The gut takes nearly all: threonine kinetics in infants[1–3]

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ABSTRACT

Background: Threonine is an essential amino acid that is abundantly present in intestinally produced glycoproteins. Animal studies show that intestinal first-pass threonine metabolism is high, particularly during a restricted enteral protein intake.

Objective: The objective of the study was to quantify intestinal first-pass threonine metabolism in preterm infants during full enteral feeding and during restricted enteral intake.

Design: Eight preterm infants ($\bar{x}$ ± SD birth weight: 1.1 ± 0.1 kg; gestational age: 29 ± 2 wk) were studied during 2 periods. During period A, 40% of total intake was administered enterally and 60% was administered parenterally. Total threonine intake was 58 ± 6 μmol · kg^{-1} · h^{-1}. During period B, the infants received full enteral feeding, and the total threonine intake was 63 ± 6 μmol · kg^{-1} · h^{-1}. Dual stable-isotope tracer techniques were used to assess splanchnic and whole-body threonine kinetics.

Results: The fractional first-pass threonine uptake by the intestine was remarkably high in both periods: 82 ± 6% during partial enteral feeding and 70 ± 6% during full enteral feeding. Net threonine retention was not affected by the route of feeding.

Conclusion: In preterm infants, the splanchnic tissues extract a very large amount of the dietary threonine intake, which indicates a high obligatory visceral need for threonine, presumably for the purposes of synthesis. *Am J Clin Nutr* 2007;86:1132–8.

KEY WORDS Threonine, preterm infants, intestine, stable isotopes, nutrition, splanchnic metabolism

INTRODUCTION

During the first few weeks of life, preterm infants are faced with the challenge of doubling their body weight (1). The high growth rate of the newborn infant puts significant pressure on the intestine to efficiently digest and absorb nutrients. This occurs at a time when the neonatal intestine is adapting to the enteral route of nutrition after a prenatal period in which amino acids (AAs) were delivered via the umbilical route. Therefore, it may be speculated that a large quantity of AAs is needed for the growth and maintenance of the premature gut to enable its optimal function and integrity.

Given the key role of the gut in the maintenance of neonatal health, there has been considerable interest in the significance of first-pass intestinal metabolism of dietary AAs (2–4). Enterally absorbed AAs can be used for incorporation into mucosal cellular proteins, for energy production, or for conversion via transamination into other AAs, metabolic substrates, and biosynthetic intermediates. It is known that, in animals, <20% of intestinal AAs are used for constitutive gut growth by the intestinal mucosa (1), and, although some essential AAs (EAAs) are known to be catabolized (3–5), the catabolism of EAAs does not account for their high utilization rate. Therefore, the synthesis of secretory glycoproteins by the enterocytes appears to be a major metabolic fate for EAAs.

Of particular interest is threonine, which is the AA that, in neonatal piglets, is used to the greatest extent by the portal-drained viscera (PDV)—ie, the intestines, pancreas, spleen, and stomach. In neonatal pigs, the splanchnic extraction of threonine ranges from 60% to 80% of the dietary intake (2, 3, 6, 7). This high intestinal requirement for threonine may reflect the use of enterally absorbed threonine for the synthesis of secretory glycoproteins as the major metabolic fate. Indeed, Roberton et al (8) found that the protein cores of secretory mucins contain large amounts of threonine. In addition, Bertolo et al (9) showed that the whole-body threonine requirement in total parenteral nutrition (TPN)–fed piglets is 40% of that observed in enterally fed piglets, which indicates that enteral nutrition itself induces metabolic processes that demand threonine, probably within the intestine. One may postulate that the compromised gut barrier function associated in humans with parenteral nutrition is caused by a sparse threonine availability combined with diminished intestinal mucin production, as has been shown in rats (10).

In view of the central role of the gut in nutrient processing and metabolism, we considered it important to investigate the effect of the amount of enteral intake on splanchnic and whole-body threonine metabolism in preterm infants. Accordingly, we simultaneously used 2 stable isotope–labeled threonine tracers—[U-^{13}C]threonine and [^{15}N]threonine—and administered them via intravenous and intragastric routes to determine the quantitative aspects of threonine metabolism in preterm neonates under both parenteral and enteral feeding conditions. This technique enabled us to measure both the first-pass intestinal threonine uptake and the whole-body threonine kinetics. Previously, our group (7) found in neonatal pigs that the considerable first-pass threonine utilization was not significantly affected by a lower

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TABLE 1
Subject characteristics[1]

Patient	GA	Sex	Birth weight	CRIB score[2]	Study weight		Postnatal age	
					Period A	Period B	Period A	Period B
	wk		*kg*		*kg*		*d*	
1	26	F	0.97	2	1.00	1.05	9	12
2	27	F	1.08	2	1.03	1.04	8	17
3	32	M	1.28	2	1.18	1.17	5	9
4	28	F	0.92	2	1.00	1.00	9	11
5	31	M	0.90	1	0.91	0.91	6	8
6	29	M	1.25	2	1.12	1.22	5	7
7	30	M	1.10	2	0.99	1.06	6	11
8	30	F	0.98	1	0.92	0.98	10	13
Mean ± SD	29 ± 2		1.06 ± 0.14	2 ± 0.5	1.02 ± 0.09	1.05 ± 0.10	7 ± 2	11 ± 3

[1] GA, gestational age; CRIB, Clinical Risk Index for Babies. $n = 8$.
[2] Maximum score, 23; minimum score, 0; with increasing scores, there is increased morbidity and mortality.

protein intake. Therefore, we hypothesized that the first-pass utilization of threonine by the splanchnic tissues would be substantial in preterm infants and would be independent of the dietary threonine intake. Hence, the present study explores the effect of the amount of enteral formula intake on various components of first-pass and whole-body threonine metabolism in neonates.

SUBJECTS AND METHODS

Subjects

Splanchnic and whole-body threonine kinetics were quantified in 8 preterm infants during 2 consecutive periods of different enteral and parenteral intakes. Patients eligible for this study were premature infants with a birth weight of 750 to 1250 g that was appropriate for gestational age according to the charts of Usher and McLean (11). Excluded from the study were infants who had major congenital anomalies or gastrointestinal or liver diseases. All infants' Clinical Risk Index for Babies (CRIB; 12) scores on the first day of life were <5. The maximum CRIB score is 23, and the minimum score is 0 (with increasing scores, there is increased morbidity and mortality). Selected relevant clinical variables for the infants studied are shown in **Table 1**. The infants received a standard nutrient regimen according to our feeding protocol: a combination of the mother's milk or formula (Nenatal; Nutricia, Zoetermeer, Netherlands; 0.024 g/mL protein) and parenteral nutrition containing glucose, AAs (Primene 10%; Clintec Benelux NV, Brussels, Belgium; 0.1 g/mL protein), and lipids (Intralipid 20%; Fresensius Kabi, Den Bosch, Netherlands). Formula feeding was given as the sole enteral nutrition 12 h before the start of the study and during the study days.

Written informed consent was obtained from the parents of the infants. The study protocol was approved by the Institutional Review Board of the Erasmus Medical Center.

Protocol

The study design consisted of 2 periods of one study day (period A: study day 1; period B: study day 2). During period A, the infants received 40% enteral feeding and 60% parenteral feeding; during period B, they received full enteral feeding. A schematic outline of the tracer-infusion studies is shown in **Figure 1**. During period A, both an arterial and an intravenous catheter were implanted in the infants for the infusion of tracers and withdrawal of blood samples. These catheters were already installed for clinical purposes. During period B, a peripheral intravenous catheter was available for the infusion of tracers, and blood samples were collected by heelstick.

To collect breath samples from these preterm infants, we employed the method described by Perman et al (13), which used a nasal tube. This method has been validated in preterm infants for the collection of expiratory carbon dioxide after the administration of ^{13}C-labeled substrates (14, 15). Briefly, a 6-Fr gastric tube (6 Ch Argyle; Sherwood Medical, Tullamore, Ireland) was carefully

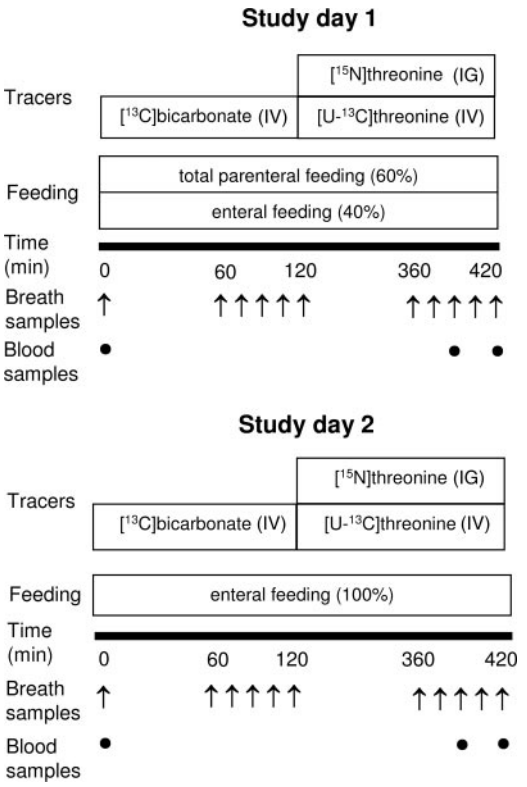

FIGURE 1. Schematic overview of study periods A (study day 1) and B (study day 2). IG, intragastric; IV, intravenous.

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placed 1.0 cm into the nasopharynx, and a 15-mL sample of end-tidal breath was slowly taken with a syringe. Duplicate aliquots of expired air from each sampling point were stored in evacuated tubes (Vacutainer; Becton Dickinson, Rutherford, NJ) for later analysis.

Three stable-isotope infusions were performed on each study day during both periods. First, a primed, continuous infusion [10.02 μmol/kg priming dose and 10.02 μmol/(kg · h) of [^{13}C]sodium bicarbonate (99 mol% ^{13}C; Cambridge Isotopes, Woburn, MA) dissolved in sterile saline was administered at a constant rate for 2 h. The ^{13}C-labeled bicarbonate infusion was immediately followed by primed, continuous infusion [14.4 μmol/kg priming dose and 14.4 μmol/(kg · h) of [U-^{13}C]threonine (97 mol% ^{13}C; Cambridge Isotopes) given intravenously and a second primed, continuous infusion [14.7 μmol/kg priming dose and 14.7 μmol/(kg · h) of [^{15}N]threonine (95 mol% ^{15}N; Cambridge Isotopes) given enterally for 5 h. This process was designed to assess whole-body and splanchnic threonine kinetics. All isotopes were tested and found to be sterile and pyrogen-free before they were used in our studies. Baseline blood and breath samples were collected at time 0. During the last hour of each tracer infusion, breath samples were collected at 15-min intervals, and blood samples were obtained at 390 and 420 min. The total amount of blood drawn on a study day was 1.5 mL, which is <2% of the blood volume of a 1000-g infant. Blood was centrifuged immediately (2500 × *g*, 4 °C, 10 min) and stored at −70 °C for further analysis.

Analytic methods

Small aliquots of plasma (100 μL) were taken for the measurement of AA concentrations by using an analyzer (Amino Acid Analyser Biochrom 20; Biochrom Ltd, Cambridge, United Kingdom). Plasma threonine enrichments were determined by gas chromatography–mass spectrometry. Briefly, 50 μL plasma was deproteinized with 50 μL of 0.24 mol sulfosalicylic acid/L. After centrifugation for 8 min at 4 °C and 14 000 × *g*, the supernatant was passed through an H^+ column (AG50W-X8; Biorad, Richmond, VA). The column was washed with 3 mL water, and the AAs were eluted with 1.5 mL of 3 mol NH_4OH/L. The eluate was dried at 70 °C under a stream of nitrogen, and, finally, derivatives of the AAs were formed by adding 350 μL acetonitril and 10 μL *N*-methyl-*N*-(*tert*-butyldimethylsilyl)-trifluoroacetamide (Pierce Omnilabo, Breda, Netherlands) to the dried AAs (16). Analyses were carried out on a Carlo Erba GC8000 gas chromatograph coupled to a Fisons MD800 mass spectrometer (both: Interscience BV, Breda, Netherlands) by injecting 1 μL with a split ratio of 50:1 on a 25-m × 0.22-mm fused silica capillary column, coated with 0.11 μm HT5 (SGE, Victoria, Australia). Natural threonine, [^{15}N]-threonine, and [U-^{13}C]-threonine were measured by selective ion monitoring of masses 404, 405, and 408, respectively (16). Breath samples were analyzed for enrichment of $^{13}CO_2$ on an isotope ratio–mass spectrometer (ABCA; Europa Scientific, Van Loenen Instruments, Leiden, Netherlands) (17).

Calculations

The rate of threonine turnover was calculated by measuring the tracer dilution at steady state as modified for stable isotope tracers, as previously described (18, 19). Plasma enrichments of threonine were used to calculate the rate of threonine turnover.

The threonine flux (intravenous or intragastric) was calculated according to the following equation:

$$Q_{\text{iv or ig}} = i_T \times [(E_i/E_p) - 1] \quad (1)$$

where $Q_{\text{iv or ig}}$ is the flux of the intravenous or intragastric threonine tracer [μmol/(kg/h)], i_T is the threonine infusion rate [μmol/(kg/h)], and E_i and E_p are the enrichments [mol percent excess (MPE)] of [U-^{13}C or ^{15}N]threonine in the threonine infusate and in plasma at steady state, respectively.

The first-pass threonine uptake was calculated according to the following equation:

$$U = [(Q_{\text{ig}} - Q_{\text{iv}})/Q_{\text{ig}}] \times I \quad (2)$$

where U is the first-pass threonine uptake, Q_{ig} is the flux of the intragastric threonine tracer, and I is the enteral threonine intake [μmol/(kg/h) for all].

In a steady state, the amount of threonine entering the plasma pool should be equal to the amount of threonine leaving the pool. Threonine can enter the pool either by being released from proteins as the result of breakdown or through the diet. Threonine may leave the pool through either oxidative disposal or nonoxidative disposal (threonine used for synthesis). To calculate the amount of threonine leaving the pool, we used the following equation:

$$Q = I + \text{TRP} = \text{Ox} + \text{NOTD} \quad (3)$$

where TRP is the amount of threonine released from protein via protein breakdown [μmol/(kg/h)], Ox is the rate of threonine oxidation [μmol/(kg/h)], and NOTD is the rate of nonoxidative disposal of threonine [a measure of protein synthesis rate, expressed as μmol/(kg/h)].

Net threonine balance, an index of protein deposition, was calculated by using the following equation:

$$\text{TBAL} = \text{NOTD} - \text{TRP} \quad (4)$$

where TBAL is threonine balance [μmol/(kg/h)].

Whole-body carbon dioxide production was estimated by using the following equation:

$$\text{Body } CO_2 \text{ production} = i_B \times [(E_{\text{iB}}/\text{breath IE}_B) - 1] \quad (5)$$

where i_B is the infusion rate of $NaH^{13}CO_3$ [μmol/(kg/h)], E_{iB} is the enrichment (MPE) of [^{13}C]bicarbonate in the bicarbonate infusate, and IE_B is the breath $^{13}CO_2$ enrichment at plateau during the $NaH^{13}CO_3$ infusion (MPE).

As described previously, threonine oxidation was calculated by multiplying the recovery of the [^{13}C]label in the expiratory air with the rate of appearance of threonine (20). The fraction of threonine oxidized was measured according to the following equation, assuming a constant rate of CO_2 production during the study, which lasted 5 h (20):

$$\text{Fraction of threonine oxidized to } CO_2 = [\text{IE}_T \times i_B]/[\text{IE}_B \times i_T \times 4] \quad (6)$$

where IE_T and IE_B are the $^{13}CO_2$ breath enrichments (MPE) at steady state during the intravenous [U-^{13}C]threonine infusion and $NaH^{13}CO_3$ infusion. The denominator is multiplied by a

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TABLE 2
Intakes of threonine, protein, carbohydrate, fat, and energy during period A and period B[1]

	Period A	Period B
Total threonine intake [μmol/(kg/h)]	58 ± 5[2]	63 ± 6
Enteral	26 ± 3	63 ± 6
Parenteral	32 ± 3	—
Total protein intake [g/(kg/d)]	3.6 ± 0.4[2]	2.8 ± 0.2
Enteral	1.1 ± 0.1	2.8 ± 0.2
Parenteral	2.5 ± 0.2	—
Total carbohydrate intake [g/(kg/d)]	11.0 ± 1.8	12.4 ± 1.7
Enteral	3.6 ± 0.4	8.9 ± 0.8
Parenteral	7.6 ± 1.7	3.4 ± 2.4
Total fat intake [g/(kg/d)]	4.6 ± 0.5	5.0 ± 0.5
Enteral	2.1 ± 0.2	5.0 ± 0.5
Parenteral	2.5 ± 0.5	—
Total energy intake [kcal/(kg/d)]	98 ± 8	106 ± 4
Enteral	38 ± 4	92 ± 9
Parenteral	61 ± 9	14 ± 10

[1] All values are $\bar{x}$ ± SD. $n = 8$.
[2] Significant difference between periods, $P < 0.05$.

factor of 4 to account for the number of C-atoms that are labeled.

Whole-body threonine oxidation was then calculated by using the following equation:

Whole-body threonine oxidation

= the product of equation *6* × the product of equation *1*

(*7*)

Statistical analysis

The data are expressed as the mean ± SD values obtained from samples taken over the last hour of each tracer infusion. We used SPSS statistical software (version 14.0; SPSS Inc, Chicago, IL) to analyze the data. Statistical comparisons were performed by using a paired Student's *t* test. $P < 0.05$ was taken as statistically significant.

RESULTS

All infants were appropriate for gestational age (mean gestational age: 29 ± 2 wk; Table 1). Seven patients were studied during both periods; in 5 of those infants, whole-body threonine oxidation was determined. During both feeding periods, 1 infant underwent mechanical ventilation, 4 infants received supplemental oxygen via a nasal prong, and 3 infants received nasal continuous positive airway pressure (CPAP), which did not allow us to obtain expired air. All infants received caffeine and were clinically stable at the time of the study. All routine blood chemistry and hematology tests (ie, electrolytes, calcium, glucose, acid base, hematocrit, thrombocyte count, and white blood cell count) were within normal limits; there were no significant changes in these variables in the periods 24 h before and after the study. Intakes of threonine, protein, carbohydrate, fat, and energy are shown in **Table 2**. We aimed to keep the different macronutrient intakes very close together during both study periods, but this was impossible because different feeds (parenteral nutrition and preterm formula) were used. No differences were found in the intakes of glucose, fat, or energy. The threonine intake was significantly lower during period A, but, in absolute amounts, the difference was only 5 μmol/(kg/d) (ie, 8% less). The protein intake during period B was significantly lower than that during period A, which was inevitable, because we aimed at comparable total threonine intakes.

Isotopic plateau

Threonine kinetics were calculated from the plateau enrichment values for plasma threonine, breath carbon dioxide, and rates of carbon dioxide production. Details of the isotopic enrichments of plasma threonine at baseline and plateau during periods A and B are given in **Figure 2**.

The background (baseline) recovery of the [^{13}C]label in expiratory air did not differ significantly between the 2 periods [period A: 1.0955 ± 0.0079 atom percent excess (APE); period B: 1.0923 ± 0.0032 APE]. The $^{13}CO_2$ enrichment in breath during [^{13}C]sodium bicarbonate infusion rose rapidly during the first hour of infusion in both periods to become constant in all infants by 120 min, with <5% variation of the plateau (CV: 1.4 ± 0.4% in period A and 3.3 ± 1.4% in period B). In **Figure 3**, the isotopic steady state of $^{13}CO_2$ excretion in expiratory air is shown both during the [^{13}C]sodium bicarbonate infusion and the [U-^{13}C]threonine infusion. Although we took 2 blood samples after 4 h of tracer infusion, we are sure that isotopic steady state was reached during the [U-^{13}C]threonine infusion because we found an isotopic plateau in carbon dioxide excretion. Before a plateau in breath is reached, a plateau has to be reached at the site of threonine oxidation—ie, intracellularly. The mean ± SD CVs of breath [^{13}C]threonine enrichment above baseline at plateau

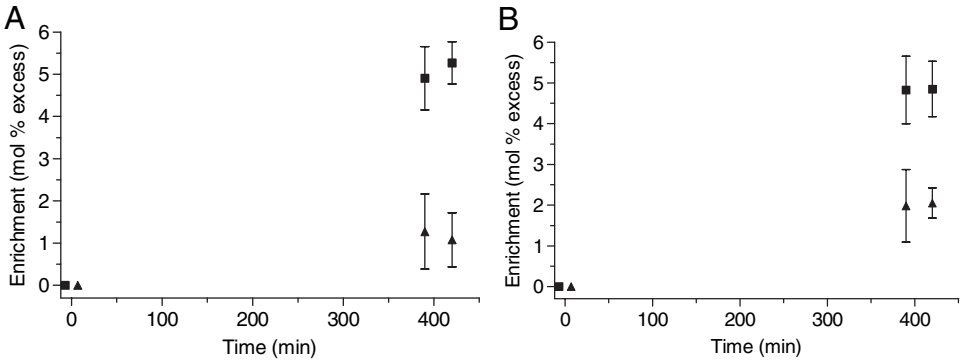

FIGURE 2. Mean (±SD) isotopic enrichments of plasma threonine at baseline and plateau during period A (A) and period B (B). ■, [U-^{13}C]threonine; ▲, [^{15}N] threonine.

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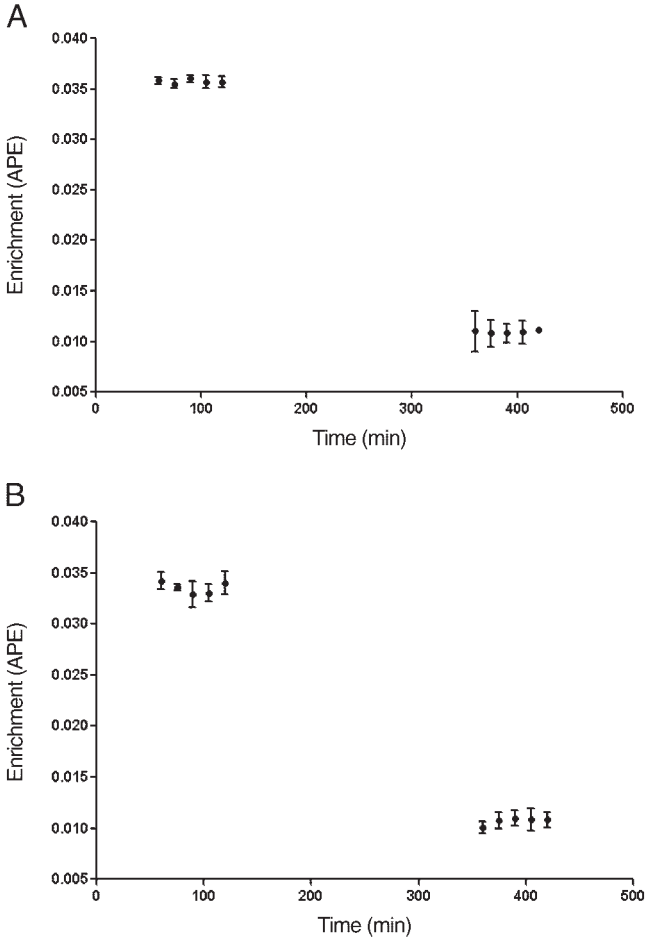

FIGURE 3. Mean (±SD) isotopic steady state in $^{13}CO_2$ excretion in expiratory air during the [^{13}C]sodium bicarbonate infusion (0–120 min) and the [U-^{13}C]threonine infusion (120–420 min). A, period A; B, period B; APE, atom percent excess.

were 3.4 ± 4.4% during period A and 8.5 ± 0.6% during period B. We considered this variation to be acceptable and to permit the detection of differences in rates of threonine oxidation between the 2 feeding periods.

Threonine kinetics

The plasma concentrations of all AAs in both feeding periods are shown in **Table 3**. We did not find a statistically significant difference in the plasma threonine concentration between the 2 feeding periods (period A: 169 ± 111 μmol/L; period B: 201 ± 98 μmol/L), but the concentrations showed a large variation. The high first-pass threonine uptake during both periods is shown in **Figure 4**. The first-pass threonine uptake, expressed as a percentage of dietary intake, was significantly higher during low enteral intake. Yet, approximately three-quarters of the dietary intake was utilized by the splanchnic tissues during full enteral feeding. However, and in contrast to our hypothesis, the first-pass threonine uptake, in absolute amounts, was significantly ($P < 0.0001$) lower during restricted enteral threonine intake [period A: 24 ± 7 μmol/(kg/h); period B: 44 ± 4 μmol/(kg/h)].

The threonine kinetics are shown in **Table 4**. There was no significant difference between the 2 feeding periods in the amount of threonine oxidized by the whole body [period A: 15 ± 9 μmol/(kg/h); period B: 17 ± 13 μmol/(kg/h)]. During both intakes, whole-body threonine oxidation accounted for ≈6% of threonine flux. The nonoxidative threonine disposal and the threonine release of protein did not differ significantly between the 2 periods. Whole-body threonine balance, calculated from the difference between nonoxidative disposal and whole-body protein degradation, was positive during both feeding periods and did not differ significantly between the 2 periods.

DISCUSSION

This study describes the splanchnic threonine kinetics in preterm infants during early postnatal life. The splanchnic tissues play an essential role in the delivery of dietary AAs to peripheral tissues. The systemic appearance of a given AA is a result of adequate digestion, transport, and intracellular metabolism. Because threonine is an EAA, high splanchnic utilization may result in the depletion of the systemic plasma pool of threonine.

Therefore, the most striking observation to emerge from this study was the very high fractional first-pass threonine uptake by the intestine in the first week of life in preterm infants during a restricted enteral intake. This observation indicates a high obligatory visceral need for threonine in neonates. Although the gastrointestinal tissues represent only 5% of body weight, because of their high rates of metabolism, they account for 15%–35% of whole-body oxygen consumption and protein turnover (22–24). Of the many factors that affect neonatal gut growth and adaptation, probably the most physiologically significant stimulus is enteral nutrition (25, 26). Enteral feeding acts directly by supplying nutrients for the growth and mucosal metabolism of epithelial cells. In the present study, we show that up to 82% of

TABLE 3
Plasma amino acid concentrations during period A and period B with a reference range in term breastfed infants[1]

	Period A	Period B	Term infants
	μmol/L		
Threonine	169 ± 111[2]	201 ± 98	70–197[3]
Cystine	25 ± 6	20 ± 11	35–69
Isoleucine	66 ± 25	52 ± 18	27–90
Leucine	112 ± 31	95 ± 27	53–169
Lysine	229 ± 118[4]	136 ± 51	80–232
Methionine	39 ± 8	34 ± 15	22–50
Phenylalanine	83 ± 13	83 ± 7	22–72
Valine	214 ± 64	154 ± 38	88–222
Alanine	235 ± 64	207 ± 108	125–647
Arginine	72 ± 27[4]	49 ± 24	42–148
Aspartic acid	18 ± 6	29 ± 25	5–51
Aspartic	41 ± 14	48 ± 13	16–81
Glutamic acid	82 ± 33	110 ± 49	24–243
Glutamine	423 ± 91[4]	326 ± 74	142–851
Glycine	292 ± 85	265 ± 131	77–376
Proline	172 ± 33	210 ± 105	83–319
Serine	153 ± 39	183 ± 110	0–326
Tyrosine	70 ± 77	75 ± 49	38–119
Total amino acids	2711 ± 767	2440 ± 865	
EAAS	911 ± 336	755 ± 219	

[1] EAAs, essential amino acids. $n = 8$.
[2] $\bar{x}$ ± SD (all such values).
[3] Reference range (all such values); from reference 21.
[4] Significant difference between periods, $P < 0.05$.

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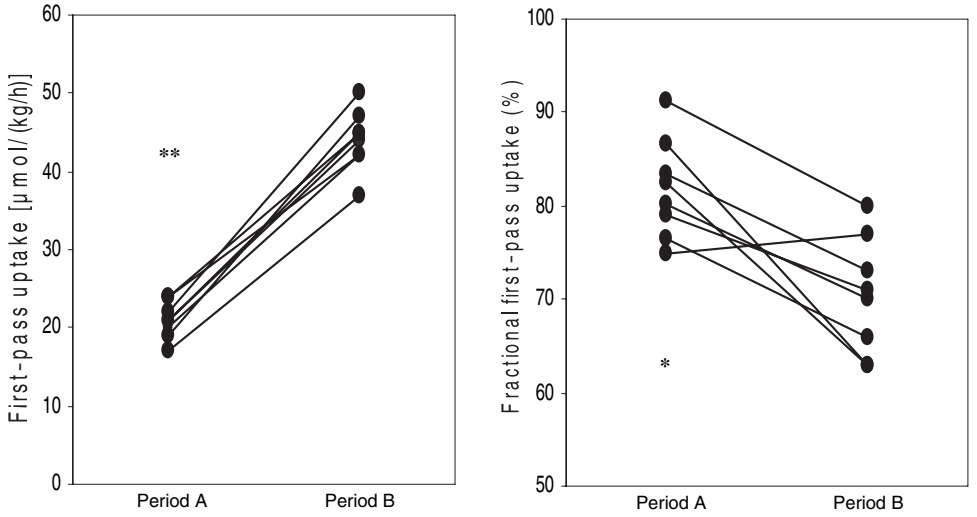

FIGURE 4. First-pass threonine uptake in 8 preterm infants during periods A and B expressed in absolute amounts [μmol/(kg/h)] and as a fraction of dietary intake. APE, atom percent excess. *,**Significant difference between periods: $^{*}P < 0.01$, $^{**}P < 0.0001$.

specific nutrients are utilized in first-pass uptake. We hypothesize that this mainly represents intestinal tissue utilization. We measured first-pass splanchnic uptake, which includes hepatic uptake. However, neonatal animal studies suggest that the intestine is the major site of utilization.

The route of administration of nutrition is a major issue in the clinical care of preterm infants, because of these infants' intolerance of enteral feeding and the associated morbidity (27). To reduce the complications of TPN and to accelerate the adaptation to full enteral feeding, many neonatologists often provide small volumes of enteral nutrition—ie, minimal enteral feeding—in combination with TPN to preterm infants in the first weeks of life (28). Studies in neonatal piglets showed that an enteral intake of 20% is necessary to prevent gut protein loss, whereas an intake of ≥40% is needed to maintain normal growth (29, 30). In the present study, preterm infants were enterally fed 40% of their total nutrient intake during period A, and the results show the fractional first-pass threonine requirements were significantly higher (82% of dietary intake) than those of fully fed infants (70% of dietary intake). Because the first-pass uptake is upregulated, even at 40% of enteral intake, this finding indicates that the enteral requirement is not yet reached.

The high enteral threonine uptake observed during both partial and full enteral feeding reflects the use of absorbed threonine for the synthesis of secretory glycoproteins, for the synthesis of mucosal cellular proteins, or for oxidative purposes (2–4). In neonatal piglets, we did not find substantial first-pass threonine oxidation, which indicates that enterally absorbed threonine is mostly used for the other 2 metabolic pathways. Although the intestinal mucosa is highly secretory and proliferatory tissue, dietary threonine is not incorporated into constitutive mucosal proteins to a great extent (4). However, the intestinal mucosa is protected by a complex network of glycoproteins, and the core proteins of the highly glycosylated domains of intestinal mucins contain large amounts of threonine (31). It is likely that a significant proportion of the utilized threonine is channeled toward mucin production. These secretory mucins play a key role in the defense of the mucosa. In fact, there is evidence that mucin production is impaired in piglets fed threonine-deficient diets, and supplying threonine parenterally cannot restore normal mucin production (10). Moreover, recent studies suggested that the restriction of dietary threonine significantly and specifically impairs intestinal mucin synthesis and consequently reduces gut barrier function (32, 33). Especially in preterm infants who are vulnerable to infections during the first weeks of life, mucus would be an important aspect of defense.

TABLE 4
Whole-body threonine kinetics quantified in 8 preterm infants during restricted enteral intake (period A) and full enteral intake (period B)[1]

	Period A	Period B
	μmol/(kg/h)	
Oxidation	15 ± 9	17 ± 13
Nonoxidative threonine disposal	231 ± 46	236 ± 41
Threonine release of protein	185 ± 35	183 ± 41
Net threonine balance	43 ± 10	44 ± 16

[1] All values are $\bar{x}$ ± SD. $n = 8$.

A second aim of this study was to determine whole-body threonine kinetics in neonates under 2 different feeding circumstances. During both partial and full enteral feeding, whole-body threonine oxidation accounted for 6% of the threonine flux, which is comparable to the fractional oxidation rates found by Darling et al (34) in breastfed preterm infants. However, Parimi et al (35) recently reported a higher fractional oxidation rate of threonine in newborn infants, although the total threonine intake was substantially lower.

In conclusion, the present study showed that the splanchnic tissues of preterm infants use the dietary threonine intake to a substantial degree—ie, more than three-quarters—irrespective of the amount of enteral threonine delivery. Furthermore, our data show that <10% of the threonine flux is oxidized, and the route of feeding does not affect this whole-body threonine oxidation. Overall, we suggest that the major metabolic fate of intestinal utilized threonine is mucosal glycoprotein synthesis.

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The authors' responsibilities were as follows—SRDvdS: recruitment of the participants, blood and breath sample collection, preparation and analysis of the data, and writing of the manuscript; DLW and JH: data analysis; JBvG (principal investigator): study design and supervision; and AV: preparation of stable isotopes. None of the authors had a personal or financial conflict of interest.

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