.2±0.4	29.6±4.9	5.4±0.8	60.3±4.4	35.8±6.3
0.86	0.92±0.05	39.3±2.8	12.2±1.2	1.0±0.2	27.8±3.1	4.6±0.5	59.9±2.3	31.0±3.5
0.94	0.99±0.05	38.3±2.5	11.9±1.2	1.1±0.2	27.9±3.5	5.1±0.4	60.1±2.5	33. 9±3.8
1.02	1.07±0.05	44.3±3.7	14.1±1.0	0.9±0.2	26.6±4.1	4.5±0.3	59.1±3.4	30.6±2.1
1.10	1.17±0.06	43.1±4.5	14.1±1.1	1.0±0.2	26.5±3.7	5.0±0.4	59.2±2.9	33.7±2.7

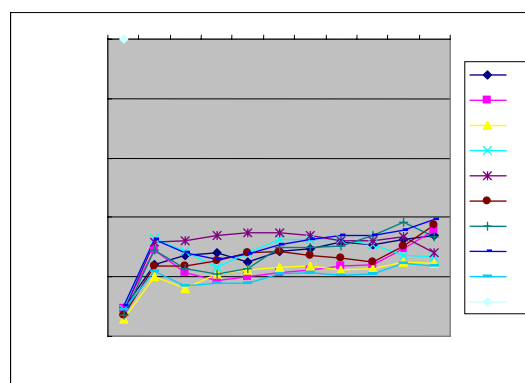**Figure 1.** Trend of δ‰ for $^{13}CO_2$ in expired air

Composition tables. The ratio of good-quality protein was ideal and the energy proportions from protein, fat and carbohydrate were reasonable according to Dietary Guidelines for Chinese Residents (Table 1).[13]

Subjects' physical features

Subjects' weights did not change significantly over the three 7day-study periods compared with the baseline (p>0.05) (baseline (kg): 55.9±4.7, period 1 (kg): 55.9±4.5, period 2 (kg): 56.1±4.7, period 3 (kg): 55.9±4.6), which provided evidence that the subjects were in energy balance during the study.

Leucine kinetics

As shown in Figure 1, steady state leucine enrichment was obtained between 210 to 270 min. Leucine flux was not significantly affected by protein intake but differed distinctly among subjects. In contrast, the rate of leucine oxidation was significantly affected by protein intake although no breakpoint could be identified visually (Table 2).

Protein requirement derived from leucine oxidation

Figure 2 shows that $F^{13}CO_2$ remained relatively stable to a point that it was close to the requirement, after which it increased significantly with the increasing protein intake. Regression analysis revealed that leucine oxidation, as measured by $F^{13}CO_2$, increased as protein intake increased from 0.91 to 1.17 g/kg·d. By using a two-phase linear regression crossover model, a breakpoint in the $F^{13}CO_2$ response curve was identified at a dietary protein intake of 0.91 g/kg·d. Similarly, by use of the two-phase linear regression crossover model, breakpoint analysis provided a mean protein requirement of 0.92 g/kg·d from

Table 2. Leucine flux, oxidation and rate of $^{13}CO_2$ production at different dietary protein levels (Mean ±SD)

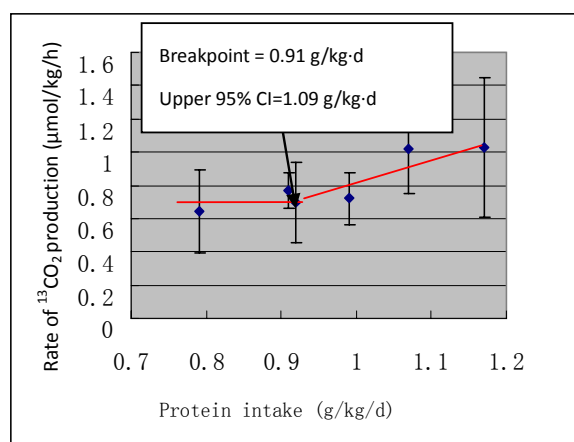| Protein level (g/kg·d)	Leucine flux (Q) (μmol/kg·h)	Leucine oxidation (O), (μmol/kg·h)	Rate of $^{13}CO_2$ released by ^{13}C-leucine ($F^{13}CO_2$), (μmol/kg·h)
0.79	43.0±10.3	9.2±2.7	0.64±0.25
0.91	37.2±7.3	10.1±4.9	0.77±0.11
0.92	48.2±20.7	11.6±5.0	0.70±0.24
0.99	49.0±10.8	10.4±4.8	0.72±0.16
1.07	46.9±10.8	13.1±6.2	1.02±0.27
1.17	49.0±5.9	16.6±6.3	1.03±0.42

Figure 2. Effect of protein intake on oxidation of L-[1-^{13}C]Leucine as determined from the rate of release of $^{13}CO_2$ ($F^{13}CO_2$). The linear regression equation for the estimated mean protein requirement is y=0.708-1.200C+1.324Cx, where C=0 for the first line and C=1 for the second line.
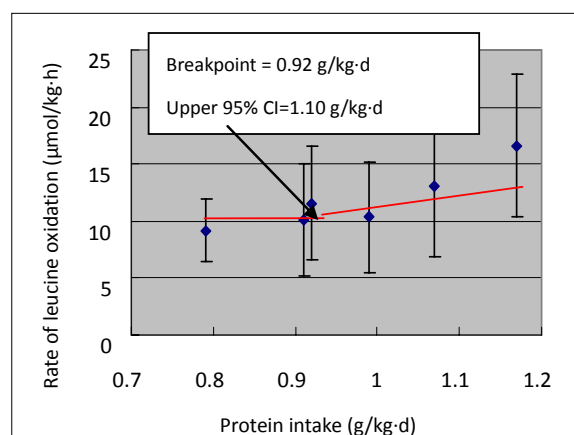

Figure 3. Effect of protein intake on oxidation of L-[1-^{13}C]Leucine determined from the rate of leucine oxidation (O). The linear regression equation for the estimated mean protein requirement is y=10.313-20.540C+22.323Cx, where C=0 for the first line and C=1 for the second line.

the oxidation rate of L-[1-^{13}C]leucine (Figure 3). Because each subject only consumed three levels of the total six protein intakes, two-phase linear regression crossover model could not be used to calculate the breakpoint for each subject, so population-safe level (upper limit of 95% confidence interval) of protein requirement could only be estimated by 1.2 times of mean protein requirement, with 1.09 g/kg·d and 1.10 g/kg·d for F^{13}CO$_2$ and oxidation rate of L-[1-^{13}C]leucine, respectively.

DISCUSSION

The IAAO method has been widely used to measure requirements of protein and necessary amino acids of adults and children.[3,14,15] But all studies used purified amino acid mixtures and not habitual diets as experimental food. Since purified amino acids are digested and absorbed more rapidly and absolutely than protein in mixed diets, those study results couldn't reflect the true protein requirement of individuals consuming the habitual diet. To avoid this problem, the present study used Chinese habitual diets as the experimental diets with accurately controlled protein intake. The adaptation period of each protein intake level was 6 days, close to the duration of nitrogen balance studies and longer than the study with amino acid mixture to get expected protein level.[2,3] The six dietary protein levels included the reference intake of FAO/WHO/UNU in 1985 (0.75 g/kg/day, 100% good quality protein) and reference intake of USA in 2002 (0.80 g/kg/day, 100% good quality protein) and close to that of Chinese in 2000 (1.16 g/kg/day, 35% good quality protein).[2] The proportion of dietary good quality protein in the present study was 40% to 45%, higher than that of Chinese reference intake but lower than that of WHO and USA reference intakes, more suitable for the Chinese customary dietary structure. The experimental dietary protein levels were given from high to low. Because subjects in a low protein state needed to first make up protein inadequacy in the body and then slowly get to the higher protein level if protein concentrations were consumed from low to high, therefore requiring a long adaptation time. Conversely, if protein was consumed from high to low concentrations, study subjects didn't need to make up the inadequacy and could directly get to the lower protein state in every experimental period. On the isotope day, the indicator amino acid L-[1-^{13}C]leucine was given with food so as to get similar digestion and absorption of protein in food. The priming NaH^{13}CO$_3$ was used to make the enrichment of ^{13}C in plasma rise to a high level rapidly and contribute the ^{13}CO$_2$ in breath to get to the steady state in a short time. NaH^{13}CO$_3$ was given only once on the start of each isotope experiment, so its effects on the enrichment of expired ^{13}CO$_2$ were the same in the six periods, and couldn't affect the trend of two-phase regression line and the breakpoint.

To date, IAAO technique has not been widely used in female protein requirement studies. Penencharz et al. reevaluated the protein requirement in young men with the IAAO method, and obtained the result of 0.93 g/kg/day.[3] But considering the purified amino acid diets in Pencharz's study, rates of digestion and absorption and efficiency of utilization of protein were higher than those of mixed diets. So if their subjects consumed mixed diets,

the result might be higher. The results of the present study, as determined from F^{13}CO$_2$ and the rate of L-[1-^{13}C]leucine oxidation, were 0.91 and 0.92 g/kg/day, respectively. However, it's worth noting that, as the subjects of the present study are healthy young women, menstruation is inevitably considered. Previous studies suggested that hormonal effects influenced nitrogen utilization at different times of the menstrual cycle.[16,17] A positive nitrogen balance was recorded around ovulation but fell significantly just before the onset of menstruation and almost all subjects were in negative nitrogen balance.[15] To ensure the consistency of results, all the subjects of the present study were not in the menstrual period on each of the isotopic day. So the results of the present study were the protein requirements of non-menstrual period. If taking into account of the menstrual period, in which protein loss is higher than that of non-menstrual period, the total protein requirements of young females should be higher than the present results, but the exact values still need to be evaluated.

Studies on protein requirement of females are limited, probably because of the difficulty of compliance of female subjects and calculating protein loss of menstruation. Only a few female protein requirement studies were examined with nitrogen balance method and the results were different.[6,18] Hannah et al. studied protein requirement of elderly women with whole good-quality protein diets, getting the result of at or above 0.76 g/kg/day. But a study of young adult Nigerian females with mixed Nigerian diets had the result of 0.6g/kg/day. Because of the different races of subjects, different experiment diets and different methods, it can't be concluded that the present result is higher than those results.

The current protein reference intake of Chinese female adults is 1.16 g/kg/day, which is based on and assumed to be equal to the nitrogen balance study of male protein requirement in the 1980's.[5] The present results are lower than the current female protein reference intake. So it is necessary to study protein requirement of individuals consuming habitual diets of both sexes with the IAAO method. And further studies are also necessary to determine protein requirement during the whole menstrual cycle in females.

REFERENCES
1. Millward DJ. Methodological considerations. Proc Nutr Soc. 2001;60:3-5.
2. FAO/WHO/UNU. Protein and amino acid requirements in human nutrition. WHO/FAO/UNU expert consultation; 2007.
3. Humayun MA, Elango R, Ball RO, Pencharz PB. Reevaluation of the protein requirement in young men with the indi-

cator amino acid oxidation technique. Am J Clin Nutr. 2007; 86:995-1002.

4. Elango R, Humayun MA, Ball RO, Pencharz PB. Lysine requirement of healthy school-age children determined by the indicator amino acid oxidation method. Am J Clin Nutr. 2007;86:360-5.

5. Taian Y, Xuanjiu Y, Jiguo B, Huang ZS, Chen XC. Protein requirements of Chinese male adults. Ying Yang Xue Bao. 1984;6:199-207.

6. Egun GN, Atinmo T. Protein requirement of young adult Nigerian females on habitual Nigerian diet at the usual level of energy intake. Br J Nutr. 1993;70:439-48.

7. Yuexin Y, Guangya W, Xingchang P. China Food Composition. Beijing: Beijing Medical University Press; 2004.

8. Riazi R, Wykes LJ, Ball RO, Pencharz PB. The total branched-chain amino acid requirement in young healthy adult men determined by indicator amino acid oxidation by use of L-[1-^{13}C] phenylalanine. J Nutr. 2003;133:1383-9.

9. Matthews DE, Motil KJ, Rohrbaugh DK, Burke JF, Young VR, Bier DM. Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-^{13}C]leucine. Am J Physiol Endocrinol Metab. 1980;238:473-9.

10. Hoerr RA, Yu YM, Wagner DA, Burke JF, Young VR. Recovery of ^{13}C in breath from NaH^{13}CO$_3$ infused by gut and vein: effect of feeding. Am J Physiol. 1989;257:426-38.

11. Littell R, Milliken G, Stroup W, Wolfinger RD. SAS system for mixed models. Cary, NC: SAS Institute; 1996.

12. Zello GA, Pencharz PB, Ball RO. Dietary lysine requirement of young adult males determined by oxidation of L-[1-^{13}C]phenylalanine. Am J Physiol Endocrinol Metab 1993; 264:677-85.

13. Chinese Nutrition Society. Dietary guidelines for Chinese residents 2007. Tibet: Tibet people's press; 2008.

14. Turner JM, Humayun MA, Elango R, Rafii M, Langos V, Ball RO, Pencharz PB. Total sulfur amino acid requirement of healthy school-aged children as determined by indicator amino acid oxidation technique. Am J Clin Nutr. 2006;83: 619-23.

15. Kurpad AV, Regan MM, Varalakshmi S, Gnanou J, Lingappa A, Young VR. Effect of cysteine on the methionine requirement in healthy Indian men determined by a 24-h indicator amino acid balance approach. Am J Clin Nutr. 2004; 80:1526-35.

16. Calloway DH, Kurzer M. Menstrual cycle and protein requirements of women. J Nutr. 1982;112:256-366.

17. Egun GN, Atinmo T. A metabolic nitrogen balance study for 40d and evaluation of the menstrual cycle on protein requirement in young Nigerian women. Br J Nutr. 1993;70: 449-57.

18. M. Hannah M, Mark DH, William JE, Wayne WC. Protein requirement of elderly women:nitrogen balance responses to three levels of protein intake. J Gerontol A Biol Sci Med Sci. 2001;56:724-30.

Original Article

Examination of Chinese habitual dietary protein requirements of Chinese young female adults by an indicator amino acid method

Ying Tian PhD[1,2], Jianmin Liu PhD[3], Yuhui Zhang BS[4], Jianhua Piao BS[1],
Lingyan Gou BS[4], Yuan Tian BS[1], Min Li PhD[1], Yibing Ji PhD[1], Xiaoguang Yang PhD[1]

[1]Laboratory of trace element nutrition MOH, Institute of nutrition and food safety, Chinese Center for Disease Control and Prevention, Beijing, China
[2]Department of nutrition, School of Tourism and Culinary Science, Yangzhou University, Yangzhou, China
[3]Hebei Medical University, Shijiazhuang, China
[4]Bethune Military Medical College, PLA Fourth Military Medical University, Shijiazhuang, China

以指標氨基酸氧化法确定中国青年女性对日常膳食蛋白质的需要量

目的：采用指標氨基酸氧化技术，确定中国青年女性对中国日常膳食蛋白质的需要量。方法：通过问卷调查、体格检查和筛选，選擇 20 名健康女性作为受试者，平均年龄 21.6（±0.9）岁。整个实验共 3 个连续的实验周期，每周期 7 天。实验膳食为中国日常混合膳食，优质蛋白占 40%−45%，共 6 个膳食蛋白质摄入水平(0.70、0.78、0.86、0.94、1.02 和 1.10 g/kg·d)。受试者被随机平均分为 2 组，每组受试者在每个实验周期摄入 1 个蛋白质水平的实验膳食，3 个周期依次为：第一组：1.10、0.86、0.78 g/kg·d；第二组：1.02、0.94、0.70 g/kg·d。每个实验周期的第 1 到 6 天为适应期，第 7 天为同位素实验日。在受试者的非月经期，应用指標氨基酸氧化法进行氨基酸代谢动力学的研究。将实验所得的 $^{13}CO_2$ 产生率、亮氨酸(leucine)氧化率两个指标，分别代入兩相回归曲线模型，确定回归曲线的拐点，即蛋白质的生理需要量。结果：通过 $^{13}CO_2$ 产生率计算的中国青年女性非月经期对中国日常混合膳食蛋白质的平均需要量是 0.91 g/kg·d，人群安全需要量是 1.09 g/kg·d；通过亮氨酸氧化率计算的平均需要量是 0.92 g/kg·d，人群安全需要量是 1.10 g/kg·d。结论：中国青年女性在非月经期对日常膳食蛋白质的需要量低于目前的膳食推荐摄入量。女性在整个月经周期中的膳食蛋白质需要量，尚需进一步研究探讨。

關鍵字：蛋白质需要量、指標氨基酸氧化法、混合膳食、安定同位素、婦女