Effect of Tyrosine Intake on the Rate of Phenylalanine Hydroxylation in Adult Males

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This study evaluated the effect of varying levels of tyrosine intake on the estimation of phenylalanine hydroxylation. Healthy men were fed 1 g protein $kg^{-1} \cdot d^{-1}$ for a 2-day period. On the third day, subjects consumed a formula diet containing 1 g protein $kg^{-1} \cdot d^{-1}$ hourly over 10 hours, and primed hourly oral doses of L-[^{15}N]phenylalanine and L-[3,3-$^{2}H_2$]tyrosine for the last 6 hours. Each subject was studied at 7 levels of tyrosine intake (3.0, 4.5, 6.0, 7.5, 9.0, 10.5, and 12.0 mg $\cdot kg^{-1} \cdot d^{-1}$) at a constant intake of phenylalanine (9 mg $\cdot kg^{-1} \cdot d^{-1}$, 4.55 μmol $\cdot kg^{-1} \cdot h^{-1}$). Phenylalanine hydroxylation was estimated from the ratio of plasma amino acid isotope enrichment of [^{15}N]phenylalanine and [^{15}N]tyrosine and the tyrosine flux estimated from [$^{2}H_2$]tyrosine enrichment. Phenylalanine and tyrosine fluxes showed no significant response to alterations in the intake of tyrosine. Linear regression analysis showed a significant response such that the rate of phenylalanine hydroxylation decreased as tyrosine intake increased (R^2 = .21; P = .003). The mean rates of phenylalanine hydroxylation were 3.89 to 8.06 μmol $\cdot kg^{-1} \cdot h^{-1}$. Given model uncertainties, the apparent protein breakdown observed at tyrosine intake levels less than 10.5 mg $\cdot kg^{-1} \cdot d^{-1}$, and the significant differences observed between the present data and our prior data, we cannot estimate the tyrosine requirement with any degree of certainty with the present hydroxylation results.

CLARKE AND BIER developed a model to measure the rate of phenylalanine hydroxylation in the fasted state using stable isotope tracers.[1] This model uses the ratio of the plasma enrichment of tyrosine to phenylalanine, representing the product and precursor pools of hydroxylation, respectively, and tyrosine flux to calculate the rate of phenylalanine hydroxylation.[1] The model was subsequently modified to allow measurements in the absence of an independent measure of tyrosine flux, using an estimate calculated from phenylalanine flux.[2] This modified model assumes a constant relationship between the ratio of tyrosine to phenylalanine from tissue protein breakdown and the flux of these amino acids.[2] Although originally developed for use in the fasted state, the Clark and Bier model and its modified version were subsequently applied to studies in the fed state in parenterally fed neonates using intravenously administered isotope infusions.[3-8] The results of these studies indicated that parenterally fed neonates have an adequate ability to hydroxylate phenylalanine. However, a recent reappraisal of our own parenterally fed neonatal piglet data questioned the quantitative validity of this measure of phenylalanine hydroxylation under certain circumstances.[9] The results[9] indicated that at low tyrosine and high phenylalanine intake, the estimated rate of hydroxylation exceeded the intake of phenylalanine, indicating net tissue protein breakdown. However, these piglets were shown to be actively growing and depositing body protein. We concluded that at a low intake of tyrosine below the mean requirement as estimated by House et al.,[10] phenylalanine hydroxylation may be overestimated.

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Submitted February 22, 1999; accepted September 7, 1999.

Supported by Medical Research Council Grant No. MT-10321.

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0026-0495/00/4904-0009$10.00/0

The Massachusetts Institute of Technology (MIT) group have also applied this model to a series of studies investigating phenylalanine requirements in both the fed and fasted states in healthy adult subjects.[11-16] Phenylalanine kinetics at 3 intake levels (18.5, 35.6, and 96.6 mg phenylalanine $\cdot kg^{-1} \cdot d^{-1}$) were compared using both intravenous and oral isotope tracers.[14,16] These studies provide a useful insight into the adequacy of these intake levels of phenylalanine. However, under conditions of varying intake of tyrosine, the rates of hydroxylation appear variable. An earlier study reported a hydroxylation rate of 3.20 μmol $\cdot kg^{-1} \cdot h^{-1}$ in the fed state following a diet containing 21.9 mg phenylalanine $\cdot kg^{-1} \cdot d^{-1}$ with no dietary tyrosine.[13] This result appears low in comparison to the rate of hydroxylation estimated by Basile-Filho et al[16] of 8.54 μmol $\cdot kg^{-1} \cdot h^{-1}$ at a phenylalanine intake of 18.5 mg $\cdot kg^{-1} \cdot d^{-1}$ and 6.79 mg tyrosine $\cdot kg^{-1} \cdot d^{-1}$ under similar study conditions. This may indicate a large variability in the estimation of phenylalanine hydroxylation in the fed state. However, this may also indicate limitations of this method in identifying the effect of changes in dietary intake. Particularly, it would appear that the intake levels of phenylalanine and tyrosine have a significant effect on the estimate of hydroxylation, and understanding this relationship is crucial to obtaining meaningful results from studies of the rate of phenylalanine hydroxylation in the fed state.

The use of the Clark and Bier model[1] has never been validated in the fed state in relation to protein status, although it has been used frequently. In addition, the influence of the intake of aromatic amino acids on the estimate of phenylalanine hydroxylation using this model has not been thoroughly investigated. Our objective was to investigate the relationship between the rates of phenylalanine hydroxylation and tyrosine intake in a group of healthy men. Phenylalanine hydroxylation was calculated from plasma amino acid enrichments, according to the current isotope model, following oral diet and isotope administration. A fixed phenylalanine intake of 9 mg $\cdot kg^{-1} \cdot d^{-1}$, the mean requirement level as determined at an excess intake of tyrosine by Zello et al,[17] was maintained in order to detect a response in the hydroxylation rate to alterations in the level of tyrosine intake. A range of tyrosine intake from 3.0 to 12.0 mg $\cdot kg^{-1} \cdot d^{-1}$ was used. The rate of hydroxylation was hypothesized to decrease with increasing tyrosine intake until

the total aromatic amino acid requirement is met. Thereafter, the rate of phenylalanine hydroxylation would be a function of phenylalanine intake only and would not be affected by changes in tyrosine intake. The level of hydroxylation at which the response reached a plateau would represent the obligatory conversion of phenylalanine to tyrosine at this phenylalanine intake. The purpose of this study was to determine whether phenylalanine hydroxylation could be used as an indicator of tyrosine requirements.

SUBJECTS AND METHODS

Subjects

Six healthy men participated in the study on an outpatient basis in the Clinical Investigation Unit at the Hospital for Sick Children (HSC), Toronto, Ontario, Canada. Subject characteristics are detailed in Table 1. None of the subjects had a history of recent weight loss, unusual dietary practices, endocrine disorders, or medication use. The design and aims of the study, as well as the potential risks involved, were fully explained to each subject and written consent was obtained. All procedures during the study were approved by the University of Toronto Human Experimentation Committee and the Human Subjects Review Committee of the HSC. Subjects received financial compensation for their participation.

Experimental Design

Subjects were randomly assigned to each of 7 dietary tyrosine levels of 3.0, 4.5, 6.0, 7.5, 9.0, 10.5, and 12.0 $mg \cdot kg^{-1} \cdot d^{-1}$. Each study consisted of 2 days on a controlled protein intake[19] of 1 $g \cdot kg^{-1} \cdot d^{-1}$ followed by a single study day for the measurement of phenylalanine hydroxylation. On the study day, phenylalanine hydroxylation was measured using L-[^{15}N]phenylalanine and L-[3,3-$^{2}H_2$]tyrosine at a protein intake of 1 $g \cdot kg^{-1} \cdot d^{-1}$. The study periods were separated by at least 1 week, with subjects completing all 7 studies within 3 months.

Diet and Energy Intake

Dietary intake was prescribed by a registered dietitian. Energy intake was based on the Food and Agriculture Organization/World Health Organization/United Nations University (FAO/WHO/UNU) predictive equations[18] multiplied by an activity factor of 1.7. The controlled protein intake prior to the study day was provided as a milkshake drink (Scandishake; Scandipharm, Birmingham, AL) supplemented with protein (Promod; Ross Laboratories, Columbus, OH) and energy (Caloreen; Clintec Nutrition, Mississauga, Ontario, Canada) to supply the prescribed requirement for both protein and energy. This was given as 4 meals spread throughout each day. No other foods or beverages, including diet products containing artificial sweeteners, were consumed during the 2-day adaptation period.

On the study day, the diet was provided as an experimental formula developed for amino acid kinetic studies, which we have previously described in detail.[20] Briefly, the diet consists of crystalline amino acids added to a protein-free base supplemented with protein-free cookies; the macronutrient balance is 55% carbohydrate, 38% fat, and 9% amino acids. Energy intakes were prescribed as before. The protein intake of 1 $g \cdot kg^{-1} \cdot d^{-1}$ was provided entirely as a crystalline amino acid mixture based on the composition of intact egg protein. The amounts of L-[^{15}N]phenylalanine and L-[3,3-$^{2}H_2$]tyrosine given during the study day were subtracted from the dietary provision of these amino acids. Phenylalanine intake was set at 9 $mg \cdot kg^{-1} \cdot d^{-1}$, which is the mean phenylalanine requirement previously determined.[17]

The total duration of each isotope study day was 10 hours. The experimental diets were prepared in the HSC research kitchen and portioned into 10 hourly meals, each providing one twelfth of the total daily intake of protein and energy. Subjects had free access to water, but no other food or beverage was ingested throughout the study day.

Table 1. Subject Characteristics

Subject No.	Age (yr)	Height (m)	Weight (kg)	Energy Intake (MJ/d)*
1	21	1.65	79.5	13.5
2	20	1.78	89.5	14.6
3	29	1.85	80.0	13.5
4	28	1.64	62.5	11.6
5	43	1.66	67.5	11.6
6	35	1.81	95.0	13.8
Mean ± SD	29 ± 8.7	1.73 ± 0.09	79.0 ± 12.4	13.1 ± 1.2

*Calculated from 1985 FAO/WHO/UNU predictive equations multiplied by an activity factor of 1.7.[18]

Tracer Protocol

The stable isotopes used in this study include L-[^{15}N]phenylalanine (99 atom percent), L-[^{15}N]tyrosine (99 atom percent), and L-[3,3-$^{2}H_2$]tyrosine (98 atom percent), all purchased from Isotec (Miamisburg, OH). Isotope solutions were prepared in deionized water and stored at −20°C. Before dispensing, the isotope solutions were sterilized through a 0.22-μm Millipore filter (Millipore, Bedford, MA). Oral priming doses of L-[^{15}N]phenylalanine (0.75 mg/kg), L-[^{15}N]tyrosine (0.18 mg/kg), and L-[3,3-$^{2}H_2$]tyrosine (0.25 mg/kg) were administered with the fifth hourly meal. Simultaneously, hourly oral dosing protocols for L-[^{15}N]phenylalanine (0.75 $mg \cdot kg^{-1} \cdot h^{-1}$) and L-[3,3-$^{2}H_2$]tyrosine (0.25 $mg \cdot kg^{-1} \cdot h^{-1}$) were commenced and continued throughout the remaining 6 hours of the study.

Sample Collection and Analysis

Baseline plasma samples were collected at 30, 20, and 10 minutes before initiation of the isotope protocol. Samples were also collected at isotopic plateau, the period from 210 to 330 minutes following commencement of the isotope protocol, at 20 minutes intervals. At the beginning of the study day, a 21-gauge needle was inserted into a superficial dorsal vein in the right hand. This remained in situ throughout the study day. The 2-mL samples of arterialized venous blood were collected into heparinized syringes (Aspirator; Marquest Medical Products, Englewood, CO) and placed on ice. Arterialized venous blood was obtained by heating the hand inside a thermostatic chamber maintained at 60°C.[21] Plasma was extracted following centrifugation at 4°C at 1,500 × *g* for 20 minutes and stored at −20°C until analysis.

The enrichment of free [^{15}N]phenylalanine, [^{15}N]tyrosine, and [3,3-$^{2}H_2$]tyrosine in plasma was measured by gas chromatography [selected ion monitoring-negative chemical ionization]–mass spectrometry (5890 Series GC, Hewlett Packard, Mississauga, Ontario, Canada; Trio-2 quadrople MS system, VG Cheshire, England). Free amino acids in 200 μL plasma were derivatized according to the method described by Patterson et al[22] to their heptafluorobutyryl *n*-propyl esters. Selected ion chromatographs were obtained by monitoring *m/z* 383 and 384 for [^{15}N]phenylalanine, corresponding to the unenriched (m) and enriched (m + 1) peaks, respectively, and *m/z* 417, 418, and 419 for tyrosine, corresponding to the unenriched (m) and enriched (m + 1 and m + 2) peaks. The areas under the peaks were integrated by a digital DECp 450_2LP computer using a Lab-Base program (VG Biotech, Altringham, England).

Estimation of Isotope Kinetics

Phenylalanine kinetics were calculated according to the stochastic model of Matthews et al,[23] previously used by Zello et al.[17] Isotopic steady state in the metabolic pool was represented by plateaux in free [^{15}N]phenylalanine, [^{15}N]tyrosine, and [3,3-$^{2}H_2$]tyrosine in plasma,

plateau being defined by the absence of a significant slope, assessed by linear regression analysis. The mean ratios of the enriched peaks (m + 1 and m + 2) to the unenriched (m) for each amino acid at both the baseline and plateau samples were used to calculate the molecules percent excess.

Phenylalanine flux (micromoles per kilogram per hour) was measured during isotopic steady state from the dilution of infused L-[1-^{15}N]phenylalanine into the plasma metabolic pool. Tyrosine flux was estimated from the dilution of infused [3,3-$^{2}H_2$]tyrosine. Phenylalanine hydroxylation was calculated from the conversion of [^{15}N]phenylalanine to [^{15}N]tyrosine and from the independent measure of tyrosine flux according to the model of Clarke and Bier,[1] $Q_{pt} = Q_t [E_t/E_p]$, where Q_{pt} is the rate of phenylalanine hydroxylation (micromoles per kilogram per hour), Q_t is the tyrosine flux (micromoles per kilogram per hour)estimated from [3,3-$^{2}H_2$]tyrosine, and E_t/E_p is the ratio of the plasma enrichment of [^{15}N]tyrosine to [^{15}N]phenylalanine.

Data Analysis

Repeated-measures ANOVA was performed to assess the effect of tyrosine intake on phenylalanine hydroxylation, phenylalanine flux, and tyrosine flux, with post hoc testing by Duncan's multiple-range test. Where an effect was identified, results were analyzed for linear and quadratic responses using repeated measures (Proc Mixed, SAS 6.12 for Windows; SAS Institute, Cary, NC, 1997). The data were also analyzed using a 2-phase linear crossover model[24] in an attempt to assign a breakpoint to the response. Results were considered statistically significant at a P value of .05 or less.

RESULTS

Plasma enrichments for [^{15}N]phenylalanine, [^{15}N]tyrosine, and [3,3-$^{2}H_2$]tyrosine are shown in Fig 1. An isotopic steady state, defined as no significant change in isotope enrichment, was reached for all 3 labeled amino acids in all subjects for the last 4 samples. Mean values for phenylalanine flux, tyrosine flux, and phenylalanine hydroxylation calculated from these isotopic plateaux are shown in Table 2. ANOVA showed no significant effect of tyrosine intake on phenylalanine flux ($P = .13$) or tyrosine flux ($P = .33$). However, a significant effect of tyrosine intake on phenylalanine hydroxylation was identified ($P = .04$), with differences between the rates of phenylalanine hydroxylation for individual tyrosine intake levels (Table 2). A linear regression response was identified such that the rate of phenylalanine hydroxylation decreased with increasing tyrosine intake ($P = .002$). No significant quadratic response was identified for the effect of tyrosine intake on phenylalanine hydroxylation ($P = .56$). Figure 2 shows individual responses in phenylalanine hydroxylation to variations in tyrosine intake and the overall linear regression response. Linear regression analysis of the effect of tyrosine intake on phenylalanine hydroxylation at a tyrosine intake from 6.0 mg · kg^{-1} · d^{-1} or 7.5 mg · kg^{-1} · d^{-1} and above indicated the absence of a significance slope ($P = .06$ or .17, respectively). However, using a 2-phase linear regression crossover model, no breakpoint in the response of phenylalanine hydroxylation to tyrosine intake could be identified.

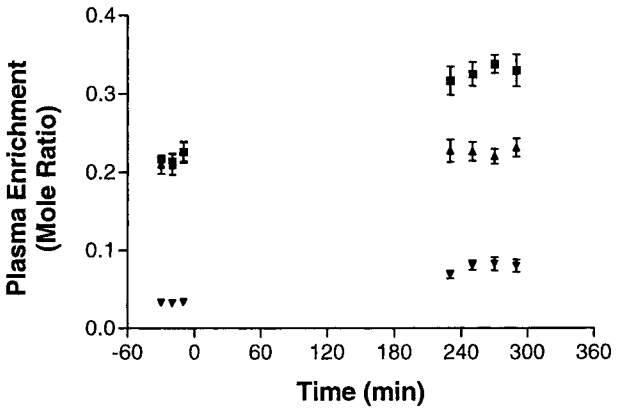

Fig 1. Plateau enrichment for [^{15}N]phenylalanine (■), [^{15}N]tyrosine (▲), and [3,3-$^{2}H_2$]tyrosine (▼). Results (mean ± SD) are shown as the baseline background values immediately before isotope administration at time 0 and plateau values at the end of isotope administration. Since, by design, plateau [^{15}N]tyrosine enrichment changes in response to tyrosine intake, the data are representative data from all 6 subjects when they were receiving a tyrosine intake of 12.0 mg · kg^{-1} · d^{-1}.

DISCUSSION

The model of phenylalanine hydroxylation developed by Clarke and Bier[1] has recently been applied to studies in parenterally fed neonates[3-8] and orally fed adult subjects.[11-16] This model was initially developed in the fasted state, and despite its frequent use in the fed state, it has never been validated under this condition. In a recent reappraisal of our own neonatal piglet data,[9] we proved that this model overestimates the rate of phenylalanine hydroxylation under certain dietary conditions; namely a high-phenylalanine, low-tyrosine intake. In the fed state, it appears that the intake of phenylalanine and tyrosine and their relative balance has a significant effect on phenylalanine hydroxylation.[9] Hydroxylation of phenylalanine has two purposes, namely to provide tyrosine and to comprise the first step in the degradation of phenylalanine.[25] In body tissue, the ratio of phenylalanine to tyrosine is approximately 55% to 45% of total aromatic amino acids.[26] A pioneering study by Rose and Wixom[27] suggested that tyrosine could meet 70% to 75% of phenylalanine needs and hence of total aromatic amino acid needs. However, our more recent studies in piglets[10,28] and in human adult males[17,29] suggest that dietary requirements for phenylalanine and tyrosine are in much the same ratio as that present in mixed tissue protein of 55% to 45%. In the study detailed here, we aimed to investigate the relationship between estimates of phenylalanine hydroxylation and tyrosine intake. At a fixed (moderate) intake of phenylalanine, the rate of hydroxylation was measured at 7 graded levels of tyrosine intake using the enrichment of plasma amino acids following oral dosing of L-[^{15}N]phenylalanine and L-[3,3-$^{2}H_2$]tyrosine.

Our results demonstrated that tyrosine intake had a significant effect on the estimate of the rate of conversion of phenylalanine to tyrosine. With increasing tyrosine intake, the rate of phenylalanine hydroxylation decreased linearly. However, a breakpoint could not be clearly identified in the data. We originally hypothesized that the rate of hydroxylation would decrease before reaching a plateau. At this point, it was expected that the provision of excess tyrosine would not spare more phenylalanine and that the phenylalanine hydroxylated to tyrosine would be oxidized and not remain within the tyrosine pool. The linear decrease suggested that a plateau was not reached. However, further analysis of our data indicated the

Table 2. Mean Phenylalanine Flux, Tyrosine Flux, and Phenylalanine Hydroxylation Results at Each Intake of Tyrosine (n = 6)

Parameter	Tyrosine Intake (mg · kg^{-1} · d^{-1})							
	3.0	4.5	6.0	7.5	9.0	10.5	12.0	SD
Phe flux (μmol · kg^{-1} · h^{-1})*	48.0	65.3	58.1	46.4	49.3	58.5	47.9	16.6
Tyr flux (μmol · kg^{-1} · h^{-1})†	29.8	39.7	32.8	32.4	33.5	32.6	32.4	6.9
Phe hydrox (μmol · kg^{-1} · h^{-1})‡	5.81^{ab}	8.06^{a}	5.89^{ab}	5.10^{b}	5.43^{b}	4.28^{b}	3.89^{b}	2.0

NOTE. Values with different superscripts are significantly different at $P < .05$.

*ANOVA showed no significant effect of tyrosine intake on phenylalanine flux ($P = .13$).

†ANOVA showed no significant effect of tyrosine intake on tyrosine flux ($P = .33$).

‡ANOVA showed a significant effect of tyrosine intake on phenylalanine hydroxylation ($P = .04$) characterized by a significant linear response ($R^2 = .21$; $P = .002$), such that the rate of hydroxylation was greater at lower intakes of tyrosine.

absence of a significant slope in phenylalanine hydroxylation for tyrosine intake levels from 6.0 mg · kg^{-1} · d^{-1} and above. This suggests that a variation in the rate of phenylalanine hydroxylation at tyrosine intake levels of 4.5 and 6.0 mg · kg^{-1} · d^{-1} may be responsible for the lack of sensitivity sufficient to observe a breakpoint. This is comparable to our indicator amino acid studies, where we found more variation in the data around the requirement level.[30] Enteral administration of the isotopes results in first-pass metabolism of the tracers in the gut and liver,[31] which might add to the variability of the hydroxylation estimates. However, when we compared the data in this experiment with results reported by those who used intravenous tracers,[14,16] as well as others[13,15,16] who also used an orally administered tracer, our hydroxylation estimates were among those with the least variance. When the requirement for tyrosine is reached, the rate of hydroxylation observed should represent only the obligatory conversion of phenylalanine to tyrosine for the oxidation of phenylalanine, which should not be affected by an increasing intake of tyrosine.

Another observation from our data is that at tyrosine intake less than 10.5 mg · kg^{-1} · d^{-1}, the rate of hydroxylation exceeded the intake of phenylalanine (9 mg · kg^{-1} · d^{-1} or 4.54 μmol · kg^{-1} · d^{-1}). According to the model of Waterlow et al,[32] this implies that the rate of tissue protein breakdown is greater than the rate of protein synthesis, suggesting that the intake of the aromatic amino acids is inadequate when tyrosine intake is less than 10.5 mg · kg^{-1} · d^{-1}. As mentioned earlier, at high phenylalanine intake but reduced tyrosine intake, we were able to show that phenylalanine hydroxylation was fallaciously high.[9] However, in the current study, we have no direct evidence that phenylalanine hydroxylation estimates are too high at lower tyrosine intakes. Based on phenylalanine balance data (intake minus hydroxylation), we can tentatively suggest a tyrosine requirement of between 9 and 10.5 mg · kg^{-1} · d^{-1}.

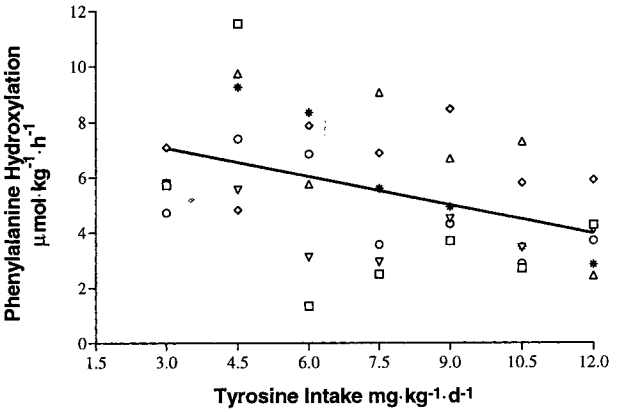

Fig 2. Phenylalanine hydroxylation in response to tyrosine intake. Subjects no. 1 (□), 2 (△), 3 (▽), 4 (◇), 5 (○), 6 (*). Linear regression analysis showed a significant effect of tyrosine intake on phenylalanine hydroxylation ($P = .002$), such that the rate of hydroxylation was higher following a lower intake of tyrosine. This relationship is defined by the equation, $y = 8.10 - 0.34 \times$ ($R^2 = .21$; SE = 2.02). Subject no. 2 data for a tyrosine intake of 3 mg · kg^{-1} · d^{-1} were technically unsatisfactory and were thus omitted; therefore, at this level of tyrosine intake, there are only 5 data points. For all other levels, there are 6 data points, for a total of 41 data points. The line indicates a level of phenylalanine intake of 4.54 μmol · kg^{-1} · h^{-1}.

We chose to use orally administered tracers since there is evidence that both tyrosine and phenylalanine are taken up in the gut.[31,33] Our reasoning is that if intravenous tracers were used, an incomplete assessment would be obtained for the effects of dietary tyrosine on phenylalanine hydroxylation. We have shown that estimates of phenylalanine flux are about 20% higher[34] in adult human subjects studied using the same protocol, where the isotope is given orally rather than intravenously. However, in the same subject studied over 7 different levels of tyrosine intake, we reason that the relative changes in hydroxylation in response to an alteration in tyrosine intake would be the same and would reflect phenylalanine and tyrosine metabolism in the whole body including splanchnic tissues.[31,33]

In our reappraisal of the parenterally fed neonatal piglet data, phenylalanine hydroxylation was calculated without an independent measure of tyrosine flux using a version of the model modified by Thompson et al.[2] We further modified the model by incorporating dietary intake into the calculation to account for this source of amino acid. However, it is still apparent that despite alterations to the calculation to account for apparent differences in the fed state, the results do not make physiological sense, as the rates of hydroxylation exceeded the intake of phenylalanine, yet we were able to show net protein accretion.[9] This indicates that sampling the plasma pool may not accurately represent the true precursor and product pool for the hydroxylation of phenylalanine when tyrosine intake is inadequate or phenylalanine intake is in excess. Indeed, previous data from an indicator amino acid study in pigs showed that the pattern of total $^{14}CO_2$ excretion in breath (representing oxidation of the labeled tracer amino acid) in response to changes in intake of the test amino acid was similar to the oxidation of the tracer amino acid calculated using the specific radioactivity of free amino acids in the liver, the site of amino acid oxidation,[35] as the precursor pool. When the rate of oxidation was calculated, using plasma amino acid specific radioactivity to represent the

precursor pool, there was less agreement with the results from the excretion of $^{14}CO_2$ in breath.

Further evidence that our estimates of phenylalanine hydroxylation and hence of phenylalanine balance may be in error is derived from a parallel study of tyrosine requirements, using indicator amino acid oxidation.[29] Using the same fixed moderate phenylalanine intake, the mean tyrosine requirement was determined to be 6 $mg \cdot kg^{-1} \cdot d^{-1}$, in contrast to the higher estimate of the tyrosine requirement in the present experiment of 9 to 10.5 $mg \cdot kg^{-1} \cdot d^{-1}$ based on phenylalanine balance.

The issue of the most appropriate amino acid pool to sample in isotope studies of amino acid metabolism, has been previously discussed.[36-38] The plasma pool has traditionally been used because it is easily accessible and has been suggested to be the best representation of whole-body amino acid kinetics. The most appropriate pool to sample for the measurement of the precursor pool for the synthesis of hepatic proteins would be the corresponding amino acyl tRNA; however, this is extremely difficult to sample. Therefore, hepatic export proteins, such as apolipoprotein B-100 (apo B-100),[36] have been suggested as alternatives for the estimation of intracellular amino acid enrichment. Studies have indicated that measurements of intracellular phenylalanine enrichment do not correspond to results obtained from the enrichment of plasma amino acids in the fasted state.[31,36] This is particularly pertinent when estimating the kinetics of an intracellular system. Phenylalanine hydroxylation occurs primarily, if not exclusively, in the hepatocyte in human subjects,[25] and therefore, plasma amino acid enrichment may not be the most appropriate pool to sample to measure the conversion of phenylalanine to tyrosine. In the fed state, these differences in the enrichment of the intracellular apo B-100 and extracellular plasma phenylalanine pools appear even greater.[36] Finally, enteral isotope administration, in the current study, further alters the relationship between plasma and intracellular apo B-100 enrichment.[38] Establishing the true response of phenylalanine hydroxylation to variations in the intake of tyrosine, using estimates of intrahepatocyte enrichment of tyrosine and phenylalanine, will provide information relevant to designing study protocols for use in the fed state. The development of a new model is crucial to improve our understanding of the regulation of the conversion of phenylalanine to tyrosine.

ACKNOWLEDGMENT

We thank Mead Johnson, Canada, for supplying the protein-free powder.

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