Rate of Phenylalanine Hydroxylation in Healthy School-Aged Children

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ABSTRACT: Hydroxylation of phenylalanine to tyrosine is the first and rate-limiting step in phenylalanine catabolism. Currently, there are data on the rate of phenylalanine hydroxylation in infants and adults but not in healthy children. Thus, the aim of the study reported here was to measure the rate of phenylalanine hydroxylation and oxidation in healthy school-aged children both when receiving diets with and without tyrosine. In addition, hydroxylation rates calculated from the isotopic enrichments of amino acids in plasma and in very LDL apoB-100 were compared. Eight healthy 6- to 10-y-old children were studied while receiving a control and again while receiving a tyrosine-free diet. Phenylalanine flux, hydroxylation, and oxidation were determined by a standard tracer protocol using oral administration of ^{13}C -phenylalanine and $^2\text{H}_2$ -tyrosine for 6 h. Phenylalanine hydroxylation rate of children fed a diet devoid of tyrosine was greater than that of children fed a diet containing tyrosine (40.25 ± 5.48 versus $29.55 \pm 5.35 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$; $p < 0.01$). Phenylalanine oxidation was not different from phenylalanine hydroxylation regardless of dietary tyrosine intake, suggesting that phenylalanine converted to tyrosine was mainly oxidized. In conclusion, healthy children are capable of converting phenylalanine to tyrosine, but the need for tyrosine cannot be met by providing extra phenylalanine. (*Pediatr Res* 69: 341–346, 2011)

Hydroxylation of phenylalanine to tyrosine is an important step for eliminating excess phenylalanine and for providing tyrosine if dietary intake of tyrosine is inadequate. Currently, phenylalanine hydroxylation is available in adults and infants but not in healthy children. The phenylalanine hydroxylation rate in adults was reported as $5\text{--}10 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ during the postabsorptive state (1–3) and $6\text{--}21 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ during the fed state (2,4,5), and in infants, it was $12\text{--}48 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ during the fed state (6–9).

Although the requirement for most amino acids in children are similar to that in adults (10–15), a recent study reported that the phenylalanine requirement of healthy children, in the absence of tyrosine intake, was only 64% of adult requirement (16). This seemingly implausible biological finding of the phenylalanine requirement of children being less than that of

adults may be due to phenylalanine hydroxylation being limited in children. It was suggested that phenylalanine may not provide the total needs for phenylalanine plus tyrosine in children fed an amino acid-based diet without tyrosine (16). Thus, further investigation of the rate of phenylalanine hydroxylation in children is necessary.

In the past, the rate of phenylalanine hydroxylation was mostly determined from phenylalanine and tyrosine isotopic enrichments measured in plasma. However, this approach may not be appropriate because it has been shown that phenylalanine hydroxylation rates were overestimated in parenterally fed piglets (17) and human neonates (18) receiving a high phenylalanine intake devoid of tyrosine, suggesting that the isotopic enrichment of plasma phenylalanine is not a good indicator of the intracellular phenylalanine precursor pool. The isotopic enrichments of amino acids contained in plasma very LDL (VLDL) apolipoprotein B-100 (apoB-100), a hepatically derived protein with a very high turnover rate, have been shown to be good indicators of the amino acid enrichments of the intrahepatic pool (19). Recently, Rafii *et al.* (20), using isotopic enrichments in apoB-100, reported that phenylalanine hydroxylation rate decreased linearly in response to increases in tyrosine intakes until a requirement was reached. This pattern was not seen when the isotopic enrichments in plasma were used. The authors concluded that apoB-100 is an accurate *in vivo* isotope model of intrahepatic enrichment of amino acids.

The objectives of the study reported here were 1) to examine the rate of phenylalanine hydroxylation and oxidation in healthy school-aged children when receiving diets containing tyrosine as were as devoid of tyrosine and 2) to compare hydroxylation rates calculated from the isotopic enrichments of amino acids in plasma and in VLDL apoB-100.

SUBJECTS AND METHODS

Subjects. Eight healthy children aged between 6 and 10 y and with NCHS percentile of body mass index between the 15th and 85th percentile were recruited for the study from the database of the Children's Nutrition Research Center (CNRC). Subjects' characteristics are described in Table 1. Those who had metabolic diseases or other conditions likely to interfere with utilization of the administered amino acids were not enrolled. Subjects with pubertal Tanner stage ≥ 2 were also excluded. The study protocol was approved by the Institutional Review Board for Human Research at Baylor College of Medicine. The purpose of the study and the potential risks involved were explained in detail to each subject and his/her responsible caregiver, and written

Received March 31, 2010; accepted October 31, 2010.

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Supported by a USDA/ARS Cooperative Agreement.

This work is a publication of the US Department of Agriculture/Agricultural Research Service Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, Texas.

The contents of this publication do not necessarily reflect the view or policies of the US Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement by the US Government.

Abbreviations: apoB, apolipoprotein B; EAR, estimated average requirement; Phe, phenylalanine; RMR, resting metabolic rate; Tyr, tyrosine

Table 1. Characteristics and energy intakes

	Range
N	8
Gender (M:F)	4:4
Age, y	6.4–10.2
Weight, kg	19.9–34.2
Height, cm	119.3–139.5
Body mass index, kg · m ⁻²	14.0–17.9
EER*, kcal · d ⁻¹	1445.6–2001.0

* EER (estimated energy requirement) = TEE + energy deposition; physical activity level at low active (21).

consent and assent were obtained from all participants and their responsible caregivers. The study participants/responsible caregivers received modest financial compensation for participating in the study.

Experimental design. At recruitment, subjects were screened for eligibility by measuring their weight and height, along with a brief medical history to confirm normal health. A dietician interviewed each subject and his/her caregiver to design the study meals. The subjects came to the metabolic research unit (MRU) on two separate occasions, approximately a month apart, for two different diet studies: 1) control diet and 2) tyrosine-free diet. Two days before each tracer study, subjects consumed meals that provided adequate energy based on their estimated energy requirement (EER) (21), ~53% energy as carbohydrate, ~35% energy as fat, and ~12% energy as protein. Food that was not consumed during those 2 d were returned and weighed for analysis.

The tracer experiment lasted 10 h for both studies. On the experimental day of the control diet, subjects had hourly meals for the first 4 h and then for every 30 min for 6 h (see Fig. 1) as a liquid nutritional supplement, PediaSure (Ross Products Division, Abbott Laboratories, OH), which contains 12% energy as protein, 53% as carbohydrate, and 35% as fat. Subjects received PediaSure at a rate of 4.3 ± 0.5 mL · kg⁻¹ · h⁻¹. The content of phenylalanine in PediaSure is 1.80 mg · mL⁻¹ for Chocolate flavor and 1.51 mg · mL⁻¹ for Vanilla and Strawberry. On the experimental day of the tyrosine-free diet, subjects had hourly meals for the first 4 h and then for every 30 min for 6 h as tyrosine-free liquid formula containing Tirez-2 (Ross Products Division, Abbott Laboratories, Columbus, OH), phenylalanine (7.18 mg · kg⁻¹ · h⁻¹; Ajinomoto Aminoscience LLC., Raleigh, NC), orange sherbet, safflower oil, beet sugar, and Kool-Aid (Kraft Food Global, Inc., Glenview, IL). Subjects consumed equal amounts of the liquid drink each hour. Each hourly meal contained ~1/12th of daily protein intake. By the end of the study, each subject had consumed 2/3 of daily energy requirement.

Tracer protocol. The labeled tracers used in the study were NaH¹³CO₃, L-[1-¹³C]phenylalanine, L-[1-¹³C]tyrosine, and L-[3,3-²H₂]tyrosine (Cambridge Isotope Laboratory, Woburn, MA).

Because the ¹³CO₂ enrichment in the breath varies depending on ¹³C enrichment of the diet and substrate oxidation (22), the oral primed-intermittent tracer administration was started after a 4-h enteral intake adaptation period (23). A primed dose of NaH¹³CO₃ (2.07 μmol · kg⁻¹), L-[1-¹³C]phenylalanine (6.55 μmol · kg⁻¹), L-[1-¹³C]tyrosine (2.77 μmol · kg⁻¹), and L-[3,3-²H₂]tyrosine (3.6 μmol · kg⁻¹) was given with the fifth meal. L-[1-¹³C]phenylalanine (5.9 μmol · kg⁻¹) and L-[3,3-²H₂]tyrosine (1.5

μmol · kg⁻¹) were given every 30 min for the next 6 h starting with the fifth meal (Fig. 1). Baseline breath and blood samples were collected between 45 and 15 min before the tracer protocol began. Additional expired breath and blood samples were collected every 30 min, beginning 4.25 h after the tracer protocol began.

The rate of CO₂ production was measured continuously for 4 h by room respiratory calorimetry after adaptation to the diet (24), and the rate of CO₂ production at rest was measured for 20 min within those 4 h.

Sample collection and laboratory analysis. The blood samples were collected in prechilled tubes containing EDTA and a cocktail of sodium azide, merthiolate and soybean trypsin inhibitor. Plasma was separated by centrifuging at 1500 × g for 10 min at 4°C and stored at -70°C until enrichment analyses.

The ¹³C enrichment of breath carbon dioxide was measured by gas-isotope ratio mass spectrometry, monitoring ions at mass-to-charge (*m/z*) ratios 44 and 45. Plasma phenylalanine and tyrosine were isolated by standard cation exchange chromatography. VLDL was isolated from plasma and its amino acids released to measure the isotopic enrichments of phenylalanine and tyrosine in apoB-100. Chylomicron was first removed from plasma by ultracentrifugation at 30,000 rpm as previously described (25). VLDL was then separated by ultracentrifugation at 100,000 rpm followed by isopropanol precipitation of apoB-100 as described previously (26). Phenylalanine and tyrosine from plasma and apoB-100 were derivatized to their *n*-propyl, heptafluorobutyramide derivatives and their isotopic enrichments determined by negative chemical ionization gas chromatography-mass spectrometry by monitoring ions at *m/z* ratios of 165 to 166 (phenylalanine) and 181 to 182/183 (tyrosine) (27).

Estimation of isotope kinetics. Whole-body phenylalanine and tyrosine fluxes were calculated by using their plasma or apo B-100 plateau tracer-tracee ratios in the following steady state equation: $Q = i(E_i/E_p)$, where Q is the rate of phenylalanine or tyrosine flux, i is the isotope infusion rate, and E_i and E_p are the enrichments of the infused isotope and plasma or apo B-100 at isotopic plateau. Endogenous flux was calculated as: $Q_{endo} = Q - (I + i)$ where I is the phenylalanine or tyrosine intake from diet. Phenylalanine hydroxylation was calculated from the conversion of the [¹³C]phenylalanine to [¹³C]tyrosine and from the independent measurement of tyrosine flux according to the model of Clarke and Bier (1) using both plasma and the apoB-100 enrichments: $Q_{phe \rightarrow tyr} = Q_{tyr}(E_{tyr}/E_{phe})$, where $Q_{phe \rightarrow tyr}$ is the rate of phenylalanine hydroxylation, Q_{tyr} is the tyrosine flux estimated from [3,3-²H₂]tyrosine, and E_{tyr}/E_{phe} is the ratio of the enrichments of [¹³C]tyrosine to [¹³C]phenylalanine in either plasma or apoB-100. Total and endogenous phenylalanine hydroxylation was calculated by using total and endogenous tyrosine flux, respectively. The rate of phenylalanine oxidation was calculated using the standard equations: $O = F^{13}CO_2(1/E_p - 1/E_i) \times 100$ where O represents phenylalanine oxidation and $F^{13}CO_2$ represents the rate of ¹³CO₂ released by phenylalanine tracer oxidation, which was calculated as $(FCO_2)(ECO_2)(44.6)(60)/(W)(0.82)(100)$, where FCO_2 is the CO₂ production rate, ECO_2 is the enrichment in expired breath at isotopic steady state (APE), the constants 44.6 μmol · mL⁻¹ and 60 min · h⁻¹ converts FCO_2 to μmol · h⁻¹, W is the weight (kg) of the subject, and the factor 0.82 is the correction for CO₂ retained in the body due to the bicarbonate fixation (28).

Statistical analyses. The differences between dietary groups were compared by paired *t* test. All statistical analyses were performed using SAS (version 9.2, SAS Institute, Cary, NC); differences were considered significant at *p* < 0.05. Values are expressed as mean ± SD.

RESULTS

Except for tyrosine intake (*p* < 0.001), protein and phenylalanine intakes on the study day were not different between the two dietary studies (Table 2). Total and endogenous phenylalanine fluxes calculated from enrichment in apoB-100 were significantly lower from those calculated from enrichment in plasma when the children were fed the control diet (*p* < 0.05; Fig. 2) but were not different when the children received the tyrosine-free diet. Total and endogenous tyrosine fluxes calculated from enrichment in apoB-100 were significantly higher than those calculated from enrichment in plasma when the children were fed the tyrosine-free diet (*p* < 0.001; Fig. 3). Total tyrosine flux was significantly higher when children were fed the control diet than the tyrosine-free diet (*p* < 0.01).

Figure 1. Schematic diagram of the study protocol. The oral-primed (*)-intermittent (■) tracer administration was started after a 4-h enteral intake adaptation period.

Table 2. Protein, phenylalanine, and tyrosine intake on the study day

	Control	Tyrosine-Free
Protein, mg · kg ⁻¹ · h ⁻¹	126.47 ± 15.43	128.16 ± 3.54
Phenylalanine, μmol · kg ⁻¹ · h ⁻¹		
Dietary	42.48 ± 6.85	43.18 ± 1.19
Tracer	11.97 ± 0.23	11.72 ± 0.31
Tyrosine, μmol · kg ⁻¹ · h ⁻¹		
Dietary	32.49 ± 5.05	0*
Tracer	2.77 ± 0.06	2.71 ± 0.08

Values are expressed as mean ± SD.

* Tyrosine intakes were different between two diet groups ($p < 0.001$).

Total and endogenous rate of phenylalanine hydroxylation of healthy children calculated from enrichments in apoB-100 were significantly higher than that calculated from enrichment in plasma when children were fed either diet ($p < 0.001$; Fig. 4). Phenylalanine oxidation calculated from enrichment in apoB-100 was significantly different from that calculated from enrichment in plasma ($p < 0.01$; Fig. 5) when the children received the control diet but was not different when children received the tyrosine-free diet. Phenylalanine oxidation calculated from FCO₂ measured during 4 h in the respiratory chamber and during the 10- to 15-min resting period in the respiratory chamber was significantly different ($p < 0.001$).

Figure 2. Phenylalanine flux of healthy children fed control (■) and tyrosine-free (□) diet. Values are mean ± SD. Differences between children fed control and tyrosine-free diets and differences between values calculated from plasma and VLDL ApoB-100 enrichments were by paired t test (* $p < 0.05$).

Figure 3. Tyrosine flux of healthy children fed control (■) and tyrosine-free (□) diet. Values are mean ± SD. Differences between children fed control and tyrosine-free diets and differences between values calculated from plasma and VLDL ApoB-100 enrichments were by paired t test (* $p < 0.05$, ** $p < 0.01$, and § $p < 0.001$).

Figure 4. Rate of phenylalanine hydroxylation of healthy children fed control (■) and tyrosine-free (□) diet. Values are mean ± SD. Differences between children fed control and tyrosine-free diets and differences between values calculated from plasma and VLDL ApoB-100 enrichments were by paired t test (* $p < 0.05$, ** $p < 0.01$, and § $p < 0.001$).

Figure 5. Phenylalanine oxidation of healthy children fed control (■) and tyrosine-free (□) diet. Values are mean ± SD. Differences between children fed control and tyrosine-free diets and differences between values calculated from plasma and VLDL ApoB-100 enrichments were by paired t test (* $p < 0.05$, ** $p < 0.01$, and § $p < 0.001$).

Phenylalanine oxidation calculated from enrichment in apoB-100 was not significantly different from total phenylalanine hydroxylation calculated from enrichment in apoB-100 when children were fed the control and tyrosine-free diets. Phenylalanine oxidation calculated from enrichment in plasma was significantly higher than total phenylalanine hydroxylation calculated from enrichment in plasma when children received either diet ($p < 0.001$).

DISCUSSION

This is the first study using stable isotope techniques to estimate the rate of phenylalanine hydroxylation in healthy 6- to 10-y-old children during the fed state. The primary aim of the study was to estimate the rate of phenylalanine hydroxylation in healthy children during the fed state when receiving diets containing tyrosine or devoid of tyrosine. The total phenylalanine hydroxylation rate estimated from the plasma enrichment in the fed state was $19.1 \pm 3.1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ when fed a control diet and $16.7 \pm 1.8 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ when fed a tyrosine-free diet. We are aware of only one other study in which phenylalanine hydroxylation was measured using the same isotope approach under similar feeding conditions (5). In a study of healthy adults receiving a generous

phenylalanine intake devoid of tyrosine and in whom the phenylalanine tracer was administered orally, it was reported that phenylalanine hydroxylation rate estimated from plasma enrichment was $\sim 26.0 \pm 7.1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ during the fed state (5). The phenylalanine hydroxylation for children obtained in this study was $\sim 35\%$ lower than that for adult, indicating that phenylalanine hydroxylation may be limiting in children fed a tyrosine-free diet.

To date, the requirements of adults and children for the branched-chain amino acids (13,14), total sulfur amino acids (10,15), and lysine (11,12) have been determined by stable isotope tracer methods. In all cases, the estimate for children was the same as for adults, lending support to the concept that the maintenance component of essential amino acid requirements in children is the same as it is in adults. Hence, it was expected that the phenylalanine plus tyrosine requirements in children would be similar to those in adults. However, an earlier study reported that phenylalanine requirement, in the absence of tyrosine, for children was $28 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ (16), which was only about two third of the adult requirements of $42 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ (29). The authors (16) suggested that phenylalanine hydroxylation may be limiting in children fed a tyrosine-free diet based on the lower urinary tyrosine/phenylalanine ratios in children at graded phenylalanine compared with adults. Our current finding that the phenylalanine hydroxylation of children are 35% lower than that of adults when subjects are studied under similar prandial conditions and using similar tracer methods does support a limitation of phenylalanine hydroxylation in children. This could be one of the reasons that the phenylalanine requirement, in the absence of tyrosine, for children determined in the earlier study (16) was 36% lower than that for adult.

The wide range of phenylalanine hydroxylation rates reported in adults ($5\text{--}21 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) is due to the different amounts of phenylalanine and tyrosine intakes, different types of tracers ($[^{13}\text{C}]$ phenylalanine *versus* $[^2\text{H}_5]$ phenylalanine) (30), and different tracer routes (5,31). For example, Marchini *et al.* (30) reported that $[^{13}\text{C}]$ phenylalanine provides a better estimate of phenylalanine hydroxylation *in vivo* and $[^2\text{H}_5]$ phenylalanine underestimates the phenylalanine hydroxylation rate by half possibly due to proton exchange. In the fed state, when the tracers were given enterally, phenylalanine hydroxylation was more than double the rate when tracers were given i.v. under the same dietary condition (5,31). It is due to the splanchnic uptake of the oral tracers. It has been reported that the first-pass disappearance of phenylalanine oral tracer was $\sim 29\%$ in the fasting state (32) and $\sim 19\%$ in the fed state (12).

The second aim of our study was to compare the rate of phenylalanine hydroxylation calculated from plasma and from VLDL apoB-100 enrichments. The enrichment of a labeled amino acid in VLDL apoB-100 has been used as a marker of the intrahepatic enrichment of the tracer in several studies (19,33,34). Recently, phenylalanine hydroxylation calculated from enrichments in apoB-100 was shown to be a more sensitive to changes compared with the corresponding values obtained from plasma enrichments (20). Because liver is one of the primary sites of phenylalanine hydroxylation (35), rate of phenylalanine hydroxylation estimated from apoB-100 en-

richments may represent the true rate occurring in the liver. This is the first study to estimate the rate of phenylalanine hydroxylation using intrahepatocytic enrichment in healthy children. The total phenylalanine hydroxylation rate in the fed state, estimated from the apoB-100 enrichment, was $29.55 \pm 5.35 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ when fed a control diet and $40.25 \pm 5.48 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ when fed a tyrosine-free diet. These values were higher than the rates estimated from the plasma enrichment. When tyrosine was present in the diet, $\sim 22\%$ of plasma tyrosine appearance was derived from plasma phenylalanine in the fed state, whereas $\sim 35\%$ of intrahepatic tyrosine appearance was derived from phenylalanine. When the tyrosine-free diet was fed, $\sim 36\%$ of plasma tyrosine appearance was from plasma phenylalanine, whereas $\sim 69\%$ of intrahepatic tyrosine appearance was derived from phenylalanine. The enrichment of $[^{13}\text{C}]$ tyrosine, derived from $[^{13}\text{C}]$ phenylalanine in apoB-100, was almost twice as high as that in plasma, indicating that the liver was a primary site for phenylalanine hydroxylation. Thus, plasma phenylalanine and tyrosine enrichments are not ideal surrogates for estimating hepatic phenylalanine hydroxylation rate for studies in which the tracer is infused for only 6 h.

In animals, when tyrosine is present in excess, tyrosine synthesized from phenylalanine was catabolized without first equilibrating with the plasma tyrosine pool (36). Shiman and Gray (37) suggested that the role of phenylalanine hydroxylase is to completely degrade phenylalanine rather than to synthesize tyrosine. In this study, phenylalanine oxidation estimated from F^{13}CO_2 and its enrichment in apoB-100 was not significantly different from phenylalanine hydroxylation, indicating that phenylalanine is channeled to oxidation regardless of dietary tyrosine. However, this was not true when oxidation and hydroxylation were estimated from plasma phenylalanine enrichment. Furthermore, the finding that phenylalanine oxidation was higher than hydroxylation calculated from plasma enrichments is an implausible phenomenon when no dietary tyrosine was given. This is further evidence that enrichments in apoB-100 are more appropriate to measure changes in phenylalanine hydroxylation.

The protein, phenylalanine, and tyrosine intake levels in this study were chosen to mimic the usual intake for children based on the Continuing Survey of Food Intakes by Individuals (CSFII; 1994–1996, 1998) (21). In this study, the subjects received $\sim 1.5 \text{ g protein} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ containing $84 \text{ mg phenylalanine} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ in both diets and $70 \text{ mg tyrosine} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ in control diet. However, phenylalanine plus tyrosine intakes in this study are higher than the FAO/WHO/UNU 2007 requirements (38). It is a distinct possibility that the high intake of phenylalanine elicited increased oxidation, thereby reducing any “sparing effect” on tyrosine. For example, in the studies of methionine and cysteine requirements, when the minimum methionine requirement has been met, adding more methionine did not spare cysteine any further (39). In addition, because the subjects received $\sim 6 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ of tyrosine in the form of the tracer, this may have reduced the need for tyrosine synthesis from phenylalanine when the tyrosine-free diet was fed. This may explain our observation that *de novo* tyrosine synthesis (difference be-

tween phenylalanine hydroxylation and oxidation) was only $\sim 3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ and no difference between phenylalanine hydroxylation and oxidation during the tyrosine-free diet.

Tyrosine is classified as a conditionally indispensable amino acid, especially during growth or in disease (40,41). Because of its poor solubility, current parenteral amino acid mixtures contain only trace amount of tyrosine or none. Generous amount of phenylalanine has been included in some solutions in the hope that its hydroxylation to tyrosine will meet phenylalanine plus tyrosine needs. Thus, from a clinical point of view, it is important to examine the rate of phenylalanine hydroxylation in the presence and absence of exogenous tyrosine. The results of our study show that, even though the rate of phenylalanine hydroxylation increased when children consumed a tyrosine-free diet, phenylalanine oxidation also increased. In addition, the rates of phenylalanine hydroxylation and oxidation were similar when children were fed both diets, indicating that the exogenous phenylalanine was converted to tyrosine and mainly oxidized. These results indicate that adding extra phenylalanine to parenteral amino acid mixtures is not a viable way to meet tyrosine needs and strongly suggest that there may be a dietary requirement for tyrosine to partially fulfill its requirement. Further investigations of the effects of generous phenylalanine intake and other cofactors, such as tetrahydrobiopterin (BH4), are also needed based on the findings that a high dose of the BH4 can be used as alternative therapy in some phenylketonuria patients (42).

In conclusion, healthy children are capable of converting phenylalanine to tyrosine, but there may also be a dietary requirement for tyrosine to partially fulfill the total requirement of tyrosine. A clinical consequence of this study is that children receiving amino acid based diets should always receive tyrosine-containing amino acid mixtures.

Acknowledgments. We thank Cynthia Boutte for recruiting subjects and coordinating the study, Ann McMeans for designing the diet, the research nurses and kitchen staff at the MRU of the CNRC for their dedicated subject care, the staff at the Calorimeter Core Laboratory for assisting with the room respiratory calorimetry, and Rebecca Newsom for administration work. We also thank Margaret Frazer, Melanie Delrosario, Shaji Chacko, and Dan Donaldson for technical assistance. Special thanks to the subjects who participated in this study and their parents.

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