Phenylalanine requirements of enterally fed term and preterm neonates

Jacomine E Hogewind-Schoonenboom, Li Zhu, Lin Zhu, Eveline CAM Ackermans, Renske Mulders, Bart te Boekhorst, Mandy Wijnen, Lianne Bijnevelt, Gardi J Voortman, Henk Schierbeek, Lisha Huang, Femke de Groof, Andras Vermes, Chao Chen, Ying Huang, and Johannes B van Goudoever

ABSTRACT

Background: Phenylalanine, which is an essential aromatic amino acid, is either used for protein synthesis or irreversibly hydroxylated to tyrosine. The provision of optimal amounts of dietary phenylalanine is not only important for growth and development but might also influence catecholamine synthesis and release rates. The current recommended aromatic amino acid requirement for infants aged 0–6 mo is based on the amino acid content of human milk.

Objective: We quantified the requirements for phenylalanine in the presence of excess tyrosine (166 or 177 mg/kg per day for term and preterm infants, respectively) for term and preterm neonates by using the indicator amino acid oxidation method with 1-[1-13C]lysine 2HCl as an indicator. Hence, we determined the minimum obligatory phenylalanine requirement.

Design: Fully enterally fed term and preterm infants received randomly graded amounts of phenylalanine (5–177 mg/kg per day) as part of an elemental formula. Data are expressed as means ± SDs.

Results: Twenty term (birth weight: 3.19 ± 0.34 kg; gestational age: 38.9 ± 1 wk) and 16 preterm (birth weight: 1.75 ± 0.17 kg; gestational age: 32.5 ± 0.6 wk) Asian infants participated at a postnatal age of 17 ± 8 d. In total, 44 studies were performed. The minimum obligatory phenylalanine requirement was 58 mg/kg per day (95% CI: 38–78 mg/kg per day) and 80 mg/kg per day (95% CI: 40–119 mg/kg per day) for term and preterm infants, respectively.

Conclusion: The determined mean phenylalanine-requirement estimates are lower than the contents of term and preterm formulas currently on the market. This trial was registered at www.trialregister.nl as NTR1610.

INTRODUCTION

Phenylalanine, which is an essential aromatic amino acid, is mainly used for protein synthesis but can also serve as a precursor for the formation of the catecholamines adrenaline, noradrenaline, and dopamine via irreversible hydroxylation to tyrosine. These catecholamines are involved in several physiologic processes in the body (e.g., glucose and lipid metabolism, blood pressure, and cardiac activity). Catecholamine synthesis and release rates in the central nervous system and sympathoadrenal cells are responsive to physiologic changes in tyrosine concentrations, which may lead to physiologic responses when neurons are actively firing (1). Dietary phenylalanine intake may influence plasma and brain concentrations of tyrosine (2–4). Therefore, the provision of optimal amounts of phenylalanine is of clear importance.

There is an absence of reliable scientific evidence of phenylalanine requirement of term and preterm neonates. In the 1950s, several nitrogen-balance studies were performed to establish amino acid requirements, but because of methodologic drawbacks, these estimates are not used for current requirement guidelines (5). Instead, the amino acid pattern of formula is modeled on the average human milk protein composition.

Because human milk is considered to be the optimal feeding for term infants, term-requirement guidelines are based on average human milk intake in the first month of life, which is 72 mg/kg per day for phenylalanine (6). However, the accuracy of these guidelines is questionable (7).

Human milk intake and content are highly variable and dependent on the time of day, maternal body composition and milk production, sex of the infant, and duration of lactation (7). The most-pronounced changes in the protein content and composition occur during the first month of lactation. The protein content drops from 2.5 to 1.3 g/100 mL, and the whey:casein ratio declines from 80:20 in colostrum to 60:40 in term milk (8–10).
Because the amino acid profiles of whey and casein protein differ, changes in the whey:casein ratio result in different amounts of essential amino acids being provided. Subsequently, these factors impede the determination of average amino acid intake, and therefore, the accuracy of requirement guidelines that are based on average human milk intake is uncertain (7).

For preterm infants, no recommendations concerning the specific amino acid requirement can be made because sole human milk does not meet their dietary needs, and empirical derived evidence is thought to be insufficient (11).

The preferred method for determining the amino acid requirement in a vulnerable population is the minimally invasive indicator amino acid oxidation (IAAO) method (12), which we previously validated for use in enterally fed neonates (13, 14).

METHODS

Subjects

All neonates were recruited from the neonatal ward of the Fudan University Children’s Hospital in Shanghai between June 2011 and April 2012. Term infants were eligible for the study when they were fully enterally fed with a gestational age of $\geq 37$ wk and birth weight of $\geq 2500$ g. Infants had to be clinically stable with a weight-gain rate $\geq 5$ g/kg per day in the preceding 3 d.

Preterm infants also had to be clinically stable with a weight-gain rate $\geq 10$ g/kg per day in the preceding 3 d. No antibiotic use was allowed at the time of study. Additional selection criteria included a corrected gestational age $\leq 37$ wk, birth weight $\leq 2200$ g, and weight $\leq 2500$ g on the study day. Exclusion criteria of both groups were congenital anomalies, gastrointestinal pathology, or sepsis.

Written informed consent was obtained from at least one parent by a native Chinese speaking pediatrician. Ethical approval was obtained from the Institutional Review Boards of the Children’s Hospital of Fudan University, Shanghai, China, and a statement of no objection was received from the Institutional Review Board of the Erasmus Medical Centre, Rotterdam, The Netherlands.

Study formula

The amino acid–based formula was manufactured for the study by SHS International with a lipid, carbohydrate, mineral, vitamin, and trace element contents as previously published (15). Term infants received 150 mL/kg per day, which contained caloric intake of 108 kcal/kg per day and an amino acid intake equal to protein intake of 2.96 g/kg per day. Each preterm participant received 160 mL/kg per day with energy intake of $\sim 115$ kcal/kg per day and an amino acid intake equal to protein intake of 3.2 g/kg per day. These intakes were maintained during the complete study. Energy, carbohydrate, fat, and amino acid content of the study formula are shown in Table 1. Its composition was identical to Neocate formula (SHS International) except for phenylalanine, lysine, and alanine contents.

The formula did not contain any phenylalanine, which allowed us to vary phenylalanine intake by adding L-phenylalanine up to the randomly assigned test intake. The average phenylalanine content of human milk in the first month of life is 72 mg/kg per day (6). Because we hypothesized that the estimated breakpoint would be comparable, we grouped our test intakes around this intake equally to maximize the power of the breakpoint analysis. The maximum test intake of phenylalanine was 166 mg/kg per day for term infants and 177 mg/kg per day for preterm infants. This amount was chosen because it represents the maximum amount of phenylalanine in regular Neocate formula when given at 150 and 160 mL/kg per day, respectively. Therefore, we considered this a safe maximum intake.

Although neonates are able to increase their hydroxylation and oxidation rates in response to increased phenylalanine intake (16), it is unclear whether they are capable of sufficient phenylalanine hydroxylation to produce enough tyrosine to support optimal protein-synthesis rates (17, 18). Therefore, a generous amount of tyrosine (166 or 177 mg/kg per day for term and preterm infants, respectively) was present during the complete study.

This amount is twice the amount in breast milk (6) and above earlier recommended values of the tyrosine requirement for

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Energy, carbohydrate, fat, and amino acid contents of the study formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component</td>
<td>Per 100 g formula</td>
</tr>
<tr>
<td>Energy, kcal</td>
<td>475</td>
</tr>
<tr>
<td>Carbohydrates, g</td>
<td>54</td>
</tr>
<tr>
<td>Fat, g</td>
<td>23</td>
</tr>
<tr>
<td>Total amino acids, g</td>
<td>13</td>
</tr>
<tr>
<td>l-Alanine$^1$</td>
<td>$\geq 0.61$</td>
</tr>
<tr>
<td>l-Arginine</td>
<td>1.08</td>
</tr>
<tr>
<td>l-Asparagine</td>
<td>1.01</td>
</tr>
<tr>
<td>l-Cyst(e)ine</td>
<td>0.4</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.95</td>
</tr>
<tr>
<td>l-Histidine</td>
<td>0.62</td>
</tr>
<tr>
<td>l-Isoleucine</td>
<td>0.95</td>
</tr>
<tr>
<td>l-Leucine</td>
<td>1.63</td>
</tr>
<tr>
<td>l-Lysine$^2$</td>
<td>0.494</td>
</tr>
<tr>
<td>l-Methionine</td>
<td>0.26</td>
</tr>
<tr>
<td>l-Phenylalanine$^3$</td>
<td>0</td>
</tr>
<tr>
<td>l-Proline</td>
<td>1.16</td>
</tr>
<tr>
<td>l-Serine</td>
<td>0.71</td>
</tr>
<tr>
<td>l-Threonine</td>
<td>0.8</td>
</tr>
<tr>
<td>l-Tryptophan</td>
<td>0.32</td>
</tr>
<tr>
<td>l-Tyrosine</td>
<td>0.73</td>
</tr>
<tr>
<td>l-Valine</td>
<td>1.04</td>
</tr>
<tr>
<td>l-Carnitine</td>
<td>0.01</td>
</tr>
<tr>
<td>Taurine</td>
<td>0.03</td>
</tr>
<tr>
<td>l-Glutamine</td>
<td>1.34</td>
</tr>
</tbody>
</table>

$^1$Molar equivalents of l-alanine were added to the test diet to compensate for varying amounts of phenylalanine to maintain an isonitrogenous diet. The study formula contained $\geq 0.61$ g l-alanine/100 g formula.

$^2$A total of 0.616 g l-lysine/100 g formula was added to the study diet on the adaptation day. An equivalent amount of l-lysine was given as stable isotope on the study day.

$^3$l-Phenylalanine was added separately depending on the test phenylalanine concentration.

$^4$Abbreviations used: APE, atom percent excess; IAAO, indicator amino acid oxidation; LNAA, large neutral amino acid.
preterm infants (19). Because the presence of excess tyrosine will lower the amount of dietary phenylalanine needed, we determined the minimum obligatory phenylalanine requirement (20).

By adding L-lysine to the formula on the adaptation day in the same amount that was given as a tracer during the study day, lysine intake was fixed during the complete study at 252 mg/kg per day for term infants and 269 mg/kg per day for preterm infants. These amounts are similar to the content of regular Neocate and well above the estimated requirement for term infants of 130 mg/kg per day shown in our earlier IAAO study (13). To keep the amount of nitrogen in the diet constant despite the varying phenylalanine content, molar equivalents of L-alanine were added.

**Experimental design**

This study was one of a series that formed a large project to determine the essential amino acid requirement of neonates by using the minimal invasive IAAO method (21) modified for enterally fed neonates and described extensively before (13–15, 22). The method is based on the assumption that amino acids, which are not used for protein synthesis, are preferably oxidized (23). The extent of protein synthesis is dependent on the most-limiting essential amino acid provided. Consequently, when a test amino acid is provided below the requirement, all other amino acids will be oxidized. Therefore, another essential amino acid labeled with a stable isotope can serve as a marker to measure oxidative disposal. This amino acid is called an indicator amino acid. The oxidation of the indicator amino acid is inversely related to the supply of test amino acid up to the point that the requirement of the test amino acid is met. Once the requirement is met, oxidation of the indicator is constant at an optimal rate. In the current study, we used L-[1-13C]lysine 2HCl as the indicator amino acid. Hence, its oxidation rates could be measured in expired air as 13CO2.

All patients were randomly assigned with respect to the amount of phenylalanine administered. Patients received the test diet, which contained the assigned amount of phenylalanine, for 24 h adaptation before initiating the study day. During the adaptation day, both preterm and term infants were bottle-fed every 2 or 3 h, respectively. On the study day, the same amount of formula was given as on the adaptation day. However, on the study day, term infants received the formula as hourly bottle-feeding. The feeding regimen of preterm infants was changed 30 min before the tracer infusion was started into a continuous drip feeding by using a gastric tube to ensure constant intake.

In some preterm infants, 2 test intakes were measured. The time between 2 protocols was ≥3 d to allow for an adequate tracer washout time before the next study day (14). To avoid infants receiving suboptimal amino acid intake for a longer period, we randomly assigned every participant who received 2 intakes to one amount above and one amount below the expected breakpoint.

Each study day included a 2.5-h (in preterm) or 3-h (in term infants) primed (14-μmol/kg) continuous (9-μmol/kg) infusion of [13C]bicarbonate [sterile, pyrogen free, 99% 13C atom percent excess (APE); Cambridge Isotopes] to quantify individual carbon dioxide production (24). This was directly followed by a primed (40-μmol/kg) continuous (36-μmol/kg) infusion of L-[1-13C]lysine 2HCl (99% 13C APE; Cambridge Isotopes) as an indicator amino acid over 4.5 h. In both term and preterm infants, tracers were given enterally by using a gastric tube.

Either phenylalanine or lysine can be used as an indicator amino acid (25). Because phenylalanine was our test amino acid, lysine was chosen as the indicator. Although lysine meets the criteria of a suitable indicator amino acid (i.e., it has no metabolic pathways other than oxidation and protein synthesis), it is considered a less-sensitive indicator than phenylalanine because of its larger and more-variable body tissue pool (25). However, the lysine indicator has been validated by comparing tryptophan requirements established with both indicators in a piglet model (26). In the first 15 infants (term: n = 10; preterm: n = 5) included in the study, the infusion time of the lysine tracer was prolonged to establish the optimal time necessary for the lysine tracer to reach a steady state. The time for lysine to reach a steady state was 3–3.5 h, which was comparable with earlier studies performed in children and neonates (27, 28).

**Sample collection and analysis**

Breath samples were collected in duplicate before isotope infusion as baseline and every 10 min during both isotopic plateaus by means of the direct sampling method described by van der Schoor et al. (29). Isotopic steady state was reached during the last 45 and 60 min of the [13C]bicarbonate and L-[1-13C]lysine 2HCl infusions, respectively. Samples were analyzed for 13C abundance in carbon dioxide by using the infrared isotope analysis technique (Helifan; Analytic Fischer Instruments), and the results were reported as the APE.

**Calculations**

The attainment of isotopic steady state was defined as ≥3 consecutive points with a slope not significantly different from zero (P ≥ 0.05). The estimated body carbon dioxide production (μmol · kg−1 · h−1) was calculated for each infant as previously described (13, 24). The rate of 13CO2 release from L-[1-13C]lysine oxidation in breath [fraction of 13CO2 recovery from [1-13C]lysine-2HCl oxidation (F13CO2)] as a percentage was calculated by using the following equation:

$$F_{13CO2} \% = \frac{(IE_{lys} \times i_B)}{i_{lys} \times IE_{B}} \times 100$$  \hspace{1cm} (I)

where $IE_{lys}$ is the 13C isotopic enrichment in expired air during [1-13C]lysine infusion (APE), $i_B$ is the infusion rate of [13C]bicarbonate (μmol/kg per hour), $i_{lys}$ is the infusion rate of [1-13C]lysine (μmol/kg per hour), and $IE_B$ is the 13C-isotopic enrichment in expired air during [13C]bicarbonate infusion (28).

Lysine flux was not obtained. However, previous studies showed that lysine flux was not affected by graded phenylalanine intakes (30, 31).

**Statistical analysis**

Descriptive data were expressed as means ± SDs. Infants who participated with 2 intakes in the study had their clinical characteristics measured twice. The effects of phenylalanine intake on the body carbon dioxide production rate were tested by using a Pearson’s correlation coefficient analysis.
The mean requirement for phenylalanine was determined by using a nonlinear regression model (26, 32). In this model, the regression equation was split into 2 parts, one part of which a negative slope was calculated and one part of which the slope was restricted to zero. The intercept between these lines, which is called the breakpoint, was estimated. This breakpoint represented the mean test amino acid requirement. The model with the best fit on the basis of the highest r$^2$ was selected. For the preterm study, an adjustment was made by using the vce option in STATA software (version 11; StataCorp LP) to account for repeated measurements within the same patients. This adjustment influenced only the SE of the estimates, not the r$^2$. The 95% CIs were calculated. On the basis of point estimates and corresponding 95% CIs, it was evaluated whether point estimates of term and preterm requirements differed significantly from each other. Data analyses were performed with STATA software (version 21.0; StataCorp Inc.). P < 0.05 was considered significant. We measured ≥20 intakes/study, which was in a similar range of the subject number used in other requirement studies (33, 34).

RESULTS

Subject characteristics

Twenty term neonates were included in the study. One test intake was studied in each infant. Clinical characteristics of the infants are presented in Table 2. Infants were admitted to the hospital ward because of unconjugated hyperbilirubinemia (n = 16), pneumonia with negative blood cultures (n = 3), and wet lung (n = 1). At the time of the study, phototherapy and respiratory support had finished. We considered these infants eligible for the study because they were gaining weight at a satisfactory rate (>5 g/kg per day for the preceding 3 d) and were discharged very shortly after the study protocol had finished.

In preterm infants, 24 test intakes were studied in 16 infants. Suspected or related reasons for premature birth were (prolonged) premature rupture of membranes (n = 4), placenta praevia (n = 3), pregnancy-induced hypertension (n = 1), and twin pregnancy (n = 1). In 5 cases, no specific explanation for premature birth could be given. At the time of the study, all preterm infants tolerated full enteral feeding, none of them received antibiotics, and respiratory support had finished. Clinical characteristics of these infants are also presented in Table 2. If infants were participating with 2 test intakes, their clinical characteristics were measured twice. All infants included were growing well (>10 g/kg per day) and were above their birth weight during the study day. Besides their prematurity, no other medical problems were present on the study day.

Two infants were excluded from all final analyses. One term infant did not reach an isotopic steady state, and one preterm infant had an F$^{13}$CO$_2$ enrichment value that far exceeded the mean +2 SDs. We were unable to identify an adequate explanation for this deviation, but because the result was so extreme, we excluded the infant from the analysis.

Table 2

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject characteristics and their protein and energy intakes during the day before the study of infants who participated in the study$^1$</td>
<td>Term</td>
<td>Preterm</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>3.19 ± 0.34$^2$</td>
<td>1.75 ± 0.17</td>
</tr>
<tr>
<td>Gestational age, wk</td>
<td>38.9 ± 1.0</td>
<td>32.5 ± 0.6</td>
</tr>
<tr>
<td>Age on study day, d</td>
<td>13 ± 6</td>
<td>20 ± 8</td>
</tr>
<tr>
<td>Weight on study day, kg</td>
<td>3.27 ± 0.40</td>
<td>1.95 ± 0.11</td>
</tr>
<tr>
<td>Weight-gain rate before study, g·kg$^{-1}$·d$^{-1}$</td>
<td>9 ± 4</td>
<td>16 ± 6</td>
</tr>
<tr>
<td>Sex, M:F</td>
<td>9:11</td>
<td>11:13</td>
</tr>
<tr>
<td>Protein intake before the study, g·kg$^{-1}$·d$^{-1}$</td>
<td>2.0 ± 0.1</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>Energy intake before the study, kcal·kg$^{-1}$·d$^{-1}$</td>
<td>103 ± 7</td>
<td>111 ± 8</td>
</tr>
</tbody>
</table>

$^1$Twenty term and 16 preterm infants were included, in whom 20 and 24 studies were performed, respectively. Clinical characteristics of infants who participated with 2 intakes were measured twice.

$^2$Mean ± SD (all such values).

Stable-isotope data

In Figure 1, the mean $^{13}$CO$_2$ enrichment and carbon dioxide production rate at the isotopic steady state of both term and preterm infants are plotted against phenylalanine intake. The baseline $^{13}$CO$_2$ enrichment was −17.12 ± 1.40 Pee Dee Belemnite for term infant and −15.21 ± 1.18 Pee Dee Belemnite for preterm infants. The mean $^{13}$CO$_2$ enrichment at isotopic steady state during [1$^{13}$C]bicarbonate infusion for term and preterm infants was 0.0376 ± 0.0054 and 0.0382 ± 0.0054 APE, respectively, and corresponding mean carbon dioxide production rates were 23.90 ± 3.03 and 23.55 ± 3.44 mmol·kg$^{-1}$·h$^{-1}$, respectively. There was no significant correlation between phenylalanine intake and the carbon dioxide production rate in both term and preterm infants (P = 0.14 and P = 0.28, respectively).

The mean $^{13}$CO$_2$ enrichment at isotopic steady state during L-[1$^{13}$C]lysine 2HCl infusion of term and preterm infants was 0.0271 ± 0.0084 and 0.0248 ± 0.0089 APE, respectively. These $^{13}$CO$_2$ enrichment values and the F$^{13}$CO$_2$ are plotted against phenylalanine intake in Figure 2. The negative slope of the high F$^{13}$CO$_2$ state before the breakpoint represents the relative excess of indicator amino acid for protein synthesis because phenylalanine is the limiting amino acid. Once the requirement for phenylalanine is met, the line of the F$^{13}$CO$_2$ graph becomes horizontal, representing the basal amount of [1$^{13}$C]lysine 2HCl oxidation when phenylalanine is not the limiting amino acid. The breakpoint represents the population mean of the phenylalanine requirement. For term infants, the breakpoint in the F$^{13}$CO$_2$, calculated by using a biphasic linear

Figure 1

The mean $^{13}$CO$_2$ enrichment and carbon dioxide production rate at the isotopic steady state of both term and preterm infants are plotted against phenylalanine intake. The baseline $^{13}$CO$_2$ enrichment was −17.12 ± 1.40 Pee Dee Belemnite for term infant and −15.21 ± 1.18 Pee Dee Belemnite for preterm infants. The mean $^{13}$CO$_2$ enrichment at isotopic steady state during [1$^{13}$C]bicarbonate infusion for term and preterm infants was 0.0376 ± 0.0054 and 0.0382 ± 0.0054 APE, respectively, and corresponding mean carbon dioxide production rates were 23.90 ± 3.03 and 23.55 ± 3.44 mmol·kg$^{-1}$·h$^{-1}$, respectively. There was no significant correlation between phenylalanine intake and the carbon dioxide production rate in both term and preterm infants (P = 0.14 and P = 0.28, respectively).

The mean $^{13}$CO$_2$ enrichment at isotopic steady state during L-[1$^{13}$C]lysine 2HCl infusion of term and preterm infants was 0.0271 ± 0.0084 and 0.0248 ± 0.0089 APE, respectively. These $^{13}$CO$_2$ enrichment values and the F$^{13}$CO$_2$ are plotted against phenylalanine intake in Figure 2. The negative slope of the high F$^{13}$CO$_2$ state before the breakpoint represents the relative excess of indicator amino acid for protein synthesis because phenylalanine is the limiting amino acid. Once the requirement for phenylalanine is met, the line of the F$^{13}$CO$_2$ graph becomes horizontal, representing the basal amount of [1$^{13}$C]lysine 2HCl oxidation when phenylalanine is not the limiting amino acid. The breakpoint represents the population mean of the phenylalanine requirement. For term infants, the breakpoint in the F$^{13}$CO$_2$, calculated by using a biphasic linear
regression crossover model occurred at an intake of 58 mg/kg per day ($r^2 = 0.62$, $P = 0.001$) with an upper CI of 78 mg/kg per day and lower CI of 38 mg/kg per day. For preterm infants, the breakpoint was estimated at 80 mg/kg per day ($r^2 = 0.48$, $P = 0.001$) with upper and lower CI of 119 and 40 mg/kg per day, respectively. The difference between the 2 requirement estimates was 21.6 mg/kg per day with upper and lower CIs of 48.1 and −4.9 mg/kg per day, respectively.

**FIGURE 1** Mean $^{13}$CO$_2$ enrichment at isotopic plateau during enteral $[^{13}$C]bicarbonate infusion of each term infant ($n = 20$) (A) and each preterm infant ($n = 24$) (B) and the carbon dioxide production rate of each term infant ($n = 20$) (C) and each preterm infant ($n = 24$) (D) plotted against phenylalanine intake. APE, atom percent excess. $n$, number of intakes studied.

**FIGURE 2** Mean $^{13}$CO$_2$ enrichment at isotopic plateau during enteral $[1-^{13}$C]lysine 2HCl infusion of each term ($n = 20$) (A) and preterm ($n = 24$) (B) infant plotted against the phenylalanine intake ($n = 24$), and the fraction of $^{13}$CO$_2$ recovery from $[1-^{13}$C]lysine 2HCl oxidation ($F_{^{13}CO_2}$) during the isotopic plateau at different phenylalanine intakes of term infants ($n = 20$) (C) and preterm infants ($n = 24$) (D). With the use of a biphasic linear regression crossover model, the mean phenylalanine requirement was estimated to be 58 mg/kg per day ($r^2 = 0.62$) with respective upper and lower CIs of 78 and 38 mg/kg per day for term infants, and 80 mg/kg per day ($r^2 = 0.48$) with respective upper and lower CIs of 119 and 40 mg/kg per day for preterm infants. APE, atom percent excess; $n$, number of intakes studied.
In this study, we determined the phenylalanine requirement of enterally fed term and preterm infants. Our study showed that the minimal obligatory phenylalanine requirement of enterally fed term neonates is 58 mg/kg per day. The preterm amino acid requirement is thought to be relatively higher than that of term infants because of higher growth and metabolic rates (35). Although NS, there was a tendency for a somewhat higher mean phenylalanine requirement for moderate preterm infants of 80 mg/kg per day.

As a result of accelerated maturation of immature enzyme systems after birth, the amino acid requirement is influenced by both gestational and postnatal ages (36). Consequently, preterm neonates form a more-heterogeneous study group than do term infants. We know that, even in homogenous adult populations, a large intrasubject variation in the amino acid requirement exist (37). We did not perform studies with different test intakes below and above the requirement intake in each infant to estimate the individual requirement and variation in our study population (38).

However, the heterogeneity of the preterm study population may have been reflected in the notable broader CI of the preterm estimate than of the term estimate. Moreover, the \( r^2 \) of the preterm estimate should be considered with a little caution because it was not corrected for the correlation of repeated measures within one individual.

Our term estimate is comparable to that in a previous study of Snyderman et al. (39) who determined the enteral phenylalanine requirement in the presence of excess tyrosine of 6 infants with ages ranging from 6 d to 9 mo. Snyderman et al. (39) measured the growth rate of all infants and the nitrogen balance of 2 infants during several different intakes of phenylalanine as part of an elemental diet. Each infant received 1–4 test intakes. The authors showed intakes that ranged from 47 to 94 mg/kg per day to be sufficient for achieving growth rates similar to those observed when the infant was fed the same complete diet. These results were not used for current amino acid requirement guidelines because the study did not meet the current methodologic standards because of limitations of the nitrogen-balance method as well as its small, heterogenic study population (6). Despite these limitations, our estimates are within the same range.

The average phenylalanine intake in the first month of life of human milk-fed infants is 72 mg/kg per day (6). Breast milk is considered to fulfill the nutritional needs for essential amino acids of all infants. We determined the mean obligatory phenylalanine requirement, which was only sufficient for 50% of the population. To define a population safe intake that covered the need of all infants, the individual variance in the phenylalanine requirement of our study population should be determined. Future studies should address this issue, but until then, a correction factor of 125% of the mean requirement as proposed by the WHO to determine the safe protein intake in early infancy should be applied (6, 22). With the application of this rule of thumb, the required safe intake is 73 mg/kg per day for term infants and 100 mg/kg per day for moderate preterm infants.

Because relative growth rates decline during the first month of life after a term birth, the protein requirement is thought to decline accordingly. Current formulas have to contain at least the amount of each essential amino acid needed during the first month of life (40). Because of the decline in requirement during the subsequent months, infants are increasingly exposed to excessive amounts of protein. In addition, the total protein content of formula is often considerably increased to compensate for the lesser bioavailability of proteins used than in human milk protein. High protein intake in the early postnatal period is thought to enhance risk of metabolic syndrome and cardiovascular diseases in adulthood (41, 42). Hence, current results contribute to optimize the protein composition to meet the requirement of formula-fed infants.

For preterm infants, amino acid requirements were previously calculated by using the factorial approach. This theoretical method is based on the assumption that the requirement is equal to the sum of the maintenance requirement plus the amount needed for growth (43). Currently, the adult requirement is believed to represent the maintenance requirement because this amount is similar in different age groups (38). A compositional analysis of corpses of aborted and stillborn infants was used as an analog for preterm amino acid accretion rates (19). The phenylalanine requirement of adults was determined to be 9 mg/kg per day by using direct amino acid oxidation (44). Widdowson et al. (45) estimated the enteral phenylalanine accretion to be 121 mg/d in fetuses with an average weight of 2.15 kg (range: 1.9–2.4 kg). If we presume that amino acid retention is 90% of total protein intake (46), we can calculate the theoretical requirement for our preterm study population to be 71 mg/kg per day (range: 65–80 mg/kg per day). Despite the limitations of this approach, in particular the drawbacks concerning the reliability of data derived from carcasses from diseased or stillborn infants, it must be appreciated that the calculated estimate is comparable to our estimate (47).

Although both our term and preterm requirement estimates are comparable to requirements that are based on nitrogen-balance techniques and the factorial approach, before extending our results to broad applicable requirement guidelines, we must first account for several limitations in our study design.

First, the single amino acids provided by elemental formulas may be differently oxidized and used for protein synthesis compared with those derived from different sources of whole protein (2, 48). We speculate that our estimates are an overestimation of requirements (48).

Furthermore, part of our study population may have been in a postinfectious state because (prior) antibiotic use was >80% in both our term and preterm populations. The aromatic amino acids, in particular phenylalanine, are an important component of acute-phase proteins, of which the formation is increased during infection (49). Hence, although all of the infants had recovered from (presumed) infection, the phenylalanine demand may have been increased modestly in our study population.

Also, the (previous) intravenous antibiotic use could have altered the quantity and diversity of intestinal commensal microbiota. The microbiota can oxidize essential amino acids or use them for the synthesis of microbial mass but can also synthesize essential amino acids, which can be made available to the host (50). Thus, the altered microbial content of the gut as a resultant of antibiotic use may have lead to an underestimate or overestimate of the determined requirement. However, Puiman et al. (51) showed neither a difference in plasma lysine and phenylalanine values nor in net body protein synthesis between control and antibiotic-treated piglets. Therefore, we expect that this effect was negligible.
Finally, graded levels of provided phenylalanine may have influenced the amino acid uptake into the brain and, thereby, our requirement estimate. All large neutral amino acids (LNAA)s compete for blood-brain barrier transport over one transporter (LAT-1), which has a high affinity for phenylalanine (52). Consequently, because of competitive uptake, even small changes in plasma phenylalanine concentrations may alter brain concentrations of all LNAA, including tyrosine (53, 54). Most LNAA are essential amino acids and, therefore, can be rate limiting for protein synthesis. Hence, dietary phenylalanine intake can influence cerebral protein and neurotransmitter synthesis. Because the IAAO method measures the requirement by considering the relative amount of the indicator amino acid incorporated into protein, we do not know whether the method takes into account the amount of phenylalanine necessary for neurotransmitter synthesis. However, the requirement for neurotransmitter synthesis is quantitatively negligible compared with that needed for growth. Therefore, we consider this effect was very small.

These limitations in our study design emphasize the need for future studies that investigate the effect of a (protein-based) formula with refined amino acid composition on growth and other physiologic variables.

The phenylalanine requirement depends on the amount of tyrosine provided because phenylalanine can be converted to tyrosine. Tyrosine in excess of the requirement can spare approximately twice that amount is provided (55). Therefore, we speculate that, in future formulae, the tyrosine content can also be lowered.

In conclusion, we determined the mean phenylalanine requirement of enterally fed term and preterm infants to be 58 and 80 mg/kg per day, respectively. The determined mean phenylalanine requirement estimates have been performed to our knowledge. However, the ideal dietary ratio of phenylalanine to tyrosine is thought to be 60:40 (20). The application of this ratio to our phenylalanine-requirement estimates results in a tyrosine requirement of 39 and 53 mg/kg per day for term and preterm infants, respectively. In currently available formulae, approximately twice that amount is provided (55). Therefore, we speculate that, in future formulae, the tyrosine content can also be lowered.

The authors’ responsibilities were as follows—JEH-S, LH, FDG, JBvG, CC, and YH: conception; JEH-S, FDG, LH, and JBvG: design; JEH-S, Li Zhu, Lin Zhu, ECAMSA, RM, BB, MW, and LB: conduction of experiments; JEH-S and JBvG: data interpretation; GJV and HS: data analysis; AV: preparation of tracers; CC, YH, and JBvG: consultation; and JEH-S and JBvG: manuscript writing. None of the authors had any competing financial or personal interests in any company or organization that sponsored the research. Study sponsors had no influence on the study design, analysis, or interpretation of data, or writing of the manuscript.

REFERENCES


Metges CC. Contribution of microbial amino acids to amino acid homeostasis of the host. J Nutr 2000;130:1857S–64S.


Metges CC. Contribution of microbial amino acids to amino acid homeostasis of the host. J Nutr 2000;130:1857S–64S.

