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Majority of Dietary Glutamine Is Utilized in First Pass in Preterm Infants

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ABSTRACT: Glutamine is a conditionally essential amino acid for very low-birth weight infants by virtue of its ability to play an important role in several key metabolic processes of immune cells and enterocytes. Although glutamine is known to be used to a great extend, the exact splanchnic metabolism in enterally fed preterm infants is unknown. We hypothesized that preterm infants show a high splanchnic first-pass glutamine metabolism and the primary metabolic fate of glutamine is oxidation. Five preterm infants (mean ± SD birth weight 1.07 ± 0.22 kg and GA 29 ± 2 wk) were studied by dual tracer ([U-^{13}C]glutamine and [$^{15}N_2$]glutamine) cross-over techniques on two study days (at postnatal week 3 ± 1 wk). Splanchnic and whole-body glutamine kinetics were assessed by plasma isotopic enrichment of [U-^{13}C]glutamine and [$^{15}N_2$]glutamine and breath $^{13}CO_2$ enrichments. Mean fractional first-pass glutamine uptake was 73 ± 6% and 57 ± 17% on the study days. The splanchnic tissues contributed for a large part (57 ± 6%) to the total amount of labeled carbon from glutamine retrieved in expiratory air. Dietary glutamine is used to a great extent by the splanchnic tissues in preterm infants and its carbon skeleton has an important role as fuel source. **(*Pediatr Res* 67: 194–199, 2010)**

After birth, very low-birth weight infants (VLBW) infants are exposed to stress-induced protein wasting and intestinal infirmity. Given the key role of the intestine in the conservation of neonatal wellness, there has been substantial interest in the magnitude of first-pass splanchnic metabolism of dietary amino acids (1–4). Dietary digested amino acids can be used for intestinal energy generation; for conversion via transamination into other amino acids, metabolic substrates, and biosynthetic intermediates; and for tissue growth. Previous studies in neonates showed a considerable intestinal amino acid metabolism, with some amino acids that are extensively catabolized, while others are presumed to be incorporated to a great extend in mucosal cellular or excreted (glyco-)proteins (5–9).

Glutamine, a conditionally essential amino acid for VLBW infants, might play a versatile role in both maintenance of gut trophicity and in nitrogen homeostasis by interorgan shuttling of carbon and nitrogen. Glutamine plays a central role as a substrate for a number of aminotransferases that are responsible for the synthesis of asparagine, glucosamine, NAD, purines, and pyrimidines (10). In animal studies, the small intestine is a major organ for glutamine utilization (11,12). Moreover, in *in vitro* experiments, glutamine has been shown to be the most important oxidative fuel source for rapidly dividing cells such as cells from gut and the immune system (13,14). In human adults, Matthews *et al.* (15,16) found that the splanchnic extraction of glutamine was 60 to 80% of the dietary intake, of which oxidation was a major metabolic fate. In preterm infants, Darmaun *et al.* (5) showed that glutamine is extensively extracted from the enteral lumen. Whether preterm infants sequester and oxidize the same amount of dietary glutamine has not yet been investigated. Such data are of particular importance in preterm infants who are vulnerable to gastrointestinal diseases such as necrotizing enterocolitis (NEC) during the first weeks of life.

Regarding this important role of glutamine in the metabolism of proliferating cells, such as enterocytes and lymphocytes, we considered it valuable to investigate the effect of full enteral feeding on splanchnic and whole-body glutamine kinetics. Consequently, glutamine dynamics in the splanchnic tissues were investigated by two different stable isotope glutamine tracers. The use of dual stable isotopically labeled tracers, administered enterally and systemically, allows us to quantify the first-pass uptake of glutamine. This is a reflection of the direct utilization rate of glutamine by the duodenum, small intestine, and liver. First, we hypothesized that the first-pass utilization of glutamine by the splanchnic tissues in fully fed preterm infants would be substantial. Second, enteral glutamine is one of the major sources of energy needed for splanchnic metabolism of rats and human adults. Hence, this study specifically explores the first-pass and whole-body metabolism of glutamine in preterm infants.

METHODS

Patients. Splanchnic and whole-body glutamine kinetics were quantified in five preterm infants during full enteral feeding. Patients eligible for this study were preterm infants with a birth weight of 750 to 1500 g, which was appropriate for GA according to Usher and McLean (17). The infants with

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Abbreviations: MPE, mole percent excess; Q_{ig}, flux of the intragastric glutamine tracer; Q_{iv}, flux of the intravenous glutamine tracer; **VLBW,** very low-birth weight

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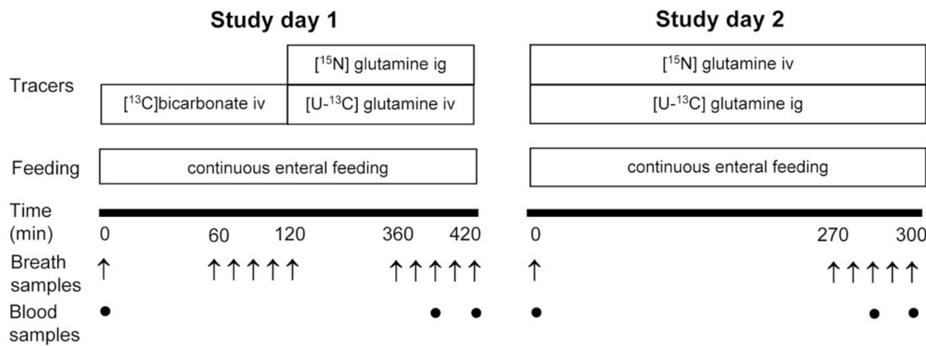

Figure 1. Study design.

major congenital anomalies, gastrointestinal, or liver diseases were excluded from this study. The infants received a standard nutrient regimen according to our feeding protocol: a combination of fortified mother's own milk (Nenatal BMF; Nutricia Nederland NV, Zoetermeer, The Netherlands) or special preterm formula (Nenatal Start; Nutricia Nederland NV; 0.024 g protein/mL). Continuous enteral feeding by nasogastrical cannula 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 VU University Medical Center, Amsterdam, The Netherlands.

Protocol. The study design consisted of two consecutive study days in which the infants received full enteral feeding. A schematic outline of the tracer-infusion studies is shown in Figure 1. During the study period, an i.v. catheter was used in the infants for the infusion of tracers. This catheter was already installed for clinical purposes such as parenteral nutrition. Blood samples were collected by heel stick. Breath samples were collected by the method described by Perman *et al.* (18). This method has been validated in preterm infants for the collection of expiratory carbon dioxide after the administration of ^{13}C-labeled substrates (19,20). Sampling of expired air was performed in duplicate and from stored in evacuated tubes (Vacutainer; Becton Dickinson, Rutherford, NJ) for later analysis. The first study day, a primed, continuous infusion [10.02 μmol/kg priming dose and 10.02 $\mu mol \cdot kg^{-1} \cdot h^{-1}$)] 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 a primed, continuous infusion [30 μmol/kg priming dose and 30 $\mu mol \cdot kg^{-1} \cdot h^{-1}$] of [U-^{13}C]glutamine (97 mol% ^{13}C; Cambridge Isotopes) given i.v. and a second primed, continuous infusion [30 μmol/kg priming dose and 30 $\mu mol \cdot kg^{-1} \cdot h^{-1}$] of [^{15}N]glutamine (95 mol% ^{15}N; Cambridge Isotopes) given enterally by nasogastric cannula for 5 h. The second study day, a primed, continuous infusion [30 μmol/kg priming dose and 30 $\mu mol \cdot kg^{-1} \cdot h^{-1}$] of [^{15}N]glutamine (95 mol% ^{15}N; Cambridge Isotopes) was given i.v. and a second primed, continuous infusion [30 μmol/kg priming dose and 30 $\mu mol \cdot kg^{-1} \cdot h^{-1}$] of [U-^{13}C]glutamine (97 mol% ^{13}C; Cambridge Isotopes) was given enterally by nasogastric cannula for 5 h. All isotopes were analyzed and found to be sterile and pyrogen-free before use. The stable isotopes solutions were aseptically and filtered through a 0.2 μm filter. Fresh solutions were prepared in the morning of each study day, because glutamine is not stable in aquatic solution. 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. Plasma enrichments of [U-$^{13}C_5$]glutamine and [$^{15}N_2$]glutamine were measured by gas chromatography-mass spectrometry (GC-MS), using *N*-ethoxycarbonylethylester derivatives. Briefly, 100 μL of plasma was deproteinated with 100 μL of 0.24 M sulfosalicyclic acid. After centrifugation (for 8 min at 4°C, and 14,000 × g), the supernatant passed over a Dowex cation-exchange resin column (AG 50 W-X8, hydrogen form, Bio-Rad Laboratories, Richmond, CA). The column was washed with 3 mL of water and the amino acids eluted with 1.5 mL M NH_4OH. The eluate was dried at room temperature in a speedvac (Savant, Thermofisher, Breda, The Netherlands) and derivatives of the amino acids were finally prepared with ethyl chloroformate as described previously (21). Analyses were performed on a MSD 5975C Agilent GCMS (Agilent Technologies, Amstelveen, The Netherlands) by injecting 0.5 μL on a PTV injector and a 30 m × 0.25-mm VF-17ms 0.25-μm coated fused silica capillary column (Varian, Middelburg, The Netherlands). Ion abundance was monitored by selective ion monitoring (SIM) at a mass to charge (*m*/*z*) for natural glutamine [M + 0], [$^{15}N_2$]glutamine [M + 2], [U-$^{13}C_5$]glutamine [M + 4], at *m*/*z* 173.1, 175.1, and 177.1 respectively. Breath samples were analyzed for $^{13}CO_2$ isotopic enrichment on an isotope ratio mass spectrometry (ABCA; Europe Scientific, Van Loenen Instruments, Leiden, The Netherlands) and expressed as atom percent excess (APE) above baseline (22). APE was plotted relative to time. Steady state was defined as three or more consecutive points with a slope not different from zero.

Calculations. The rate of glutamine turnover was calculated by measuring the tracer dilution at steady state as modified for stable isotope tracers, as previously described (23,24). Plasma enrichments of glutamine were used to calculate the rate of glutamine turnover. The glutamine flux (i.v. or intragastric) was calculated according to the following equation:

$$Q_{\mathrm{iv\ or\ ig}} = iG \times [(Ei/Ep) - 1] \quad (1)$$

where $Q_{\mathrm{iv\ or\ ig}}$ is the flux of the i.v. or intragastric glutamine tracer [$\mu mol \cdot kg^{-1} \cdot h^{-1}$], i_G is the glutamine infusion rate [$\mu mol \cdot kg^{-1} \cdot h^{-1}$], and E_i and E_p are the enrichments [mol percent excess (MPE)] of [U-^{13}C or ^{15}N]glutamine in the glutamine infusate and in plasma at steady state.

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

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

where *U* is the first-pass glutamine uptake, and *I* is the enteral glutamine intake [$\mu mol \cdot kg^{-1} \cdot h^{-1}$].

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

$$\text{Body } CO_2 \text{ production} = i_B \times [E_{iB}/\text{breath } IE_B] - 1 \quad (3)$$

where i_B is the infusion rate of $NaH^{13}CO_3$ [$\mu mol \cdot kg^{-1} \cdot h^{-1}$], 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).

The carbon skeleton of glutamine is degraded to α-ketoglutarate before it enters the tricarboxylic acid (TCA) cycle as acetyl CoA. The amount of [^{13}C]label that finally appears as $^{13}CO_2$ was calculated by multiplying the recovery of the [^{13}C]label in the expiratory air with the rate of appearance of glutamine (6,8,25). The fraction of glutamine oxidized using the enrichment of the i.v. [U-^{13}C]glutamine infusion on study d 1 and intragastric [U-^{13}C]glutamine on study d 2 was calculated as according to the following equation, assuming a constant rate of CO_2 production during the study, which lasted 5 h:

$$\text{Fraction of carbon skeleton of glutamine oxidized to } CO_2 = [IE_{Giv} \times i_B]/[IE_B \times i_G \times 5] \quad (4)$$

where IE_{Giv} are the $^{13}CO_2$ breath enrichments (MPE) at steady state during the i.v. [U-^{13}C]glutamine infusion. The denominator is multiplied by a factor of 5 to account for the number of C-atoms that are labeled.

Whole-body oxidation of the carbon skeleton of glutamine was calculated by using the following equation:

$$\text{Whole-body oxidation of the carbon skeleton of glutamine} = \text{product of equation 4} \times \text{product of equation 1} \quad (5)$$

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Whole-body oxidation of the carbon skeleton of glutamine from intragastric [U-^{13}C]glutamine on study d 2 was calculated by using the following equation:

Fraction of carbon skeleton of glutamine oxidized to CO_2

$$= [IE_{Gig} \times i_B]/[IE_B \times i_G \times 5] \quad (6)$$

where IE_{Gig} are the $^{13}CO_2$ breath enrichments (MPE) at steady state during the intragastric [U-^{13}C]glutamine infusion. The denominator is multiplied by a factor of 5 to account for the number of C-atoms that are labeled.

Whole-body oxidation of the carbon skeleton of glutamine was then calculated by using the following equation:

Whole-body oxidation of the carbon skeleton of glutamine

$$= \text{product of equation 6} \times \text{product of equation 1} \quad (7)$$

where in eq 1 the i.v. tracer enrichments and infusion rates are used on the second study day.

The amount of $^{13}CO_2$ release in expiratory air derived from the systemic circulation was estimated by using the following equation:

Oxidation of the carbon skeleton of glutamine

in the systemic circulation to $^{13}CO_2$ on day 2

$$= [IE_{Gig}]/[IE_{Giv}] \times \text{equation 5} \quad (8)$$

where IE_{Gig} and IE_{Giv} are the $^{13}CO_2$ breath enrichments (MPE) at steady state during the intragastric (study d 2) and i.v. (study d 1) [U-^{13}C]glutamine infusion.

First-pass glutamine oxidation was calculated by using the following equation:

$$\text{First-pass glutamine oxidation} = \text{equation 7} - \text{equation 8} \quad (9)$$

Total whole-body glutamine oxidation was calculated by using the following equation:

$$\text{Total whole-body glutamine oxidation} = \text{equation 9} + \text{equation 5} \quad (10)$$

Statistical analysis. Data are expressed as mean ± SD values obtained from samples taken over the last hour of each tracer infusion. Differences in background and steady state enrichments between the two study days were analyzed by paired *t* tests. A value of $p < 0.05$ was considered to be statistically significant. Statistical analyses were performed using SPSS version 14.0 (SPSS, Chicago, IL).

RESULTS

Characteristics of the five preterm infants are shown in Table 1. Their mean GA was 29 ± 1 wk and mean birth weight was 1.07 ± 0.22 g. All were clinically stable at the time of the study. On both study days, four infants received oxygen by nasal prongs, and one infant did not receive supplemental oxygen. The background (baseline) values of the ^{13}C label in expiratory air did not differ between the two study days (study d 1: 1.0997 ± 0.0036 and study d 2: 1.0956 ± 0.0001 APE; $p = 0.3$) and were in the same range as we have found previously (8,20). Similarly, baseline enrichments of [U-^{13}C]glutamine and [$^{15}N_2$]glutamine in plasma were not significantly different ([U-^{13}C]glutamine 0.062 *versus* 0.028 MPE, $p = 0.25$; [^{15}N]glutamine 0.064 *versus* 0.068, $p = 0.94$). The $^{13}CO_2$ enrichment in breath during [^{13}C]bicarbonate infusion was constant in all infants by 120 min, with less than 5% variation of plateau (variation coefficient; CV: 2.3 ± 0.6% in study d 1). The CO_2 production was 28.3 ± 3.9 $mmol \cdot kg^{-1} \cdot h^{-1}$), similar to previous studies (6,8).

To determine splanchnic and whole body glutamine kinetics, we used plateau glutamine enrichment values from plasma and labeled carbon dioxide in expiratory air (Tables 2 and 4). Although we withdrew only two blood samples during the tracer infusions, we are convinced that isotopic steady state was reached during the glutamine tracer infusions, because we found an isotopic plateau in carbon dioxide excretion. The mean ± SD variation coefficient of breath [^{13}C]glutamine enrichment above baseline at plateau were 2.0 ± 1.7% at study d 1 and 0.4 ± 0.2% at study d 2.

The whole-body and splanchnic glutamine kinetics are shown in Tables 3 and 4. As expected, whole-body glutamine fluxes were higher for the enteral than for the i.v. tracer group during both study days (study d 1: 2559 ± 1130 *versus* 634 ± 91 $\mu mol \cdot kg^{-1} \cdot h^{-1}$, study d 2: 1734 ± 227 *versus*. 834 ± 139). The fractional first-pass uptake is the fraction of dietary glutamine, directly absorbed through the splanchnic tissues. The mean first-pass glutamine uptake was 65 ± 15%. The total amount of ^{13}C label from glutamine that was recovered in expired breath was calculated from the flux of the i.v. infused [U-^{13}C]glutamine on study d 1. This was 765 ± 160 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ and accounted for 87% of the glutamine turnover. The splanchnic tissues contributed for a large part (57 ± 6%) to the total amount of labeled carbon retrieved in expiratory air.

DISCUSSION

The main purpose of this study was to measure splanchnic glutamine kinetics in preterm infants during early postnatal life to gain insight into the significance of the splanchnic tissues in relation to dietary glutamine metabolism. Our data show that in clinically stable fully fed preterm infants more than half of the dietary glutamine is used by the splanchnic region. Therefore, less than 50% of the enteral glutamine passed intact through the splanchnic bed and was available to systemic tissues. In addition, half of the labeled glutamine carbon recovered in expired breath derived from the splanchnic tissues, indicating that the carbon skeleton of glutamine is an important fuel substrate for the splanchnic tissues.

Table 1. *Characteristics of the patients who were on full continuous enteral feedings during both study days*

Patient	GA (wk)	BW (kg)	SA 1, (d)	SA 2, (d)	SW 1, (kg)	SW 2, (kg)	Glutamine intake, ($\mu mol \times kg^{-1} \times h^{-1}$)
1	26.3	0.73	40	41	1.15	1.15	211
2	31.6	1.10	15	18	1.17	1.17	178
3	30.0	1.34	11	12	1.44	1.44	218
4	29.4	1.09	10	11	1.12	1.12	248
5	29.4	1.10	10	11	1.11	1.11	250
Mean ± SD	29.3 ± 1.92	1.07 ± 0.22	17 ± 13	19 ± 13	1.20 ± 0.14	1.20 ± 0.16	221 ± 30

BW, birth weight; SA 1, postnatal age at study d 1; SA 2, postnatal age at study d 2; SW 1, weight at study d 1; SW 2, weight at study d 2.

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Table 2. *Steady-state isotopic enrichments of [U_{13}-C]glutamine and [$^{15}N_2$]glutamine in plasma on study d 1 and 2*

	[$U_{13}C$]glutamine (mol% excess)						[$^{15}N_2$]glutamine (mol% excess)					
	D1			D2			D1			D2		
Patient	4.5 h	5 h	Mean	4.5 h	5 h	Mean	4.5 h	5 h	Mean	4.5 h	5 h	Mean
1	3.94	3.74	3.84	1.19	1.28	1.24	1.39	1.20	1.30	3.31	3.27	3.29
2	4.32	4.63	4.48	1.86	1.90	1.88	1.46	1.22	1.34	2.90	3.05	2.98
3	3.68	3.52	3.60	1.36	1.33	1.35	1.12	1.17	1.15	2.93	2.57	2.75
4	4.67	4.80	4.74	1.58	1.36	1.47	1.83	1.41	1.62	3.88	4.13	4.01
5	3.66	3.84	3.75	1.35	1.40	1.38	1.25	1.55	1.40	3.18	3.31	3.25
Mean	4.05	4.11	4.08	1.47	1.45	1.46	1.41	1.31	1.36	3.24	3.27	3.25
SD	0.44	0.57	0.50	0.26	0.25	0.25	0.27	0.16	0.17	0.40	0.57	0.47

Table 3. *Whole-body and splanchnic glutamine kinetics in preterm infants (n = 5)*

		Q_{IV}		Q_{IG}		FFP		AFP		Mean	
Patient	I	D1	D2	D1	D2	D1	D2	D1	D2	FFP	AFP
1	211	741	1057	4520	5892	0.84	0.82	176	173	0.83	175
2	178	554	879	2021	1409	0.73	0.38	129	67	0.56	98
3	218	690	992	2377	1864	0.71	0.47	155	102	0.59	128
4	248	526	660	1652	1747	0.68	0.62	169	154	0.65	162
5	250	659	807	2226	1914	0.70	0.58	176	145	0.64	160
Mean	221	634	879	2559	2565	0.73	0.57	161	128	0.65	145
SD	30	91	165	1130	1870	0.06	0.17	20	43	0.15	36

I, glutamine intake [μmol $\times$ kg^{-1} $\times$ h^{-1}] and was equal on D1 and D2; Q, flux [μmol $\times$ kg^{-1} $\times$ h^{-1}]; FFP, fractional first-pass uptake (%); AFP, absolute first-pass uptake [μmol $\times$ kg^{-1} $\times$ h^{-1}]; D1, study d 1; D2, study d 2; mean FFP and AFP, average of D1 and D2.

Table 4. *Whole-body and splanchnic glutamine oxidation rates in preterm infants*

	$^{13}CO_2$ enrichments		Whole-body glutamine oxidation	Splanchnic glutamine oxidation
Patient	D1 (iv)	D2 (ig)		
1	0.275	0.371	952	600
2	0.310	0.335	707	344
3	0.265	0.317	914	516
4	0.263	0.396	663	405
5	0.286	0.350	587	313
Mean	0.279	0.350	765	436
SD	0.006	0.008	160	120

On D1, [U-^{13}C]glutamine infusion is given intravenously; on D2, [U-^{13}C]glutamine infusion is given intragastricly; $^{13}CO_2$ enrichments at plateau are given in APE; and glutamine oxidation rates are given in μmol $\times$ kg^{-1} $\times$ h^{-1}.

These data are qualitatively consistent with those from animal and human studies (1,15,16). In human adults, approximately three-quarters of the dietary glutamine intake were sequestered in the splanchnic tissues (15,16,26). The splanchnic utilization rates of glutamine observed in our study are comparable to those observed in 10 d-old preterm infants (5). However, the magnitude of the glutamine extraction by the intestinal tissues was somewhat lower compared with studies performed in 5-wk-old preterm infants (27). A similar high splanchnic utilization was observed in our previous studies in preterm infants regarding glutamate and threonine (7,8).

The reason for the high extraction might be 3-fold: degradation to glutamate, incorporation into newly synthesized protein, or irreversible oxidation to CO_2. Three enzymes, *i.e.* glutamate dehydrogenase, glutamine synthetase, and glutaminase, participate in the metabolism of glutamine. Glutamine is synthesized from amination of glutamate via glutamine synthetase and catabolized via deamination back to glutamate via glutaminase. Glutamine carbon enters the TCA cycle as α-ketoglutarate and is used for energy or for formation of new glucose (28). Glutamate itself can be used as precursor for glutathione, the major intracellular anti-oxidant. In human adults, only 17% of the dietary used glutamine was retained for incorporation into new proteins or conversion to glucose (16). However, degradation with subsequent oxidation within the TCA cycle seems an important fate in preterm fully enterally fed infants as found in our study. However, one cannot directly speak of glutamine oxidation because there is a rapid exchange of carbon atoms within the TCA cycle, although ultimately $^{13}CO_2$ is derived from the carbon skeleton of the administered labeled glutamine. A similar finding was observed in preterm infants regarding glutamate oxidation. Almost 90% of the used glutamate in the first pass was oxidized, while essential amino acids such as leucine and lysine were not oxidized in preterm infants (6). Furthermore, glucose oxidation (10 kcal $\cdot$ kg^{-1} $\cdot$ d^{-1}) contributed five times as much as glutamate oxidation (1.7 kcal $\cdot$ kg^{-1} $\cdot$ d^{-1}) to splanchnic CO_2 production, indicating that glucose is the major source of energy in the human neonatal intestine (29). The oxidation of the carbon skeleton of glutamine in first pass yielded another 6.5 kcal $\cdot$ kg^{-1} $\cdot$ d^{-1}, indicating the importance of nonessential amino acids as energy source when proteins are delivered at large amounts to the intestines.

Glutamine, a conditionally essential amino acid for VLBW infants, gained a lot of interest in recent years because of its important role in immunity, metabolism, and intestinal function. Parenteral administration of glutamine in critically ill adults reduced mortality while in preterm infants it did not decrease mortality or the incidence of late-onset sepsis when administered early in life (30,31). Previously, we found that glutamine-enriched enteral nutrition did not improve feeding

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tolerance or short-term outcome in VLBW infants, but infectious morbidity was significantly decreased in infants who received glutamine-enriched enteral nutrition (32). As glutamine is a source of "respiratory fuel" for gut-associated lymphoid tissue, it has been suggested to contribute to barrier function of the gut (33), by preservation of transepithelial resistance and/or decreasing intestinal permeability. These effects may result in less bacterial translocation and subsequently sepsis. In an infant rodent model (pup in the cup), dietary glutamine significantly decreased lipopolysaccharide-induced inflammatory mediators in the intestine, liver, and lung (34). Furthermore, low plasma glutamine levels have been found in preterm infants with NEC (35). The available literature reveals that septic neonates have increased muscle proteolysis, decreased protein synthesis, and a negative nitrogen balance during critical illness (36). Becker *et al.* (35) showed that infants with NEC have certain plasma amino acid deficiencies, *i.e.* arginine and glutamine. It is likely that these deficiencies are a result of intestinal consumption of these amino acids in the gastrointestinal tract during intestinal inflammation. Up till now, no dose response studies are available in preterm infants, but studies in neonatal pigs show that a higher enteral intake of amino acids results in a higher "consumption" of amino acids by the portal drained viscera (*i.e.* intestine, pancreas, spleen, and stomach) (2). Recently, Li *et al.* (37) showed that neither glutamine nor glutamate were able to rescue the somatic and intestinal growth retarding effects of decreased protein intake in rat pups. Whether splanchnic glutamine oxidation is further increased in infants with NEC or infants with sepsis who are partially enterally fed remains to be investigated. Interestingly, studies in neonatal piglets show that during protein restriction visceral amino acid oxidation is substantially suppressed.

To our knowledge, this is the first report in which preterm infants have been infused on the same day by both nasogastric and i.v. routes with a carbon and nitrogen labeled glutamine tracer. By using this dual stable isotope methodology, we were able to measure the first pass utilization of glutamine, to determine intermediates of glutamine in the TCA cycle, and to quantify the contribution of the abovementioned substrates to $^{13}CO_2$ production. We found a small difference in the fractional first pass glutamine utilization during both study days. This might be due to our study protocol of two consecutive study days and thereby shortening of the washout period of all isotopes. However, baseline plasma enrichments and $^{13}CO_2$ were not significantly different between the two study days, indicating that the tracer washout period between the two study days was appropriate. The small number of patients and the interindividual variability might explain this difference. Therefore, we believe that the mean fractional first-pass uptake of both study days gives an actual depiction.

Although, the glutamine kinetics was examined in five preterm infants only, the data consistently demonstrate that the splanchnic tissues of preterm infants use more than half of the dietary glutamine. We hypothesized that the intestine has a high first-pass effect and is a major site of oxidation in preterm infants. The results of this study show that glutamine is extracted in great amounts by the splanchnic tissues and nearly 60% of the whole-body glutamine oxidation occurs in the splanchnic tissues, stressing its important role as oxidative fuel source for the enterocytes. In conclusion, the intestine is not merely an organ of nutrient assimilation, but one that uses substantial quantities of glutamine to maintain functions of critical importance, already in the first weeks of life in preterm infants.

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