Threonine requirement of parenterally fed postsurgical human neonates\textsuperscript{1–3}

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ABSTRACT

Background: The threonine requirement of human neonates who receive parenteral nutrition (PN) has not been determined experimentally.

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

Design: Nine postsurgical neonates were randomly assigned to 16 threonine intakes ranging from 10 to 100 mg \textcdot \text{kg}^{-1} \textcdot \text{d}^{-1}. Breath and urine samples were collected at baseline and at plateau for \textsuperscript{13}CO\textsubscript{2} and amino acid enrichment, respectively. The mean threonine requirement was determined by applying a 2-phase linear regression crossover analysis to the measured rates of \textsuperscript{13}CO\textsubscript{2} release (F\textsuperscript{13}CO\textsubscript{2} and \text{L-[\textsuperscript{1-\textsuperscript{13}}C]phenylalanine oxidation.

Results: The mean threonine parenteral requirement determined by using phenylalanine oxidation was 37.6 mg \textcdot \text{kg}^{-1} \textcdot \text{d}^{-1} (upper and lower confidence limits, respectively: 29.9 and 45.2 mg \textcdot \text{kg}^{-1} \textcdot \text{d}^{-1}) and by using F\textsuperscript{13}CO\textsubscript{2} oxidation was 32.8 mg \textcdot \text{kg}^{-1} \textcdot \text{d}^{-1} (upper and lower confidence limits, respectively: 29.7 and 35.9 mg \textcdot \text{kg}^{-1} \textcdot \text{d}^{-1}). Graded intakes of threonine had no effect on phenylalanine flux.

Conclusion: This is the first study to report on the threonine requirement for human neonates receiving PN. We found that the threonine requirement for postsurgical PN-fed neonates is 22–32\% of the content of threonine that is presently found in commercial PN solutions (111–165 mg \textcdot \text{kg}^{-1} \textcdot \text{d}^{-1}). Am J Clin Nutr 2009; 89:134–41.

INTRODUCTION

Threonine is an indispensable amino acid (AA) that must come from dietary sources. It is critical in the production of mucins in the gut (1, 2) and contributes significantly to collagen, elastin, and tooth enamel formation (3–5). Too little exogenous threonine produced aberrant anorectic dietary behavior in rats and decreased bovine serum albumin antibody concentrations in piglets (6–8). Prolonged dietary excess of threonine fed to rats, however, was neurotoxic (9) or had negative behavioral consequences (9, 10).

There are no defined parenteral threonine requirements for human neonates. The amount of threonine in 3 commonly used commercial parenteral nutrition (PN) formulations is based on patterns of AAs in human breast milk, infant plasma, or human cord blood. Current neonatal PN AA solutions provide intakes of threonine between 111 and 165 mg \textcdot \text{kg}^{-1} \textcdot \text{d}^{-1} (11); these intakes are greater than an infant’s enteral intake from breast milk, which has been measured at 76 mg \textcdot \text{kg}^{-1} \textcdot \text{d}^{-1} (12). As a result, PN-fed infants receive more threonine when fed intravenously than they do when fed orally.

As early as 1986, investigators have known that the route of feeding has an impact on protein metabolism in neonates (13). Since that time, a solid body of evidence has been established in animals and humans that confirms splanchnic bed uptake and use of certain indispensable AAs during first-pass metabolism (14–16). Threonine is selectively retained (=55\% uptake by the splanchnic bed) during first-pass metabolism as shown by Bertolo et al (17) in piglets. The use of the piglet model (18) has enabled us to define AA requirements in humans. The application of data from the piglet model was first validated in neonates when the tyrosine requirement was defined in 2001 (19); more recently, the total sulfur AA requirement was determined in PN-fed human neonates (20), and this was found to agree with the data extrapolated from the piglet model (21). On the basis of our piglet data (17), we predicted that the threonine requirement of PN-fed infants would be =38 mg \textcdot \text{kg}^{-1} \textcdot \text{d}^{-1}.

Neonates may sustain serious metabolic disturbances if dietary protein is provided in excess of their needs (22). If PN-fed neonates only receive =45\% of the amount of threonine fed enterally, as shown by our piglet research, but receive the same amount parenterally, the plasma threonine concentrations will be elevated. When plasma threonine concentrations increased, formula-fed hospitalized neonates had a lower capacity to oxidize...
threonine (23). Those neonates who require PN after gastrointestinal surgery (eg, for gastrochisis) may be exposed to increased plasma concentrations of threonine for prolonged periods, which potentially leads to neurotoxicity (9, 10). Therefore, our objective was to determine the parenteral threonine requirement for human neonates using the minimally invasive indicator AA oxidation (IAAO) technique.

SUBJECTS AND METHODS

Subjects

Nine neonates (4 boys, 5 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 7 April and 22 December 2007.

Infants were included in the study if they were >35 wk gestational age, appropriate for gestational age, >1.5 kg at birth, were <28 postnatal days old, and if ≥3 d had passed since surgery. Infants who are studied ≥3 d after surgery do not differ in AA metabolism from infants who do not undergo surgery (24). The neonates were also required to be clinically stable as determined by normal blood values and vital signs. With respect to their diet, the infants had their daily enteral protein intake limited to <10% during the 48-h study while receiving a total protein intake ≥2.5 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 receiving medications that would influence protein and AA metabolism (eg, corticosteroid therapy).

Experimental design and study diet

The study design used the minimally invasive IAAO model developed in adults and applied in children (25, 26) and neonates (20). The IAAO design is based on the concept that, because AAs are not stored in the body, the intake of dietary essential AAs relative to their use for protein synthesis determines whether they are oxidized or incorporated into protein. When the intake of one indispensable AA is restricted, the other indispensable AAs are in excess and are oxidized because they cannot be incorporated into protein as was described in detail previously (27).

Sixteen studies were performed in 9 neonates, and each study took place over a 48-h period (Figure 1). During the first 24 h of the study, the adaptation day, all infants received the same PN solution. During the second 24-h phase, the test study day, infants received a PN solution with different concentrations of threonine. Nutrient intake was prescribed by the NICU physicians and dietitians according to standard clinical procedures and guidelines as recommended by the SickKids Nutrition Team (28). All infants were fed intravenously via a central line and received a fluid intake of 120–160 mL · kg⁻¹ · d⁻¹.

During the first 24 h of adaptation (study day 1), all neonates were prescribed the same commercial AA solution (Primene; Baxter Corporation, Mississauga, Canada) plus dextrose and a 20% lipid solution (Intralipid; Fresenius Kabi, Uppsala, Sweden). The purpose of study day 1 was for the infants to adapt to the solution and to ensure that all infants received appropriate amounts of protein and nonprotein energy. The PN solution used on study day 1 has an AA profile similar to cord blood and is a standard parenteral solution used routinely in our NICU. It is also widely used in Europe and produces plasma AA concentrations similar to most of the plasma AA concentrations found in breastfed infants. On study day 1, the threonine intake from the standard PN formulation was 109.3 ± 10.8 mg · kg⁻¹ · d⁻¹. Vitamins and minerals were also added to the solutions before delivery to the infant. The vitamins and minerals were supplied as a commercial solution, which consisted of a blend of water and fat-soluble vitamins prepared specifically for pediatric parenteral use (Multi-12K1; Baxter Corporation, Mississauga, Canada). The mineral solution contained calcium, phosphorus, magnesium, zinc, copper, manganese, iodine, chromium, and selenium. The vitamin and mineral contents of the solution met current hospital and American Society for Parenteral and Enteral Nutrition (29) standards. On study day 2, the second 24-h

FIGURE 1. Procedures and samples for threonine on study days 1 and 2. All arrows indicate the times of infusions. The solid circles designate the times breath samples were taken and the open circles show the times when urine samples were collected. The solid diamond specifies the time when VCO₂ was measured. The X’s indicate the times when anthropometric measurements were taken. Sol’n, solution.
period, the 9 infants were randomly assigned according to 16 dietary levels of threonine ranging from 10 to 100 mg · kg⁻¹ · d⁻¹. Our 1998 study (17) found that piglets had a mean threonine requirement of 190 mg · kg⁻¹ · d⁻¹. Because piglets grow at a rate ~5 times that of human neonates, we calculated one-fifth of this value (38 mg · kg⁻¹ · d⁻¹) and arranged our threonine intakes around the predicted breakpoint. The graded intakes of threonine delivered parenterally were as follows: 10, 15, 17, 20, 25, 30, 35, 37, 40, 50, 60, 75, 80, 90, 95, and 100 mg · kg⁻¹ · d⁻¹.

Three separate AA solutions were made for study day 2. The first solution, which contained the bulk of the AAs, was patterned on the AA profile of the solution used on study day 1 but with modifications made to ensure correct delivery of the test AA. Although the total amount of phenylalanine the infants received was 3.7 g/100 g, only 1.9 g/100 g was provided by the test solution; the remainder was given as stable isotope L-[1⁻¹⁳C]phenylalanine. The dipeptide glycyl-L-tyrosine, at 4 g/100 g AA solution, provided an excess amount of soluble tyrosine. An excess quantity of tyrosine (120 mg · kg⁻¹ · d⁻¹) was provided in the test solution to facilitate the channeling of tyrosine, which was synthesized from phenylalanine, to oxidation (30). This step increases the sensitivity of the IAAO approach. The amount of arginine was increased slightly to account for its higher parental requirement (31, 32) compared with the amount in commercial PN products. To balance the increase in nitrogen from arginine, the amount of aspartate was decreased proportionately. The AA concentrations of the parenteral solutions on days 1 and 2 are shown in Table 1.

Threonine was not added to the first solution. A second solution of threonine and sterile water was made 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 sterile and free of bacterial growth after 7 d in culture and pyrogen free after Limulus amebocyte lysate testing (33). The AA composition of the 3 solutions (Table 1) was verified by HPLC with an AA column (P580LPG; Dionex Corporation, Sunnyvale, CA). The solutions were stored at 4°C in the Research Pharmacy at the hospital until reconstituted on the study day.

All infants received an adequate protein intake (34, 35), and nonprotein nitrogen 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 or 88.3 ± 4.1 kcal · kg⁻¹ · d⁻¹ (range: 336.6–392.3 kJ · kg⁻¹ · d⁻¹ and 80.4–93.7 kcal · kg⁻¹ · d⁻¹, respectively). A protein intake of 2.7–3.5 g · kg⁻¹ · d⁻¹ resulted in nitrogen retention similar to that in the uterine environment (35). Currently, the practice in our NICU is to provide a protein intake of 2.6–3.5 g · kg⁻¹ · d⁻¹ and an energy intake of 334.9–460.5 kJ · kg⁻¹ · d⁻¹ (80–100 kcal · kg⁻¹ · d⁻¹). The PN prescribed on study day 2 brought the total amount of amino acids to 100%.

### Table 1

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<thead>
<tr>
<th>Amino acid</th>
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<th>Day 2</th>
</tr>
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<tbody>
<tr>
<td>Aspartate</td>
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</tr>
<tr>
<td>Glycine</td>
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</tr>
<tr>
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<td>L-Phenylalanine</td>
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<td>L-Isoleucine</td>
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<td>3.8</td>
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<td>L-Serine</td>
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<td>L-Taurine</td>
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<td>0.6</td>
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<td>L-Alanine</td>
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<td>L-Lysine</td>
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<td>Tyrosine</td>
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<td>0²</td>
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<td>L-Cysteine</td>
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<td>L-Ornithine</td>
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</tr>
<tr>
<td>L-Proline</td>
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</tr>
<tr>
<td>Totals</td>
<td>100.6</td>
<td>95.6</td>
</tr>
</tbody>
</table>

¹ 6.1 g Glycyl-L-tyrosine = 4 g tyrosine and 1.6 g glycine.
² Remainder of phenylalanine was used as tracer.
³ Additional alanine was added because the amount of threonine varied to keep the solution isonitrogenous.
⁴ Delivered as 13.6 g lysine HCl.
⁵ Solution on day 2 represented 95.55% of the total amino acid provided. The addition of threonine and alanine solutions to the first solution on study day 2 brought the total amount of amino acids to 100%.

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. The isotope was reconstituted in normal saline (Baxter Corporation), sterilized by passage through a 0.22-μm filter, and stored at 4°C by the Research Pharmacy at the Hospital for Sick Children. All solutions were sterile and free of bacterial growth over 7 d in culture and pyrogen free as indicated by Limulus amebocyte lysate testing. d-[1-13C]Phenylalanine in the isotope was minimally detected at 0.1% as indicated by analysis with liquid chromatography mass spectrometry/mass spectrometry with a Chirobiotic T Chiral column (Sigma Aldrich Ltd, St Louis, MO).

The intravenous isotope infusion was begun at the same time as the study day 2 PN solution was given. A priming dose of L-[1-13C]phenylalanine was delivered at 15.6 mg·kg⁻¹·d⁻¹ over a 15-min period after which the isotope was delivered at 13 mg·kg⁻¹·d⁻¹ was continuously infused for 23.75 h. The tracer dose required to achieve a measurable expired 13CO₂ was determined in previous studies (19, 20); 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 was used previously in studies of children and infants (19, 20). Isotopic steady state in the tracer enrichment at baseline and plateau was represented by the lack of difference in L-[1-13C]phenylalanine values 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 AA 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. Blood samples were monitored to provide information on the clinical status of the neonate. Breath and urine samples were collected at baseline and plateau. The infants had reached isotopic steady state by 12 h of tracer infusion. The timing for procuring samples is dependent on clinical care of the infants; collection of sufficient numbers of samples of urine can take ≤8 h. The total parenteral nutrition and tracer were started at ≈1500 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 to 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 (eg, X-rays, dressing changes, and ultrasound).

Expired carbon dioxide, which was collected by using a ventilated-hood system, was measured directly with a portable carbon dioxide analyzer (Servomex, 1400 series; Westech Industrial Ltd, Mississauga, Canada) and a mass flow meter (5860 series; Brooks Trillium Measurement and Control, Stouffville, Canada). Infants slept under a clear plastic hood through which room air flowed at ≈1–2 L·kg⁻¹·min⁻¹. The carbon dioxide concentration in the hood was monitored closely and kept between 0.35% and 0.45% to minimize the variability in the ratio of 13CO₂ to 12CO₂ during measurements. Three to 5 baseline breath samples were obtained before the start of the isotope infusion, and a second set of samples was collected after 12 h of L-[1-13C]phenylalanine infusion. Each sample was collected over a 10-min period by bubbling the carbon dioxide effluent into a reflux condenser with 10 mL NaOH. The mixture of carbon dioxide and sodium hydroxide formed sodium bicarbonate, which was injected into evacuated tubes (Becton Dickinson and Co, Franklin Lakes, NJ) and stored at −20°C until analyzed.

Previously, Bross et al (39) reported that urinary and plasma enrichments of L-[1-13C]phenylalanine are highly correlated at isotopic steady state. To keep the study minimally invasive for this vulnerable pediatric population, urine rather than plasma was collected for measuring AA enrichment and flux. Urine samples were collected by placing sterile rayon balls (Dunmex Medical, Marietta, GA) on the inside of the diapers on both study days 1 and 2 for the measurement of AA enrichment. Three to 5 baseline urine samples were collected before the start of the isotope infusion, and a second set of urine samples was collected after 12 h of the start of the isotope infusion. The urine was stored at −20°C until analyzed.

The enrichment of L-[1-13C]phenylalanine in urine was analyzed with a triple quadrupole mass analyzer (API 4000; Applied Biosystems/MDI Sciex, Concord, Canada), which was coupled to an Agilent 1100 HPLC system (Agilent, Mississauga, Canada) as previously described (40). A Chirobiotic T Chiral column (Sigma Aldrich Ltd) was used to separate the d-[1-13C]phenylalanine from the L-[1-13C]phenylalanine. Selected ion chromatograms were obtained by monitoring the mass-to-charge ratios of the product ions of 165 and 166 for [1-13C]phenylalanine corresponding to the unenriched (M) and enriched (M+1) peaks, respectively. Isotopic enrichment was expressed as mole percent excess and was calculated from peak area ratios at isotopic steady state at baseline and at plateau.

The liberation of carbon dioxide from sodium bicarbonate by combining 200 μL of the sodium bicarbonate sample with 200 μL phosphoric acid in an evacuated tube preceded the breath 13C analysis. Breath 13C enrichment was measured by continuous-flow isotope ratio mass spectrometry (CF-IRMS20/20 isotope analyzer; PDZ Europa Ltd, Cheshire, United Kingdom). Enrichments were expressed as APE and compared with a reference standard of compressed carbon dioxide gas.

Statistical analysis

The effect of threonine intake on phenylalanine flux, oxidation, and F13CO₂ was tested by using analysis of variance with the PROC GLM procedure (SAS version 9.1; SAS Institute Inc, Cary, NC). Estimates of the mean threonine intake were derived by breakpoint analysis of the rate of release of 13CO₂ (F13CO₂) data with the use of a 2-phase linear regression crossover model, as described previously (41, 42). The breakpoint was calculated by using the mixed models and regression procedure of SAS, and the slope of the second line was not significantly different from zero. Statistical significance was established at P ≤ 0.05. Regression analysis variables were threonine intake as the independent variable and F13CO₂ and phenylalanine oxidation as dependent variables. Selection of the best model was determined by factors relating to fit (significance of the model and r²) and estimates of variation about the model (CV and SE of the estimate). The population Recommended Dietary Allowance was...
TABLE 2
Characteristics of parenterally fed neonates who received varying intakes of threonine

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
<th>Range</th>
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<tbody>
<tr>
<td>Gestational age (wk)</td>
<td>37.6 ± 1.2</td>
<td>35–40</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>2.8 ± 0.3</td>
<td>2.4–3.5</td>
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<tr>
<td>Study weight (kg)</td>
<td>2.9 ± 0.3</td>
<td>2.6–3.5</td>
</tr>
<tr>
<td>Age on study day 2 (d)</td>
<td>16 ± 6</td>
<td>9.0–27</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>48.5 ± 2.2</td>
<td>45–52</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>32.9 ± 1.06</td>
<td>32–36</td>
</tr>
</tbody>
</table>

1 Indicates that birth weight was regained or surpassed by time of isotopic infusion.
2 \(n = 9\) neonates over 16 studies: gastroschisis (7), ileal atresia (6); Hirschsprung disease (n = 1).

Estimated by determining the upper 95% confidence limits of the breakpoint estimate (42).

RESULTS

Clinical characteristics and nutrient intake

Clinical characteristics and diagnoses for the subjects across 16 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 2–3 d after surgery, and if little or no edema was present as assessed by the nurse practitioner or registered nurse. Weights and other study parameters changed over the study periods for those infants who were tested at 2 levels of threonine intake; 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 356.9 ± 17.7 kJ · kg⁻¹ · d⁻¹ (85.4 ± 4.2 kcal · kg⁻¹ · d⁻¹) and 2.9 ± 0.3 g · kg⁻¹ · d⁻¹, respectively. Average intakes of the carbohydrates and lipids provided were 12.8 ± 1.1 and 3.0 ± 0.2 g · kg⁻¹ · d⁻¹, respectively. In 8 of the 16 studies, the neonates were “nil per os” and had no enteral intake. Enteral threonine intakes in 8 infants who were fed expressed breast milk or formula were not sufficient to alter their requirement. All subjects had normal concentrations of sodium, potassium, calcium, and phosphorous and normal pH levels. All values reported are means ± SDs.

In adherence to clinical practice in our NICU (28), all infants were prescribed energy intakes of between 100 and 80 kcal · kg⁻¹ · d⁻¹ (35). In 14 of the 16 studies, the infants had intakes within this range and met their energy requirements. In 2 studies the infants were prescribed energy intakes at the lower end of the range. No infant received an excess energy intake. There was no indication of metabolic intolerance in any of the infants. Blood values for chemical and hematologic testing were in the normal clinical range for all infants (eg, blood glucose and urea). The effect of energy intake on the requirement was optimized by delivering appropriate energy intakes that were well tolerated by the neonates.

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 \(^{13}C\)-[1]phenylalanine at plateau was <9%, and the variation in expired \(^{13}CO_2\) enrichment within the plateau was <0.2%. There was no detectable \(d^{-13}C\)-phenylalanine present in the PN solution and only minimal detection (0.1%) in the isotope used in the study.

Phenylalanine kinetics

Phenylalanine flux was not affected by threonine intake. The phenylalanine flux of the infants was 118.6 ± 22.9 \(\mu\)mol · kg⁻¹ · d⁻¹, and there was no significant relation between threonine

TABLE 3
Intakes in subjects on study day 2

<table>
<thead>
<tr>
<th>Study</th>
<th>Subject</th>
<th>Threonine</th>
<th>Amino acid</th>
<th>Lipid</th>
<th>Carbohydrate</th>
<th>Energy</th>
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<tr>
<td></td>
<td></td>
<td>mg · kg⁻¹ · d⁻¹</td>
<td>g · kg⁻¹ · d⁻¹</td>
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intake and phenylalanine flux (P = 0.08). Phenylalanine oxidation was significantly affected by varying threonine intakes (Figure 2). A decrease in phenylalanine oxidation was observed as the threonine intake increased from 9.5 to 36.8 mg·kg⁻¹·d⁻¹. Increasing the threonine intake from 39 to 98.0 mg·kg⁻¹·d⁻¹ produced no further change in phenylalanine oxidation. In a 2-phase linear regression crossover model, the breakpoint for phenylalanine oxidation was defined as 37.6 mg·kg⁻¹·d⁻¹ (P < 0.0001, r² = 0.79). The 95% upper and lower confidence limits were 45.2 and 29.9 mg·kg⁻¹·d⁻¹, respectively.

Varying the threonine intake also had a significant effect on F₁³CO₂ (Figure 3). A decrease in F₁³CO₂ was observed as the threonine intake increased from 9.5 to 29.2 mg·kg⁻¹·d⁻¹. Increasing the threonine intake from 34.5 to 98.0 mg·kg⁻¹·d⁻¹ produced no further decline in F₁³CO₂. Similar to our method for defining the breakpoint for phenylalanine oxidation, we used a 2-phase linear regression crossover model; the breakpoint for F₁³CO₂ was defined as 32.8 mg·kg⁻¹·d⁻¹ (P < 0.0001, r² = 0.93). The 95% upper and lower confidence limits were 35.9 and 29.7 mg·kg⁻¹·d⁻¹, respectively. The decline in F₁³CO₂ until the breakpoint indicated the responsiveness of the direct measurements of tracer in breath. The F₁³CO₂ values did not change after the breakpoint, which signified that the test AA (threonine) was no longer limiting protein synthesis.

**DISCUSSION**

To our knowledge, this is the first study to report a mean threonine requirement in human neonates receiving PN. Using the IAAO technique, we were able to define the mean threonine neonatal PN requirement as 32.8 mg·kg⁻¹·d⁻¹ (upper and lower confidence limits, respectively: 29.9 and 35.9 mg·kg⁻¹·d⁻¹). Given a normal distribution, this mean estimate would meet the needs of only 50% of the neonatal population (equivalent to the estimated average requirement). On the basis of the upper 95% confidence limit of the breakpoint estimate, the requirement for this population is 35.9 mg·kg⁻¹·d⁻¹ (equivalent to the Recommended Dietary Allowance). These data show that the threonine requirement (35.9 mg·kg⁻¹·d⁻¹) for post-surgical PN-fed neonates is only 22–32% of the amount of threonine currently in PN solutions (111–165 mg·kg⁻¹·d⁻¹) (11). An excess of threonine in the current PN solutions could increase neurotoxicity (9) or cause behavioral anomalies (10) in neonates who receive PN over a prolonged period of time. We recommend that the amount of threonine available in commercial PN solutions be amended in light of this experimentally derived requirement.

A preliminary estimate of the human neonatal threonine requirement was derived from the use of the IAAO technique in piglets. Bertolo et al (17) established the parenteral threonine requirements for piglets by feeding graded concentrations of threonine (50–800 mg·kg⁻¹·d⁻¹) and measuring the oxidation of [¹⁴C]phenylalanine. The piglets required a mean parenteral threonine intake of 190 mg·kg⁻¹·d⁻¹. Piglets grow at a rate =5 times that of human neonates; therefore, to extrapolate this finding to the requirement for human neonates, the piglet requirement (190 mg·kg⁻¹·d⁻¹) was divided by a factor of 5, which resulted in an estimated mean requirement of 38 mg·kg⁻¹·d⁻¹. The current study identified the mean threonine requirements for parenterally fed human neonates as 32.8 mg·kg⁻¹·d⁻¹, which is comparable with the predicted estimate. The similarity among the predicted and measured requirements for threonine (present study), methionine (20), and tyrosine (19) clearly shows that the piglet data are transferable to human infants when adjustments are made for the rapid growth of piglets.

In this study the values derived from the F₁³CO₂ data are believed to more accurately represent the threonine requirement, although we present both AA (phenylalanine) oxidation and label (F₁³CO₂) oxidation. The phenylalanine oxidation data provide a slightly higher mean threonine requirement (37.6 mg·kg⁻¹·d⁻¹; upper and lower confidence limits, respectively: 29.9 and 45.2 mg·kg⁻¹·d⁻¹), which may be a result of a greater variability in the data. The variance is significantly higher for AA oxidation (r² = 0.79) than for the label oxidation (r² = 0.93) as shown in Figure 3. In addition, we recently reported that the oxidation based on plasma measurements of enrichment do not truly reflect the first step in phenylalanine oxidation, namely...
phenylalanine hydroxylation (43). The study, carried out in healthy young adults, determined that the breakpoint for IAAO measured by using $\text{F}^{3}\text{C}_{2}\text{O}_{2}$ was similar to the breakpoint for phenylalanine hydroxylation measured by using apolipoprotein B-100, a hepatic export protein that is synthesized from intrahepatocyte AAs. AA oxidation measurements using plasma enrichments may be unreliable because plasma is not the true precursor pool from which protein synthesis takes place. $\text{F}^{3}\text{C}_{2}\text{O}_{2}$ is more representative in studies in which relative rates are being compared, as was the case in the present study. We have also shown (44) that $\text{F}^{3}\text{C}_{2}\text{O}_{2}$ is not affected by route of tracer administration (intravenous compared with oral). Therefore, label oxidation ($\text{F}^{3}\text{C}_{2}\text{O}_{2}$) provides more scientifically justifiable results than does AA oxidation, which uses plasma enrichment to calculate oxidation rate.

Phenylalanine flux was not affected by threonine intake, and there was no significant relation between threonine intake and phenylalanine flux ($P = 0.08$). The lack of significant change in flux indicates balanced partitioning of AAs 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.

No previous studies have determined a parenteral threonine requirement, and only one study proposed an enteral threonine requirement in infants. Pratt et al (45) attempted to define an enteral threonine requirement in healthy human infants using the nitrogen balance technique and the measurement of growth and total serum protein. They fed infants graded intakes of threonine (0, 30, 45, 47, 48, 50, 70, 173, and 180 mg $\cdot$ kg$^{-1}$ $\cdot$ d$^{-1}$) for a period of $\approx 1$ wk per intake amount and fed from 2 to 5 intake amounts to each infant. Infants who received 0 mg $\cdot$ kg$^{-1}$ $\cdot$ d$^{-1}$ for 1 wk had impaired nitrogen retention, failed to gain weight, had increased loss of nitrogen in the urine, and experienced glossitis and a reddening of the buccal mucosa. They concluded that 60 mg $\cdot$ kg$^{-1}$ $\cdot$ d$^{-1}$ was the minimum requirement for infants aged 1–6 mo. Their findings were used as a basis for the proposed infant threonine requirement of 87 mg $\cdot$ kg$^{-1}$ $\cdot$ d$^{-1}$ in the 1985 Energy and Protein Requirements publication by the Food and Agriculture Organization, the World Health Organization, and the United Nations University (FAO/WHO/UNU) (46). However, the Pratt data were not used in the determination of the threonine requirement in infants by the FAO/WHO/UNU in 2007 (12) nor were they considered when establishing the 2005 Dietary Reference Intakes (47) because of the limited, highly variable data set.

In our study of threonine requirements of piglets, an important finding was that 55% of enteral threonine fed to the piglet was retained by the splanchnic bed (17). As yet, we have not experimentally identified an enteral requirement in human neonates. We do know, however, that a 1-mo-old infant who is breastfed has a mean enteral threonine intake of 76 mg $\cdot$ kg$^{-1}$ $\cdot$ d$^{-1}$, which is calculated from the AA content of proteins in breast milk (12). Our mean parenteral estimate of 32.8 mg $\cdot$ kg$^{-1}$ $\cdot$ d$^{-1}$ is 43% of the enteral threonine intake in healthy growing infants. This relation, in which the PN requirement is 43% of the enteral requirement, corroborates the similar finding (45%) in neonatal piglets (17). Law et al (2) reported that the proportion of enterally fed threonine taken up by the gut (≈55%) is used for the production of mucin in piglets. They found that a substantial and constant supply of threonine was critical in mucin synthesis and was necessary to maintain gut function and structure. In a study with human neonates, van der Schoor et al (14) reported that splanchnic tissues extract a large amount of enteral threonine intake, which infers a high visceral need for threonine. It may be assumed, given the current findings in humans and piglets, that the human infant has the same or similar enteral requirements for mucin production as does the piglet. We are probably overfeeding many AAs to neonates parenterally because intakes have been primarily patterned after adequate oral intakes.

In conclusion, this study is the third in a series of neonatal parenteral AA requirement studies performed by our laboratory to determine whether our findings in the neonatal piglet can be applied to the human infant. In each of the 3 studies, the human estimate has approximated the values predicted from the piglet model. Roberts et al (19) showed that glycyll-L-tyrosine is an effective means of providing the mean parenteral tyrosine requirement (74 mg $\cdot$ kg$^{-1}$ $\cdot$ d$^{-1}$). Courtney-Martin et al (20) recently established the mean parenteral methionine requirement (49 mg $\cdot$ kg$^{-1}$ $\cdot$ d$^{-1}$) for postsurgical neonates. The current study determined the mean parenteral threonine requirement for postsurgical human neonates to be 32.8 mg $\cdot$ kg$^{-1}$ $\cdot$ d$^{-1}$ (upper and lower confidence limits, respectively: 29.7 and 35.9 mg $\cdot$ kg$^{-1}$ $\cdot$ d$^{-1}$). To promote optimum metabolic and neurologic growth in neonates, we believe that current parenteral solutions need to be revised to incorporate the population-safe requirements of the 3 indispensable AAs as shown by our experiments.

We are grateful for the interest, support, and participation of the families in this study; their cooperation was invaluable. We especially thank Jacob Langer, Nicole de Silva, and all of the doctors and nurses in the NICU at the Hospital for Sick Children for their help with patient recruitment and data collection. We are indebted to Mark Bedford and the TPN Research Pharmacy at the Hospital for Sick Children for their expertise in PN solution design and production.

The authors’ responsibilities were as follows—KPC: study design, subject recruitment, data collection, sample and data analysis, and manuscript writing; GC-M: data collection and manuscript writing; AMM: subject recruitment and NICU study setup; ROB and PBP: study design, data analysis, and manuscript writing. The authors had no conflict of interest.

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