**Laurence D van der Schoor, P J Reeds, F Stellaard, J D L Wattimena, P J J Sauer, H A Büller, J B van Goudoever**

**SMALL INTESTINE**

**Lysine kinetics in preterm infants: the importance of enteral feeding**

**Introduction:** Lysine is the first limiting essential amino acid in the diet of newborns. First pass metabolism by the intestine of dietary lysine has a direct effect on systemic availability. We investigated whether first pass lysine metabolism in the intestine is high in preterm infants, particularly at a low enteral intake.

**Patients and methods:** Six preterm infants [birth weight 0.9 (0.1) kg] were studied during two different periods: period A (n = 6): 40% of intake administered enteraly, 60% parenterally; lysine intake 92 (6) \(\mu\)mol/(kg·h); and period B (n = 4): 100% enteral feeding; lysine intake 100 (3) \(\mu\)mol/(kg·h). Dual stable isotope tracer techniques were used to assess splanchnic and whole body lysine kinetics.

**Results:** Fractional first pass lysine uptake by the intestine was significantly higher during partial enteral feeding (period A 32 (10)% \(v\) period B 18 (7)%; \(p < 0.05\)). Absolute uptake was not significantly different. Whole body lysine oxidation was significantly decreased during full enteral feeding (period A 44 (9)% \(v\) period B 17 (3)% \(\mu\)mol/(kg·h); \(p < 0.05\)) so that whole body lysine balance was significantly higher during full enteral feeding (period A 52 (25)% \(v\) period B 83 (3)% \(\mu\)mol/(kg·h); \(p < 0.05\)).

**Conclusions:** Fractional first pass lysine uptake was much higher during partial enteral feeding. Preterm infants receiving full enteral feeding have lower whole body lysine oxidation, resulting in a higher net lysine balance, compared with preterm infants receiving partial enteral feeding. Hence parenterally administered lysine is not as effective as dietary lysine in promoting protein deposition in preterm infants.

**METHODS**

**Patients**

The study protocol was approved by the Erasmus University Institutional Review Board. Written informed consent was obtained from parents. Splanchnic and whole body lysine kinetics were quantified in preterm infants during partial enteral feeding (period A) and full enteral feeding (period B). Patients eligible for this study were premature infants with a birth weight ranging from 750 g to 1250 g who were appropriate for gestational age according to the charts of

**Abbreviations:** LRP, lysine release from protein breakdown; NOLD, non-oxidative lysine disposal; PDB, Pee Dee Belemnite
Schematic overview of study days 1 and 2 during period A, and study days 3 and 4 during period B.

Figure 1

Lysine kinetics in preterm infants

Excluded from the study were infants who had congenital anomalies, or gastrointestinal or liver diseases. CRIB scores on the first day of life were all below 5. The clinical characteristics of the neonates are shown in Table 1. They received a nutrient regimen according to our feeding protocol: a combination of breast feeding or formula (Nenatal; Nutricia, Zoetermeer, the Netherlands) and parenteral nutrition containing glucose, amino acids (Primene 10%; Clintec Benelux NV, Brussels, Belgium), and lipids (Intralipid 20%; Fresenius Kabi, Den Bosch, the Netherlands). Nenatal was given as sole enteral nutrition 12 hours before the start of the study and during the study days.

Protocol

The study design consisted of two periods of two consecutive study days (period A—study days 1 and 2, and period B—study days 3 and 4) (Fig 1). During period A, infants received 40% enteral and 60% parenteral feeding, and during period B they received full enteral feeding. During period A, infants were implanted with both an arterial and intravenous catheter for infusion of tracers and withdrawal of blood samples. During period B, a peripheral intravenous catheter was available for infusion of tracers; blood samples were collected by heelstick. Withdrawing blood via a heelstick is obtaining blood from arterial capillaries while the heel is heated before collection to ensure easy collection. We assume that similar to the heated hand box technique, there is no difference in plasma enrichment.

In order to collect breath samples from these infants, we used the method described by Perman et al who used a nasal tube.22 This method has been used in children and preterm infants for collection of expiratory CO₂ recovery after administration of [¹³C] labelled substrates.23 24 We have validated this technique for the use of oxidation studies in preterm infants (Van der Schoor, unpublished data). Briefly, a 6 Fr gastric tube (6 Ch Argyle; Sherwood Medical, Tullamore, Ireland) was placed 1–1.5 cm into the nasopharynx, and a sample of 15 ml end tidal breath was taken slowly with a syringe. Duplicate aliquots of expired air from each sampling point were stored in vacutainers for analysis.

Three different stable isotope infusions were performed during each study day. Firstly, a primed two hour infusion (10.02 μmol/kg and 10.02 μmol/(kg·h)) of [¹³C]bicarbonate during each study day. Firstly, a primed two hour infusion (10.02 μmol/kg and 10.02 μmol/(kg·h)) of [¹³C]bicarbonate
and 9.31 mol\/(kg h)). First pass lysine uptake as a fraction of dietary lysine intake was significantly higher during restricted enteral lysine intake (period A 32 (10)\% v period B 18 (7)\%; p<0.05). However, splanchnic lysine uptake in absolute amounts was not significantly different during full enteral intake (period A 10 (3) v period B 18

### Table 2  Lysine, protein, carbohydrate, fat, and energy intakes during periods A and B

<table>
<thead>
<tr>
<th></th>
<th>Period A</th>
<th>Period B</th>
</tr>
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<tbody>
<tr>
<td><strong>Total lysine intake</strong></td>
<td>92 (13)</td>
<td>100 (6)</td>
</tr>
<tr>
<td>Enteral</td>
<td>33 (5)</td>
<td>100 (6)</td>
</tr>
<tr>
<td>Parenteral</td>
<td>59 (15)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total protein intake</strong></td>
<td>3.4 (0.3)</td>
<td>3.5 (0.2)</td>
</tr>
<tr>
<td>Enteral</td>
<td>1.2 (0.2)</td>
<td>3.5 (0.2)</td>
</tr>
<tr>
<td>Parenteral</td>
<td>2.2 (0.6)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total carbohydrate intake</strong></td>
<td>10.6 (2.2)</td>
<td>11.5 (0.7)</td>
</tr>
<tr>
<td>Enteral</td>
<td>3.7 (0.6)</td>
<td>11.5 (0.7)</td>
</tr>
<tr>
<td>Parenteral</td>
<td>6.9 (2.0)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total fat intake</strong></td>
<td>4.1 (0.9)*</td>
<td>6.5 (0.4)</td>
</tr>
<tr>
<td>Enteral</td>
<td>2.0 (0.3)</td>
<td>6.5 (0.4)</td>
</tr>
<tr>
<td>Parenteral</td>
<td>2.0 (0.9)</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total energy intake</strong></td>
<td>92 (11)*</td>
<td>123 (4)</td>
</tr>
<tr>
<td>Enteral</td>
<td>38 (7)</td>
<td>123 (4)</td>
</tr>
<tr>
<td>Parenteral</td>
<td>52 (10)</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are means (SD); n = 6 in period A and n = 4 in period B.  
*p<0.05
Whole body lysine oxidation was significantly lower during full enteral feeding (fig 2). Whole body lysine oxidation accounted for 50 (19)% of total lysine intake during partial enteral nutrition whereas only 17 (3)% of total lysine intake was oxidised during full enteral feeding (p<0.05). The non-oxidative disposal of lysine (NOLD; period A 222 (36) µmol/(kg·h)) and lysine release of protein breakdown (LRP; period A 214 (39) µmol/(kg·h)) were not significantly different during both periods. The resulting net lysine balance was significantly higher during full enteral feeding (fig 2).

DISCUSSION

The main purpose of this study was to measure lysine kinetics in preterm infants to gain insight into the importance of splanchnic tissues in relation to whole body lysine kinetics. In addition, we wished to quantify first pass lysine metabolism during both full enteral feeding and significantly lower enteral intake. Our data demonstrated that in preterm infants receiving full enteral feeding, whole body lysine oxidation was significantly lower and lysine balance significantly higher than in preterm infants receiving partial enteral feeding. Approximately one third of dietary lysine is removed by the splanchnic region during partial enteral feeding compared with a lower fraction (18%) during full enteral feeding. Furthermore, we could not detect any first pass lysine oxidation by splanchnic tissues in either feeding period.

It is of interest to note our results in neonatal pigs receiving a low protein diet, with regard to the high fractional first pass lysine uptake observed under a restricted enteral amino acid supply in preterm infants. The high splanchnic extraction as a fraction of dietary intake in preterm infants receiving partial enteral feeding is also consistent with the high values for protein turnover observed in immature rats, with a maximal contribution of the intestine to whole body protein synthesis attained at weaning.

A high first pass uptake may also be caused by incomplete digestion and absorption of dietary proteins and amino acids. However, studies in adults and infants have shown a high digestibility of approximately 98% of milk proteins and cereal protein. This indicates that probably all dietary milk proteins are absorbed by the intestinal mucosa and are not lost in faeces. Thus lysine that does not appear in the systemic circulation is utilised by the intestine, and is not lost via faeces. Indeed, Stoll et al have demonstrated in neonatal pigs that splanchnic tissues have high requirements for amino acids and are proving to be an important modulator of whole body amino acid availability. High splanchnic lysine extraction in preterm infants in their first weeks of life may be necessary to provide amino acids for their high intestinal protein synthesis during a period of rapid adaptation to enteral feeding. It is well known, for example, that the presence of nutrients in the intestinal lumen provides a marked stimulus to intestinal growth.

In contrast with our finding in piglets, intraluminal lysine or lysine taken up by splanchnic tissues is apparently not oxidised, implying that intestinal lysine metabolism in preterm infants only includes protein synthesis. In other words, these results suggest that the premature gut has a...
most efficient use of the essential amino acid lysine, and plays an important role in channelling dietary amino acids to peripheral tissues.

Our second aim was to determine whole body lysine kinetics in relation to two different enteral lysine supplies. Surprisingly, in preterm infants on full enteral feeding, whole body lysine oxidation was significantly lower in comparison with partial enteral feeding. Decreased whole body lysine oxidation suggests a protein sparing mechanism during full enteral feeding in preterm infants. Consequently, lysine balance increased significantly as dietary lysine intake was augmented. In concordance with these oxidation results, plasma lysine concentration was significantly higher during partial enteral feeding. During this feeding period, amino acids were administered via both the venous circulation and the stomach, in contrast with full enteral feeding. The higher plasma lysine concentration during partial enteral feeding may be a result of our direct intravenous infusion and significantly lower first pass lysine uptake by the gut. In addition, lower whole body lysine oxidation during partial enteral feeding may have contributed to a higher plasma lysine level. Another explanation for this observation may be that total amino acid intake was nutritionally better balanced during full enteral feeding, which resulted in an improved lysine balance. In other words, it might indicate that lysine is not the first limiting essential amino acid in the diet under the conditions of partial enteral feeding. Otherwise, lysine oxidation would have been lower during partial enteral feeding.

The apparent lower whole body lysine oxidation and higher lysine balance during full enteral feeding were similar to values obtained by Duffy and Pencharz.33 Their study compared the effect of feeding route (intravenous or enteral) on protein metabolism of the neonate and concluded that orally fed neonates utilise amino acids for synthesis more efficiently than total parenterally fed infants.33 Early parenteral supply of amino acids is given to prevent protein loss but the present study clearly shows that enteral feeding is a far more efficient way of feeding preterm infants. Unfortunately, intestinal immaturity often impairs early full enteral feeding but our data suggest that maximal effort should be paid to initiate enteral feeding as soon as possible in preterm infants.34

There were some limitations in our study design. Energy intake is one of the factors that influence protein synthesis rate. Although lysine intakes during both feeding periods were almost identical, energy intake was significantly different between the feeding periods. Intake of metabolic energy by partial enterally fed infants was 74% of the intake of enterally fed infants. Parenterally fed neonates appear to require only 75% of the energy of enterally fed infants to achieve the same growth rate because of the lower energy expenditure of the splanchnic tissues during parenteral nutrition. In addition, enterally fed infants have some energy losses in stool due to malabsorption. In a previous study, we found no significant effect of reducing energy intake from 120 to 100 kcal/(kg×day) on nitrogen excretion and protein deposition in preterm infants.35 Van Goudoever et al. showed in preterm infants that the overall pattern of fuel utilisation was unaffected by the mode of feeding.36 Therefore, we speculate that the higher energy intake during full enteral feeding did not significantly influence lysine kinetics.

Another issue relevant to the study design is the different postnatal ages at both study periods. Infants were older during period B, which may have influenced lysine kinetics. However, it was not feasible in our study design to have infants on full enteral feeding on day 7. On the other hand, it was not ethically justified to have infants on partial enteral feeding at a postnatal age of three weeks for a period of at least five days in order for them to adjust to a reduced enteral feeding rate. In addition, we wanted to evaluate lysine kinetics in preterm infants who were treated according to standard neonatal intensive care feeding regimens.

In conclusion, we have shown that splanchnic tissues have a high rate of metabolism and use almost one third of dietary lysine intake in preterm infants undergoing partial enteral feeding. However, although lysine is catabolised by the whole body to a great extent, especially during partial enteral feeding, it is not catabolised by splanchnic tissues during first pass in preterm infants. Furthermore, lysine balance was much higher during full enteral feeding compared with partial enteral feeding. Taken together, the results indicate that parenterally administered lysine is not as effective as dietary administered lysine in promoting protein deposition in preterm infants in their first weeks of life.

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APPENDIX
Lysine flux was calculated as:

\[ Q_v = I \times [ (E_i/E_p) - 1 ] \]  

where \( Q_v \) is flux of the intravenous lysine tracer (\( \mu \text{mol/(kg} \times \text{h}) \)), \( I \) is the lysine infusion rate (\( \mu \text{mol/kg} \times \text{h}) \)), and \( E_i \) and \( E_p \) are the enrichments (mol% excess) of [\(^{13}\text{C}\) lysine or \(^2\text{H}_4\) lysine] in the lysine infusate and in plasma at steady state, respectively.

First pass lysine uptake was calculated as:

\[ U = [(Q_{lg} - Q_v)/Q_{lg}] \times 1 \]

where \( U \) is first pass lysine uptake (\( \mu \text{mol/kg} \times \text{h}) \)), \( Q_{lg} \) is flux of the intragastric lysine tracer (\( \mu \text{mol/kg} \times \text{h}) \)), and \( I \) is enteral lysine intake (\( \mu \text{mol/kg} \times \text{h}) \)).

At steady state, the amount of lysine entering the plasma pool should be equal to the amount of lysine leaving the pool. Lysine can enter the pool either by being released from proteins as result of breakdown or through diet. Lysine leaving the pool may be either oxidative disposal or non-oxidative disposal.

\[ Q = I + LRP = Ox + NOLD \]

where \( Q \) is flux of the lysine tracer (\( \mu \text{mol/kg} \times \text{h}) \)), LRP is the amount of lysine released from protein via protein breakdown (\( \mu \text{mol/kg} \times \text{h}) \)), \( Ox \) is the rate of lysine oxidation (\( \mu \text{mol/kg} \times \text{h}) \)), and \( NOLD \) is the rate of non-oxidative lysine disposal (\( \mu \text{mol/kg} \times \text{h}) \)).
Net lysine balance, an index of protein deposition, was calculated as:

\[ B = NOLD - LRP \]  

(4)

where \( B \) is lysine balance (\( \mu \text{mol/(kg.h}) \)).

Whole body CO₂ production was estimated as:

\[ \text{Body CO}_2 \text{ production} = i_k \times (E_{EB}/E_{EB}) - 1 \]  

(5)

where \( i_k \) is the infusion rate of \([^{13}\text{C}]\)sodium bicarbonate (\( \mu \text{mol/(kg.h}) \)), \( E_{EB} \) is enrichment (mol% excess) of \([^{13}\text{C}]\)bicarbonate in the bicarbonate infusate and \( E_{EB} \) is \([^{13}\text{C}]\)CO₂ enrichment at plateau during the \([^{13}\text{C}]\)sodium bicarbonate infusion (mol% excess). This equation does not correct for retention of \([^{13}\text{C}]\)bicarbonate in different body pools and will overestimate CO₂ production rate. However, the same correction factor has to be applied to quantify the lysine oxidation rate (eqn (6)). Consequently, lysine oxidation rate is not overestimated.26

As described previously, lysine oxidation was calculated by multiplying recovery of \([^{13}\text{C}]\)lysine in expiratory air with the rate of appearance of lysine.27 The fraction of lysine oxidised was measured according to the following equation, assuming a constant VCO₂ (rate of CO₂ production) during the study, that lasted seven hours:

\[ \text{Fraction of lysine oxidised} = \frac{[E_{E1} \times x_{A1}]/[E_{EB} \times x_{B1}]}{[E_{E2} \times x_{B2}]} \times 100 \]  

(6)

where \( E_{E1} \) and \( E_{E2} \) are \([^{13}\text{C}]\)enrichments (mol% excess) at steady state during intravenous \([^{13}\text{C}]\)lysine and a constant VCO₂ during intravenous \([^{13}\text{C}]\)lysine and intravenous \([^{2}\text{H}_4]\)lysine tracer flow rates and level of protein hydrolysis in the human jejunum after \([^{15}\text{N}]\)milk flow rates and level of protein hydrolysis in the human jejunum after \([^{15}\text{N}]\)milk ingestion.

The metabolism of enterally administered \([^{13}\text{C}]\)lysine to CO₂ is complicated by the fact that some of the lysine tracer is absorbed and thereby labels the arterial blood. The non-first pass oxidation of \([^{13}\text{C}]\)labelled lysine that was administered enterally but was not absorbed and entered the body at study day 2 can be calculated as:

\[ \text{Non-first pass oxidation of enterally administered lysine} = \frac{[E_{E1C} \times x_{I1C}]}{[E_{EB} \times x_{B1}]} \times 100 \]  

(7)

where \( E_{E1C} \) and \( E_{E2C} \) is the \([^{13}\text{C}]\)enrichment (mol% excess) in plasma during intragastric \([^{13}\text{C}]\)lysine infusion on study day 2, and \( E_{EB} \) is the \([^{13}\text{C}]\)enrichment in plasma during intravenous \([^{13}\text{C}]\)lysine infusion on study day 1.

Total lysine oxidation on study day 2 is calculated as described previously:

\[ \text{Total lysine oxidation} = \text{Recovery} \times Q_o \]  

(8)

where \( Q_o \) is the flux of the intravenous \([^{2}\text{H}_4]\)lysine tracer (\( \mu \text{mol/(kg.h}) \)).

First pass lysine oxidation can thus be calculated as:

First pass lysine oxidation = eqn (9) – eqn (8).  

(10)