Original article

Assessment of the lysine requirement of healthy young male adults through albumin and IgG in plasma

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Abstract. *Background and aim:* The lysine requirement from the indicator amino acid oxidation (IAAO) method has not been validated with the measurement of health. The aim of the study was to assess the lysine requirement of healthy young male adults from the IAAO technique through the concentrations and the fractional synthesis rates (FSRs) of albumin and IgG in the plasma. *Methods:* Five healthy young men participated in the self-controlled study. The intakes of lysine, weight and body composition, concentrations and FSRs of albumin and IgG in the plasma of the subjects were determined both in the free-living, self-selected diets and in the controlled lysine diets (controlled for 10 weeks, lysine intake was equal to the lysine requirement from the IAAO method of 70 $mg \cdot kg^{-1} \cdot d^{-1}$). *Results:* Although the intakes of lysine, plasma concentrations of IgG and FSRs of albumin and IgG were all significantly lower in the controlled lysine diets ($P<0.05$), the plasma concentrations of albumin and IgG were still within the normal ranges, and the weight and body composition of the subjects did not change significantly ($P>0.05$). *Conclusions*: A lysine intake of 70 $mg \cdot kg^{-1} \cdot d^{-1}$ from the IAAO method was sufficient to keep the plasma concentration of albumin and IgG of the young male adults in the normal range for at least 10 weeks.

Key words: Lysine requirement, Plasma concentration, Fraction synthesis rate, Albumin, IgG

Introduction

The requirement for lysine has received attention because lysine is an essential amino acid for human and is the first limiting amino acid in cereals, especially wheat and rice, which are staple foods in many countries. The methods for determining the necessary amino acid requirements have progressed from the classical nitrogen balance to the current stable isotope-based methods (1). Indicator amino acid oxidation (IAAO) method has been more widely used for adults, children and neonates because of its advantages such as minimal invasion and relatively simple procedures (2,3). In our previous study, we examined the lysine requirement of Chinese healthy young male adults with a 6-day habitual dietary adaptation period for each lysine level by the IAAO method and got the upper 95% confidence interval of lysine requirements 70 $mg \cdot kg^{-1} \cdot d^{-1}$(4).

However, a central problem with most isotopic studies is the difficulty in determining the enrichments of labelled precursors at the sites of their oxidation (5). To date, no method is perfect to provide comprehensive data on amino acid requirements (5). It must be recognized that the requirements derived by the stable isotope-based methods have not been validated in any entirely satisfactory way, i.e. in long-term studies at the requirement intakes with measurement of health (6,7). Lysine is indispensable because it cannot be synthesized de novo in mammals, and is essential for the synthesis of any protein in the body (8-11). Therefore,

it is necessary to evaluate the lysine requirement derived from the IAAO method from the whole-body or tissue-specific protein synthesis and metabolism (12).

Proteins and antibodies in blood are relatively more responsive to dietary protein and amino acids, particularly in humans (8,13). Plasma concentrations of albumin and IgG are usually taken as indicators of the effects of dietary lysine on health (13,14). Human studies have shown that the fractional synthesis rate (FSR) of plasma albumin decreases if dietary protein is deficient, but that the plasma concentration of albumin does not change (15). Similar results were showed in our previous rat experiment (16). We found that the plasma concentration of IgG in the low lysine group of the male adult Sprague–Dawley rats was significantly lower than that in the normal lysine group, but there was no significant difference in the plasma concentration of albumin between the two groups (16). In the meantime, the FSRs of albumin and IgG in plasma labelled by heavy water (^2H_2O) had a positive correlation with the dietary intake of lysine of the rats, suggesting that the FSRs of albumin and IgG in plasma were more sensitive to dietary intake of lysine than their concentrations (16). In addition, albumin and IgG are both high in the blood, with the albumin accounting for 60% and IgG accounting for 15% of all the plasma proteins in mammals, respectively (17,18). The abundances of the two plasma proteins in the blood and the well-developed methods for isolation and purification of them make them more readily accessible (19,20).

Therefore, in the present self-controlled study, we examined the concentrations and FSRs of albumin and IgG in plasma of the healthy young male subjects both in the free-living, self-selected diets and in the controlled lysine diets. We sought to ascertain if the lysine requirement of the Chinese healthy young male adults determined by the IAAO method could meet the synthesis metabolism of the two plasma proteins.

Materials and methods

Subjects

Eleven adult male volunteers from Yangzhou University were screened for study recruitment. 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 and blood tests (activity of liver enzymes, concentrations of albumin, hemoglobin, urea, creatinine and uric acid). Exclusion criteria were as follows: picky eaters, taking any nutritional supplements in the past three months, smoking or alcohol consumption in the past three months, weight loss (>5% change) or insomnia in the past three months, regular physical exercise, chronic disease and endocrine disorder. Five eligible volunteers were finally recruited for study participation. 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, Yangzhou University was also obtained (NO. 20140305). The study protocol was approved by the Committee of the National Natural Science Foundation of China (NO. 81472963). All procedures were carried out in accordance with the standards stated in the 2013 version of the Helsinki Declaration.

Study design

There were two stages in this self-controlled study. In the first stage (from November to December, 2016), the subjects were allowed to eat and drink in free living conditions and maintain a light physical activity level for 5 weeks. They were weighed and examined for body composition (EX/COM DF850 body composition analyser, Japan) based on the bioelectrical impedance analysis every weekend. On the first morning of the experiment, a 10-hour fasting venous blood was collected as baseline samples. Then the subjects were asked to consume the loading dose of ^2H_2O (60 ml of 70 atom % ^2H_2O each time, Cambridge Isotope Laboratories) at 8:00 (after breakfast), 10:00, 12:00 (after lunch), 14:00, 16:00 and 18:00 (after dinner), respectively. Over the following 5 weeks, the subjects drank 60 ml 70% ^2H_2O twice a day (after breakfast and dinner, respectively). A 10-hour fasting venous blood samples were collected every weekend for the 5 weeks of ^2H_2O intake.

A 12-month washout period preceded the second ^2H_2O labelling stage (from November to December, 2017). In the second stage of 10 weeks, the lysine intake

of every subject was limited to 70 $mg \cdot kg^{-1} \cdot d^{-1}$. The first five weeks were the adaptation period, and the last five weeks were ^2H_2O labelling period, the labelling method was the same as in the first stage. Food and blood samples were collected and weight and body composition of the subjects were measured, also as in the first stage.

Diets

In the first stage, the subjects were asked to have duplicate portions of meals, beverages and snacks for three consecutive days each week. One portion was consumed by the subjects and the other was collected for laboratory analysis. All foods and drinks in each meal were weighed accurately and recorded both before and after consumption by each subject to determine the actual intake. Each food sample was analysed for lysine, total nitrogen, fat, carbohydrate, water and ash.

Diets at the second stage were provided in the form of Chinese habitual mixed foods according to the Chinese dietary guidelines (21) with a six-day rotation of menus (including meat, fish, shrimp, egg, milk, soy products, vegetables, fruits, rice and wheat flour) (Table 1). The dietary structure was similar to that in our previous IAAO study (4). The everyday lysine intake of each subject 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 the Schofield equations for prediction of the basal metabolic rate of Chinese adults based on the individual's age, sex and weight. Basal metabolic rate value was multiplied by an activity coefficient of 1.5 to obtain an appropriate energy intake. The proportions of total energy intake provided by carbohydrates, fats and proteins were 50% - 65%, 20% - 30% and 10% - 20%, respectively (21). Proteins from animal foods and soy products made up 40% - 50% of the total protein. The weight of each food on the menu was calculated according to the data of lysine content in the China Food Composition (22). Food samples were weighed and tested in the same way as in the first stage.

Physical activity record

During the two stages, the subjects were instructed to record their physical activities in 24h every day. The duration of each activity was recorded, and the energy expenditure per unit time of the activity was multiplied by the duration of it to get the total energy expenditure of the activity. The energy expenditure of every physical activity during the day was added to obtain the total physical activity energy expenditure in a day.

Food and blood analysis

Foods

Food samples were stored at −20 °C until analyses. The total lysine in the food samples was examined using a high-performance liquid chromatography (Agilent Technologies 1100), nitrogen content was determined by Kjeldahl analysis, fat content was measured by Soxhelt extraction method and acid hydrolyzation method, the contents of water and ash were detected by weighing method and carbohydrate content was determined by the subtraction method. The energy intake was calculated according to the formula: Energy intake (kcal/d) = protein intake (g/d) × 4 kcal/g + fat intake (g/d) × 9 kcal/g + carbohydrate intake (g/d) × 4 kcal/g (22).

Blood

Blood samples (15 mL) were collected in ethylenediamine tetraacetic acid (EDTA) anticoagulant tubes. Plasma was separated by low-speed centrifugation and stored at −80 °C until analyses. The blood samples were examined as described previously (16). In brief, the concentration of lysine in plasma was determined by high-performance liquid chromatography (Agilent Technologies 1100). The content of lysine were determined by the external standard method (23). The content of albumin, IgG and total protein in plasma was measured using enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems) according to the manufacturer's instructions. Albumin in the plasma was isolated by the trichloroacetic acid-ethanol method (16,24). IgG in the plasma was purified on the chromatography system (BioLogic DuoFlow™ 10, Bio-Rad Laboratories) connected to a HiTrap Protein G HP column (GE Healthcare) according to the manufacturer's instructions. Purity of the albumin and IgG was

Table 1. Parts of the Menus of the 3rd Week in the Two Stages for Subject NO.5.

		Stage 1	Stage 2
Day 1	Breakfast (g)	Oatmeal with milk added (270)	Soybean milk (200) Deep-fried dough sticks (80) Boiled egg (50)
	Lunch (g)	Barbecue (176) Rice (300)	Braised beef (120) with potatoes (80) Stir fried vegetables with mushrooms (120) Rice (400) Winter jujube (50)
	Supper (g)	Barbecue (312) Rice (300) Instant noodles (662) Ham sausage (86) Boiled egg (37)	Braised pork (20) with eggplant (80) Stir fried shredded pork (45) with green pepper (135) Rice (350) Pear (250) Lotus root soup (100)
Day 2	Breakfast (g)	Jellied bean curd (412)	Soybean milk (130) Yogurt (110) Boiled egg (50) Steamed buns (110) Banana (160)
	Lunch (g)	Chicken and cauliflower (235) Steamed egg (139) Rice (343)	Stir fried diced chicken (50) with pleurotus eryngii (50) Stir fried cauliflower (80) Rice (400) Apple (200) Lotus root soup (50)
	Supper (g)	Braised chicken (604) Rice (295)	Stir fried pork liver (40) with carrot (40) Stir fried pork (60) with lotus root slices (120) Rice (400)
Day 3	Breakfast (g)	Ham sausage (80) Boiled egg (41) Noodles (317)	Soybean milk (200) Yogurt (160) Boiled egg (50) Steamed buns (120) Steamed sweet potato (60) Orange (140)
	Lunch (g)	Braised chicken (250) Rice (334)	Braised prawns with oil (60) Sauteed celery (100) with dried tofu slices (50) Rice (400) Apple (200)
	Supper (g)	Jellied bean curd (502) Vegetable buns (170)	Stir fried pork (55) with garlic bolt (110) Stir fried shredded potato with green pepper (200) Rice (400)

verified in randomly selected samples using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS–PAGE) (19,25). The ^{2}H enrichments in alanine from albumin, IgG and free alanine in plasma were assayed by the gas chromatography-masss spectrometry (GC-MS) (Trace DSQ II, Thermo Fisher Scientific) with a DB17-MS capillary column (30 m × 0.25 mm × 0.25 μm) (Agilent Technologies) after acid hydrolysis of albumin and IgG and derivation of alanine (25-27).

Calculations

The enrichment of ^{2}H-alanine derivatives was given by (24):

$$\frac{\text{Peakarea of } {}^2\text{H}-\text{alanine derivatives}}{\text{Peakarea of H}-\text{alanine derivatives} + \text{peakarea of } {}^2\text{H}-\text{alanine derivatives}} \times 100\%$$

A = ^{2}H enrichment of free alanine in plasma,

B = ^{2}H enrichment of alanine in plasma albumin or IgG,
C = ln (A – B)

The values of C and time (week) were fitted to a linear regression line. The slope of the line was equal to the FSR of the plasma albumin or IgG, i.e., the fraction of the albumin pool or IgG pool synthesized per week as expressed in %/week by multiplied by 100%.

Statistical analysis

Results are expressed as the means ± standard error of the differences (SED). Paired-*t* tests were performed to compare the body weights, body compositions, the intakes of dietary energy and nutrients, the concentrations of blood lysine and proteins and the FSRs of albumin and IgG of the first and second stages. Statistical significance was set at $P<0.05$. Data were analysed using SPSS Statistics (version 20.0, IBM).

Results

Subject characteristics

The characteristics of the subjects were: age 20.0 ± 1.7 years, weight 66.2 ± 6.4 kg, height 1.73 ± 0.04 m and BMI 21.99 ± 1.72 kg/m^2.

Intakes of dietary nutrients and energy

The intake of lysine in the stage 1 was significantly higher than that in the stage 2 ($P<0.05$), while the intakes of protein, fat and energy were significantly lower in the first stage ($P<0.05$). There was no significant difference in the two stages for the percentage of energy from protein ($P>0.05$), but the percentages of energy from fat and carbohydrate were significantly different ($P<0.05$) (Table 2). The energy proportions from protein, fat and carbohydrate in the second stage were all reasonable according to the Chinese Dietary Guidelines (21), while the percentage of energy from fat was below the acceptable macronutrient distribution ranges (AMDR) (20%-30%) and the energy proportion from carbohydrate was beyond the AMDR (50%-65%) in the first stage (28).

Weights and body compositions

As shown in Table 3, there were no significant differences for the weights and body compositions of the subjects in the two stages ($P>0.05$).

Physical activity energy expenditure

The subjects expended 327.94±24.71 kcal/d and 330.99±27.46 kcal/d for physical activity in stage 1 and stage 2, respectively. There was no significant difference in the two stages ($P>0.05$).

Table 2. Intakes of Lysine, Macronutrients and Energy of the Subjects.

	Stage 1	**Stage 2**	**Stage1-Stage2**	*P*
Lysine (mg·kg^{-1}·d^{-1})	74.21±5.86	68.06±1.02	6.14±3.52	0.02
Protein (g/d)	74.84±3.83	87.40±2.23	-18.23±11.47	0.02
Percentage of energy from protein (%)	13.52±0.27	13.34±0.12	-0.62±1.60	0.43
Fat (g/d)	37.83±1.89	71.35±1.70	-42.55±10.98	0.00
Percentage of energy from fat (%)	15.39±0.41	24.50±0.28	-12.72±3.95	0.00
Carbohydrate (g/d)	386.47±14.94	407.24±10.92	-50.31±48.74	0.08
Percentage of energy from carbohydrate (%)	69.98±1.09	62.15±0.37	13.36±4.45	0.00
Energy (kcal/d)	2210.30±86.55	2632.40±48.88	-570.55±325.86	0.02

Values are the mean ± SED, n = 5. Statistical significance is set at $P<0.05$.

Table 3. Weights and Body Composition of Subjects.

	Stage 1	Stage 2	Stage1-Stage2	*P*
Weight (kg)	66.18±2.85	69.00±2.01	-2.81±5.29	0.30
Muscle mass (kg)	25.33±0.22	24.93±1.16	0.41±2.77	0.76
Percentage of muscle (%)	38.24±1.41	37.00±2.23	1.22±2.89	0.40
Fat mass (kg)	13.86±0.10	15.48±1.11	-1.62±2.46	0.21
Percentage of fat (%)	20.48±2.54	22.60±1.97	-2.10±4.14	0.32

Values are the mean ± SED, n = 5. Statistical significance is set at $P<0.05$.

Table 4. 10-hour Fasting Plasma Concentrations of Lysine, Albumin, IgG and Total Protein of the Subjects.

	Stage 1	Stage 2	Stage1-Stage2	*P*
Lysine (mmol/L)	56.63±0.59	52.79±1.24	4.05±0.04	0.03
Albumin (g/L)	36.96±3.42	35.69±1.47	1.27±6.76	0.70
IgG (g/L)	9.23±0.29	7.86±0.43	1.14±0.33	0.00
Total protein (g/L)	70.65±3.25	72.41±2.83	-1.76±4.43	0.42

Values are the mean ± SED, n = 5. Statistical significance is set at $P<0.05$.

Table 5. ^{2}H Enrichment in Free Alanine in Plasma (A value) and in Alanine from Albumin and IgG in Plasma (B Value), the C Value of Albumin and IgG and the FSRs of Albumin and IgG in Plasma of the Subjects.

	Stage 1	Stage 2	Stage1-Stage2	*P*
A Value (%)	12.59±0.47	12.33±0.18		
B Value (%)				
albumin	9.75±0.18	9.82±0.28		
IgG	10.77±0.18	10.91±0.36		
C Value (%)				
albumin	-379.66±16.13	-380.56±13.15		
IgG	-437.34±22.84	-443.89±31.03		
FSR (%/week)				
albumin	47.46±3.24[a]	28.24±1.89[b]	0.19±0.14	0.00
IgG	59.76±6.07[a]	26.27±3.78[b]	0.33±0.07	0.00

Values are the mean ± SED, n = 5. Statistical significance is set at $P<0.05$.

Concentrations of lysine and proteins in the plasma

The concentrations of lysine and IgG in the plasma in the stage 2 were significantly lower than those in the stage 1 ($P<0.05$). There were no significant differences for the plasma concentrations of albumin and total protein in the two stages ($P>0.05$) (Table 4).

The FSRs of albumin and IgG

Table 5 shows that the FSRs of albumin and IgG in the plasma were both significantly lower in the stage 2 than those in the stage 1 ($P < 0.05$).

Discussion

There are some environmental factors affecting amino acids requirements, for example, the low or high ambient temperatures (5). Because the utilization and deposition of proteins are energy-dependent at all stages of amino acid transport and transformation, protein synthesis and proteolysis, the changes of the ambient temperature can affect the energy consumptions, and then affect the metabolism of protein and amino acid in the subjects (6). Therefore, in this self-controlled study, the $^{2}H_2O$ labelling period in the two stages were both designed at the same season (from

November to December) for two consecutive years (2016-2017) to eliminate the effects of climate and temperature on the energy metabolism of the subjects. There was no significant difference in temperature of November between the two years (11.2°C±4.4°C in 2016 vs. 11.6°C±3.6°C in 2017, *P*=0.67), while there was significant difference in temperature of December between the two years (6.7°C±3.2°C in 2016 vs. 5.0°C±2.5°C in 2017, *P*=0.02). However, because the subjects could adjust their clothing and spent most of the day indoors with air conditioning at a constant temperature of 20°C to maintain comfort during the study, the additional energy cost of thermoregulation rarely had an appreciable effect on total energy expenditure (29) and so the effect of ambient temperature on the metabolism of amino acids could be ignored.

All the subjects had the same eating habits in the stage 1. As shown in table 1, the food types of one of the subjects in his self-selected meals (stage 1) were much fewer than those in the controlled lysine diets (stage 2). He ate much meat, but seldom ate vegetables, fruits, tubers and dairy products in the stage 1. The main reasons were: firstly, the subjects did not know how to achieve a balanced diet and chose food only according to their dietary preferences; secondly, the subjects bought food at their own expense and could not afford some expensive food such as fruits and dairy productions. On the contrary, the menus of the stage 2 were designed according to the Chinese dietary guidelines21 and the dietary structure was similar to that in our previous IAAO study. All foods in the stage 2 were provided to the subjects free of charge, so the dietary structure and the proportions of energy from protein, fat and carbohydrate of the subjects were more reasonable than those in the stage 1. In addition, the intake of meat in the stage 1 were more than two times of that in the stage 2. Because meat such as pork, beef and chicken is rich in lysine (30), eating more meat resulted in significantly higher lysine intake in the stage 1 compared to that in the stage 2. Moreover, with the increasing intakes of vegetables, fruits and tubers in the stage 2, the intake of dietary fiber increased which could interfere with protein digestion and lysine absorption. Accordingly, the concentrations of lysine and IgG and the FSRs of albumin and IgG in the plasma were all significantly higher in the stage 1. It suggested that the difference in the intake of dietary fiber in the two studies might have an effect on the absorption of lysine. So the relationship between dietary fiber intake and lysine absorption should be further studied. Furthermore, although the actual lysine intake in the stage 2 (68 $mg \cdot kg^{-1} \cdot d^{-1}$) were somewhat lower than the designated level (70 $mg \cdot kg^{-1} \cdot d^{-1}$), there was no significant difference between the two lysine intakes by one-sample T test (*P*=0.13).

Muscles of healthy individuals exist in dynamic equilibrium whereby the influx of amino acids associated with the intake of dietary proteins canceling out the efflux of amino acids from muscle protein breakdown that occurs between meals (31). It can be seen from table 2 and table 3 that the lysine intake of the subjects in the stage 2 were significantly lower than that in the stage 1, while the muscle mass and percentage of muscle of the subjects in the two stages were not significantly different. The protein intakes in the two stages were both above the recommended protein intake of 65g/d for the Chinese male adults aged from 18 to 50 years old (28), indicating that the protein intakes of the subjects were adequate. Therefore, the results suggested that lysine was preferred to maintain a stable muscle mass rather than supporting protein synthesis in plasma such as albumin and IgG when lysine intake was reduced. The results of the present study were consistent with our previous animal study, which showed that the FSR of quadriceps femoris protein in the low lysine rat group was similar to that in the normal lysine rat group. The result was partly responsible for the decrease of the FSRs of albumin and IgG in the plasma in the stage 2. Moreover, it is known that increased intake of energy can lead to weight gain. The intakes of energy and fat of the subjects in the stage 2 were significantly higher than those in the stage 1, and there was no significant difference for the physical activity energy expenditure in the two stages, but there were no significant differences in the body weight, fat mass and percentage of body fat between the two stages, in despite of a tendency to increase in the stage 2. This might because the intervention time in the stage 2 was not long enough.

It can be seen from table 2 and table 5 that the lower lysine intake resulted in the significantly lower FSRs of albumin and IgG in plasma in the stage 2 compared to those in the stage 1. The present results were consistent with those in our previous rat

experiment that decreasing dietary intake of lysine could reduce the FSRs of albumin and IgG in plasma (16). The FSR of a protein is the rate at which the enrichment of an isotope-labelled amino acid is incorporated into the protein relative to the total enrichments of the amino acid pool per unit time (32). Because amino acids are the anabolic constituents of proteins, the decrease of FSR means the decrease of protein synthesis in the body. This was probably the main reason for the significantly lower concentration of IgG in plasma at the lysine intake of 70 $mg \cdot kg^{-1} \cdot d^{-1}$. The concentration of albumin in the plasma did not decreased significantly, probably because the fractional decomposition rate of albumin decreased and the half-life of albumin prolonged when the intake of lysine was reduced (33,34). It was reported in a human experiment that lysine-fortified wheat flour significantly increased the plasma concentration of IgG in Chinese men consuming diets in which 58% to 67% of the protein originated from wheat, but there was no significant difference between the control and the lysine fortification group in albumin concentration (14). Both the results of the above study and the present study suggested that the plasma concentration of IgG might be a sensitive indicator to intake of dietary lysine.

Although the FSRs of albumin and IgG in the plasma were significantly lower at the lysine intake of 70 $mg \cdot kg^{-1} \cdot d^{-1}$, the plasma concentrations of the two proteins were still within the normal ranges. And the body weights and body compositions of the subjects did not change significantly. Therefore, the results suggested that the lysine intake of 70 $mg \cdot kg^{-1} \cdot d^{-1}$ determined by the IAAO method was sufficient to the subjects during the 10 weeks of the experiment. But it is not certain that the plasma concentrations of the two proteins, especially IgG, will remain stable if the intake of lysine at 70 $mg \cdot kg^{-1} \cdot d^{-1}$ is maintained over a longer period of time.

For financial reasons, the sample size of the present study was small, and there were some limitations in the self-controlled study, for example, the environmental temperatures were different in the two stages. So it is necessary to further expand the sample size and adopt cross-over study (35) to determine whether lysine requirement from IAAO method can meet human health needs.

Conclusion

The lysine requirement of 70 $mg \cdot kg^{-1} \cdot d^{-1}$ determined by the IAAO method was sufficient for the healthy young male adult subjects for at least 10 weeks, and the plasma concentration of IgG might be a sensitive indicator to intake of dietary lysine.

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Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

Abbreviations:

^2H_2O: heavy water
AMDR: acceptable macronutrient distribution ranges
EDTA: ethylenediamine tetraacetic acid
ELISA: enzyme-linked immunosorbent assay
FSR: fractional synthesis rate
GC-MS: gas chromatography-masss spectrometry
IAAO: Indicator amino acid oxidation
SDS–PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SED: standard error of the differences

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