Effect of enteral glutamine or glycine on whole-body nitrogen kinetics in very-low-birth-weight infants

Prabhu S Parimi, Srisatish Devapatla, Lourdes L Gruca, Saeid B Amini, Richard W Hanson, and Satish C Kalhan

ABSTRACT

Background: Glutamine is a critical amino acid for the metabolism of enterocytes, lymphocytes, and other proliferating cells. Although supplementation with glutamine has been suggested for growing infants, its effect on protein metabolism has not been examined.

Objective: The objective was to examine the effect of enteral glutamine or glycine on whole-body kinetics of glutamine, phenylalanine, leucine, and urea in preterm infants.

Design: Infants at <32 wk of gestation were given formula supplemented with either glutamine (0.6 g · kg−1 · d−1; n = 9) or isonitrogenous amounts of glycine (n = 9) for 5 d. Eight infants fed unsupplemented formula served as control subjects. Glutamine, phenylalanine, leucine nitrogen flux, leucine carbon flux, and urea kinetics were quantified during a basal fasting period and in response to nutrient intake.

Results: Growing preterm infants had a high weight-specific rate of appearance of glutamine, phenylalanine, and leucine nitrogen flux. When compared with the control treatment, enteral glutamine resulted in a high rate of urea synthesis, no change in the plasma glutamine concentration, and no change in the rate of appearance of glutamine. Glycine supplementation resulted in similar changes in nitrogen metabolism, but the magnitude of change was less than that in the glutamine group. In the nonsupplemented infants, the rate of appearance of leucine nitrogen flux was negatively correlated (ρ = −0.72) with urea synthesis. In contrast, the correlation (ρ = 0.75) was positive in the glutamine group.

Conclusion: Enteral glutamine supplementation in growing preterm infants is entirely metabolized in the gut and does not have a discernable effect on whole-body protein and nitrogen kinetics. Am J Clin Nutr 2004;79:402–9.

KEY WORDS  Glutamine, glycine, premature infants, leucine, urea, phenylalanine, reamination, growth

INTRODUCTION

Glutamine, a nonessential amino acid, is the most abundant amino acid in the blood and in the free amino acid pool in the body. It is synthesized by virtually every tissue in the body, although only certain tissues (eg, skeletal muscle, brain, and lung) release it into the circulation in significant quantities (1–4). Glutamine plays an important role in interorgan shuttles of nitrogen and carbon and has been shown to be the primary oxidative fuel for dividing cells such as enterocytes and lymphocytes (1, 3). In addition, glutamine is a key substrate for ammonia production by the kidney (4), is a precursor for purine and pyrimidine synthesis, and is suggested to play a role in the regulation of protein synthesis (5–7). Many studies in adults and animals have examined the metabolism of glutamine and its relation to gluconeogenesis and to whole-body protein metabolism. However, few studies in the literature have examined the effect of growth and nitrogen accretion on whole-body glutamine and nitrogen kinetics. These data are confounded by a lack of consistency in the route of nutrient intake (parenteral compared with enteral), the quantity of protein intake, and the inclusion of appropriate control groups. In addition, the effect of enteral glutamine on whole-body glutamine and nitrogen metabolism has not been evaluated in infants, particularly during periods of rapid growth.

Data in healthy adults show that almost 74% of enterally administered glutamine is extracted by the splanchnic compartment during the first pass (8–11). The fraction of glutamine uptake is lower when glutamine is given in larger quantities (8). Enteral glutamine did not appear to have any effect on the systemic rate of appearance (Ra) of leucine, although it did result in a decrease in the systemic rate of glutamine turnover (9). Whether growing neonates show similar responses to enteral glutamine has not been examined. Such data are critically important because many investigators have proposed the use of supplemental glutamine to enhance growth and protein synthesis and to improve certain cellular and tissue functions, particularly in preterm low-birth-weight infants (12, 13). These recommendations are based either on the data from studies in adults or on certain clinical benefits, such as a shorter length of hospital stay and possibly a lower incidence of sepsis in preterm infants (12, 13).

The purpose of the present study was to examine the relation between the rate of glutamine turnover, leucine nitrogen turnover, and urea synthesis in growing preterm infants. In addition, we examined the effect of enteral administration of glutamine on the above parameters and on whole-body protein (phenylala-
The infants required minimal oxygen support. The control group consisted of nonsupplemented infants and a group of infants administered glycine in isonitrogenous amounts.

**SUBJECTS AND METHODS**

Preterm infants (n = 26) born at <32 wk gestation and who weighed between 693 and 1846 g were recruited for the study (Table 1). The infants required minimal oxygen support. The infants were randomly assigned to either the glutamine group (n = 9) or the glycine group (n = 9), and 8 infants served as control subjects. The study protocol was initiated only after the infants were receiving ≥120 kcal · kg⁻¹ · d⁻¹, or 150 mL · kg⁻¹ · d⁻¹ of 24 kcal/30 mL formula (PF 24; Ross Laboratories, Columbus, OH) for premature infants at the time of entry to the study nursery in the General Clinical Research Center. The infants were recruited into the study protocol between 10 and 74 d of age; most of the infants were older than 23 d. Two infants were studied early: 1 control subject on day 10 and 1 infant in the glutamine group on day 18. The conceptional age of all of the infants at the time of study was ≈34 wk; their weight range was 1504–2440 g (Table 1). The investigators were not responsible for the clinical care of these infants. The protocol was reviewed and approved by the Institutional Review Board, MetroHealth Medical Center, Case Western Reserve University, Cleveland. Written informed consent was obtained from both parents or from the parents and guardians after the procedure was fully explained.

The effect of enteral glutamine (0.6 g · kg⁻¹ · d⁻¹) or glycine (0.6 g · kg⁻¹ · d⁻¹) administered for 5 d on whole-body leucine, phenylalanine, glutamine, and urea kinetics was examined with the use of stable isotopic tracers. Glutamine or glycine (Ajinomoto USA, Inc, Paramus, NJ) was administered mixed in the infants’ prescribed formula in equally divided doses throughout the day. The control group continued to receive their regular formula. The glutamine group received additional oral glutamine, ≈167 μmol · kg⁻¹ · h⁻¹, whereas the glycine group received additional oral glycine (≈330 μmol · kg⁻¹ · h⁻¹) (Table 2). The tracer isotope study was performed on day 6, ie, after 5 d of glutamine or glycine administration. 1-[1-¹³C,¹⁵N]leucine (99% [¹³C,¹⁵N]), [¹⁵N₂]phenylalanine (98% [¹⁵N]), and [¹⁵N₂]urea (99% [¹⁵N]) were purchased from Merck & Co (Dorval, Canada), and 1-[¹⁵N]glutamine (99% [¹⁵N]) was purchased from Isotec Inc (Miamisburg, OH).

Three hours after the last meal, the infants were transferred to the study nursery in the General Clinical Research Center. The design for the tracer study is displayed in Figure 1. Two intravenous cannulas were placed in the infants, one in the dorsum of the hand to infuse the isotopic tracers and the other in the saphenous vein to draw blood samples. The sampling site was kept patent by a continuous infusion of 0.9% NaCl at 2–3 mL/h. Weighed amounts of the isotopic tracers were mixed in 0.45% NaCl and sterilized by Millipore (Bedford, MA) filtration as described previously (14). The tracer solution was infused at 3 mL/h. The actual rate of infusion was determined gravimetrically on completion of the study by using the same infusion tubing, cannula, and infusion pump. The isotopic tracers were administered as prime-constant-rate infusions as follows: 1-[1-¹³C,¹⁵N]leucine [7.5 μmol/kg (prime) and 7.5 μmol · kg⁻¹ · h⁻¹ (constant)]; [⁵-¹⁵N]glutamine [30 μmol · kg⁻¹ · h⁻¹ (prime) and 30 μmol · kg⁻¹ · h⁻¹ (constant)]; [¹⁵N₂]urea [33 μmol/kg (prime) and 3.3 μmol · kg⁻¹ · h⁻¹ (constant)]; and [¹⁵N₂]phenylalanine [6 μmol/kg (prime) and 4 μmol · kg⁻¹ · h⁻¹ (constant)]. Blood samples (0.5 mL, depending on the weight of the infant) were obtained in heparin-containing syringes be-

**TABLE 1**

Clinical characteristics of the study infants

<table>
<thead>
<tr>
<th></th>
<th>Birth weight</th>
<th>Gestation</th>
<th>Age at study entry</th>
<th>Corrected age at study entry</th>
<th>Weight at study entry</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g</td>
<td>wk</td>
<td>d</td>
<td>wk</td>
<td>g</td>
</tr>
<tr>
<td>Glutamine group (n = 9)</td>
<td>1216 ± 330</td>
<td>28 ± 2</td>
<td>41 ± 20</td>
<td>34 ± 1.3</td>
<td>1888 ± 307</td>
</tr>
<tr>
<td>Glycine group (n = 9)</td>
<td>1082 ± 334</td>
<td>29 ± 3</td>
<td>46 ± 16</td>
<td>35 ± 2.5</td>
<td>2031 ± 199</td>
</tr>
<tr>
<td>Control group (n = 8)</td>
<td>1161 ± 340</td>
<td>29 ± 3</td>
<td>39 ± 20</td>
<td>34 ± 0.8</td>
<td>1827 ± 330</td>
</tr>
</tbody>
</table>

¹ x ± SD. There were no significant differences between groups.

**TABLE 2**

Growth and nutritional intake of the study infants

<table>
<thead>
<tr>
<th></th>
<th>Energy kcal · kg⁻¹ · d⁻¹</th>
<th>Protein g · kg⁻¹ · d⁻¹</th>
<th>Fat g · kg⁻¹ · d⁻¹</th>
<th>Leucine μmol · kg⁻¹ · h⁻¹</th>
<th>Phenylalanine μmol · kg⁻¹ · h⁻¹</th>
<th>Glutamine μmol · kg⁻¹ · h⁻¹</th>
<th>Glycine μmol · kg⁻¹ · h⁻¹</th>
<th>Weight gain g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamine group (n = 9)</td>
<td>122 ± 6</td>
<td>3.3 ± 0.2</td>
<td>6.7 ± 0.4</td>
<td>105 ± 6</td>
<td>31 ± 2</td>
<td>167 ± 3</td>
<td>35 ± 4</td>
<td>19.9 ± 6.7</td>
</tr>
<tr>
<td>Glycine group (n = 9)</td>
<td>127 ± 21</td>
<td>3.4 ± 0.5</td>
<td>6.9 ± 1.1</td>
<td>109 ± 17</td>
<td>32 ± 5</td>
<td>—</td>
<td>361 ± 34</td>
<td>19.6 ± 4.7</td>
</tr>
<tr>
<td>Control group (n = 8)</td>
<td>123 ± 16</td>
<td>3.3 ± 0.3</td>
<td>6.7 ± 0.8</td>
<td>107 ± 14</td>
<td>31 ± 4</td>
<td>—</td>
<td>35 ± 2</td>
<td>18.7 ± 4.3</td>
</tr>
</tbody>
</table>

¹ x ± SD. There were no significant differences between groups.

² Does not include additionally supplemented glutamine or glycine.

³ Estimated glycine content of the formula.

⁴ No supplemented glutamine.
fore the start of tracer infusion and at 150, 165, and 180 min. At 180 min, the infants were fed the infant formula (24 kcal/30 mL) at a rate of 10 mL · kg⁻¹ · h⁻¹ for the next 2 h. The glutamine and glycine groups were given glutamine or glycine mixed with the formula. The infants were offered formula every 30 min, and the ingested volume was recorded. Additional blood samples were obtained at 270, 285, and 300 min; the blood was mixed with cold trichloroacetic acid (10%) and centrifuged (2000 × g, 4 °C, 20 min), and the separated plasma was stored at −70 °C until analyzed. Blood glucose concentrations were monitored in all infants at the bedside throughout the study and remained in the normal range.

**Analytic procedures**

Plasma glucose and urea nitrogen concentrations were measured by the glucose oxidase and urease methods, respectively, with the use of commercial analyzers (Beckman Instruments, Fullerton, CA). Plasma amino acids were measured with a high-pressure liquid chromatograph equipped with fluorescent detector using α-phthalaldehyde derivative and precolumn derivatization (15). The concentration of plasma insulin and glucagon-like peptide 1 (GLP-1) in plasma were measured with a commercially available enzyme-linked immunoassay kit (Linco Research Inc, St Charles, MO).

The mass spectrometric methods used to measure the isotopic enrichment of leucine, α-ketoisocaproic acid (KIC), and urea were described previously in publications from our laboratory (16–18). Amino acids and urea were separated from the plasma by preparatory ion-exchange chromatography with a minicolumn. An N-acetyl, N-propyl ester derivative of leucine and phenylalanine was prepared according to the method of Adams (19) with certain modifications (16). A model 5973 or model 5870 gas chromatography–mass spectrometry system (Hewlett-Packard, Palo Alto, CA) was used. Methane chemical ionization was used, and mass-to-charge ratios of 216 and 218, which represented unlabeled and di-labeled leucine, respectively, were monitored with the use of selected ion-chromatography software. For phenylalanine, mass-to-charge ratios of 250 and 255 were monitored, which represented unlabeled and [H₅]₀-labeled phenylalanine, respectively. The ¹³C enrichment of plasma KIC was measured using the quinoxaline derivative (17). Standard solutions of known isotopic enrichment were run along with unknowns to correct for analytic and instrumental variations.

Glutamine in the amino acid eluate were derivatized according to the method of Haisch et al (10). A tri-tert-butylidimethylsilyl derivative was prepared by adding 50 μL MTBSTFA plus 1% N-methyl-N(tert-butylidimethylsilyl) trifluoroacetamide plus 1% tert-butylidimethylchlorosilane and 50 μL acetonitrile to the dry eluate. Gas chromatography–mass spectrometry analysis was performed in electron impact ionization mode with the use of a 0.25-mm internal diameter, 30-m HP-1 column (Agilent Inc, Palo Alto, CA) with a film thickness of 0.25 μm. Helium was used as the carrier gas. Glutamine eluted at ≈8 min. Ion clusters with mass-to-charge ratios of 431 and 432, which represented unlabeled and [¹⁵N]glutamine, respectively, were monitored for quantification of the ¹⁵N enrichment of glutamine. [¹⁵N]Enrichment of urea was quantified by using trifluoroaceto-hydroxypropyrimidine derivative in the electron impact mode as described previously (18).

**Calculations**

The Ra values for leucine, phenylalanine, glutamine, and urea were calculated by tracer dilution with the use of steady state kinetics.

\[
Ra = I \times \left[ \frac{(E_p - E)}{E_p} \right] \tag{1}
\]

where I is the rate of infusion of the tracer (μmol · kg⁻¹ · h⁻¹), and \(E_p\) and \(E\) represent the enrichment of the infusate and of plasma amino acids at steady state, respectively. The CV for enrichment data for various tracers in individual subjects was between 3% and 5%; the slope was not different from zero.

The kinetic data collected between 150 and 180 min were designated as “fasting” data and those collected between 270 and 300 min were designated as “fed” data. In newborns fed every 3 h, the fasting period (ie, 5.5–6 h after the last feeding) may not be comparable with the postabsorptive period in adults because of the large variability in gastric emptying and gut motility. However, for clinical and ethical considerations, the infants could not be starved for more prolonged periods.

Leucine carbon flux (\(Q_L\)) was calculated by using ¹³C enrichment of plasma KIC, whereas leucine nitrogen flux (\(Q_{LN}\)) was calculated by using the \(M+2\) enrichment, \([¹⁵C]{¹⁵N}\), of plasma leucine during isotopic steady state (20). During fasting, \(Q_{LN}\) is the sum of leucine released from protein breakdown and that formed from the reamination of KIC. \(Q_{LN}\), in contrast, is predominantly due to protein breakdown because leucine carboxyl carbon \([¹⁵C]\) is not lost during transamination of leucine to and from KIC. The difference between \(Q_{LN}\) and \(Q_{LN}\) provides an estimate of the rate of reamination of leucine KIC (20, 21). As discussed previously (21), inasmuch as \([¹³C]\)leucine enrichment is measured in the plasma, calculated \(Q_{LN}\) is an underestimation because the intracellular enrichment would be less than that in the plasma.

The contribution of glutamine nitrogen to urea nitrogen was calculated from the \(M+1\) enrichment of urea during isotopic steady state by using the precursor-product relation. This estimate also will include a small, or no, amount of \(¹⁵N\) reincorporated into urea after the hydrolysis of infused \([¹⁵N]\)urea in the gut. However, theoretical estimates suggest that such reincorporation of \(¹⁵N\) will be negligible (22, 23). Because \([¹⁵N]\)urea was infused at a rate corresponding to 1.5–2% of the endogenous rate of urea synthesis, the maximal contribution of the recycled nitrogen would be only 0.04 μmol · kg⁻¹ · h⁻¹, or 0.02% of urea synthesized (assuming the rate of urea synthesis to be ≈200 μmol · kg⁻¹ · h⁻¹ and a maximal 20% rate of hydrolysis in the gut). Nevertheless, the hydrolysis of urea into ammonia in the gut...
and the reincorporation of this ammonia into urea in the liver of newborns, particularly in those receiving enteral nutrition, has not been confirmed (22–24). The M+1 enrichment of urea could also be the result of 15N incorporation from alanine and aspartate as a result of transamination from labeled leucine. This is also anticipated to be small because of the large dilution of 15N from leucine in the intermediate pools. For these reasons, the M+1 enrichment of urea mostly represents the incorporation of the amide 15N of glutamine into urea.

The Ra of glutamine in the plasma was measured by [5-15N]glutamine tracer dilution. The M+1 enrichment of glutamine measured by tri-tert-butyldimethylsilyl derivative includes both the amide as well as the amino nitrogen. Because the contribution of 15N from the infused [1-13C15N]leucine to M+1 (amino) glutamine is anticipated to be small, as a result of the transamination of leucine with multiple amino acids, the measured M+1 enrichment of glutamine is mostly due to the infused (amide) glutamine tracer. The Ra of glutamine in plasma as measured here is the sum of the de novo synthesis of glutamine and the glutamine released from protein breakdown. Assuming that glutamine represents a fixed proportion of whole-body protein, the rate of release of glutamine from protein breakdown was calculated as follows:

\[
\text{B Glutamine} = \text{Ra of phenylalanine} \times 1.07
\]

where the fraction 1.07 represents the relation between glutamine and phenylalanine in mixed muscle protein (25). The rate of de novo synthesis of glutamine is the difference between the Ra of glutamine and B glutamine.

Statistical analysis

All data are reported as means ± SDs. Statistical analyses were performed by using commercial software (Statistix 7.0; Analytic Software, Tallahassee, FL). The data were initially analyzed for skewness and kurtosis by using descriptive statistics. The difference among the groups and the effect of time (fasting compared with fed) were analyzed by using repeated-measure analysis of variance. When the interaction between the groups and time was significant, the data were analyzed by repeated measure within each group. Because the sample sizes were small, we also repeated the same analysis by using mean corrected ranks instead of the raw data; however, it did not have any effect on the analysis. Plasma amino acid concentrations were analyzed by using one-way analysis of variance and Kruskal-Wallis nonparametric statistics. Both Bonferroni and Tukey corrections were used in post hoc comparisons of the groups whenever there was a significant group effect. Spearman correlations were done for linear regression analysis.

RESULTS

At the time of entry into the study, all infants had recovered from their acute illnesses, none of the infants required significant support, none of the infants were taking antibiotics, all of the infants were of similar conceptional age (≈35 wk), all of the infants were gaining weight (≈20 g · kg⁻¹ · day⁻¹; Table 2), and all of the infants were tolerating enteral feeding. The infants’ calorie and macronutrient intakes, except for supplemental glutamine (0.6 g · kg⁻¹ · day⁻¹, or 167 μmol · kg⁻¹ · h⁻¹) or glycine (0.6 g · kg⁻¹ · day⁻¹, or 330 μmol · kg⁻¹ · h⁻¹), were not significantly different.

Insulin and glucagon-like peptide 1

No significant differences in plasma insulin concentrations were observed between the groups during fasting (control group: 1.73 ± 0.61 μU/mL; glutamine group: 1.78 ± 0.57 μU/mL; glycine group: 1.67 ± 0.60 μU/mL). In response to feeding, the infants who received supplemental glycine had slightly higher (NS) plasma insulin concentrations than did the control and glutamine groups (control group: 12.37 ± 3.16 μU/mL; glutamine group: 12.73 ± 5.53 μU/mL; glycine group: 17.33 ± 7.38 μU/mL). Plasma GLP-1 concentrations were also not significantly different between the 3 groups, and no significant increase in plasma GLP-1 was observed in response to feeding (data not shown).

Phenylalanine and leucine kinetics

During fasting, the Ra of phenylalanine in the plasma—a measure of the whole-body rate of proteolysis—was not significantly different between the control group and the 2 supplemented groups (Table 3). In response to feeding, a significant increase in phenylalanine Ra was observed in all 3 groups (P < 0.001). In response to feeding, there was a significant increase in the Ra of Qe in the entire study population (time effect: \( P = 0.01 \)). Qe kinetics, as measured by the dilution of tracer leucine in the KIC pool, were not significantly different between the 3 groups and did not change during feeding. The rate of reamination of leucine,

**Table 3**

Phenylalanine and leucine kinetics

<table>
<thead>
<tr>
<th>Phenylalanine Ra</th>
<th>Leucine nitrogen flux Ra</th>
<th>Leucine carbon flux Ra</th>
<th>Reamination of leucine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting</td>
<td>Fed</td>
<td>Fasting</td>
<td>Fed</td>
</tr>
</tbody>
</table>

\( P^1 \) and \( P^2 \) are for comparison between groups. NS indicates no significant difference.

\( ^1 \) ± SD; \( n \) in brackets.

\( ^2 \) Repeated-measures ANOVA for the difference between groups (\( P^1 \)); comparison of time, fasting compared with fed (\( P^2 \)); and the interaction between group and time (\( P^3 \)).
Glutamine kinetics

- The Ra of glutamine during fasting was not significantly different between the 3 groups (Table 4), although it was slightly higher than the values reported previously by us in healthy term infants soon after birth (539 ± 99 μmol·kg⁻¹·h⁻¹) (14). In response to feeding, there was a significant decrease in glutamine Ra (P < 0.001). As shown, the decrease in glutamine Ra was the consequence of a decrease in the de novo synthesis of glutamine. Our calculation of the glutamine released from protein breakdown is higher than the actual amount released because the present data do not allow a true estimate of proteolysis during the fed state because of the entry of phenylalanine from enteral feeding.

Glutamine kinetics

- The concentration of plasma urea nitrogen was higher in the glutamine group during fasting than in the control or glycine group (Table 5). There was no significant change in plasma urea nitrogen concentration during feeding. The Ra of urea, measured by ¹⁵N₂ urea tracer dilution, was 132 μmol·kg⁻¹·h⁻¹ in the control infants. The urea Ra was significantly higher during fasting in the glutamine and glycine groups. In response to feeding, there was a significant decrease in urea Ra (P < 0.05). The fraction of urea derived from plasma glutamine ranged from 2% to 5% during fasting and increased to a range of 3–7% during feeding.

Amino acids

As anticipated, plasma concentrations of serine were higher in the glycine-supplemented infants than in the other 2 groups of infants (Table 6). Plasma arginine, citrulline, and ornithine concentrations were higher in the glutamine- and glycine-supplemented infants than in the control infants.

Correlations

A negative correlation was observed between the Ra of QN and the rate of urea synthesis in the control group (ρ = −0.72; Figure 2). In contrast, a positive correlation between the Ra of QN and urea synthesis was seen in the glutamine group (ρ = 0.75). The glycine group showed no correlation between the Ra of QN and urea Ra.

DISCUSSION

Data from the present study showed that, in prematurely born infants who are gaining weight normally, enteral administration of glutamine in combination with formula feeding does not affect the systemic Ra of glutamine (Table 4). Glutamine appears to be metabolized primarily in the splanchnic (gut) compartment and is associated with an increased rate of urea synthesis (Table 5). In addition, glutamine and glycine supplementation resulted in an

### Table 4

<table>
<thead>
<tr>
<th>Glutamine kinetics</th>
<th>Glutamine Ra</th>
<th>Glutamine de novo synthesis</th>
<th>Glutamine from proteolysis</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Fasting</td>
<td>Fed</td>
<td>Fasting</td>
</tr>
<tr>
<td></td>
<td>μmol·kg⁻¹·h⁻¹</td>
<td>μmol·kg⁻¹·d⁻¹</td>
<td></td>
</tr>
<tr>
<td>Glutamine group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 9)</td>
<td>599 ± 120</td>
<td>102 ± 12</td>
<td>538 ± 92</td>
</tr>
<tr>
<td>Glycine group</td>
<td>678 ± 112</td>
<td>108 ± 13</td>
<td>604 ± 111</td>
</tr>
<tr>
<td>Control group</td>
<td>655 ± 145</td>
<td>101 ± 16</td>
<td>529 ± 117</td>
</tr>
<tr>
<td>P&lt;sup&gt;2&lt;/sup&gt;</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>P&lt;sup&gt;gt&lt;/sup&gt;</td>
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<td>&lt;0.005</td>
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</tr>
<tr>
<td>P&lt;sup&gt;gt&lt;/sup&gt;</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
<td>—</td>
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</tbody>
</table>

### Table 5

<table>
<thead>
<tr>
<th>Urea kinetics</th>
<th>Plasma urea nitrogen</th>
<th>Urea Ra</th>
<th>Percentage of urea nitrogen from glutamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasting</td>
<td>Fed</td>
<td>Fasting</td>
</tr>
<tr>
<td>Glutamine group (n = 9)</td>
<td>2.35 ± 0.92</td>
<td>301 ± 110</td>
<td>4.66 ± 3.06</td>
</tr>
<tr>
<td>Glycine group (n = 9)</td>
<td>2.04 ± 0.48</td>
<td>201 ± 31</td>
<td>2.18 ± 0.57</td>
</tr>
<tr>
<td>Control group (n = 8)</td>
<td>0.88 ± 0.69</td>
<td>132 ± 50</td>
<td>2.33 ± 1.49</td>
</tr>
<tr>
<td>P&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.005</td>
<td>&lt;0.001</td>
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<tr>
<td>P&lt;sup&gt;gt&lt;/sup&gt;</td>
<td>NS</td>
<td>&lt;0.05</td>
<td>—</td>
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<tr>
<td>P&lt;sup&gt;gt&lt;/sup&gt;</td>
<td>NS</td>
<td>NS</td>
<td>—</td>
</tr>
</tbody>
</table>

1 ± SD.
2 Repeated-measures ANOVA for the difference between groups (P<sub>P</sub>); comparison of time, fasting compared with fed (P<sub>P<sub>gt</sub></sub>); and the interaction between group and time (P<sub>P<sub>gt</sub></sub>).
attenuation of the meal-associated increase in reamination of leucine (Table 3).

All infants in the present study were gaining weight normally and had recovered from their acute illnesses and therefore represented a healthy population of prematurely born infants. The data in the control group, when compared with our previously published data for full-term infants (14), showed that the preterm infants had a higher weight-specific Ra of phenylalanine, $Q_N$, and a lower rate of urea synthesis (term infants: $200.0 \pm 83.5 \mu mol \cdot kg^{-1} \cdot h^{-1}$; preterm infants: $132 \pm 50 \mu mol \cdot kg^{-1} \cdot h^{-1}$). In addition, the rate of reamination of leucine was also higher in the preterm control group than in the full-term infants (term infants: $104 \pm 32 \mu mol \cdot kg^{-1} \cdot h^{-1}$; preterm infants: $269 \pm 41 \mu mol \cdot kg^{-1} \cdot h^{-1}$). These data suggest that growth and nitrogen accretion (and possibly prematurity) are characterized by a lower rate of urea synthesis, a higher rate of branched-chain amino acid reamination, and a higher weight-specific rate of whole-body protein turnover. Because the full-term infants were studied soon after birth and were not yet gaining weight and because the infants in the present study were studied 5–6 wk after birth, the present data represent growth and nitrogen accretion. As with other parameters of nitrogen metabolism, the rate of turnover of glutamine was also higher in growing preterm infants. The higher rate of glutamine turnover was due to both higher rates of glutamine released from proteolysis and higher rates of de novo synthesis of glutamine. The responses of these growing preterm infants to feeding were qualitatively similar to those of full-term infants and adults (14, 26, 27), except for the increase in the Ra of phenylalanine, which suggests a lower rate of splanchnic extraction of phenylalanine. In contrast, there was no effect of feeding on the Ra of leucine (KIC) in growing infants or in full-term infants (studied during neonatal transition), which suggests a high rate of extraction of leucine and KIC in the splanchnic compartment.
Response to enteral glutamine

The concentration of glutamine and the tracer-measured Ra of glutamine in the plasma during fasting were not significantly different between the control and glutamine groups. These data suggest that enterally administered glutamine was entirely metabolized by the gut in the first pass and did not enter the circulation. These data are qualitatively consistent with those of other studies in adults and in neonatal animals and humans (8, 9, 26-28). The magnitude of splanchnic extraction of glutamine in healthy adults was reported to be ≈60–80% (10, 11). The higher glutamine metabolism by the gut in our study may have been related to the low dose of glutamine administered (18) and to the rapid rate of growth (weight gain) and nitrogen accretion in infants.

Orally administered glutamine did not have a significant effect on the whole-body rate of protein turnover. The effect of glutamine on skeletal muscle protein turnover has been shown to be related to an increase in the tissue glutamine concentration. Thus, the observed lack of effect of glutamine on protein turnover could be related to the lack of change in plasma or tissue glutamine concentrations as a result of the extensive metabolism of glutamine in the gut. The unchanged rate of systemic glutamine turnover in response to enteral glutamine, observed in the present study and in healthy adults in other studies (8, 29, 30), is not easily explained.

Our data also suggest that glutamine taken up by the gut is rapidly metabolized locally in the gut, as evidenced by the increase in plasma concentrations of urea, citrulline, arginine, ornithine, and alanine and by the increase in the rate of urea synthesis. The higher rate of urea synthesis was surprising and suggests a rapid metabolism of glutamine in the intestine and the splanchnic compartment. On the basis of these data, we propose the scheme shown in Figure 3 for the metabolism of glutamine in the splanchnic compartment. Glutamine taken up by the gut is converted to glutamate and ammonia by glutaminase. Glutamate serves as a precursor for citrulline, ornithine, and alanine and is also a respiratory fuel for the gut. Additionally, glutamate could serve as a precursor of aspartate in the liver, which, along with the ammonia, resulted in the observed increase in the rate of urea synthesis.

Glycine group

We elected to use glycine as the isonitrogenous control, although its appropriateness as the control can be argued because of the significant differences in the metabolism of glycine nitrogen and glutamine nitrogen. A mixture of alanine, proline, asparagine, serine, and glycine—as used by some investigators in animal studies—may be more appropriate (31, 32).

Isonitrogenous administration of glycine had no effect on the whole-body rate of protein turnover or on $Q_N$ turnover. However, oral glycine was associated with a significant increase in the rate of urea synthesis. Unlike in the glutamine group, the fraction of urea nitrogen derived from glutamine was unchanged in the glycine group, even though there was a 95% increase in the total rate of urea synthesis. Because glycine serves as a major nitrogen donor (sink), these data suggest that the catabolism of glycine via the glycine cleavage system resulted in the availability of ammonia, which was used for the increased urea synthesis and the formation of glutamate from $\alpha$-ketoglutarate, which results in a small increase in glutamine turnover in both the fasting and fed states. The increase in hepatic amino nitrogen from glycine may have also decreased glutamine uptake by the liver and, together with the increase in glutamine Ra, resulted in an increased plasma concentration of glutamine in the blood (Table 6). Glycine could also serve as a source for serine, which could be used for protein synthesis when the supply of the latter is limiting. As anticipated, glycine administration resulted in an increased plasma concentration of serine, glutamine, citrulline, and arginine. A scheme for glycine metabolism in the splanchnic compartment is shown in Figure 4. There was no significant change in plasma insulin or GLP-1 concentrations. Gannon et al (33) recently observed no effect of oral glycine on plasma glucose and insulin concentrations in healthy adult volunteers.

The correlations between the Ra of $Q_N$ and urea synthesis in the 3 groups reflect the metabolism of glutamine because the Ra of glutamine is positively related to the Ra of $Q_N$ (14). In the control group, a high rate of $Q_N$ turnover, and therefore of glutamine turnover, is the consequence of the high rate of whole-body nitrogen turnover for various synthetic processes. In the glutamine group, the data suggest an increased participation of glutamine in urea synthesis (Figure 2). In contrast, because glycine nitrogen does not participate in transamination, there was no relation between $Q_N$ turnover and urea synthesis.

In summary, data from the present study show that enteral administration of glutamine and glycine in growing infants results in an increased rate of urea synthesis as a consequence of their unique metabolism in the splanchnic compartments. Oral glutamine appears to be entirely metabolized in the gut (and liver), with little or no effect on the whole-body rate of protein turnover. Oral glycine is also extensively metabolized in the splanchnic compartment (liver) and results in increased plasma concentrations of several nonessential amino acids and an in-
creased rate of urea synthesis. Finally, growth and nitrogen accretion in preterm infants are characterized by a lower rate of urea synthesis and a higher rate of whole-body nitrogen turnover.

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