Lysine requirement in parenterally fed postsurgical human neonates

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ABSTRACT

Background: The lysine requirement of human neonates receiving parenteral nutrition (PN) has not been determined experimentally.

Objective: The objective was to determine the parenteral lysine requirement for human neonates by using the minimally invasive indicator amino acid oxidation technique with l-[1-13C] phenylalanine as the indicator amino acid.

Design: Eleven postsurgical neonates were randomly assigned to 15 lysine intakes ranging from 50 to 260 mg · kg⁻¹ · d⁻¹. Breath and urine samples were collected at baseline and at plateau for [13CO₂] and amino acid enrichment, respectively. The mean lysine requirement was determined by applying a 2-phase linear regression crossover analysis to the measured rates of [13CO₂] release and l-[1-13C]phenylalanine oxidation.

Results: The mean parenteral lysine requirement determined by [13CO₂] release oxidation was 104.9 mg · kg⁻¹ · d⁻¹ (upper and lower CIs: 120.6 and 89.1 mg · kg⁻¹ · d⁻¹, respectively). The mean lysine parenteral requirement determined by phenylalanine oxidation was 117.6 mg · kg⁻¹ · d⁻¹ (upper and lower CIs: 157.5 and 77.6 mg · kg⁻¹ · d⁻¹, respectively). Graded intakes of lysine had no effect on phenylalanine flux.

Conclusion: We recommend a mean lysine requirement for the effect on phenylalanine flux.

We recommend a mean lysine requirement for the human neonate of 104.9 mg · kg⁻¹ · d⁻¹ (11.0, 8.6, and 8.2 g/100 g) (17). These intakes are greater than an infant’s enteral intake from breast milk, which has been reported as 119 mg · kg⁻¹ · d⁻¹ (18); this means that the PN-fed infant receives more lysine when fed intravenously than when fed orally.

Human neonates have been shown to exhibit serious metabolic disturbances when dietary protein is provided in excess of their requirements (19) but studies into the ingestion of excessive amounts of lysine in animals have shown mixed results. Teubuku et al (20) found no observed adverse effects when rats were fed up to 4 g lysine · kg⁻¹ · d⁻¹. However, an infusion of 4.5 g lysine hydrochloride · kg⁻¹ · d⁻¹ over 3 d in dogs led to nephrotoxicity (21); in young males, an excess enteral intake of lysine inhibited renal tubular protein reabsorption (22). Rats fed a 5% increase in lysine enteral intake had an increase in liver total lipid, triacylglycerol, and cholesterol concentrations (23).

The use of the piglet model (24) has enabled us to define amino acid requirements in human neonates. In each of our 3 previous neonatal parenteral amino acid studies (1–3), the experimentally determined requirement was found to agree with data extrapolated from our piglet model. Therefore, the lysine requirement for human neonates receiving requirement intakes of protein and energy is predicted to be ~158 mg · kg⁻¹ · d⁻¹ based on the results from our piglet research (25). This predicted requirement is significantly less than the amount neonates are currently receiving in their parenteral amino acid solution (17). Neonates who require PN after gastrointestinal surgery (eg, ileal atresia) may be exposed to increased plasma concentrations of lysine.
(19), which could potentially lead to metabolic anomalies. Hence, our objective was to use the minimally invasive indicator amino acid oxidation (IAAO) technique to determine the parenteral lysine requirement for human neonates.

SUBJECTS AND METHODS

Subjects

Eleven neonates (9 boys, 2 girls) admitted to the neonatal intensive care unit (NICU) in the Hospital for Sick Children (Toronto, Canada) participated in the study. All study procedures were approved by the research ethics board at the hospital, and permission to enroll the neonates in the study was obtained from the attending surgeons. Written informed consent was obtained from one or both parents. Recruitment of the infants took place between 27 July 2008 and 27 May 2009. Infants were included in the study if they were >35 wk gestational age, appropriate for gestational age, >1.5 kg at birth, ≤28 postnatal days old, and >3 d postoperative. Infants who were studied ≥3 d postoperatively do not differ in amino acid metabolism from infants who do not undergo surgery (26). The neonates were also required to be clinically stable as determined by normal blood values and vital signs. Infants were included in the study if they were receiving PN as part of their clinical treatment and if their enteral protein intake was <5% of their total protein intake during the study day. They were required to have a total protein intake ≥2.7 g · kg⁻¹ · d⁻¹ and an energy intake of ≥334.9 kJ · kg⁻¹ · d⁻¹ (≥80 kcal · kg⁻¹ · d⁻¹). Neonates were excluded from the study if they were receiving supplemental oxygen, were mechanically ventilated, had any endocrine or genetic anomalies, or were taking medications that would influence protein and amino acid metabolism, eg, corticosteroid therapy.

Experimental design and study diets

The study design used the minimally invasive IAAO model developed in adults and applied in children (14, 27) and neonates (2, 3), which was described in detail previously (11). Fifteen studies were performed in 11 neonates; 4 infants were tested at 2 intakes of lysine. Each study took place over a 48-h period as previously described (3). During the first 24-h of the study, all infants were prescribed the same commercial amino acid solution (Primene; Baxter Laboratories) plus dextrose (Baxter Laboratories) and a 20% lipid solution (Intralipid; Fresenius Kabi, Uppsala, Sweden). The PN solution used on study day 1 has an amino acid profile similar to that of cord blood and is the parenteral solution in the NICU. It is also widely used in Europe and produces plasma amino acid concentrations similar to most of the plasma amino acid concentrations found in breastfed infants (28). The purpose of study day 1 was to ensure that all infants received appropriate amounts of protein and nonprotein energy. Adaptation periods of longer duration (eg, 7–10 d) are required during nitrogen balance studies because of the necessity of equilibrating the large and slow changing urea pool. With the IAAO method, prior adaptation is minimal because the endpoint is amino acid oxidation, which adapts in a matter of hours. In a recently published study (29), different adaptation times (8 h, 3 d, or 7 d) were compared over a wide range of lysine intakes to observe whether there were any effects on the oxidation of the indicator amino acid l-[1-¹³C]phenylalanine to ¹³CO₂ (³¹⁵CO₂). They determined that the minimally invasive IAAO model, in which participants are adapted to an adequate protein intake for 2 d followed by study day adaptation to the test amino acid intake for 8 h, was sufficient to estimate individual amino acid requirements in healthy young men.

For study day 2, the second 24-h period, 3 separate amino acid solutions were prepared at the Hospital for Sick Children. The first solution, which contained the bulk of the amino acids (Ajinomoto AminoScience LLC, Raleigh, NC), was patterned on the amino acid profile of the solution used on study day 1 but with modifications made to ensure correct delivery of the test amino acid. Lysine hydrochloride (Ajinomoto) was added to the first or base solution in the amount of 1.04 g/100 g (50 mg · kg⁻¹ · d⁻¹), which is significantly less than that found in the commercial preparation. This was done to ensure an appropriate pH for the stability of cysteine (Ajinomoto) in the hospital-prepared solution. Although the total amount of phenylalanine received by the infants was 3.7 g/100 g (111 mg · kg⁻¹ · d⁻¹), only 1.9 g/100 g (57 mg · kg⁻¹ · d⁻¹) was provided by the test solution; the remainder was given as stable isotope l-[1-¹³C]phenylalanine. The dipeptide glycy1-l-tyrosine (Evonik Degussa Canada Inc, Burlington Canada), at 4 g/100 g amino acid (120 mg · kg⁻¹ · d⁻¹) solution, provided an excess amount of soluble tyrosine. An excess quantity of tyrosine was provided in the test solution to facilitate the channeling of tyrosine, synthesized from phenylalanine, to oxidation (30). The amount of arginine (Ajinomoto) was increased slightly to account for its higher parental requirement (31, 32) compared with the amount in the study day 1 solution. To balance the increase in nitrogen from arginine, the amount of aspartate (Ajinomoto) was decreased proportionately. The amino acid concentrations of the parenteral solutions on days 1 and 2 are found in Table 1.

A second solution was prepared with lysine hydrochloride and sterile water (Baxter) at a concentration of 50 mg/mL and was added back to the first solution on study day 2 to make it equivalent to the test concentration that was randomly assigned to the subject. A third solution containing alanine (Ajinomoto) and sterile water (Baxter), at a concentration of 50 mg/mL, was also made and added to the first solution on study day 2 to make it isonitrogenous to the study day 1 formulation. The 3 solutions were filter sterilized (0.22 µm filter; Alaris Medical Systems, San Diego, CA) in the Research Pharmacy at The Hospital for Sick Children. The solutions were shown to be sterile and free of bacterial growth after 7 d in culture and pyrogen free after limulus amebocyte lysate testing (33). The amino acid composition of the 3 solutions was verified by HPLC with an amino acid column (P580LPG; Dionex, Sunnyvale, CA). The solutions were stored at 4°C in the Research Pharmacy at the hospital until reconstituted on the study day.

On study day 2, the 11 infants were randomly assigned across 15 dietary lysine intakes ranging from 50 to 260 mg · kg⁻¹ · d⁻¹. Our 1998 study (25) found that piglets had a mean lysine requirement of 790 mg · kg⁻¹ · d⁻¹. Because piglets grow at a rate =5 times that of human neonates, we calculated one-fifth of this value (158 mg · kg⁻¹ · d⁻¹) and arranged our lysine intakes around the predicted breakpoint. The graded intakes of lysine delivered parenterally were 50, 70, 80, 90, 100, 110, 120, 140, 150, 160, 170, 200, 225, 250, and 260 mg · kg⁻¹ · d⁻¹.

On both study days 1 and 2, nutrient intakes were prescribed by the NICU physicians and diétitians according to standard clinical
Procedures and guidelines as recommended by the SickKids Nutrition Team (34). All infants were fed intravenously via a central line and received a fluid intake of 120 to 160 mL·kg⁻¹·d⁻¹ by physician’s orders; the lysine intake from the standard PN formulation was 142.3 ± 28.2 mg·kg⁻¹·d⁻¹. Vitamins and minerals were also added to the solutions before delivery to the infant as in previous studies (2, 3). All infants received an adequate protein intake (35, 36), and nonprotein energy was supplied as dextrose and a 20% lipid solution. Protein was prescribed for the infants at 3.1 ± 0.2 g·kg⁻¹·d⁻¹ (range: 2.8–3.5 g·kg⁻¹·d⁻¹) and energy at 369.7 kJ·kg⁻¹·d⁻¹ [88.3 ± 4.1 kcal·kg⁻¹·d⁻¹; range: 336.6–392.3 kJ·kg⁻¹·d⁻¹ (80.4–93.7 kcal·kg⁻¹·d⁻¹)]. A protein intake of 2.7 to 3.5 g·kg⁻¹·d⁻¹ has been shown to result in nitrogen retention similar to the uterine environment (36). Currently, the practice in our NICU for term and preterm infants is to provide a protein intake of 2.7 to 3.5 g·kg⁻¹·d⁻¹ and an energy intake of 334.9 to 460.5 kJ·kg⁻¹·d⁻¹ (80 to 100 kcal·kg⁻¹·d⁻¹). The PN prescribed on study days 1 and 2 met these requirements.

All neonates received the study day 2 solution until the end of the 24-h study day period, when administration of the PN formulation they had received before the study started was resumed. For infants who received 2 intakes of lysine, the time between studies ranged from 28 to 48 h.

The highest and lowest amounts of lysine were studied in the first 3 infants enrolled in the study to establish that the correct range of intakes had been chosen. For each infant who received 2 different intakes of lysine in 2 separate studies, 1 intake was randomized to an amount above the anticipated breakpoint and the second intake was randomized to an amount below the predicted breakpoint. Randomization in this manner was done to prevent infants participating in 2 studies from receiving suboptimal amounts of lysine for prolonged periods.

### Tracer protocol and kinetics

L-[1-¹³C]Phenylalanine [99 atom percent excess (APE); Cambridge Isotope Laboratories, Andover, MA] was the isotope used in the tracer study to measure phenylalanine kinetics. Quality control tests were performed by the manufacturers as described previously (3). The intravenous isotope infusion was begun at the same time as the study day 2 PN solution. A priming dose of L-[1-¹³C]phenylalanine was delivered at 15.6 mg/kg over a 15-min period after which the infusion was delivered at 13 mg·kg⁻¹·d⁻¹ and was continuously infused for 23.75 h. The tracer dose required to achieve a measurable expired ¹³CO₂ was determined in previous studies (1–3); the period for isotope infusion was set to ensure adequate urine sample collection from the neonates.

The stochastic model of Matthews et al (37) was used to calculate the isotopic kinetics and has been used previously in studies with children (14, 27) and infants (1–3). Isotopic steady state at baseline and plateau was represented by the lack of difference in the values of L-[1-¹³C]phenylalanine in urine and ¹³CO₂ in breath. At plateau, the APE was calculated by subtracting the mean baseline breath ¹³CO₂ enrichments from the mean plateau enrichments. Whole-body phenylalanine flux (in μmol·kg⁻¹·h⁻¹) was calculated from dilution of the isotope in the body’s amino acid pool at isotopic steady state (38) by using urinary isotopic enrichment as a representation of plasma enrichment (39). Equations to determine whole-body phenylalanine flux, phenylalanine oxidation, and F¹³CO₂ were described by Matthews et al (37).

### Sample collection and analysis

Weight, length, and head circumference were measured on or immediately before study day 1 for each 48-h period of study. Infants who participated in 2 intakes of lysine had their anthropometric measurements taken separately for each study. Blood samples were monitored to provide information on the clinical status of the neonates. Breath and urine samples were collected at baseline and plateau. The infants had reached isotopic steady state by 12 h of tracer infusion as determined in previous studies (1–3). The timing for procuring samples was dependent on the clinical care of the infants; collection of sufficient numbers of samples of urine took ≤8 h. The PN and tracer were started at ~1500 h on study day 2, and the infants had reached steady state by 0300 the next day. Urine collection began at ~0400, and samples were collected every 2–3 h until 1200. Concurrent with urine collection, breath samples were collected between 0500 and 1200; sample collection was organized around the tests and procedures planned for each infant during the day (X-rays, dressing changes, and ultrasound).

Expired carbon dioxide was collected from the neonates by using a ventilated-hood system, the details of which were reported previously (3). Urine samples were collected by placing

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### TABLE 1

Amino acid concentration of parenteral solutions administered to neonates on day 1 (adaptation) and day 2 (study)

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Day 1</th>
<th>Day 2</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>g/100 g</td>
<td>g/100 g</td>
</tr>
<tr>
<td>Aspartate</td>
<td>6.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Glycine</td>
<td>4.0</td>
<td>2.3</td>
</tr>
<tr>
<td>l-Tryptophan</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>10.0</td>
<td>9.9</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>4.2</td>
<td>1.9</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>6.7</td>
<td>6.7</td>
</tr>
<tr>
<td>L-Histidine</td>
<td>3.8</td>
<td>3.8</td>
</tr>
<tr>
<td>L-Serine</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>L-Taurine</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>L-GLutamate</td>
<td>10.0</td>
<td>9.9</td>
</tr>
<tr>
<td>L-Valine</td>
<td>7.6</td>
<td>7.6</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>3.7</td>
<td>3.7</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>8.4</td>
<td>9.7</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>8.4</td>
<td>7.9</td>
</tr>
<tr>
<td>L-Lysine HCl</td>
<td>11.0</td>
<td>2.1[^1]</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.5</td>
<td>0.0[^2]</td>
</tr>
<tr>
<td>Glycyl-l-tyrosine</td>
<td>0.0</td>
<td>6.1[^3]</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>1.9</td>
<td>1.9</td>
</tr>
<tr>
<td>L-Ornithine</td>
<td>2.5</td>
<td>0.0</td>
</tr>
<tr>
<td>L-Proline</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Total</td>
<td>100.6</td>
<td>90.5[^4]</td>
</tr>
</tbody>
</table>

[^1]: 6.1 g glycyl-l-tyrosine = 4 g tyrosine and 1.6 g glycine.
[^2]: Remainder of phenylalanine was provided as tracer (1.8 g/100 g).
[^3]: Minimum amount of lysine in first or bulk solution to promote stability of cysteine; additional amounts of lysine as added as the intake was varied. Supplementary alanine was added according to the variation in lysine intakes to keep the solution isomoticogenous; 2.08 g lysine HCl provides 1.665 g lysine.
[^4]: The first or bulk solution on day 2 represented 90.5% of the total amino acid provided.
Effects of lysine, protein, and energy intakes on \( ^{13}\text{CO}_2 \) were derived by breakpoint analysis of the rate of release of \( ^{13}\text{CO}_2 \). PROC MIXED procedure (SAS version 9.1; SAS Institute Inc., Cary, NC). Estimates of the mean lysine requirements were derived by breakpoint analysis of the rate of release of \( ^{13}\text{CO}_2 \) data with the use of a 2-phase linear regression crossover model, as described previously (40, 41). The breakpoint was calculated by using the mixed-models and regression procedure of SAS. This model selects for the minimum residual SE in a stepwise partitioning of data points between 2 regression lines. The first regression line has a slope, and the second line is horizontal with minimal or no slope. Statistical significance was established at \( P \leq 0.05 \).

The regression analysis variables were lysine intake as the independent variable and \( ^{13}\text{CO}_2 \) and phenylalanine oxidation as the dependent variables. The final model that identified the “breakpoint” estimate or the requirement for lysine was determined by factors relating to correlation (significance of the model and \( r^2 \)) and estimates of variance (CV and the SEE) (41). Effects of lysine, protein, and energy intakes on \( ^{13}\text{CO}_2 \) were examined by using Pearson correlation coefficient analysis.

RESULTS

Clinical characteristics and nutrient intake

Clinical characteristics and diagnoses for the subjects across 15 studies are presented in Table 2. All infants were appropriate for gestational age and were studied when they had either regained or were above their birth weight, which indicated that the infants were in a positive growth phase. The studies took place \( \geq 3 \) d after surgery if little or no edema was present in the infants, as assessed by the nurse practitioner or registered nurse.

TABLE 2
Characteristics of parenterally fed neonates who received various intakes of lysine (\( n = 11 \) over 15 studies)

<table>
<thead>
<tr>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (wk)</td>
<td>36.6 ± 1.8</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>2.9 ± 0.5</td>
</tr>
<tr>
<td>Study weight (kg)(^1)</td>
<td>3.0 ± 0.5</td>
</tr>
<tr>
<td>Age on study day 2 (d)</td>
<td>12.1 ± 3.9</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>48.6 ± 2.7</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>33.0 ± 1.0</td>
</tr>
</tbody>
</table>

\(^1\) Indicates that birth weight was regained or surpassed by the time of isotope infusion.

For those infants who were tested at a second lysine intake, the weight on day 2 of the second study was the weight used in the calculations for the second study; therefore, each study was considered as a separate entity. Nutrient intakes varied with each study and were dependent on the total volume of PN infused as prescribed for each infant at the outset of every study (Table 3). The mean (±SD) energy and protein intakes were 354.7 ± 18.0 \( \text{kJ} \cdot \text{kg}^{-1} \cdot \text{d}^{-1} \) (85.5 ± 4.3 \( \text{kJ} \cdot \text{kg}^{-1} \cdot \text{d}^{-1} \)) and 3.1 ± 0.2 \( \text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1} \), respectively. Average intakes of the carbohydrates and lipids provided were 13.3 ± 1.1 and 2.7 ± 0.3 \( \text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1} \), respectively. In 13 of the 15 studies, the neonates were “nil per os” and had no enteral intake. Of the 2 infants who consumed enteral feeds, 1 had an ileostomy and the output from the stoma was greater than his enteral intake; we concluded that the enteral feeds had no effect on his lysine intake. The second infant’s intake of expressed breast milk was not sufficient (<5%) to alter his lysine requirement, as determined in a previous study (1). All subjects had normal sodium, potassium, calcium, phosphorous, and pH. All values reported are means ± SD.

All infants were prescribed energy intakes between 80 and 100 \( \text{kcal} \cdot \text{kg}^{-1} \cdot \text{d}^{-1} \) (36), which was in line with the clinical practice in our NICU (34). In 14 of the 15 studies, the infants had intakes within this range and met their energy requirements. In one study, the infant was prescribed an energy intake at the lower end of the range. No infant received an excess energy intake, and there was no effect of energy on \( ^{13}\text{CO}_2 \) (\( P = 0.653 \)). There was also no indication of metabolic intolerance in any of the infants. Blood values for chemical and hematological testing were in the normal clinical range for all infants (blood glucose, urea, etc.).

Variance of the hospitalized neonate’s intake (Table 3; protein 7.6%, 5% of energy) is to be expected because of the interruption of their total PN for medication and treatments. On analysis, however, there was no effect of energy (\( P = 0.6533 \)) or protein (\( P = 0.861 \)) intakes on the end product \( ^{13}\text{CO}_2 \). As expected, there was a significant effect of lysine intake on \( ^{13}\text{CO}_2 \) (\( P = 0.002 \)).

Expired carbon dioxide and urinary amino acid enrichment

Isotopic steady state (plateau) was achieved for all neonates by 12 h after the start of the isotope infusion and was defined by the absence of a significant slope between the data points at plateau. The variation in urinary \( \text{L-}[1-^{13}\text{C}] \)phenylalanine at plateau was <5% and the variation in expired \( ^{13}\text{CO}_2 \) enrichment within the plateau was <0.2%. There was no detectable \( \text{D-}[1-^{13}\text{C}] \)phenylalanine present in the PN solution and only a minimal amount (0.1%) in the isotope used in the study.

Phenylalanine kinetics

Phenylalanine flux was not affected by lysine intake. The mean (±SD) phenylalanine flux of the infants was 115.4 ± 27.7 \( \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{d}^{-1} \), and there was no significant relation between lysine intake and phenylalanine flux (\( P = 0.48 \)). There was also no evidence of tracer recycling between studies for those infants because the \( ^{13}\text{C}_{/12}\text{C} \) isotope ratio in each infant’s breath had returned to baseline when compared with the values obtained at baseline on study day 1 of their first study. In addition, for each of the 15 studies, there was no evidence of tracer recycling.
during the data collection period of the study as observed by analysis of the isotopic enrichment at steady state. This finding is supported by Sleven and Waterlow (42).

Varying the lysine intake had a significant effect on $^{13}$CO$_2$ (Figure 1). A decrease in $^{13}$CO$_2$ was observed as the lysine intake increased from 41.5 to 97.1 mg·kg$^{-1}$·d$^{-1}$. Increasing the lysine intake from 109.1 to 256.6 mg·kg$^{-1}$·d$^{-1}$ produced no further decline in $^{13}$CO$_2$. In a 2-phase linear regression crossover model, the breakpoint for $^{13}$CO$_2$ was defined as 104.9 mg·kg$^{-1}$·d$^{-1}$ ($P < 0.0001$, $r^2 = 0.84$). The 95% upper and lower CIs were found to be 120.6 and 89.1 mg·kg$^{-1}$·d$^{-1}$, respectively. The decline in $^{13}$CO$_2$ until the breakpoint indicated the responsiveness of the direct measurements of tracer in breath. The $^{13}$CO$_2$ values did not change after the breakpoint, which signified that the test amino acid (lysine) was no longer limiting protein synthesis.

Phenylalanine oxidation was also significantly affected by varying amounts of lysine intake (Figure 2). A decrease in phenylalanine oxidation was observed as the lysine amount increased from 41.5 to 110.5 mg·kg$^{-1}$·d$^{-1}$. Increasing the lysine intake from 139.4 to 256.6 mg·kg$^{-1}$·d$^{-1}$ produced no further change in phenylalanine oxidation. Similar to our method for defining the breakpoint for $^{13}$CO$_2$, we used a 2-phase linear regression crossover model. The breakpoint for phenylalanine oxidation was defined as 117.6 mg·kg$^{-1}$·d$^{-1}$ ($P < 0.0041$, $r^2 = 0.65$). The 95% upper and lower CIs were found to be 157.5 and 77.6 mg·kg$^{-1}$·d$^{-1}$, respectively.

**DISCUSSION**

This is the first study to report a mean lysine requirement in human neonates receiving PN. Using the IAAO technique and with a mean ($\pm$SD) neonatal protein intake of 3.1 ± 0.2 g·kg$^{-1}$·d$^{-1}$ and energy intake of 354.7 ± 18.0 kJ·kg$^{-1}$·d$^{-1}$ (85.5 ± 4.3 kcal·kg$^{-1}$·d$^{-1}$), we were able to define the mean lysine neonatal PN requirement as 104.9 mg·kg$^{-1}$·d$^{-1}$ (upper

<table>
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<th>Study</th>
<th>Subject</th>
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<th>Protein</th>
<th>Lipid</th>
<th>Carbohydrate</th>
<th>Energy</th>
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</tr>
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Mean $\pm$ SD — — 3.1 ± 0.2 2.7 ± 0.3 13.3 ± 1.1 354.7 ± 18.0

**FIGURE 1.** Effect of 15 increasing lysine intakes on $^{13}$CO$_2$ release ($^{13}$CO$_2$) in parenteral nutrition-fed neonates ($n = 11$) over 15 studies. Lysine intake had a significant effect on phenylalanine $^{13}$CO$_2$ ($P < 0.0001$, ANOVA). In a 2-phase linear regression crossover model, the breakpoint (BP, or mean lysine requirement) was estimated to be 104.9 mg·kg$^{-1}$·d$^{-1}$ ($r^2 = 0.84$). The upper 95% CI of the breakpoint estimate was 120.6 mg·kg$^{-1}$·d$^{-1}$, and the lower CI was 89.1 mg·kg$^{-1}$·d$^{-1}$, as indicated by the vertical dashed lines.
and lower CIs: 120.6 and 89.1 mg · kg⁻¹ · d⁻¹, respectively (17). An excess of lysine in the current PN solutions could lead to nephrotoxicity (21, 22) in the neonate who receives PN over a prolonged period of time. We recommend that the amount of lysine available in commercial PN solutions be reviewed in light of this experimentally derived requirement.

The F¹³CO₂ derived mean requirement of 104.9 mg · kg⁻¹ · d⁻¹ has been chosen over the phenylalanine oxidation mean requirement of 117.6 mg · kg⁻¹ · d⁻¹ for 2 reasons. First, we recently reported that plasma does not represent the precursor pool for phenylalanine oxidation (43). The F¹³CO₂ does not have the same limitations. Therefore, label oxidation (F¹³CO₂), which uses the end product in breath to determine oxidation, defines a more scientifically acceptable requirement than amino acid oxidation, which uses urinary enrichment to calculate the oxidation rate.

Second, the lysine estimate from F¹³CO₂ is biologically more appropriate. The current study identified the mean lysine requirements for parenterally fed human neonates as 104.9 mg · kg⁻¹ · d⁻¹. The mean lysine intake of infants who are exclusively breast fed is 119 mg · kg⁻¹ · d⁻¹ and is recommended as a lysine intake for infants up to 1 mo of age (18).

Studies suggest that the first-pass metabolism of oral lysine in the gastrointestinal tract is ≈30–42% in humans and piglets (44–47), which would indicate that the parenteral requirement should be less than the enteral requirement. The requirement of 104.9 mg · kg⁻¹ · d⁻¹, as experimentally derived in human neonates, is less than the recommended enteral intake of 119 mg · kg⁻¹ · d⁻¹, which suggests a splanchic uptake in humans of 12%.

Finally, the variability of the data also had an effect on the choice of the requirement as determined by label oxidation (F¹³CO₂). The variability of the amino acid oxidation (r² = 0.65) was significantly higher than for the label oxidation (r² = 0.84).

We used our animal model to provide us with a preliminary estimate of human neonatal lysine requirement using the IAAO technique in piglets. House et al (25) established the parenteral lysine requirements for piglets by feeding graded concentrations of lysine (400–1300 mg · kg⁻¹ · d⁻¹) and measuring the oxidation of [¹⁴C]phenylalanine. The piglets required a mean parenteral lysine intake of 790 mg · kg⁻¹ · d⁻¹. Piglets grow at a rate of ≈5 times that of human neonates; therefore, to extrapolate this finding to the requirement for human neonates, the piglet requirement (790 mg · kg⁻¹ · d⁻¹) was divided by a factor of 5, which resulted in an estimated mean requirement of 158 mg · kg⁻¹ · d⁻¹. The similarity among the predicted and measured requirements for threonine (3), methionine (2), and tyrosine (1) clearly shows that the piglet data are mostly transferable to the human infant when adjustments are made for the rapid growth of piglets. However, it appears that human requirements for lysine are proportionately less than those in the piglet.

There was no significant relation between lysine intake and phenylalanine flux (P = 0.48), which is needed for indicator oxidation to be valid (24). The lack of significant change in flux indicates reciprocal partitioning of amino acids between oxidation and protein synthesis. With the IAAO technique, when the intake is constant, the change in phenylalanine used for protein synthesis is matched by the change in phenylalanine oxidation; thus, the flux stays the same. The constant intake is maintained by providing phenylalanine, in both the test solution and the isotopic tracer, for a total of 3.7 g/100 g (111 mg · kg⁻¹ · d⁻¹).

No previous studies have determined a parenteral lysine requirement, and only one study was found that proposed an enteral lysine requirement in infants. In 1959, Snyderman et al (48) conducted a study to define an enteral lysine requirement in clinically stable human infants. The infants were 1–5 mo of age, and they used the nitrogen balance technique, growth, and total serum protein measurements to determine a requirement. They prescribed graded intakes of lysine (0, 55, 70, 75, 85, 90, 105, 205, 215, and 225 mg · kg⁻¹ · d⁻¹) for ≈1 to 3 wk per intake amount and fed from 2 to 5 intake amounts to each infant. Infants who received between 0 and 85 mg · kg⁻¹ · d⁻¹ for ≥1 wk were found to have impaired nitrogen retention, weight loss or failure to gain weight, diarrhea, and/or infections (eg, upper

FIGURE 2. Effect of 15 increasing lysine intakes on phenylalanine (Phe) oxidation in parenteral nutrition–fed neonates (n = 11) over 15 studies. Lysine intake had a significant effect on phenylalanine oxidation (P < 0.0041, ANOVA). In a 2-phase linear regression crossover model, the breakpoint (or mean lysine requirement) was estimated to be 117.6 mg · kg⁻¹ · d⁻¹ (r² = 0.65). The upper 95% CI of the breakpoint estimate was 157.5 mg · kg⁻¹ · d⁻¹, and the lower CI was 77.6 mg · kg⁻¹ · d⁻¹, as indicated by the vertical dashed lines.

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respiratory tract infection). They concluded that a range of 90 to 105 mg \( \cdot \) kg\(^{-1}\) \( \cdot \) d\(^{-1}\) was the minimum lysine enteral requirement for infants 1–6 mo of age. Their findings were used as a basis for the proposed infant lysine requirement of 103 mg \( \cdot \) kg\(^{-1}\) \( \cdot \) d\(^{-1}\) in the 1985 FAO/WHO/UNU publication Energy and Protein Requirements (49). However, the Snyderman data were not used in the determination of the infant lysine requirement by the WHO/FAO/UNU in 2007 (18), nor was it considered when establishing the 2005 Dietary Reference Intakes (50) because of the highly variable limited data set.

In conclusion, this study is the fourth in a series of neonatal parenteral amino acid requirement studies performed by our laboratory. Roberts et al (1) showed that, with the use of the dipeptide glycyl-tyrosine, the mean parenteral lysine requirement is 74 mg \( \cdot \) kg\(^{-1}\) \( \cdot \) d\(^{-1}\). Courtney-Martin et al (2) and Chapman et al (3) recently established the mean parenteral methionine requirement to be 49 mg \( \cdot \) kg\(^{-1}\) \( \cdot \) d\(^{-1}\) and the mean threonine parenteral requirement to be 33 mg \( \cdot \) kg\(^{-1}\) \( \cdot \) d\(^{-1}\) for postsurgical neonates. The current study determined the mean parenteral lysine requirement for postsurgical human neonates to be 104.9 mg \( \cdot \) kg\(^{-1}\) \( \cdot \) d\(^{-1}\), which is 32% of the lysine content of one of the commercially prepared formulations. To promote optimum metabolic and neurologic growth in the neonate, we believe that current PN solutions need to be reviewed in light of these experimentally derived requirements.

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The authors’ responsibilities were as follows—KPC: study design, subject recruitment, data collection, sample and data analysis, and manuscript writing; GC-M: sample analysis and manuscript writing; JCL, AMM, and CT: subject recruitment, data collection, sample and data analysis, and manuscript writing; none of the authors had a conflict of interest.

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