Splanchnic Oxidation Is the Major Metabolic Fate of Dietary Glutamate in Enterally Fed Preterm Infants

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ABSTRACT: The intestine is a major site of amino acid metabolism, especially in neonates. The energy needed for the metabolic processes in neonatal animals is derived from dietary glucose and amino acids. No data are available showing that dietary amino acids function as intestinal fuel source in human neonates as well. We hypothesized that preterm infants show a high splanchnic first-pass glutamate metabolism and the primary metabolic fate of glutamate is oxidation. Five preterm infants (birth weight 1.2 ± 0.2 kg, gestational age 29 ± 1 wk) were studied by dual tracer ($[U-^{13}C]$ glutamate and $[D_3]$ glutamate) techniques on two study days (within postnatal d 14–19). Splanchnic and whole-body glutamate kinetics were assessed by plasma isotopic enrichment of $[U-^{13}C]$ glutamate and $[D_3]$ glutamate and breath $^{13}CO_2$ enrichment. Fractional first-pass glutamate uptake was $77 \pm 18\%$ on d 1, and $70 \pm 7\%$ on d 2, mean $74 \pm 13\%$. Almost all ($86 \pm 7\%$) of the glutamate used in the first pass is directed toward oxidation. There is a high splanchnic fractional first-pass uptake and a high oxidation rate of glutamate in preterm infants. Glutamate is an important source of energy for the splanchnic tissues in preterm infants receiving full enteral feeding. (*Pediatr Res* 62: 468–473, 2007)

The splanchnic tissues have much higher energy expenditure and protein synthesis rates than expected based on their weight. Studies in neonatal animals found that up to 50% of the dietary protein intake in the portal drained viscera (intestines, pancreas, spleen, and stomach) is used for protein turnover and biosynthetic pathways; from 10% to 20% is used for whole-body energy expenditure (1–3). Humans also show considerable intestinal amino acid metabolism (4,5) and the metabolic rate is even higher in preterm infants. Previous studies showed that first-pass metabolism of leucine in newborns is twice that of adults (6) and that in preterm infants, a great amount of dietary lysine is used in first pass as well (7).

Glutamate plays a pivotal role in human metabolism. It provides a critical link between carbohydrate and amino acid metabolism. Glutamate is the obligatory precursor of glutamine; it plays a central role in all transamination reactions in the body and also functions as a neurotransmitter. In addition,

dietary glutamate is a major substrate for glutathione (GSH) synthesis (8). Animal studies showed that the gut of infant piglets metabolizes virtually all the enteral glutamate during absorption (9) and that glutamate is an important oxidative substrate for the intestinal mucosa (10). In human adults, most of the dietary glutamate (88%) is metabolized in first-pass as well (11) and is oxidized to a great extent in the splanchnic region (12). These studies in animals and adult humans suggest that dietary glutamate serves as an important metabolic fuel source in the intestine. If so, this may have a particular impact on the nutritional needs of preterm infants. The intestine of preterm infant is immature and has a low tolerance to enteral feeding. Yet, enteral feeding is a must for intestinal maturation and the growth and development of other organs. Much effort should be directed toward understanding the nutritional needs of the intestine of preterm infants. The present study was developed to extend the previously mentioned observations made in animals and adult humans and thus gain more insight into the nutritional needs of infants.

In the present study, we, therefore, investigated 1) the effect of first-pass splanchnic uptake of glutamate and 2) the metabolic fate of the used glutamate in the splanchnic bed of enterally fed preterm infants. Glutamate kinetics in the splanchnic region were studied by dual, primed, constant i.v. and enteral infusions of amino acids labeled with stable isotopes and preceded by an i.v. labeled sodium bicarbonate infusion as described previously (2,7). We hypothesized that preterm infants would show significant splanchnic first-pass uptake and oxidation of glutamate.

METHODS

Patients. The Erasmus MC Institutional Review Board approved the study. Written informed consent was obtained from parents or legal guardians. Eligible for this study were preterm infants admitted to the Neonatal Intensive Care Unit of the Erasmus MC-Sophia Children's Hospital, Rotterdam, The Netherlands. Inclusion criteria were birth weight between 750 and 1500 g, appropriate for gestational age according to the charts of Usher and Mclean and with CRIB scores <5 on the first day of life (13). Exclusion criteria were congenital anomalies and gastrointestinal and liver diseases. All enrolled infants received full enteral feeding at the time of the study and were clinically stable. Neonatal (Nutricia Zoetermeer, The Netherlands) was the sole enteral nutrition from 10 h before start of the study and during both study days.

Protocol. To investigate splanchnic and whole-body glutamate kinetics, a crossover study design was applied on two consecutive study days. During the

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Abbreviations: APE, atom percent excess; GSH, glutathione; ORS, oral rehydration solution

Figure 1. Study design.

tracer infusion, infants were fed continuously through a nasogastric tube. All received the same feeding regimen on both study days. A peripheral i.v. catheter was available for infusion of tracers during both days. Blood samples were obtained by heel stick. Breath samples were obtained by the direct sampling method as described previously (14,15). Briefly, in mechanically ventilated neonates, a syringe was connected to the ventilator tubing and breath samples were taken slowly during expiration with a total volume of 15 mL. When breathing was spontaneous, a 6-Fr gastric tube (6 Ch Argyle; Cherwood Medical, Tullamore, Ireland) was inserted 1.0–1.5 cm into the nasopharynx and end-tidal breath samples were taken slowly with a syringe connected at the end. Collected air was transferred into 10 mL sterile, nonsilicon-coated evacuated glass tubes (Van Loenen Instruments, Zaandam, The Netherlands) and stored at room temperature until analysis (15).

The study design is depicted in Figure 1. Three different stable isotopes were infused during study d 1. First, a primed (10 $\mu\text{mol/kg}$) constant [10 $\mu\text{mol}/(\text{kg} \cdot \text{h})$] i.v. infusion of [¹³C]bicarbonate (99.0 mol% ¹³C; Cambridge Isotopes, Woburn, MA) dissolved in sterile saline was administered i.v. for 2 h. This was immediately followed by primed 5-h infusions [30 $\mu\text{mol/kg}$ and 30 $\mu\text{mol}/(\text{kg} \cdot \text{h})$] of [U-¹³C]glutamate (95.0 mol% ¹³C; Cambridge Isotopes) and [30 $\mu\text{mol/kg}$ and 30 $\mu\text{mol}/(\text{kg} \cdot \text{h})$] of [2,2,4-D₃]glutamate (97.0 mol% D₃; Cambridge Isotopes). [U-¹³C]glutamate was given *via* an i.v. catheter and [D₃]glutamate *via* the intragastric catheter. On d 2, we switched the i.v. and intragastric routes. All isotopes were tested and found to be sterile and pyrogen free before use. On each day, baseline blood and breath samples were collected at time 0. During the last half hour of each tracer infusion, breath samples were collected at 15-min intervals and two blood samples were obtained after 4.5- and 5-h tracer infusion. The total amount of blood withdrawn per study day was 1.5 mL, which is less than 2% of the blood volume in a 1000-g infant. Blood was centrifuged immediately, and plasma was stored at -80°C for further analysis.

Analytical methods. Plasma enrichments of [U-¹³C]glutamate and [D₃]glutamate were measured by gas chromatography-mass spectrometry (GC-MS), using *N*-methyl-*N*-(tert-butyl)dimethylsilyltrifluoroacetamide (MTBSTFA) derivatives. Briefly, 50 μL of plasma was deproteinized with 50 μL 0.24 M sulfosalicylic acid. After centrifugation (for 8 min at 4°C , and

14,000 $\times g$), the supernatant passed over a Dowex cation-exchange resin column (AG 50 W- $\times 8$, 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 3 M NH_4OH . The eluate was dried at 70°C under nitrogen and derivatives of the amino acids were finally formed by adding 35 μL acetonitrile and 10 μL of MTBSTFA (Pierce, Omnilabo, Breda, The Netherlands) and incubation at 60°C for 30 min. Analyses were performed on a Carlo Erba GC8000 gas chromatograph coupled with a Fisons MD800 mass spectrometer (Interscience BV, Breda, The Netherlands) by injecting 1 μL with a split ratio of 50:1 on a 25 m $\times$ 0.22-mm fused silica capillary column coated with 0.11 μm HT5 (SGE, Victoria, Australia). Ion abundance was monitored by selected ion monitoring, and mass to charge for natural glutamate [$M + 0$] was 432 and for D₃-glutamate [$M + 3$] and [U-¹³C]glutamate [$M + 5$], 435 and 437, respectively.

¹³CO₂ isotopic enrichment in expired air was measured by isotope ratio mass spectrometry (ABCA; Europe Scientific, Van Loenen Instruments, Leiden, The Netherlands) and expressed as atom percent excess (APE) above baseline. APE was plotted relative to time. Steady state was defined as three or more consecutive points with a slope not different from zero.

Calculations. We studied amino acid turnover according to Waterlow's stable isotopic dilution model (16). Whole-body CO₂ kinetics were calculated as described previously (2). Substrate turnover was calculated by using dual stable isotope tracer techniques. Tracer dilutions were measured during steady state as described previously (7,17,18). The equations used to obtain the results are detailed in the Appendix.

Statistics. Data are expressed as mean $\pm$ 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 by using SPSS version 14.0 (SPSS, Chicago, IL).

RESULTS

Characteristics of the five enrolled infants are listed in Table 1. Their mean gestational age was 29 ± 1 wk and mean birth weight 1.18 ± 0.23 kg. All were clinically stable at the time of the study. On d 1, four infants needed supplemental oxygen by a nasal prong; on d 2, only two infants needed supplemental oxygen. They all needed caffeine to support breathing, but no other medication was prescribed. Baseline ¹³C enrichment in expired breath on d 1 (1.0867 ± 0.0005 APE) did not differ from that on d 2 (1.0889 ± 0.0029 APE) ($p = 0.17$) and were in the same range as we have found previously (15). Also, baseline enrichments of [U-¹³C]glutamate and [D₃]glutamate in plasma did not significantly differ for the two study days.

To calculate splanchnic and whole-body glutamate kinetics, we used plateau glutamate enrichment values from plasma (Table 2) and labeled carbon dioxide in expired breath. Although we obtained only two blood samples during the tracer infusions, a steady state must have been reached at the time we drew the plasma samples because at that time, the ¹³CO₂ excretion was at isotopic plateau. As shown in Figure 2,

Table 1. Patient characteristics

Patient	GA, wk	BW, kg	SA 1	SA 2	SW 1, kg	SW 2, kg	Kcal
1	31	1.29	9	11	1.25	1.28	108
2	27	0.92	13	19	0.94	1.12	123
3	29	1.45	15	17	1.46	1.48	106
4	29	1.30	21	22	1.41	1.41	109
5	30	0.96	12	25	0.96	0.99	101
Mean	29	1.18	14	19	1.20	1.26	109
SD	1	0.23	4	5	0.25	0.20	8

GA, gestational age; 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; Kcal, kilocalories/(kg $\cdot$ d).

Table 2. Steady-state isotopic enrichments of [U-¹³C]glutamate and [D₃]glutamate in plasma on study d 1 and 2

Patient	[U- ¹³ C]glutamate (mol% excess)						[D ₃]glutamate (mol% excess)					
	D 1 Δ			D 2 Δ			D 1 Δ			D 2 Δ		
	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	0.82	0.91	0.87	0.50	—	0.72	0.41	0.63	0.52	1.63	—	1.63
2	3.99	4.06	4.01	1.23	1.45	1.61	0.53	0.51	0.52	3.57	3.65	3.60
3	3.19	3.15	3.17	0.44	0.44	0.68	0.50	0.46	0.48	1.72	1.68	1.70
4	1.54	1.77	1.66	0.19	0.15	0.38	0.43	0.43	0.43	0.72	0.78	0.75
5	2.22	2.27	2.25	0.18	0.18	0.50	0.40	0.45	0.43	1.24	1.28	1.26
Mean	2.35	2.43	2.39	0.51	0.56	0.52	0.45	0.50	0.48	1.78	1.85	1.79
SD	1.27	1.22	1.23	0.43	0.61	0.47	0.06	0.08	0.05	1.08	1.26	1.08

Figure 2. Enrichments of ¹³C in expired breath during i.v. infusion of [U-¹³C]glutamate on d 1 (left) and intragastric [U-¹³C]glutamate infusion (right) on d 2. Rates are expressed as mean APE ± SD (*n* = 5).

steady state of ¹³CO₂ during enteral and i.v. [U-¹³C]glutamate infusion was achieved within 2 h of infusion. The slope of the line from time point 120 min to 300 min was not different from zero (*p* = 0.40). As expected, plateau enrichment (mole % excess) of the enterally administered tracer was much lower than that of the tracer administered i.v.

Table 3 shows the results of whole-body and splanchnic glutamate kinetics. The mean estimated whole body CO₂ production was 32.3 ± 5.3 mmol/(kg · h). These values are in the same range as we have reported previously (7,15). The rate of appearance of [U-¹³C]glutamate or flux (Q) during i.v. administration on d 1 was 1287 ± 864 μmol/(kg · h). The [D₃]glutamate flux during i.v. infusion on d 2 was 1900 ± 1061 μmol/(kg · h). The Q_{IV} on study d 1 did not significantly differ from Q_{IV} on d 2 (*p* = 0.34). Based on the rate of appearance during i.v. administration of [U-¹³C]glutamate, the mean whole-body oxidation of glutamate was 1052 ± 721 μmol/(kg · h). The fractional first-pass uptake is the fraction of dietary glutamate, directly absorbed through the splanchnic system. It amounted to 77 ± 18% and 70 ± 7% on the first

and second study days, respectively. Whole-body glutamate oxidation accounted for 82 ± 7% of the glutamate turnover, and the fraction of dietary glutamate that was oxidized by the splanchnic bed in first pass was 86 ± 7%. This latter finding emphasizes that glutamate is an important source of oxidation for the splanchnic system.

DISCUSSION

The aim of our study was to quantify the need for glutamate in the splanchnic tissues of premature infants by measuring splanchnic first-pass uptake and oxidation during full enteral feeding. Splanchnic first-pass glutamate uptake and oxidation rates were found to be very high. Approximately three fourths of the dietary glutamate intake was consumed in the first pass. Most of the extracted glutamate was then oxidized, indicating that oxidation is the metabolic fate of dietary glutamate in the premature infant.

Our results agree with those from animals (9) and human studies (11,12). Additionally, and in agreement with our re-

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

Patient	Q _{IV}			Q _{IG}		FFP		AFP		Mean	
	I	D 1	D 2	D 1	D 2	D 1	D 2	D 1	D 2	FFP	AFP
1	185	2736	1645	5084	5001	0.46	0.67	86	123	0.57	104
2	188	584	661	5003	1684	0.88	0.61	185	101	0.75	143
3	181	720	1607	5700	5298	0.87	0.70	159	125	0.79	142
4	186	1357	3575	6320	13,164	0.79	0.73	146	136	0.76	141
5	173	1036	2012	6132	10,705	0.83	0.81	146	138	0.82	142
Mean	183	1287	1900	5648	7170	0.77	0.70	144	125	0.74	134
SD	6	864	1061	596	4656	0.18	0.07	36	15	0.13	28

I, glutamate intake [μmol/(kg · h)] and was equal on d 1 and 2; Q, flux [μmol/(kg · h)]; FFP, fractional first-pass uptake (%); AFP, absolute first-pass uptake [μmol/(kg · h)]; d 1, study d 1; d 2, study d 2; mean FFP and AFP, average of d 1 and 2.

sults, in this issue, Haÿs *et al.* demonstrate that the primary fate of dietary glutamate in premature infants is splanchnic extraction (19). This finding, which indicates that dietary glutamate is not a significant gluconeogenic substrate, is not surprising, seeing that we showed that oxidation is the primary metabolic pathway of enterally absorbed glutamate. The present data demonstrate a wide range in the mean glutamate flux, which expectedly was higher than observed in human adults. Both the i.v. and intragastric flux on the second study day were higher than on d 1, despite the fact that the infant's glutamate intake showed little variation. Clearly, the difference is large, yet not significant due to a small number of subjects and therefore a wide variance. However, as the first-pass splanchnic uptake showed a very small range, we may assume that the data are reliable. No such differences have been reported in animal studies (9,20,21). An explanation of the small range in outcome in animal studies could be the identical experimental setting or genetic similarity because animals used in these studies usually are descended from the same litter. Identical experimental setting is hard, if not impossible, to organize in human studies.

When glutamate is delivered enterally, it must pass through the enterocytes during absorption and could be used at that stage for transamination to the formation of α -ketoglutarate. The labeled α -ketoglutarate enters the Krebs cycle where the first ^{13}C label is lost by decarboxylation, thereby producing $^{13}\text{CO}_2$. However, it does not necessarily lead to complete glutamate oxidation; the remaining carbon atoms could also be used for synthesis of other metabolic compounds, *e.g.* pyruvate, alanine, and lactate. Animal studies reported that only a minor proportion of labeled pyruvate, derived from intragastrically administered $[\text{U-}^{13}\text{C}]\text{glutamate}$, was converted to alanine and lactate and released into venous blood (1,20). Apart from oxidation, glutamate can also act as a precursor for proline, arginine, and GSH. In rats, approximately 64% of the glutamate was released as CO_2 and 10% was incorporated in amino acids such as proline, ornithine, and alanine; the latter accounted only for 3% of the carbon label incorporation (20). We were not able to determine the quantity of labeled alanine, lactate, and pyruvate. Thus, the contribution of $^{13}\text{CO}_2$ from oxidation from these products could not be quantified. We found a fractional glutamate oxidation of 86%, which indicates that complete oxidation is unlikely. However, from animal data, we hypothesize that the contribution of labeled CO_2 from these amino acids is relatively small and that the fractional glutamate oxidation is not overestimated. Reeds *et al.* (9) showed in infant piglets infused with $[\text{U-}^{13}\text{C}]\text{glutamate}$ $[\text{M} + 5]$ that lower mass isotopomers appeared in plasma and determined the degree to which the ^{13}C label is recycled in intermediary metabolism: 11% of the labeled glutamate appeared in $[\text{M} + 1]$ and $[\text{M} + 3]$ isotopomers. We, however, were not able to determine the present abundance of plasma $[\text{M} + 3]$ because the $[\text{D}_3]\text{glutamate}$ tracer has a mass of $[\text{M} + 3]$ as well and thus could not be distinguished from the $[\text{M} + 3]$ glutamate produced from intermediary metabolism. Nevertheless, plasma $[\text{D}_3]\text{glutamate}$ enrichment on d 1 was very low (0.48 MPE) and contribution of 3-carbon prod-

ucts $(\text{M} + 3)$ from $[\text{U-}^{13}\text{C}]\text{glutamate}$ was minimal. The same held true for the second study day.

Several animal studies have shown that enterally derived glutamate is preferentially metabolized above systemically derived glutamate (9,20). This could have important consequences for preterm infants receiving full parenteral feeding, with nutrients bypassing the intestine. In these infants, only systemically derived glutamate is available and uptake by the enterocytes will be diminished, resulting in a decrease in glutamate as an energy substrate. Conversely, they have lower energy requirements $[90 \text{ kcal}/(\text{kg} \cdot \text{d})]$ than preterm infants who receive full enteral feeding $[120 \text{ kcal}/(\text{kg} \cdot \text{d})]$ (22). This difference may be due to the energy needs of the splanchnic tissues, loss of nutrients, or intestinal (glyco-)protein synthesis and thus energy storage during enteral nutrition. In general, approximately 8–14 kcal/(kg · d) is lost in stools (23). Fuel sources used by the gut to generate energy for synthesis purposes could be glucose, amino acids, or fatty acids. We showed earlier that glucose is an important intestinal energy source for preterm infants (18). The splanchnic tissues use one third of the dietary glucose in the first pass and approximately 80% of this amount is oxidized; thus, most of the glucose is used for energy generation. The same pattern is found for glutamate, with 86% of the dietary glutamate oxidized in the first pass; approximately 14 mmol glucose/(kg · d) is oxidized in the splanchnic tissues, accounting for 10 kcal/(kg · d). Glutamate first-pass oxidation yields an additional 1.7 kcal/(kg · d). Thus, besides glucose, glutamate oxidation by the splanchnic tissues is an important source of energy. It seems likely that the glutamate demand depends on other available oxidative substrates as well and that the splanchnic glutamate oxidation rate is related to the dietary glutamate load. In piglets, we found that the intestinal glutamate oxidation was significantly lower during low protein feeding compared with normal protein intake (10). We, therefore, expect that the splanchnic fraction of glutamate oxidation will increase if more dietary glutamate is provided.

Although glutamate is a nonessential amino acid, its availability has many clinical implications. Apart from oxidation and incorporation into proteins, glutamate is a precursor of the synthesis of several other amino acids and for GSH synthesis. This is a tripeptide consisting of glutamate-cysteine-glycine and is the most important intracellular antioxidant; especially in preterm infants receiving supplemental oxygen causing increased oxidative stress. Mucosal and luminal GSH both play an evident role against oxidative damage in the mucosa resulting from peroxidative damage and dietary toxins (24). Reeds *et al.* (8) showed that enterally derived glutamate is the preferential source for mucosal GSH synthesis in neonatal piglets. Approximately 85% of the mucosal GSH-glutamate derived from dietary glutamate, and at 500%–600% per day, the fractional GSH synthesis rate was extremely high. If the intestine oxidizes almost all dietary glutamate, the quantity of glutamate left may be insufficient to maintain the preterm infant's demand for GSH production. About 135 μmol glutamate/(kg · h) is used by the enterocytes in first-pass and 116 μmol glutamate/(kg · h) thereof is oxidized. Thus, a maximum of 19 μmol glutamate/(kg · h) is left for intestinal GSH syn-

thesis or for other proteins. In neonatal piglets GSH synthesis required the provision of 25 μmol glutamate/(kg $\cdot$ h) with a four times higher intake. The minimal glutamate requirement for GSH synthesis during stress and illness in human neonates is not yet known. Animal data, however, strongly indicate that mucosal GSH-glutamate derives mainly from dietary glutamate. This could have implications for parenterally fed infants as they lack supply of luminal (dietary) glutamate during parenteral feeding. The intestinal GSH synthesis may be restricted and therefore limit protection against oxidative injury.

Another important question is whether glutamate should be added to oral rehydration solutions (ORS). The standard ORS solutions (World Health Organization recommended) nowadays contain glucose and electrolytes but not glutamate. However, if the gut is at risk during diarrhea, glutamate might play an important role in restoring essential functions of the enterocytes as an energy source and a precursor of protein synthesis and possibly also of increased GSH production. However, glutamate added to ORS will increase osmolality. Clinical studies are needed to determine the precise effects of added specific amino acids such as glutamate.

A limitation of this study is that we determined glutamate kinetics in only five premature infants. However, the results demonstrate clearly that the splanchnic bed sequesters most of the enteral glutamate tracer in the first pass. We hypothesized that the intestine has a very high first-pass effect and is a major site of oxidation in neonates. The results of this study demonstrate that the splanchnic tissues are indeed a major site of metabolism and oxidation.

APPENDIX

Glutamate flux during i.v. infusion was calculated as follows (7):

$$Q_{IV} = I_{\text{glu}_{iv}} \times [(IE_i/IE_p) - 1], \quad (1)$$

where Q_{IV} is the flux of the i.v. infused tracer [$\mu\text{mol}/(\text{kg} \cdot \text{h})$], $i_{\text{glu}_{iv}}$ is the i.v. glutamate infusion rate [$\mu\text{mol}/(\text{kg} \cdot \text{h})$], and IE_i and IE_p are the isotopic enrichments (mol% excess) of i.v. administered [$U\text{-}^{13}\text{C}$ or D_3]glutamate in the infusate or in plasma at steady state, respectively.

The flux for the intragastrically administered tracer was calculated as follows:

$$Q_{IG} = I_{\text{glu}_{ig}} \times [(IE_i/IE_p) - 1], \quad (2)$$

where Q_{IG} is the flux of the intragastrically infused tracer [$\mu\text{mol}/(\text{kg} \cdot \text{h})$], $i_{\text{glu}_{ig}}$ is the intragastric glutamate infusion rate [$\mu\text{mol}/(\text{kg} \cdot \text{h})$], and IE_i and IE_p are the isotopic enrichments (mol% excess) of intragastrically administered [$U\text{-}^{13}\text{C}$ or D_3]glutamate in the infusate or in plasma at steady state, respectively.

First-pass glutamate uptake was calculated as:

$$U = [(Q_{IG} - Q_{IV})/Q_{IG}] \times I, \quad (3)$$

where U is the first-pass glutamate uptake [$\mu\text{mol}/(\text{kg} \cdot \text{h})$], Q_{IG} is the flux of the intragastrically administered glutamate tracer [$\mu\text{mol}/(\text{kg} \cdot \text{h})$], Q_{IV} is the flux of the i.v. administered glutamate tracer [$\mu\text{mol}/(\text{kg} \cdot \text{h})$], and I is the intake of enteral glutamate [$\mu\text{mol}/(\text{kg} \cdot \text{h})$].

Whole-body CO_2 production was estimated as:

$$\text{CO}_2 \text{ Production} = I_B \times [(IE_{iB}/IE_B) - 1], \quad (4)$$

where i_B is the infusion rate of [^{13}C]sodium bicarbonate [$(\mu\text{mol}/(\text{kg} \cdot \text{h}))$], IE_{iB} is the enrichment (mol% excess) of [^{13}C]bicarbonate in the bicarbonate infusate, and IE_B is the $^{13}\text{CO}_2$ enrichment at steady state during the labeled bicarbonate infusion (mol% excess). Splanchnic and whole-body glutamate oxidation rates were determined under the assumption that CO_2 production during [^{13}C]bicarbonate infusion equals the labeled glutamate tracer infusion. Assuming a constant CO_2 production rate during the 7 h of infusion, it is not necessary to use a correction factor for retention of [^{13}C]bicarbonate in the body (25).

Glutamate oxidation was calculated by multiplying recovery of [^{13}C]glutamate in expiratory air with the rate of appearance of glutamate (7,25). The fraction of glutamate oxidized using the enrichment of the i.v. [$U\text{-}^{13}\text{C}$]glutamate infusion on study d 1 and intragastric [$U\text{-}^{13}\text{C}$]glutamate on study d 2 was calculated as:

Fractional glutamate oxidation

$$= [IE_{\text{glu}} \times i_B] / [IE_B \times i_{\text{glu}} \times 5], \quad (5)$$

where IE_{glu} and IE_B are $^{13}\text{CO}_2$ enrichments (mol% excess) in expired breath at steady state during i.v. (d 1) or intragastric (d 2) [$U\text{-}^{13}\text{C}$]glutamate and [^{13}C]bicarbonate infusion. The i_{glu} is multiplied by a factor of 5 to account for the number of labeled C atoms.

Whole-body glutamate oxidation (day 1) was calculated as:

Whole body glutamate oxidation

$$= \text{Eqn. (5)}_{IV} \times \text{Eqn. (1)}_{IV}. \quad (6)$$

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