Tyrosine requirements in children with classical PKU determined by indicator amino acid oxidation

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Departments of 1Nutritional Sciences and 3Paediatrics, University of Toronto, Toronto, Ontario M5S 3E2; 2The Research Institute, The Hospital for Sick Children, Toronto, Ontario M5G 1X8; and 4Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2P5

Bross, Rachelle, Ronald O. Ball, Joe T. R. Clarke, and Paul B. Pencharz. Tyrosine requirements in children with classical PKU determined by indicator amino acid oxidation. Am. J. Physiol. Endocrinol. Metab. 278: E195–E201, 2000.—Tyrosine (Tyr) is an essential amino acid in phenylketonuria (PKU) because of the limited hydroxylation of phenylalanine (Phe) to Tyr. The recommended intake for Tyr in PKU are at least five times the recommended phenylalanine intakes. This suggests that Phe and Tyr contribute ~20 and 80%, respectively, of the aromatic amino acid (AAA) requirement (REQ). In animals and normal humans, dietary Tyr was shown to spare 40–50% of the Phe requirement, proportions that reflect dietary and tissue protein composition. We tested the hypothesis that the Tyr REQ in PKU would account for 45% of the total AAA REQ by indicator amino acid oxidation (IAAO). Tyr REQ was determined in five children with PKU by examining the effect of varying dietary Tyr intake on lysine oxidation and the appearance of $^{13}$CO$_2$ in breath (F $^{13}$CO$_2$) under dietary conditions of adequate energy, protein (1.5 g·kg$^{-2}$·day$^{-1}$), and phenylalanine (25 mg·kg$^{-2}$·day$^{-1}$). Lysine oxidation and F $^{13}$CO$_2$ were determined using a primed 4-h oral equal-dose infusion of L-[1-$^{13}$C]lysine. Lysine oxidation and F $^{13}$CO$_2$ decreased linearly as Tyr intake increased, to a break point that was interpreted as the mean dietary Tyr requirement (16.3 and 19.2 mg·kg$^{-2}$·day$^{-1}$, respectively). At Tyr intakes of >16.3 and 19.2 mg·kg$^{-2}$·day$^{-1}$, lysine oxidation and F $^{13}$CO$_2$, respectively, were low and constant. This represents 40.4 and 44.4%, respectively, of the total AAA intake. The current recommendations for Tyr intake in PKU patients appear to be overestimated by a factor of ~5. This study is the first application of the IAAO technique in a pediatric population and in humans with an inborn error of metabolism.

The aim of dietary treatment of PKU is to maintain plasma concentrations of phenylalanine, tyrosine, and other amino acids within the normal range, thereby allowing for optimum growth and brain development. Treatment consists of supplying adequate energy, other amino acids and nutrients, while phenylalanine intake is restricted and tyrosine intake is supplemented (8). At present, the dietary management of patients with PKU is empirical because it is based on monitoring plasma amino acid concentrations, blood urea nitrogen, and growth indexes, and not on direct measures of tyrosine and phenylalanine requirements.

The recommended daily aromatic amino acid (phenylalanine plus tyrosine) intakes in healthy infants and children (9) are shown in Table 1, as are the currently recommended levels for phenylalanine and tyrosine for children with PKU (8) in these three age groups. In PKU, the supplemental tyrosine intake, when added to the phenylalanine intake, far exceeds the recommendations for aromatic amino acid intake in the general, healthy population (8, 9). In fact, the median tyrosine recommended intake across the different age groups represents five to seven times the corresponding phenylalanine intake. This suggests that, of the total aromatic amino acid requirement, phenylalanine contributes ~20% and tyrosine ~80%, proportions that are significantly different from the relative contributions of phenylalanine and tyrosine in animals (11, 12, 19, 31, 36) and normal humans (5), in which dietary tyrosine was shown to spare 40–50% of the phenylalanine requirement. This almost equivalent contribution of phenylalanine and tyrosine to total aromatic amino acid intake is consistent with the plasma (20, 27) and mixed body protein (23) ratio of phenylalanine to tyrosine.

This study reports the first use of the recently developed technique of indicator amino acid oxidation (4, 41) in children with an inborn error of metabolism. This technique allows a determination of tyrosine requirements by use of lysine as the indicator amino acid. On the basis of ratios of phenylalanine to tyrosine described above, we hypothesized that the tyrosine requirement in PKU would account for ~45% of the total aromatic amino acid requirement.

**Phenylketonuria (PKU)** is a disorder of aromatic amino acid metabolism in which phenylalanine cannot be converted to tyrosine, or only to a very limited extent (18, 24, 33). Thus tyrosine is an indispensable amino acid in PKU because it is not supplied endogenously via phenylalanine hydroxylation (18, 27), or only to a very limited degree (33).

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PKU, phenylketonuria. Data for daily aromatic acid (phenylalanine and tyrosine) requirements are expressed in mg·kg⁻¹·day⁻¹.

*FAO/WHO/UNU, 1985 (Ref. 9). †Elsas and Acosta, 1994 (Ref. 8).

METHODS

Study subjects. Five children (mean ± SE age, 7.6 ± 0.5 yr) with classical PKU, treated at the Hospital For Sick Children (HSC), Toronto, participated on an outpatient basis. Classical PKU was defined as a plasma phenylalanine concentration ≥1,200 µM at diagnosis. All subjects were treated by dietary phenylalanine restriction from early infancy. None of the subjects had a recent history of weight loss, endocrine disorders, or medication use. Subject characteristics at the start of the study are summarized in Table 2. The purpose of the study, the benefits, and the potential risks involved were explained to the subjects and their parents. Written consent was obtained from the subjects’ parents, and detailed information sheets about the protocol were provided. All procedures used in the study were approved by the University of Toronto Human Experimentation Committee and the Human Subjects Review Committee of the HSC.

Study design. Each subject was studied for six nonconsecutive days during a 2-mo period between March and September, 1996. To be able to repeatedly study 6- to 9-yr-old children by use of amino acid oxidation techniques, we modified the standard models used in adults (6, 17, 40): specifically, oral administration of the isotope and ingestion of the study diet only on the six study days, and sampling of plasma amino acid isotope enrichment of arterial blood by measuring amino acid oxidation study is analyzed by a two-phase linear crossover model, where the crossover (break) point is the mean requirement estimate (see Data analysis). For optimal statistical analysis, the oxidation levels should be evenly distributed above and below the predicted break point. In this case, the predicted break point was based on consideration of two values: 1) the average phenylalanine intake of the subjects; and 2) the published recommendations for tyrosine intakes (90–160 mg·kg⁻¹·day⁻¹) for the subjects (which in this case were used to define the maximum tyrosine level). The average of the phenylalanine intakes of all five subjects (24 mg·kg⁻¹·day⁻¹) was predicted to equal 55% of the total aromatic amino acid requirement. The predicted mean tyrosine requirement (19.6 mg·kg⁻¹·day⁻¹) was therefore calculated as the difference between the total aromatic amino acid requirement (43.6 mg·kg⁻¹·day⁻¹) and the average phenylalanine intake.

Experimental diet. The experimental diet was based on an amino acid mixture developed for amino acid kinetic studies (37). A flavored liquid formula (Protein-Free Powder, Product 80056, Mead Johnson, Evansville, IN) and protein-free cookies (HSC Research Kitchen) supplied the main source of energy in the diet. A crystalline amino acid mixture, based on the amino acid composition of egg protein, was consumed at 1.5 g·kg⁻¹·day⁻¹ and provided the only source of amino nitrogen in the diet. This level of protein was chosen because it met or exceeded the recommended level (9) and was of a similar magnitude to the children’s habitual protein intake of 1.9 ± 0.2 g·kg⁻¹·day⁻¹. The approximate macronutrient composition of the experimental diet, expressed as a percentage of dietary energy, was 53% carbohydrate, 38% fat, and 9% protein. The macronutrient composition of the subject’s usual diet was 55% carbohydrate, 35% fat, and 10% protein. The diets were prepared and portioned into eight isenergetic, isonitrogenous meals. The diet was consumed as hourly meals, and each meal represented one-eighth of the subject’s total daily requirement. Total energy intakes were based on each subject’s calculated resting metabolic rate (9), multiplied by an activity factor of 1.5. Subjects were instructed to maintain their usual level of physical activity and to fast for 10–12 h overnight before the study. Within 1 wk of the first study day and on the morning of each subsequent study day, each subject had his height and weight measured. Standing height was measured without shoes, to the nearest 0.1 cm, by use of a wall-mounted stadiometer. Body weight was measured to the nearest 0.1 kg on a balance scale (Toledo Scale, model 2020, Windsor, ON, Canada) while subjects wore light clothing and were without shoes, after an overnight fast, and after voiding. Body weight and height from one study day were used to calculate the total energy and protein (amino acid) content of the experimental diet for the next study day. Subjects were not adapted to the experimental diet, because it was possible to match the protein and phenylalanine requirements in healthy children and those with PKU.

Table 1. Recommended daily aromatic acid requirements in healthy children and those with PKU

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Infants</th>
<th>Preschool</th>
<th>Older Children</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy*</td>
<td>25–90</td>
<td>69</td>
<td>22</td>
</tr>
<tr>
<td>PKU†</td>
<td>15–70</td>
<td>15–40</td>
<td>15–35</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>250–350</td>
<td>115–200</td>
<td>90–160</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>265–420</td>
<td>130–240</td>
<td>105–195</td>
</tr>
</tbody>
</table>

PKU, phenylketonuria. Data for daily aromatic acid (phenylalanine and tyrosine) requirements are expressed in mg·kg⁻¹·day⁻¹.

*FAO/WHO/UNU, 1985 (Ref. 9). †Elsas and Acosta, 1994 (Ref. 8).

Table 2. Subject characteristics of the children with PKU who participated in the tyrosine requirement study

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Sex</th>
<th>TN</th>
<th>LF</th>
<th>AK</th>
<th>MJ</th>
<th>TJ</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>9</td>
<td>7</td>
<td>7.6 ± 0.51</td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>40.0</td>
<td>31.8</td>
<td>29.3</td>
<td>40.6</td>
<td>38.0</td>
<td>35.9 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>Height, cm</td>
<td>129.7</td>
<td>137.0</td>
<td>115.3</td>
<td>147.6</td>
<td>135.9</td>
<td>133.1 ± 5.3</td>
<td></td>
</tr>
<tr>
<td>FFM – BIA, kg</td>
<td>29.8</td>
<td>26.7</td>
<td>21.8</td>
<td>33.8</td>
<td>28.2</td>
<td>28.0 ± 1.9</td>
<td></td>
</tr>
<tr>
<td>FFM – SF, kg</td>
<td>27.9</td>
<td>27.6</td>
<td>19.5</td>
<td>32.1</td>
<td>26.3</td>
<td>26.7 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>Phe intake, mg·kg⁻¹·day⁻¹</td>
<td>36.0</td>
<td>22.0</td>
<td>25.0</td>
<td>17.0</td>
<td>21.0</td>
<td>24.2 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>Tyr intake, mg·kg⁻¹·day⁻¹</td>
<td>485.0</td>
<td>235.0</td>
<td>457.0</td>
<td>150.0</td>
<td>102.0</td>
<td>285.8 ± 78.7</td>
<td></td>
</tr>
</tbody>
</table>

IBW, ideal body weight; actual height was classified by percentile derived from growth curves, IBW was the weight at the same percentile as the actual height, and actual weight was divided by IBW × 100% = %IBW. FFM – BIA, fat free mass determined by bioelectrical impedance analysis. FFM – SF, fat free mass determined by multiple skinfold thickness. Phe and Tyr intake represents habitual daily intakes.
intakes of the experimental diet to the usual intakes of the subjects who followed a highly regulated diet (see Table 2). In addition, the protein source for these subjects with PKU was composed of crystalline amino acids, similar to the amino acid mix used in the experimental diet. The phenylalanine intake provided by the experimental diet on the study day was 24 mg/kg. The lysine intake on the study day was 64 mg/kg. This value reflected the upper limit of the recommended lysine intake for children aged 2–12 (9).

Oral isotope infusion studies. Each isotope study took 8 h (480 min) and was divided into a 4-h period to allow background isotope equilibration to the experimental diet (3) and a 4-h period after the isotope administration was started. Time 0 was defined as when isotope administration was started, with 240 min before and after time 0. Hourly meals were consumed beginning at time –240 min. The level of lysine in each meal was the same. This was achieved by reducing the dietary lysine content of the last four meals by an amount that corresponded to the amount of [13C]lysine administered. The level of amino nitrogen in the diet was kept constant despite the different tyrosine test levels by the addition of molar equivalents of L-alanine.

L-[1-13C]lysine with an enrichment of 99% (Cambridge Isotopes Laboratories, Woburn, MA) was used in this study. The chemical and isotopic purity of the labeled amino acid was confirmed by gas chromatography-mass spectrometry. Isometric purity (<0.2% d-isomer) was assessed by chiral HPLC. The stock solution of [13C]lysine (10 mg/ml) was prepared with sterile water by passage through a 0.22-µm filter (Millipore, Bedford, MA) under a laminar flow hood and then dispensed into multiple-dose vials. Each subject received a priming dose of 17.1 µM/kg at time 0 min and eight subsequent equal oral infusion doses of 4.79 µM/kg at 30-min intervals beginning 15 min after the prime. The isotope was taken orally and was followed by water to rinse the tube that had contained the isotope.

At least 150 ml of water were consumed with each meal to ensure a steady production of urine. Three baseline samples of urine were collected between –60 and 0 min, and five plateau urine samples were collected during the last 120 min of the study. Urine samples were separated into 500-µl aliquots and stored at –20°C until analysis. This meal and oral isotope protocol had been shown to achieve a satisfactory isotopic steady state in ~2 h after start of the [13C]lysine isotope (3) in adult volunteers. The same was seen in the present study (the first with children), as is shown in Fig. 1.

Three baseline breath samples were collected between –21 and 0 min, and four plateau breath samples were collected during the last 120 min of the study. One 20-min measure of the CO2 production rate (VCO2, ml/min) was determined between 0 and 120 min by use of a 2900 Metabolic Cart (Senoromedics, Anaheim, CA). Each breath sample was collected while the subjects lay in a semirecumbent position on a hospital bed. The subjects breathed in a normal fashion while wearing a ventilated face mask (Scott 80216730, Sensormedics). Once the subjects’ airflow had stabilized (CO2 concentration = 0.5–0.8%), the expired breath was collected using a vacuum extraction system (Pump VB0025, Vortex Blower, Spencer Turbine, Windsor, CT) combined with a mass flowmeter. To fully trap the respiratory CO2, the expired breath was bubbled at a rate of 500 ml/min through 10 ml of a 1 M NaOH solution in a modified reflux condenser for 7 min (35, 36). The resulting NaH13CO3 solution was then injected (Monojet, Sherwood Medical, St. Louis, MO) into Vacutainer glass tubes (brand 6441, 100 × 16 mm, Becton-Dickinson (Mississauga, ON, Canada)). The vacutainers were evacuated of any air introduced during the injection and were frozen at –20°C until analysis.

VCO2 was determined by continuous indirect calorimetry with the use of a ventilated hood. The 2900 Metabolic Cart was equipped with a paramagnetic O2 analyzer and an infrared CO2 analyzer. Before any VCO2 measurement, gas analyzers were calibrated with standard gases (76% nitrogen, 20% oxygen, and 4% carbon dioxide; Linde Medical Gas, Union Carbide, Toronto, ON, Canada). CO2 production was expressed under standard conditions (STPD): dry gas at 0°C and 760 mmHg. Measured VCO2 took place under ambient conditions, and the 2900 corrected the gas volumes to standard conditions.

Analytical procedures. Amino acids in 500 µl of urine were derivatized by the method described by Patterson et al. (25) to their N-heptafluorobutyryl-n-propyl esters. Isotopic enrichment for urinary free [13C]lysine was measured by gas chromatography-selected ion monitoring-negative chemical ionization-mass spectrometry [Hewlett-Packard model 5890 Series II GC (Mississauga, ON) VG Trio-2 quadrupole mass spectrometer system]. Selected ion chromatograms were obtained by monitoring mass-to-charge ratios of 560 and 561 for [13C]lysine, corresponding to the unenriched (m) and enriched (m+1) peaks, respectively. The areas under the peaks were...
integrated by a Digital DECP 450D2LP computer by use of a Lab-Base program (VG Biotech, Altringham, UK).

The trapped $^{13}$CO$_2$ was released from the NaOH by addition of an equal volume (0.25 ml) of 85% phosphoric acid in a twin-limed preevacuated Rittenberg Tube (13). The percentage enrichment of the expired $^{13}$CO$_2$ was measured on a dual-inlet magnetic sector isotope ratio mass spectrometer (VG Micromass 602D, Cheshire, UK) by use of techniques described in earlier work (13). Breath enrichments from baseline samples and from those taken during the isotope infusion were expressed as atoms percent excess (APE) $^{13}$CO$_2$ over a reference standard of compressed CO$_2$ gas.

Data analysis. Results are presented as means ± SE. A stochastic model was used to evaluate lysine kinetics (35). Isotopic steady state in the metabolic pool was represented by plateaus in $^{13}$CO$_2$ enrichment in breath and in $[^{13}$C]lysine enrichment in urine. This state was achieved in breath and urine by 120 min from the start of the isotope infusion and was maintained to the end of the study at 240 min. Plateaus in urine and breath isotopic enrichments were defined first by visual inspection (Fig. 1) followed by regression analysis demonstrating that the slope was not different from zero. The mean breath isotopic enrichment values of the three baseline samples and the four plateau samples were used to determine APE above baseline at isotopic steady state. The mean ratio of the enriched peak ($m+1$) to the unenriched peak ($m$) in urine for both baseline and plateau samples was used to calculate molecules percent excess (MPE) for $[^{13}$C]lysine.

Lysine kinetics were estimated from breath and urine enrichment data with standard equations (17). Examples of the use of these equations in indicator amino acid oxidation are described in detail elsewhere (40). Briefly, lysine flux ($Q$) was calculated using the following equation

$$Q = i[(E_i /E_p) - 1]$$  \hspace{1cm} (1)

where $i$ is the mass of the isotope; $E_i$ is the enrichment of the isotope; and $E_p$ is the enrichment of the $[^{13}$C]lysine at plateau. The rate of $^{13}$CO$_2$ released in breath ($F^{13}$CO$_2$) from oxidation of the $[^{13}$C]lysine tracer ($\mu$mol·kg$^{-1}$·h$^{-1}$) was calculated as follows

$$F^{13}$CO$_2 = (F_{CO2})(ECO2)(44.6)(60)/(W)(0.82)(100) \hspace{1cm} (2)$$

where $F_{CO2}$ is the CO$_2$ production rate measured by indirect calorimetry; $ECO2$ is the $^{13}$CO$_2$ enrichment in breath at the isotopic steady state (APE); and $W$ is the weight of the subject (kg). The constants are 44.6, to convert gas volume to moles; 60, to show time per hour; 0.82, to allow for delay of excretion into breath of the label from the bicarbonate pools; and 100, to convert APE to a fraction (40).

Results

The mean lysine flux was 114.0 ± 7.9 µmol·kg$^{-1}$·day$^{-1}$, data not shown. Lysine flux was not affected by the level of test tyrosine intake ($P = 0.89$). However, there were differences between individual subjects in their lysine fluxes ($P = 0.0001$); conversely, the sex of the subject (male vs. female) had no effect ($P = 0.14$) on the estimate of lysine flux.

Statistical analysis was performed on primary and derived values. ANOVA (26) was used to assess the relationship of lysine flux, lysine oxidation, and lysine $F^{13}$CO$_2$ to the experimental variables: 1) tyrosine test intake, 2) subject, and 3) sex of subject. The Least Squares Difference multiple range test was used to test the significance of specific differences between variables grouped according to tyrosine test intake. Results were considered to be statistically significant at $P \leq 0.05$.

The mean requirement for tyrosine was determined by break point analysis by use of a two-phase linear regression cross-over model (28) similar to that described previously (7, 15, 38, 40). For both the $F^{13}$CO$_2$ data and the lysine oxidation data, the tyrosine intakes of 0, 8, 12, and 16 mg·kg$^{-1}$·day$^{-1}$ were partitioned to the first regression line, and tyrosine intakes of 32, 64, and 130 mg·kg$^{-1}$·day$^{-1}$ were partitioned to the second line, because the overall error for the two-phase linear regression crossover model was lowest for this separation of data. All other combinations resulted in higher error and lower correlation. The 95% confidence limits for the level of tyrosine intake corresponding to the intersection of the two straight lines were determined using Fieller’s theorem (28). The estimates set by the upper confidence interval of the linear regression model have been suggested to closely represent the requirement to meet the needs of 95% of the population (40). Individual tyrosine requirements were estimated by visual inspection of the break point from the $F^{13}$CO$_2$ and oxidation responses.

Table 3. Effect of tyrosine intake on the rate of $^{13}$CO$_2$ released from $L$-[*$^{13}$C] lysine oxidation in children with PKU

<table>
<thead>
<tr>
<th>Subject</th>
<th>Tyrosine Intake, mg·kg$^{-1}$·day$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>TN</td>
<td>2.29</td>
</tr>
<tr>
<td>LF</td>
<td>2.17</td>
</tr>
<tr>
<td>AK</td>
<td>1.65</td>
</tr>
<tr>
<td>MJ</td>
<td>2.68</td>
</tr>
<tr>
<td>TJ</td>
<td>1.85</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>2.13 ± 0.18</td>
</tr>
</tbody>
</table>

$^{13}$CO$_2$ released from $L$-[*$^{13}$C] lysine oxidation ($F^{13}$CO$_2$) is expressed as µmol·kg$^{-1}$·h$^{-1}$. By analysis of variance, tyrosine intake significantly affected $F^{13}$CO$_2$ (means with different superscripts are significantly different, $P < 0.05$); however, subject and sex of subject did not significantly affect the $F^{13}$CO$_2$ released.
whereas the sex of the subjects did not (P = 0.20). However, the sex of the subjects did not significantly affect lysine oxidation.

Figure 2 shows the effect of tyrosine intake on the mean rate of lysine oxidation between tyrosine intakes of 0 and 12 mg·kg⁻¹·day⁻¹. There was a significant decrease in the mean rate of lysine oxidation (Table 4). There was a significant decrease in the mean rate of lysine oxidation between tyrosine intakes of 0 and 12 mg·kg⁻¹·day⁻¹. The individual subject had a significant effect on lysine oxidation (P = 0.0001), whereas the sex of the subjects did not (P = 0.20). Figure 2 shows the effect of tyrosine intake on the mean rates of lysine oxidation. A break point in the lysine oxidation curve occurred at a dietary intake of 16.3 mg·kg⁻¹·day⁻¹ (95% confidence limits of 5.8–26.8).

DISCUSSION

Our study was prompted by the lack of direct measurement of aromatic amino acid requirements in individuals with PKU. Up until the present, tyrosine requirement estimates were based on plasma tyrosine levels (the timing of which in relationship to meals was not standardized) as well on growth measurements and blood urea nitrogen levels (1, 8). It was further prompted by the observation that amino acid homeostasis, although disturbed in untreated PKU, is not fully normalized in treated PKU (10). If untreated, patients with PKU show low-to-normal plasma tyrosine concentrations (14). Other authors have reported low plasma tyrosine concentrations in treated PKU patients after an overnight fast (29). Treated patients with PKU have both lower than healthy control and higher than healthy control postprandial plasma tyrosine concentrations while consuming the current recommended tyrosine enriched amino acid mixtures (34). The clinical implications of low fasting plasma tyrosine concentrations are not known; however, low plasma tyrosine levels have been the basis for tyrosine supplementation in PKU. As shown in Table 1, the current recommendations for aromatic amino acids in PKU far exceed the recommended intake for the general population (9). The source of this difference is the supplemental tyrosine provided to PKU patients. We regarded the current recommendation for tyrosine, which is 80% of total aromatics, as being too high, and we hypothesized it would be ~45% (of total aromatic amino acid needs), as in the healthy population.

Indicator amino acid oxidation is based on the principle that when one amino acid is in limited supply for protein synthesis, other essential amino acids will be in relative excess and hence will be oxidized (4, 41). It overcomes most of the limitations of direct amino acid oxidation, in that 1) the precursor pool for oxidation does not change as the level of the test amino acid is varied; and 2) all amino acids can be studied, not only those whose carboxyl group is directly released to the bicarbonate pools (41). Indicator oxidation was developed and validated in growing piglets, and the short-term indicator oxidation estimates of requirement were shown to correspond to classical longer-term measurements such as nitrogen balance, growth, and changes in body composition (2, 4). When we first used the indicator oxidation technique to determine amino acid requirements of humans, we used the classical amino acid metabolism model (17) with intravenous isotope administration, sampling of tracer enrichment in plasma, and adaptation to an amino acid-based diet for a period of ≤8 days (38). With the objective of adapting the indicator amino acid oxidation technique to be acceptable in children, we developed a minimally invasive model (3), which we then applied, for the first time in children, in the present study. The estimate of tyrosine requirement that was derived in these children with PKU is one for optimal protein synthesis and, hence, growth. Tyrosine’s other role is as a precursor for the adrenergic neurotransmitters. A recent study has

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**Table 4. Effect of tyrosine intake on the rate of lysine oxidation in children with PKU**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Tyrosine Intake, mg·kg⁻¹·day⁻¹</th>
<th>Tyrosine Intake, mg·kg⁻¹·day⁻¹</th>
<th>Tyrosine Intake, mg·kg⁻¹·day⁻¹</th>
<th>Tyrosine Intake, mg·kg⁻¹·day⁻¹</th>
<th>Tyrosine Intake, mg·kg⁻¹·day⁻¹</th>
<th>Tyrosine Intake, mg·kg⁻¹·day⁻¹</th>
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<tbody>
<tr>
<td>TN</td>
<td>38.30</td>
<td>36.89</td>
<td>24.69</td>
<td>29.10</td>
<td>26.10</td>
<td>36.24</td>
</tr>
<tr>
<td>LF</td>
<td>17.94</td>
<td>10.38</td>
<td>12.57</td>
<td>8.14</td>
<td>7.79</td>
<td>13.42</td>
</tr>
<tr>
<td>AK</td>
<td>21.16</td>
<td>18.43</td>
<td>15.78</td>
<td>17.31</td>
<td>13.09</td>
<td>10.62</td>
</tr>
<tr>
<td>MJ</td>
<td>28.47</td>
<td>19.93</td>
<td>11.95</td>
<td>7.54</td>
<td>8.83</td>
<td></td>
</tr>
<tr>
<td>TJ</td>
<td>17.44</td>
<td>12.99</td>
<td>16.25</td>
<td>12.90</td>
<td>13.16</td>
<td>10.19</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>26.7 ± 3.9a</td>
<td>19.7 ± 4.6bc</td>
<td>14.1 ± 2.2bc</td>
<td>16.5 ± 2.8bc</td>
<td>15.1 ± 3.9bc</td>
<td>14.0 ± 4.2c</td>
</tr>
</tbody>
</table>

Lysine oxidation is expressed as µmol·kg⁻¹·h⁻¹. By analysis of variance, tyrosine intake and subject significantly affected lysine oxidation (means with different superscripts are significantly different, P < 0.05); however, sex of the subject did not significantly affect lysine oxidation.
investigated whether tyrosine supplementation of 100 mg·kg⁻¹·day⁻¹, in addition to their dietary tyrosine intake (total tyrosine intakes were ~130 mg·kg⁻¹·day⁻¹), had any effect on neuropsychological performance, and none was found (30). Because this was a randomized controlled trial with adequate numbers, the possibility that higher levels of tyrosine are needed to enhance brain function is currently unproven.

This study demonstrates the first use of lysine as an indicator for amino acid requirements in a human population. Both phenylalanine and lysine were used as indicators in a study of tryptophan requirements in piglets (2). Because of the enzyme defect in PKU, lysine was used as the indicator amino acid in this study. The pattern of F¹³CO₂, or lysine oxidation, was similar to those observed in previous human studies in which phenylalanine was used as the indicator (7, 15, 40). Both F¹³CO₂ and lysine oxidation decreased and then reached a plateau as tyrosine intake increased from deficient levels to levels above requirement. The results from this study demonstrate that [¹³C]lysine can be used as an indicator amino acid in the estimation of amino acid requirement in humans.

Lysine flux was not affected by changes in tyrosine intakes. The lysine flux observed in the current PKU group (81.7–128.2 µmol·kg⁻¹·h⁻¹) was in an expected range given the levels reported from studies in adults (3, 6, 21, 22, 39). An important assumption of the indicator oxidation method is that changes in intake of the test amino acid do not alter the flux of the indicator (4, 38).

The mean tyrosine requirement in children with classical PKU was estimated at 19.2 and 16.3 mg·kg⁻¹·day⁻¹ by the F¹³CO₂ and oxidation response curves, respectively. From the F¹³CO₂ and oxidation data, the upper 95% confidence interval of the breakpoint estimate was calculated as 25.2 and 26.8 mg·kg⁻¹·day⁻¹, respectively. The mean requirement estimates determined by F¹³CO₂ released and by lysine oxidation represent 44.4 and 40.4%, respectively, of the total aromatic amino acid intake and not 80%, as is suggested by the current recommendations used in clinical practice. The requirement estimates derived from these two data sets are similar and support the hypothesis that tyrosine contributes ~45% of the total aromatic amino acid requirement in PKU, if we assume that the mean habitual phenylalanine intake of 24 mg·kg⁻¹·day⁻¹ determined by clinical monitoring is a reasonable estimate of the true phenylalanine requirement of this population.

The proportion of tyrosine to phenylalanine determined in the present experiment in PKU children is consistent with the animal literature, in which tyrosine was shown to spare 40–46% of phenylalanine requirement by both nitrogen balance (19, 31, 36) and tracer oxidation methods (11, 12). This proportion is also consistent with the requirements estimated in normal humans by nitrogen balance (5, 16, 32) and is also in keeping with human plasma phenylalanine-to-tyrosine ratios (27) and with the ratio in mixed piglet body protein (23). Finally, these results are consistent with an estimate of the tyrosine requirement of 25 mg·kg⁻¹·day⁻¹ in children with hypertryrosinemia, aged 9–10 yr (1). Given the agreement between our data and the literature on animals, healthy humans, and children with hypertryrosinemia, we believe that our data are a reasonable estimate of the true requirement and that current clinical practice is in error.

Each subject was studied at six test tyrosine levels in the present study, thus uniquely enabling an estimate of his individual requirement. Significant differences among individuals were observed with respect to the lysine oxidation response, but not with respect to the F¹³CO₂ response to changes in tyrosine intake. The individual patterns of lysine oxidation were, however, consistent with both the group oxidation data and with the F¹³CO₂ data. The oxidation pattern observed in these individual measurements suggests an inflection in oxidation occurring between 15.0 and 19.0 mg·kg⁻¹·day⁻¹ of tyrosine intake. Individual tyrosine requirements based on inflections in the F¹³CO₂ curves ranged from 16.0 to 25.0 mg·kg⁻¹·day⁻¹.

In the present study, the mean tyrosine requirement was estimated to be 16.3 to 19.2 mg·kg⁻¹·day⁻¹, which represents 40.4–44.4% of the total aromatic amino acid intake in children with PKU. However, direct measurement of the phenylalanine requirement in PKU may be required to refine the relative contribution of tyrosine and phenylalanine to the total aromatic amino acid requirement in PKU. Therefore, the current recommendations for tyrosine intake in PKU patients are overestimated by a factor of ~5. The findings of this study have significant implications with respect to the dietary treatment of individuals with PKU.

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REFERENCES

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