Tyrosine requirement of healthy men receiving a fixed phenylalanine intake determined by using indicator amino acid oxidation[1–4]

Susan A Roberts, Jane M Thorpe, Ronald O Ball, and Paul B Pencharz

ABSTRACT

Background: The currently accepted total aromatic amino acid requirement for adults is based on nitrogen balance measurements in individuals who received their intake of aromatic amino acids solely as phenylalanine.

Objective: The objective of this study was to determine the requirement for the amino acid tyrosine in healthy men receiving an adequate, but not excessive, intake of phenylalanine (9 $mg \cdot kg^{-1} \cdot d^{-1}$).

Design: The effect of a graded intake of tyrosine was determined in 6 healthy men consuming energy-sufficient diets containing 1 g protein $\cdot kg^{-1} \cdot d^{-1}$. The tyrosine requirement was determined by using indicator amino acid oxidation methodology with L-[1-^{13}C]lysine as the indicator. Subjects were studied at each of 7 tyrosine intakes.

Results: A graded intake of tyrosine had no effect on lysine flux. The mean tyrosine requirement was determined from the response of the oxidation of L-[1-^{13}C]lysine to breath $^{13}CO_2$. A 2-phase linear regression crossover analysis of breath $^{13}CO_2$ identified the breakpoint and upper 95% confidence limit, which represents the mean and safe intakes, to be 6.0 and 7.0 $mg \cdot kg^{-1} \cdot d^{-1}$, respectively.

Conclusions: The safe intake of total aromatic amino acids calculated from the present results for tyrosine and our previous estimate for phenylalanine is estimated to be 21 $mg \cdot kg^{-1} \cdot d^{-1}$. This intake is 1.5 times the currently recommended total aromatic amino acid intake of the FAO/WHO/UNU (1985), 14 $mg \cdot kg^{-1} \cdot d^{-1}$. Furthermore, the absolute aromatic amino acid requirement may be dependent on the proportional balance of these amino acids in the diet. *Am J Clin Nutr* 2001;73:276–82.

KEY WORDS Amino acid metabolism, aromatic amino acids, stable isotopes, adult amino acid requirements, phenylalanine, tyrosine, men

INTRODUCTION

In the past decade there has been a resurgence of interest in aromatic amino acid requirements (1–9). These studies focused largely on the phenylalanine requirement in persons consuming a diet either lacking or low in tyrosine (2–4, 8). From the results of these studies, a total aromatic amino acid requirement was estimated; however, it was assumed that phenylalanine can provide the entire tyrosine requirement as well as can a balanced intake of phenylalanine and tyrosine.

To our knowledge, the study by Zello et al (1) is the only investigation in adult humans that examined the body's minimum phenylalanine requirement under conditions of excess tyrosine intake. In the presence of excess tyrosine, a phenylalanine intake above that required is believed to be preferentially oxidized before first equilibrating with the whole-body tyrosine pool (10, 11). Zello et al's design isolated the component of phenylalanine requirement from that of total aromatic amino acids. The estimated mean requirement and safe (upper 95% confidence limit) phenylalanine intake derived from this study were 9.1 and 14 $mg \cdot kg^{-1} \cdot d^{-1}$, respectively.

Tyrosine is a conditionally indispensable amino acid. Although it is indispensable in neonates (12), it is a dispensable amino acid in healthy adults because it can be synthesized through hepatic phenylalanine hydroxylation (13) when sufficient phenylalanine is provided in the diet. However, in conditions known to impair aromatic amino acid metabolism, such as hepatic and renal disease, a preformed balanced source of the amino acid may be required to minimize aromatic amino acid excess and catabolism.

The goal of this study was to estimate, for the first time, tyrosine requirements in adults at fixed and adequate, but not excessive, phenylalanine intakes. An indicator amino acid oxidation technique was used, with L-[1-^{13}C]lysine as the indicator. This study was the first to use the indicator amino acid oxidation technique to determine the requirements of a dispensable amino acid. In addition, this study was the first to use lysine as an indicator of amino acid requirements in adult humans; we previously used

[1] From the Departments of Nutritional Sciences and Paediatrics, University of Toronto; The Research Institute, The Hospital for Sick Children, Toronto; and the Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton, Canada.

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[4] Address reprint requests to PB Pencharz, Division of Gastroenterology and Nutrition, The Hospital for Sick Children, 555 University Avenue, Toronto, Canada M5G 1X8. E-mail: paul.pencharz@sickkids.on.ca.

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TABLE 1
Individual characteristics and energy intakes of healthy men

Subject	Age	Weight	Height	Energy intake[1]
	y	*kg*	*m*	*MJ/d*
1	21	79.5	1.65	13.5
2	35	95.0	1.81	13,5
3	29	80.0	1.85	13.5
4	20	89.5	1.78	14.6
5	43	67.5	1.66	11.6
6	28	62.5	1.64	11.6
$\bar{x}$ ± SD	29 ± 9	79 ± 12	1.73 ± 0.1	13.1 ± 1.2

[1] Calculated by using WHO/FAO/UNU (1985) predictive equations with an activity factor of 1.7.

lysine as an indicator to determine tyrosine requirements in children with phenylketonuria (9). In addition to estimating the tyrosine requirement, we used our previous estimated minimum phenylalanine requirement (1) to provide an estimate of total aromatic amino acid requirements.

SUBJECTS AND METHODS

Subjects

Six healthy men with a mean (±SD) age of 29 ± 9 y were enrolled in the study. Subjects participated in the study as outpatients; all studies were carried out in the Clinical Investigation Unit at The Hospital for Sick Children, Toronto. Subjects were excluded from the study if they had a chronic disease, had a history of weight loss, had an endocrine disorder, or were receiving drug therapy. The purpose of the study and the potential risks associated with the protocol were explained in detail to the subjects. Participants were encouraged to maintain their usual physical activities throughout the study period. Written, informed consent was obtained from all subjects. The study protocol was approved by both the University of Toronto Human Experimentation Committee and the Human Review Committee of The Hospital for Sick Children. All volunteers were remunerated for their participation. Subject characteristics are summarized in **Table 1**.

Experimental design and dietary intake

In our earlier study (1) we established a mean phenylalanine requirement of 9.1 $mg \cdot kg^{-1} \cdot d^{-1}$ and an upper 95% CI of 14 $mg \cdot kg^{-1} \cdot d^{-1}$ under conditions of excess tyrosine intake (40 $mg \cdot kg^{-1} \cdot d^{-1}$). In the present study we chose to use a fixed phenylalanine intake of 9.0 $mg \cdot kg^{-1} \cdot d^{-1}$, which approximates the average minimal phenylalanine requirement (1). We reasoned that although a lower or a higher phenylalanine intake would alter the estimate of tyrosine needs, the average phenylalanine intake should allow extrapolation of the data to an estimate of total aromatic amino acid requirements.

Each subject was allocated to receive each of 7 different tyrosine intakes in random order. Studies were separated by ≥1 wk and all studies were carried out within 3 mo. For 2 d before each study day, subjects consumed a controlled formula that provided adequate energy and protein intakes. The basal metabolic rate was determined first by using predictive equations for each subject (14). The basal metabolic rate for each subjects was multiplied by 1.7 to determine total daily energy needs and hence the amount of energy to provide the subjects. Protein was provided at an intake of 1 $g \cdot kg^{-1} \cdot d^{-1}$. The formula was a low-protein milk shake (Scandishake; Scandipharm, Inc, Birmingham, AL) prepared with skim milk and added carbohydrate (Caloreen; Baxter Corporation, Mississauga, Canada) and protein (Promod; Ross Laboratories, Columbus, OH) to tailor the formula to the exact energy and protein intakes required. The diet was given as 4 meals spread throughout the day. Except for water, no additional food or beverages were consumed.

Starting at 0600 on the study day, subjects consumed the same protein and energy intakes in the form of a liquid formula and protein-free cookies (15). Meals were provided hourly for 12 h, each meal providing one-twelfth of the daily requirement. The diet provided 37% of energy as fat, 53% as carbohydrate, and 10% as protein. The main source of energy came from a nonprotein liquid formula (product 80056, Protein-Free Powder; Mead Johnson, Evansville, IN). The nitrogen component was provided as crystalline L-amino acids with an amino acid profile reflecting that of egg protein. The only amino acids that diverged from this profile were phenylalanine, lysine, tyrosine, and alanine. The phenylalanine intake was fixed at 9.0 $mg \cdot kg^{-1} \cdot d^{-1}$. The phenylalanine intake was chosen to represent the estimated mean adult requirement based on direct amino acid oxidation methods (1). The intake of the indicator amino acid for the study, lysine, was fixed at 45 $mg \cdot kg^{-1} \cdot d^{-1}$, the estimated safe intake for men (14, 15). Subjects received each of 7 tyrosine intakes (3.0, 4.5, 6.0, 7.5, 9.0, 10.5, and 12.0 $mg \cdot kg^{-1} \cdot d^{-1}$) in random order. Alanine intake was adjusted to maintain a constant nitrogen intake.

Tracer protocol

The isotopically labeled compounds used in the study were $NaH^{13}CO_3$ (99% ^{13}C; Isotec Inc, Miamisburg, OH) and L-[1-^{13}C]lysine (99% ^{13}C; Isotec Inc). Quality-control tests were performed by the manufacturer of the isotopes. Chemical purity and the specified isotopic enrichment and position were confirmed by nuclear magnetic resonance, whereas the isomeric purity was determined with gas chromatography–mass spectrometry (GC-MS). Solutions of each tracer were prepared in deionized water and stored at −20°C until used. Before being dispensed, isotope solutions were sterilized by passage through a 0.22-μm filter (Millipore Corporation, Bedford, MA).

The oral tracer-infusion protocol began at 1000 on the study day after the subjects underwent adaptation to enteral intake for 4 h, which ensured sufficient time for $^{13}CO_2$ background equilibration in expired carbon dioxide (16). A bolus dose of 0.172 mg $NaH^{13}CO_3$/kg and 2.4 mg L-[1-^{13}C]lysine/kg was given to prime the bicarbonate and lysine pools, respectively. In addition to the priming dose, the subjects were given 1.2 mg L-[1-^{13}C]lysine/kg hourly throughout the remaining 6 h of the study. Isotopes were given with the hourly meals.

Sample collection

Three baseline blood and expired breath $^{13}CO_2$ samples were collected 10, 20, and 30 min before the isotope protocol began. Blood and breath samples were collected at isotopic steady state (plateau) every 30 min during the period 210–330 min after the isotope protocol began. Blood specimens were sampled from a 21-gauge needle inserted into a superficial dorsal vein on the hand. The blood in the hand region was arterialized by placing the subject's hand in a warming device (17) maintained at 60°C for 15 min before sample collection. Blood specimens (2 mL)

were drawn into heparin-containing syringes (Aspirator; Marquest Medical Products, Inc, Englewood, CO) and immediately placed on ice until centrifuged. Plasma was separated from the sample by centrifugation at 1500 × *g* for 20 min at 4 °C and then was transferred into microcentrifuge tubes and stored at −20 °C until analyzed for L-[1-^{13}C]lysine enrichment and phenylalanine and tyrosine concentrations.

The production rate of expired carbon dioxide ($\dot{V}CO_2$) was measured during the baseline period of each study day with an indirect calorimeter (2900 Computerized Energy Measurement System; Sensormedics, Yorba Linda, CA). Carbon dioxide was collected by bubbling a sample (500 mL/min) of the expired carbon dioxide collected from a ventilated mask through a modified reflux container containing 10 mL NaOH for 7 min (18). The sample was subsequently transferred into collection tubes (Vacutainer 6441, 100 × 16 mm; Becton Dickinson Inc, Mississauga, Canada) for storage at −20 °C until analyzed for ^{13}C enrichment.

Analytic procedures and calculations

The enrichment of plasma amino acids was analyzed by the method of Patterson et al (19) by using gas chromatography–mass spectrometry. Briefly, plasma samples (200 μL) were deproteinized with an equal volume of 20% (wt:vol) trichloroacetic acid followed by centrifugation at 9000 × *g* for 5 min at 23 °C in microcentrifuge tubes. The supernate was transferred from the tubes to columns containing a cationic ion exchange resin (1.5 mL) (Dowex 50W-X8, 100–200 mesh, H^+ form; Bio-Rad Laboratories, Hercules, CA) for amino acid separation. The effluent solution was freeze-dried (Freezone 12 L; Labconco Corp, Kansas City, MO) before derivatization to its *N*,*O*-heptafluorobutyryl *n*-propyl esters.

The first step of the derivatization process involved esterification of the amino acids by adding 500 μL acetylpropanol prepared fresh each day by reacting acetyl chloride with propanol over ice at a ratio of 1:5. Samples were vortex mixed, heated at 110 °C for 1 h, and dried under a steady stream of nitrogen gas at <40 °C. After being dried, 50 μL of the derivative heptafluorobutyric anhydride was added to the samples, vortex mixed, and heated at 60 °C for 20 min. Samples were then dried, topped with nitrogen gas, and stored at −20 °C until analyzed. When ready for analysis, samples were reconstituted in 100 μL hexane.

Amino acid separation was performed on a gas chromatograph (GC model 5890 Series 2; Hewlett Packard, Mississauga, Canada) attached to a quadrupole mass spectrometer (Trio-2; Vacuum Generator Micromass, Cheshire, United Kingdom). Derivatized amino acids were splitlessly introduced into the instrument by automatic injector (model 7673 injector; Hewlett Packard). Separation was performed on a 30 m × 0.32 mm (inner diameter) × 1.0 μm (film thickness) fused silica capillary column (model HP-5; Hewlett-Packard) with helium serving as a carrier gas.

The GC column was coupled directly to the ion source set at a temperature of 160 °C and operated under conditions of negative chemical ionization. Ammonia was used as reactant gas. Selected ion chromatograms were obtained by monitoring the mass-to-charge ratio of 360 and 361 for the *M* and *M*+1 species of lysine, respectively. Areas under the peaks were integrated by using a Digital DECp 450D$_2$LP computer (Digital Instruments, Santa Barbara, CA) with Lab-Base software (Biotech, Cheshire, United Kingdom). Concentrations of phenylalanine and tyrosine were determined by GCMS by using the internal standard technique described by Wolfe (20).

Expired $^{13}CO_2$ enrichment was measured by isotope ratio mass spectrometry as described previously (18). $\dot{V}CO_2$, collected from a ventilated-hood system was measured indirectly by using a carbon dioxide analyzer (1400 series; Servomex; Westech Industrial Ltd, Mississauga, Canada) and mass flow meter (5860 series, Brooks; Trillium Measurement and Control, Stouffville, Canada). $^{13}CO_2$ enrichment was measured with a dual-inlet magnetic sector isotope ratio mass spectrometer (model 602D; Vacuum Generator Micromass, Cheshire, United Kingdom). Trapped carbon dioxide in the form of Na_2CO_3 was released by reacting a 0.5-mL sample with an equal volume of 85% phosphoric acid within an evacuated Rittenburg tube. The carbon dioxide gas was isolated from the aqueous phase by immersing the tube in a bath of methanol and dry ice to freeze the liquid, without restricting movement of the gas. Enrichment measures were expressed as atoms percent excess (APE) $^{13}CO_2$ over a reference standard of compressed carbon dioxide gas. Regular repeat analysis of a standard from the National Institute of Standards and Technology (NBS no. 20, PDB limestone; Gaithersburg, MD) gave a CV of 0.2%.

Model of amino acid metabolism and calculations

Whole-body lysine flux was calculated from the dilution of isotope in the body amino acid pool at isotopic steady state (21):

$$Q_{lys} = i\,[E_i/E_p - 1] \qquad (1)$$

where Q_{lys} is the rate of lysine flux, i is the isotope infusion rate ($\mu mol \cdot kg^{-1} \cdot h^{-1}$), E_i is the enrichment of the infused isotope (APE) and E_p is the enrichment of the amino acid in the plasma at isotopic steady state (plateau, in units of APE).

The rate of lysine oxidation was calculated as described by Matthews et al (21):

$$O_{lys} = \dot{V}^{13}CO_2(1/E_{lys} - 1/E_i) \times 100 \qquad (2)$$

where O_{lys} represents lysine oxidation and $\dot{V}^{13}CO_2$ represents the rate of $^{13}CO_2$ released by lysine tracer oxidation ($\mu mol \cdot kg^{-1} \cdot h^{-1}$) calculated with the following equation:

$$\dot{V}^{13}CO_2 = (\dot{V}CO_2)\,(ECO_2)\,(44.6)\,(60)/(W)\,(RF)\,(100) \qquad (3)$$

where $\dot{V}CO_2$ is in cm^3/min, ECO_2 is $^{13}CO_2$ enrichment in expired breath at isotopic steady state (APE), the constants 44.6 μmol/cm^3 and 60 min/h convert $\dot{V}CO_2$ to μmol/h, the factor of 100 changes APE to a fraction, W is the weight (kg) of the subject, and $^{13}CO_2$ retained by the body as bicarbonate is corrected by using a retention factor (RF).

Statistical analyses

The study followed a completely randomized design with tyrosine intake as the independent variable. A three-way analysis of variance was carried out to determine the effect of individual, order of study, and tyrosine intake on $\dot{V}^{13}CO_2$, oxidation, and lysine flux. Regression models (linear, quadratic, and linear regression crossover) (22) were fitted to the data to determine the relation that best described the response of the dependent variables $\dot{V}^{13}CO_2$ and lysine oxidation to increasing tyrosine intakes. The regression analyses were performed by the method of least squares by using the regression procedure of SAS (23). The best model was determined on the basis of factors relating to model fit and variation. Plasma amino acid concentrations were analyzed by two-way analysis of variance with tyrosine intake and

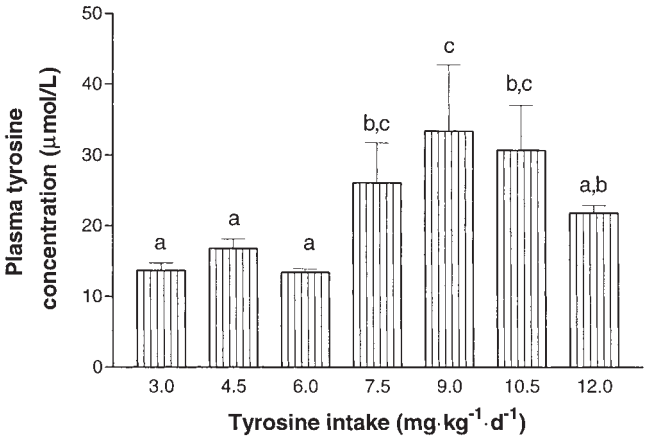

FIGURE 1. Mean (±SEM) plasma tyrosine concentrations in healthy men after 8 h of consuming the study diet. Bars with different superscript letters are significantly different, $P < 0.05$.

subject included in the model as main effects. When warranted, post hoc analysis was performed with Duncan's new multiple-range test. All statistical analyses and data modeling were performed by using SAS (23).

RESULTS

There were significant differences in plasma tyrosine concentrations after 8 h of feeding, which were highest at tyrosine intakes >6 mg·kg^{-1}·d^{-1}, except for the highest tyrosine intake (12 mg·kg^{-1}·d^{-1}), at which plasma tyrosine concentrations were not significantly different from those at an intake of 6 mg·kg^{-1}·d^{-1} (**Figure 1**). Although there were significant differences in the phenylalanine concentration after 8 h of feeding at different tyrosine intakes, there was no trend (**Figure 2**).

Lysine flux estimates are shown in **Table 2**. Tyrosine intake and order of study day did not affect lysine flux significantly within individuals. There was, however, a subject effect on lysine flux. There was a main effect of tyrosine intake ($P < 0.003$) on the estimate of $\dot{V}^{13}CO_2$, but not of individual or order. Data for the tyrosine intake of 3 mg·kg^{-1}·d^{-1} is missing for one subject because of a technical difficulty with the sample collection. The mean (±SEM) $\dot{V}^{13}CO_2$ values at each tyrosine intake are shown in **Figure 3**. In general, $\dot{V}^{13}CO_2$ decreased from tyrosine intakes of 3.0 to 6.0 mg·kg^{-1}·d^{-1}, after which point there was no change (slope not significantly differently different from zero) with further increases in tyrosine intake. Regression analysis showed that the two-phase linear regression crossover model was the best fit to the data. The breakpoint estimate, representative of the mean requirement, was estimated to be 6.0 mg·kg^{-1}·d^{-1}. The upper 95% CI, which was calculated from variation about the estimate and represented the safe population requirement, was estimated to be 7.0 mg·kg^{-1}·d^{-1}.

The oxidation rates of individual subjects are shown in **Table 3**. In general, the oxidation estimate, calculated from plasma lysine enrichment, was more variable than were the $\dot{V}^{13}CO_2$ values that were measured directly. Nevertheless, analysis of variance showed a significant effect of tyrosine intake and subject on lysine oxidation. However, the two-phase linear regression crossover analysis did not result in a significant model; therefore, estimates of requirements were not made with use of the oxidation estimate.

DISCUSSION

The present study estimated the mean tyrosine requirement of healthy men receiving an average intake of dietary phenylalanine to be 6 mg·kg^{-1}·d^{-1}. The safe population estimate of tyrosine intake calculated from the upper 95% confidence limit of the breakpoint analysis was 7 mg·kg^{-1}·d^{-1}. These data are important because they represent the first estimate of requirement of a dispensable amino acid in adult humans. The provision of excess tyrosine was shown to cause excess phenylalanine to be directly oxidized as opposed to equilibrating with the whole-body tyrosine pool (10, 11) and hence was available to meet tyrosine needs. This characteristic of phenylalanine metabolism within the hepatocyte enabled the need for phenylalanine to be distinguished from that for total aromatic amino acids. Fixing the phenylalanine intake at the average requirement provided an adequate phenylalanine intake without an excess that could potentially be used as a source of tyrosine. This phenylalanine intake results in an inadequate total aromatic amino acid intake until an adequate intake of tyrosine is provided. This design, in turn, allowed for the isolation of tyrosine requirements from that for phenylalanine.

A breakpoint was identified when the data points of $\dot{V}^{13}CO_2$ from the oxidation of L-[1-^{13}C]lysine were partitioned between 2 linear regression equations. $\dot{V}^{13}CO_2$ is the primary outcome measure used for determining amino acid requirements (24) because it was shown consistently to be less variable and more sensitive than the respective amino acid oxidation estimate based on plasma enrichment of the test amino acid (25, 26). The greater variation of the oxidation estimate is believed to be due to the observation that plasma amino acid enrichment is an inaccurate estimate of the intracellular pool for oxidation (27). Because $^{13}CO_2$ can only arise from the enriched amino acid at the site of oxidation, it is considered to be a more sensitive and more specific responder to changes in the test amino acid intake (26, 28–30).

This is the first time that lysine has been used as an indicator amino acid in adult humans. To serve as an indicator, the amino acid must be indispensable and the labeled carboxyl carbon must be irreversibly oxidized to carbon dioxide. Furthermore, the pool and flux of the indicator should not be altered by changing the intake of the test amino acid (25, 31). Because the flux of the indicator is partitioned between oxidation and incorporation into protein, the total flux remains the same (31).

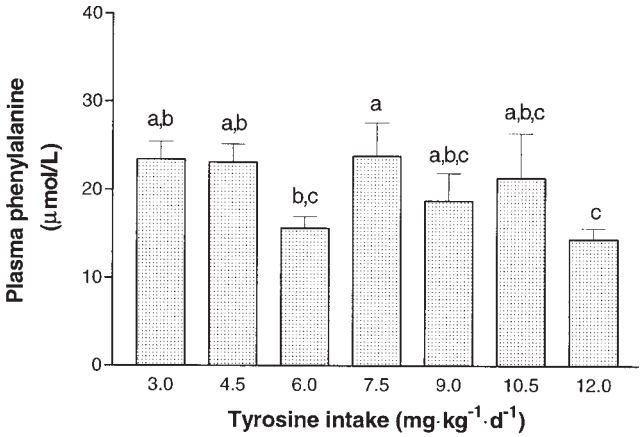

FIGURE 2. Mean (±SEM) plasma phenylalanine concentrations in healthy men after 8 h of consuming the study diet. Bars with different superscript letters are significantly different, $P < 0.05$.

TABLE 2
Effect of tyrosine intake and subject on lysine flux[1]

Subject	Tyrosine intake ($mg \cdot kg^{-1} \cdot d^{-1}$)							$\bar{x} \pm SD$[2]
	3.0	4.5	6.0	7.5	9.0	10.5	12.0	
	$\mu mol \cdot kg^{-1} \cdot h^{-1}$							$\mu mol \cdot kg^{-1} \cdot h^{-1}$
1	82.9	91.7	82.6	84.8	82.5	87.2	98.4	87.2 ± 6.0[a]
2	112.1	134.6	120.0	128.7	120.8	100.9	116.5	119.1 ± 11.0[b]
3	88.1	73.9	99.6	110.3	93.6	81.5	75.5	89 ± 13.3[a]
4	80.4	70.3	81.1	99.6	100.4	87.2	85.8	86.4 ± 10.8[a]
5	131.4	164.9	147.3	133.0	124.5	134.5	129.4	137.9 ± 13.8[c]
6	115.0	143.7	158.7	127.2	138.6	111.6	149.4	134.9 ± 17.6[c]
$\bar{x} \pm SD$	101.7 ± 20.8	113.2 ± 39.8	114.9 ± 32.9	113.9 ± 19.1	110.1 ± 21.3	100.5 ± 20.0	109.2 ± 27.9	109.1 ± 5.8

[1] There were no significant effects of tyrosine intake or order on lysine flux; however, there was a significant effect of subject on flux ($P < 0.0001$).
[2] Means with different superscript letters are significantly different, $P < 0.05$.

Although phenylalanine has proven to be an effective indicator for amino acid requirement studies in animals (28–30) and humans (24–26), the present study provides evidence that lysine is also an effective indicator amino acid.

At a fixed phenylalanine intake of 9.0 $mg \cdot kg^{-1} \cdot d^{-1}$, the mean and safe tyrosine requirements in men were found to be 6.0 and 7.0 $mg \cdot kg^{-1} \cdot d^{-1}$, respectively. The resultant average requirement and safe total aromatic amino acid intake were 15 and 21 $mg \cdot kg^{-1} \cdot d^{-1}$, respectively. The balance of aromatic amino acids determined from the mean estimates of phenylalanine and tyrosine requirements was 60:40 (phenylalanine:tyrosine). This balance of aromatic amino acids was similar to that determined in neonatal pigs (54:46) (5, 6), piglet tissue (57:43) (32), and human fetal tissue (59:41) (33). These data suggest that the ideal balance of aromatic amino acids, which supports the maximum efficiency of amino acid utilization, is within a narrow range of phenylalanine and tyrosine intakes. These findings could benefit individuals with conditions associated with altered aromatic metabolism such as hepatic (34, 35) or renal (36, 37) disease.

Sanchez et al (2, 3) and Basile-Filho et al (4, 8) carried out extensive 24-h amino acid balance studies using stable isotope tracers of phenylalanine and tyrosine to examine aromatic amino acid metabolism and the adequacy of phenylalanine intake. This approach defines the requirement as the minimum intake needed to equal obligatory amino acid catabolism. These authors used 2 catabolic indexes, phenylalanine hydroxylation and oxidation, and studied subjects receiving 1 of 3 phenylalanine intakes (21.9, 39, and 100 $mg \cdot kg^{-1} \cdot d^{-1}$) in a diet that contained little tyrosine. The collective evidence from these studies suggests that an intake of 21.9 $mg \cdot kg^{-1} \cdot d^{-1}$ was inadequate as indicated by the negative phenylalanine balance estimated from the difference between phenylalanine input and oxidation or hydroxylation. Individuals who received phenylalanine intakes of 100 and 39 $mg \cdot kg^{-1} \cdot d^{-1}$, in contrast, were generally in positive phenylalanine balance. Therefore, the data from those studies suggest that the total aromatic amino acid requirement is between 21.9 and 39 $mg \cdot kg^{-1} \cdot d^{-1}$, which is higher than the requirement estimated in the present study. The mean total aromatic amino acid requirement for men estimated previously by Rose et al (38) was 13.3 $mg \cdot kg^{-1} \cdot d^{-1}$; the safe intake (+2 SD) was estimated to be 17.1 $mg \cdot kg^{-1} \cdot d^{-1}$. Comparable estimates in women ranged from 14 to 18 $mg \cdot kg^{-1} \cdot d^{-1}$ (39). These estimated requirements are lower than our estimated safe intake of 21.0 $mg \cdot kg^{-1} \cdot d^{-1}$. It is important to note that neither of the 2 previous nitrogen balance studies included miscellaneous nitrogen losses. A recent reanalysis of lysine requirements determined on the basis of nitrogen balance showed that when miscellaneous nitrogen losses are accounted for, the estimated lysine requirement increases significantly (40).

There are several possible reasons that our results differ from those of Sanchez et al (2, 3) and of Basile-Filho et al (4, 8). First, provision of aromatic amino acids in an ideal balance, such as in the present experiment, likely maximized the utilization efficiency of the aromatic amino acids such that the estimated total aromatic amino acid requirement was lower than what would have been estimated if all aromatic amino acids were given as phenylalanine. Sanchez et al (3) discussed the likelihood that intake of a preformed source of tyrosine may improve aromatic amino acid homeostasis. Second, their estimates of phenylalanine catabolism were based on either phenylalanine oxidation or hydroxylation. Both estimates are dependent on precursor pool enrichment estimates, which, as discussed above, contribute considerable variation to the estimates and can result in incorrect results for phenylalanine hydroxylation (7). Third, the 24-h aromatic amino acid balance studies included a several-day adaptation period to the test amino acid, whereas, apart from a 2-d adaptation to a protein intake of 1 $g \cdot kg^{-1} \cdot d^{-1}$, our subjects were adapted only for a matter of 6–8 h on the study day to the test amino acid. During the 2-d adaptation

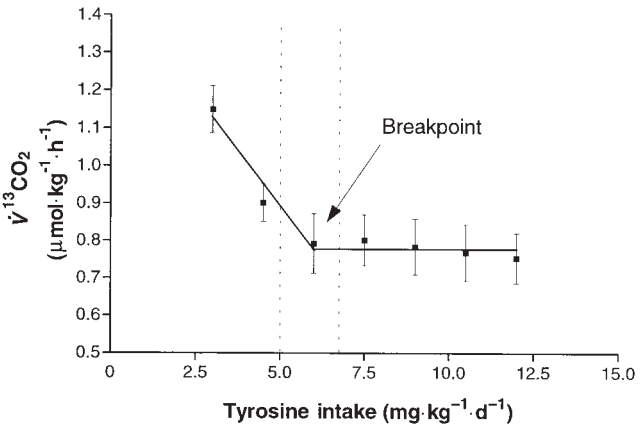

FIGURE 3. Mean (±SEM) production of $^{13}CO_2$ ($\dot{V}^{13}CO_2$) from the oxidation of L-[1-^{13}C]lysine at graded tyrosine intakes. Dotted vertical lines represent the 95% confidence limits of the breakpoint (6.0 $mg \cdot kg^{-1} \cdot d^{-1}$) estimate ($r^2 = 0.4$, $P < 0.001$).

TABLE 3
Effect of tyrosine intake and subject on lysine oxidation[1]

Subject	Tyrosine intake ($mg \cdot kg^{-1} \cdot d^{-1}$)							$\bar{x} \pm SD$[2]
	3.0	4.5	6.0	7.5	9.0	10.5	12.0	
	$\mu mol \cdot kg^{-1} \cdot h^{-1}$							$\mu mol \cdot kg^{-1} \cdot h^{-1}$
1	12.3	11.6	7.8	10.1	10.6	10.9	12.8	$10.9 \pm 1.7^{a,b}$
2	15.4	17.4	13.5	11.9	10.0	10.7	10.9	$12.8 \pm 2.7^{a,c}$
3	14.2	8.9	6.1	11.5	10.4	7.9	6.0	$9.3 \pm 3.0^{b,d}$
4	NA	7.4	10.5	6.3	7.5	7.5	7.5	7.8 ± 1.4^{d}
5	15.3	17.3	13.7	15.8	14.2	14.0	13.0	14.8 ± 1.5^{c}
6	17.8	12.8	16.4	13.0	10.5	6.3	10.8	$12.5 \pm 3.9^{a,c}$
$\bar{x} \pm SD$[2]	13.0 ± 5.2^{c}	$12.6 \pm 4.2^{a,c}$	$11.3 \pm 3.9^{a,b}$	$11.4 \pm 3.2^{a,b}$	$10.5 \pm 2.1^{a,b}$	9.6 ± 2.8^{b}	$10.2 \pm 2.9^{a,b}$	11.2 ± 1.2

[1] There were no significant effects of order on lysine oxidation, but there was a significant effect of tyrosine intake ($P < 0.001$) and subject ($P < 0.006$) on lysine oxidation. NA, not available.

[a–d] Means within rows and columns with different superscript letters are significantly different, $P < 0.05$.

to protein intake, we calculated that subjects were also receiving ≈43 mg phenylalanine$\cdot kg^{-1} \cdot d^{-1}$ and 39 mg tyrosine$\cdot kg^{-1} \cdot d^{-1}$. On the day of each indicator oxidation study, the subjects consumed the test intake of tyrosine and the fixed phenylalanine intake of 9 $mg \cdot kg^{-1} \cdot d^{-1}$. It was assumed that this lack of prior adaptation would result in a higher estimated requirement (41–43); however, the total aromatic amino acid requirement determined in the present study was actually slightly lower than that derived from the 24-h balance studies. The requirement, however, was higher than the earlier nitrogen balance–based estimates, which included adaptation periods of ≥5–7 d. Finally, the tyrosine requirement was estimated by partitioning the oxidation of the indicator amino acid, lysine, to a 2-phase linear regression crossover analysis. This model involves the study of 6–7 test amino acid intakes carried out in the same subject, which allows for a precise mathematic estimate of requirement. The 24-h balance studies, because of their cost, difficulty, and complexity, have only studied 3 aromatic amino acid intakes, making an exact estimate of requirement difficult. Nonetheless, future work is needed to determine whether prior adaptation to tyrosine would have altered the estimates obtained in this experiment.

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