The Effect of Graded Intake of Glycyl-L-Tyrosine on Phenylalanine and Tyrosine Metabolism in Parenterally Fed Neonates with an Estimation of Tyrosine Requirement

SUSAN A. ROBERTS, RONALD O. BALL, AIDEEN M. MOORE, ROBERT M. FILLER, AND
PAUL B. PENCHARZ

*Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada, M5G 1X8 [S.A.R., A.M.M.,
R.M.F., P.B.P.]; Departments of Paediatrics [A.M.M., P.B.P.], Nutritional Sciences [S.A.R., R.O.B.,
P.B.P.], and Surgery [R.M.F.], University of Toronto, Toronto, Ontario, Canada, M5S 1A1; and
Department of Agricultural, Food, and Nutritional Sciences, University of Alberta, Edmonton, Alberta,
Canada, T6G 2P5 [R.O.B.]*

ABSTRACT

Although tyrosine is considered indispensable during the neonatal period, its poor solubility has limited its inclusion in parenteral amino acid solutions to less than 1% of total amino acids. Dipeptides of tyrosine are highly soluble, have been shown to be well used and safe in animal models and humans, and, therefore, may be used as an effective means of providing tyrosine in the parenterally fed neonate. The goal of the present study was to determine the tyrosine requirement of the parenterally fed neonate receiving graded intakes of glycyl-L-tyrosine as a source of tyrosine. Thirteen infants receiving adequate energy (340 ± 38 kJ·kg^{-1}·d^{-1}) and protein (2.4 ± 0.4 g·kg^{-1}·d^{-1}) were randomized to receive parenteral nutrition with one of five graded levels of glycyl-L-tyrosine. The mean requirement and safe level of intake were estimated using a 1-^{13}C-phenylalanine tracer and linear regression cross-over analysis that identified a break point in the response of label appearance in breath CO_2 ($F^{13}CO_2$) and phenylalanine oxidation to graded tyrosine intake. Based on the mean estimates of whole-body phenylalanine oxidation, the tyrosine mean requirement and safe level of intake were found to be 74 mg·kg^{-1}·d^{-1} and 94 mg·kg^{-1}·d^{-1}, respectively. This represents 3.1 and 3.9% of total amino acids, respectively, considerably higher than levels found in present commercially available pediatric amino acid solutions. These data raise concern regarding the adequacy of aromatic amino acid intake in the parenterally fed neonate. (*Pediatr Res* **49: 111–119, 2001**)

Abbreviations
TPN, total parenteral nutrition
FCO$_2$, breath carbon dioxide production
F^{13}CO$_2$, breath ^{13}CO$_2$ production
GT, glycyl-L-tyrosine

Nearly three decades ago, Snyderman [1] suggested that the neonate requires a preformed source of tyrosine. Presently, the main impediment to meeting total aromatic amino acid requirements in the parenterally fed neonate is the poor solubility of tyrosine (0.453 g/L in H_2O at 25°C) [2], limiting its inclusion in parenteral amino acid solutions to <1% of total amino acids. When compared with neonatal reference proteins such as human milk or human fetal tissue, which contain approximately equal quantities of phenylalanine and tyrosine (3.7–4.1 and 2.9–4.6 g/100 g protein, respectively) [3–5], the levels provided by these synthetic nutrient solutions appear grossly inadequate. To circumvent this problem, increased phenylalanine has been included in some solutions in hope that the neonate will endogenously hydroxylate sufficient phenylalanine to tyrosine to meet total aromatic amino acid needs. However, elevated plasma phenylalanine levels have been observed with this approach [6–11], even though it has been shown that increased phenylalanine intake is associated with an absolute greater rate of *in vivo* phenylalanine hydroxylation [12].

Derivatives of tyrosine have also been investigated as soluble precursors of tyrosine. N-acetyl-tyrosine, for example, has been included in one of the commercially available pediatric amino acid solutions, TrophAmine (McGaw Inc., Irvine, CA, U.S.A.) [13]. Unfortunately, N-acetyl-tyrosine has been shown

to be poorly used in the parenterally fed piglet, which excreted 65% of intake (14), and in the human neonate who also has demonstrated poor utilization through the excretion of large quantities of the derivative (13, 15–17). In contrast, there is evidence that dipeptides of tyrosine can serve as a soluble source of tyrosine in pigs (14, 18, 19), primates (20), and adult humans (21–25). To improve the utilization of the presently available pediatric amino acid solutions, a soluble source of tyrosine must be included. At present, dipeptides seem to be the most likely candidate to meet this objective.

Using graded intakes of tyrosine in the form of GT, House *et al.* (18) estimated the mean requirement and safe tyrosine intake (95% upper confidence limit) in the piglet receiving a fixed phenylalanine intake of 4.1% of total amino acids to be 2.7 and 3.2% of total amino acids, respectively. This is significantly higher than the level of tyrosine in currently available pediatric amino acid solutions (0.9% of total amino acids). The lower level of total aromatic amino acids is responsible for the reduced growth and nitrogen retention in piglets receiving the pediatric amino acid solution Vaminolact (Pharmacia and Upjohn, Stockholm) *versus* the general purpose solution Vamin (Pharmacia and Upjohn, Stockholm) (26).

Due to the immaturities in the neonatal tyrosine catabolic enzyme pathway, tolerance of tyrosine intakes at levels greatly over requirement is limited (27). In addition, due to the known neurologic impairment caused by hypertyrosinemia to the developing brain as assessed by lower IQ and psychologic tests, excess intakes must be avoided (28, 29).

The tyrosine requirement in the neonate receiving parenteral nutrition has not been determined. In the present study, this was determined by partitioning the oxidation of phenylalanine to graded intake of tyrosine at a fixed phenylalanine intake.

METHODS

Patients and nutrient intake. Thirteen parenterally fed neonates (Table 1) who had been referred to the Neonatal Intensive Care Unit at The Hospital for Sick Children were enrolled into the study. All infants were clinically stable during the study period and were maintained in standard servo-controlled incubators or infant cribs. Intestinal atresia, necrotizing enterocolitis, gastroschisis, and malrotation were the diagnoses of infants enrolled in the study (Table 1). The study protocol was reviewed and approved by the Human Subject Review Committee of The Hospital for Sick Children, and written informed consent was obtained from one or both parents.

Study subjects were enrolled into the study when receiving adequate amino acid and energy intake (30); targeted minimal intakes were 2 $g \cdot kg^{-1} \cdot d^{-1}$ of amino acid and 270 $kJ \cdot kg^{-1} \cdot d^{-1}$. Infants were randomized to receive TPN containing a pediatric amino acid solution, Primene (Baxter, Deerfield, IL, U.S.A.), with one of five levels of GT supplement (ICN Biomedicals, Mississauga, Ontario) as the source of tyrosine for 2 d. The base amino acid profile of Primene is shown in Table 2. The five levels of GT were 0.18, 0.35, 0.52, 0.68, and 1.01 mg/mL of parenteral amino acid solution. Nutrient intake was as prescribed by the attending physician. Actual individual intakes of amino acid, lipid, carbohydrate, phenylalanine, and tyrosine are described in Table 3. There were no enteral feeds consumed by the subjects.

Study protocol. Isotopes used for the tracer study were L-[1-^{13}C]phenylalanine (99%, ^{13}C, Tracer Technologies, Sommerville, MA, U.S.A.) and L-[3,3-^{2}H$_2$]tyrosine (98%, ^{2}H$_2$, Cambridge Isotope Laboratories, Andover, MA, U.S.A.). Quality control tests were performed by the manufacturers. Chemical purity, specified isotopic enrichment, and position were confirmed by nuclear magnetic resonance, whereas isomeric purity and a second confirmation of isotopic enrichment were performed by gas chromatography mass spectrometry. Solutions of each tracer amino acid in 3.3% dextrose and 0.3% NaCl were prepared by the Manufacturing Pharmacy at The Hospital for Sick Children. Isotope solutions were sterilized by passage through a 0.22-μm filter before being dispensed into single-use vials for storage at 4°C until use. All solutions were demonstrated to be sterile and free of bacterial growth over 7 d in culture and to be pyrogen-free by the *Limulus* amebocyte lysate test (31).

Table 1. *Subject information of parenterally fed neonates receiving varying intake of GT as a source of tyrosine*

	Mean	SD
n	13	
Birth weight (kg)	2.5	0.9
Gestational age (wk)	37	3
Postnatal age (d)	12	11
Postconceptional age (wk)	40	8
Gender (F:M)	10:3	
Study weight (kg)	2.6	0.8
Study length (cm)	49	6
Head circumference (cm)	34	3
Size for gestational age (A:S, A = appropriate, S = small)	11:2	
Diagnoses		
Necrotizing enterocolitis	2	
Intestinal atresia	5	
Malrotation	2	
Gastroschisis	4	

Table 2. *Amino acid profile of Primene (g/100 g amino acid)**

Isoleucine	6.7
Leucine	9.9
Valine	7.6
Lysine	10.9
Methionine	2.4
Cysteine	1.9
Phenylalanine	4.2
Tyrosine	0.9
Threonine	3.7
Tryptophan	2.0
Histidine	3.8
Arginine	8.4
Glycine	4.0
Alanine	7.9
Aspartate	6.0
Glutamate	9.9
Proline	3.0
Serine	4.0
Taurine	0.6
Ornithine	2.2

* Primene 6.5% amino acid concentration (Baxter, Deerfield, IL, U.S.A.).

Table 3. *Individual subject tyrosine, phenylalanine, amino acid, lipid and carbohydrate, and energy intakes*

Subject	Tyrosine*	Phenylalanine	Amino Acid†	Lipid‡	CHO§	Energy
	mg·kg^{-1}·d^{-1}			g·kg^{-1}·d^{-1}		kJ·kg^{-1}·d^{-1}
1	28.8	78.1	1.9	1.9	9.9	268
2	32.2	87.4	2.1	2.4	11.0	305
3	48.8	93.2	2.2	2.1	15.2	360
4	52.5	124.9	3.5	3.0	11.1	356
5	60.3	115.1	2.7	2.5	15.1	389
6	64.2	78.1	1.9	3.0	9.9	310
7	66.6	96.6	2.3	2.8	12.4	347
8	71.8	105.8	2.5	2.3	13.8	356
9	72.4	106.7	2.5	2.1	13.7	347
10	81.4	99.1	2.4	3.4	12.4	377
11	101.5	90.7	2.2	2.0	11.4	297
12	104.3	93.2	2.2	2.9	15.0	393
13	108.1	96.6	2.3	2.3	12.1	318
Mean	—	97.4	2.4	2.5	12.4	339
SD	—	13.4	0.4	0.5	1.8	38

* Total tyrosine intake (includes the tyrosine component of GT and that from Primene).

† Amino acid energy equivalent = 16.7 kJ/g.

‡ Intralipid (Pharmacia and Upjohn, Stockholm, Sweden); energy equivalent = 41.8 kJ/g.

§ CHO = carbohydrate as dextrose; energy equivalent = 15.7 kJ/g.

The phenylalanine dose required to achieve a measurable expired $^{13}CO_2$ was determined in a previous study (12). At least 24 h after the start of the infusion of TPN, phenylalanine flux, hydroxylation, oxidation, percent dose oxidized, and tyrosine flux were determined using a primed i.v. 24-h constant infusion of L-[1-^{13}C]phenylalanine (15.6 μmol/kg and 13 μmol·kg^{-1}·h^{-1}, respectively) and L-[3,3-^{2}H$_2$]tyrosine (3.6 μmol/kg and 3.0 μmol·kg^{-1}·h^{-1}, respectively). The priming dose was infused over the first 15 min of isotope infusion. Urine samples were collected for the measurement of background and steady state tracer amino acid enrichment and creatinine. Urine was used for measuring amino acid enrichment, as it is considered to be a valid representation of plasma enrichment (32, 33), provided the isotopically labeled amino acids are D-isomer-free (34). One to three baseline urine samples were collected before parenteral isotope infusion, followed by one sample every 3–4 h throughout the infusion. Urine was stored at −20°C until analyses were performed. Expired CO$_2$ was collected, and the production rate measured before and after 17–20 h of constant isotope infusion.

Analytical procedures and calculations. Urine was analyzed for phenylalanine and tyrosine enrichment as described previously (12).

Urinary creatinine was measured by the Jaffe reaction by using a Kodak Ektachem 700 Analyzer (Johnson and Johnson, Rochester, NY, U.S.A.). Urinary phenylalanine, tyrosine, glycine, and GT concentrations were analyzed by ion-exchange chromatography (Beckman 7300 Amino Acid Analyzer), expressed in micromoles per millimole of creatinine. Urinary excretion was determined using standards of known amounts of amino acid or dipeptide. The concentration, standardized to creatinine excretion, was determined using an external standard and was calculated with the following equation:

[(area of sample peak/area of peak of external standard)

$$\times \text{(dilution factor)} \times 5 \ \mu g/\mu L]/\text{creatinine mmol/L} \quad (1)$$

The concentration of the external standard was 5 μg/μL. S$_2$-aminoethyl-cysteine (400 μmol/L) was used as internal standard.

Expired CO$_2$ production collected from a ventilated hood system was measured directly using a portable CO$_2$ analyzer (1400 series, Servomex, Westech Industrial Ltd., Mississauga, Ontario) and mass flowmeter (5860 series, Brooks, Trillium Measurement and Control, Stouffville, Ontario). Expired $^{13}CO_2$ enrichment was determined using isotope-ratio mass spectrometry as previously described (12).

The model of amino acid metabolism used in this study is a modification of the Waterlow *et al.* (35) model:

$$Q = B + I = S + PAH \quad (2)$$

where Q is the rate of phenylalanine flux (μmol·kg^{-1}·h^{-1}); S is the rate of phenylalanine nonoxidative disposal, a measure of the rate of phenylalanine incorporation into body protein; PAH is the rate of phenylalanine hydroxylation to tyrosine; B is the rate of phenylalanine release from body protein; and I is the rate of exogenous phenylalanine intake.

Whole-body phenylalanine and tyrosine turnover was calculated from dilution of the isotope in the body amino acid pool at isotopic steady state (32, 33, 36):

$$Q_{phe} = I[E_i/E_p - 1] \quad (3)$$

where Q_{phe} is the rate of phenylalanine or tyrosine (Q_{tyr}) flux, i is the isotope infusion rate (μmol·kg^{-1}·h^{-1}), E^i is the enrichment of the infused isotope, and E_p is the enrichment of the amino acid in the urine at the isotopic steady state.

To measure the rate of phenylalanine hydroxylation, the model of Clarke and Bier (37) with the modification of Thompson *et al.* (38) was used.

$$Q_{phe \rightarrow tyr} = Q_{tyr}[E_{tyr}/E_{phe}][Q_{phe}/i_{phe} + Q_{phe}] \quad (4)$$

where E_{tyr} and E_{phe} are the urinary enrichments of L-[1-^{13}C]tyrosine and L-[1-^{13}C]phenylalanine, respectively; and i_{phe} is the rate of infusion of the labeled phenylalanine.

The rate of phenylalanine oxidation was calculated as described by Matthews *et al.* (36):

$$O_{phe} = F^{13}CO_2(1/E_{phe} - 1/E_i) \cdot 100 \qquad (5)$$

where O_{phe} represents phenylalanine oxidation, and $F^{13}CO_2$ represents the rate of $^{13}CO_2$ release by phenylalanine tracer oxidation (μmol·kg^{-1}·h^{-1}) calculated from the following equation:

$$F^{13}CO_2 = (FCO_2)(ECO_2)(44.6)(60)/(W)(RF)(100) \qquad (6)$$

where FCO_2 is the CO_2 production rate (cm^3/min), and ECO_2 is the $^{13}CO_2$ enrichment in expired breath at the isotopic steady state. The constants 44.6 μmol/cm^3 and 60 min/h convert FCO2 to micromoles per hour, and the factor of 100 changes the enrichment in atoms percent into a fraction. W is the weight (kg) of the infant. $^{13}CO_2$ retained by the body as bicarbonate is corrected for by using a retention factor calculated from a regression equation, using energy intake as the predictor variable (39).

Statistical analyses. The study followed a completely randomized design with tyrosine intake as the independent variable. Regression models (linear, quadratic, linear regression cross-over model) were fitted to the data to determine the relationship that best described the response of the dependent variables phenylalanine hydroxylation, phenylalanine F$^{13}CO_2$ and oxidation, and tyrosine flux to increasing tyrosine intake. The regression analyses were performed by the method of least squares by use of the regression procedure of SAS (40). Selection of the best model was determined by factors relating to fit (significance of the model and r^2) and estimates of variation about the model (coefficient of variation and SE of the estimate).

A two-phase regression cross-over model (41) was found to be the most appropriate analysis for the data (phenylalanine F$^{13}CO_2$ and oxidation), and a break point was objectively identified. As described previously (42, 43), the analysis involves partitioning the response of the dependent variables to tyrosine intake into data points fitting either a flat line (zero slope) representing obligatory phenylalanine oxidation when tyrosine intake is inadequate or a sloped line representing phenylalanine oxidation when tyrosine intake is above requirement. The break point of the two linear regression lines is considered representative of the group mean tyrosine requirement. The equation for the statistical model is:

$$Y = A1 + B1x + (A2 - A1)D + (B2 - B1)(Dx) + E \qquad (7)$$

where Y is the individual observation of the dependent variable phenylalanine F$^{13}CO_2$ or oxidation; *A1* and *A2* are the intercepts of the first and second line, respectively; *B1* and *B2* are the slopes of the first and second lines, respectively; *D* equals 0 if the observation is from the first line and 1 if it is from the second line; *x* is tyrosine intake; and *E* is the residual error associated with the model. The safe population intake was estimated by determining the upper 95% confidence limits of the break point estimate (41).

RESULTS

Clinical characteristics and nutrient intake. Subject information including clinical characteristics, nutrient intake, and diagnoses are presented in Table 1. Parenteral nutrition was as prescribed by the attending physician, and, therefore, the exact nutrient intakes were dependent on the total volume of parenteral nutrition infused. The average energy and protein intakes were 340 ± 38 kJ·kg^{-1}·d^{-1} (81 ± 9 kcal·kg^{-1}·d^{-1}) and 2.4 ± 0.4 g·kg^{-1}·d^{-1}, respectively (Table 3). Mean phenylalanine intake was 97 ± 13 mg·kg^{-1}·d^{-1}. The tyrosine intake reflected the level of GT to which the neonate was assigned and ranged from 29 to 108 mg·kg^{-1}·d^{-1}, whereas the phenylalanine intake was that present in the base parenteral amino acid solution (Table 3).

Urinary amino acid and expired CO_2 enrichment. Plateau in amino acid enrichment was achieved for all neonates by 9 h and was defined by the absence of a significant slope. The variation in phenylalanine and tyrosine amino acid enrichment within the plateau was <12%. Individual enrichments of L-[1-^{13}C]phenylalanine, L-[1-^{13}C]tyrosine, L-[3,3-^{2}H$_2$]tyrosine, and expired $^{13}CO_2$ are shown in Table 4.

Phenylalanine and tyrosine kinetics. There was no significant relationship between tyrosine intake and phenylalanine flux (Table 5). There was, however, a significant quadratic response between phenylalanine hydroxylation and tyrosine intake ($p < 0.004$, $r^2 = 0.59$; Table 5) with hydroxylation being lowest and equal at the lowest and highest tyrosine intake levels. There was also a significant linear relationship between tyrosine intake and tyrosine flux ($p < 0.006$, $r^2 = 0.51$). The two-phase linear regression cross-over model proved to be the best fit for the F$^{13}CO_2$ data and the phenylalanine oxidation data. A break point in phenylalanine F$^{13}CO_2$ at 66 mg·kg^{-1}·d^{-1} ($p < 0.008$, $r^2 = 0.7$; Fig. 1) was identified, and in phenylalanine oxidation at 82 mg·kg^{-1}·d^{-1} (Fig. 2). Increasing tyrosine intake above the break point level resulted in increased F$^{13}CO_2$ and oxidation. These break points, objectively identified by the two-phase linear regression cross-over model, are representative of the mean tyrosine requirement for the neonate. The 95% upper confidence limits were found to be 90 and 97 mg·kg^{-1}·d^{-1} for the F$^{13}CO_2$ and oxidation estimate, respectively (Figs. 1 and 2).

Urinary amino acid concentrations. Urinary phenylalanine, tyrosine, and glycine excretion were generally within the normal range for neonates (phenylalanine = 0–151 μmol/mmol creatinine, tyrosine = 0–200 μmol/mmol creatinine, glycine = 254–2341 μmol/mmol creatinine) (4). However, subject 4, receiving a tyrosine intake of 52.5 mg·kg^{-1}·d^{-1}, excreted an above average amount of phenylalanine and tyrosine in the urine, suggesting that the plasma concentration of these amino acids may also have been elevated (Table 6). This neonate did not demonstrate any evidence of abnormal liver function based on results of serum alanine aminotransferase or serum aspartate aminotransferase measurements performed near the time of the study. The individual receiving the highest tyrosine intake (108.1 mg·kg^{-1}·d^{-1}) demonstrated the greatest excretion of ty-

Table 4. *Individual subject L-[1-^{13}C]phenylalanine, L-[1-^{13}C]tyrosine, L-[3,3-^{2}H$_2$]tyrosine, and ^{13}CO$_2$ enrichment*

Subject	TYR* Intake	^{13}CPHE†	^{13}CTYR†	^{2}H$_2$TYR†	^{13}CO$_2$
	mg·kg^{-1}·d^{-1}		MPE		APE
1	28.6	0.0828	0.0133	0.0349	0.003754
2	32.2	0.0955	0.0162	0.0385	0.003054
3	48.8	0.0837	0.0157	0.0340	0.004067
4	52.5	0.1142	0.0224	0.0406	0.003447
5	60.3	0.0726	0.0187	0.0355	N/A‡
6	64.2	0.1137	0.0279	0.0417	0.002438
7	66.6	0.0949	0.0128	0.0277	0.002231
8	71.8	0.1185	0.0199	0.0254	0.004260
9	72.4	0.0820	0.0179	0.0348	0.003472
10	81.4	0.0833	0.0114	0.0182	0.004833
11	101.5	0.0886	0.0120	0.0280	0.004642
12	104.3	0.1062	0.0081	0.0213	0.007795
13	108.1	0.1057	0.0087	0.0203	0.004950

* TYR = tyrosine; total tyrosine intake (includes the tyrosine component of GT and that from Primene).
† ^{13}CPHE = L-[1-^{13}C]phenylalanine, ^{13}CTYR = L-[1-^{13}C]tyrosine, ^{2}H$_2$TYR = L-[3,3-^{2}H$_2$]tyrosine.
‡ Consent was not given for breath collection in this subject, therefore, these data were not available.
MPE indicates molecules percent excess; APE, atoms percent excess.

Table 5. *Individual subject phenylalanine and tyrosine kinetics*

Subject	TYR* Intake	PHE Flux	PHEOH†	PHE F^{13}CO$_2$	PHE Oxidation	TYR Flux‡
	mg·kg^{-1}·d^{-1}			μmol·kg^{-1}·h^{-1}		
1	28.8	143	13	0.3759	6.00	82
2	32.2	122	13	0.4884	4.60	74
3	48.8	141	16	0.8374	9.80	85
4	52.5	100	14	0.5511	4.28	70
5	60.3	164	21	N/A§	N/A§	81
6	64.2	100	17	0.4932	3.84	68
7	66.6	123	14	0.4951	4.02	104
8	71.8	96	19	0.8063	5.47	114
9	72.4	144	18	0.4986	5.45	82
10	81.4	142	20	1.3620	8.06	161
11	101.5	132	14	1.1281	8.40	103
12	104.3	108	10	1.4245	12.21	136
13	108.1	109	12	1.0985	8.93	143
Mean	—	125	—	—	—	100
SD	—	21	—	—	—	30

† PHEOH = phenylalanine hydroxylation; quadratic relationship with tyrosine intake ($p < 0.004$; $r^2 = 0.59$).
‡ Linear relationship between tyrosine intake and tyrosine flux ($p < 0.006$; $r^2 = 0.51$).
§ Consent was not given for breath collection in this subject; therefore, these data were not available.

rosine in the urine, although levels remained within the expected ranges for the neonate (Table 6). The additional glycine intake from the dipeptide increased the glycine intake from 4.2 to 5.3% total amino acids. Urinary glycine excretion did not differ in relation to the level of GT intake (Table 6). Four (subjects 3, 6, 9, 11) of the thirteen subjects demonstrated a small amount of dipeptide in the urine, in each case representing <10 μmol/mmol creatinine (Table 6).

DISCUSSION

Using nitrogen balance and growth measures, Snyderman (1) identified that the enterally fed neonate may require a preformed source of tyrosine at levels ranging from 50 to 120 mg·kg^{-1}·d^{-1}. The estimate of the tyrosine requirement for the enterally fed neonate does not necessarily apply to neonates fed parenterally because recent evidence has demonstrated that the splanchnic organs play a major role in amino acid metabolism, and its bypass can significantly alter the needs of individual amino acids (44–50). Starting with a piglet model, we have studied the aromatic amino acid requirements of the parenterally fed neonate. First, we showed in the piglet that the current pediatric TPN amino acid mixtures are limiting in total aromatic amino acids (14), a finding which we have recently confirmed in human neonates (12). Further, we showed that both the piglet and human neonates have active hydroxylation of phenylalanine to tyrosine (12, 14). Further, in the piglet, we showed that phenylalanine was an effective source of tyrosine for protein synthesis and growth measured by nitrogen balance, but at the cost of high plasma phenylalanine levels as well as elevated urinary excretion of alternate catabolites of phenylalanine and tyrosine (14). We have recently confirmed this observation in human neonates (12). In the piglet, we have determined both tyrosine (18) and phenylalanine (19) requirements by using amino acid oxidation techniques. The present
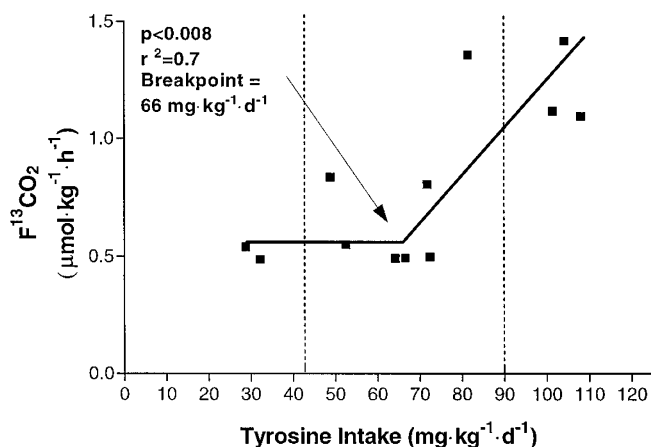
Figure 1. The effect of increasing tyrosine intake *via* GT on $F^{13}CO_2$ production from phenylalanine oxidation. The *dotted vertical lines* represent the 95% confidence limits of the break point estimate.
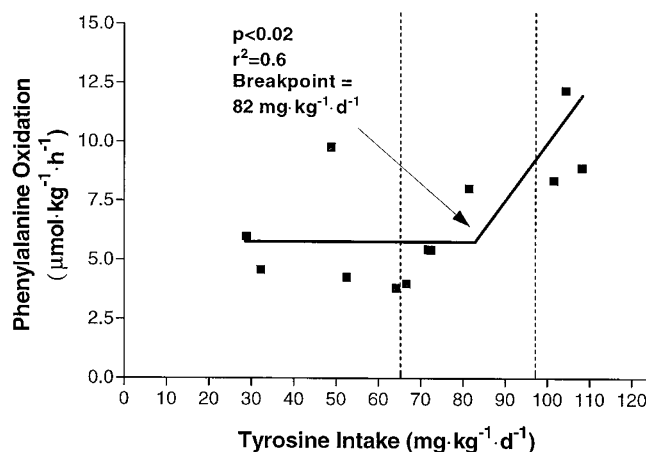

Figure 2. The effect of increasing tyrosine intake *via* GT on phenylalanine oxidation. The *dotted vertical lines* represent the 95% confidence limits of the break point estimate.

work was designed to determine whether the human neonate used GT and to obtain an estimate of tyrosine requirements at the moderate phenylalanine intake level in a commercial pediatric TPN mixture. The isotope model we used took advantage of the fact (based on our piglet work) that the phenylalanine concentration in Primene was modestly higher than requirement. After sufficient tyrosine was given, the excess phenylalanine would be metabolically channeled within the liver to oxidation (51). Therefore, the break point in either the $F^{13}CO_2$ or phenylalanine oxidation *versus* tyrosine intake (Figs. 1 and 2) would be an estimate of the population average tyrosine needs, and the 95% confidence interval would cover 95% of the population and, hence, would be a safe level for the population as a whole.

The present data are, to our knowledge, the first estimate of tyrosine requirement in the parenterally fed human neonate. Using a linear regression cross-over analysis, the estimated average tyrosine requirement at a fixed and adequate but not excessive phenylalanine intake of 4.0% of total amino acids was 66 and 82 mg·kg^{-1}·d^{-1} for the estimates based on $^{13}CO_2$ production ($F^{13}CO_2$) and phenylalanine oxidation, respectively. These intakes represent 2.8 and 3.4% of total amino acid intake, respectively. The upper 95% confidence limit, representative of the safe population intake, was estimated as 90 and 97 mg·kg^{-1}·d^{-1} for phenylalanine $F^{13}CO_2$ and oxidation data, respectively. These intakes represent between 3.8 and 4.0% of total amino acids. Another way of examining these results is to compare the net protein deposition occurring in the three infants receiving tyrosine intake above the 95% confidence interval with the three infants receiving the lowest intakes of tyrosine. Net protein deposition was calculated from phenylalanine balance (intake − hydroxylation) and assuming there are 272 μmol of phenylalanine per gram of mixed body protein (52). Net protein deposition in the three infants receiving the highest tyrosine intakes was 50% higher (1.02 g·kg^{-1}·d^{-1}) than the group receiving the lowest tyrosine intake (0.68 g·kg^{-1}·d^{-1}). By using the value of 272 μmol of phenylalanine per gram of mixed body protein, it is also possible to convert the mean phenylalanine flux rate of 125 μmol·kg^{-1}·h^{-1} to a whole-body

protein flux (turnover) rate of 11.0 g·kg^{-1}·d^{-1}. This value is fairly similar to an earlier estimate of 9.5 g·kg^{-1}·d^{-1} that we obtained for parenterally fed neonates by using ^{15}N-glycine as a tracer and ^{15}N-urea as an end-product (53).

The present results are supported by similar estimates of tyrosine in the parenterally fed piglet. The estimated mean requirement and safe level of intake in the piglet model was found to represent 2.1 and 2.4% total amino acids at a fixed phenylalanine intake of 4.0% of total amino acids. Further, the present results are within the estimated range of tyrosine requirement in the orally fed infant receiving adequate phenylalanine intake (50–120 mg·kg^{-1}·d^{-1}) (1). Nonetheless, the method used in the present study is novel and has not been previously used. It would, therefore, be desirable in future studies to confirm our estimate of tyrosine requirements by using other established methods such as indicator amino acid oxidation (42), which directly reflects the use of the test amino acid (in this case tyrosine) for whole-body protein synthesis.

Based on the data of the present study, the proportional balance of the mean aromatic amino acid requirements is 56% phenylalanine and 44% tyrosine. This ratio is similar to that found in the piglet model of the neonate (59:41) (18, 19) and to the proportional phenylalanine to tyrosine content of fetal tissue (59:41) (52). Provision of aromatic amino acids in this recommended balance should lower the incidence of hyperphenylalaninemia and hypertyrosinemia observed in neonates receiving high intakes of phenylalanine (6–8, 11, 26, 54, 55) or tyrosine (14). Because the long-term neurologic effects of low or elevated tyrosine intake are unknown, efforts should be made to provide intakes that meet needs without great excess.

The present estimate of tyrosine requirement was determined at a phenylalanine intake of 97 ± 13 mg·kg^{-1}·d^{-1}, representing a phenylalanine intake of 4% of total amino acids. This intake is above the level determined by House *et al.* (18) in the piglet model to be the safe level of phenylalanine requirement (3.2% of total amino acids). The impact of such a small excess of phenylalanine on the tyrosine requirement is unknown but may be that the tyrosine requirement was underestimated, because phenylalanine can be hydroxylated through

Table 6. *Individual subject urinary creatinine, phenylalanine, tyrosine, GT, and glycine concentrations*

Subject	Tyrosine Intake*	Creatinine	Phenylalanine	Tyrosine Concentrations	GT	Glycine
	$mg \cdot kg^{-1} \cdot d^{-1}$	$\mu mol/L$		$\mu mol/mmol$ creatinine		
1	28.8	1722	ND	41	ND‡	1005
2	32.2	718	32	14	ND	1224
3	48.8	609	25	35	7	2292
4	52.5	640	206	116	ND	2683
5	60.3	488	ND	39	ND	969
6	64.2	1131	23	28	0.8	743
7	66.6	495	34	70	ND	2752
8	71.8	830	27	92	ND	2666
9	72.4	896	27	69	6.4	2799
10	81.4	1320	26	90	ND	2105
11	101.5	687	27	34	2.5	1227
12	104.3	731	31	115	ND	2743
13	108.1	523	40	141	ND	2356
Mean	—	830	45	68	—	1966
SD	—	363	53	40	—	801

* Total tyrosine intake (includes the tyrosine component of GT and that from Primene).

‡ ND = not detected.

hepatic phenylalanine hydroxylase to form tyrosine. Whether this is so remains to be determined; however, because the phenylalanine excess was small, any underestimation of tyrosine needs should also be small. In addition, a small excess of phenylalanine would not change the estimate of total aromatic amino acid requirements, only the proportion of phenylalanine to tyrosine. Also, because the estimated balance of phenylalanine to tyrosine is so similar to the piglet data and the composition of human fetal tissue, the phenylalanine excess must have been very small.

Although a significant quadratic response of phenylalanine hydroxylation to increasing tyrosine intake was observed, the estimate of phenylalanine hydroxylation did not provide meaningful results toward estimating the tyrosine requirement. The response of phenylalanine hydroxylation to graded tyrosine intake was unexpected. The rate of phenylalanine hydroxylation was similar at both the lowest and highest intake of tyrosine. The predicted response was that hydroxylation would be higher at the lowest intake of tyrosine and decrease as tyrosine intake approached requirement. These data reveal that the estimate of phenylalanine hydroxylation by use of plasma amino acid enrichments and the Clarke and Bier (37) model as modified by Thompson *et al.* (38) cannot be used as an index of the adequacy of tyrosine intake in the neonate. Phenylalanine hydroxylation estimates have been previously shown to poorly reflect net tissue phenylalanine and tyrosine accumulation in the parenterally fed piglet receiving a high level of phenylalanine intake (56). Such discrepancies may be attributed to limitations of the model, possibly due to the fact that plasma (or urine as a surrogate) enrichment does not accurately reflect the precursor pool enrichment. As new approaches to quantify intrahepatocyte precursor pool enrichment become available, such as using the rapid turnover hepatic protein apolipoprotein B100 (57), the hydroxylation estimate will need to be reassessed as a measure of the adequacy of tyrosine intake.

Urinary excretion of phenylalanine, tyrosine, glycine, and GT were monitored to provide some insight into the metabolic handling of the doses of aromatic amino acids and GT. Plasma levels were not measured to minimize the invasiveness of the study protocol. In general, the excretion of the three amino acids was within normal ranges, and the excretion of GT was either nondetectable or appeared in trace amounts. These data suggest that the intakes of these amino acids were not at levels that cause overload to the neonate's metabolic pathways. The lack of significant excretion of the dipeptide, even at the highest intake of GT supplementation, suggests that it was rapidly hydrolyzed into its respective amino acids.

One subject (subject 4) who received a tyrosine intake below the estimated requirement level (GT = 52.5 $mg \cdot kg^{-1} \cdot d^{-1}$) experienced urinary phenylalanine and tyrosine levels that were above expected norms for the neonate. This suggests that the plasma concentrations of phenylalanine and tyrosine were elevated (4). The greater than normal excretion of phenylalanine and tyrosine with a modest aromatic amino acid intake in conjunction with the lack of evidence of impaired liver function from liver enzyme tests suggests that this neonate may have had an immaturity in the tyrosine catabolic pathway. In fact, tyrosine aminotransferase (E.C. 2.6.1.5) (27, 58) and 4-hydroxyphenylpyruvate dioxygenase (E.C. 1.13.11.27) (58, 59) are considered to be immature during the neonatal period.

Most of the subjects had undetectable excretion of the dipeptide GT in the urine. Four individuals excreted trace amounts of GT, and, within the subjects with dipeptide excretion, the presence of the dipeptide was not related to the intake. In fact, the two individuals with the greatest intake of the dipeptide did not present with detectable amounts of GT in the urine. This efficient handling of the dipeptide provides further evidence that GT is well used in the human neonate.

Additional glycine associated with the dipeptide was infused. This amounted to 4 to 29 $mg \cdot kg^{-1} \cdot d^{-1}$ and reflected an increase of glycine intake from 4.2 to 5.3% of total amino acids. Although the glycine intake was increased, the excretion of glycine did not correlate with increasing GT infused, suggesting that the additional glycine load did not greatly impact the plasma glycine pool.

In conclusion, this is the first estimate of tyrosine requirement in the parenterally fed human neonate. These data specifically demonstrate that the mean tyrosine requirement of the parenterally fed neonate lies between 66 and 82 mg·kg^{-1}·d^{-1}, representing 2.8 to 3.4% of total amino acids. The corresponding safe estimate of intake was 90 to 97 mg·kg^{-1}·d^{-1}, corresponding to 3.8 to 4.0% of total amino acids. The ideal balance of phenylalanine to tyrosine was found to be 56:44 based on the mean requirement estimate. This balance of aromatic amino acid intake is similar to that found in the piglet model of the neonate (59:41) (18, 19) as well as that of fetal tissue composition (59:41) (52). Therefore, the ratio may be similar even for lower-birth-weight infants. However, because the total amino acid (protein) needs of the very-low-birth-weight infant are higher (52, 60), the total safe aromatic amino acid intake may also be higher. On the basis of the present research, the total safe aromatic amino acid intake that meets the needs of the neonate is approximately 7.9% of total amino acids. This total aromatic amino acid intake is below the intake of the phenylalanine-supplemented solutions such as Vamin, which contain 8.6% of the total amino acids as phenylalanine and tyrosine. In contrast, the requirement estimate in the present experiment is well above that found in current pediatric amino acid solutions (5.1% of total amino acids). These data provide valuable information toward the development of a parenteral amino acid solution that better meets the needs of the neonate.

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