

Phenylalanine and Tyrosine Kinetics in Critically Ill Children with Sepsis

LETICIA CASTILLO, Y. MING YU, J. SERGIO MARCHINI, THOMAS E. CHAPMAN,
MELCHOR SANCHEZ, VERNON R. YOUNG, AND JOHN F. BURKE

Laboratory of Human Nutrition, Clinical Research Center and School of Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139 [L.C., Y.M.Y., T.E.C., M.S., V.R.Y.]; Shriners Burns Institute, Boston, Massachusetts 02114 [L.C., Y.M.Y., T.E.C., V.R.Y.]; Pediatric and Neonatal Intensive Care Units, Children's Service, Massachusetts General Hospital and Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02114 [L.C., M.S.]; Division of Nutrition-School of Medicine, Ribeirao Preto, SP 14049-900, Brazil [J.S.M.]; and Department of Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts 02114 [J.F.B.]

ABSTRACT. To better understand the impact of severe illness on the amino acid economy and nutritional needs of pediatric patients, we studied plasma phenylalanine and tyrosine kinetics in eleven critically ill patients (six full-term newborns and five young infants). Within 48 h of the diagnosis of sepsis they were given primed constant i.v. infusions of L-[1-¹³C]phenylalanine and L-[3,3-²H₂]tyrosine for 4 h. Routine nutritional support continued during this period by parenteral administration of dextrose, lipid emulsion, and an amino acid mixture low in tyrosine. Phenylalanine and tyrosine fluxes and rate of phenylalanine hydroxylation did not differ significantly between the two age groups, and so the data were combined for evaluation. For the entire group, values ($\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$; mean \pm SD) for phenylalanine and tyrosine fluxes and rate of phenylalanine hydroxylation were 132 ± 24 , 66 ± 16 , and 29 ± 12 , respectively. Plasma phenylalanine to tyrosine concentration ratio was 1.67 ± 0.6 . From a comparison of the rate of phenylalanine hydroxylation with measured phenylalanine intakes, it was concluded that their routine, clinical nutritional support was inadequate to achieve body phenylalanine balance. In comparison with published data, the relative rate of phenylalanine hydroxylation appears to be high. We speculate that tyrosine is a conditionally indispensable amino acid under these conditions; it would be desirable to establish the intake levels and ratio of phenylalanine to tyrosine that effectively support aromatic amino acid balance in these critically ill patients. (*Pediatr Res* 35: 580-588, 1994)

quantitative changes in nutrient metabolism, and the mechanisms responsible for them, under these disease conditions.

In this study we examined plasma kinetics of phenylalanine and tyrosine metabolism in critically ill neonates and infants with sepsis. These amino acids are substrates for protein synthesis and precursors of physiologically important metabolites, such as catecholamines and thyroid hormones. We hypothesized that the standard parenteral nutritional support given during the acute stage of critical illness to our patients might be insufficient to meet the aromatic amino acid requirement of these patients and that tyrosine, usually considered to be a nutritionally dispensable amino acid, becomes "conditionally" indispensable during hypermetabolic states. Therefore, an adequate exogenous supply of tyrosine would be necessary to support fully and effectively the nutritional needs of the patient. To explore these hypotheses, we investigated plasma phenylalanine and tyrosine fluxes in 11 critically ill newborns and young infants with [¹³C]phenylalanine, [¹³C]tyrosine, and [²H₂]tyrosine as labeled tracers. Our findings are compared with published data derived in healthy newborns using similar tracer protocols (7).

MATERIALS AND METHODS

Patients. Eleven critically ill patients (six full-term newborns and five young infants) were studied. Their clinical characteristics are described in Table 1. All the newborns had appropriate weight for gestational age. The body weights of the five infants were below the 5th percentile for age (8). All patients had a diagnosis of sepsis and were admitted to the Neonatal and Pediatric Intensive Care Units at the Massachusetts General Hospital. Patients were studied within 48 h of the diagnosis of sepsis once hemodynamic stability was achieved. Sepsis in newborns was defined by the presence of two or more of the following: history of maternal fever or chorioamnionitis, rupture of membranes greater than 18 h, signs of respiratory distress, temperature instability, changes in level of consciousness or lethargy, shock, white blood cell count greater than 20 000 cells/mm³ or less than 5000 cells/mm³, a left shift with more than 10% band forms or greater than 60% polymorphonuclear forms, or a positive blood culture or evidence of clinical sepsis syndrome (9). Sepsis in infants was defined as bacteremic if there was a positive blood culture, or it was defined as clinical sepsis syndrome if three or more of the following were found: clinical evidence of infection (pneumonia, meningitis, for example), fever or hypothermia, tachypnea, tachycardia, or symptoms of decreased organ perfusion, such as oliguria, lethargy, or increased lactate level (10).

In the newborn group of six patients, three of them had positive blood cultures at diagnosis. Patient 2 had a positive blood culture

Adequate nutritional support of critically ill patients represents an essential component of medical care (1, 2), with well-nourished patients having better outcome and a shorter hospital stay (3, 4). Although current nutritional regimens provided to critically ill children are based largely on the nutritional requirements for healthy children (5, 6), these patients are often hypermetabolic and show profound changes in the pattern of energy substrate and amino acid use. It is likely that more effective nutritional therapies will evolve after a better understanding of the

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Correspondence: Leticia Castillo, M.D., Pediatric and Neonatal Intensive Care Units, Ellison 317, Massachusetts General Hospital, Fruit St., Boston, MA 02114.

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Table 1. Clinical characteristics of patients studied for phenylalanine and tyrosine kinetics*

Group	Patient	Age	Wt (kg)	Diagnosis	Severity score		
					NPSI	TISS	PSI
Newborns	1	4 d	3.72	Clinical sepsis syndrome; perinatal asphyxia	3	24	
	2	4 d	2.82	Group B streptococci sepsis	5	46	
	3	11 d	3.67	<i>Staphylococcus epidermidis</i> sepsis; S/P tetralogy of Fallot repair	6	33	
	4	3 d	4.0	Septic shock; NEC; positive culture for <i>Escherichia coli</i> and <i>Clostridium perfringens</i>	6	53	
	5	8 d	4.0	Clinical sepsis syndrome	5	22	
	6	2 d	2.84	Clinical sepsis syndrome; pulmonary hemorrhage	8	31	
	Mean ± SD	5.3 ± 2.7	3.5 ± 0.5		5.5 ± 1.5	29 ± 9	
Infants	7	4 mo	4.20	ARDS; <i>Streptococcus pneumoniae</i> sepsis		42	8
	8	5 mo	4.70	Failure to thrive; <i>E. coli</i> sepsis; <i>Pneumocystis carinii</i> pneumonia; HIV negative		46	6
	9	13 mo	7.80	Stevens Johnson syndrome; clinical sepsis syndrome; ARDS		77	19
	10	14 mo	7.85	Clinical sepsis syndrome		20	6
	11	6 mo	4.10	<i>Haemophilus influenzae</i> sepsis		39	9
	Mean ± SD	8.6 ± 4.6	5.7 ± 1.7		44.8 ± 18	9.6 ± 4.84	

* NPSI, Neonatal Physiologic Stability Index; PSI, Physiologic Stability Index; TISS, Therapeutic Intervention Score Systems; S/P, status post; NEC, necrotizing enterocolitis; ARDS, adult respiratory distress syndrome; HIV, human immunodeficiency virus.

to group B streptococci, patient 3 had a positive peripheral blood culture to *Staphylococcus epidermidis*, as well as positive blood culture to the same organism obtained from the central line placed for cardiac surgery. Patient 4 had a positive blood culture to *Escherichia coli* and positive culture to *Clostridium perfringens*, *Haemophilus parainfluenzae*, and *E. coli* from peritoneal fluid obtained during colectomy and ileostomy for necrotizing enterocolitis. Patient 5 came to the emergency department with shock, seizures, thrombocytopenia, leukopenia, and metabolic acidosis. Viral and bacterial cultures remained negative, and a workup for metabolic diseases was also negative. Patients 1 and 6 had negative blood cultures; however, both received antibiotics at the referring hospital before blood cultures were obtained, and the mother of patient 1 was treated with antibiotics for chorioamnionitis. These three patients (patients 1, 5, and 6), met the criteria for clinical sepsis syndrome. All patients had hemodynamic instability and required inotropic support with one (dopamine in patients 1, 3, and 5), two (dopamine and dobutamine in patients 2 and 6), or three (dopamine, dobutamine, and epinephrine in patient 4) inotropic agents. All patients required multiple fluid bolus for resuscitation. Disseminated intravascular coagulation was present in four patients (patients 2, 3, 4, and 6). All patients had variable degrees of metabolic acidosis and respiratory distress. Multiorgan failure (renal, hepatic, CNS, and respiratory) occurred in patient 4. Leukopenia was present in patients 4 and 5.

In the pediatric group, patients 7 and 10 had pneumonia and adult respiratory distress syndrome (patient 7 had a positive blood culture to *Streptococcus pneumoniae*; patient 10 had a negative bacterial blood culture at the time of the investigation and severe respiratory failure). Patient 8 had *Pneumocystis carinii* pneumonia, diagnosed by lung biopsy, and a positive blood culture to *E. coli*. Her HIV status was negative at the time of her discharge from the intensive care unit, although her mother tested HIV positive. Patient 9 had Stevens-Johnson syndrome resulting from bactrim treatment for otitis media. Adult respiratory distress syndrome and multiorgan failure developed in

this patient, but a blood culture was negative at the time of the study. Patient 11 had *H. influenzae* sepsis and severe bronchopulmonary dysplasia. Patients 9 and 10 met criteria for clinical sepsis syndrome.

All infant patients had variable degrees of multiorgan involvement; their lungs were mechanically ventilated, and they required inotropic support and had arterial and central venous lines in place. The newborn patients required a servocontrol radiant warmer to maintain skin temperature at 36.5°C. Infants were studied in a room with ambient temperature of about 25°C and relative humidity of 40%.

Physiologic Stability Index (11), Neonatal Physiologic Stability Index (12), and Therapeutic Intervention Score System (13) scores were obtained in each patient. These three systems objectively assess severity of disease and predict outcome (chances of death or survival). The Physiologic Stability Index and Neonatal Physiologic Stability Index estimate the degree of derangement in seven major physiologic systems (cardiovascular, respiratory, neurologic, hematologic, renal, gastrointestinal, and metabolic). After the degree of abnormality is assessed, each variable is assigned a score of 1, 3, or 5, indicating the clinical importance of the derangement. A score of 3 in a given variable mandates a change in therapy; a score of 5 indicates a life-threatening situation. The Therapeutic Intervention Score System evaluates the degree of intervention that a patient requires. Therapeutic interventions are classified from 1 to 4. Score 1 indicates routine intensive care interventions (ECG monitoring, supplemental oxygen, i.v. antibiotics, for example); score 2 indicates more invasive therapeutic or monitoring interventions, such as central venous pressure line and more than two i.v. lines or hemodialysis; score 3 indicates invasive therapeutic or monitoring interventions that may be lifesaving, such as endotracheal intubation. Score 4 indicates interventions in response to major events, such as cardiopulmonary resuscitation, pulmonary artery catheter placement, and cardiac pacing. These scores are routinely used in intensive care units for assessing severity of illness, with higher scores indicating a greater severity of the disease.

Study protocol. The study protocol was approved by the Subcommittee on Human Studies at the Massachusetts General Hospital. Written informed consent was obtained from the patient's parents or guardians. We used the arterial line as the sampling source and the central venous line for tracer infusion. Isotope tracers were infused by means of a calibrated syringe pump (Harvard Apparatus, Natick, MA). Verification of isotopic purity was obtained before the infusion study. Both tracer solutions (see below) were infused at a rate of $4.7 \text{ mL} \cdot \text{h}^{-1}$, except for infants 9 and 10, who received an infusion rate of $10.74 \text{ mL} \cdot \text{h}^{-1}$ because of their higher body weight. All patients received priming tracer doses of $5.4 \mu\text{mol} \cdot \text{kg}^{-1}$ of L-[1- ^{13}C]phenylalanine (99 atoms percent), and $3.6 \mu\text{mol} \cdot \text{kg}^{-1}$ of L-[3,3- $^2\text{H}_2$]tyrosine (98 atoms percent). The [^{13}C]phenylalanine-derived tyrosine pool was primed with $2.5 \mu\text{mol} \cdot \text{kg}^{-1}$ of L-[1- ^{13}C]tyrosine (98 atoms percent). Tracers were purchased from Tracer Technologies, Inc. (Somerville, MA). These primes were followed by 4-h constant i.v. infusions with [^{13}C]phenylalanine and [$^2\text{H}_2$]tyrosine at rates of 4.1 and $3.0 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, respectively. Labeled tracers were confirmed to be sterile and pyrogen free before use.

Blood samples for determinations of plasma phenylalanine and tyrosine isotopic enrichment and free plasma amino acid concentrations were obtained at 0, 180, 190, 200, 210, and 240 min in all patients. The samples were placed in tubes containing heparin and centrifuged immediately, and the plasma was stored at -80°C until used for analysis. The total amount of blood withdrawn during the entire study period in each patient was less than 5 mL.

Nutritional support. Nutritional support could not be manipulated experimentally in this investigation and so was provided as indicated by the routine clinical care of these critically ill infants. Hence, the levels of energy and protein intake varied between subjects. Mean energy intakes for neonates and infants were 35 and $40 \text{ kcal} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ respectively, provided principally as a 10–20% dextrose solution and a soybean lipid emulsion (Intralipid 10 or 20%, KabiVitrum Inc., Franklin, OH). The protein needs of each patient were supplied by a 1.5–2.5% amino acid mixture of Novamine (Novamine, 11.4%; Clintec, Deerfield, IL). The amino acid composition of the mixture is shown in Table 2. None of the patients received nutrients by the oral route. The duration of nutritional support before the infusion study was variable, ranging in the neonatal group from 0 to 32 h and in the infant group for 0 to 18 h. From the time of

admission, some neonates and infants were never parenterally or enterally fed before the infusion study.

Analytical methods. Amino acid concentrations in plasma and in the infusates of tracers were analyzed by means of a Hewlett-Packard Amino Quant (Palo Alto, CA), which involved pre-column derivatization with orthophthaldehyde and quantitation by fluorescence.

For isotopic analysis of phenylalanine and tyrosine, $200 \mu\text{L}$ plasma was used. To this 1 mL 2 N acetic acid was added, and then it was placed on an ion exchange column (BioRad AG50-X2 resin, Richmond, CA) (14). The fraction containing the amino acids was eluted with ammonium hydroxide and then evaporated to dryness. A tertiary butyldimethylsilyl derivative of the amino acids was prepared in a one-step procedure by heating the residue for 1 h at 60°C with $50 \mu\text{L}$ of MTBSTFA [*n*-methyl-*N*-(*tert*butyldimethylsilyl)trifluoroacetamide] (Pierce, Rockford, IL) and $50 \mu\text{L}$ of acetonitrile (15). Two to $3 \mu\text{L}$ of mixture were sufficient for gas chromatography/mass spectrometry analysis.

All measurements of plasma amino acid isotope dilutions were carried out on a Hewlett-Packard 5890 Series II gas chromatograph coupled to a Hewlett-Packard 5988A quadrupole mass spectrometer and a Hewlett-Packard RTE-A data system. Electron impact ionization was carried out at 120 eV.

Gas chromatography/mass spectrometry analysis of phenylalanine and tyrosine was carried out in one run on a $30 \text{ m} \times 0.25 \text{ mm}$ DB 1301 fused silica column (J and W Scientific, Folsom, CA) that was temperature programmed from 200°C to 300°C at $15^\circ\text{C}/\text{min}$. Phenylalanine and tyrosine eluted at 5.6 and 7.8 min, respectively. Selective ion monitoring of both amino acids was carried out with the M-57 fragment ion that corresponded to loss of the *t*-butyl group (15). Selective ion monitoring was carried out at m/z 336 and m/z 337 for natural and [^{13}C]phenylalanine, respectively. Ions at m/z 466, m/z 467, and m/z 468 were monitored for natural, [^{13}C], and [$^2\text{H}_2$]tyrosine, respectively.

Because the ion clusters of the tyrosine tracee and the [^{13}C] and [$^2\text{H}_2$] tracers overlap, estimation of sample enrichments was based on multiple linear regression, where the various mass spectrometer signals were used as dependent variables and the sample enrichments served as unknown parameters. The coefficients of the regression equations were derived from standard mixtures ranging from 0 to 8% mole fraction for [^{13}C]phenylalanine and [$^2\text{H}_2$]tyrosine and 0 to 6% mole fraction for [^{13}C]tyrosine. A further set of five standard mixtures was also prepared containing both [^{13}C] and [$^2\text{H}_2$]tyrosine. Each sample was measured in duplicate and multiple samples measured during the isotopic plateau. Additional details concerning the determination of plasma isotope enrichments of phenylalanine and tyrosine are given in the appendix.

Enantiomeric purity of the tracers was verified by gas chromatographic analysis on a Chirasil-Val capillary column (Chrom Pack, Middelburg, The Netherlands) as the pentafluoropropionyl propyl esters (16).

Calculations. The model of phenylalanine-tyrosine metabolism used here follows that described by Clarke and Bier (17) with modifications proposed by Thompson *et al.* (18). Briefly, the amino acid (phenylalanine or tyrosine) flux (Q_P or Q_T , respectively; $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) is calculated by isotope dilution, as previously described for leucine (19):

$$Q = i \cdot \left(\frac{E_i}{E_p} - 1 \right) \quad (1)$$

where i is the rate of tracer infusion ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) and E_i and E_p are the isotopic enrichments of the infusate and plasma amino acid, respectively.

The rate of phenylalanine conversion to tyrosine ($Q_{P \rightarrow T}$; $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) (phenylalanine hydroxylation) was derived as follows:

Table 2. Composition of amino acid solution used for parenteral administration*

Amino acid	Unit of measure		Nitrogen (g/L)
	g/L	g/100 g amino acids	
Isoleucine	5.7	5.0	6.09
Leucine	7.9	6.9	8.44
Lysine	9.0	7.9	11.50
Methionine	5.7	5.0	5.35
Phenylalanine	7.9	6.9	6.70
Threonine	5.7	5.0	6.70
Tryptophan	1.9	1.7	2.61
Valine	7.3	6.7	8.73
Alanine	16.5	14.5	25.94
Arginine	11.2	9.8	29.77
Histidine	6.8	6.0	18.41
Proline	6.8	6.0	8.28
Serine	4.5	3.9	6.00
Glycine	7.9	6.9	14.74
Tyrosine	0.3	0.3	0.23
Glutamic acid	5.7	5.0	5.43
Aspartic acid	3.3	2.9	3.47
Total	114.1		168.39

* Novamine 11.4% (composition data provided by Clintec, Deerfield, IL).

$$Q_{P \rightarrow T} = Q_T \cdot \left(\frac{Et}{Ep} \right) \left[\frac{Q_P}{(ip + Q_P)} \right] \quad (2)$$

where Q_T and Q_P are the tyrosine and phenylalanine fluxes, respectively, estimated from the primed constant infusions of [$^2\text{H}_2$]tyrosine and 1-[^{13}C]phenylalanine; Et and Ep are the plasma enrichments of 1-[^{13}C]tyrosine and 1-[^{13}C]phenylalanine, respectively; ip is the rate of infusion of the labeled phenylalanine. As discussed by Thompson *et al.* (18), the term $Q_P/(ip + Q_P)$ corrects for the contribution of the phenylalanine tracer infusion to $Q_{P \rightarrow T}$.

The rate of phenylalanine disappearance by incorporation into proteins (Phe_S) was estimated by subtracting the rate of phenylalanine hydroxylation ($Q_{P \rightarrow T}$) from phenylalanine flux (Q_P):

$$\text{Phe}_S = Q_P - Q_{P \rightarrow T} \quad (3)$$

Phenylalanine entering the plasma pool by protein breakdown (Phe_B) was determined from phenylalanine flux and phenylalanine intake (pi):

$$\text{Phe}_B = Q_P - pi \quad (4)$$

Thus, from equations 3 and 4, the body balance of phenylalanine (Phe_{BAL}) can be obtained:

$$\text{Phe}_{BAL} = \text{Phe}_S - \text{Phe}_B \quad (5)$$

The rates of whole body protein synthesis and breakdown were obtained according to Thompson *et al.* (18) assuming the phenylalanine content of whole body mixed proteins to be $280 \mu\text{mol/g}$ protein. We have expressed these rates as $\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$. Thus:

$$\text{Prot}_S = \frac{\text{Phe}_S \times 24}{280} \quad (6)$$

$$\text{Prot}_B = \frac{\text{Phe}_B \times 24}{280} \quad (7)$$

and

$$\text{Prot}_{BAL} = \text{Prot}_S - \text{Prot}_B \quad (8)$$

Data analysis. Results are summarized as means \pm SD as a measure of dispersion of the individual data points. Primary and derived values were tested for significance between infant and newborn groups using analysis of covariance with age, weight, and severity of disease as variables. Adjusted means were tested for significance with a least-squares mean procedure. Dependent variables included phenylalanine flux (Q_P), tyrosine flux (Q_T), phenylalanine hydroxylation ($Q_{P \rightarrow T}$), and protein turnover (Prot_S , Prot_B , and Prot_{BAL}). Because no statistically significant differences were observed between newborns and infants, the results for both groups were combined for purposes of comparing these values with those reported in the literature (7). A $p < 0.05$ was chosen as being statistically significant. Relationships among variables were tested for significance using Pearson's product moment correlation. The relationship between phenylalanine and tyrosine intake with phenylalanine and protein balance was evaluated by least-squares linear regression analysis. An SAS program (Cary, NC) was used for the statistical analysis (20).

RESULTS

Energy and protein intakes for the patients during the period of study are shown in Table 3. The levels of intake were lower than those recommended for healthy newborns and infants (21), which generally reflects our patients' critical clinical status. Critically ill patients often require infusion of multiple drugs, blood products, and fluids at the expense of their nutritional support. Furthermore, fat administration in the presence of respiratory failure or sepsis is often restricted by attending physicians because of concerns about possible lipid-induced impairment of respiratory and immune function (22–24).

The Plasma-free phenylalanine and tyrosine concentrations during the tracer study period are shown in Table 4. The phenylalanine/tyrosine ratio in these patients with sepsis was increased (1.67) when compared with values reported for healthy newborns (0.71) (25) and adults (0.82) (26, 27).

A steady state level of isotopic enrichment in plasma of our patients was achieved during the 4-h continuous tracer infusion period as determined by lack of a statistically significant slope in the plasma pools of phenylalanine and tyrosine isotopologs (Fig. 1). For the entire group of patients, the mean coefficients of variation for plasma isotope enrichment plateaus were 7, 5, and 11% for [^{13}C]phenylalanine, [$^2\text{H}_2$]tyrosine, and [^{13}C]tyrosine, respectively. Their kinetics and the rates of whole body protein turnover were calculated from these isotopic enrichments of plasma phenylalanine and tyrosine.

Phenylalanine and tyrosine fluxes and rates of phenylalanine hydroxylation were 137 ± 17 , 63 ± 13 , and $34 \pm 6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ in the neonates and 126 ± 29 , 70 ± 18 , and $24 \pm 14 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ in the infants (Table 5). Considerable variation existed within each group, and differences between group means were not statistically significant ($p > 0.1$). Therefore, for our purposes, we considered it appropriate to combine the results into one data set for the further comparisons given below. The combined group mean phenylalanine flux was $132 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, with a mean phenylalanine hydroxylation rate of $29 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$, representing 22% of the phenylalanine flux.

The components of phenylalanine flux are presented in Table 6. Tyrosine intakes were very low (mean, $0.6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$). Intakes of phenylalanine ranged widely, with the mean being $17.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. Extrapolated to a daily basis, this amounts to a mean intake of $69 \text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, which is in the requirement range for healthy infants when tyrosine intakes are generous (28), but it presumably would be less than adequate (21) for tyrosine intakes that are low, as in the present study. Also, because the mean phenylalanine hydroxylation rate was $29 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ (equivalent to $115 \text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) (Table 5), the group of patients as a whole were in negative body phenylalanine balance. In Table 6, we summarized the rates of disappearance of phenylalanine from plasma by protein synthesis (Phe_S) and entry into plasma by protein degradation (Phe_B). Again, these rates reflect the extent of the negative body phenylalanine balance, which was a mean of $-14 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ for the combined groups.

The derived values for body protein turnover are presented in Table 7. Mean protein synthesis and breakdown rates were 8.6 and $9.81 \text{g} \text{protein} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, respectively, for the combined two study populations.

The following statistical correlations were observed in these children with sepsis: body protein balance was negatively correlated ($R^2 = 0.5$; $p < 0.01$) with the rate of phenylalanine hydroxylation to tyrosine and positively correlated ($R^2 = 0.4$, $p < 0.04$) with phenylalanine intake (Fig. 2).

DISCUSSION

We have investigated the kinetics of whole body phenylalanine and tyrosine metabolism in critically ill children with sepsis. Despite the importance of phenylalanine as an indispensable amino acid and as precursor for tyrosine, relatively few studies address phenylalanine and tyrosine kinetics in relationship to the metabolic needs and nutritional status of pediatric patients (29–31). To our knowledge, no similar studies appear to have been conducted previously in critically ill children with sepsis. Because we were not able to investigate a healthy cohort, our combined data for phenylalanine and tyrosine kinetics, as well as the estimates obtained for whole body protein turnover in critically ill young infants with sepsis are compared here with those of Denne *et al.* (7), who have reported similar data for seven normal, term infants studied at approximately 21 d of age. In their study, phenylalanine and tyrosine fluxes and rate of phenylalanine hydroxylation were 76 ± 12 , 53 ± 12 , and 12 ± 3 , respectively,

Table 3. Energy and macronutrient intakes by parenteral feeding in critically ill newborns and infants

Patient and group	Glucose (mg·kg ⁻¹ ·min ⁻¹)	Protein (g·kg ⁻¹ ·d ⁻¹)	Lipid (g·kg ⁻¹ ·d ⁻¹)	Energy (kcal·kg ⁻¹ ·d ⁻¹)
Newborns				
1	6.9	1.3	0	32.8
2	5.8	1.5	0	29.2
3	6.5	1.0	1.0	32.4
4	3.0	0	0	26.0
5	13.0	2.4	2.0	61.6
6	5.0	1.0	1.0	30.3
Mean ± SD	6.7 ± 3	1.2 ± 0.7	0.5 ± 0.7	35 ± 11.9
Infants				
7	6.1	1.0	1.0	30.5
8	6.0	1.0	1.0	29.9
9	6.0	0	0	24.0
10	14.9	2.6	0.5	70.9
11	8.9	1.6	1.0	44.0
Mean ± SD	8.4 ± 3.5	1.2 ± 0.8	0.7 ± 0.4	39.9 ± 16.8

Table 4. Plasma-free phenylalanine and tyrosine concentrations in newborns and infants with sepsis

	Phenylalanine (μmol/L)	Tyrosine (μmol/L)
Neonates		
1	55.1	30.7
2	125.3	68.8
3	163.8	109.2
4	149.4	61.5
5	69.0	55.5
6	134.7	106.9
Mean ± SD	116 ± 44	72 ± 31
Phenylalanine/tyrosine ratio	1.6 ± 0.05	
Infants		
7	66.7	49.0
8	64.4	41.0
9	74.3	35.2
10	77.3	52.7
11	79.6	23.2
Mean ± SD	72.5 ± 7	40.2 ± 12
Phenylalanine/tyrosine ratio	1.8 ± 0.9	
Combined data		
Phenylalanine/tyrosine ratio	1.7 ± 0.6	

for the fasted state and 86 ± 13 , 46 ± 8 , and 10 ± 3 (mean \pm SD; $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$), respectively, for the fed state. These investigators used [²H₅]phenylalanine and [²H₂]tyrosine as tracers. Thus, phenylalanine fluxes are higher in our patients with sepsis when compared with the healthy newborns, as might be anticipated for a hypermetabolic, catabolic condition. When the intravenously administered phenylalanine intake is subtracted from phenylalanine flux, the "corrected," mean phenylalanine flux is $115 \pm 24 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$, remaining higher than that in healthy newborns (7). This correction for i.v. intake of phenylalanine is necessary, particularly because Wykes *et al.* (31) found that phenylalanine flux was greatly affected by the parenteral phenylalanine supplied. Tyrosine fluxes in our group of critically ill infants with sepsis were similar or perhaps somewhat higher than those in healthy neonates (7), who were also receiving no tyrosine by i.v. feeding. Also, the hydroxylation of phenylalanine, expressed either as an absolute rate or as a percentage of the phenylalanine flux, was higher in our patients than was phenylalanine hydroxylation in newborn infants as reported by Denne

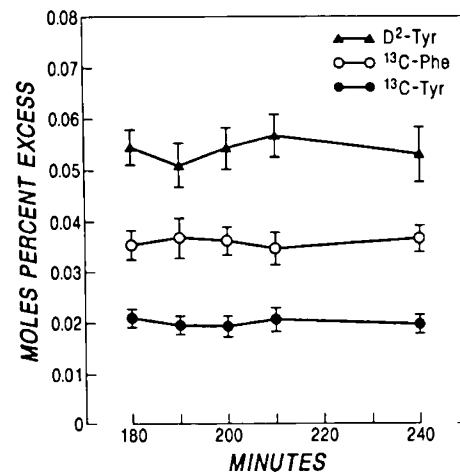


Fig. 1. Results for plasma isotopic enrichment of ¹³C-phenylalanine (¹³C-Phe), [²H₂]tyrosine (*D*₂-Tyr), and ¹³C-tyrosine (¹³C-Tyr) during the 4-h i.v. tracer infusion study. Vertical lines represent \pm SD for the entire group of 11 patients.

et al. (7). Some difficulty exists, however, in drawing a strict comparison between the present hydroxylation data and those of Denne *et al.* (7) because pentadeuterated phenylalanine was used as tracer by these latter investigators; we have shown recently in healthy adults that this tracer underestimates hydroxylation rates *in vivo* by about a factor of 2.0 (32). For this reason, the estimate of $10\text{--}12 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ for phenylalanine hydroxylation in healthy newborns (7) might be lower than the actual rates, although that is speculative and a matter for further study.

The values for Prot_S and Prot_B in our patients with sepsis are somewhat higher than the 7 and 6 $\text{g}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$, respectively, reported for healthy infants (25, 33), although perhaps they are not as high as might be anticipated for hypermetabolic or acute catabolic states (34).

Despite these difficulties, in fully comparing the present values with those appearing in the published literature, the present data point to changes in kinetics and in the relationship between phenylalanine and tyrosine metabolism in infants with sepsis versus normal infants. For example, phenylalanine fluxes in our critically ill infants are considerably higher than those reported for normal babies (7). Furthermore, the flux ratio of phenylalanine to tyrosine was higher in our infants than in the normally growing newborns studied by Denne *et al.* (7). This finding is consistent with previous reports (35) showing an increased plasma phenylalanine/tyrosine concentration ratio in patients

Table 5. Plasma phenylalanine and tyrosine kinetics in children with sepsis*

Group	Flux		Hydroxylation (Q_{P-T})	$Q_{P-T}/Q_P \times 100$
	Phenylalanine	Tyrosine		
Newborns				
1	158	65	44	28
2	114	82	28	25
3	118	78	37	31
4	140	51	25	18
5	157	52	37	24
6	132	50	35	27
Mean \pm SD	137 \pm 17	63 \pm 13	34 \pm 6	25 \pm 4
Infants				
7	161	75	24	15
8	114	97	48	42
9	76	73	11	14
10	146	63	26	18
11	134	40	9	7
Mean \pm SD	126 \pm 29	70 \pm 18	24 \pm 14	19 \pm 12
Combined data	132 \pm 24	66 \pm 16	29 \pm 12	22 \pm 9

* Values are $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$.

Table 6. Phenylalanine and tyrosine intake, disappearance by protein synthesis, appearance by protein breakdown, and body phenylalanine balance

Group	Intake				
	Phenylalanine	Tyrosine	PHE _S	PHE _B	PHE _{BAL}
Newborns					
1	8	0.26	114	151	-36.00
2	27	0.95	86	87	-0.86
3	14	0.47	81	104	-22.80
4	0	0.00	116	141	-24.17
5	37	1.20	120	120	-0.07
6	18	0.60	97	115	-17.38
Mean \pm SD	17.2 \pm 12	0.58 \pm 0.4	102.5 \pm 15	119.5 \pm 21	-17 \pm 13*
Infants					
7	18	0.60	137	143	-6.25
8	7	0.23	65	107	-41.19
9	0	0.00	64	76	-10.86
10	47	1.60	98	99	-0.92
11	17	0.58	125	117	7.76
Mean \pm SD	17.7 \pm 16	0.59 \pm 0.5	97.9 \pm 29	108.2 \pm 22	-10 \pm 17
Combined mean \pm SD	17.4 \pm 14	0.59 \pm 0.47	100.4 \pm 23	114 \pm 22	-13.9 \pm 15

* Values are $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$. PHE_S, phenylalanine disappearance by protein synthesis; PHE_B, phenylalanine appearance by protein breakdown; PHE_{BAL}, body balance of phenylalanine.

with sepsis, suggesting an increased rate of muscle breakdown, reduced rate of incorporation of phenylalanine into muscle proteins, and/or an alteration in the rate of hepatic conversion of phenylalanine to tyrosine (26, 27, 35, 36). Also, during protein deficiency, phenylalanine use decreases, leading to a relative increase in the plasma phenylalanine level, which again is possibly due to a decreased enzymatic conversion of phenylalanine to tyrosine (37, 38). Of course, the low content of tyrosine in the parenteral amino acid mixture would also be expected to raise the plasma-free phenylalanine/tyrosine ratio, although the ratio was also high in the two patients (patients 4 and 9) who were receiving glucose alone. This finding suggests an altered aromatic amino acid homeostasis in our patients and possibly a change in the coupling between phenylalanine kinetics, hydroxylation, and tyrosine balance and oxidation. We speculate that because of the stressful or catabolic state combined with the low tyrosine intake, an impaired balance exists among phenylalanine retention, hydroxylation, and tyrosine homeostasis in these patients with sepsis. Thus, phenylalanine hydroxylation rates may be adaptively increased in these patients but with the net effects of

impairing phenylalanine balance and inadequately meeting the metabolic needs for tyrosine. It is not unusual for critically ill patients with sepsis to develop supranormal values of physiologic parameters such as cardiac output or oxygen delivery that are still insufficient to meet their needs (39). We speculate that a similar situation may occur at the metabolic level, whereby multiple factors, such as increased protein breakdown and changes in the pattern of amino acid utilization and requirements, lead to increased rates of phenylalanine hydroxylation that are still insufficient to meet the tyrosine requirements. This hypothesis will need to be verified in follow-up investigations in which different intakes and ratios of phenylalanine and tyrosine intake are examined for their effects on phenylalanine and tyrosine kinetics. Alternatively, the altered rates of phenylalanine hydroxylation and their relationships to phenylalanine kinetics and intake might be a more general reflection of an impairment and changes in amino acid catabolism, which frequently occurs in sick and stressed patients (40, 41).

The clinically determined standard amount of total parenteral nutrition formulation routinely used in our critically ill patients

Table 7. Whole body protein turnover (synthesis and breakdown) in critically ill infants

Group	Synthesis (g·kg ⁻¹ ·d ⁻¹)	Breakdown (g·kg ⁻¹ ·d ⁻¹)	Protein balance (g·kg ⁻¹ ·d ⁻¹)
Newborns			
1	9.81	12.91	-3.11
2	7.36	7.44	-0.07
3	6.98	8.94	-1.95
4	9.93	12.04	-2.11
5	10.31	10.32	-0.01
6	8.34	9.83	-1.49
Mean ± SD	8.8 ± 1.3	10.2 ± 1.83	-1.5 ± 1.11
Infants			
7	11.71	12.24	-0.54
8	5.63	9.16	-3.53
9	5.55	6.48	-0.93
10	8.37	8.45	-0.08
11	10.71	10.05	0.67
Mean ± SD	8.4 ± 2.53	9.3 ± 1.89	-0.88 ± 1.43
Mean (combined) ± SD	8.6 ± 1.97	9.81 ± 1.92	-1.2 ± 1.3

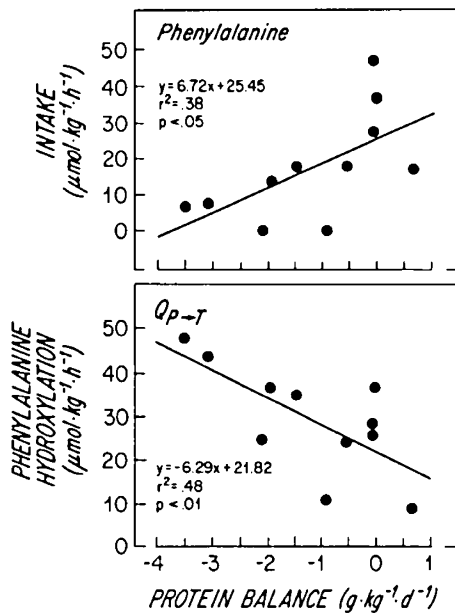


Fig. 2. Relationship between body protein balance and phenylalanine intake (top panel) and phenylalanine hydroxylation (bottom panel).

appears to be inadequate to support their aromatic amino acid needs. From the data shown in Table 6, intakes in the range of about 30–40 $\mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ permit neutral body phenylalanine balances, suggesting that this intake level (equivalent to about 138 mg phenylalanine·kg⁻¹·d⁻¹) is probably an appropriate target for critically ill patients during the acute stage of their disease and in the virtual absence of a tyrosine intake.

Critically ill newborns and infants with sepsis who receive limited parenteral protein and energy intakes, as dictated by routine clinical practice (34, 42), are unable to maintain phenylalanine homeostasis, and because of the link between phenylalanine and tyrosine metabolism, it is to be expected that a deterioration in tyrosine homeostasis will occur. Indeed, tyrosine may become a “conditionally” indispensable amino acid under these circumstances despite an apparently generous supply of phenylalanine. The reason for this is that the formation of tyrosine from phenylalanine occurs principally in the liver, where this tyrosine may or may not be made as easily available in times

of metabolic stress as an exogenous source of tyrosine would be for meeting the tyrosine needs of the body organs and tissues. Because of its poor solubility, most parenteral amino acid mixtures contain only a trace of tyrosine, while being designed to provide a generous phenylalanine intake; the assumption here is that the supply of phenylalanine will be sufficient to maintain an adequate availability of tyrosine where it is required. This assumption may be, and probably is, an overly simplistic assessment of phenylalanine-tyrosine interrelationships, particularly if the regulation of tissue and organ pools of phenylalanine and tyrosine occurs by way of separate systems and mechanisms and if these are, in turn, affected differently by stressful stimuli, including infection. Although another commonly used mixture (Trophamine, Kendall-McGaw Laboratories, Irvine, CA), has been supplemented with acetyl-tyrosine, the target intake level has been designed to support plasma amino acid concentrations similar to those observed in healthy full-term, breast-fed, 1-month-old infants (43, 44). Although a possibly useful criterion, it is important to assess whether “normal” serum values represent an appropriate nutritional goal for the critically ill patients (45, 46).

Current estimates of amino acid requirements in human subjects are based largely on nitrogen balance studies (21); in the case of children, these values are supplemented with data from measurements of growth rates in response to specific nutritional treatments (21, 47). In the case of critically ill neonates and infants, these procedures are not feasible, and we suggest, from the present experience, that application of stable isotope tracer methods might be a useful way to investigate the quantitative requirements for specific amino acids in these patients. Indeed, ¹³C-enriched amino acid tracers have been used successfully in adults to estimate minimum intakes required to achieve body balance of the irreversible oxidative losses of nutritionally indispensable amino acids (48). However, we recognize that for critically ill children with mechanically ventilated lungs it might be difficult to obtain accurate measurements of total ¹³C-labeled CO₂ and CO₂ production by indirect calorimetry, especially under conditions of the high air flow rates used to meet their ventilatory needs. Measurements of ¹³CO₂ in plasma is possible, but precise estimation of rates of total CO₂ production in these patients remains difficult. For these reasons, we chose to apply the present phenylalanine-tyrosine tracer model as described by Thompson *et al.* (18), except that we used [1-¹³C]phenylalanine instead of [2-³H-ring]phenylalanine as tracer, thereby obviating the need for breath collections and measurement of respiratory gas exchange rates.

In summary, we describe here, apparently for the first time, phenylalanine and tyrosine kinetics in critically ill pediatric patients with sepsis. From these observations we conclude that current clinical practice of total parenteral administration during critical illness is insufficient to meet the aromatic amino acid requirements of these patients. Under these circumstances, we also speculate that tyrosine may be a “conditionally” indispensable amino acid. Hence, consideration should now be given to defining adequate intakes of the aromatic acids and, in particular, the ratio between phenylalanine and tyrosine. We fully appreciate that the present study is limited by sample size, the variable clinical condition of our patients, lack of a concurrent control group of healthy infants, and the different, clinically determined intakes of macronutrients among the infants. Although these difficulties are commonly faced by investigators who are interested in carrying out precise metabolic studies in critically ill infants, we considered it important to begin to establish in quantitative terms whether the nutritional component of these infants' care was appropriate to the metabolic changes that accompany their disease state. Our findings indicate the desirability of now investigating the impact of the level of aromatic amino acid intake, including the exogenous supply of tyrosine possibly given in dipeptide form, on phenylalanine and tyrosine kinetics and balance and their functional significance in critically ill children with sepsis.

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APPENDIX

Calculations regarding [¹³C] and [²H₂]tyrosine enrichments. The isotopomers of tyrosine seen arising at m/z 466[M-57]⁻ in the electron impact mass spectrum of the tertiary butyldimethylsilyl derivative at the m + 1 (m/z 467) and m + 2 (m/z 468) channels have abundances of 20% and 44%, respectively, relative to the main ion (m) at m/z 466. The isotopomers of the tracee therefore overlap with those of the less abundant [¹³C] and [²H₂] tyrosine tracers present in the labeled plasma samples.

If only one tracer was present in the samples to be analyzed, then standards containing mixtures of natural and [¹³C] or [²H₂] tyrosine could be measured and a calibration graph constructed. The regression coefficient (slope) and y intercept (constant) could then be calculated by single linear regression. These could be used to solve for the unknown enrichments in plasma. However, in these studies two tracers are present, and their isotopomers overlap. The ¹³C-tracer that is found at m/z 467 has isotopomers arising from it that contribute 44% of its signal to the channel at m/z 468 where the [²H₂]tyrosine is predominantly found. Also, the [²H₂]tyrosine tracer has isotopomers arising from it at m/z 468 that are 10% of its signal and contribute to the channel at m/z 467 where the ¹³C-tracer is predominantly found. If the contributions of these isotopomers are not taken into account, then an overestimate of both enrichments will be made, resulting in an underestimate of tyrosine flux and an overestimate of phenylalanine hydroxylation. For instance, in these studies the corrected mean plasma [¹³C] and [²H₂]tyrosine enrichments were about 1.5 and 5%, respectively. However, because of the 44% spill-up of the [¹³C]tyrosine tracer into the ²H₂-tracer channel at m/z 468, the uncorrected enrichment would have been 5.7%. Also, because of the [²H₂]tyrosine spill-down into the [¹³C]

tyrosine channel at m/z 467, the uncorrected enrichment would have been 2.0%.

To accurately calculate the enrichment of both the [^{13}C] and [$^2\text{H}_2$]tyrosine enrichments that allows the signals at both m/z 467 and m/z 468 to be taken into account, we carried out multiple linear regression of the ion ratios obtained from the standard mixtures as follows. First, the data obtained from the first set of standards containing only [^{13}C]tyrosine and the second set of standards containing only [$^2\text{H}_2$]tyrosine were plotted by assigning the [^{13}C] or [$^2\text{H}_2$]tyrosine mole fractions to the x axis and m/z 467/ m/z 466 and m/z 468/ m/z 466 to the y axis. These two calibrations were checked for linearity and outliers. Then the data obtained from the [^{13}C] and [$^2\text{H}_2$]tyrosine mixtures were combined with the two sets of standards containing only one labeled tracer. Multiple linear regression was carried out by regressing mole fraction [^{13}C]tyrosine against the ratios m/z 467 / m/z 466 and m/z 468 / m/z 466. The equation for [$^2\text{H}_2$]tyrosine

mole fraction was then also obtained by regressing the mole fraction [$^2\text{H}_2$]tyrosine against the ratios m/z 467 / m/z 466 and m/z 468 / m/z 466. The following equations from the multiple regression least squares analysis were obtained:

$$\text{Mole fraction } [^{13}\text{C}]\text{tyrosine} = C^1_0 + C^1_1N_1 + C^1_2N_2$$

$$\text{Mole fraction } [^2\text{H}_2]\text{tyrosine} = C^2_0 + C^2_1N_1 + C^2_2N_2$$

where C^1_0 and C^2_0 are the derived constants for the [^{13}C] and [$^2\text{H}_2$]tyrosine, respectively. C^1_1 and C^2_1 are the derived regression coefficients for [^{13}C] and [$^2\text{H}_2$]tyrosine at m/z 467 / m/z 466, respectively. C^1_2 and C^2_2 are the derived regression coefficients for [^{13}C] and [$^2\text{H}_2$]tyrosine at m/z 468 / m/z 466, respectively. These would then be used to calculate the mole fraction enrichments of the [^{13}C] and [$^2\text{H}_2$]tyrosine from the measured ratios (N_1 and N_2) found in the plasma samples at m/z 467 / m/z 466 and m/z 468 / m/z 466, respectively.