INTESTINAL AMINO ACID METABOLISM IN NEONATES

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INTESTINAL AMINO ACID METABOLISM IN NEONATES

Aminozuur metabolisme door de darm bij neonaten

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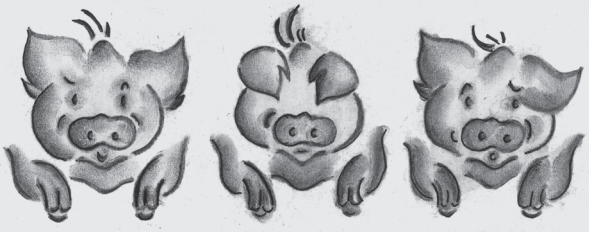
INTRODUCTION



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Introduction

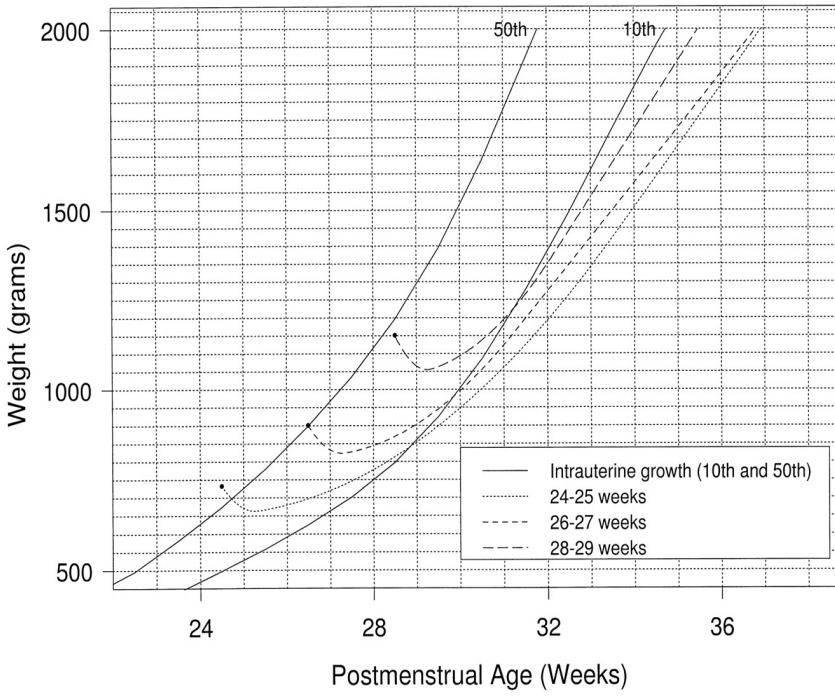
Preterm infants and postnatal growth

At birth infants can be classified on the basis of their gestational age or on their weight. Infants born before 37 weeks of gestation are premature, those born with a weight less than 2500 g are low birth weight infants (LBW), those weighing less than 1500 g are very low birth weight infants (VLBW), and those less than 1000 g are extremely low birth weight infants (ELBW). If a neonate weights less than the 5th percentile for its gestational age, it is small for gestational age (SGA), if not, it is appropriate for gestational age (AGA). Intrauterine growth retardation (IUGR) is often used interchangeably with SGA, although its meaning is subtly different. To be IUGR implies that the infant's full growth in utero was not achieved, for example, due to placental inadequacy. A SGA infant born to parents who are both less than the fifth centile for height may have achieved its full genetic potential in utero and is not IUGR. Similarly, LBW infants may be term IUGR, term SGA, appropriately grown preterm, SGA preterm, or IUGR preterm.

Over the past two decades neonatal populations and nutritional standards have changed and the number of preterm infants with a birthweight <1000 g has increased. Furthermore, the advances in the ventilatory support, resuscitation, and pharmacological management, have increased the survival rate of preterm infants from 15 to 75%. [1-6] However, the neonatologists involved with the care of these newborns, are now faced with a constellation of problems, which include prevention of morbidity. The importance of the gastrointestinal system in meeting these challenges is well recognized. According to the recommendations of the American Academy of Pediatrics, weight gain should occur at the same rate as intra-uterine weight gain (14-20 g/(kg·d) [7,8], but several studies have shown that the growth rate of preterm infants does not reach intra-uterine growth rates, as is shown in Figure 1. [9-11] Postnatal linear growth retardation (body length <10th percentile of the Lubchenco's curves at discharge) [12] was observed in 22% of the AGA preterm infants at birth in an epidemiological study including approximately 800 preterm infants with a birthweight

<1500g. [13] The postnatal lag of growth is related to long term growth and neurodevelopmental delays at least through schoolage [14-18] and possibly, into adulthood. [19] Despite all this, information about protein and energy requirements of VBLW infants is sparse. The observed postnatal lag of growth defines critical needs for further studies about the nutritional requirements for growth of preterm infants, how they should be fed, and whether improved growth and developmental outcomes can be achieved with for instance earlier and more sophisticated postnatal nutrition.

Figure 1. Average body weight versus postmenstrual age in weeks for infants with gestational ages 24 to 25 weeks (dotted line), 26 to 27 weeks (short dashes), and 28 to 29 weeks (long dashes) from Ehrenkranz et al. [10] The reference intrauterine growth curves were plotted using the smoothed 10th and 50th percentile birth weight data reported by Alexander et al. [8]



Still, nutritional regimens differ greatly from one neonatal intensive care unit to another, particularly for neonates with critical illness or VLBW infants in the first weeks of life. In these neonates, total parenteral nutrition is often gradually advanced over the first 2 weeks of life because of concerns of feeding intolerance and eventually necrotizing enterocolitis (NEC), despite the fact that parenteral nutrition for preterm infants theoretically should simulate intrauterine feeding. The presumed incapability to metabolize nutrients is often ascribed to the stress of birth, immature metabolic pathways, and pathophysiologic processes associated with different disease states, such as infection. Specifically, the introduction of amino acids in the first days of life in ill and premature infants is often limited due to concerns over these infants' inability to metabolize specific amino acids, which could result in uremia, hyperaminoacidemia, and metabolic acidosis. [20] However, several studies have examined the issue of early initiation of parenteral nutrition, and they have shown that the preterm infant is capable of metabolizing parenterally administered amino acids from birth onwards. [21,22] There were no recognizable metabolic derangements, like hyperammonemia, metabolic acidosis or distorted plasma amino acids patterns. A rise in blood urea nitrogen, which is often observed after the start of total parenteral nutrition, is not an adverse effect or sign of toxicity. Rather, an increase in urea nitrogen is a normal accompaniment of an increase in the intake of amino acids. [23]

Evidence is accumulating that the amino acid requirements for neonates receiving total parenteral nutrition are significantly lower (25-50%) than those for oral feeding. [24-26] On the other hand, the high rates of weight gain we are trying to establish in preterm infants are only possible with a well-developed gut that is able to absorb and digest a high intake per mass unit. A well-developed gut function reduces the need for prolonged parenteral nutrition, consequently reducing morbidity and will, therefore, have a significant health and economic impact.

A growing body of evidence suggests that the gut modulates amino acid flux and inter-organ relationships in various metabolic states. This may be particularly true during the absorptive period, when the intestine: *1)* controls the amino acid absorption, *2)* may modulate the different metabolic fates of absorbed amino acids, and *3)* consequently influences the availability of amino acids for the peripheral tissues. For example, dietary amino acids are major fuels for the small intestinal mucosa (e.g. glutamine), [27-30] and are essential precursors for intestinal synthesis of glutathione, nitric oxide, polyamines, purine and pyrimidine nucleotides, and are obligatory for maintaining the intestinal mucosal mass and integrity. [31-33]

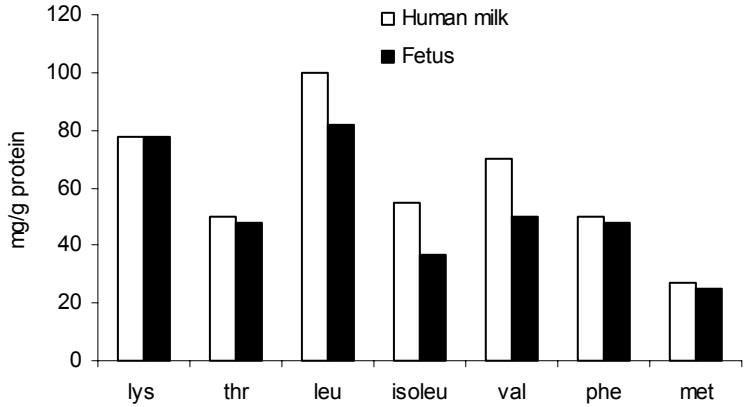
Given the key role of the intestine in the maintenance of neonatal health, there has been considerable interest in the intestinal amino acid requirements. In specific, our interest is on the developmental and physiological aspects of intestinal metabolism in relation to enteral feeding in preterm infants. Of the many factors that affect neonatal gut growth, probably the most physiologically stimulus is enteral nutrition. Oral nutrients act directly by supplying nutrients for growth and oxidative metabolism of mucosal epithelial cells, and indirectly by triggering the release of gut hormones, local growth factors, and activating neural pathways. Because of the intestinal absorptive and secretory functions, the energy requirements of the intestine are high. Moreover, the gut requires energy for the synthesis of digestive enzymes, and the renewal of enterocytes. In line with these high intestinal substrate requirements, enteral nutrient deprivation is known to adversely affect gut DNA and protein mass, cell proliferation, villus height, and protein synthesis. [34-36]

Regarding the different metabolic fates of intestinally absorbed amino acids; the catabolic and anabolic utilization of amino acids within the gut might significantly influence the requirements for specific amino acids. The impact of intestinal metabolism is particularly important for rate-limiting essential amino acids, i.e. lysine in the formula fed neonate (Figure 2), or for amino acids such as threonine that play a critical role in the secretory function of the gut. [37,38] Besides

non-essential amino acids, there is limited knowledge of the essential amino acids required for intestinal growth and function. Moreover, little is known about the interaction between intestinal essential amino acid requirements and the dietary protein intake. A recent study with piglets, however, showed that lysine oxidation in the portal-drained viscera (PDV; intestine, pancreas, spleen and stomach) is virtually absent in piglets during protein restriction, whereas the total lysine use of the PDV is largely unaffected by a low protein diet. [39]

The focus of this thesis is to evaluate the splanchnic metabolism, which includes the intestine and the liver, of two essential amino acids in particular, e.g. lysine and threonine. Because of limitations in studying nutrient metabolism in preterm neonates, several studies in neonatal animals have been performed to quantify protein metabolism in vivo.

Figure 2. Amino acid composition of human milk and the fetus



Lys, lysine; thr, threonine; leu, leucine; isoleu, isoleucine; val, valine; phe, phenylalanine; met, methionine.

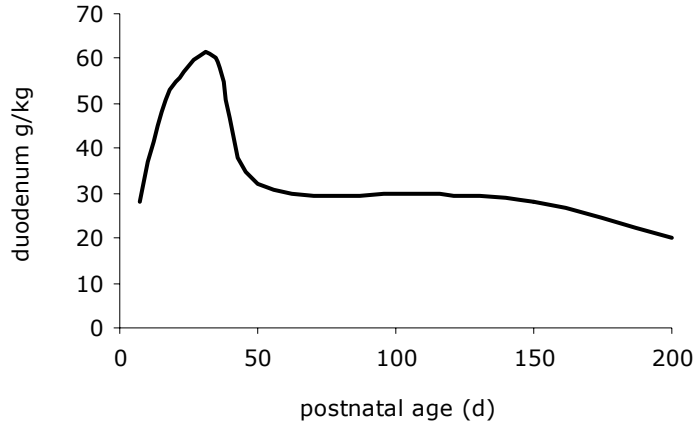
The neonatal pig compared to the human neonate

Clinical research in the human neonate (especially the preterm neonate) is generally limited by several ethical constraints and factors:

1) ethical and practical restrictions that limit manipulation of feeding regimens and design of experiments, *2)* a heterogeneous population with different illnesses that complicate metabolic demands, *3)* validation of nutritional assessment techniques in this particular age group, *4)* given the limited access to most organ systems, the approaches used must allow extrapolation from the sampled data to events in otherwise inaccessible areas, and *5)* the maximal information possible must be obtained from a study owing the need for studying the smallest number of subjects necessary to evaluate adequately the proposed hypothesis.

The use of an animal model permits experimental studies involving a healthy population, specifically designed feeding regimens, and more invasive and sensitive methods that would not be accepted in humans. Such a model can provide a comprehensive and profound picture of many fields in the neonatal nutrition research.

Figure 3. Duodenal growth rate of neonatal pigs, expressed as a fraction of whole-body weight



Ideally, an animal model for the study of nutrient metabolism should meet the following criteria: *1)* similarities in nutrient metabolism, *2)* reasonable cost, *3)* easy to obtain and house, and *4)*

permissibility for invasive studies. The neonatal pig fulfills these requirements. The neonatal piglet is considered to be an appropriate model for the human infant due to similarities in anatomy, physiology and metabolism. In specific, the amino acid metabolism is comparable between the piglet and the human infant, because both species show similar patterns of indispensable amino acid requirements. [40-42] The average gestational age and birth weight of the term infant are approximately twice those of the piglet. [43] In contrast, the piglet grows and develops more rapidly than the preterm infant (Table 1). [44]

Table 1. Comparison of the growth rates between the neonatal pig and the human infant

Species	*Gestation*	*Birth Weight*	*Growth Rate*
	d	*g*	*g/(kg·d)*
Infant	280	3500	12.5
Pig	120	1500	4.2

Regarding the gastrointestinal tissues, the intestine grows rapidly in the first weeks of life, and undergoes a profound increase in weight compared to the total body weight (Figure 3). The gastrointestinal development and physiology of the neonatal pig is similar in preterm infants. [45-49] And finally, term piglets and preterm human infants also have many similarities in body composition of protein and fat; the greatest number of similarities occurs when the human infant is born between 23 and 31 weeks of gestation (Table 2).

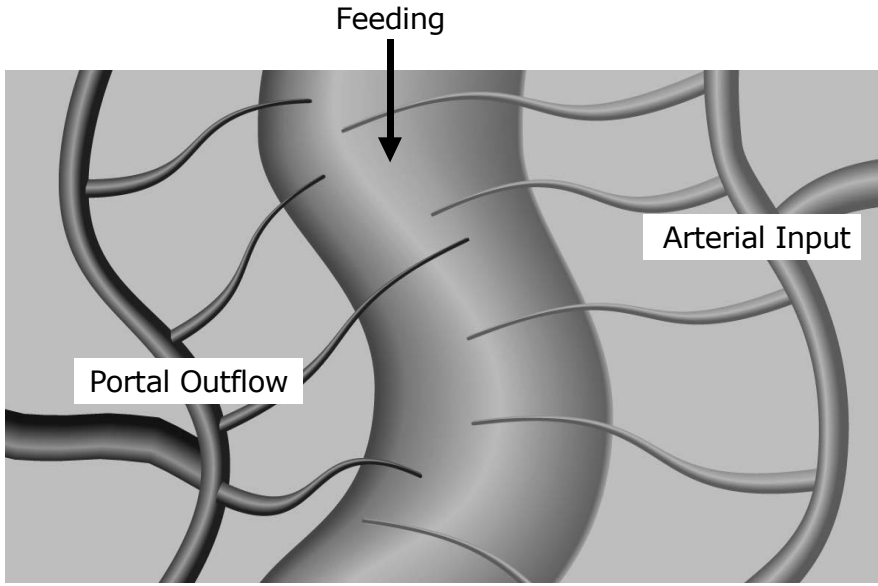
Table 2. Protein and fat composition of the neonatal pig and the human infant

	Pig	*Infant*	*Infant*	*Infant*	*Infant*	*Infant*
	At term	*23 wk*	*26 wk*	*31 wk*	*37 wk*	*40 wk*
Protein (%)	11.3	8.8	8.8	10.6	11.8	11.9
Fat (%)	1.1	1.0	1.0	2.3	7.4	16.0

As a result of the reported similarities between the two species, the neonatal piglet has been used as a model for growth and development of the gut [50-53] and as a model for the TPN fed human infant. [50,54-56]

A complicating factor in studying intestinal metabolism is that the intestinal mucosa receives and utilizes nutrients from two sources, from the diet (brush border membrane) and the systemic circulation (basolateral membrane) (Figure 4). [57-59] Therefore, we have developed an arterial and portal catheterized piglet model to study visceral amino acid metabolism in growing, fed piglets. [60] We performed studies in their first weeks of life, because the impact of intestinal substrate metabolism on whole-body substrate metabolism should be maximal during this period due to the relatively high growth rate. In these studies on intestinal protein metabolism, we used stable isotope labeled tracers of several different amino acids and glucose.

Figure 4. Schematic representation of the gut in the piglet model



Stable isotope techniques

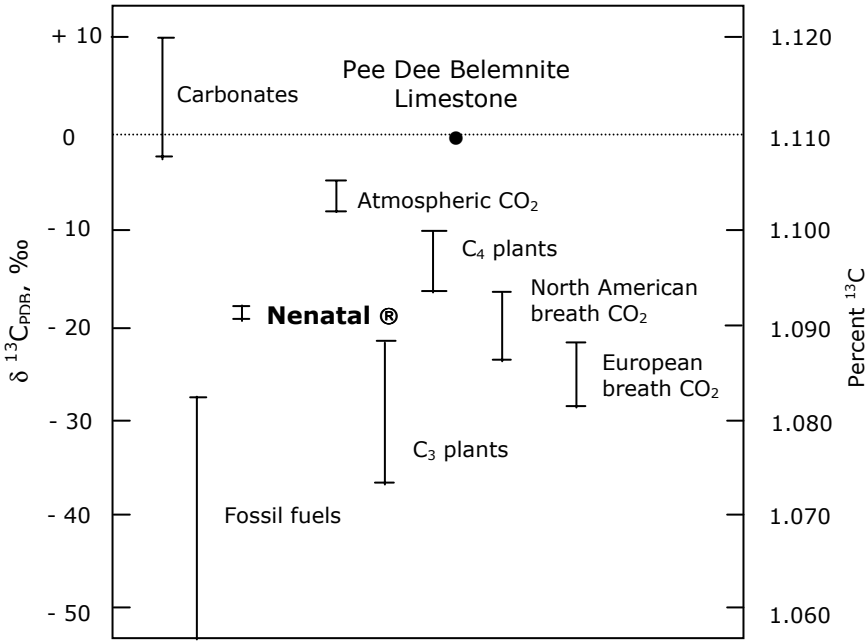
To study metabolic pathways and turnover in animals and humans, stable isotopes have been used for the last 65 years. Stable isotopes take advantage of being non-radioactive, and thus, can safely be used in preterm infants. Moreover, the primed constant intravenous infusion of amino acids labeled with stable isotopes has become the reference method for studying whole-body protein dynamics. [61-63] Importantly, the use of portal vein catheterization has demonstrated the important contribution of intestinal and hepatic tissues to whole-body protein metabolism. [64,65] To assess splanchnic amino acid metabolism in a non-invasive manner in neonates, the constant gastric or intraduodenal infusion of a labeled amino acid can be used in combination with the intravenous infusion of different labeled tracer of the same amino acid.

This dual route of infusion allows the calculation of "first-pass splanchnic extraction"; a reflection of the intestinal and hepatic utilization of a specific amino acid. With the assumption that there is no malabsorption of the tracer and substrates, the comparison between plasma enrichments of the enterally and intravenously infused tracers will allow the calculation of the first-pass splanchnic uptake. It has been demonstrated in several studies that there is hardly any malabsorption of tracers. First, less then 1% of [^{14}C]phenylalanine was found in stools after oral [^{14}C]phenylalanine administration. [66] Second, studies in adults have shown a high digestibility of approximately 98% of milk proteins, [67] and finally studies in infants showed that even intact proteins are rapidly and almost completely digested and absorbed in the distal region of the ileum. [68] Thus, dual tracer studies seem very useful in assessing splanchnic utilization and metabolic rates of different substrates.

The antecedents of clinical applications of $^{13}CO_2$ measurements lie in the use of [^{14}C]labeled compounds for research and diagnosis in departments of nuclear medicine in the early seventies. In a number of instances, [^{13}C]labeled substrates have been used to study metabolic and physiological conditions in which the recovery of $^{13}CO_2$ in breath provided useful information. These studies have been designated

collectively as breath tests, i.e. for fat malabsorption, disaccharidase deficiency. [69-72] These tests share the common characteristics of requiring a substrate whose structure contains a labeled functional group that may be enzymatically cleaved under specific conditions. [73] This may occur during the migration of the substrate down the gastrointestinal tract, during its absorption or subsequent to its transport to a specific organ, terminating in the release of labeled carbon dioxide from the lungs.

Figure 5. Natural variation in the abundance of ^{13}C expressed both as percent ^{13}C and as relative enrichment or depletion relative to Pee Dee Belemnite limestone. [74]



As shown in Figure 5, the natural abundance of ^{13}C in nature is approximately 1.1%. [74] The natural enrichment of stable isotopes is normally accounted for by taking baseline samples prior to the start of the isotope infusion, and then subtracting the enrichment of the

baseline sample from all the samples obtained during and/or after the isotope infusion. This is satisfactory for studies in which no change in metabolic status occurs during the study. By keeping the nutrient intake constant in the study subjects as is done in the studies presented in this thesis, it can be assumed that variation in background enrichment of expired CO_2 is negligible.

To assess splanchnic and whole-body protein kinetics with the use of different stable isotope labeled substrates, specific calculations are needed.

Substrate metabolism calculations

After the start of the stable isotope infusion, the labeled substrate will enter a metabolic pool by continuous infusion, and eventually an isotopic plateau will be achieved. Injecting a priming dose of tracer just before the start of the infusion can shorten the time it requires to reach an apparent isotopic equilibrium.

The measurement of the quantity of excess $^{13}CO_2$ over baseline values requires extremely precise techniques; therefore the isotopic enrichment of a labeled substrate is measured by mass spectrometry. Depending on the substrate an isotope ratio mass spectrometry (IRMS), a gas chromatograph mass spectrometry (GCMS) or a gas chromatograph isotope ratio mass spectrometry (GC-IRMS) can be used. For example, after an infusion of [^{13}C]sodium bicarbonate, the ratio of the ion intensity of mass 45 to mass 44 ($^{13}CO_2$/ $^{12}CO_2$) is measured for the sample and compared with the identical ratio for the standard CO_2 sample of known isotopic composition. The difference between the sample and the standard is given with the following calculation:

$$\delta \text{‰} = [(R_u - R_s / R_s] \times 1000$$

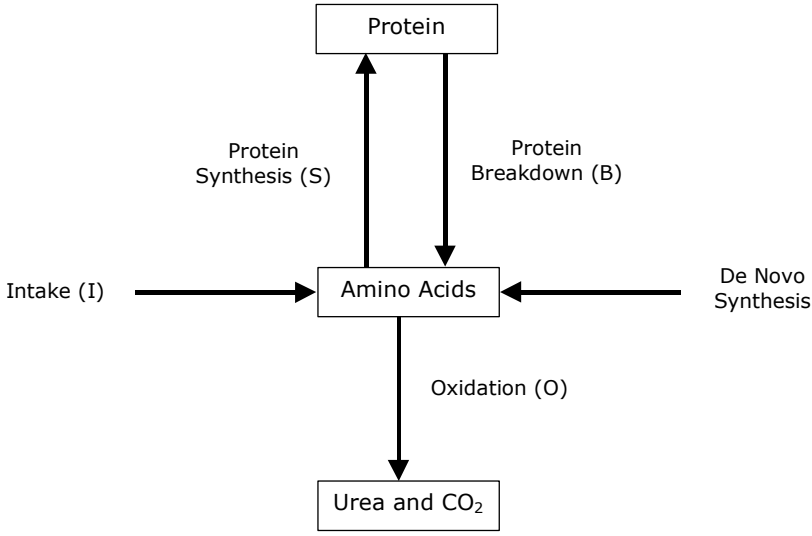
where R_u is the sample and R_s is the standard ratio. The quantities are so small that they are multiplied by 1000; called per mil. To facilitate interlaboratory comparisons, the results are expressed relative to CO_2 delivered from the PDB limestone, a Belemnite limestone of well-defined isotopic abundance ($^{13}C/^{12}C = 0.011237$). [75]

The stable isotope technique for the in vivo assessment of protein kinetics is based on a model [76,77] which can be summarized as follows (Figure 6): amino acids from the diet (I) and from protein breakdown (B) enter a metabolic pool of amino acids. Amino acids are withdrawn from this pool for protein synthesis (S) or for amino acid oxidation (O). Assuming that this pool is constant in size, the input of amino acids is equal to the output and corresponds to Q, which is the rate of amino acid flux through the amino acid pool:

$$\mathbf{Q = I + B = S + O}$$

Changes in the rates of these systems permit an adjustment in nitrogen balance, which might remain in equilibrium or detoriate in a negative balance depending upon the degree of change in protein intake.

Figure 6. Model of protein kinetics



Oxidation rates of substrates are calculated as the product of the flux rate and the percentage of the ^{13}C-label transferred from the infusate to the breath. By measuring the rate of amino acid oxidation,

an estimate of the net retention of the amino acid (i.e. rate of protein synthesis) can be derived by subtracting the oxidation rate from the intake of the amino acid. Briefly summarized, with the use of [^{13}C]labeled tracers it is possible to determine rates of substrate oxidation from expired breath $^{13}CO_2$ measurement, whereas plasma [^{13}C] enrichment at steady state allows for the calculation of the substrate appearance rate.

For the calculations of substrate oxidation in preterm infants the CO_2 production rate should be known. Several methods are known to quantify VCO_2, but closed circuit indirect calorimetry is the most commonly performed method and serves as the gold standard for the estimation of VCO_2. However, particular circumstances in preterm infants make the use of the results obtained from indirect calorimetry difficult. Another method to estimate the CO_2 production and calculate substrate oxidation is the use of the previously mentioned [^{13}C]sodium bicarbonate infusion. But, this tracer technique uses a relatively time consuming and complicated method to collect expired air.

We aimed to develop an alternative method to collect expired air in preterm infants based on the previously described breath test by Perman et al. [69] This direct naso-pharyngeal sampling technique offers the advantage of being simple, easy to perform, and non-time consuming, and is therefore possibly appropriate for the use in preterm infants in combination with a [^{13}C]bicarbonate infusion for the calculation of substrate oxidation.

Aims of the thesis

The overall purpose of the work presented in this thesis is to quantify intestinal substrate metabolism in both the preterm neonate and the neonatal pig, and its relationship with the level of enteral intake. With the determination of the intestinal substrate metabolism and the response to an increase in enteral intake, a better understanding of the obligatory intestinal need of substrates in the first weeks of life of preterm infants is possible, ultimately leading to an improved feeding strategy to support growth. Overall, the ability to establish and maintain normal gastrointestinal function has a critical influence on the clinical care and nutritional support of neonates, especially those born preterm.

Part one of this thesis describes the substrate metabolism of the portal-drained viscera in neonatal piglets under different feeding circumstances.

The metabolism of dietary essential amino acids by the gut has a direct effect on their systemic availability and potentially limits whole-body growth. The relative contribution of dietary and systemic utilization of threonine is an important question with regulatory implications, particularly in relation to nutritional effects. **The first aim** of this thesis is to determine the relative rates of arterial and dietary first-pass threonine metabolism during both a high protein and a low protein intake in neonatal piglets.

The second aim is to quantify the net intestinal utilization of all amino acids, and glucose during a 12-h feeding period. Furthermore, we quantified the pattern of secretory (glyco-)protein recycling by the intestinal tissues during the following period of 12-h of fasting.

The third aim is to answer the question whether the intestinal tissues of neonatal pigs have an obligatory requirement for amino acids as energy generating substrates, and what role they play under conditions of a low protein intake.

Part two of this thesis describes the confirmation of the results obtained in neonatal piglets regarding intestinal substrate metabolism,

in comparison with splanchnic substrate metabolism in preterm human neonates.

In clinical human studies, the metabolism of [^{13}C]labeled substrates to CO_2 and the measurement of the appearance of an excess of ^{13}C in respiratory CO_2 have progressed to an increasingly common method. However, the collection of respiratory CO_2 currently used; trapping in NaOH, is not well suited for application in a clinical situation. In order to collect breath samples from preterm infants in a suitable manner, we employed the direct naso-pharyngeal sampling technique previously described by Perman et al. used in older children.[69] **The fourth aim** of this thesis is to validate our direct sampling method for the collection of expiratory air in preterm infants.

Lysine is the first limiting essential amino acid in the diet of newborns. Thus, if the pattern of amino acids in the diet is not ideal, the rate of protein synthesis and growth will be determined by lysine. Previously, we have found in piglets under normal high-protein-feeding conditions (22 g/(kg·d) protein) that about half of the dietary lysine intake was utilized by the portal-drained viscera (PDV: i.e. intestine, pancreas, spleen and stomach), and that this utilization fraction even increased during dietary protein restriction. [39] Apart from the first-pass lysine utilization, the magnitude of lysine catabolism is also important to the nutrition of preterm newborns. **The fifth aim** of this thesis is to measure lysine kinetics in preterm infants in order to gain insight into the importance of the splanchnic tissues, in particular the intestine, in relation to whole-body lysine kinetics.

Threonine is an essential amino acid that is abundantly present in intestinally produced (glyco-)proteins. **Aim number six** is to answer the question whether the first-pass threonine metabolism by the intestine is substantial irrespective of the enteral intake, in preterm infants during their first weeks of life.

Under both high and low protein feeding conditions, glucose is a major oxidative substrate for intestinal energy generation in neonatal piglets. **The final aim** of this thesis is to investigate the first-pass

uptake and oxidation of glucose by the intestinal tissues in newborn infants during a low and full enteral intake.

References

1. Msall M, DiGaudio K, Rogers B, LaForest S, Catanzaro NL, Campbell J, Wilczenski F, Duffy LC. The functional independence measure for children (WeeFIM): Conceptual basis and pilot use in children with developmental disabilities. Clin. Pediatr. 1994;33:421-430.
2. The Victorian infant collaborative study group: Improvement of outcome for infants of birth weight under 1000 g. Arch. Dis. Child. 1991;66:765-769.
3. Hack M, Friedman MA, Faranoff AA. Outcomes of extremely low birth weight infants. Pediatrics. 1996;98:931-937.
4. The Victorian infant collaborative study group: Improved outcome into the 1990s for infants of birth weight 500-999 g. Arch. Dis. Child. 1997;77:F91-F94.
5. Stevenson DK, Wright LL, Lemons JA Oh W, Korones SB, Papile LA, Bauer CR, Stoll BJ, Tyson JE, Shankaran S, Fanaroff, AA, Donovan EF, Ehrenkranz RA, Verter J. Very low birth weight outcomes of the National Institute of Child Health and Development Neonatal Research Network, January 1993 through December 1994. Am. J. Obstet. Gynecol. 1998;178:1632-1639.
6. Richardson DK, Gray JE, Gortmaker SL, Goldmann DA, Pursley DM, McCormick MC. Declining severity adjusted mortality: Evidence improving neonatal intensive care. Pediatrics. 1998;102:893-899.
7. American Academy of Pediatrics Committee on Nutrition: Nutritional needs of preterm infants. In: Kleinman RE (ed) Pediatric Nutrition Handbook. American Academy of Pediatrics, Elk Grove Village, IL, 1998:55–87.
8. Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M. A United States national reference for fetal growth. Obstet. Gynecol. 1996;87:163-168.
9. Pauls J, Bauer K, Versmold H. Postnatal body weight curves for infants below 1000 g birth weight receiving early enteral and parenteral nutrition. Eur. J. Pediatr. 1998;157:416-421.
10. Ehrenkranz RA, Younes N, Lemons JA, Fanaroff AA, Donovan EF, Wright LL, Katsikiotis V, Tyson JE, Oh W, Shankaran S, Bauer CR, Korones SB, Stoll BJ, Stevenson DK, Papile LA. Longitudinal growth of hospitalized very low birth weight infants. Pediatrics. 1999;104:280-289.
11. Embleton NE, Pang N, Cooke RJ. Postnatal malnutrition and growth retardation: an inevitable consequence of current recommendations in preterm infants. Pediatrics. 2001;107:270-273.

12. Lubchenko LO, Hansman C, Boyd E. Intrauterine growth in length and head circumferences as estimated from life births at gestational ages from 26 to 42 weeks. Pediatrics. 1966;47:403-408.
13. Rigo J, Boboli H, Franckart G, Pieltain C, De Curtis M. Surveillance de l'ancien prématuré: Croissance et nutrition. Arch. Pédiatr. 1998;5:449-453.
14. Ross G, Lipper EG, Auld PA. Growth achievement of very low birth weight premature children at school age. J. Pediatr. 1990;117:307-309.
15. Kitchen WH, Doyle LW, Ford GW, Callanan C. Very low birth weight and growth to age 8 years. I: Weight and height. Am. J. Dis. Child. 1992;146:40-45.
16. Hack M, Breslau N, Weissman B, Aram D, Klein N, Borawski E. Effect of very low birth weight and subnormal head size on cognitive abilities at school age. N. Engl. J. Med. 1991;325:231-237.
17. Kitchen WH, Doyle LW, Ford GW, Callanan C, Rickards AL, Kelly E. Very low birth weight and growth to age 8 years. II: Head dimensions and intelligence. Am. J. Dis. Child. 1992;146:46-50.
18. Lucas A, Morley R, Cole TJ. Randomised trial of early diet in preterm babies and later intelligence quotient. BMJ. 1998;317:1481-1487.
19. Allen LH. The nutrition CRSP: what is marginal malnutrition, and does it affect human function? Nutr. Rev. 1993;51:255-267.
20. Hanning RM, Zlotkin SH. Amino acid and protein needs of the neonate: effects of excess and deficiency. Semin. Perinatol. 1989;13:131-141.
21. Van Lingen RA, Van Goudoever JB, Luijendijk IHT Wattimena JL, Sauer PJ. Effects of early amino acid administration during total parenteral nutrition on protein metabolism in pre-term infants. Clinical. Science. 1992;82:199-203.
22. Van Goudoever JB, Colen T, Wattimena JL, Huijmans JG, Carnielli VP, Sauer PJ. Immediate commencement of amino acid supplementation in preterm infants: effect on serum amino acid concentrations and protein kinetics on the first day of life. J. Pediatr. 1995;127:458-465.
23. Thureen PJ, Hay WW Jr. Intravenous nutrition and postnatal growth of the micropremie. Clin. Perinatol. 2000;27:197-219.
24. House JD, Pencharz PB, Ball RO. Lysine requirement of neonatal piglets receiving total parenteral nutrition as determined by oxidation of the indicator amino acid L-[1-^{14}C]phenylalanine. Am. J. Clin. Nutr. 1998;67:67-73.
25. Bertolo RF, Chen CZ, Law G, Pencharz PB, Ball RO. Threonine requirement of neonatal piglets receiving total parenteral nutrition is considerably lower

than that of piglets receiving an identical diet intragastrically. J. Nutr. 1998;128:1752-1759.
26. Elango R, Pencharz PB, Ball RO. The branched-chain amino acid requirement of parenterally fed neonatal piglets is less than the enteral requirement. J. Nutr. 2002;132:3123-3129.
27. Matthews DE, Marano MA, Campbell RG. Splanchnic bed utilization of glutamine and glutamic acid in humans. Am. J. Physiol. 1993;264:E848-E454.
28. Battezzati A, Brillon DJ, Matthews DE. Oxidation of glutamic acid by the splanchnic bed in humans. Am. J. Physiol. 1995;269:E269-E276.
29. Darmaun D, Roig JC, Auestad N, Sager BK, Neu J. Glutamine metabolism in very low birth weight infants. Pediatr. Res. 1997;41:391-396.
30. Haisch M, Fukagawa NK, Matthews DE. Oxidation of glutamine by the splanchnic bed in humans. Am. J. Physiol. Endocrinol. Metab. 2000;278:E593-E602.
31. Blachier F, Selamnia M, Robert V, M'Rabet-Touil H, Duee PH. Metabolism of L-arginine through polyamine and nitric oxide synthase pathways in proliferative or differentiated human colon carcinoma cells. Biochim. Biophys. Acta. 1995;1268:255-262.
32. Reeds PJ, Burrin DG, Stoll B, Jahoor F, Wykes L, Henry J, Frazer ME. Enteral glutamate is the preferential source for mucosal glutathione synthesis in fed piglets. Am. J. Physiol. 1997;273:E408-E415.
33. Wallace JL, Miller MJ. Nitric oxide in mucosal defense: a little goes a long way. Gastroenterology. 2000;119:512-520.
34. Goldstein RM, Hebiguchi T, Luk GD et al. The effects of total parenteral nutrition on gastrointestinal growth and development. J. Pediatr. Surg. 1985;20:783-791.
35. Stoll B, Chang X, Fan MZ, Reeds PJ, Burrin DG. Enteral nutritient intake determines the rate of intestinal protein synthesis and accretion in neonatal pigs. Am. J. Physiol. 2000;279:G288-G294.
36. Burrin DG, Stoll B, Jiang R Chang X, Hartmann B, Holst JJ, Greeley GH Jr, Reeds PJ. Minimal enteral nutrient requirements for intestinal growth in neonatal piglets: How much is enough? Am. J. Clin. Nutr. 2000;71:1603-1610.
37. Reeds PJ. Milk proteins and tissue nitrogen equilibrium. In: Barth CA (ed) Milk Proteins, Steinkopff Verlag Darmstadt 1989:37-48.
38. Roberton AM, Rabel B, Harding CA, Tasman-Jones C, Harris PJ, Lee SP. Use of the ileal conduit as a model for studying human small intestinal mucus glycoprotein secretion. Am. J. Physiol. 1991;261:G728-G734.

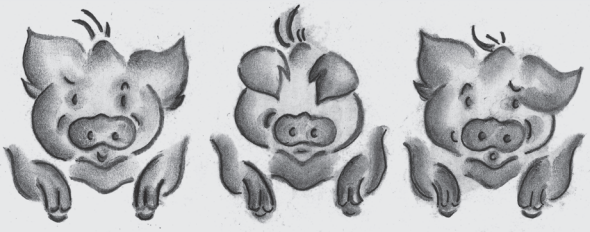
39. Van Goudoever JB, Stoll B, Henry JF, Burrin DG, Reeds PJ. Adaptive regulation of intestinal lysine metabolism. Proc. Nat. Acad. Sci. 2000;97:11620-11625.
40. FAO/WHO/UNU 1985, NRC 1988.
41. Wu G, Ott TL, Knabe DA, Bazer FW. Amino acid composition of the fetal pig. J. Nutr. 1999;129:1031-1038.
42. Ball RO, House JD, Wykes LJ, Pencharz P. A piglet model for neonatal amino acid metabolism during TPN. In: Tumbleson ME, Schook LB (ed) Advances in Swine in Biomedical Research, Plenum Press, New York 1996:713-731.
43. Widdowson EM. In: Assali NS (ed) Biology of Gestation, Academic Press NY, 1968;2:1-49.
44. Book SA, Bustad LK. The fetal and neonatal pig in biomedical research. J. Anim. Sci. 1974;38:997-1002.
45. Pond WG. In: Pond WG Houpt KA (ed) The Biology of the Pig, Cornell University Press, Ithaca, New York 1978:13-64.
46. Miller ER, Ullrey DE. The pig as a model for human nutrition. Annu. Rev. Nutr. 1987;7:361-382.
47. Moughan PJ, Birtles MJ, Cranwell PD, Smith WC, Pedraza M. The piglet as a model animal for studying aspects of digestion and absorption in milk-fed human infants. World. Rev. Nutr. Diet. 1992;67:40-113.
48. Rowan AM, Moughan PJ, Wilson MN, Maher K, Tasman-Jones C. Comparison of the ileal and faecal digestibility of dietary amino acids in adult humans and evaluation of the pig as a model animal for digestion studies in man. Br. J. Nutr. 1994;71:29-42.
49. Darragh AJ, Moughan PJ. The three-week-old piglet as a model animal for studying protein digestion in human infants. J. Pediatr. Gastroenterol. Nutr. 1995;21:387-393.
50. Shulman RJ, Fiorotto ML, Sheng HP, Garza C. Effect of different total parenteral nutrition fuel mixes on the body composition of infant miniature pigs. Pediatr. Res. 1984;18:261-265.
51. Goldstein RM, Hebiguchi T, Luk GD, Taqi F, Guilarte TR, Franklin FA Jr, Niemiec PW, Dudgeon DL. The effects of total parenteral nutrition on gastrointestinal growth and development. J. Pediatr. Surg. 1985;20:785-791.
52. Morgan W 3rd, Yardley J, Luk G, Niemiec P, Dudgeon D. Total parenteral nutrition and intestinal development: a neonatal model. J. Pediatr. Surg. 1987;22:541-545.

53. Shulman RJ, Henning SJ, Nichols BL. The miniature pig as an animal model for the study of intestinal enzyme development. Pediatr. Res. 1988;23:311-315.
54. Fiorotto ML, Shulman RJ, Sheng HP, Garza C. The effects of different total parenteral nutrition fuel mixes on skeletal muscle composition of infant miniature pigs. Metabolism. 1986;35:354-359.
55. Shulman RJ. Effect of different total parenteral nutrition fuel mixes on small intestinal growth and differentiation in the infant miniature pig. Gastroenterology. 1988;95:85-92.
56. Wykes LJ, Ball RO, Pencharz PB. Development and validation of a total parenteral nutrition model in the neonatal piglet. J. Nutr. 1993;123:1248-1259.
57. MacRae JC, Bruce LA, Brown DS, Calder AG. Amino acid use by the gastrointestinal tract of sheep given lucerne forage. Am. J. Physiol. 1997;273:G1200-G1207.
58. Yu YM, Burke JF, Vogt JA, Chambers L, Young VR. Splanchnic and whole body L-[1-^{13}C,^{15}N]leucine kinetics in relation to enteral and parenteral amino acid supply. Am. J. Physiol. 1992;262:E687-E694.
59. Alpers DH. Protein synthesis in intestinal mucosa: the effect of route of administration of precursor amino acids. J. Clin. Invest. 1972;51:167-173.
60. Ebner S, Schoknecht P, Reeds P, Burrin D. Growth and metabolism of gastrointestinal and skeletal muscle tissues in protein-malnourished neonatal pigs. Am. J. Physiol. 1994;266:R1736-R1743.
61. Matthews DE, Marano MA, Campbell RG. Splanchnic bed utilization of leucine and phenylalanine in humans. Am. J. Physiol. 1993;264:E109-E118.
62. Hoerr RA, Matthews DE, Bier DM, Young VR. Leucine kinetics from [$^{2}H_3$]- and [^{13}C]leucine infused simultaneously by gut and vein. Am. J. Physiol. 1991;260:E111-E117.
63. Matthews DE, Motil KJ, Rohrbaugh DK, Burke JF, Young VR, Bier DM. Measurement of leucine metabolism in man from a primed, continuous infusion of L-[^{13}C]leucine. Am. J. Physiol. 1980;238:E473-E479.
64. Yu YM, Wagner DA, Tredget EE, Walaszewski JA, Burke JF, Young VR. Quantitative role of splanchnic region in leucine metabolism: L-[1-^{13}C,^{15}N]leucine and substrate balance studies. Am. J. Physiol. 1990;259:E36-E51.
65. Yu YM, Burke JF, Vogt JA, Chambers L, Young VR. Splanchnic and whole body L-[1-^{13}C,^{15}N]leucine kinetics in relation to enteral and parenteral amino acid supply. Am. J. Physiol. 1992;262:E687-E694.

66. Biolo G, Tessari P, Inchiostro S, Bruttomesso D, Fongher C, Sabadin L, Fratton MG, Valerio A, Tiengo A. Leucine and phenylalanine kinetics during mixed meal ingestion: a multiple tracer approach. Am. J. Physiol. 1992;262:E455-E463.
67. Gaudichon C, Mahe S, Luengo C, Laurent C, Meaugeais P, Krempf M, Tome D. A ^{15}N-leucine-dilution method to measure endogenous contribution to luminal nitrogen in the human upper jejunum. Eur. J. Clin. Nutr. 1996;50:261-268.
68. Shulman RJ, Gannon N, Reeds PJ. Cereal feeding and its impact on the nitrogen economy of the infant. Am. J. Clin. Nutr. 1995;62:969-972.
69. Perman JA, Barr RG, Watkins JB. Sucrose malabsorption in children; a noninvasive diagnosis by interval breath hydrogen determination. J. Pediatr. 1978;93:17-22.
70. Veereman-Wauters G, Ghoos Y, Van der Schoor S, Maes B, Hebbalkar N, Devlieger H, Eggermont E. The ^{13}C-octanoic acid breath test: a noninvasive technique to assess gastric emptying in preterm infants. J. Pediatr. Gastroenterol. Nutr. 1996;23:111-117.
71. Van den Driessche M, Van Malderen N, Geypens B, Ghoos Y, Veereman-Wauters G. Lactose-[^{13}C]ureide breath test: a new, noninvasive technique to determine orocecal transit time in children. J. Pediatr. Gastroenterol. Nutr. 2000;31:433-438.
72. Van Dijk-Van Aalst K, Van den Driessche M, Van der Schoor S, Schiffelers S, Van't Westeinde T, Ghoos Y, Veereman-Wauters G. ^{13}C mixed triglyceride breath test: a noninvasive method to assess lipase activity in children. J. Pediatr. Gastroenterol. Nutr. 2001;32:579-585.
73. Hofmann AF, Lauterburg BH. Breath test with isotopes of carbon: progress and potential. J. Lab. Clin. Med. 1977;90:405-411.
74. Schoeller DA, Klein PD, Watkins JB, Heim T, MacLean WC Jr. ^{13}C abundances of nutrients and the effect of variations in ^{13}C isotopic abundances of test meals formulated for $^{13}CO_2$ breath tests. Am. J. Clin. Nutr. 1980;33:2375-2385.
75. Nier AO. A redetermination of the relative abundances of the isotopes of carbon, nitrogen, oxygen, argon and potassium. Phys. Rev. 1950;77:789.
76. Waterlow JC. Lysine turnover in man measured by intravenous infusion of L-[U-^{13}C]lysine. Clin. Sci. Lond. 1967;33:507-515.
77. Waterlow JC, Garlick PJ, Millward DJ. Protein turnover in mammalian tissues and in the whole-body. In: Elsevier North-Holland, Amsterdam 1978.

Part I

INTESTINAL METABOLISM IN NEONATAL PIGS



Chapter 2

THE HIGH OBLIGATORY VISCERAL NEED FOR THREONINE

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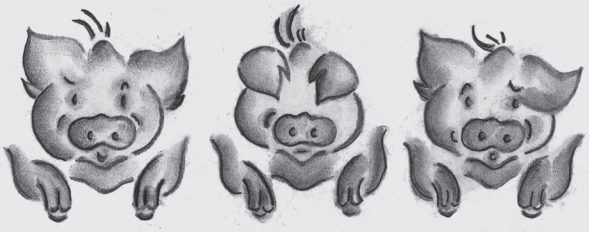

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Background & Aims: The whole-body threonine requirement in TPN-fed piglets is substantially lower than the requirement in enterally fed piglets, indicating that enteral nutrition induces intestinal processes in demand of threonine. Regarding the significant contribution of threonine to intestinal (glyco-)proteins, we hypothesized that the threonine utilization by the portal-drained viscera (PDV; intestine, pancreas, spleen and stomach is independent of the dietary protein intake. Methods: Twenty-three growing piglets were fed an isocaloric diet with either a high-protein (HP) or a low-protein (LP) content. Intraduodenal and intravenous infusions of [U-^{13}C]threonine were used to determine whole-body and first-pass kinetics of threonine by the PDV. Results: In HP-fed pigs, both dietary and systemic threonine were utilized by the PDV in equimolar amounts [dietary utilization: 459 ± 53 vs. systemic utilization: 497 ± 93 μmol/(kg·h)]. The total threonine utilization was significantly affected by protein restriction [HP: 765 ± 57 vs. LP: 355 ± 48 μmol/(kg·h), $P < 0.001$]. However, the equivalent of the total dietary threonine intake was extracted by the PDV during LP-feeding. During high protein feeding conditions, both dietary and systemic threonine were oxidized by the PDV, and the first-pass threonine oxidation accounted for approximately one-third of the whole-body threonine oxidation [FP oxidation: 42 ± 14 vs. WB oxidation: 117 ± 18 μmol/(kg·h)]. The whole-body threonine oxidation was significantly decreased by protein restriction, in contrast to the first-pass threonine oxidation which was not significantly affected. Similar to HP-feeding, both systemic and dietary threonine were oxidized in first-pass by the PDV. Conclusion: We conclude that, in vivo, the PDV have a high obligatory visceral requirement for dietary threonine, especially during a low protein intake.

The small intestinal mucosa is one of the most metabolically active tissues in the body. For example, the PDV in neonatal pigs accounts for 4 to 6% of the whole-body mass, but are responsible for about 25% of the total whole-body CO_2 production, and for 20-50% of the total protein turnover. [1-5]

Studies in animals have shown that more than 70% of the first-pass metabolism of some essential amino acids by the splanchnic tissues occur in the intestine. [6-8] For some non-essential amino acids, notably glutamate, the first-pass splanchnic extraction exceeds 90% of the dietary intake, both in humans and in pigs. [9,10] In humans, the splanchnic tissues retain between 20 to 50% of the dietary intake of specific essential amino acids. [11] Altogether, these studies indicate that the small intestine has a substantial amino acid metabolism.

In this context, threonine is of critical nutritional importance, because it is the single most used essential amino acid by the PDV. The retention of threonine by the PDV ranges from 60-80% of the dietary intake under normal feeding circumstances. [12-14] A major metabolic fate of threonine might be incorporation into secretory (glyco-)proteins, because threonine is particular dominant in intestinal mucins, with threonine constituting 20% of amino acids in the core proteins of mucins.[15] Interestingly and in a way confirming the above mentioned hypothesis is that whole-body threonine requirements are reduced by 60% in piglets receiving total parenteral nutrition as compared to enteral nutrition. [16] Furthermore, dietary threonine does not seem to be incorporated in constitutive mucosal proteins to a large extend. [14]

The degree, to which the first-pass utilization of amino acids is dependent on the nutrient composition, and dietary protein intake in particular, is an important question. Although there have been few investigations concerning this question, the available data are contradictory. In a previous study with growing pigs, we showed that during protein restriction the intestinal growth is preserved, apparently at the expense of skeletal muscle growth. [17,18] In addition, we have found that a prolonged period of protein restriction lowers the fractional rate of mucosal protein synthesis by only 25%. [19] Furthermore, we

reported that the total lysine utilization by the PDV was largely unaffected by low-protein feeding. [12] The investigation of the response of first-pass threonine utilization by the PDV to a low protein intake was the first objective of our study.

Uniquely, the mucosal cells receive substrates directly both from the diet and from the mesenteric circulation and previous studies showed a compartmentalization of amino acids by the intestinal tissues. [8,10,12,20,21] The proportion of visceral threonine metabolism derived from the luminal and the arterial site is not known. Therefore, the determination of the relative rates of arterial and dietary first-pass threonine metabolism in the PDV was the second objective of this study. Once taken up by intestinal cells, substrates can be used for different metabolic fates, including catabolism for intestinal energy generation. If the predominant fate of dietary threonine in the enterocyte is oxidation, then the first-pass metabolism by the intestine is a source of nutritional inefficiency. The first-pass oxidation of threonine by the PDV was the third aim of this study.

In summary, we report the utilization of dietary and systemic threonine by the PDV in 4-week old piglets receiving iso-energetic diets containing either a high (HP)- or low-protein (LP) content. By using the dual isotope methodology, we were able to measure: *1)* first-pass intestinal uptake of dietary threonine, *2)* systemic threonine uptake by the PDV, *3)* first-pass oxidation of threonine by the PDV, and *4)* the response in intestinal and whole-body threonine metabolism to protein restriction.

Methods

Animals

The Baylor College of Medicine Animal Protocol Review Committee approved the study. Housing and care of the animals conformed to the USDA guidelines. The study involved 23 four-week-old female crossbred piglets (Large White x Hampshire x Duroc) purchased from the Texas Department of Criminal Justice (Huntsville, TX). The pigs were received at the CNRC when they were 2 weeks old and were fed a liquid milk

replacer (Litterlife, Merrick, Union, WI) at a rate of 50 g/(kg·d) body weight. The composition (per kg dry matter) of the milk replacer was 500 g lactose, 100 g fat and 250 g protein. The calculated energy density was 18 MJ gross energy per kg dry matter.

Study design

The design has been described previously. [12,21-23] Briefly, at a postnatal age of 3 weeks, piglets underwent surgery after an overnight fast. Catheters were implanted into the stomach, the duodenum, the portal and jugular vein and the common carotid artery. An ultrasonic blood flow probe (Transonic Systems Inc., Ithaca, NY) was placed around the portal vein. Following surgery, the piglets received complete intravenous nutrition for 24-36 h. The pigs then received either regular Litterlife (HP diet: 12.7 g protein, 5.1 g lipid and 25.5 g carbohydrates per kilogram per day) or a diet that contained only 40% of the protein present in Litterlife (LP diet: 5.1 g protein, 7.5 g lipid and 30.4 g carbohydrates per kilogram per day). The protein intake during LP feeding was deliberately set at a rate to provide enough protein for body nitrogen maintenance. The diets were made iso-caloric by adding lactose (Sigma Chemical, St. Louis. MO) and corn oil in the same ratio as in the control (HP) diet. At postnatal d 28, whole body CO_2 production was measured with an infusion of [^{13}C]bicarbonate. The [U-^{13}C]threonine infusion protocols were carried out on postnatal days 30 to 32, at which the animals had received full enteral feeding of the same diet (either LP or HP) for at least 8 days.

Experimental protocol

After an overnight fast, the pigs consumed a meal that supplied one-seventh of the preceding daily intake, to restore intestinal motility. Immediately thereafter, a continuous gastric infusion of diet was started at a rate that provided one-fourteenth of the preceding daily intake each hour. On postnatal day 28, [^{13}C]bicarbonate (99%, Cambridge Isotope Laboratories, Inc, Andover, MA) was infused into the jugular catheter at a rate of 10 μmol/(kg·h). Arterial and portal blood samples (1mL) were

taken 15-min intervals from 75 to 120 min of infusion. On postnatal days 30 to 32, [U-^{13}C]threonine 97%, Cambridge Isotope Laboratories, Inc, Andover MA) was infused either via the duodenal or the jugular catheter at a rate of 10.8 µmol/(kg·h) for 5 h. During the last hour of the tracer infusion, four arterial and portal blood samples were drawn at 15-min intervals. The animals were killed with an arterial injection of sodium pentobarbital (50 mg/kg) and sodium phenytoin (5 mg/kg) (Beutanasia-D; Schering-Plough Animal Health, Kenilworth, NJ).

Analysis of samples

Small aliquots (0.2 mL) were taken for direct determination of the concentrations of blood gases (Chiron Diagnostics, Halstead, Essex, UK), glucose and lactate (YSI analyzer, Yellow Springs, OH). An aliquot of whole blood (0.2 mL) was taken for subsequent analysis of amino acid concentrations by reverse-phase high performance liquid chromatography of their phenylisothiocyanate derivatives (Pico Tag; Waters). An aliquot of whole blood (1.0 mL) was placed in a 10-mL vacutainer (Becton Dickinson, Franklin Lakes, NJ) and 0.5 mL perchloric acid (10% wt/wt) was added. The vacutainer was placed on ice. Room air (8 mL), filtered through soda lime (Sodasorb, Grace container products, Lexington, MA) to remove carbon dioxide, was injected into the vacutainer and removed into a gas tight syringe. This air was transferred to a second vacutainer for subsequent analysis of isotopic enrichment of carbon dioxide on a continuous flow gas isotope ratio mass spectrometer (ANCA, Europa Instruments, Crewe, UK). The blood/perchloride acid mixture was centrifuged at 3,000 x g for 10 min and the supernatant stored at –20°C. For analysis, the supernatant was thawed and brought to pH≤4 with KOH (4 mol/L). After centrifugation, the amino acids in the supernatant were bound to a 1 mL column of Dowex 50 Wx8 (H^+ form), eluted with 2 mL of 3 mol/L NH_4OH, and dried under vacuum.

Mass spectrometric analysis of threonine was conducted with a tri-fluoro-acetyl-methylester derivative. [24,25] The isotopic enrichment was measured on a gas chromatograph (Hewlett Packard, Palo Alto, CA) connected to a combustion oven (850°C) an isotope ratio mass

spectrometer (Europa Instruments, Crewe, UK). The atom percent enrichment was converted to mol percent threonine enrichment, after accounting for the 1.5-fold dilution of carbon in the derivative and the measured ^{13}C-abundance (97%) of the threonine tracer.

Calculations

Previous studies from our and other laboratories have used a steady state, whole-body model of amino acid metabolism that was developed as described by Waterlow et al. [26] This model assumes a common metabolic amino acid pool through which all amino acids move, either as dietary amino acids or from protein breakdown, or to exit for protein synthesis or oxidation. This movement through the metabolic pool is called flux. From the measurements of arterial and portal enrichments of isotopically labeled tracers, arterial and portal amino acid concentrations, and portal blood flow, the uptake of substrates across the PDV can be calculated. [12,21-23] A schematic model of the different fluxes through the PDV is shown in Figure 1. To obtain the results we used the following equations:

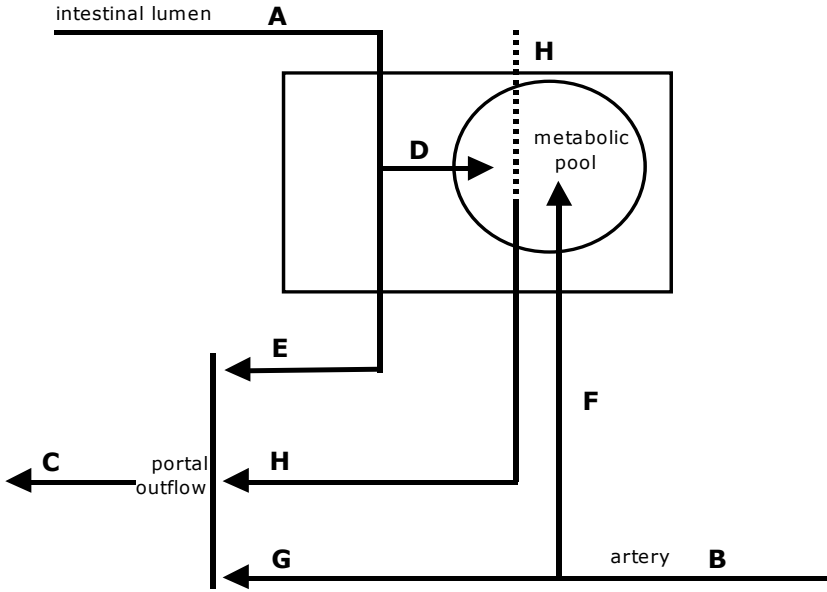
Net portal mass balance across the PDV = C - B
Net utilization of threonine = A – (C – B)
First-pass appearance of dietary threonine intake (E) = A – D
Arterial utilization of tracer (F) = G – B
Recycling (H) = C – E – G

The equations are detailed in the Appendix.

Statistics

All concentrations, isotopic enrichments and portal balances are presented as the mean values for samples taken over the last hour of the tracer study ± SEM. Balances were tested against zero by one-tailed t-tests. Fractional balances are the means of the ratios. Differences between the balances of the animals fed the HP or LP diet were tested by two-tailed t-tests. A value of $P < 0.05$ was taken as significant.

Figure 1. Schematic representation of the metabolic fate of dietary and arterial threonine by the PDV



A, dietary intake of threonine; B, arterial flux of threonine through the PDV; C, portal outflow of threonine; D, unidirectional, first-pass uptake of dietary threonine by the PDV; E, dietary threonine that is not metabolized in first-pass by the PDV; F, unidirectional uptake of arterial threonine by the PDV ; G, arterial threonine that is not metabolized by the PDV; H, recycled threonine that is derived from proteolysis or reabsorption of previously synthesized and secreted endogenous proteins but not derived from the diet.

Results

Before surgery, the pigs were gaining weight at a rate of 56 ± 12 g/(kg·d). There was no difference in weight at the day of surgery between both feeding groups [mean: 5.46 ± 0.62 kg]. Following surgery, the average weight gain in the HP-piglets was 46 ± 7 g/(kg·d) vs. 21 ± 7 g/(kg·d) in LP-piglets (P < 0.0001), which resulted in a significant

difference in body weight at postnatal day 32 when the pigs were killed [HP: 8.95 ± 1.24 vs. LP: 7.07 ± 0.44 kg, $P < 0.0001$].

Neither the whole-body CO_2 production [mean: 59.7 ± 8.0 mmol/(kg·h)], nor the CO_2 production by the PDV [mean: 7.5 ± 3.5 mmol/(kg·h)], were significantly affected by protein restriction. On average, the PDV accounted for 14% of the total whole-body CO_2 production.

The arterial threonine concentrations were significantly lower during LP-feeding as compared to HP-feeding. The portal mass balance of threonine was significantly lower in LP-fed piglets, and even more interestingly, was not different from zero [mean HP: 140 ± 49 µmol/(kg·h) vs. mean LP: -16 ± 55 µmol/(kg·h), $P < 0.05$].

Table 1. The portal blood flows (PBF), doses, and arterial and portal enrichments in piglets receiving an HP- or a LP-diet in combination with an intravenous (iv) or intraduodenal (id) infusion of [U-^{13}C]threonine

Tracer experiment	*PBF L/(kg·h)*	*Arterial IE MPE*	*Portal IE MPE*	*Tracer balance µmol/(kg·h)*
HP iv n=6	4.8 ± 0.8	0.776 ± 0.051	0.681 ± 0.041	-4.87 ± 1.70
LP iv n=5	4.9 ± 0.4	0.899 ± 0.049	0.863 ± 0.043	-0.68 ± 0.57
HP id n=7	4.0 ± 0.7	0.811 ± 0.092	0.833 ± 0.088	2.19 ± 1.13
LP id n=5	4.0 ± 0.4	1.071 ± 0.136	1.088 ± 0.137	-0.35 ± 1.21

Values are expressed as mean ± SEM; MPE, mole percent excess.

Table 1 summarizes the results obtained during the intravenous and intraduodenal [U-^{13}C]threonine tracer infusions in both feeding groups. During both protein intakes and intravenous tracer administration, the portal isotopic enrichment of threonine was lower than the arterial isotopic enrichment, and higher during the enteral tracer administration. Thus, there was net uptake of systemic threonine during both feeding periods. The fractional utilization of systemic threonine by the PDV was 10% during HP-feeding, and decreased to 3% during LP-feeding.

Table 2. The threonine intake, first-pass and systemic utilization of threonine by the PDV, the threonine recycling, and the total threonine utilization by the PDV in piglets receiving either a high-protein (HP) or a low-protein (LP) diet

Diet	*Intake*	*First-pass utilization*	*Systemic utilization*	*Recycling*	*Total utilization*
	μmol/(kg·h)	*μmol/(kg·h)*	*μmol/(kg·h)*	*μmol/(kg·h)*	*μmol/(kg·h)*
HP	934	459 ± 53	497 ± 93	162 ± 94	765 ± 57
LP	374	367 ± 19	61 ± 30	37 ± 41	355 ± 48
Diet effect		NS	P < 0.01	NS	P < 0.001

Values are expressed as mean ± SEM.

Table 2 shows the threonine kinetics by the PDV during both feeding intakes. During HP-feeding, the PDV utilized threonine both from the diet and from the systemic circulation in almost equimolar amounts. The total threonine utilization by the PDV was significantly lower during protein restriction [HP: 765 ± 57 vs. LP: 355 ± 48 μmol/(kg·h)]. However, the first-pass threonine utilization from the diet was not significantly affected by a lower protein intake, almost 98% of the total utilization derived from the diet. In other words, the equivalent of the total dietary threonine intake is retained by the PDV during LP-feeding. The amount of threonine in the portal vein that derived from intracellular proteolysis or from re-absorbed, previously secreted intestinal (glyco-)proteins, so called recycled threonine contributed for approximately 2% to the overall threonine outflow from the PDV in both feeding groups [HP: 2 ± 1 vs. LP: 2 ± 2%].

In order to determine the metabolic fate of the utilized threonine, we examined the threonine oxidation rates by the PDV (Table 3). During HP-feeding, the PDV oxidized both dietary and systemic threonine in almost equimolar amounts. The total whole-body threonine oxidation was 117 ± 18 μmol/(kg·h); thus the intestinal threonine oxidation represented one-third of the whole-body threonine oxidation. The whole-body threonine oxidation was significantly affected by protein restriction,

and decreased significantly as a result of the lower protein intake [LP: 61 ± 6 µmol/(kg·h), $P < 0.05$]. In contrast, the PDV maintained to oxidize both dietary and systemic threonine during a LP-feeding.

Table 3. Effect of a low protein intake on first-pass threonine oxidation by the PDV

	First-pass oxidation		
Diet	*dietary*	*systemic*	*total*
	µmol/(kg·h)	*µmol/(kg·h)*	*µmol/(kg·h)*
HP	23 ± 7	15 ± 7	42 ± 14
LP	6 ± 3	28 ± 10	34 ± 12
Diet effect	NS	NS	NS

Values are expressed as mean ± SEM.

Neither the whole-body non-oxidative threonine disposal (NOTD; an estimate of protein synthesis) nor the whole-body protein degradation (TRP) was significantly affected by a lower protein intake [NOTD HP: 1,316 ± 140 vs. NOTD LP: 1,188 ± 61 µmol/(kg·h), TRP HP: 958 ± 102 vs. TRP LP: 1,236 ± 40 µmol/(kg·h), Table 4]. Despite the lower protein intake, the threonine utilization by the gut is still roughly 30 to 34% of the whole-body protein synthesis. Also, even though the TRP is not statistically different, the value is 30% in the LP than HP pigs. However, the whole-body threonine balance (WB bal), calculated from the difference between non-oxidative disposal and whole-body degradation, was significantly affected by protein restriction, and was not significantly different from zero during a LP-diet [WB bal HP: 358 ± 18 vs. WB bal LP: -54 ± 4 µmol/(kg·h)].

Discussion

Threonine is an important limiting amino acid for growth and maintenance in diets for pigs. [27] Moreover, its oxidation represents an irreversible loss for whole-body proteins, as it cannot be synthesized de novo. Several investigators studying protein metabolism concluded that

the utilization of a limiting amino acid is not constant over a wide range of intakes. [28-30] Therefore, conservation of threonine from oxidation is important to maintain whole-body protein homeostasis during restricted dietary protein intake. In addition, threonine is of critical importance for the intestinal function because it is essential to the structural protein mucous layer lining of the gastrointestinal tract. The aim in the present study was to investigate the effect of protein restriction on first-pass intestinal and whole-body threonine metabolism in piglets. We found that, irrespective of the dietary protein intake, the PDV extracted a very large amount of dietary threonine. Furthermore, whole-body threonine oxidation and balance were significantly decreased by a lower protein intake, while the threonine oxidation by the PDV was not affected by protein restriction.

Table 4. Effect of a low protein intake on whole-body threonine kinetics

Diet	*NOTD* *μmol/(kg·h)*	*TRP* *μmol/(kg·h)*	*Oxidation* *μmol/(kg·h)*	*Balance* *μmol/(kg·h)*
HP	1,316 ± 140	958 ± 102	117 ± 18	358 ± 18
LP	1,188 ± 61	1,236 ± 40	61 ± 6	-54 ± 4
Diet effect	NS	NS	$P < 0.05$	NS

Values are expressed as mean ± SEM. NOTD indicates non-oxidative disposal of threonine, TRP indicates threonine release of proteins.

For an effective nutritional support in neonates with respect to growth, it is necessary to evaluate the amount of amino acids that is actually available from the diet for absorption. This is not an easy task, as the flow of amino acids delivered to the small intestines is comprised of microbial proteins, and proteins secreted by the intestinal mucosa cells. The contributions of endogenous secretions account for 15-25% of the total protein outflow of the lumen. In addition, the profile of amino acids ultimately appearing into the portal blood is altered by gut metabolism. There are four possible fates of dietary amino acids during

the absorption process: *1)* direct transport into the systemic circulation, *2)* use for gut protein synthesis, *3)* complete oxidation and *4)* conversion to other metabolites.

In recent years, there has been growing recognition that a very large proportion of certain non-essential amino acids (e.g. aspartate, glutamate, and glutamine) from the diet are utilized by the intestine and do not appear in the systemic circulation. [9,10] Likewise, we previously showed in piglets that the net portal balance of lysine is significantly less than the dietary intake, indicating that the PDV tissues utilize a considerable amount of dietary lysine. [12] Moreover, we showed that in HP-fed pigs, most of the lysine used by the PDV is derived from the systemic circulation, but this shifts to an increased first-pass use during protein restriction. In contrast to these studies in lysine, the current studies imply that the first-pass utilization of dietary threonine represents nearly half the total PDV use in HP-fed pigs and is nearly the exclusive source of PDV threonine use in protein restricted pigs. The total PDV threonine utilization represents 30 to 34% of the whole-body protein synthesis, regardless of the dietary protein intake. These findings strongly suggest that there is a high obligatory requirement for threonine by the PDV tissues, mainly the gut.

It is noteworthy that intestinal secretions are rather rich in threonine and that intestinal oxidation in HP-fed pigs represented only 6% of the absorbed threonine. Therefore, it appears that most threonine used by the intestine is for tissue and secretory protein synthesis. Considering the relatively large surface of the small intestine with its substantial mucus layer, dietary threonine seems to be obligatory for maintaining intestinal mucosal mass and integrity. Law et al. reported that pigs fed a threonine deficient diet had a lower gut weight, villus height, mucin content, and overall had diarrhea. [31,32] Furthermore, there is evidence that mucin production is impaired in pigs fed a threonine deficient diet, and administration of parenteral threonine does not restore normal mucin production. [33,34] It is conceivable that a lower mucin production leads to an impairment of intestinal mucosa disease resistance, and a higher risk of infections. Thus, as the first-pass threonine utilization

increased during a lower protein intake, the threonine needs may even be greater during periods of recovery from gut stress, such as infection, or chemotherapy.

In terms of amino acid economy, systemic amino acid availability will not be impaired as long as the sloughed cells and secretions are efficiently reabsorbed. In a previous study we showed that intestinal recycling of amino acids contributes significantly to the systemic availability of amino acids. [23] During the present 5-h study we measured only a small amount of threonine recycling by the PDV. This might be an underestimation, as the degradation process of mucins takes a considerable longer time. Therefore, we assume that the threonine utilized for intestinal (glyco-)protein synthesis eventually becomes available for the peripheral tissues through an efficient reabsorption process.

The second observation in this study that requires comment is the first-pass and whole-body threonine oxidation. Threonine is catabolized mainly by two routes: *1)* through threonine dehydratase which oxidizes threonine to ketobutyric acid to propionic acid and CO_2, and *2)* through threonine dehydrogenase that catabolizes threonine to glycine and acetyl CoA. Threonine is traditionally considered not to be oxidized by the intestinal mucosa because of the reported absence or negligible activity of initial or other rate-controlling enzymes, e.g. threonine 3-dehydrogenase, threonine dehydratase and threonine aldolase. [35] As suggested by Wu et al., the validity of the reported lack of catabolic enzymes in the intestinal tissues should be re-examined, because of the used preparation techniques. [36] The present results indicate that the intestinal oxidation of threonine under normal feeding conditions accounts for one-third of the whole-body threonine oxidation, and this first-pass threonine oxidation is not suppressed during protein restriction. In contrast to our previous findings regarding intestinal lysine oxidation, the PDV oxidizes both threonine from the mesenteric artery and from the luminal site. [12] Based upon these results, and previous work [14] there seems to be a intestinal channeling of amino acids to specific metabolic end-products depending whether the amino acid

enters the enterocyte from the basolateral membrane or the luminal side. These findings challenge the traditional concept that threonine is not catabolized by the intestinal mucosa, and therefore further enzymological work is required to establish biochemical bases for intestinal catabolism of dietary threonine.

Regarding whole-body threonine oxidation, our results are in contrast to the data reported by Chu and Hegsted. They observed no changes in threonine dehydratase activity in rats fed protein-free or threonine-free diets, and concluded that no adaptation is achieved in whole-body protein metabolism when threonine is specifically lacking. [28,37] In contrast to their findings, we found a substantially decreased whole-body threonine oxidation during protein restriction. However, the measurement of ^{13}C-labeled CO_2 probably underestimates threonine oxidation because of sequestration of two C-atoms of threonine into glycine as shown by Ballevre et al. who developed and validated a multitracer method in pigs allowing the calculation of the partition of both threonine pathways. [38] Nevertheless, the whole-body threonine oxidation was significantly lowered by protein restriction, suggesting a protein sparing adaptive mechanism.

In conclusion, the present study demonstrates that during protein restriction the PDV maintain a high rate of metabolism and continue to consume the equivalent of the total dietary threonine intake. We suggest that the considerable first-pass utilization of dietary threonine contributes significantly to the threonine-rich intestinal mucins. The results also show that the level of protein intake affects the site of threonine utilization by the PDV, as it switches from dual threonine use (i.e. dietary and systemic) to nearly exclusive luminal utilization of threonine when dietary protein becomes limiting. In addition, the threonine oxidation by the PDV accounted for one-third of the whole-body threonine oxidation. In contrast, this first-pass threonine oxidation was completely suppressed during protein restriction. Taken together, the results highlight the important role of the intestine in modulating dietary amino acid availability to the body and points to the obligatory intestinal requirement of threonine for maintaining intestinal integrity.

Appendix

Threonine calculations

Arterial thr input [μmol/(kg·h)] = arterial thr conc x PBF **[1]**

in which thr concentration is expressed in μmol/liter, and PBF is expressed in liter/(kg·h).

Portal thr output [μmol/(kg·h)] = portal thr conc x PBF **[2]**

Net portal thr balance [μmol/(kg·h)] = Eq.**2** - Eq.**1** **[3]**

Net utilization of thr by PDV [μmol/(kg·h)] =

dietary intake - Eq.**3** **[4]**

Fractional utilization of thr by PDV (fraction of intake) =

$$\frac{\text{Eq.}\mathbf{4}}{\text{dietary intake}} \quad \textbf{[5]}$$

Substituting the threonine concentration with the tracer concentration will give the tracer kinetics:

Arterial thr tracer input [μmol/(kg·h)] =

arterial thr tracer conc x PBF **[6]**

Portal thr tracer output [μmol/(kg·h)] =

portal thr tracer conc x PBF **[7]**

Portal thr tracer balance = Eq.**7** - Eq.**6** **[8]**

The percentage of arterial thr that is utilized by the PDV is measured during the intravenous tracer study and is calculated as follows:

$$\text{Fractional arterial thr utilization} = \frac{\text{Eq.}\mathbf{6} - \text{Eq.}\mathbf{7}}{\text{Eq.}\mathbf{6}} \quad \textbf{[9]}$$

The amount of arterial thr that is utilized by the PDV is calculated with the following equation:

Arterial utilization of thr by the PDV [μmol/(kg·h)] =

Eq.**9** x Eq.**1** **[10]**

The amount of dietary threonine utilized in first-pass by the intestine has to be corrected by the amount of dietary threonine that appears in the portal vein (and thus is not metabolized by the PDV), and then re-enters the PDV, but now from the arterial site. This threonine will be utilized by the PDV in the same proportion as the intravenous administered tracer (Eq.**9**). Thus, during the intraduodenal tracer infusion, the equation is:

Fract ent thr utilization =

$$\frac{\text{dietary tracer intake - Eq.}\mathbf{7}\text{ - [(1 - Eq.}\mathbf{9}\text{) x Eq.}\mathbf{6}\text{]}}{\text{dietary tracer intake}} \qquad \mathbf{[11]}$$

Thus, the amount of dietary thr that is utilized by the PDV is calculated by:

First-pass thr utilization by PDV [μmol/(kg·h)] =

Eq.**11** x dietary intake **[12]**

Direct absorption of dietary threonine is not the sole source of portal threonine, as threonine can also be released from intracellular proteolysis and from the digestion and re-absorption of secreted (glyco-)proteins previously synthesized by the intestines. It is also possible that threonine synthesized by the gut flora might contribute to portal threonine. In the present paper, we term threonine appearing in the portal vein that is not derived from the diet as recycled threonine (H in Fig. 1). It was calculated as follows, with results from both the intravenous and the intraduodenal tracer studies:

Recycled thr appearing in portal vein [μmol/(kg·h)] =

Eq.**2** - (dietary intake - Eq.**12**) - (Eq.**1** - Eq.**10**) **[13]**

References

1. McNurlan MA, Garlick PJ. Contribution of rat liver and gastrointestinal tract to whole-body protein synthesis in the rat. Biochem. J. 1980;186:381-383.
2. Burrin DG, Ferrell CL, Britton RA, Bauer M. Level of nutrition and visceral organ size and metabolic activity in sheep. Br. J. Nutr. 1990;64:439-448.
3. Lobley GE, Milne V, Lovie JM, Reeds PJ, Pennie K. Whole body and tissue protein synthesis in cattle. Br. J. Nutr. 1980;43:491-502.
4. Hoerr RA, Matthews DE, Bier DM, Young VR. Effects of protein restriction and acute refeeding on leucine and lysine kinetics in young men. Am. J. Physiol. 1993;264:E567-E575.
5. Hoerr RA, Matthews DE, Bier DM, Young VR. Leucine kinetics from [$^{2}H_{3}$]- and [^{13}C]leucine infused simultaneously by gut and vein. Am. J. Physiol. 1991;260:E111-E117.
6. Yu YM, Wagner DA, Tredget EE, Walaszewski JA, Burke JF, Young VR. Quantitative role of splanchnic region in leucine metabolism: L-[1-

^{13}C,^{15}N]leucine and substrate balance studies. Am. J. Physiol. 1990;259:E36-E51.
7. Yu YM, Burke JF, Vogt JA, Chambers L, Young VR. Splanchnic and whole body L-[1-^{13}C,^{15}N]leucine kinetics in relation to enteral and parenteral amino acid supply. Am. J. Physiol. 1992;262:E687-E694.
8. Stoll B, Burrin, DG, Henry JF, Yu H, Jahoor F, Reeds PJ. Substrate oxidation by the portal-drained viscera of fed piglets. Am. J. Physiol. 1999;277:E168-E175.
9. Battezzati A, Brillon DJ, Matthews DE. Oxidation of glutamic acid by the splanchnic bed in humans. Am. J. Physiol. 1995;269:E269-E276.
10. Reeds PJ, Burrin DG, Stoll B, Jahoor F, Wykes LJ, Henry J, Frazer ME. Enteral glutamate is the preferential source for mucosal glutathione synthesis in fed piglets. Am. J. Physiol. 1996;270:E413-E418.
11. Biolo G, Tessari P, Inchiostro S, Bruttomesso D, Fongher C, Sabadin L, Fratton MG, Valerio A, Tiengo A. Leucine and phenylalanine kinetics during mixed meal ingestion: a multiple tracer approach. Am. J. Physiol. 1992;262;E455-E463.
12. Van Goudoever JB, Stoll B, Henry JF, Burrin DG, Reeds PJ. Adaptive regulation of intestinal lysine metabolism. Proc. Nat. Acad. Sci. 2000;97:11620-11625.
13. Stoll B, Burrin DG, Henry J, Yu H, Jahoor F, and Reeds PJ. Dietary amino acids are the preferential source of hepatic protein synthesis in piglets. J. Nutr. 1998;128:1517-1524.
14. Stoll B, Henry JF, Reeds PJ, Yu H, Yahoor F, Burrin DG. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein fed piglets. J. Nutr. 1998;128:606-614.
15. Roberton AM, Rabel B, Harding CA, Tasman-Jones C, Harris PJ, Lee SP. Use of the ileal conduit as a model for studying human small intestinal mucus glycoprotein secretion. Am. J. Physiol. 1991;261:G728-G734.
16. Bertolo RF, Chen CZ, Law G, Pencharz PB, Ball RO. Threonine requirement of neonatal piglets receiving total parenteral nutrition is considerably lower than that of piglets receiving an identical diet intragastrically. J. Nutr. 1998;128:1752-1759.
17. Seve B, Reeds PJ, Fuller MF, Cadenhead A, Hay SM. Protein synthesis and retention in some tissues of the young pig as influenced by dietary protein intake after early-weaning. Possible connection to the energy metabolism. Reprod. Nutr. Dev. 1986;26:849-861.

18. Dudley MA, Wykes L, Dudley AW Jr, Fiorotto M, Burrin DG, Rosenberger J, Jahoor F, Reeds PJ. Lactase phlorizin hydrolase synthesis is decreased in protein-malnourished pigs. J. Nutr. 1997;127:687-693.
19. Ebner S, Schoknecht P, Reeds P, Burrin D. Growth and metabolism of gastrointestinal and skeletal muscle tissues in protein-malnourished neonatal pigs. Am. J. Physiol. 1994;266:R1736-R1743.
20. Beauteloup-Demange C, Boirie Y, Dechelotte P, Gachon P, Beaufrere B. Gut mucosal protein synthesis in fed and fasted humans. Am. J. Physiol. 1998;274:E541-546.
21. Stoll B, Burrin DG, Henry JF, Jahoor F, Reeds PJ. Dietary and systemic phenylalanine utilization for mucosal and hepatic constitutive protein synthesis in pigs. Am. J. Physiol. 1999;276:G47-G59.
22. Van der Schoor SRD, Van Goudoever JB, Stoll B, Henry JF, Rosenberger JF, Burrin DG, Reeds PJ. The pattern of intestinal substrate oxidation is altered by protein restriction in pigs. Gastroenterology. 2001;121:1167-1175.
23. Van der Schoor SRD, Reeds PJ, Stoll B, Henry JF, Rosenberger JR, Burrin DG, Van Goudoever JB. The high metabolic cost of a functional gut. Gastroenterology. 2002;123:1931-1940.
24. Matthews DE, Ben-Galim E, Bier DM. Determination of stable isotopic enrichment in individual plasma amino acids by chemical ionization mass spectrometry. Anal. Chem. 1979;51:80-84.
25. Stalling DL, Gehrke CW, Zumwalt RW. A new silylation reagent for amino acids bis(trimethylsilyl)trifluoroacetamide (BSTFA). Biochem. Biophys. Res. Commun. 1968;23:616-622.
26. Waterlow JC. Lysine turnover in man measured by intravenous infusion of L-[U-^{14}C]lysine. Clin. Sci. Lond. 1967;33:507-515.
27. Fuller MF, McWilliam R, Wang TC, Giles LR. The optimum dietary amino acid pattern for growing pigs. 2. Requirements for maintenance and for tissue protein accretion. Br. J. Nutr. 1989;62:255-267.
28. Hegsted DM, Neff R. Efficiency of protein utilization in young rats at various levels of intake. J. Nutr. 1970;100:1173-1179.
29. Baker DH. Partitioning of nutrients for growth and other metabolic functions: efficiency and priority considerations. Poult. Sci. 1991;70:1797-1805.
30. Bikker P, Verstegen MW, and Bosch MW. Amino acid composition of growing pigs is affected by protein and energy intake. J. Nutr. 1994;124:1961-1969.
31. Law G, Chen CZL, Bertolo RFP, Pencharz PB, Ball RO. Threonine requirement in weaned piglets is affected by gut function. In: Advances in Pork Production 1999;10:27.

32. Law G, Adjiri-Awere A, Pencharz PB, Ball RO. Mucins in piglets are dependent upon dietary threonine. In: Advances in Pork Production 2000;11:10.
33. Iiboshi Y, Nezu R, Kennedy M, Fujii M, Wasa M, Fukuzawa M, Kamata S, Takagi Y, and Okada A. Total parenteral nutrition decreases luminal mucous gel and increases permeability of small intestine. J. Parenter. Enteral. Nutr. 1994;18:346-350.
34. Ball RO, Law G, Bertolo RFP et al. Adequate oral threonine is critical for mucin production and mucosal growth by the neonatal piglet,. In VIIIth International Symposium on Protein Metabolism and Nutrition: Wageningen, The Netherlands, Wageningen Pers, 1999:31.
35. Le Floc'h N, Thibault JN, Seve B. Tissue localization of threonine oxidation in pigs. Br. J. Nutr. 1997;77:593-603.
36. Wu G. Synthesis of citrulline and arginine from proline in enterocytes of postnatal pigs. Am. J. Physiol. 1997;272:G1382-G1390.
37. Chu SH, Hegsted DM. Adaptive response of lysine and threonine degrading enzymes in adult rats. J. Nutr. 1976;106:1089-1096.
38. Ballevre O, Cadenhead A, Calder AG, Rees WD, Lobley GE, Fuller MF, Garlick PJ. Quantitative partition of threonine oxidation in pigs: effect of dietary threonine. Am. J. Physiol. 1990;259:E483-E491.

Chapter 3

THE HIGH METABOLIC COST OF A FUNCTIONAL GUT

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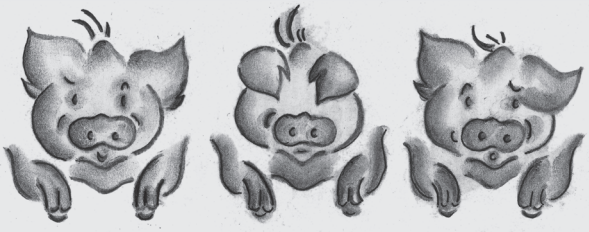
[3] This paper is dedicated to the memory of Peter J Reeds who died recently. It is an enormous loss for all of them who have worked with him. He was a great mentor and inspirator to all of us.



Gastroenterology 2002;123:1931-1940.

Background & Aims: Animal studies have shown that more than half of the dietary protein intake is utilized by the gut and that a large proportion of this utilization is devoted to (glyco-)protein synthesis. Recycling of these secretions may play a critical role in the regulation of overall dietary amino acid bioavailability. Methods: Four piglets (age 32 d, 8-10 kg) bearing portal, arterial and duodenal catheters and a portal flow probe were infused with a complete diet via the duodenum for 12 h, followed by 12 h of fasting. The portal balance of glucose and amino acids was measured throughout the 24-h period. The animals also received duodenal and intravenous infusions of different lysine and threonine tracers. Measurements of intestinal tracer utilization and reappearance in the portal blood were used to calculate intestinal amino acid utilization and recycling. Results: From 0 to 6 h one-third of the protein intake appeared in the portal blood. As feeding continued, the portal glucose balance (60% of intake) was constant, but the net amino acid portal balance became progressively more positive. Significant net amino acid absorption continued for at least 6 h after the cessation of feeding. Over 24 h, 52% of the dietary protein intake appeared in the circulation and one-third of this derived from recycled intestinal secretions. Conclusions: Intestinal recycling of amino acids contributes significantly to their systemic availability and may be a critical factor in amino acid nutrition.

The portal-drained viscera (PDV; i.e. the intestine, pancreas, spleen and stomach) have a high rate of both energy expenditure and protein synthesis. [1-3] Therefore, these tissues have high nutrient requirements, one result being the disproportionately high utilization of the dietary protein intake by the gut itself. Because the rate of amino acid utilization by the intestine determines the amino acid systemic availability, intestinal metabolism can thus be an important regulator of whole-body growth.

In previous studies we have shown that although milk proteins are efficiently digested, [4,5] not all the dietary amino acids appear in the portal circulation during the first hours of feeding. [6,7] This low systemic amino acid availability reflects the use of absorbed amino acids for the synthesis of mucosal cellular proteins, for oxidative purposes, and for secretion of (glyco-)proteins. In contrast to our earlier conclusions, [7] we postulate that the majority of the utilized amino acids are incorporated into (glyco-)proteins that are secreted into the intestinal lumen. After secretion into the lumen, the synthesized proteins are either digested and reabsorbed, or they are passed to the colon, where they are degraded, fermented and lost from the body. Previous studies in piglets [8,9] showed that 12% of the intestinal endogenous secreted (glyco-)proteins were lost to the colon following secretion, implying that a large majority of the secreted (glyco-)proteins might be digested and reabsorbed. However, given that dietary amino acids are utilized in first-pass by the intestine, there is no assurance that reabsorbed, previously secreted amino acids will appear in the portal circulation. This is important information because the nutrient requirements of the portal-drained viscera might have a critical impact on the systemic availability of nutrients.

Studying nutrient use by the gut, and especially the intestinal mucosa, is particularly difficult. A complicating factor is that the intestinal mucosa receives nutrients from two sources, the diet (brush border membrane) and the systemic circulation (basolateral membrane). We have developed a portal catheterized piglet model [10] to study visceral metabolism in growing, conscious and fed animals. In

previous studies, we have used this model, in combination with enteral and intravenous infusions of stable isotopically labeled tracers, to quantify the first-pass metabolism of dietary amino acids. [6,11,12] In order to evaluate the contribution of recycling to overall systemic amino acid availability, we used our arteriovenous organ tracer-balance piglet model in a 24-h study protocol in the present study. The experiment was based on the combination of the intravenous and intraduodenal infusions of stable isotopically amino acid tracers with the quantification of the amino acid mass (to measure total net absorption) and tracer (to measure either luminal or arterial absorption of two specific amino acids: lysine and threonine) balance across the PDV of fed and fasted piglets.

Our main objective was to determine the magnitude and pattern of secretory (glyco-)protein recycling. Therefore, we quantified the net portal appearance of all amino acids, glucose and lactate during a 12-h feeding period and a subsequent 12-h fasting period. The reason underlying the experimental design was that if the secreted (glyco-)proteins are transferred to the systemic circulation as amino acids then *(a)* there will be a progressive increase in the portal appearance of amino acids during prolonged continuous feeding and *(b)* this portal amino acid appearance will continue for a considerable time following the end of feeding. In addition, in order to determine the exact rate of recycling of two amino acids of specific nutritional importance, lysine and threonine, we simultaneously administered six stable isotopically labeled tracers of lysine and threonine by both intravenous and intraduodenal routes. By using these different tracers at different times during the feeding period and by measuring the net portal tracer mass balances, we were able to calculate the extent of recycling of lysine and threonine by the PDV.

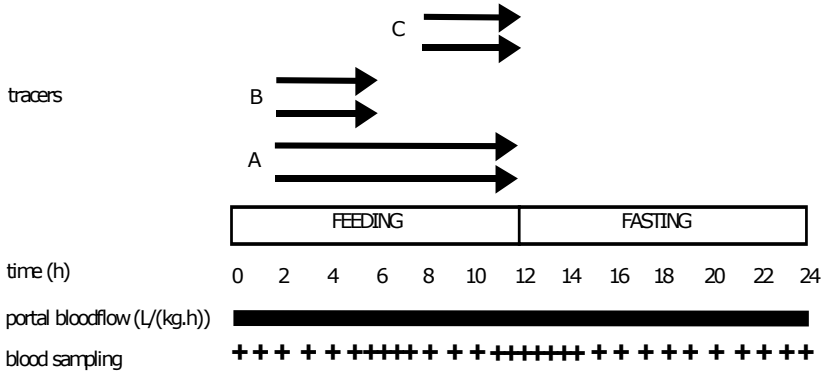
Methods

Animals

The Baylor College of Medicine Animal Protocol Review Committee approved the study. Housing and care of the animals conformed to

current US Department of Agriculture guidelines. The study involved four 4-week-old female crossbred piglets (Large White x Hampshire x Duroc) obtained from the Texas Department of Criminal Justice, Huntsville, TX. Two-week-old pigs were received at the Children's Nutrition Research Center and fed a liquid milk replacer diet (Litter Life, Merrick, Middleton, WI) at a rate of 50 g/(kg·d) body weight. The composition (per kg dry matter) of the milk replacer was 500 g lactose, 100 g fat and 250 g protein.

Figure 1. Schematic outline of the 24-h protocol



A, represents [^{15}N]threonine and [^{2}H4]lysine intravenously; B, [U-^{13}C]threonine and [U-^{13}C]lysine intraduodenally and C, [U-^{13}C, ^{15}N]threonine and [e-^{15}N]lysine intraduodenally.

Study Design

At a postnatal age of 3 weeks, the piglets underwent surgery after an overnight fast. Under isoflurane anesthesia and strict aseptic conditions, catheters were implanted into the duodenum, the portal and the common carotid artery. An ultrasonic flow probe (Transonic Systems Inc, Ithaca, NY) was implanted around the common portal vein. This procedure allowed us to sample from the arterial and portal blood and to measure the blood flow through the portal-drained viscera. Furthermore, by means of continuous duodenal infusion, we

could control the exact dietary intake independent of gastric emptying. The piglets received complete parenteral nutrition for 24 h and achieved full enteral feeding on the second postoperative day. All piglets were weighed daily, and sampling catheters were flushed with sterile, heparinized saline to maintain patency. Piglets and cages were cleaned daily.

The 24-h protocol (Figure 1) was carried out 8 days after surgery when the piglets weighed 8.8 ± 0.9 kg. The 24-h study period was divided into two 12-h metabolic periods; 12 h of continuous feeding (a liquid milk replacer diet supplying 5.7 mmol/(kg·h) lactose and 8.3 mmol/(kg·h) amino acids) and 12 h of fasting. This design was implemented to achieve two requirements; first we wanted to obtain portal mass balances of all amino acids and glucose during the fasted and the fed state and, second, we wanted to determine the exact rate of recycling of two specific essential amino acids, lysine and threonine by using different stable isotopically labeled tracers. During the 12-h feeding period, constant intravenous and intraduodenal infusions of labeled lysine and threonine were given as described below. Our purpose was to minimize the potential for recycling of the tracer (i.e., its return to plasma via protein proteolysis) by using different tracers at different time points.

The animals were deprived of food from 18.00 h the night preceding the study. After this overnight fast, baseline arterial and portal blood samples were drawn at 7.00 h, after which the pigs received a single bolus meal that supplied two-fourteenths of their preceding daily intake (186 ml/(kg·d) Litter Life). This meal served to initiate an adequate pattern of intestinal motility. Immediately thereafter, a continuous duodenal infusion of the diet was started at an hourly rate of one-fourteenth of their preceding daily intake for the next 12 h. At 19.00 h, the duodenal infusion was stopped, the duodenal contents were removed by aspiration and the animals were fasted for a further 12 h. At the end of the 24-h study, the pigs were killed with an intra-arterial injection of pentobarbital sodium (50 mg/kg

body wt) and sodium phenytoin (5 mg/kg; Beutanasia-D; Schering-Plough Animal Health, Kenilworth, NJ).

Tracer and Amino Acids Protocol

[$^{2}H_4$]lysine ([H_4]lysine, 98% ^{2}H), [U-^{13}C]lysine ([$^{13}C_6$]lysine, 97% ^{13}C), and [e-^{15}N]lysine ([e-^{15}N]lysine, 98% ^{15}N), [^{15}N]threonine ([^{15}N]threonine, 98% ^{15}N), [U-^{13}C]threonine ([U-$^{13}C_4$]threonine, 97% ^{13}C), and [U-^{13}C,^{15}N]threonine ([^{13}C,^{15}N]threonine, 97% ^{13}C,^{15}N) were purchased from Cambridge Isotopes (Woburn, MA). Just before the start of each infusion, a prime dose of [$^{2}H_4$]lysine (7 μmol·kg^{-1}), [^{15}N]threonine (50 μmol/kg), [U-^{13}C]lysine (5 μmol/kg), [U-^{13}C]threonine (5 μmol/kg), [e-^{15}N]lysine (30 μmol/kg), and [U-^{13}C,^{15}N]threonine (50 μmol/kg) was administered over ≈ 1 min. Immediately thereafter an intravenous or intraduodenal infusion of [$^{2}H_4$]lysine, [^{15}N]threonine, [U-^{13}C]lysine, [U-^{13}C]threonine, [e-^{15}N]lysine, and [U-^{13}C,^{15}N]threonine (7, 50, 5, 5, 30, and 50 μmol/(kg·h), respectively) were started by intravenous or intraduodenal route. Figure 1 depicts the design of the 24-h tracer protocol, including the different time points for the different tracers; [D_4]lysine and [^{15}N]threonine intravenously from 2 tot 12 h of feeding, [U-^{13}C]lysine and [U-^{13}C]threonine intraduodenally from 2 to 6 h of feeding, and [e-^{15}N]lysine and [U-^{13}C,^{15}N]threonine intraduodenally from 8 to 12 h of feeding. For all piglets, the prime and infusion doses were nominally the same (although the exact infusion rate was determined for each infusion from the amount of infusate infused and the concentration of each label).

During the 24-h period, portal blood flow was measured constantly. Blood samples (0.4 ml) for amino acids, glucose and lactate analysis were collected hourly for the whole 24-h period. In addition, three half hourly samples were withdrawn from 5.5 to 6.5 h of feeding, three half hourly from 11 to 12 h of feeding and seven blood samples over the first 2 h of fasting.

Sample analysis

Small aliquots (0.2 ml) were taken for the determination of glucose and lactate (YSI analyzer; YSI Incorporated, Yellow Springs, OH). For analysis of amino acid concentrations, an aliquot of whole blood (0.2 mL) was mixed with an equal volume of an aqueous solution of methionine sulfone (0.5 mmol/L) and centrifuged at 10,000 x g for 10 min at room temperature through a 3-kDa cutoff filter. The filtrate was dried and the amino acids were analyzed by reverse-phase high performance liquid chromatography of their phenylisothiocyanate derivatives (Pico Tag, Waters, Woburn, MA). Mass spectrometric analysis of lysine and threonine was conducted with the tri-fluoro-acetyl-methylester derivative. [13,14] The ^{13}C-isotopic enrichment was measured on a gas chromatograph (Hewlett-Packard, Palo Alto, CA) connected to a combustion oven (850°C) and an isotope ratio mass spectrometer (ANCA, Europa Instruments, Crewe, UK) The atom percentage ^{13}C-enrichment was converted to mole percentage of [^{13}C]lysine and [^{13}C]threonine enrichment, after accounting for the dilution of carbon in the derivative and the measured ^{13}C abundance (97%) of the tracers. The atom percentage ^{15}N-enrichment and $^{2}H_4$-enrichment was converted to mole percentage of [^{15}N]lysine, [$^{2}H_4$]lysine, and [^{15}N]threonine enrichment, after accounting for the dilution of nitrogen and hydrogen in the derivative and the measured ^{15}N and $^{2}H_4$ abundance (98%) of the tracers.

Calculations

Previous studies from our and other laboratories have used a steady-state, whole-body model of amino acid metabolism that was developed as described by Waterlow et al. [15] This model assumes a common metabolic amino acid pool through which all amino acids move, either as dietary amino acids or from protein breakdown, or to exit for protein synthesis or oxidation. This movement through the metabolic pool is called flux. From the measurements of arterial and portal enrichments of [^{13}C, ^{15}N or $^{2}H_4$]tracers, arterial and portal amino acid concentrations, and portal blood flow, the uptake of substrates across

the PDV can be calculated. [6] A schematic model of the different fluxes through the PDV is shown in Figure 4. To obtain the results we used the following equations:

Net portal mass balance across the PDV = C - B
Net utilization of lysine/threonine = A – (C – B)
First-pass appearance of dietary intake (E) = A – D
Arterial utilization of tracer (F) = G – B
Recycling (H) = C – E – G

The equations are detailed in the Appendix.

Statistics

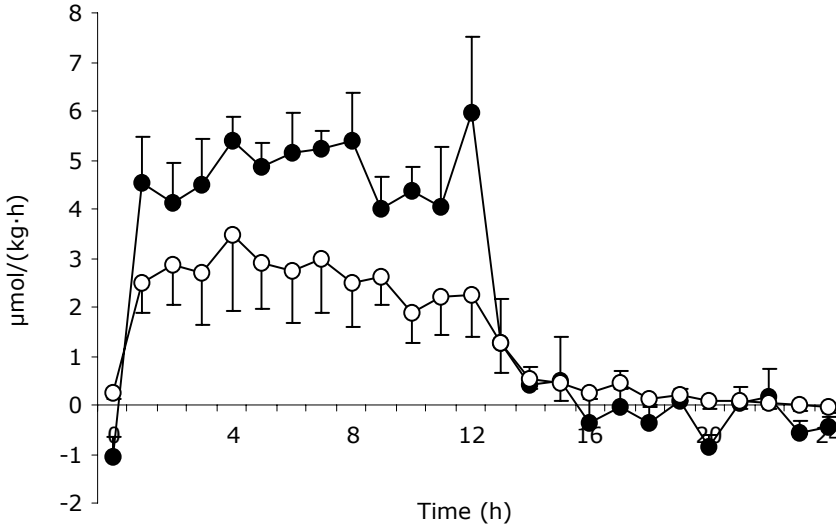
All concentrations and portal balances are presented as mean values plus or minus the inter-animal SEM. Balances were tested against zero by one-tailed *t* tests. For balances that are expressed as a proportion of intake, the values are the mean of the ratios. A value of $P < 0.05$ was taken as statistically significant.

Results

Glucose and lactate utilization

The portal blood flow did not differ significantly between the fed and the fasted state (fed 6.09 ± 0.59 vs. fasted 5.40 ± 0.75 L/(kg·h)). Immediately before the start of feeding (i.e. after an overnight fast) the net portal mass balance across the PDV of glucose was negative. As shown in Figure 2, within one hour after the onset of the continuous 12-h feeding, both the net glucose and lactate portal mass balances reached a steady state, which was maintained throughout the feeding period. Over a 24-h period, 60 ± 3% of the glucose intake appeared in the portal circulation. Within two hours after the end of the feeding the net portal glucose mass balance across the PDV fell to zero again. The net portal lactate mass balance did not reach zero until 4 h after the end of feeding.

Figure 2. The mean net portal mass balance of glucose and lactate during a 24-h period



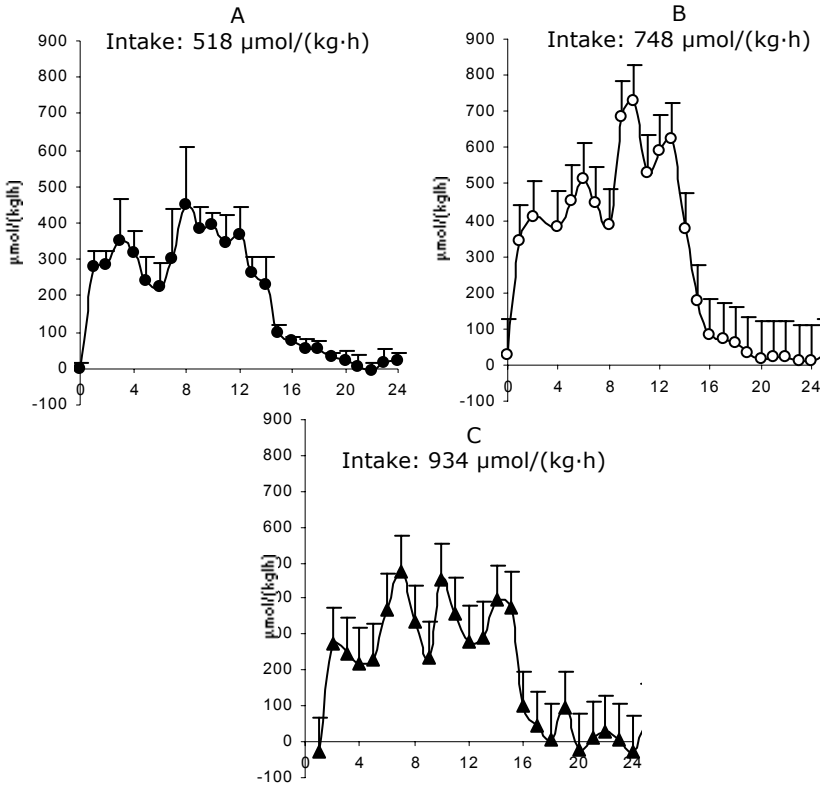
Amino acid utilization

The 24-h pattern of appearance in the portal circulation of three individual amino acids, lysine, leucine and threonine, is shown in Figure 3. The appearance of both essential and not-essential amino acids in the portal vein rose immediately after the feeding was started. However, in contrast to glucose (net utilization rate of >30%), the net utilization rate of non-essential and essential amino acids during the first 6-h of continuous feeding, was 61% and 70% of the dietary protein intake, respectively. There was a also significant ($P < 0.01$) and progressive (2% per h) increase in the portal appearance of the amino acids from 6 to 12 h of feeding, for the total amino acids. Sixty percent of dietary amino acid intake became systemically available during the last period of feeding.

In agreement with our previous data, [6] there was a wide variation in the net utilization rate of specific essential amino acids during continuous feeding. This ranged from approximately 75% of the intake

for threonine and isoleucine during the first 6 h of feeding, to only 20% of the intake for leucine and methionine during the second 6 h of feeding. In marked contrast to glucose, the mean portal mass balance of all essential amino acids remained significantly greater than zero for at least 8 h after the cessation of feeding.

Figure 3. The mean net portal mass balance of lysine, leucine and threonine during a 24-h period



Values are means ± SEM, n = 4. Figure 3A represents lysine, Figure 3B represents leucine, and Figure 3C represents threonine.

Regarding the non-essential amino acids, the utilization rates by the PDV expressed as a percentage of intake also varied widely during

both 6-h intervals (Table 1). On average, 61% of the total non-essential amino acid intake was utilized by the PDV during the first 6 h of feeding. As feeding progressed, the fraction of total non-essential amino acid intake utilized by the PDV decreased to 41%, although this decrease did not reach statistical significance. As we have found consistently in previous studies, almost all aspartate, glutamate and glutamine was utilized by the gastrointestinal tissues (on average more than 90% of dietary intake, Table 1). On the other hand, the fraction of intake utilized by the PDV of some amino acids after 12 h of continuous feeding (alanine, arginine, glycine and tyrosine) exceeded 100%, indicating a net production by the PDV.

As shown in Table 2, the proportional contribution to the total appearance in the portal circulation during 24-h of each specific amino acid and glucose was calculated for the different periods. For each essential amino acid, this fractional appearance increased as feeding progressed, specifically for methionine, whose fractional appearance had nearly doubled by the end of the feeding period. Tryptophan utilization values as a fraction of intake are not reported, but only as a fraction of total 24-h portal mass balance, because tryptophan is destroyed by acid hydrolysis prior to analysis of the formula. [16]

The total net portal mass balance of the essential amino acids across the PDV increased by 12% during the last 6-h of feeding when expressed relative to the total net portal mass balance over 24-h. During the fasting period, the portal appearance of essential amino acids accounted for a further 14% of the total essential amino acid portal mass balance over 24-h. On average for all essential amino acids, this suggests that recycling was responsible for 26% of the total 24-h appearance of essential amino acids. In other words, approximately one-third (35%) of the utilized essential amino acids was recycled.

Table 1. Systemic availability of amino acids and glucose by the PDV during 12-h of feeding

	Systemic availability		*Systemic availability*	
	µmol/(kg·6h)	*%*	*µmol/(kg·6h)*	*%*
	0-6 h		*6-12 h*	
Isoleu	1,345 ± 168	22 ± 3 *	1,535 ± 91	33 ± 2
Leu	2,564 ± 483	43 ± 8 *	3,545 ± 652	79 ± 15
Lys	1,564 ± 248	38 ± 6 *	2,236 ± 347	72 ± 11
Met	354 ± 69	38 ± 7 *	644 ± 74	82 ± 5
Phe	613 ± 70	30 ± 3	823 ± 141	54 ± 9
Tryp	311 ± 94		288 ± 25	
Thr	1,757 ± 337	24 ± 5	1,965 ± 278	35 ± 5
Val	1,768 ± 237	29 ± 4	2,875 ± 903	63 ± 20
Ala	4,764 ± 384	78 ± 6 *	5,045 ± 505	110 ± 11
Arg	715 ± 96	86 ± 12 *	1,029 ± 82	165 ± 13
Asp	187 ± 29	3 ± 1	288 ± 66	7 ± 2
Glu/Gln	440 ± 107	6 ± 1	705 ± 190	12 ± 3
	-407 ± 199	-5 ± 3	93 ± 223	2 ± 4
Gly	1,484 ± 537	37 ± 13	3,119 ± 1,598	103 ± 53
Pro	1,263 ± 238	30 ± 6 *	1,687 ± 319	54 ± 10
Ser	1,388 ± 119	33 ± 3 *	1,949 ± 331	62 ± 11
Tyr	534 ± 51	95 ± 9	920 ± 239	219 ± 57
Total AA	20,333 ± 2,040	31 ± 3	28,456 ± 5,080	60 ± 12
Gluc	24,317 ± 1,531	53 ± 3	23,360 ± 1,932	70 ± 7

*Data are expressed in micromoles per kilogram per 6-h and expressed as a percentage of intake during a 6-h feeding period. Values are means ± SEM; * indicates significant difference between 0–6 h and 6–12 h at P < 0.05; n = 4. Isoleu, isoleucine; leu, leucine; lys, lysine; met, methionine; phe, phenylalanine; tryp, tryptophan; thr, threonine; val, valine; ala, alanine; arg, arginine; asp, aspartate; glu, glutamate; gln, glutamine; gly, glycine; pro, proline; ser, serine; tyr, tyrosine; total AA, total amino acids; gluc, glucose.*

Table 2. Proportional contribution to overall systemic availability of amino acids and glucose for the different time periods

	Total availability μmol/(kg·d) 0–24 h	*Total availability % 0–24 h*	*Contribution to overall availability % 0-6 h*	*6-12 h*	*12-24 h*
Isoleu	3,276 ± 336	30 ± 3	41 ± 3	48 ± 4 §	11 ± 5
Leu	6,925 ± 1,212	66 ± 12	37 ± 4 *	51 ± 2 §	12 ± 2
Lys	4,665 ± 543	64 ± 7	33 ± 8 *	48 ± 4 §	19 ± 3
Met	1,227 ± 172	75 ± 10	29 ± 3 *	55 ± 9 §	17 ± 8
Phe	1,735 ± 210	49 ± 6	36 ± 4	47 ± 3 §	17 ± 4
Thr	4,273 ± 833	33 ± 6	42 ± 3	50 ± 8 §	8 ± 10
Tryp	613 ± 143		50 ± 4	49 ± 7	1 ± 4
Val	5,431 ± 1,076	51 ± 10	34 ± 5	50 ± 5 §	16 ± 4
Ala	12,005 ± 1,172	112 ± 11	40 ± 4	42 ± 1 §	18 ± 4
Arg	2,264 ± 259	156 ± 18	31 ± 2 *	46 ± 2 §	23 ± 3
Asp	581 ± 102	6 ± 1	33 ± 3 *	48 ± 3 §	19 ± 4
Glu/Gln	1,438 ± 293	10 ± 2	30 ± 2	46 ± 8	24 ± 9
	-2,234 ± 256	-16 ± 2	16 ± 9	6 ± 11 §	91 ± 18
Glyc	6,301 ± 2169	89 ± 31	23 ± 1 *	43 ± 7	34 ± 7
Pro	3,183 ± 599	43 ± 8	40 ± 1 *	53 ± 2 §	7 ± 1
Ser	3,735 ± 499	51 ± 7	38 ± 3 *	52 ± 3 §	10 ± 2
Tyr	1,645 ± 283	168 ± 29	34 ± 4 *	54 ± 4 §	12 ± 2
Total AA	56,452 ± 7,641	48 ± 7	37 ± 3	50 ± 3 §	14 ± 3
Gluc	47,751 ± 2422	60 ± 3	51 ± 3	49 ± 2 §	0 ± 3

*Data are expressed in micromoles per kilogram per day and as a fractional appearance in the portal circulation of total 24-h appearance of amino acids and glucose; values are means ± SEM; * indicates significant difference between 0–6 h and 6–12 h at P < 0.05; § indicates significant difference between 6-12 h and 12-24 h at P < 0.05; n = 4.*

We found a comparable pattern for the non-essential amino acids. During the last 6-h of feeding, there was a 13% increase in the portal appearance of non-essential amino acids. During the fasting period, the

portal appearance of essential amino acids accounted for a further 13% of the total non-essential amino acid portal mass balance over 24-h. Thus, as with the essential amino acids, recycling was responsible for 26% of the total 24-h appearance of non-essential amino acids, with glutamine being the only exception. Virtually all the glutamine, when expressed as a fraction of the total 24-h appearance, appeared in the portal circulation during fasting.

Table 3. Arterial and portal enrichments and portal balances of different tracers

Tracer Infused	*Tracer/tracee, mol%*		*Tracer Balance*	
	Arterial	*Portal*	*μmol/(kg·h)*	*% of input*
[D_4]lys	0.0125 ±	0.0109 ±	-2.8 ± 1.6	-7 ± 6
iv 2-12 h	0.0017	0.0018		
[U-^{13}C]lys	0.0075 ±	0.0081 ±	4.7 ± 1.3	45 ± 13
id 2-6 h	0.0008	0.0010		
[^{15}N]lys	0.0484 ±	0.0515 ±	34.3 ± 18.4	71 ± 38 *
id 8-12 h	0.0061	0.0037		
[^{15}N]thr	0.0784 ±	0.0723 ±	-13.9 ± 18.7	-3 ± 4
iv 2-12 h	0.0112	0.0106		
[U-^{13}C]thr	0.0065 ±	0.0070 ±	4.2 ± 2.3	39 ± 21
id 2-6 h	0.0010	0.0009		
[U-^{13}C,^{15}N]thr	0.0551 ±	0.0581 ±	38.0 ± 15.7	65 ± 27 *
id 8-12 h	0.0067	0.0064		

*Values are means over last three hours of tracer infusion ± SD, * indicates significant difference between 4–6 h and 10–12 h at P < 0.05; n = 4. id, intraduodenal; iv, intravenous; mol%, mole percent excess.*

Tracer utilization

As described in the Methods section, we administered six stable isotopically labeled tracers of lysine and threonine by both intravenous and intraduodenal routes to quantify the actual rate of recycling of these two amino acids. The arterial and portal enrichments and portal balances of the six different tracers are shown in Table 3. The net

absorption of both the enteral lysine tracers as the enteral threonine tracers increased significantly as feeding progressed.

A schematic representation of the recycling rate of lysine in the PDV at two different periods is depicted in Figure 4. Approximately 50% of the lysine exiting to the portal vein was derived from recycling after 6-h of continuous feeding. At the end of the feeding period, 61% of the lysine appearing in the portal vein was derived from recycling. Interestingly, no labeled threonine was found to be recycled to any extent over the 15 hours of direct measurement of the threonine isotopic enrichment in the portal vein. This is of substantial quantitative significance, since 67% of the total daily threonine intake was utilized by the PDV.

Discussion

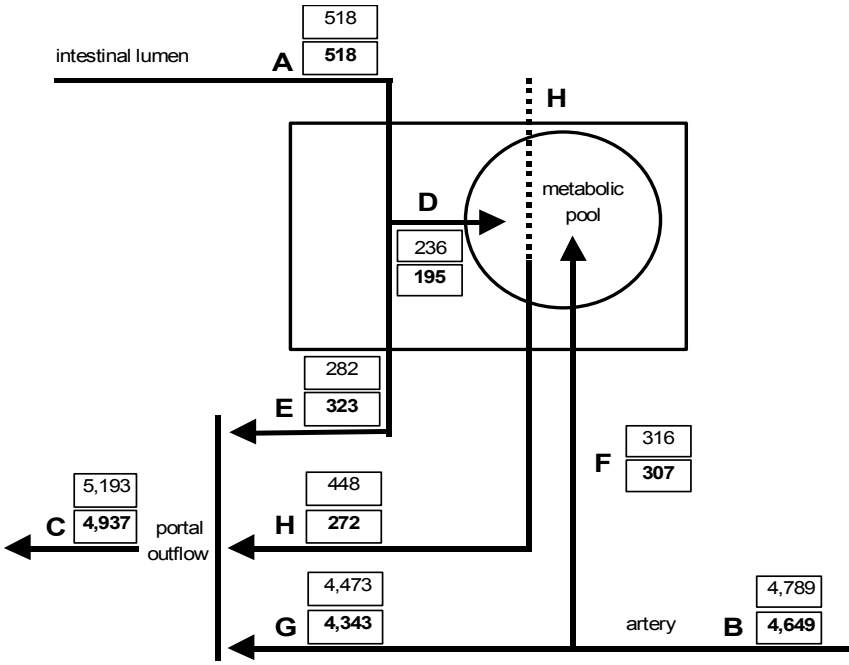
The present study utilizing dual mass and tracer balance was conducted to investigate the 24-h kinetics and the recycling pattern of amino acids. The first conclusion to be reached from this study is that approximately 50% of the dietary amino acid intake is utilized by the PDV during a 24-h period including 12-h of feeding and 12-h of fasting. In the first hours after the start of feeding only 30% of the dietary protein intake appeared in the portal circulation and hence was available for whole-body growth. The portal appearance of amino acids showed a significant and continuous rise during prolonged continuous feeding, resulting in a fractional systemic availability as a percentage intake of 57% during the last 6-h of feeding.

Our second conclusion is that, over a 24-h period including a fed and a fasted state, 26% of the dietary protein intake reappeared in the portal vein by way of recycling of previously secreted (glyco-) proteins. So, intestinal recycling of amino acids contributes significantly to their overall systemic availability.

A critical factor in interpreting the physiological significance of our results, is the metabolic fate of amino acids once they are absorbed by the intestinal cells. Absorbed amino acids can either be used for de

novo synthesis of proteins, for catabolic processes, or for incorporation into proteins.

Figure 4. Schematic representation of the metabolic fate of enteral and systemic lysine in the PDV at t = 6 h and t = 12 h



A, dietary intake of lysine; B, arterial flux of lysine through the PDV; C, portal outflow of lysine; D, unidirectional, first-pass uptake of dietary lysine by the PDV; E, dietary lysine that is not metabolized by the PDV in first-pass; F, unidirectional uptake of arterial lysine by the PDV ; G, arterial lysine that is not metabolized by the PDV; H, recycled lysine that is derived from proteolysis or the reabsorption of previously synthesized and secreted endogenous proteins, but not derived from the diet. Values are means and expressed in μmol/(kg·h), n = 4. The bold numbers are values at t = 6 h and the normal numbers are values at t = 12 h.

We found previously that less than 20% of intestinal amino acid utilization was used for constitutive gut growth by the intestinal mucosa. [7] Thus, for nutritionally indispensable amino acids, secretory (glyco-)protein synthesis appears to be a major metabolic fate. Moreover, although some essential amino acids are known to be catabolized, [6,11,17] the catabolism of essential amino acids does not account for their high utilization rate. The question of intestinal (glyco-)protein secretion and recycling is of particular importance with regards to the protective function of the gut in general and for the nutrition of threonine in particular. Structurally, the mucosa is protected by a complex network of (glyco-)proteins (mucus), of which two mucins MUC-2 and MUC-3 are important components. [18] The highly glycosylated domains of small intestinal mucins have been isolated, and the core-proteins of these molecules contain large amounts of threonine. In view of this, it is, perhaps, not surprising that the systemic availability of threonine is remarkably low. More than two-third of the enteral threonine intake was utilized by the PDV, a result that we have obtained in all of our previous studies of intestinal amino acid utilization, [6,7] and this high utilization rate of threonine represents in our view the consequence of a significant mucin production. Our results are in agreement with a study done by Bertolo et al., which investigated the threonine requirement during both parenteral and enteral feeding in neonatal piglets. [19] That study demonstrated that the threonine requirement of neonatal piglets is reduced by 45% during parenteral nutrition, and support the concept that threonine is required especially by a functional gut that secretes a lot of mucins. It appears therefore, that intestinal (glyco-)protein secretion probably makes a substantial contribution to maintenance amino acid needs in general, and to threonine needs in particular.

Perhaps more importantly, we were not able to detect any statistically significant recycling of threonine from mucins the whole study period, suggesting either that mucins are resistant to digestion or that threonine released from mucins degradation is immediately, and quantitatively, reincorporated into secretory (glyco-)proteins.

In contrast to what we found for threonine, after 6 h of feeding 46% of the lysine in the portal vein was derived from secreted protein recycling. It is, of course, possible that the first-pass lysine utilization is underestimated by tracer techniques. Specifically, separate studies in rats, pigs and humans reveal that there is a significant microbial synthesis of lysine within the gastrointestinal tract. [20-23] This lysine is made available to the host through release via microbial protein breakdown and its subsequent intestinal uptake. Lysine produced via this way will also appear in the portal vein and will, of course, be accounted for as "non-utilized" lysine in our model. Under the assumption that this occurs, the measured recycling rate would not include a contribution made by intestinally synthesized lysine and therefore our isotopically labeled tracer technique would underestimate the recycling rate of lysine. To fully resolve this issue, similar studies on the metabolic fates of the essential amino acids entering the gastrointestinal tract and the contribution made by the microbial flora to host tissue amino acid metabolism will be required in animals without micro bacterial flora.

Given the high rate of intestinal protein metabolism, it is essential that as the enterocytes are initially exposed to the diet, their requirements are met first. From different studies we know that intestinal energy production is largely derived from the oxidation of glutamate, glutamine, aspartate and glucose. [7,11,12] In addition, some essential amino acids (lysine, leucine, and phenylalanine) are known to be used for oxidative purposes by the intestinal tissues as well, [6,11] which is a nutritional loss. Furthermore, results from studies done in piglets have shown that the portal outflow varies widely among amino acids, indicating a heterogeneity in the intestinal metabolic fate among essential amino acids during the absorptive process. [6] For example, the portal appearance of phenylalanine as a fraction of intake was significantly lower as compared to the portal appearance of tyrosine as a fraction of intake. Phenylalanine oxidation is associated with its hydroxylation to tyrosine as a first step. The total 24-h phenylalanine utilization by the PDV (51%) determined in this study is similar to that

found in a study involving dual intravenous/intragastric infusions of labeled phenylalanine in fed humans (58% of dose), [24] but is lower than that measured in the postprandial state (29%). [25] We concluded from that study on splanchnic phenylalanine metabolism in pigs that catabolism is an important metabolic fate of enteral phenylalanine in the intestinal mucosa. [26] Our observed low appearance of phenylalanine and the high appearance of tyrosine supports these results, indicating a high rate of hydroxylation of phenylalanine to tyrosine.

Interestingly, almost all aspartate, glutamate and glutamine were extracted by the gastrointestinal tissues (on average more than 90% of dietary intake). Apart from the indication that these three amino acids are extensively utilized within the splanchnic tissues, the results also highlight the fact that systemic aspartate, glutamate and glutamine are almost exclusively derived from synthesis within the body. Based upon previous studies, we assume that a large part of these utilized amino acids are metabolized by the PDV for oxidative purposes and energy generation. [11,12] Glutamate, together with cysteine and glycine, also serves as a precursor for the biosynthesis of glutathione, [27] and for mucosal nucleic acids. [28] On the other hand, the utilization of some amino acids expressed as a percentage of intake after 12-h of continuous feeding (alanine, arginine, glycine and tyrosine) exceeded their intake, indicating that the intestinal tissues are nutritionally significant site of de novo synthesis of alanine, arginine, glycine and, especially, tyrosine. Indeed, studies in vitro [29] and in vivo [12] have found net arginine synthesis in the small intestine of piglets and there is also indirect evidence for this in humans. [30] The physiological and nutritional importance of this arginine pathway is strikingly illustrated by the fact that massive gut resection renders arginine a fully essential amino acid. [31]

Limited experimental information exits about the appearance of amino acids in the portal circulation during a prolonged feeding period and during fasting in piglets. Thus, measurements of net portal amino acid balance during a 24-h period gives information on the proportion

and, perhaps more importantly, the pattern of dietary amino acids that are available for the support of whole-body metabolism. As the feeding period progressed, the portal appearance of amino acids showed a significant increase while the food intake was not changed. This observation holds regardless of the decelerating effect of gastric emptying on the absorption of amino acids by the intestine. By feeding the piglets continuously with an intraduodenal catheter we avoided that the slow gastric emptying would delay the absorption of dietary nutrients. Following cessation of the continuous feeding the mean portal mass balance of all essential amino acids remained significantly different from zero for an 8-h period. The continuation of a net positive amino acid mass balance in the portal circulation during fasting for several hours presumably reflects an ongoing release of amino acids from recycled proteins. The only two exceptions were glutamine and tryptophan, nearly all the utilized glutamine appeared in the portal circulation during fasting. During fasting protein breakdown is increased which leads to a higher nitrogen release, and because glutamine plays an instrumental role in nitrogen homeostasis, the high net release of glutamine by the PDV during fasting could be an exit of metabolic nitrogen into glutamine as found by Remesy et al. [32] Our balance study showed that tryptophan was not recycled within the gut, since the portal mass balance was not significantly different from zero during the fasted state. From a study done by Cvitkovic et al., it seems that tryptophan requirement is not increased during enteral nutrition as compared to parenteral nutrition, indicating that there is no additional specific need for tryptophan during feeding. [33] This again explains the lack of recycling in our study, presumably indicating that there is hardly any tryptophan incorporated in (glyco-)proteins that are secreted into the intestinal lumen. The utilized tryptophan might well be utilized as a precursor for the biosynthesis of serotonin within the intestinal epithelium since this tissue is highly innervated. [34]

In conclusion, we have shown that during a prolonged feeding period, the PDV maintain a high rate of metabolism by continuing to use a disproportionately large amount of the dietary protein intake.

The results also show that during a fasted state the portal mass balance of all essential amino acids remains significantly greater than zero for at least 8-h. Our findings strongly support the idea that intestinal metabolism has a substantial effect on the availability of dietary amino acids for the support of whole-body growth, and that intestinal recycling of amino acids is an important regulatory mechanism of the gut for systemic amino acid availability. In other words, the intestine is not "merely" an organ of nutrient assimilation, but one that uses substantial quantities of amino acids to maintain functions of critical physiological importance.

Appendix

Arterial amino acid (AA) input [μmol/(kg·h)] =

arterial AA conc x PBF **[1]**

in which AA means amino acid which can be substituted by lysine or threonine, the amino acid concentration is expressed in μmol/liter, and PBF is expressed in liter/(kg·h).

Portal AA output [μmol/(kg·h)] =

portal AA conc x PBF **[2]**

Net portal AA balance [μmol/(kg·h)] = Eq.**2** - Eq.**1** **[3]**

Net utilization of AA by PDV [μmol/(kg·h)] =

dietary intake - Eq.**3** **[4]**

Fractional utilization of AA by PDV

(fraction of intake) = $\frac{\text{Eq.}\mathbf{4}}{\text{dietary intake}}$ **[5]**

Substituting the amino acid concentration with the tracer concentration will give the tracer kinetics.

Arterial tracer AA input [μmol/(kg·h)] =

arterial tracer AA conc x PBF **[6]**

Portal tracer AA output [μmol/(kg·h)] =

portal tracer AA conc x PBF **[7]**

Portal tracer AA balance = Eq.**7** - Eq.**6** **[8]**

The percentage of arterial AA that is utilized by the PDV is measured during the intravenous tracer study and is calculated as follows.

$$\text{Fractional arterial AA utilization} = \frac{\text{Eq.}\mathbf{6} - \text{Eq.}\mathbf{7}}{\text{Eq.}\mathbf{6}} \quad \mathbf{[9]}$$

The amount of arterial AA that is utilized by the PDV is calculated with the following equation.

$$\text{Arterial utilization of AA by the PDV [μmol/(kg·h)]} = \text{Eq.}\mathbf{9} \times \text{Eq.}\mathbf{1} \quad \mathbf{[10]}$$

The amount of dietary lysine or threonine utilized in first-pass by the intestine has to be corrected by the amount of dietary lysine or threonine that appears in the portal vein (and thus is not metabolized by the PDV) and then reenters the PDV but now from the arterial site. This lysine or threonine will be utilized by the PDV in the same proportion as the intravenous administered tracer (Eq.**9**). Thus, during the intraduodenal tracer infusion, the equation is

$$\text{Fract enteral AA utilization} = \frac{\text{dietary tracer intake} - \text{Eq.}\mathbf{7} - [(1 - \text{Eq.}\mathbf{9}) \times \text{Eq.}\mathbf{6}]}{\text{dietary tracer intake}} \quad \mathbf{[11]}$$

Thus, the amount of dietary AA that is utilized by the PDV is calculated by

$$\text{First-pass AA utilization by PDV [μmol/(kg·h)]} = \text{Eq.}\mathbf{11} \times \text{dietary intake} \quad \mathbf{[12]}$$

As stated in Figure 4, some of the lysine or threonine appearing in the portal vein is neither derived from the diet nor from the artery. This so-called recycled lysine or threonine is derived from proteolysis, either intracellular or luminal, or from bacterial production. It was calculated as follows, with results from both the intravenous and the intraduodenal tracer studies.

$$\text{Recycled AA appearing in portal vein [μmol/(kg·h)]} = \text{Eq. } \mathbf{2} - (\text{dietary intake} - \text{Eq.}\mathbf{12}) - (\text{Eq.}\mathbf{1} - \text{Eq.}\mathbf{10}) \quad \mathbf{[13]}$$

References

1. McNurlan MA, Garlick PJ. Contribution of rat liver and gastrointestinal tract to whole-body protein synthesis in the rat. Biochem. J. 1980;186:381-383.
2. Lobley GE, Milne V, Lovie JM, Reeds PJ, Pennie K. Whole-body and tissue protein synthesis in cattle. Br. J. Nutr. 1980;43:491-502.

3. Burrin DG, Ferrell CL, Britton RA, Bauer M. Level of nutrition and visceral organ size and metabolic activity in sheep. Br. J. Nutr. 1990;64:439-448.
4. Shulman RJ, Gannon N, Reeds PJ. Cereal feeding and its impact on the nitrogen economy of the infant. Am. J. Clin. Nutr. 1995;62:969-972.
5. Gaudichon C, Roos N, Mahe S, Sick H, Bouley C, Tomé D. Gastric emptying regulates the kinetics of nitrogen absorption from ^{15}N-labeled milk and ^{15}N-labeled yogurt in miniature pigs. J. Nutr. 1994;124:1970-1977.
6. Van Goudoever JB, Stoll B, Henry JF, Burrin DG, Reeds PJ. Adaptive regulation of intestinal lysine metabolism. Proc. Nat. Acad. Sci. 2000;97:11620-11625.
7. Stoll B, Henry JF, Reeds PJ, Yu H, Yahoor F, Burrin DG. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein fed piglets. J. Nutr. 1998:128;606-614.
8. Huisman J. True protein digestibility and amounts of endogenous protein measured with the ^{15}N-dilution technique in piglets fed on peas (Pisum sativum) and common beans (Phaseolus vulgaris). Br. J. Nutr. 1992:68:101-110.
9. Lien KA, Sauer WC, Mosenthin R, Souffrant WB, Dugan ME. Evaluation of the ^{15}N-isotope dilution technique for determining the recovery of endogenous protein in ileal digestion of pigs: effect of dilution in the precursor pool for endogenous nitrogen secretion. J. Anim. Sci. 1997;75:148-158.
10. Ebner S, Schoknecht P, Reeds P, Burrin D. Growth and metabolism of gastrointestinal and skeletal muscle tissues in protein-malnourished neonatal pigs. Am. J. Physiol. 1994;266:R1736-R1743.
11. Van der Schoor SRD, Van Goudoever JB, Stoll B, Henry JF, Rosenberger JF, Burrin DG, Reeds PJ. The pattern of intestinal substrate oxidation is altered by protein restriction in pigs. Gastroenterology. 2001;121:1167-1175.
12. Stoll B, Burrin, DG, Henry JF, Yu H, Jahoor F, Reeds PJ. Substrate oxidation by the portal-drained viscera of fed piglets. Am. J. Physiol. 1999;277:E168-E175.
13. Stalling DL, Gehrke CW, Zumwalt RW. A new silylation reagent for amino acids bis(trimethylsilyl)trifluoroacetamide (BSTFA). Biochem. Biophys. Res. Commun. 1968:23; 616-622.

14. Matthews DE, Ben-Galim E, Bier DM. Determination of stable isotopic enrichment in individual plasma amino acids by chemical ionization mass spectrometry. Anal. Chem. 1979:51;80-84.
15. Waterlow JC. Lysine turnover in man measured by intravenous infusion of L-[U-^{13}C]lysine. Clin. Sci. Lond. 1967;33:507-515.
16. McKenzie HA. Amino acid, peptide, and function group analyses. In: Milk Proteins: Chemistry and Molecular Biology (McKenzie HA eds.) Academic Press, New York, NY. 1970:181-192.
17. Benevenga NJ, Radcliffe BC, Egan AR. Tissue metabolism of methionine in sheep. Aust. J. Biol. Sci. 1983;36:475-485.
18. Bengmark S, Jeppsson B. Gastrointestinal surface protection and mucosa reconditioning. J. Parent. Enteral. Nutr. 1995:19;410-415.
19. Bertolo RF, Chen CZ, Law G, Pencharz PB, Ball RO. Threonine requirement of neonatal piglets receiving total parenteral nutrition is considerably lower than that of piglets receiving an identical diet intragastrically. J. Nutr. 1998;128:1752-1759.
20. Torrallardona D, Harris CI, Milne E, Fuller MF. The contribution of intestinal microflora to amino acid requirements in pigs. Proceedings of VIth International Symposium on Protein Metabolism and Nutrition (Souffrant WB, Hagemeister H, eds.) EAAP Publication No. 80. Bad Doberan, Germany, EAAP. 1994:245-248.
21. Torrallardona D, Harris CI, Milne E, Fuller MF. Site absorption of lysine synthesized by the gastrointestinal microflora of pigs. Proceedings of VIIth International Symposium on Protein Metabolism and Nutrition (Nunes AF, Portugal AV, Costa JP, Ribeiro JR, eds.) EAAP Publication No. 81. Santarém, Portugal, EAAP. 1996:369-371.
22. Torrallardona D, Harris CI, Coates ME, Fuller MF. Microbial amino acid synthesis and utilization in rats: incorporation of ^{15}N from $^{15}NH_4Cl$ into lysine in the tissues of germ-free and conventional rats. Br. J. Nutr. 1996:76;689-700.
23. Metges CC, El-Khoury AE, Henneman L, Petzke KJ, Grant I, Bedri S, Pereira PP, Ajami AM, Fuller MF, Young VR. Availability of intestinal microbial lysine for whole body lysine homeostasis in human subjects. Am. J. Physiol. 1999:277;E597-E607.
24. Biolo G, Tessari P, Inchiostro S, Bruttomesso D, Fongher C, Sabadin L, Fratton MG, Valerio A, Tiengo A. Leucine and phenylalanine kinetics during mixed meal ingestion: a multiple tracer approach. Am. J. Physiol. 1992:262;E455-E463.
25. Matthews DE, Marano MA, Campbell RG. Splanchnic bed utilization of

leucine and phenylalanine in humans. Am. J. Physiol. 1993:264;E109-E118.
26. Stoll B, Burrin DG, Henry J, Jahoor F, Reeds PJ. Phenylalanine utilization by the gut and liver measured with intravenous and intragastric tracers in pigs. Am. J. Physiol. 1997:273;G1208-G1217.
27. Reeds PJ, Burrin DG, Stoll B, Jahoor F, Wykes LJ, Henry J, Frazer ME. Enteral glutamate is the preferential source for mucosal glutathione synthesis in fed piglets. Am. J. Physiol. 1996:270;E413-E418.
28. Perez JF, Reeds PJ. A novel stable isotopic approach enables the simultaneous determination of protein and RNA synthesis in vivo. J. Nutr. 1998:128;1562-1569.
29. Wu G, Knabe DA. Arginine synthesis in enterocytes of neonatal pigs. Am. J. Physiol. 1995:269;R621-R629.
30. Berthold HK, Reeds PJ, Klein PD. Isotopic evidence for the differential regulation of arginine and proline synthesis in man. Metabolism. 1995:44;466-473.
31. Wakabayashi Y, Yamada E, Yoshida T, Takahashi N. Effect of intestinal resection and arginine-free diet on rat physiology. Am. J. Physiol. 1995:269;G313-G318.
32. Remesy C, Moundras C, Morand C, Demigne C. Glutamine or glutamate release by the liver constitutes a major mechanism for nitrogen salvage. Am. J. Physiol. 1997:272;G257-G264.
33. Cvitkovic S, Pencharz P, Ball R. The intravenous tryptophan requirement of neonatal piglets is similar to oral requirements. Proceedings of 42nd Annual Meeting of the Canadian Federation of Biological Sciences 1999;77.
34. Meyer T, Brinck U. Differential distribution of serotonin and tryptophan hydroxylase in the human gastrointestinal tract. Digestion. 1999:60;63-68.

Chapter 4

THE PATTERN OF INTESTINAL SUBSTRATE OXIDATION IS ALTERED BY PROTEIN RESTRICTION IN PIGS

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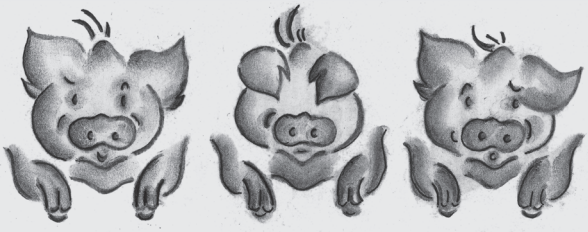

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Background & Aims: Previous studies indicate that amino acids and glucose are the major oxidative substrates for intestinal energy generation. We hypothesized that low protein feeding would lower the contribution of amino acids to energy metabolism, thereby increasing the contribution of glucose. Methods: Piglets, implanted with portal, arterial and duodenal catheters and a portal flow probe, were fed isocaloric diets of either a high protein (0.9 g/(kg·h) protein, 1.8 g/(kg·h) carbohydrate, and 0.4 g/(kg·h) lipid) or a low protein (0.4 g/(kg·h) protein, 2.2 g/(kg·h) carbohydrate and 0.5 g/(kg·h) lipid) content. They received enteral or intravenous infusions of [1-^{13}C]leucine (n=17), [U-^{13}C]glucose (n=15) or enteral [U-^{13}C]glutamate (n=8). Results: CO_2 production by the splanchnic bed was not affected by the diet. The oxidation of leucine, glutamate and glucose accounted for 82% of the total CO_2 production in high protein-fed pigs. Visceral amino acid oxidation was substantially suppressed during a low protein intake. Although glucose oxidation increased to 50% of the total visceral CO_2 production during a low protein diet, this increase did not compensate entirely for the fall in amino acid oxidation. Conclusions: Although low protein feeding increases the contribution of enteral glucose oxidation to total CO_2 production, this adaptation is insufficient. To compensate for the fall in amino acid oxidation other substrates become increasingly important to intestinal energy generation.

Even though the portal-drained viscera (PDV; the intestines, pancreas, spleen and stomach) contribute less than 5% of body weight, they utilize between 10 and 20% of whole-body energy expenditure and contribute up to 50% of the whole-body turnover of some essential amino acids. [1-3]

Furthermore, because the small intestinal mucosa is the first tissue with the capability of amino acid metabolism to be exposed to the diet, dietary amino acid utilization by the intestine could have a substantial effect on the systemic availability of essential amino acids. Intestinal metabolism could therefore have a regulatory influence on whole-body protein deposition. It is also clear from studies in a number of mammalian species, including human beings, that dietary essential amino acids are directly utilized by the intestines for protein synthesis and other biosynthetic pathways. [4-6]

The most extensive information on intestinal carbon metabolism in vivo was obtained in a series of experiments with isolated, in situ, vascularly perfused loops of rat small intestines. [7-11] In summarizing these experiments, Windmueller and Spaeth [12] concluded that aspartate, glutamate and glutamine were the major oxidative substrates used by the small intestinal mucosa and that glucose was a minor oxidative substrate in animals that had been fed up to the initiation of the study. Recently, we performed studies in pigs using ^{13}C-labeled tracers of glutamate, glutamine and glucose. [13] In confirmation of earlier studies, [7-11] we concluded that dietary glutamate was the single most important source of energy for the portal-drained viscera in the fully-fed conscious animal. Glucose accounted for approximately 30% and glutamine for 15% of total CO_2 production by the PDV.

However, the oxidation of glutamate, glutamine and glucose accounted for neither the total CO_2 production nor the production of alanine and ammonia by the PDV. Subsequently, we found that intestinal lysine oxidation accounted for one-third of whole-body lysine oxidation. [14] When this result was taken together with our previous observations and unpublished results on leucine oxidation, we

concluded that amino acids in general are the major contributors to mucosal oxidative energy generation in adequately nourished animals.

An important question posed by these results is, whether the intestinal tissues have an obligatory requirement for amino acids as energy generating substrates or whether the results that we had obtained reflected the prior (protein-rich) diet of the experimental animals. If the intestinal catabolism of amino acids is obligatory, then this pathway would have a critical effect on amino acid nutrition under conditions of a low protein intake. Therefore, the first objective of the present study was to quantify the effects of low protein intake on the oxidation of dietary glutamate and leucine as representatives of amino acids which metabolism occurs via different portions of central pathways of intermediary metabolism. [15] On the basis of some recent results in vitro [16] we hypothesized firstly, that the amino acid oxidation in the intestinal tissues adapts to a low protein intake and secondly, under this condition that there is an up-regulation of glucose catabolism to compensate for the lower rate of the amino acid oxidation.

One unique feature of the intestinal metabolism is that in the fed state the mucosal cells receive substrates directly from both the mesenteric circulation and from the diet. Previous studies [16-19] have shown that both systemic and dietary amino acids and glucose are utilized by the PDV, but the relative contribution of systemic and first-pass utilization of dietary amino acids and glucose has received relatively little attention even though it has potentially regulatory implications, particularly in relation to systemic availability. The second objective of the experiments was to quantify the effect of low protein feeding on the relative contributions of the metabolism of systemic and enteral substrates to the overall intermediary metabolism of the PDV.

In the present experiments we have measured, with enteral and intravenous infusions of ^{13}C-labeled glucose, leucine or glutamate, the oxidative metabolism by the PDV in 4-week-old piglets receiving iso-energetic diets and either a high (HP 12.7 g/(kg·d)) or a low (LP 5.1 g/(kg·d)) protein intake. The low protein intake was deliberately set at

40% of the normal intake, in an attempt to keep the animals in nitrogen equilibrium. We used piglets because of their well-established homology to infants in terms of gastrointestinal development and function, body composition, and metabolism. [20-22]

The results generally confirmed our primary hypothesis by showing a substantial suppression of visceral amino acid oxidation under conditions of a low protein intake. However, although there was an increase in the contribution of glucose oxidation to total CO_2 production, this incompletely compensated for the fall in the contribution of amino acid oxidation in order to generate energy to the PDV.

Methods

Animals

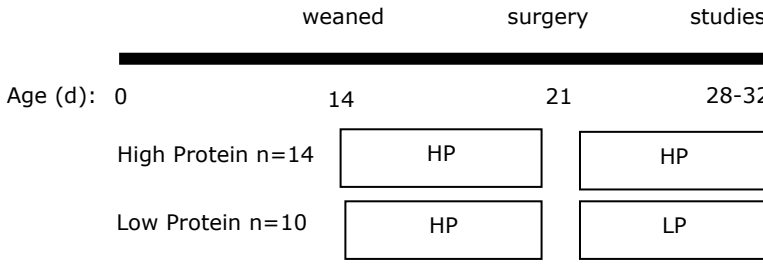
The Baylor College of Medicine Animal Protocol Review Committee approved the study. Housing and care of the animals conformed to the current USDA guidelines. The study used 4-week-old female crossbred piglets (Large White x Hampshire x Duroc) obtained from the Texas Department of Criminal Justice, Huntsville, TX. Two-week-old pigs were received at the CNRC and for a 7 d adjustment period they were fed a liquid milk replacer diet (Litter Life, Merrick, Middleton, WI) at a rate of 50 g/(kg·d) body weight. The composition (per kg dry matter) of the milk replacer is 500 g lactose, 100 g fat and 250 g protein.

Study design

The study design is depicted in Figure 1. At postnatal age of 3 weeks, the piglets underwent surgery after an overnight fast. Catheters were implanted into the stomach, duodenum, the portal and jugular vein and the common carotid artery. An ultrasonic flow probe (Transonic Systems Inc, Ithaca, NY) was implanted around the common portal vein. Following surgery, the piglets received total parental nutrition for 24-36 h. Following this period of parenteral nutrition, the piglets received either regular Litter Life (HP diet) or a diet that contained only 40% of the protein present in Litter Life (LP diet). The low protein fed

piglets were deliberately set at a feeding rate that on the basis of current recommendations would be expected to maintain body protein balance and homeostasis. [23] The diets were made isocaloric by adding lactose (Sigma Chemical, St Louis, MO) and corn oil in the same ratio as in the HP diet. The pigs were offered 50% of their preceding daily intake on the second postoperative day and resumed full feed intake on the third postoperative day. Three piglets did not survive the direct postoperative period. The animals were weighed daily for one week prior to surgery and during the whole post surgical period. At a postnatal age of 4 weeks, all animals (n = 24) received an intravenous infusion of $NaH^{13}CO_2$ in order to measure whole-body CO_2 production. Animals were randomly assigned to receive the infusions of [U-^{13}C]glutamate (HP n = 4, LP n = 4), [1-^{13}C]leucine (HP n = 9, LP n = 8) and [U-^{13}C]glucose (HP n = 8, LP n = 7) on postnatal days 29-31.

Figure 1. Schematic presentation of study design



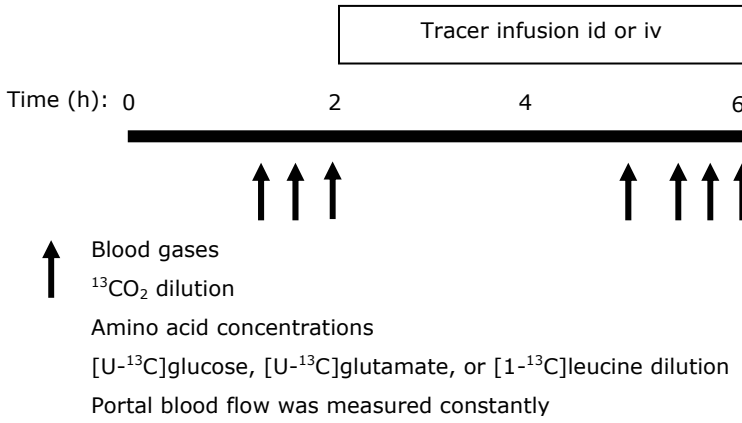
Surgery indicates placement of catheters in jugular and portal vein, carotid artery, stomach and duodenum and placement of ultrasonic flow probe around portal vein.

Tracer Protocol

The tracer protocol is shown in Figure 2. After an overnight fast, arterial and portal baseline blood samples were obtained, and the pigs were offered a single bolus meal that supplied one-fourteenth of their daily intake (186 ml/(kg·d) Litter Life). This meal served to initiate an

adequate pattern of gastric emptying. Immediately thereafter, a continuous duodenal infusion of diet was started at a rate of one-fourteenth of their daily intake for the next 12 h. This feeding protocol allowed us to feed the animals for 12 h and fast them the other 12 h before starting the next study on the following day. Thus, the piglets fed the HP diet received 14.4 kcal/(kg·h), 0.9 g/(kg·h) protein, 1.8 g/(kg·h) carbohydrate, and 0.4 g/(kg·h) lipid, while the piglets fed the LP diet received 14.4 kcal/(kg·h), 0.4 g/(kg·h) protein, 2.2 g/(kg·h) carbohydrate, and 0.5 g/(kg·h) lipid. Tracer infusion commenced 2 h after the initiation of feeding. The enteral tracers were administered via an intraduodenal or intragastric catheter. On postnatal day 28, [1-^{13}C]bicarbonate (99 mol% ^{13}C; Cambridge Isotopes,Woburn, MA) was infused into the jugular catheter at a rate of 10 µmol/(kg·h). Arterial and portal blood samples (3 mL) were drawn after 90, 105 and 120 min and at 15-min intervals during the last hour of tracer infusion.

Figure 2. Schematic presentation of tracer protocol. Numbers of piglets per specific tracer infusion: [U-^{13}C]glucose HP n = 8 vs. LP = 7; [U-^{13}C]glutamate HP n = 4 vs. LP n = 4 and [1-^{13}C]leucine HP n = 9 vs. LP n = 8



On postnatal day 29, randomly assigned piglets received a constant infusion of [U-^{13}C]glutamate (91 mol% U-^{13}C; Ajinomoto, Tokyo, Japan) providing approximately 30 μmol/(kg·h) via the duodenal catheter. On postnatal day 30, randomly assigned piglets received a constant infusion of [U-^{13}C]glucose (92 mol% U-^{13}C); Cambridge Isotopes, Woburn, MA) and [$^{2}H_2$]glucose (99 mol% D_2; Cambridge Isotopes, Woburn, MA) at a rate of 180 μmol/(kg·h) via either the duodenal catheter or the jugular catheter. On postnatal day 31, randomly assigned piglets received a constant infusion of [1-^{13}C]leucine (98.6 mol% ^{13}C; Cambridge Isotopes, Woburn, MA) providing approximately 8 μmol/(kg·h) via either the intragastric catheter or the jugular catheter. On postnatal days 29-31, arterial and portal blood samples (3 mL) were drawn after 90, 105 and 120 min and at 15-min intervals during the last hour of tracer infusion. Blood gases (Chiron Diagnostics, Halstead, Essex, UK), glucose and lactate (YSI analyzer, Yellow Springs, OH) were determined immediately in all samples. An aliquot of whole blood (0.2 mL) was mixed with an equal volume of an aqueous solution of methione sulfone (0.5 mmol/L) and directly frozen in liquid nitrogen. This was taken for subsequent analysis of amino acid concentrations. Another aliquot of EDTA-blood (0.5 mL) was taken for the analysis of its ammonia concentration, and 0.7 mL of whole blood was taken to measure blood $^{13}CO_2$, as described before. [14]

The animals were then killed with an intra-arterial injection of pentobarbital sodium (50 mg/kg body wt) and sodium phenytoin (5 mg/kg; Beutanasia-D; Schering-Plough Animal Health, Kenilworth, NJ) after 6 h of tracer infusion and the intestines were removed and weighed.

Sample analysis

Plasma ammonia was measured with the Beckman spectrophotometer (Raichem/Sigma Diagnostics, St Louis, USA) with the manufacturer's protocol. Blood samples were prepared for amino acid analysis and mass spectrometry as described previously.[5,13,14] Gas chromatography-

mass spectrometry was performed with the pentacetate derivative of glucose, the hepta-fluoro-butyramide derivative [24,25] of glutamate and the tri-fluoro-acetyl-methylester derivative [14] of leucine. The between-sample standard deviation for gas chromatograph mass spectrometer measurements was 0.1 MPE. $^{13}CO_2$ enrichment was measured on isotope ratio mass spectrometer as described before. [14] The between-sample standard deviation of isotope ratio mass spectrometer measurements was 0.001 atom percent excess (1‰). Amino acid concentrations were measured with reverse-phase high performance liquid chromatography of their phenyl-isothiocyanate derivatives (Pico Tag, Waters, Woburn, MA). The ^{13}C enrichments are reported as mole percent excess after subtracting the baseline ^{13}C content measured from samples obtained before the start of the tracer infusion.

Calculations

Previous studies from this and other laboratories have used a steady state, whole-body model of amino acid metabolism that developed in large part from work by Waterlow et al. [26] This model assumes a common metabolic amino acid pool through which all amino acids move, either to enter as dietary amino acids or from protein breakdown, or to exit for protein synthesis or oxidation. This movement through the metabolic pool is called flux. From the measurements of arterial and portal enrichments of the [^{13}C]tracers, arterial and portal amino acid concentrations, and portal blood flow, the uptake of substrates across the PDV can be calculated. [14] Corrections were made for the recycling of the enteral administered tracer that entered the PDV from the systemic circulation. By measurement of the $^{13}CO_2$ production across the PDV in a similar fashion, the oxidation of ^{13}C-labelled substrates can be calculated. [14] Again a correction was made for the recycling of the [^{13}C]tracer that was originally administered enterally, and subsequently underwent oxidation. Because the present experimental conditions were identical as those used previously [14] (except for the different tracers used, e.g.

glucose, glutamate and leucine instead of lysine), we used the same equations.

Statistics

The data are expressed as the mean values obtained from samples taken over the last hour of tracer infusions ± the inter-animal SD. Balances were tested against zero by one-tailed *t*-tests. The differences in proportion of the dietary and systemic amino acids and glucose oxidized by the splanchnic bed were tested with a two-tailed grouped *t*-test. A value of $P < 0.05$ was taken as statistically significant.

Table 1. Mean body weights, gut masses, and CO_2 production in piglets fed the two different diets

Diet	*Body weight*	*Gut mass*	*CO_2 production PDV*	*CO_2 production whole-body*	*% CO_2 production PDV*
	kg	*g/kg*	*mmol/(kg·h)*	*mmol/(kg·h)*	
HP	7.8 ± 1.0	44 ± 7	8.13 ± 2.41	64.0 ± 16.9	13.0 ± 3.9
LP	7.0 ± 1.1	42 ± 5	6.88 ± 3.44	59.6 ± 7.2	11.2 ± 6.8
Diet effect	NS	NS	NS	NS	NS

Values are means ± SD.

Results

The portal blood flow, body weights, gut masses, CO_2 production by the PDV and total CO_2 production are shown in Table 1. Prior to surgery the rate of weight gain was not significantly different between the two study groups (HP: 50 ± 4 g/(kg·d) vs. LP: 52 ± 7 g/(kg·d)). The daily weight gain over the postoperative period (10 ± 1 days) differed ($P < 0.001$) between the two groups (HP: 45 ± 3 g/(kg·d) vs. LP: 26 ± 5 g/(kg·d)). At the time of the tracer infusions, the piglets weighed 7.8 ± 1.0 kg (HP) and 7.0 ± 1.1 kg (LP), the difference

between the mean weight of the animals at the time of the study periods were related to their growth rates. The contribution of the intestines to total body weight was not significantly different (HP: 44 ± 7 vs. LP: 42 ± 5 g/kg). Low protein feeding did not significantly affect portal blood flow (PBF) and whole-body CO_2 production (HP: 64 ± 17 vs. LP: 60 ± 7 mmol/(kg·h)) as measured by [1-^{13}C]bicarbonate infusion. As found in our earlier studies, the CO_2 production by the PDV accounted for more than 10% of the total CO_2 production and was not significantly affected by the diet composition (HP: 8.1 ± 2.4 vs. LP: 6.9 ± 3.4 mmol/(kg·h)).

Mass balance of substrates and their products

Table 2 shows the net portal balances of the primary substrates over the last hour of the study. The net portal mass balances and the fractional mass balance (mass balance divide by intake) were significantly lower in the LP group for leucine and glutamate.

Table 2. Arterial and portal concentrations and net portal mass balance of primary substrates

Substrate	*Flux*		*Portal mass balance*	
	Arterial	Portal		
	mmol/(kg·h)	Mmol/(kg·h)	mmol/(kg·h)	% of input
Leu HP	2.49 ± 0.80	3.03 ± 0.88	0.48 ± 0.12	68 ± 16
Leu LP	0.91 ± 0.17	1.07 ± 0.23	0.14 ± 0.04	50 ± 13
Diet effect	*P* < 0.001	*P* < 0.001	*P* < 0.001	*P* < 0.05
Glu HP	1.57 ± 0.49	1.74 ± 0.59	0.10 ± 0.02	10 ± 2
Glu LP	0.68 ± 0.12	0.70 ± 0.12	0.02 ± 0.02	5 ± 4
Diet effect	*P* < 0.05	*P* < 0.05	*P* < 0.001	*P* < 0.05
Gluc HP	45.61 ± 8.74	51.21 ± 8.91	5.21 ± 1.92	92 ± 34
Gluc LP	28.19 ± 3.80	30.22 ± 6.74	4.98 ± 1.00	74 ± 15
Diet effect	*P* < 0.01	*P* < 0.05	NS	NS

Values are means ± SD; leu, leucine; glu, glutamate; gluc, glucose.

Table 3 summarizes the portal mass balances of ammonia, alanine, aspartate, glutamine and lactate in both groups. In the HP group the ammonia production accounted for 11% of the total nitrogen intake, while the fraction of ammonia production of the total nitrogen intake was decreased by nearly 50% in the LP group. During LP feeding the portal appearance of alanine was significantly decreased. The fall in ammonia and alanine production both indicate a markedly lower rate of amino acid oxidation by the PDV. The aspartate balance was essentially zero in both groups. Both during a high and a low protein intake the net portal balance of glutamine was slightly negative, indicating a net uptake of glutamine by the PDV. For lactate, the appearance in the portal circulation was decreased by nearly 80% during LP feeding.

Table 3. Portal mass balance of metabolic end products of glutamate and glucose metabolism

Product	*Flux*		*Portal Balance*
	Arterial	*Portal*	
	mmol/(kg·h)		*mmol/(kg·h)*
Amm HP	0.72 ± 0.07	1.79 ± 0.97	1.15 ± 0.05
Amm LP	0.07 ± 0.35	0.38 ± 0.44	0.27 ± 0.01
Diet effect	*P* < 0.0001	*P* < 0.0001	*P* < 0.0001
Ala HP	4.02 ± 1.97	4.70 ± 2.34	0.65 ± 0.33
Ala LP	2.62 ± 0.98	3.01 ± 1.14	0.32 ± 0.13
Diet effect	NS	NS	*P* < 0.01
Asp HP	0.25 ± 0.07	0.29 ± 0.07	0.04 ± 0.01
Asp LP	0.09 ± 0.04	0.10 ± 0.02	0.01 ± 0.00
Diet effect	*P* < 0.01	*P* < 0.01	*P* < 0.01
Gln HP	3.44 ± 2.07	3.32 ± 1.81	-0.01 ± 0.10
Gln LP	1.07 ± 0.49	1.02 ± 0.46	- 0.06 ± 0.04
Diet effect	*P* < 0.05	*P* < 0.01	*P* < 0.01
Lact HP	6.30 ± 3.88	8.09 ± 5.04	1.73 ± 1.25
Lact LP	5.48 ± 2.29	5.92 ± 2.55	0.37 ± 0.21
Diet effect	NS	NS	*P* < 0.05

Values are means ± SD; amm, ammonia; ala, alanine; asp, aspartate; gln, glutamine; lact, lactate.

The arterial and portal enrichments of the of [^{13}C]tracers is shown in Table 4. We detected no [U-^{13}C]glutamate in the portal or arterial circulation both during HP and LP feeding, indicating a complete first-pass removal of the glutamate tracer. The net absorption of enteral [1-^{13}C]leucine did not differ significantly between the groups. Ninety percent of the enteral dose of [U-^{13}C]glucose appeared in the portal circulation and while the portal appearance of [U-^{13}C]glucose (72% of infusion) was lower during a low protein intake, the difference was not significant.

Glutamate oxidation

The glutamate metabolism by the portal-drained viscera is depicted in Figure 3. During a generous protein intake 47% of the enteral dose of [U-^{13}C]glutamate appeared as portal $^{13}CO_2$, whereas 32% of the enteral dose was oxidized during an low protein intake. Subsequently, and due to the lower glutamate intake during low protein feeding, the oxidation of glutamate in the LP group was significantly lower as compared to the HP group and the contribution of enteral glutamate to total CO_2 production by the splanchnic bed dropped significantly (HP: 32 ± 15% vs. LP: 10 ± 3%, $P < 0.05$) during LP feeding (Table 5).

Figure 3. Glutamate metabolism by the PDV. The white bar represents the HP group and the black bar represents the LP group; * indicates a significant difference between HP- and LP-fed piglets at $P < 0.05$

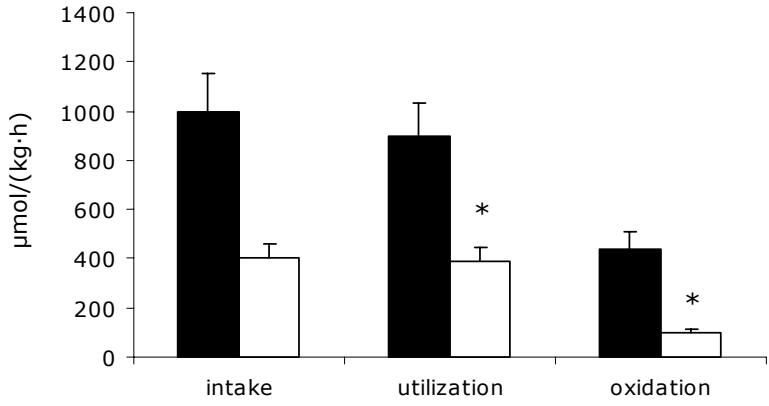


Table 4. Arterial and portal enrichments and portal balances of [^{13}C]tracers

Tracer	*Arterial enrichment MPE*	*Portal enrichment* MPE	*Tracer balance µmol/(kg·h)*	*% of input*
[1-^{13}C]leu id HP	1.0460 ± 0.1306	1.2298 ± 0.1588	794 ± 214	46 ± 21
[1-^{13}C]leu id LP	1.5998 ± 0.2037	1.7728 ± 0.1703	499 ± 151	37 ±10
Diet effect	*P* < 0.05	*P* < 0.05	NS	NS
[1-^{13}C]leu iv HP	1.0858 ± 0.2956	0.8638 ± 0.2957	60 ± 213	2 ± 12 *
[1-^{13}C]leu iv LP	1.4985 ± 0.2029	1.2505 ± 0.0544	-75 ± 153	-
Diet effect	*P* < 0.05	*P* < 0.05	*P* < 0.05	*P* < 0.05
[U-^{13}C]glu id HP	0.0004 ± 0.0003	0.0003 ± 0.0003	0.3 ± 0.5	-
[U-^{13}C]glu id LP	0.0018 ± 0.004	0.0056 ± 0.011	-5 ± 16	1 ± 2
Diet effect	*P* < 0.05	NS	NS	NS
[U-^{13}C]gluc id HP	0.0256 ± 0.001	0.0252 ± 0.001	160 ± 51	90 ± 29
[U-^{13}C]gluc id LP	0.0163 ± 0.0002	0.0175 ± 0.0004	129 ± 46	72 ± 26
Diet effect	*P* < 0.05	*P* < 0.05	NS	NS
[U-^{13}C]gluc iv HP	0.0216 ± 0.002	0.0245 ± 0.002	-84 ± 56	-
[U-^{13}C]gluc iv LP	0.0231 ± 0.003	0.0185 ± 0.003	-33 ± 25	-
Diet effect	NS	NS	NS	NS

*Values are means ± SD; id, intraduodenal; iv, intravenous; MPE, mole percent excess; leu, leucine; glu, glutamate; gluc, glucose; * expressed as a percentage of input of arterial [1-^{13}C]leucine by the PDV.*

Leucine oxidation

Details of the leucine metabolism are shown in Figure 4. During HP feeding both arterial and enteral leucine were oxidized in almost equal amounts by the PDV. Total leucine oxidation contributed for 18% to overall CO_2 production by the PDV (Table 5). The oxidation of both arterial and enteral leucine oxidation by the PDV fell to zero in the LP group. Leucine oxidation by the splanchnic tissues accounted for 50% of whole-body leucine oxidation during a high protein intake, while whole-body leucine oxidation was significantly lower during LP feeding as compared to HP feeding (HP: 252 ± 64 vs. LP: 200 ± 42 μmol/(kg·h), $P < 0.01$).

Table 5. Oxidation and fraction of contribution of different substrates to CO_2 production by the PDV

	HP		*LP*	
	Oxidation	*Fract CO_2 prod by PDV*	*Oxidation*	*Fract CO_2 prod by PDV*
	μmol/(kg·h)	*%*	*μmol/(kg·h)*	*%*
PDV CO_2 prod	8,129 ± 2,408	100	6,881 ± 3,975	100
Leu enteral	696 ± 508	12 ± 10	55 ± 307	-
Leu systemic	432 ± 702	6 ± 8	-70 ± 81	-
Glu enteral	2,349 ± 1,363	32 ± 15	771 ± 604	10 ± 3
Gluc enteral	-1,754 ± 556		1,983 ± 2,681	25 ± 30
Gluc systemic	3,714 ± 851	39 ± 10	1,170 ± 500	25 ± 7
Miscellaneous		11		40

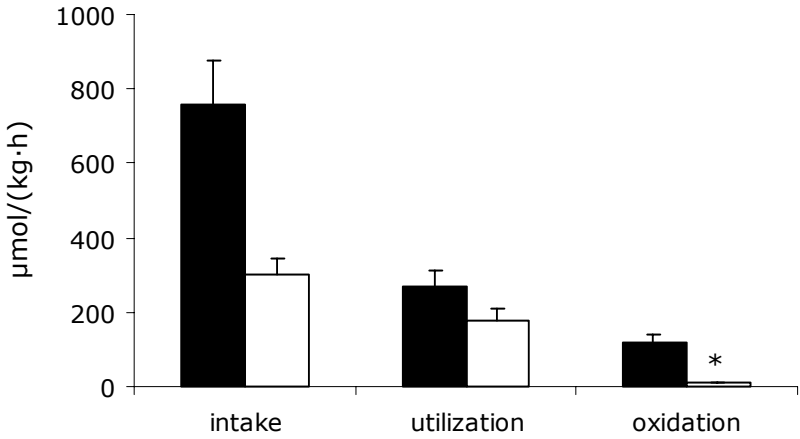
Values are means ± SD.

Glucose oxidation

In the HP group, the intestine oxidized arterial glucose at a rate of 619 ± 142 μmol/(kg·h), and there was no net oxidation of enteral glucose after correction of the secondary metabolism of the recycled [U-^{13}C]glucose (Figure 5). The oxidation rate from arterial glucose decreased by more than 75% to 209 ± 60 μmol/(kg·h) in response to a

low protein diet. However, in the LP group there was an increase in the first-pass oxidation of enteral glucose in the LP fed pigs (330 ± 447 μmol/(kg·h)), so that the total glucose oxidation by the PDV in the LP group (540 μmol/(kg·h)) was not significantly different from the total oxidation in the HP group.

Figure 4. The leucine metabolism by the PDV. The white bar represents the HP group and the black bar represents the LP group; * indicates a significant difference between HP- and LP-fed piglets at *P* < 0.05

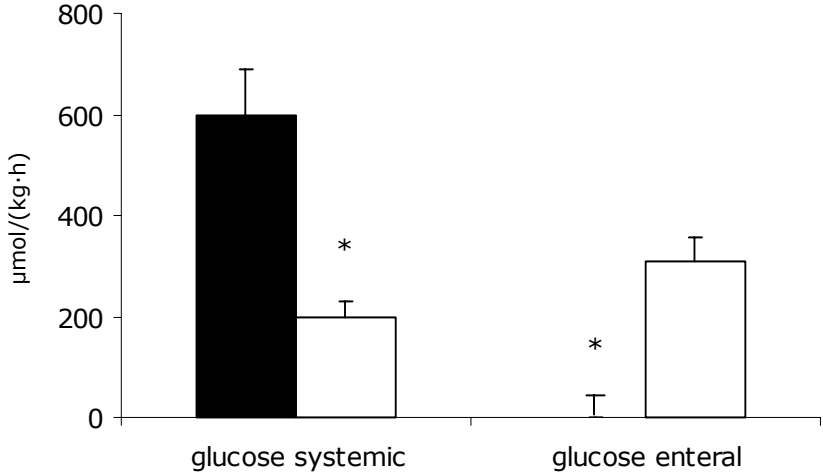


CO_2 production by the different substrates

The contributions of the different substrates to total CO_2 production by the splanchnic tissues are presented in Table 5. During HP feeding arterial glucose and enteral glutamate were the most important sources of energy (glucose iv 39% and glutamate id 32%) and a high proportion (89%) of the total CO_2 production by the PDV could be accounted for by direct measurements. During the low protein intake period, amino acid oxidation accounted for only 10% of the total CO_2 production by the PDV. Although the fractional contribution of the glucose oxidation rose to 50%, the sum of amino acid and glucose

oxidation accounted for no more than 60% of the measured CO_2 production by the PDV.

Figure 5. The glucose oxidation rates by the PDV. The white bar represents the HP group and the black bar represents the LP group. * indicates a significant difference between the HP-and the LP-fed pigs at *P* < 0.05



Discussion

In our previous work, we showed that under high protein feeding conditions, intestinal energy production is largely derived from the oxidation of glucose, glutamate, glutamine and aspartate. [13] In this previous study, dietary glutamate was the single most important oxidative substrate for the intestinal mucosa. This is in contrast to the general believe that glutamine is the most important energy substrate, a conclusion that is based upon the earlier reports of measurements in isolated loops of rat. [7,8,11] However, much of the more recent literature on splanchnic metabolism has ignored two other observations made by same authors, i.e., first, that the metabolism of luminal glutamate was even more extensive than that of arterial glutamine; and second, that the presence of high concentrations of glutamate in the intestinal

lumen had only a small (25%) effect on the intestinal utilization of glutamine. [9] In other words, these two closely related amino acids may have different functional roles in the splanchnic tissues. In subsequent studies [14] we showed that the PDV also oxidize lysine and the present study extends these observations to leucine, a result that confirms Yu's work in the dog. [17] The present study was designed primarily to test two hypotheses *(I)* that when protein becomes the limiting nutrient then there is an adaptation in the oxidation of amino acids by the PDV and *(II)* that under this circumstance an up-regulation of glucose oxidation compensates for the fall in the contribution of amino acids to intestinal energy generation.

In discussing the results it is important to emphasize two aspects of the study design. First, the lower level of protein intake was set at a value that held the animals in whole-body nitrogen equilibrium. [27] Second, that the overall energy intake of the low protein fed animals was kept constant, and that also the relative contribution of carbohydrate (lactose) and lipid (corn oil) was unaltered. The first result of interest was that although the overall nitrogen balance across the PDV of the LP group was close to zero, this appeared to be the resultant of continuing protein gain in the intestinal tissues and a small protein loss from the rest of the body. This confirms previous work by us [27] and suggests that the continuing weight gain of the low protein fed animals consisted largely of body lipid. In addition, as we have shown in a previous report, [14] the impact of low protein feeding on the overall utilization of dietary protein by the intestinal tissues was minimal, and presumably because the intestine was supplied with adequate quantities of non-protein energy substrates, the rates of visceral CO_2 production and oxygen consumption were not significantly different between the high and low protein-fed animals. However, despite the maintenance of normal rates of energy expenditure, the low protein intake was associated with a virtually complete suppression of leucine oxidation and, on the basis of the portal ammonia and alanine outflow, a 75% decrease in overall amino acid catabolism. Nevertheless, it was of considerable interest that the utilization of

glutamine continued in proportion to intestinal tissue mass and that the fall in glutamate oxidation was in proportion to the reduction in glutamate intake. In other words, the metabolic importance of both glutamate and glutamine was maintained, even though protein was in substantial deficit.

With regards to intestinal glucose metabolism the present data in the HP group confirmed our previous observations [13] in demonstrating that in this group the oxidation of arterial glucose supported about 40% of intestinal CO_2 production. In addition, the present results also confirmed the complete lack of first-pass oxidation of enteral glucose in high protein-fed animals. It was notable however, that despite the higher lactose intake of the LP group, the portal balance of glucose was essentially the same in the two feeding groups. Furthermore, because only 30% of the utilized glucose by the PDV appeared as CO_2, the PDV of the LP group were utilizing more glucose for anabolic purposes, presumably for lipid and glycoprotein synthesis.

It was of considerable interest that in the LP group not only the contribution of glucose oxidation to total CO_2 production was increased to some extent, but that the increase associated with low protein feeding was a reflective of an increase in the oxidation of enteral glucose in first pass. This "switch" towards an increased reliance in first-pass metabolism was also observed in our previous study of intestinal lysine utilization in the low protein fed animals. Together the results imply that there are adaptive changes in both the apical and basolateral amino acid and glucose transporters associated with the prolonged consumption of a diet which contains less protein. Studies in rats showed that during a low protein intake the expression of the CAT-1 transportersystem, an intestinal amino acid transportersystem, was up-regulated in liver cells. [28] It remains to be investigated to what extent the amino acid transport is handled in first-pass by the transporters in the apical and basolateral membrane of the intestinal enterocytes during a low protein intake.

Although the contribution of the oxidation of enteral glucose to the total CO_2 production was somewhat higher in the low protein fed

animals, the increase did not compensate for the fall in overall amino acid catabolism by the PDV. In fact, while in the HP group more than 89% of the output of CO_2 by the PDV derived from the oxidation of glucose, glutamate and leucine, in the LP group the measurements could only account for 60% of the output of CO_2 by the PDV. Thus, other substrates must have assumed an increased importance. In this context it is important to emphasize the fact that the dietary substitution consisted of a combination of lactose and corn oil so that the overall lipid intake of the LP animals was also increased and presumably the PDV became more reliant on lipid oxidation. Whether this represents the oxidation of free fatty acids, ketones or short-chain fatty acids is unknown, although it should be noted that the intestinal tissues are capable of oxidizing all three alternative substrates. [11,29] Moreover work in mice receiving isocaloric diets deferring in the lipid:carbohydrate ratio has suggested that the intestinal mucosal cells are able to readily substitute these substrates to potential sources of ATP.

In conclusion, we have shown that during a low protein intake the splanchnic tissues maintain a high rate of energy expenditure by increasing the oxidation rate of dietary glucose. Under the conditions of the present study, intestinal adaptation appears to favor the systemic availability of amino acids by reducing the amino acid oxidation, although the anabolic utilization of essential amino acids is altered to only a small extent. However, the oxidation of glucose and amino acids does not provide enough substrate for the energy need of the PDV. Therefore the gut has to use other substrates like fatty acids or ketone bodies to keep up the high mucosal metabolism. At this stage, the mechanism that underlies the preferential choice of the gut for substrates as fuel source during a low protein supply is not clear and merits further examination.

References

1. McNurlan MA, Garlick PJ. Contribution of rat liver and gastrointestinal tract to whole-body protein synthesis in the rat. Biochem. J. 1980;186:381-383.

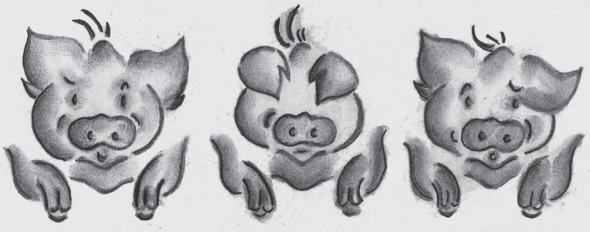
2. Burrin DG, Ferrell CL, Britton RA, Bauer M. Level of nutrition and visceral organ size and metabolic activity in sheep. Br. J. Nutr. 1990;64:439-448.
3. Lobley GE, Milne V, Lovie JM, Reeds PJ, Pennie K. Whole body and tissue protein synthesis in cattle. Br. J. Nutr. 1980; 43:491-502.
4. Hoerr RA, Matthews DE, Bier DM, Young VR. Effects of protein restriction and acute refeeding on leucine and lysine kinetics in young men. Am. J. Physiol. 1993;264:E567-E575.
5. Stoll B, Henry J, Reeds PJ, Yu H, Farook J, Burrin DG. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. J. Nutr. 1998;128:606-614.
6. MacRae JC, Bruce LA, Brown DS, Calder AG. Amino acid use by the gastrointestinal tract of sheep given lucerne forage. Am. J. Physiol. 1997;273:G1200-G1207.
7. Windmueller HG. Glutamine utilization by the small intestine. Adv. Enzymol. Relat. Areas. Mol. Biol. 1982;53:201-237.
8. Windmueller HG, Spaeth AE. Uptake and metabolism of plasma glutamine by the small intestine. J. Biol. Chem. 1974;249:5070-5079.
9. Windmueller HG, Spaeth AE. Intestinal metabolism of glutamine and glutamate from the lumen as compared to glutamine from blood. Arch. Biochem. Biophys. 1975;171:662-672.
10. Windmueller HG, Spaeth AE. Metabolism of absorbed aspartate, asparagine, and arginine by rat small intestine in vivo. Arch. Biochem. Biophys. 1976;175:670-676.
11. Windmueller HG, Spaeth AE. Identification of ketone bodies and glutamine as the major respiratory fuels in vivo for postabsorptive rat small intestine. J. Biol. Chem. 1978;253:669-676.
12. Windmueller HG, Spaeth AE. Respiratory fuels and nitrogen metabolism in vivo in small intestine of fed rats. Quantitative importance of glutamine, glutamate, and aspartate. J. Biol. Chem. 1980;255:107-112.
13. Stoll B, Burrin DG, Henry JF, Yu H, Jahoor F, Reeds PJ. Substrate oxidation by the portal drained viscera of fed piglets. Am. J. Physiol. 1999;277:E168-E175.
14. Van Goudoever JB, Stoll B, Henry JF, Burrin DG, Reeds PJ. Adaptive regulation of intestinal lysine metabolism. Proc. Nat. Acad. Sci. 2000;97:11620-11625.
15. Jungas RL, Halperin ML, Brosnan JT. Quantitative analysis of amino acid oxidation and related gluconeogenesis in humans. Physiol. Rev. 1992;72:419-448.

16. Fleming SE, Zambell KL, Fitch MD. Glucose and glutamine provide similar proportions of energy to mucosal cells of rat small intestine. Am. J. Physiol. 1997;273:G968-G978.
17. Yu YM, Burke JF, Vogt JA, Chambers L, Young VR. Splanchnic and whole body L-[1-^{13}C,^{15}N]leucine kinetics in relation to enteral and parenteral amino acid supply. Am. J. Physiol. 1992;262:E687-E694.
18. Fellows FC, Lewis MH. Lysine metabolism in mammals. Biochem. J. 1973;136:329-334.
19. Alpers DH. The relation of size to the relative rates of degradation of intestinal brush border proteins. J. Clin. Invest.1972;51:2621-2630.
20. Shulman RJ. The piglet can be used to study the effects of parenteral and enteral nutrition on body composition. J. Nutr. 1993;123:395-398.
21. Darragh AJ, Moughan PJ. The three-week-old piglet as a model animal for studying protein digestion in human infants. J. Pediatr. Gastroenterol. Nutr. 1995;21:387-393.
22. House JD, Pencharz PB, Ball RO. Lysine requirement of neonatal piglets receiving total parenteral nutrition as determined by oxidation of the indicator amino acid L-[1-^{14}C]phenylalanine. Am. J. Clin. Nutr. 1998;67:67-73.
23. Reeds PJ, Burrin DG, Jahoor F, Wykes L, Henry J, Frazer EM. Enteral glutamate is almost completely metabolized in first pass by the gastrointestinal tract of infant pigs. Am. J. Physiol. 1996;270:E413-E418.
24. Matthews DE, Ben-Galim E, Bier DM. Determination of stable isotopic enrichment in individual plasma amino acids by chemical ionization mass spectrometry. Anal. Chem. 1979;51:80-84.
25. Waterlow JC. Lysine turnover in man measured by intravenous infusion of L-[U-^{14}C]lysine. Clin. Sci. Lond. 1967;33:507-515.
26. Fuller MF, Reeds PJ, Cadenhead A, Seve B, Preston T. Effects of the amount and quality of dietary protein on nitrogen metabolism and protein turnover of pigs. Br. J. Nutr. 1987;58:287-300.
27. Ebner S, Schoknecht P, Reeds PJ, Burrin DG. Growth and metabolism of gastrointestinal and skeletal muscle tissues in protein-malnourished neonatal pigs. Am. J. Physiol. 1994;266:R1736-R1743.
28. Morens C, Gaudichon C, Metges CC, Fromentin G, Baglieri A, Even PC, Huneau JF, Tomé D. A high-protein meal exceeds anabolic and catabolic capacities in rats adapted to a normal protein diet. J. Nutr. 2000;130:2312-2321.

29. Fleming SE, Fitch MD, DeVries S, Liu LM, Kight C. Nutrient utilization by cells isolated from rat jejunum, cecum and colon. J. Nutr. 1991;121:G869-G978.

Part II

SPLANCHNIC METABOLISM IN PRETERM INFANTS



Chapter 5

VALIDATION OF THE DIRECT NASO-PHARYNGEAL SAMPLING METHOD FOR THE COLLECTION OF EXPIRED AIR AFTER A [^{13}C] BICARBONATE INFUSION IN PRETERM NEONATES

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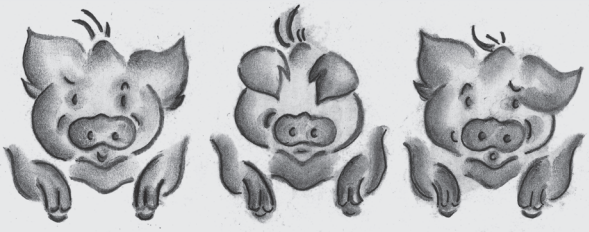

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Background & Aims: In clinical studies, the oxidation of [^{13}C]labeled substrates to $^{13}CO_2$ and the measurement of the appearance of excess $^{13}CO_2$ in expiratory air has progressed to an increasingly common method as it is noninvasive and lacks the radiation exposure associated with the use of ^{14}C. The collection of respiratory CO_2 currently used, occurs via trapping of CO_2 in sodium hydroxide (trapping method) sometimes in conjunction with indirect calorimetry. The aim of the present study was to determine the accuracy of our direct naso-pharyngeal sampling method for the collection of breath samples in preterm infants compared with the currently used trapping method. Methods: We present a method that simplifies the collection of breath samples in preterm infants. Seven preterm infants with a gestational age of 26-29 wk were studied on different postnatal days (range: 8-52 days) while receiving full enteral feeding. A primed constant 3-h intragastric infusion of [^{13}C]bicarbonate was given and breath samples were collected by means of direct naso-pharyngeal sampling and by a sodium hydroxide trap simultaneously. Results: The ^{13}C isotopic enrichment in breath rose rapidly to reach a plateau by 120 min with <5% variation of plateau in both methods. $^{13}CO_2$ breath isotopic enrichments obtained by the direct naso-pharyngeal sampling method correlated highly (r = 0.933 and *P* < 0.0001) with the trapping method. The Bland-Altman analysis showed no significant variability between both methods and demonstrated that the 95% confidence interval is within ± 4.68 δ‰. Conclusion: These findings validate the simple method of direct naso-pharyngeal sampling of expired air in neonates.

Over the last three decades, stable isotopes have been extensively used to investigate whole-body protein metabolism in adults, children and newborns. [1-3]

To determine the rate of substrate oxidation, the excretion of $^{13}CO_2$ in expired air must be quantified. The rate of oxidation is calculated by multiplying the enrichment of $^{13}CO_2$ in breath by the total rate of CO_2 excreted, correcting for the incomplete recovery of ^{13}C during an infusion of [^{13}C]bicarbonate. [1] This correction factor has to be used to adjust for the fractional recovery of CO_2. [4,5] In addition, the total CO_2 production (VCO_2) needs to be measured.

Several methods are known to quantify VCO_2, but closed circuit indirect calorimetry is the most commonly performed method and serves as gold standard for the estimation of VCO_2. This procedure requires the patient to breathe via a mouthpiece with the nares occluded or via a hood that catches all expired CO. [6] However, particular circumstances make the use of the results obtained from indirect calorimetry difficult. For instance, results are difficult to interpret when there is an unmeasured loss of expired gasses due to the use of uncuffed leaking endotracheal tubes in mechanically ventilated neonates. [7] In addition, there is an increased possibility of error in the indirect calorimeter determination of VO_2 and VCO_2 with the low volumes of respiratory gas exchanged in small neonates, [8] and the higher FiO_2 used in critically ill patients. [9] This error is of such a significant degree that the use of indirect calorimetry is not recommended in patients receiving more than 0.60 FiO_2. [10]

Another method for measuring VCO_2 involves the infusion of a [^{14}C]labeled substrate and the measurement of its specific activity and the rate of excretion of $^{14}CO_2$ in breath over a period at isotopic steady state. [11] However, the use of radioactive isotopes in children is prohibited.

Numerous studies described the use of isotopic dilution of CO_2 during the infusion of [^{13}C]bicarbonate to predict CO_2 production, which avoids the quantification of total expired air. [4,12] However, this tracer technique uses a relatively complicated method to collect expired air.

Breath samples must be collected using a closely fitted facemask and an inlet/outlet system with a low dead volume. Thereafter, the collected expired air has to pass an all-glass spiral condenser, containing sodium hydroxide. After liberating CO_2 by adding phosphoric acid to the solution, the CO_2 can be stored in a septum-capped tube until analysis. [13] During all these procedures to liberate CO_2, the fraction of $^{13}CO_2$ might be decreased, resulting in an underestimation of the substrate oxidation.

To collect breath samples from preterm infants in a suitable manner, we applied the direct naso-pharyngeal sampling technique previously described by Perman et al. [14] This method has been used in older children for the non-invasive diagnosis of sucrose or lactose malabsorption by a H_2 breath test, and in preterm infants for the collection of expiratory air after administration of ^{13}C-labeled substrates. [14-16] However in preterm infants, the direct naso-pharyngeal sampling method has never been validated for the use in substrate oxidation studies. The direct sampling approach offers the advantage of being simple and non-time consuming, therefore, possibly appropriate for the use in preterm infants in combination with [^{13}C]bicarbonate stable isotope dilution technique for the estimation of CO_2 production. Validation of such a method would provide a technique that could be utilized in a variety of clinical investigations in neonates and children.

The purpose of this study was to determine whether the direct naso-pharyngeal sampling technique for the collection of expired air could be used to estimate CO_2 production in preterm infants. We validated this technique to the method using CO_2 trapping in sodium hydroxide for collection of expiratory air after [^{13}C]bicarbonate infusion in preterm infants.

Methods

Subjects

Subjects included seven preterm infants admitted to the neonatal intensive care unit of the Erasmus MC-Sophia Children's Hospital. The study protocol was reviewed and approved by the Erasmus-MC

Institutional Review Board and written and informed consent was obtained from the parents of the preterm infants.

Direct naso-pharyngeal sampling method and trapping method

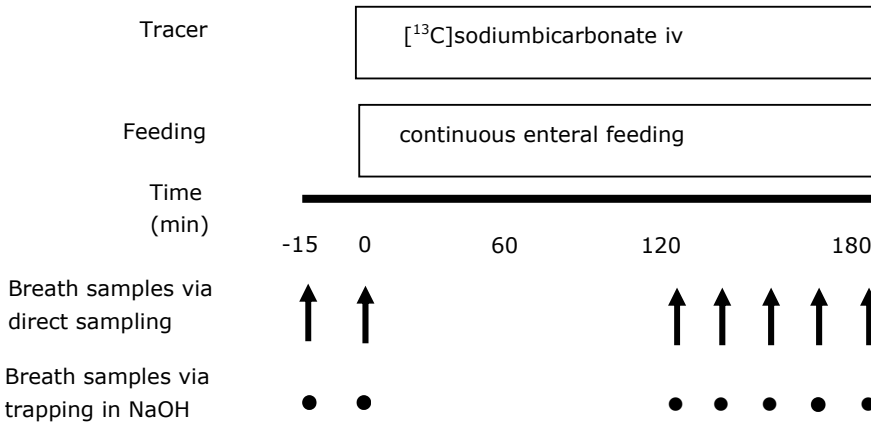
The collection of expired air was obtained by two different methods, i.e. the direct naso-pharyngeal sampling method and the trapping method. In the direct naso-pharyngeal sampling technique for the collection of expired air, we used a 6 Fr gastric tube (6 Ch Argyle; Sherwood Medical, Tullamore, Ireland) as a collection device which was placed carefully for 1 to 1.5 cm into the nasopharynx. When the infant received supplemental oxygen by nasal prong, the oxygen air had to pass a sodalime filter (Erich Jaeger GmbH & Co. KG, Wurzburg, Germany) before entering the nasopharynx in order to supply CO_2 devoid air. While observing the patient's normal breathing pattern, the examiner repeatedly aspirated 1 ml of each tidal volume late in the expiratory phase from the nasal prong connected to a 10 ml plastic syringe until 10 ml was obtained. Aliquots were transferred by syringe to a 10 ml vacutainer (Van Loenen Instruments, Zaandam, The Netherlands).

The trapping method has been described for CO_2 production measurements in neonates. [13,17] In this method, breath samples are collected via a closely fitted facemask or canopy which is placed around the head and chest of the neonate. The baby's head and neck were placed under the transparent perspex canopy, and a partial seal is created by tucking the attached flexible material beneath the body and mattress in order to prevent any air leaks. We used a canopy as is used for indirect calorimetry studies in infants. [18] An inlet/outlet system with a low dead volume is used to avoid dilution of the carbon dioxide <1.5-2.0%. Next, the collected expired air has to pass a sample of air leaving the ventilated hood through an all-glass spiral condenser, containing 10 ml of 1 M sodium hydroxide (Merck, Darmstadt, Germany). After liberating CO_2 by adding phosphoric acid (Merck, Darmstadt, Germany) to the solution, the CO_2 can be stored in septum-capped tubes until analysis.

Validation of the collection method

To demonstrate that the isotopic enrichment in the expired air collected by the direct naso-pharyngeal sampling method showed a consistent relationship and to show that this collection of expired air is a reliable technique, comparison with the trapping method was performed. Validation of direct naso-pharyngeal sampling technique and the trapping method was achieved by obtaining simultaneously duplicate paired samples of expired air in seven clinically stable preterm infants without ventilatory support.

Figure 1. Schematic overview of study protocol



Tracer protocol

After baseline expired breath samples were obtained according both methods (Figure 1), a primed (10.02 μmol/kg), 3-h continuous intragastric infusion (10.02 μmol/(kg·h)) of sterile pyrogen-free [^{13}C]sodium bicarbonate (99 mol% ^{13}C; Cambridge Isotopes, Woburn, MA) was administered. Five sets of duplicate CO_2 breath samples were collected at 15-min intervals over the last hour of infusion by using both sampling techniques. Breath isotopic enrichment was plotted against

time, and the plateau defined according to the convention of taking four or more consecutive points with a coefficient of variation of <5%.

Calculations

Isotopic enrichment of expired CO_2 was measured by monitoring ions at m/e 44 and 45 with a continuous flow isotope ratio mass spectrometer (ABCA; Europa Scientific, Van Loenen Instruments, Leiden, The Netherlands). [19] The ratio of the signals from the ionized species $^{13}CO_2$ and $^{12}CO_2$ each focused on one of the dual spectrometers is recorded and compared with the natural abundance of CO_2 gas of known isotopic composition relative to the standard Pee Dee Belemnite (PDB). The results of this differential measurement are expressed as the del per mil (δ‰) difference between the $^{13}CO_2/^{12}CO_2$ ratio of the sample and standard. [20]

$$\delta^{13}C\ (‰) = \frac{R_u - R_s}{R_u} \times 1{,}000 \qquad \textbf{[1]}$$

where R_u is the difference between the $^{13}CO_2/^{12}CO_2$ ratio of the sample, and R_s is the difference between the $^{13}CO_2/^{12}CO_2$ ratio of the standard.

Steady state values were obtained by determining the average CO_2 isotopic enrichment after reaching plateau as previously described. [21] $RaCO_2$ was calculated using the standard steady state equation: [22]

$$RaCO_2 = i_B \times [(IE_I / IE_B) - 1] \qquad \textbf{[2]}$$

where i_B is the infusion rate of [^{13}C]sodium bicarbonate (μmol/(kg·h)), IE_I is the enrichment (mole percent excess) of [^{13}C]bicarbonate in the bicarbonate infusate and IE_B is the $^{13}CO_2$ enrichment in expiratory air at plateau during the [^{13}C]sodium bicarbonate infusion (mole percent excess).

Statistics

All values are expressed in means ± SD. Statistical analyses were performed by ANOVA, t-test, and Pearson correlation. Significance was determined as $P < 0.05$. Bland-Altman analysis was performed to test the variability between the two sampling methods. [23]

Results

All infants were appropriate for gestational age (median gestational age 28 wk, range: 26 –29 wk, Table 1). Seven patients who breathed spontaneously were studied, six of them received supplemental oxygen by a nasal prong. All infants were clinically stable at the time of the study. The median body weight at the time of study period was 1.2 kg (range: 1.0 – 1.5 kg), and the median postnatal age was 28 d (range: 8 – 52 d).

Table 1. Clinical characteristics

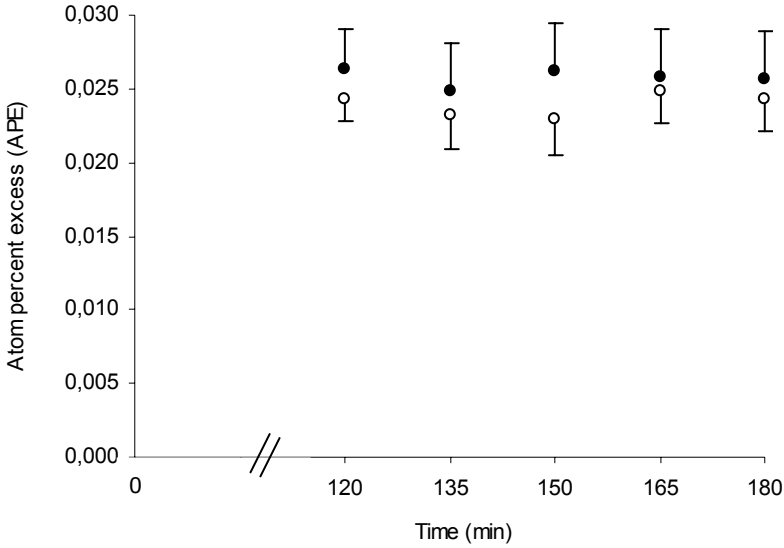
Patient	*Gender*	*Birth weight*	*GA*	*Study weight*	*Postnatal age*	*Ventilation*
		kg	wk	G	d	
1	F	0.62	29	1.06	52	None
2	M	0.96	26	1.48	52	Nasal prong
3	F	1.11	28	1.38	29	Nasal prong
4	F	0.46	27	0.60	18	Nasal prong
5	F	0.88	28	0.98	21	Nasal prong
6	M	1.29	28	1.19	16	Nasal prong
7	M	0.99	27	1.35	36	Nasal prong
Median		1.01	28	1.18	28	

GA, gestational age.

Isotopic steady state was achieved during $NaH^{13}CO_3$ infusion with a coefficient of variation of 4.1% in the direct sampling method and 4.8% in the trapping method (Figure 2). More importantly, the del per mil (δ‰) difference determined from the direct naso-pharyngeal sampling method correlated highly ($r = 0.933$, $P < 0.0001$) with the del per mil (δ‰) difference obtained by the trapping technique. As shown in Figure 3, a linear regression equation relating the measurement of del per mil (δ‰) from the [^{13}C]bicarbonate infusion quantified with the direct naso-pharyngeal sampling technique and the dynamic trapping method was found to be highly significant. Furthermore, Bland-Altman analysis

(Figure 4) demonstrated agreement between the two methods to within ± 4.68 δ‰, i.e. 95% confidence interval.

Figure 2. Isotopic plateau of $^{13}CO_2$ enrichment in breath following a primed constant intragastric [1-^{13}C]bicarbonate infusion collected with the direct naso-pharyngeal sampling method (dark circles) and collected with the trapping method (white circles). An isotopic steady state was achieved by 120 min with less than 5% variation of the plateau in both methods.

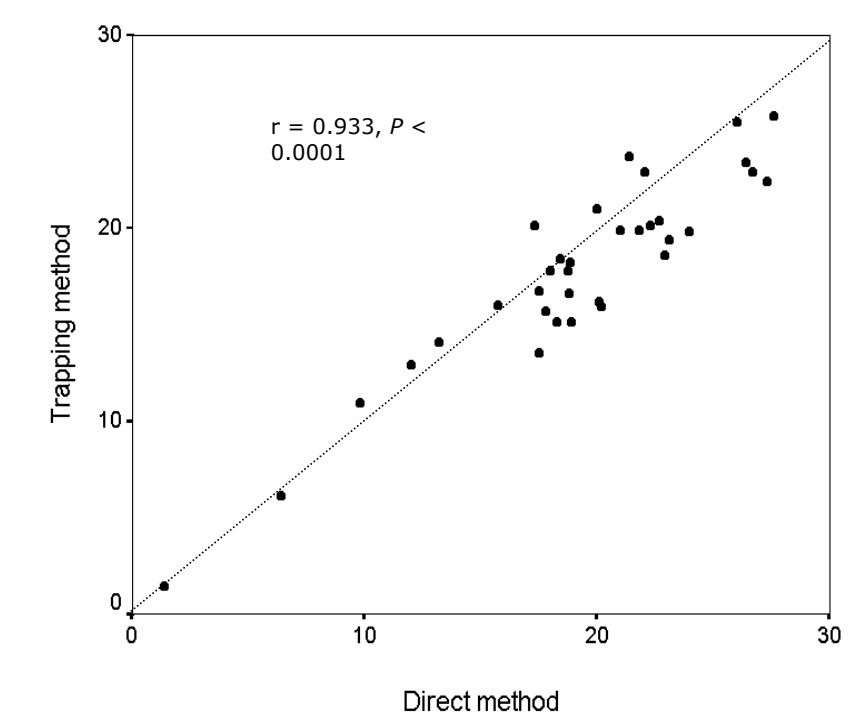


Discussion

The present study describes a methodology for the collection of expired air in preterm infants after administration of ^{13}C-labeled material. In preterm neonates, isotopic steady state of $^{13}CO_2$ in breath obtained by our direct naso-pharyngeal sampling technique after a [^{13}C]bicarbonate infusion is accurately related to isotopic steady state obtained by the trapping technique. This is demonstrated by the highly significant correlation between the measured values acquired from both methods. Therefore, in neonates the direct naso-pharyngeal sampling technique

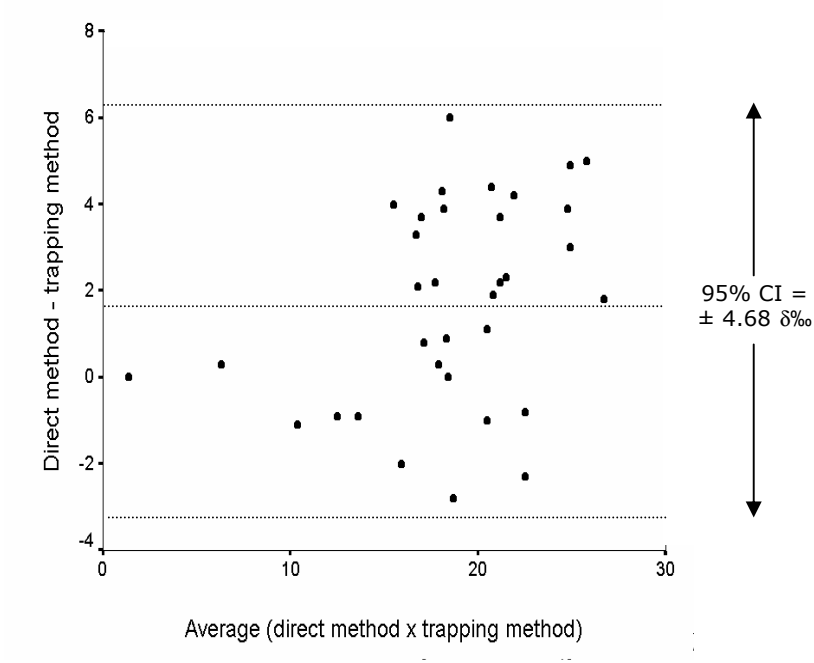
can be used to obtain expired air for the estimation of CO_2 production and the quantification of oxidation of labeled substrates.

Figure 3. Linear regression analysis between $^{13}CO_2$ enrichment in breath following a primed constant infusion of $NaH^{13}CO_3$ by the direct naso-pharyngeal sampling technique and by the trapping in NaOH method in seven preterm infants



In order to determine the accuracy and precision of our technique in each individual subject in comparison with the trapping method we used the Bland-Altman analysis. Furthermore, by using this analysis we could assess the variability between both methods. [23] As shown in Figure 4 the 95% confidence interval of the two methods vary within an acceptable ± 4.68 δ‰. In other words, there is less then 5% probability that the two methods differ more than this amount.

Figure 4. Bland-Altman analysis showing the difference between the direct naso-pharyngeal sampling method and the trapping method measurements of $^{13}CO_2$ enrichment in breath is in agreement to within ± 4.68 δ‰. CI indicates confidence interval



Whole-body flux and oxidation rates of nutrients can be quantified in adults and infants from primed constant infusion studies using ^{13}C-labeled substrates with the collection of expired air. [1-3] The methodology for measuring in vivo oxidation of substrates with the use of [^{13}C]labeled material in clinical studies has been developed with isotope ratio mass spectrometry. The excretion of labeled CO_2 in breath after administration of [^{13}C]labeled substrates is a function of the physical parameters involved in CO_2 metabolism, the kinetics of metabolism, and the delay associated with the passage through the whole-body bicarbonate pool before its expiration in the breath. [24] For example, the labeled CO_2 is temporarily retained in the bicarbonate pool of the bone, into larger organic molecules, and in small portions in feces, sweat and urine. [24] That this bicarbonate pool is limited is shown by an almost complete

recovery of CO_2 during an extended infusion (24-48h) and collection of expired air. [25] In short lasting studies in neonates, the CO_2 retention varies between 70 and 84% and is correlated with the energy intake, metabolic rate and CO_2 production. [5] Therefore, to determine the rate of oxidation of a labeled substrate, a correction factor is commonly used in the calculations. [5] However, in previous studies in preterm infants without signs of infection or respiratory distress, we have shown that in case of a constant CO_2 production over a few hours and a combination of a [^{13}C]bicarbonate and a [^{13}C]labeled amino acids infusion no correction factor is needed. [13,17]

We conclude that the presently described direct naso-pharyngeal sampling method for the collection of expired air in preterm neonates is as good as the previously described methods for the calculation of substrate oxidation. This method is simple to use, and should provide new opportunities to study metabolism of various substrates in preterm infants, children, and adults.

References

1. Matthews DE, Motil KJ, Rohrbaugh DK, Burke JF, Young VR, Bier DM. Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-^{13}C]leucine. Am. J. Physiol. 1980;238:E473-E479.
2. Denne SC, Kalhan SC. Leucine metabolism in human newborns. Am. J. Physiol. 1987; 253:E608-E615.
3. Van Goudoever JB, Sulkers EJ, Halliday D, Degenhart HJ, Carnielli VP, Wattimena JL, Sauer PJ. Whole-body protein turnover in preterm appropriate for gestational age and small for gestational age infants: comparison of [^{15}N]glycine and [1-^{13}C]leucine administered simultaneously. Pediatr. Res. 1995;37:381-388.
4. Kien CL. Isotopic dilution of CO_2 as an estimate of CO_2 production during substrate oxidation studies. Am. J. Physiol. 1989;257:E296-E298.
5. Van Aerde JE, Sauer PJ, Pencharz PB, Canagarayar U, Beesley J, Smith JM, Swyer PR. The effect of energy intake and expenditure on the recovery of $^{13}CO_2$ in the parenterally fed neonate during a 4-hour primed constant infusion of $NaH^{13}CO_3$. Pediatr. Res. 1995;19:806-810.
6. Ferrannini E. The theoretical bases of indirect calorimetry: a review. Metabolism. 1988;37:287-301.

7. Forsyth JS, Crighton A. Low birthweight infants and total parenteral nutrition immediately after birth. I. Energy expenditure and respiratory quotient of ventilated and non-ventilated infants. Arch. Dis. Child. Fetal. Neonatal. Ed. 1995;73:F4-F7.
8. Bauer K, Pasel K, Uhrig C, Sperling P, Versmold H. Comparison of face mask, head hood, and canopy for breath sampling in flow-through indirect calorimetry to measure oxygen consumption and carbon dioxide production of preterm infants < 1500 grams. Pediatr. Res. 1997;41:139-144.
9. Ultman JS, Bursztein S. Analysis of error in the determination of respiratory gas exchange at varying FIO_2. J. Appl. Physiol. 1981;50:210-216.
10. Mayfield SR. Technical and clinical testing of a computerized indirect calorimeter for use in mechanically ventilated neonates. Am. J. Clin. Nutr. 1991;54:30-34.
11. Classic KL, Schenk WF, Haymond MW. Radiobiological half-lives for carbon-14 and hydrogen-3 leucine in man. Proc 4th Int Radiopharm Dosimetry Symp 1986;681-687.
12. Spear ML, Darmaun D, Sager BK, Parsons WR, Haymond MW. Use of [^{13}C]bicarbonate infusion for measurement of CO_2 production. Am. J. Physiol. 1995;268:E1123-E1127.
13. Van Goudoever JB, Sulkers EJ, Kalhan SC, Sauer PJ. Glucose metabolism in a term infant with transient hyperinsulinism and high carbohydrate intake. Eur. J. Pediatr. 1993;152:343-347.
14. Perman JA, Barr RG, Watkins JB. Sucrose malabsorption in children; a noninvasive diagnosis by interval breath hydrogen determination. J. Pediatr. 1978;93:17-22.
15. Veereman-Wauters G, Ghoos Y, Van der Schoor S, Maes B, Hebbalkar N, Devlieger H, Eggermont E 1996 The ^{13}C-octanoic acid breath test: a noninvasive technique to assess gastric emptying in preterm infants. J. Pediatr. Gastroenterol. Nutr. 1996;23:111-117.
16. Van Dijk-Van Aalst K, Van den Driessche M, Van der Schoor S, Schiffelers S, van't Westeinde T, Ghoos Y, Veereman-Wauters G. ^{13}C mixed triglyceride breath test: a noninvasive method to assess lipase activity in children. J. Pediatr. Gastroenterol. Nutr. 2001;32:579-585.
17. Van Goudoever JB, Colen T, Wattimena JL, Huijmans JG, Carnielli VP, Sauer PJ. Immediate commencement of amino acid supplementation in preterm infants: effect on serum amino acid concentrations and protein kinetics on the first day of life. J. Pediatr. 1995;127:458-465.

18. Kingdon CC, Mitchell F, Bodamer OA, Williams AF. Measurement of carbon dioxide production in very low birth weight babies. Arch. Dis. Child. Fetal. Neonatal. Ed. 2000;83:F50-F55.
19. Zuijdgeest-van Leeuwen SD, Van den Berg JW, Wattimena JL, Van der Gaast A, Swart GR, Wilson JH, Dagnelie PC. Lipolysis and lipid oxidation in weight-losing cancer patients and healthy subjects. Metabolism. 2000;49:931-936.
20. Craig H. Isotopic standards for C and O and correction factors for mass-spectrometric analysis of CO_2. Geochim. Cosmochim. Acta. 1957;12:133-149.
21. Jahoor F, Burrin DG, Reeds PJ, Frazer M. Measurement of plasma protein synthesis rate in infant pig: an investigation of alternative tracer approaches. Am. J. Physiol. 1994;267:R221-R227.
22. Tserng K, Hay WJ. Calculation of substrate turnover rate in stable isotope tracer studies. Am. J. Physiol. Endocrinol. Metab. 1983;245:E308-E311.
23. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;1:307-310.
24. Winchell HS, Wiley K. Considerations in analysis of breath $^{14}CO_2$ data. J. Nucl. Med. 1970;11:708-710.
25. Elia M, Fuller NJ, Murgatroyd PR. Measurement of bicarbonate turnover in humans: applicability to estimation of energy expenditure. Am. J. Physiol. 1992;263:E676-E687.

Chapter 6

LYSINE KINETICS IN PRETERM INFANTS: THE IMPORTANCE OF ENTERAL FEEDING

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[2] This paper is dedicated to the memory of Peter J Reeds who died recently. It is an enormous loss for all of them who have worked with him. He was a great mentor and inspirator to all of us.
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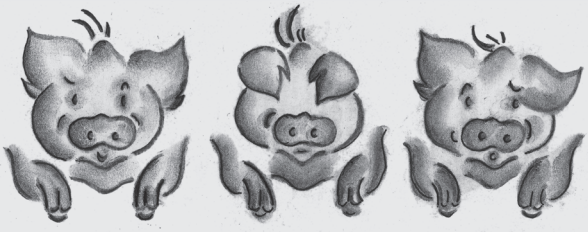


Accepted for publication by Gut.

Background & Aims: Lysine is the first limiting essential amino acid in the diet of newborns. The first-pass metabolism by the intestine of dietary lysine has a direct effect on the systemic availability. We investigated whether first-pass lysine metabolism in the intestine is high in preterm infants, particularly at a low enteral intake. Methods: Six preterm infants (birthweight 0.9 ± 0.1 kg) were studied during two different periods [A (n=6): 40% of intake administered enterally, 60% parenterally; lysine intake 92 ± 6 μmol/(kg·h), and B (n=4): 100% enteral feeding; lysine intake 100 ± 3 μmol/(kg·h)). Dual stable isotope tracer techniques were used to assess splanchnic and whole-body lysine kinetics. Results: The fractional first-pass lysine uptake by the intestine was significantly higher during partial enteral feeding [A: 32 ± 10 vs. B: 18 ± 7%, *P* < 0.05]. The absolute uptake was not significantly different. Whole-body lysine oxidation was significantly decreased during full enteral feeding [A: 44 ± 9 vs. B: 17 ± 3 μmol/(kg·h), *P* < 0.05] so that whole-body lysine balance was significantly higher during full enteral feeding [A: 52 ± 25 vs. B: 83 ± 3 μmol/(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 a 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.

The primed constant intravenous infusion of amino acids labeled with stable isotopes has become the reference method for studying whole-body protein dynamics. [1-3]

By measuring oxidation rates of specific amino acids directly, conclusions can be drawn about the excessive supply of a particular amino acid. In healthy infants, an excessive intake of a specific amino acid will lead to a high oxidation rate. Because of limitations in studying nutrient metabolism in preterm neonates, several studies in neonatal animals have been performed to quantify protein metabolism in vivo. These studies have shown that the portal-drained viscera (PDV: intestine, pancreas, spleen and stomach) utilize more than 50% of the dietary protein intake for protein synthesis and biosynthetic pathways, [4-7] and is a major site of lysine oxidation. [8] Of particular importance is the utilization rate by the intestine of essential amino acids. A high utilization rate of essential amino acids by the intestine results subsequently in a lower systemic availability of essential amino acids and could perhaps influence whole-body growth. We were specifically interested in lysine, an essential amino acid that is the first limiting amino acid in milk-fed mammalian newborns. [9] Thus, if the pattern of amino acids in the diet is not ideal, the rate of protein synthesis and growth will be determined by lysine. Previously, we have found in piglets under normal high-protein-feeding conditions that about half of the dietary lysine intake was utilized by the PDV, and that this utilization fraction even increased during dietary protein restriction. [8] Subsequently, the systemic availability of dietary lysine decreased significantly during a restricted dietary protein supply.

The neonatal piglet is generally considered to be a good model for the human infant with respect to rate and pattern of gastrointestinal tract development. [10] In addition, amino acid patterns are similar between piglets and infants with respect to both plasma concentrations and requirements. [11,12] However, no data on lysine kinetics in infants or children are available, while in adults the splanchnic lysine uptake has been found to vary between 5% and 35% of the lysine intake. [7,13]

Largely as a result of their immature gastrointestinal tract, all preterm infants face a period of compromised enteral intake in their first weeks of life, and they frequently receive a combined parenteral and enteral nutrient supply. Based upon our finding that the absolute lysine utilization by the intestine of neonatal pigs appears to be independent of their lysine intake, [8] we postulated that the fractional first-pass lysine utilization would be much higher during partial enteral feeding than during full enteral feeding. Importantly, this would result in a lower systemic availability of dietary lysine. To evaluate this hypothesis, we measured the first-pass lysine uptake in response to two different enteral intakes in neonates.

Apart from the first-pass lysine utilization, the magnitude of intestinal lysine catabolism is also important to the nutrition of preterm newborns. The energy needed to sustain the high rate of protein turnover in the gut is largely derived from the oxidation of non-essential amino acids. [4,14] However, some essential amino acids, including lysine, are oxidized as well, which leads to an irreversible loss. [8,15,16] Despite the general belief that the intestinal mucosa does not possess lysine catabolic enzymes, [17,18] we showed that the intestinal oxidation of dietary lysine in pigs receiving a high protein diet accounted for approximately 30% of the whole-body lysine oxidation. [8] Whether dietary lysine is oxidized by the splanchnic tissues in humans has not yet been investigated. Neither has the effect of increasing the dietary protein supply on whole-body lysine oxidation been studied. We hypothesized that the catabolic utilization of lysine within the splanchnic tissues would significantly influence the requirements of lysine. Therefore, quantification of the response of splanchnic and whole-body lysine oxidation on the level of enteral protein intake in preterm infants was the second objective of this study.

Methods

Subjects

The study protocol was approved by the Erasmus University Institutional Review Board. Written, informed consent was obtained

from the parents. Splanchnic and whole-body lysine kinetics were quantified in preterm infants during partial enteral feeding (period A) and during full enteral feeding (period B). Patients eligible for this study were premature infants with a birthweight ranging from 750 to 1250 g who were appropriate for gestational age according to the charts of Usher and McLean. [19] Excluded from the study were infants who had congenital anomalies, or gastrointestinal or liver diseases. CRIB-scores [20] 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 neonatal intensive care 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%, Fresensius Kabi, Den Bosch, The Netherlands). Nenatal® was given as sole enteral nutrition 12 h before the start of the study and during the study days.

Protocol

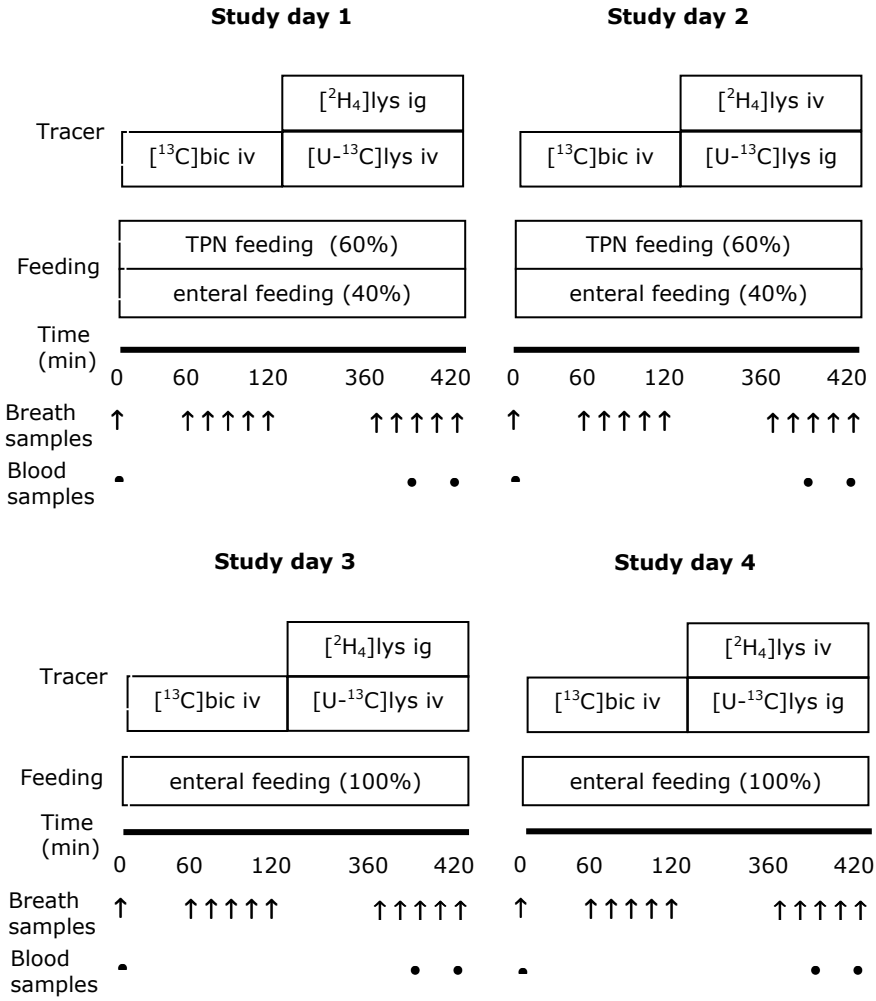
The study design consisted of two periods of two consecutive study days (Figure 1; period A: study days 1 and 2, and period B: study days 3 and 4); during period A the infants received 40% enteral feeding and 60% parenteral feeding, and during period B they received full enteral feeding. During period A the infants were implanted with both an arterial and an intravenous catheter for the infusion of tracers and withdrawal of blood samples. During period B, a peripheral intravenous catheter was available for the infusion of tracers, blood samples were collected by heelstick. Withdrawing blood via an heelstick is withdrawing blood from arterial capillaries, while the heel is heated before collection in order to assure easy collection. Therefore, we assume that similarly to the heated hand box technique there is no difference in plasma enrichment. [21]

In order to collect breath samples from these infants, we employed the method described by Perman et al., who used a nasal tube. [22] This method has been used in children for a non-invasive

diagnosis of sucrose malabsorption by H_2 breath tests, [22] and in preterm infants for the collection expiratory CO_2 recovery after administration of ^{13}C-labeled 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 for 1 to 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 in both periods. First, a primed, continuous 2-h infusion [10.02 μmol/kg and 10.02 μmol/(kg·h)] of [^{13}C]sodium bicarbonate (99.0 mol% ^{13}C; Cambridge Isotopes, Woburn, MA) dissolved in sterile saline was administered at a constant rate. The ^{13}C-labeled bicarbonate infusion was immediately followed by two primed, continuous 5-h infusions [9.77 μmol/kg and 9.77 μmol/(kg·h)] of [U-^{13}C]lysine (97.0 mol% ^{13}C; Cambridge Isotopes, Woburn, MA) and [9.31 μmol/kg and 9.31 μmol/(kg·h)] of [$^{2}H_4$]lysine (98.0 mol% ^{2}H; Cambridge Isotopes, Woburn, MA) designed to assess whole-body and splanchnic lysine kinetics. During both period A and period B we administered the tracers in the following order: On study days 1 and 3, [U-^{13}C]lysine was given via an intravenous catheter and [$^{2}H_4$]lysine via the intragastric catheter. On study days 2 and 4 the intravenous and intragastric routes were switched; [$^{2}H_4$]lysine was infused intravenously and [U-^{13}C]lysine was given intragastrically. All isotopes were tested and found to be sterile and pyrogen-free before use in our studies. At time zero, baseline blood and breath samples were collected. During the last hour of each tracer infusion, breath samples were collected at 15-min intervals (T 60, 75, 90, 105, 120, 360, 375, 390, 405, 420 min), and blood samples were obtained at 390 and 420 min. The total amount of blood withdrawn during a study day was 1.5 mL, which is less than 2% of blood volume in a 1000-g infant. Blood was centrifuged immediately and stored at −70°C for further analysis.

Figure 1. Schematic overview of study days 1 and 2 during period A, and study days 3 and 4 during period B



[^{13}C]bic, [^{13}C]sodiumbicarbonate; [$^{2}H_4$]lys, [$^{2}H_4$]lysine; [U-^{13}C]lys, [U-^{13}C]lysine; iv, intravenous; and ig, intragastricly.

Analytical methods

Small aliquots of plasma (100 µL) were taken for the measurement of plasma lysine concentrations by the Amino Acid Analyser (Amino Acid Analyser, Biochrom Ltd, Cambridge, England). Enrichments of [U-^{13}C]lysine and [$^{2}H_4$]lysine in plasma were determined by GCMS as the N(O,S)-methoxycarbonylmethylester (MCM) derivative according to Husek [25] with minor modifications. Breath samples were analyzed for enrichment of $^{13}CO_2$ on an isotope ratio mass spectrometer (ABCA; Europa Scientific, Van Loenen Instruments, Leiden, The Netherlands).[26]

Calculations

The rate of lysine turnover was calculated by measuring the tracer dilution at steady state as modified for stable isotope tracers, as previously described. [27,28]

The lysine flux was calculated according to the following equation:

Eq.**1**

$$Q_{iv} = i_L \times [(E_i / E_p) - 1] \qquad \textbf{[1]}$$

where Q_{iv} is flux of the intravenous lysine tracer (µmol/(kg·h)), i_L is the lysine infusion rate (µmol/kg·h)), E_i and E_p are the enrichments (mol percent excess) of [U-^{13}C or $^{2}H_4$]lysine in the lysine infusate and in plasma at steady state, respectively.

The first-pass lysine uptake was calculated according to the expression:

Eq.**2**

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

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

In a 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 the diet. Lysine leaving the pool may be either oxidative disposal or non-oxidative disposal (lysine used for synthesis). In equation:

Eq.**3**

$$Q = I + LRP = Ox + NOLD \quad \textbf{[3]}$$

where Q is flux of the lysine tracer (μmol/(kg·h)), LRP is the amount of lysine released from protein via protein breakdown (μmol/(kg·h)), Ox is the rate of lysine oxidation (μmol/(kg·h)) and NOLD is the rate of non-oxidative lysine disposal (a measure of protein synthesis rate, μmol/(kg·h)).

Net lysine balance, an index of protein deposition, was calculated as follows:

Eq.**4**

$$B = NOLD - LRP \quad \textbf{[4]}$$

where B is lysine balance (μmol/(kg·h)).

Whole-body CO_2 production was estimated as follows:

Eq.**5**

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

where i_B is the infusion rate of [^{13}C]sodium bicarbonate (μmol/(kg·h)), E_{iB} is the enrichment (mol percent excess) of [^{13}C]bicarbonate in the bicarbonate infusate and IE_B is the $^{13}CO_2$ enrichment at plateau during the [^{13}C]sodium bicarbonate infusion (mol percent excess). This equation does not correct for retention of [^{13}C]bicarbonate in different body pools and will overestimate CO_2 production rate. However, the same correction factor has to be applied to quantify the lysine oxidation rate (Eq.**6**). Consequently, lysine oxidation rate is not overestimated. [29]

As described before, lysine oxidation was calculated by multiplying the recovery of the [^{13}C]lysine in the expiratory air with the rate of appearance of lysine. [25] The fraction of lysine oxidized was measured according to the following equation, assuming a constant VCO_2 (rate of CO_2 production) during the study, that lasted 7 hours: [29]

Eq.**6**

$$\text{Fraction of lysine oxidized} = [IE_L \times i_B] / [IE_B \times i_L \times 6] \quad \textbf{[6]}$$

where IE_L and IE_B are the $^{13}CO_2$ enrichments (mol percent excess) at steady state during the intravenous [U-^{13}C]lysine and [^{13}C]sodium

bicarbonate infusion. The i_L is multiplied by factor 6 to account for the number of C-atoms that are labeled.

Whole-body lysine oxidation was then calculated as follows:

Eq.**7**

Whole-body lysine oxidation = Eq.**6** x Eq.**1** **[7]**

The calculation of the metabolism of enterally administered [U-^{13}C]lysine to CO_2 is complicated by the fact that some of the lysine tracer is absorbed and thereby labels the arterial pool. Thus, the non-first pass oxidation of [^{13}C]labeled lysine that was administered enterally but was absorbed and entered the body at study day 2 can be calculated as follows:

Eq.**8**

Non-first pass oxidation of enterally administered lysine =

$(IE_{13C\ ig} / IE_{13C\ iv})$ x Eq.**7** **[8]**

where $IE_{13C\ ig}$ is the ^{13}C enrichment (mol percent excess) in plasma during the intragastric [^{13}C]lysine infusion at study day 2, and $IE_{13C\ iv}$ is the ^{13}C enrichment (mol percent excess) in plasma during the intravenous [^{13}C]lysine infusion at study day 1.

The total lysine oxidation at study day 2 is calculated as described before:

Eq.**9**

Total lysine oxidation = Recovery (Eq.**6**) x Q_{iv} **[9]**

where Q_{iv} is the flux of the intravenous [$^2H_4$]lysine tracer (μmol/(kg·h)).

The first-pass lysine oxidation can thus be calculated with the following equation:

Eq.**10**

First-pass lysine oxidation = Eq.**9** - Eq.**8** **[10]**

Statistics

The data are expressed as the mean values obtained from samples taken over the last hour of each tracer infusion ± SD. Statistical comparisons were performed with a Student *t* test. A value of $P < 0.05$ was taken as statistically significant.

Results

Six patients were studied during period A, whereas four of them were studied during period B (Table 1). The body weight at the time of period A was 1.0 ± 0.1 kg, and 1.3 ± 0.2 kg during period B. All infants were clinically stable, and they had no clinical signs of septicaemia. Routine blood chemistry and hematology were all within normal limits, and there were no significant changes in these parameters for 24 h before and after the study. During period A five out of six infants received supplemental oxygen by a nasal prong, while during period B none of the four infants needed supplemental oxygen.

Table 1. Subject characteristics

S	*GA*	*BW*	*CRIB score*	*PA A*	*PA B*	*SW A*	*SW B*
	wk	kg		d	d	kg	kg
1	26	1.05	2	7	39	1.1	1.5
2	30	0.92	1	11	22	0.9	1.0
3	29	1.24	2	5	12	1.1	1.5
4	31	1.22	1	4	9	1.1	1.2
5	29	0.95	4	10		0.8	
6	26	0.90	1	5		0.9	
	29 ± 2	0.9 ± 0.3	2 ± 2	7 ± 4	21 ± 13	1.0 ± 0.1	1.3 ± 0.2

Values are means ± SD. S, subject; GA, gestational age; BW, birth weight; PA, postnatal age; SW, study weight; A, Period A; and B, Period B.

The mean lysine, protein, glucose, fat and energy intakes during period A and B are presented in Table 2. By design, the total lysine, total protein, and total glucose intake was not significantly different between both periods. The energy intake differed significantly among both periods [A: 92 ± 4 vs. B: 123 ± 2 kcal/(kg·d), $P < 0.05$], because of a significant higher fat intake.

Table 2. Lysine intake (µmol/(kg·h)), protein intake (g/(kg·d)), carbohydrate intake (g/(kg·d)), fat intake (g/(kg·d)), and energy intake (kcal/(kg·d)) during period A and period B

	Period A		*period B*	
Total lysine intake	92 ± 13		100 ± 6	
enteral		33 ± 5		100 ± 6
parenteral		59 ± 15		-
Total protein intake	3.4 ± 0.3		3.5 ± 0.2	
enteral		1.2 ± 0.2		3.5 ± 0.2
parenteral		2.2 ± 0.6		-
Total carbohydrate intake	10.6 ± 2.2		11.5 ± 0.7	
enteral		3.7 ± 0.6		11.5 ± 0.7
parenteral		6.9 ± 2.0		-
Total fat intake	4.1 ± 0.9 *		6.5 ± 0.4	
enteral		2.0 ± 0.3		6.5 ± 0.4
parenteral		2.0 ± 0.9		
Total energy intake	92 ± 11 *		123 ± 4	
enteral		38 ± 7		123 ± 4
parenteral		52 ± 10		-

*Means ± SD, n = 6 in period A and n = 4 in period B, and * indicates significant difference at P < 0.05.*

Isotopic plateau

Lysine kinetics were calculated from the plateau enrichment values for plasma lysine and breath carbon dioxide (Table 3). The background recovery of the [^{13}C]label in expiratory air was not significantly different between both periods [A: –19.80 ± 1.28 vs. B: –20.24 ± 0.99 ^{13}C Pee Dee Belemnite]. There was also no difference in the background recovery of the [^{13}C]label in expiratory air between the two consecutive study days of each period (period A: day 1: -20.61 ± 1.23 vs. day 2: –18.99 ± 1.43 PDB, and period B: day 1: -21.58 ± 0.35 vs. day 2: -18.89 ± 2.59 PDB). Although we took two blood samples after 4 hours of tracer infusion, we are sure that isotopic steady state was

reached since we found an isotopic plateau in CO_2 excretion. Before a plateau in breath is reached, a plateau has to be reached at the site of lysine oxidation, i.e. intracellularly. The $^{13}CO_2$ enrichment in breath during [^{13}C]sodium bicarbonate infusion rose rapidly during the first hour of infusion in both periods, to become constant in all infants by 120 min, with <5% variation of the plateau [A: 4.1 ± 1.0 (n=6) vs. B: 3.4 ± 0.8% (n=4)]. The coefficient of variation of breath [^{13}C]lysine enrichment above baseline at plateau was 6.2 ± 1.5% (n=6) during period A and 6.8 ± 1.6% (n=4) during period B.

Table 3. Baseline and steady-state plasma isotopic enrichments on study days 1 and 2 during period A and on study days 3 and 4 during period B

SD	*[U-^{13}C]lysine*		*[$^2H_4$]lysine*	
	baseline	Plateau	baseline	Plateau
	mol %	mol %	mol %	mol %
1	0.0010 ± 0.0006	0.0345 ± 0.0042	0.0019 ± 0.0008	0.0201 ± 0.0045
2	0.0019 ± 0.0007	0.0315 ± 0.0040	0.0042 ± 0.0019	0.0360 ± 0.0067
3	0.0018 ± 0.0005	0.0372 ± 0.0100	0.0028 ± 0.0008	0.0324 ± 0.0061
4	0.0059 ± 0.0065	0.0362 ± 0.0074	0.0031 ± 0.0005	0.0461 ± 0.0094

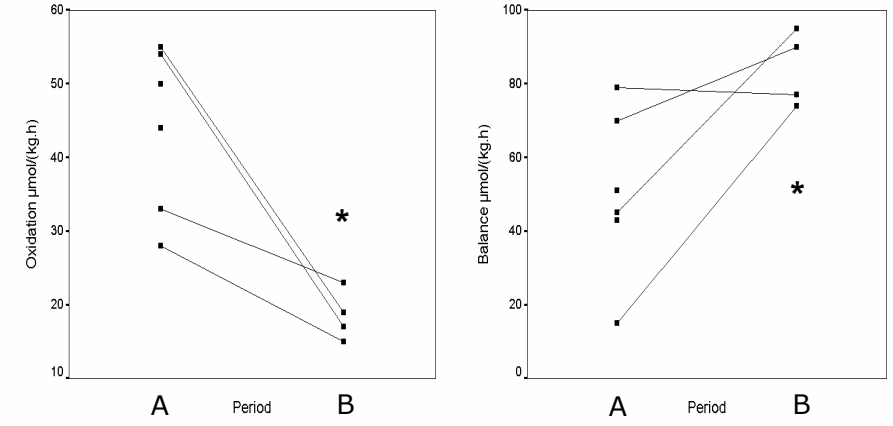
Values are means ± SD, n = 6 at study days 1 and 2, and n = 4 at study days 3 and 4, and SD, study days. On study days 1 and 3 [U-^{13}C]lysine was administered intravenously and [$^2H_4$]lysine intragastricly, and on study days 2 and 4 [U-^{13}C]lysine was given intragastricly and [$^2H_4$]lysine intravenously.

Lysine kinetics

The plasma lysine concentration was significantly higher during partial enteral feeding compared to full enteral feeding [A: 231 ± 108 vs. B: 133 ± 48 μmol/(kg·h), $P < 0.05$]. As expected, the whole-body lysine fluxes were higher for the oral than for the intravenous tracer group during both periods [A: flux ig 396 ± 49 vs. flux iv 267 ± 35 μmol/(kg·h), B: flux ig 279 ± 42 vs. flux iv 231 ± 40 μmol/(kg·h)]. The first-pass lysine uptake as a fraction of dietary lysine intake was significantly higher during a restricted enteral lysine intake [A: 32 ± 10

vs. B: 18 ± 7%, $P < 0.05$]. However, the splanchnic lysine uptake in absolute amounts was not significantly different during a full enteral intake [A: 10 ± 3 vs. B: 18 ± 6 µmol/(kg·h), $P = 0.17$]. The splanchnic tissues did not oxidize dietary lysine, neither during partial enteral feeding nor during full enteral feeding [A: -4 ± 23 vs. B –18 ± 20 µmol/(kg·h)].

Figure 2. Whole-body lysine oxidation and balance in absolute amounts (µmol/(kg·h))



*During period A: n=6, and during period B: n=4. * Indicates a significant difference at P < 0.05*

Whole-body lysine oxidation was significantly lower during full enteral feeding (Figure 2). The whole-body lysine oxidation accounted for 50 ± 19% of the total lysine intake during partial enteral nutrition, whereas only 17 ± 3% of the total lysine intake was oxidized during full enteral feeding ($P < 0.05$). The non-oxidative disposal of lysine [NOLD; A: 222 ± 36 vs. B: 169 ± 36 µmol/(kg·h)] and lysine release of protein breakdown [LRP; A: 214 ± 39 vs. B; 131 ± 38 µmol/(kg·h)] were not significantly different during both periods. The resulting net lysine balance was significantly higher during full enteral feeding (Figure 2).

Discussion

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

It is of interest to note our results in neonatal pigs receiving a low protein diet [8] 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 with partially enteral feeding is also consistent with the high values of protein turnover observed in immature rats, [30] with a maximal contribution of the intestine to whole-body protein synthesis attained at weaning.

A high first-pass uptake might as well be caused by an 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. [31,32] This indicates that probably all dietary milk proteins are absorbed by the intestinal mucosa and are not lost in the faeces. And thus, lysine that does not appear in the systemic circulation, is utilized by the intestine, and is not lost via the faeces.

Indeed, Stoll et al. have demonstrated in neonatal pigs that the splanchnic tissues have high requirements for amino acids and are proving to be an important modulator of whole-body amino acid availability. [6] A possible explanation for our high fractional first-pass

lysine extraction, is the considerable secretion of (glyco-)proteins and high proliferation rate of the gut mucosa of newborns. The high splanchnic lysine extraction in preterm infants in their first weeks of life might 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. [33]

In contrast to our finding in piglets, intraluminal lysine or lysine taken up by the splanchnic tissues is apparently not oxidized, 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 channeling dietary amino acids to the peripheral tissues.

Our second aim was to determine the 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 infants fed partially enteral. The decreased whole-body lysine oxidation suggests a protein sparing mechanism during full enteral feeding in preterm infants. Consequently, the lysine balance increased significantly as the dietary lysine intake was augmented. In concordance with these oxidation results, the plasma lysine concentration was significantly higher during partial enteral feeding. During this feeding period, amino acids were administered both via the venous circulation and via the stomach, in contrast to full enteral feeding. The higher plasma lysine concentration during partial enteral feeding might be a result of our direct intravenous infusion and a significant lower first-pass lysine uptake by the gut. In addition, the observed lower whole-body lysine oxidation during partial enteral feeding may have contributed to a higher plasma lysine level. Another explanation for this observation might be that the 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 circumstances of partial enteral feeding. Otherwise, the lysine oxidation would have been lower during partial enteral feeding.

It is of interest to note that the apparent lower whole-body lysine oxidation and higher lysine balance during full enteral feeding is similar to the values obtained by Duffy et al. [34] Duffy's study compared the effect of feeding route (iv or enteral) on protein metabolism of the neonate and concluded that orally fed neonates utilize amino acids for synthesis more efficiently than TPN fed infants. [34] 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 to feed 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. [35]

There are some limitations with respect to the study design that should be discussed. Energy intake is one of the factors that influence protein synthesis rate. As shown in Table 2, although lysine intakes during both feeding periods were virtually identical, the energy intake was significantly different between both feeding periods. The intake of metabolic energy by partial enteral 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, [36] 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 the energy intake from 120 to 100 kcal/(kg.d) on nitrogen excretion and protein deposition in preterm infants. [37] Denne et al., using [1-^{13}C]leucine in preterm neonates with enteral or parenteral feeding, showed that the overall pattern of fuel utilization was unaffected by the mode of feeding. [38] Therefore, we speculate that the higher energy intake during full enteral feeding did not significantly influence the net lysine oxidation and balance.

Another issue relevant to the different feeding periods is the different postnatal ages at both study periods. The infants were older at period B, which might influence the lysine kinetics. However, it was not feasible in our study design to put the infants on full enteral feeding already at day 7. On the other hand, it was not ethically justified to put them on partial enteral feeding at a postnatal age of 3 weeks for a period of at least 5 days in order to adjust them to the reduced enteral feeding rate and to perform the stable isotope studies. In addition, we wanted to evaluate lysine kinetics in preterm infants who were treated according to the standard neonatal intensive care feeding regiments.

In conclusion, we have shown that the splanchnic tissues have a high rate of metabolism and use almost a third of dietary lysine intake in preterm infants with partial enteral feeding. However, although lysine is catabolized by the whole-body to a great extent especially during partial enteral feeding, it is not catabolized by the splanchnic tissues in first-pass in preterm infants. Furthermore, the lysine balance was much higher during full enteral feeding as compared to 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.

References

1. Matthews DE, Marano MA, Campbell RG. Splanchnic bed utilization of leucine and phenylalanine in humans. Am J Physiol 1993;264:E109-E118.
2. Hoerr RA, Matthews DE, Bier DM, Young VR. Leucine kinetics from [$^{2}H_{3}$]- and [^{13}C]leucine infused simultaneously by gut and vein. Am J Physiol 1991;260:E111-E117.
3. Matthews DE, Motil KJ, Rohrbaugh DK, Burke JF, Young VR, Bier DM. Measurement of leucine metabolism in man from a primed, continuous infusion of L-[^{13}C]leucine. Am J Physiol 1980;238:E473-E479.
4. Van der Schoor SRD, Van Goudoever JB, Henry JF, Henry JF, Rosenberger JR, Burrin DG, Reeds PJ. The pattern of intestinal substrate oxidation is

altered by protein restriction in pigs. Gastroenterology. 2001;121:1167-1175.
5. MacRae JC, Bruce LA, Brown DS, Calder AG. Amino acid use by the gastrointestinal tract of sheep given lucerne forage. Am. J. Physiol. 1997;273:G1200-G1207.
6. Stoll B, Henry JF, Reeds PJ, Yu H, Jahoor F, Burrin DG. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein fed piglets. J Nutr. 1998;128:606-614.
7. Hoerr RA, Matthews DE, Bier DM, Young VR. Effects of protein restriction and acute refeeding on leucine and lysine kinetics in young men. Am. J. Physiol. 1993;264:E567-E575.
8. Van Goudoever JB, Stoll B, Henry JF, Burrin DG, Reeds PJ. Adaptive regulation of intestinal lysine metabolism. Proc. Nat. Acad. Sci. 2000;97:11620-11625.
9. Davis TA, Nguyen HV, Costa DP, Reeds PJ. Amino acid composition of pinniped milk. Comp. Biochem. Physiol. B. Biochem. Mol. Biol. 1995;110:633-639.
10. Moughan PJ, Birtles MJ, Cranwell PD, Smith WC, Pedraza M. The piglet as a model animal for studying aspects of digestion and absorption in milk-fed human infants. World. Rev. Nutr. Diet. 1992;67:40-113.
11. Wu G, Ott TL, Knabe DA, Bazer FW. Amino acid composition of the fetal pig. J. Nutr. 1999;129:1031-1038.
12. Ball RO, House JD, Wykes LJ, Pencharz P. A piglet model for neonatal amino acid metabolism during TPN. In Advances in Swine in Biomedical Research, Tumbleson ME, Schook LB (eds). Plenum Press, New York.1996:713-731.
13. Metges CC, El-Khoury AE, Henneman L, Petzke KJ, Grant I, Bedri S, Pereira PP, Ajami AM, Fuller MF, Young VR. Availability of intestinal microbial lysine for whole body lysine homeostasis in human subjects. Am. J. Physiol. 1999;277:E597-E607.
14. Stoll B, Burrin DG, Henry JF, Yu H, Jahoor F, Reeds PJ. Substrate oxidation by the portal drained viscera of fed piglets. Am. J. Physiol. 1999;277:E168-E175.
15. Yu YM, Burke JF, Vogt JA, Chambers L, Young VR. Splanchnic and whole body L-[1-^{13}C,^{15}N]leucine kinetics in relation to enteral and parenteral amino acid supply. Am. J. Physiol. 1992;262:E687-E694.
16. Benevenga NJ, Radcliffe BC, Egan AR. Tissue metabolism of methionine in sheep. Aust. J. Biol. Sci. 1983;36:475-485.
17. Chu SH, Hegsted DM. Adaptive response of lysine and threonine degrading enzymes in adult rats. J. Nutr. 1976;106:1089-1096.

18. Hutzler J, Dancis J. Lysine-ketoglutarate reductase in human tissues. Biochim. Biophys. Acta. 1975;23:42-51.
19. Usher RH, McLean F. Intrauterine growth of live-born Caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J. Pediatr. 1969;74:901-910.
20. Rautonen J, Makela A, Boyd H, Apajasalo M, Pohjavuori M. CRIB and SNAP: assessing the risk of death for preterm neonates. Lancet. 1994;343:1272-1273.
21. Copeland KC, Kenney FA, Nair KS. Heated dorsal hand vein sampling for metabolic studies: a reappraisal. Am J Physiol 1992;263:E1010-E1014.
22. Perman JA, Barr RG, Watkins JB. Sucrose malabsorption in children; a noninvasive diagnosis by interval breath hydrogen determination. J. Pediatr. 1978;93:17-22.
23. Veereman-Wauters G, Ghoos Y, Van der Schoor S, Maes B, Hebbalkar N, Devlieger H, Eggermont E. The ^{13}C-octanoic acid breath test: a noninvasive technique to assess gastric emptying in preterm infants. J. Pediatr. Gastroenterol. Nutr. 1996;23:111-117.
24. Van Dijk-van Aalst K, Van den Driessche M, Van der Schoor S, Schiffelers S, Van't Westeinde T, Ghoos Y, Veereman-Wauters G. ^{13}C mixed triglyceride breath test: a noninvasive method to assess lipase activity in children. J. Pediatr. Gastroenterol. Nutr. 2001;32:579-585.
25. Husek P. Rapid derivatization and gas chromatographic determination of amino acids. J. Chromatogr. 1991;552:289-299.
26. Zuijdgeest-Van Leeuwen SD, Van den Berg JW, Wattimena JL, Van der Gaast A, Swart GR, Wilson JH, Dagnelie PC. Lipolysis and lipid oxidation in weight-losing cancer patients and healthy subjects. Metabolism. 2000;49:931-036.
27. Steele R. Influence of glucose loading and of injected insulin on hepatic glucose output. Proc .NY. Acad. Sci. 1959;82:420-430.
28. Tserng K, Hay WJ. Calculation of substrate turnover rate in stable isotope tracer studies. Am. J. Physiol. 1983;245:E308-E311.
29. Van Goudoever JB, Sulkers EJ, Chapman TE, Carnielli VP, Efstatopoulos T, Degenhart HJ, Sauer PJ. Glucose kinetics and glucoregulatory hormone levels in ventilated, preterm infants on the first day of life. Pediatr. Res. 1993;33:553-559.
30. Goldspink DF, Lewis SEM, Kelly FJ. Protein synthesis during developmental growth of the small and large intestine of the rat. Bioch. J. 1984;217:527-534.

31. Gaudichon C, Mahe S, Roos N, Benamouzig R, Luengo C, Huneau JF, Sick H, Bouley C, Rautureau J, Tome D. Exogenous and endogenous nitrogen flow rates and level of protein hydrolysis in the human jejunum after [^{15}N]milk and [^{15}N]yoghurt ingestion. Br. J. Nutr. 1995;74:251-260.
32. Shulman RJ, Gannon N, Reeds PJ. Cereal feeding and its impact on the nitrogen economy of the infant. Am. J. Clin. Nutr. 1995;62:969-972.
33. Shulman RJ, Henning, SJ, Nichols BL. The miniature pig as an animal model for the study of intestinal enzyme development. Pediatr. Res. 1988;23:311-315.
34. Duffy B, Pencharz P. The effect of feeding route (IV or oral) on the protein metabolism of the neonate. Am. J. Clin. Nutr. 1986;43:108-111.
35. Lafeber HN. Are there beneficial effects of rapid introduction of enteral feeding in very low birth-weight infants, even in those with severe intra-uterine growth retardation (IUGR)? J. Pediatr. Gastroenterol. Nutr. 2002;35:137-138.
36. Heim T. Homeothermy and its metabolic cost. In Scientific foundations of paediatrics Davis JA, Dobbing J (eds). London: Heinemann, 1981:91-128.
37. Denne SC, Karn CK, Liu YM, Leitch CA, Liechty EA. Effect of enteral versus parenteral feeding on leucine kinetics and fuel utilization in premature newborns. Pediatr. Res. 1994;36:429-435.
38. Van Goudoever JB, Sulkers EJ, Lafeber HN, Sauer PJ. Short-term growth and substrate use in very-low-birth-weight infants fed formulas with different energy contents. Am. J. Clin. Nutr. 2000;71:816-821.

Chapter 7

THREONINE KINETICS IN PRETERM INFANTS: THE GUT TAKES NEARLY ALL

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[2] This paper is dedicated to the memory of Peter J Reeds. His passing in late 2002 represented an enormous loss for everyone who had the privilige of working with him. Dr Reeds was a great mentor and noble role model, who continues to provide inspiration to his former colleagues.

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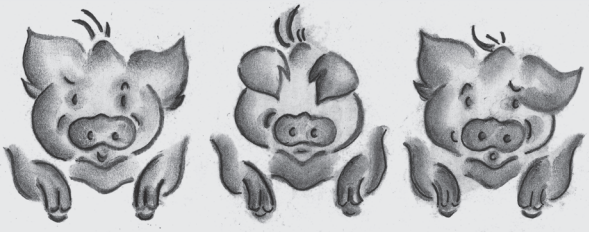

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Background & Aims: Threonine is an essential amino acid that is abundantly present in intestinally produced (glyco-)proteins. We hypothesized that intestinal first-pass threonine metabolism is high in preterm infants, particularly during a restricted enteral protein intake. Methods: Eight infants (birthweight 1.1 ± 0.1 kg, gestational age 29 ± 2 weeks) were studied during two different periods [A: 40% of total intake administered enterally 60% parenterally; total threonine intake 58 ± 6 μmol/(kg·h), and B: full enteral feeding; total threonine intake 63 ± 6 μmol/(kg·h)]. Dual stable isotope tracer techniques were used to assess splanchnic and whole-body threonine kinetics. Results: The fractional first-pass threonine uptake by the intestine was remarkably high under both circumstances: 70 ± 6% during full enteral feeding, and 82 ± 6% (*P* < 0.01) during partial enteral feeding. Hardly any threonine was oxidized in first-pass during either period [A: 0 ± 8 vs. B 3 ± 4 μmol/(kg·h)]. Whole-body threonine balance was not affected by the route of feeding. Conclusion: In preterm infants, the splanchnic tissues extract a very large amount of the dietary threonine intake, which is not oxidized. This indicates a high obligatory visceral need for threonine presumably for purposes of synthesis.

During the first few weeks of life, preterm infants are faced with the challenge of doubling their body weight. [1] The relatively high growth rate of the newborn infant puts significant pressure on the intestine to digest and absorb nutrients efficiently.

This occurs at a time when the neonatal intestine is adapting to the enteral route of nutrition after a prenatal period in which amino acids were delivered via the umbilical route. Therefore, it might be speculated that a large quantity of amino acids is needed for the growth and maintenance of the premature gut, for optimal function and integrity.

Given the key role of the gut in the maintenance of neonatal health, there has been considerable interest in the significance of first-pass intestinal metabolism of dietary amino acids.[2-5] Enterally absorbed amino acids can be used for incorporation into mucosal cellular proteins, for catabolic processes, or for conversion via transamination into other amino acids, metabolic substrates and biosynthetic intermediates. It is known in animals that less than 20% of intestinal amino acid utilization is used for constitutive gut growth by the intestinal mucosa, [1] and although some essential amino acids are known to be catabolized, [3,4,6] the catabolism of essential amino acids does not account for their high utilization rate. Therefore, the synthesis of secretory proteins by the enterocytes appears to be a major metabolic fate for nutritionally indispensable amino acids.

Of particular interest is threonine, which in neonatal piglets is the amino acid that is used to the greatest extent by the portal-drained viscera (PDV: the intestines, pancreas, spleen and stomach). In neonatal pigs, the splanchnic extraction of threonine ranges from 60% to 80% of the dietary intake. [2,6] This high intestinal requirement for threonine might reflect the use of enterally absorbed threonine for the synthesis of secretory (glyco-)proteins as the major metabolic fate, besides its use for the synthesis of mucosal cellular proteins and for oxidative purposes. Indeed, Roberton et al. found that the protein cores of secretory mucins contain large amounts of threonine. [7] Consistent with this finding, Bertolo et al. showed that the whole-body

threonine requirement in TPN-fed piglets is 40% of that observed in enterally fed piglets,[8] indicating that enteral nutrition induces intestinal processes in demand of threonine. One might postulate that in humans the compromised gut barrier function associated with parenteral nutrition is caused by a sparse threonine availability combined with diminished intestinal mucin production, as has been shown in rats. [9]

In view of the central role of the gut in nutrient processing and metabolism, we considered it important to investigate the impact of the amount of enteral intake on splanchnic and whole-body threonine metabolism in preterm infants. Accordingly, we used two different stable isotope-labeled threonine tracers, i.e., [U-^{13}C]threonine and [^{15}N]threonine simultaneously, administered via intravenous and intragastric routes to determine the quantitative aspects of threonine metabolism in preterm neonates under both parenteral and enteral feeding conditions. This array of techniques enabled us to measure both the first-pass intestinal threonine uptake and oxidation and whole-body threonine kinetics. Previously, we found in neonatal pigs that the considerable first-pass threonine utilization was not significantly affected by a lower protein intake (Van Goudoever, unpublished data). Therefore, we hypothesized that the first-pass utilization of threonine by the splanchnic tissues would be substantial in preterm infants and would be independent of the dietary threonine intake. Hence, the present study explores the impact of the amount of enteral protein intake on various components of first-pass and whole-body threonine metabolism in neonates.

Methods

Subjects

The study protocol was approved by the Erasmus-MC Institutional Review Board. Written, informed consent was obtained from the parents. Splanchnic and whole-body threonine kinetics were quantified in eight preterm infants during two consecutive periods of different enteral and parenteral intakes. Patients eligible for this study were premature infants, with a birth weight ranging from 750 to 1250 g,

who were appropriate for gestational age according to the charts of Usher and McLean. [10] Excluded from the study were infants who had major congenital anomalies, or gastrointestinal or liver diseases. CRIB -scores [11] on the first day of life were all below 5. Selected relevant clinical variables for the infants studied are shown in Table 1. The infants received a standard nutrient regimen according to our neonatal intensive care feeding protocol: a combination of breast or formula feeding (Nenatal®, Nutricia, Zoetermeer, The Netherlands; 0.024 g/ml protein) and parenteral nutrition containing glucose, amino acids (Primene® 10%, Clintec Benelux NV, Brussels, Belgium; 0.1 g/ml protein), and lipids (Intralipid® 20%, Fresensius Kabi, Den Bosch, The Netherlands). Nenatal® was given as sole enteral nutrition 12 h before the start of the study and during the study days.

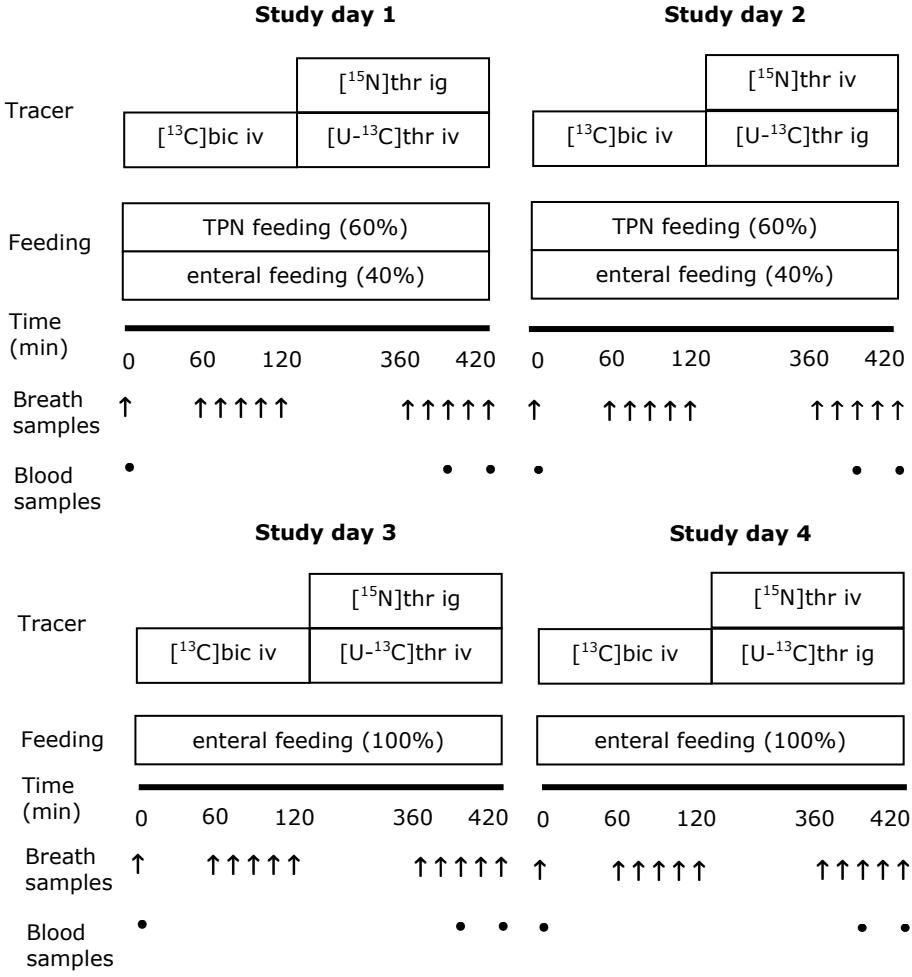
Protocol

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). During period A, the infants received 40% enteral feeding and 60% parenteral feeding, and during period B, they received full enteral feeding. A schematic outline of the tracer-infusion studies is shown in Figure 1. During period A the infants were implanted with both an arterial and an intravenous catheter for the infusion of tracers and withdrawal of blood samples. During period B, a peripheral intravenous catheter was available for the infusion of tracers, blood samples were collected by heelstick.

In order to collect breath samples from these tiny infants, we employed the method described by Perman et al., who used a nasal tube. [12] This method has been validated and used in children for the non-invasive diagnosis of sucrose malabsorption by H_2 breath tests, [12] and in preterm infants for the collection of expiratory CO_2 recovery after administration of ^{13}C-labeled substrates. [13,14] Briefly, a 6-Fr gastric tube (6 Ch Argyle; Sherwood Medical, Tullamore, Ireland) was carefully placed 1 to 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 later analysis.

Figure 1. Schematic overview of study periods A and B during the four study days



[13C]bic, [13C]sodiumbicarbonate; [15N]thr, [15N]threonine; [U-13C]thr, [U-13C]threonine; iv, intravenous; and ig, intragastricly.

Three different stable-isotope infusions were performed during each study day in both periods. First, a primed, continuous 2-h infusion [10.02 μmol/kg and 10.02 μmol/(kg·h)] of [^{13}C]sodium bicarbonate (99 mol% ^{13}C; Cambridge Isotopes, Woburn, MA) dissolved in sterile saline was administered at a constant rate. The purpose of this first isotope infusion was to determine the approximate rate of whole-body CO_2 production. The ^{13}C-labeled bicarbonate infusion was immediately followed by two primed, continuous 5-h infusions [14.4 μmol/kg and 14.4 μmol/(kg·h)] of [U-^{13}C]threonine (97 mol% ^{13}C; Cambridge Isotopes, Woburn, MA) and [14.7 μmol/kg and 14.7 μmol/(kg·h)] of [^{15}N]threonine (95 mol% ^{15}N; Cambridge Isotopes, Woburn, MA) designed to assess whole-body and splanchnic threonine kinetics. During both period A and period B we administered the tracers in the following order: On study day 1, [U-^{13}C]threonine was given via an intravenous catheter and [^{15}N]threonine via the intragastric catheter. On study day 2 the intravenous and intragastric routes were switched; [^{15}N]threonine was infused intravenously and [U-^{13}C]threonine was given intragastrically. All isotopes were tested and found to be sterile and pyrogen-free before use in our studies. At time zero, baseline blood and breath samples were collected. During the last hour of each tracer infusion, breath samples were collected at 15-min intervals (T 60, 75, 90, 105, 120, 360, 375, 390, 405, 420 min), and blood samples were obtained at 390 and 420 min. The total amount of blood withdrawn during a study day was 1.2 mL, which is less than 2% of blood volume in a 1000-g infant. Blood was centrifuged immediately and stored at -70°C for further analysis.

Analytical methods

Small aliquots of plasma (100 μL) were taken for the measurement of amino acid concentrations by the Amino Acid Analyser (Amino Acid Analyser Biochrom 20, Biochrom Ltd, Cambridge, England). Plasma threonine enrichment was determined by gaschromatography-mass spectrometry. Briefly, 50 μL plasma was deproteinized with 50 μL 0.24 M sulfosalicylic acid. After centrifugation for 8 min at 4°C and 14,000 g,

the supernatant was passed through an AG50W-X8, H^+-column (Biorad, Richmond, Virginina, USA). The column was washed with 3 ml water and the amino acids eluted with 1.5 ml 3 M NH_4OH. The eluate was dried at 70°C under a stream of nitrogen and finally derivatives of the amino acids were formed by adding 350 µL acetonitril and 10 µL N-methyl-N-(*tert*-butyldimethylsilyl)-trifluoroacetamide (Pierce, Omnilabo Breda, The Netherlands) to the dried amino acids. [15] Analyses were carried out on a Carlo Erba GC8000 gaschromatograph coupled to 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 x 0.22 mm fused silica capillary column, coated with 0.11 µm HT5 (SGE, Victoria, Australia). Natural threonine, [^{15}N]-threonine and [U-^{13}C]-threonine were measured by selective ion monitoring of masses 404, 405, and 408, respectively. [15] Breath samples were analyzed for enrichment of $^{13}CO_2$ on an isotope ratio mass spectrometer (ABCA; Europa Scientific, Van Loenen Instruments, Leiden, The Netherlands).[16]

Calculations

Plasma enrichments of threonine were used to calculate the rate of threonine turnover. The rate of threonine turnover was calculated by measuring the tracer dilution at steady state as modified for stable isotope tracers, as previously described. [17,18]

The threonine flux (iv or ig) was calculated according to the following equation:

Eq.**1**

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

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

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

Eq.**2**

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

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

In a steady state the amount of threonine entering the plasma pool should be equal to the amount of threonine leaving the pool. Threonine can enter the pool either by being released from proteins as the result of breakdown or through the diet. Threonine leaving the pool may be either oxidative disposal or non-oxidative disposal (threonine used for synthesis) (Figure 2). We used the equation:

Eq.**3**

$$Q = I + TRP = Ox + NOTD \quad \textbf{[3]}$$

where TRP is the amount of threonine released from protein via protein breakdown (μmol/(kg·h)), Ox is the rate of threonine oxidation (μmol/(kg·h)) and NOTD is the rate of non-oxidative disposal of threonine (a measure of protein synthesis rate, μmol/(kg·h)).

Net threonine balance, an index of protein deposition, was calculated as follows:

Eq.**4**

$$B = NOTD - TRP \quad \textbf{[4]}$$

where B is threonine balance (μmol/(kg·h)).

Whole-body CO_2 production was estimated as follows:

Eq.**5**

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

where i_B is the infusion rate of $NaH^{13}CO_3$ (μmol/(kg·h)), E_{iB} is the enrichment (mol percent excess) 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 (mol percent excess).

As described previously, threonine oxidation was calculated by multiplying the recovery of the [^{13}C]label in the expiratory air with the rate of appearance of threonine. [19] The fraction of threonine oxidized was measured according to the following equation, assuming a constant VCO_2 (CO_2 production) during the study which lasted 7 h.[19]

Eq.**6**

$$\text{Fraction of threonine oxidized} =$$

$$[IE_T \times i_B] / [IE_B \times i_T \times 4] \quad \textbf{[6]}$$

where IE_T and IE_B are the $^{13}CO_2$ enrichments (mol percent excess) at steady state during the intravenous [U-^{13}C]threonine infusion and $NaH^{13}CO_3$ infusion. The denominator is multiplied by a factor of 4 to account for the number of C-atoms that are labeled.

Whole-body threonine oxidation was then calculated as follows:

Eq.**7**

Whole-body threonine oxidation =

Eq.**6** x Eq.**1** **[7]**

The calculation of the metabolism of enterally administered [U-^{13}C]threonine to CO_2 is complicated by the fact that some of the threonine tracer is absorbed and transferred to the systemic circulation, and thereby labels the arterial pool. Thus, the non-first-pass oxidation of [^{13}C]labeled threonine that was administered enterally, but was absorbed and entered the body at study day 2, can be calculated as follows:

Eq.**8**

Non-first-pass oxidation of the enteral administered [U-^{13}C]threonine = ($IE_{13C\ ig}$ / $IE_{13C\ iv}$) x Eq.**7** **[8]**

where $IE_{13C\ ig}$ is the ^{13}C enrichment (mol percent excess) in plasma during the intragastric [^{13}C]threonine infusion at study day 2, and $IE_{13C\ iv}$ is the ^{13}C enrichment (mol percent excess) in plasma during the intravenous [^{13}C]threonine infusion at study day 1.

The total threonine oxidation at study day 2 is calculated as described previously:

Eq.**9**

Total threonine oxidation =

Recovery (Eq.**6**) x Q_{iv} **[9]**

where Q_{iv} is the flux of the intravenous [^{15}N]threonine tracer (μmol/(kg·h)).

The first-pass threonine oxidation can thus be calculated with the following equation:

Eq.**10**

First-pass threonine oxidation = Eq.**9** - Eq.**8** **[10]**

Statistics

The data are expressed as the mean values obtained from samples taken over the last hour of each tracer infusion ± SD. Statistical comparisons were performed with a paired Student *t* test. A value of $P < 0.05$ was taken as statistically significant.

Table 1. Subject characteristics

Patient	*GA*	*BW*	*CRIB Score*	*SW A*	*SW B*	*PA A*	*PA B*
	wk	kg		kg	Kg	d	d
1	26	0.97	2	1.00	1.05	9	12
2	27	1.08	2	1.03	1.04	8	17
3	32	1.28	2	1.18	1.17	5	9
4	28	0.92	2	1.00	1.00	9	11
5	31	0.90	1	0.91	0.91	6	8
6	29	1.25	2	1.12	1.22	5	7
7	30	1.10	2	0.99	1.06	6	11
8	30	0.98	1	0.92	0.98	10	13
	29 ± 2	1.1 ± 0.1	2 ± 0.5	1.0 ± 1.0	1.1 ± 0.1	7 ± 2	11 ± 3

Values are means ± SD. GA, gestational age; BW, birth weight; SW, study weight; PA, postnatal age; A, Period A; and B, Period B.

Results

All infants were appropriate for gestational age (mean gestational age 29 ± 2 wk, Table 1). Eight patients were studied during both periods; in five of those infants, splanchnic and whole-body threonine oxidation was determined. During both feeding periods, one infant was mechanically ventilated, four infants received supplemental oxygen via a nasal prong, and three infants received nasal continuous positive airway pressure (CPAP) which did not allow us to obtain expired air. All infants received caffeine, and were clinically stable at the time of the study. Routine blood chemistry and hematology (electrolytes, calcium, glucose, acid base, hematocrit, thrombocyte count and white blood cell

count) were all within normal limits; there were no significant changes in these parameters for 24 h before and after the study. Intakes of threonine, protein, carbohydrate, fat and energy are shown in Table 2. No differences were found in glucose, fat and energy intake. The threonine intake was significantly lower during period A, but the difference was only 5 μmol/(kg·d) in absolute amounts. The protein intake during period B was significantly lower than that during period A, which was inevitable since we aimed at comparable total threonine intakes.

Table 2. Threonine intake (μmol/(kg·h)), protein intake (g/(kg·d)), carbohydrate intake (g/(kg·d)), fat intake (g/(kg·d)), and energy intake (kcal/(kg·d)) during period A and period B

	period A		*period B*	
Total threonine intake	58 ± 5 *		63 ± 6	
enteral		26 ± 3		63 ± 6
parenteral		32 ± 3		-
Total protein intake	3.6 ± 0.4 *		2.8 ± 0.2	
enteral		1.1 ± 0.1		2.8 ± 0.2
parenteral		2.5 ± 0.2		-
Total carbohydrate intake	11.0 ± 1.8		12.4 ± 1.7	
enteral		3.6 ± 0.4		8.9 ± 0.8
parenteral		7.6 ± 1.7		3.4 ± 2.4
Total fat intake	4.6 ± 0.5		5.0 ± 0.5	
enteral		2.1 ± 0.2		5.0 ± 0.5
parenteral		2.5 ± 0.5		
Total energy intake	98 ± 8		106 ± 4	
enteral		38 ± 4		92 ± 9
parenteral		61 ± 9		14 ± 10

*Means ± SD, n = 8, and * indicates significant difference at P < 0.05.*

Isotopic plateau

Threonine kinetics were calculated from the plateau enrichment values for plasma threonine, breath carbon dioxide, and rates of carbon dioxide production. Details of the isotopic enrichments of plasma threonine at baseline and plateau during periods A and B are given in Table 3.

Table 3. Baseline and steady-state plasma isotopic enrichments on study days 1 and 2 during period A and on study days 3 and 4 during period B

D	*[U-^{13}C]threonine*		*[^{15}N]threonine*	
	baseline	*plateau*	*baseline*	*plateau*
	mol %	*mol %*	*mol %*	*mol %*
1	0.0075 ± 0.0006	0.0585 ± 0.0064	0.2608 ± 0.0011	0.2726 ± 0.0077
2	0.0123 ± 0.0003	0.0554 ± 0.0015	0.2624 ± 0.0009	0.2833 ± 0.0094
3	0.0097± 0.0018	0.0549 ± 0.0174	0.2615 ± 0.0006	0.2772 ± 0.0074
4	0.0164 ± 0.0055	0.0509 ± 0.0021	0.2641 ± 0.0001	0.2781 ± 0.0047

Values are means ± SD; n = 8; and D, study day. On study day 1 and 3 [U-^{13}C]threonine was administered intravenously and [^{15}N]threonine intragastrically; on study days 2 and 4 [U-^{13}C]threonine was given intragastrically and [^{15}N]threonine intravenously.

The background (baseline) recovery of the [^{13}C]label in expiratory air was not significantly different between the two periods (A: 1.0955 ± 0.0079 vs. B: 1.0923 ± 0.0032 APE). There was also no difference in the background recovery of the [^{13}C]label in expiratory air between the two consecutive study days of each study period (A: study day 1: 1.0894 ± 0.0001 vs. study day 2: 1.0924 ± 0.0001 APE, and B: study day 3: 1.0896 ± 0.0002 vs. study day 4: 1.0922 ± 0.0002 APE). The $^{13}CO_2$ enrichment in breath during [^{13}C]sodium bicarbonate infusion rose rapidly during the first hour of infusion in both periods, to become constant in all infants by 120 min, with <5% variation of the plateau (1.4 ± 0.4% in period A vs. 3.3 ± 1.4% in period B). The route of tracer administration did not affect the time it took to achieve plateau.

The coefficient of variation (mean ± SD) of breath [^{13}C]threonine enrichment above baseline at plateau was 3.4 ± 4.4% during period A and 8.5 ± 0.6% during period B. We considered this variation to be acceptable and to permit the detection of differences in rates of threonine oxidation between the two feeding periods.

Threonine kinetics

The plasma concentrations of all amino acids in both feeding periods are presented in Table 4.

Table 4. Plasma amino acid levels during period A and period B with a reference range of term breast-fed infants

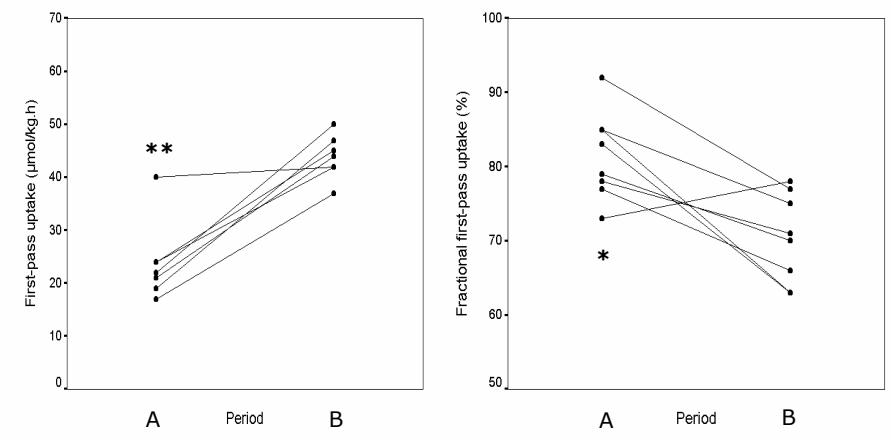
	Period A	*Period B*	*Term infants*
Threonine	169 ± 111	201 ± 98	70 - 197
Cystine	25 ± 6	20 ± 11	35 - 69
Isoleucine	66 ± 25	52 ± 18	27 - 90
Leucine	112 ± 31	95 ± 27	53 - 169
Lysine	229 ± 118 *	136 ± 51	80 - 232
Methionine	39 ± 8	34 ± 15	22 - 50
Phenylalanine	83 ± 13	83 ± 7	22 - 72
Valine	214 ± 64	154 ± 38	88 - 222
Alanine	235 ± 64	207 ± 108	125 - 647
Arginine	72 ± 27 *	49 ± 24	42 - 148
Aspartic acid	18 ± 6	29 ± 25	5 - 51
Aspartic	41 ± 14	48 ± 13	16 - 81
Glutamic acid	82 ± 33	110 ± 49	24 - 243
Glutamine	423 ± 91 *	326 ± 74	142 - 851
Glycine	292 ± 85	265 ± 131	77 - 376
Proline	172 ± 33	210 ± 105	83 - 319
Serine	153 ± 39	183 ± 110	0 - 326
Tyrosine	70 ± 77	75 ± 49	38 - 119
Total AA	2,711 ± 767	2,440 ± 865	
EAA	911 ± 336	755 ± 219	

*Values are means ± SD and are expressed in µmol/L. * indicates significant difference at P < 0.05. AA, amino acids; and EAA, essential amino acids.*

We did not find a statistically significant difference in the plasma threonine concentration between the two feeding periods [period A: 169 ± 111 vs. period B: 201 ± 98 µmol/L].

As expected, the whole-body threonine fluxes were higher for the oral than for the intravenous tracer group during the two periods [period A: flux ig 1,473 ± 710 vs. flux iv 237 ± 38 µmol/(kg·h); period B: flux ig 860 ± 253 vs. flux iv 246 ± 43 µmol/(kg·h), Table 5]. This was due to the first-pass disappearance of the intragastrically administered threonine tracer within the splanchnic region. Figure 2 illustrates the very high first-pass threonine uptake during both periods.

Figure 2. First-pass threonine uptake in absolute amount (µmol/(kg·h)) and expressed as a fraction of dietary intake during period A and B



*During both periods n=8, * indicates a significant difference at P < 0.01, and ** indicates a significant difference at P < 0.0001.*

From the data presented in Figure 2, it is clear that the first-pass threonine uptake, expressed as a percentage of dietary intake, was significantly higher during a low enteral intake. Yet, approximately three-quarters of the dietary intake was utilized by the splanchnic

tissues during full enteral feeding. In absolute amounts, the first-pass threonine uptake was significantly lower during a restricted enteral threonine intake [period A: 24 ± 7 vs. period B: 44 ± 4 μmol/(kg·h), $P < 0.0001$]. Despite this high first-pass threonine utilization, the splanchnic tissues did not oxidize dietary threonine during partial enteral feeding and the first-pass threonine oxidation during full enteral feeding was not significantly different from zero [period A: 0 ± 8 vs. period B: 3 ± 4 μmol/(kg·h), NS].

Table 5. Whole-body intravenous and intragastric fluxes of threonine, whole-body oxidation and balance of threonine in absolute amounts (μmol/(kg.h)) and as a fraction of threonine flux during period A and period B

	Period A		*Period B*	
	μmol/(kg·h)	*%*	*μmol/(kg·h)*	*%*
Flux iv	237 ± 38		246 ± 43	
Flux ig	1,473 ± 710		860 ± 253	
Threonine oxidation	15 ± 9	6 ± 4	17 ± 13	6 ± 2
Threonine balance	43 ± 10		44 ± 16	

Values are means ± SD, n = 5.

There was no significant difference in the amount of threonine oxidized by the whole-body between the two feeding periods (Table 5). During both intakes, whole-body threonine oxidation accounted for approximately one-tenth of threonine flux [period A: 6 ± 4% vs. period B: 6 ± 2%]. We determined the incorporation of tracer into glycine (Figure 3); however, the enrichments we obtained were very low. The non-oxidative threonine disposal [NOTD; period A: 231 ± 46 vs. period B: 236 ± 41 μmol/(kg·h)] and the threonine release of protein breakdown [TRP; period A: 185 ± 35 vs. period B: 183 ± 41 μmol/(kg·h)] were not significantly different between the two periods. Whole-body threonine balance, calculated from the difference between non-oxidative disposal and whole-body protein degradation, was

positive during both feeding periods, but was not significantly different between the two periods [period A: 43 ± 10 vs. period B: 44 ± 16 μmol/(kg·h), Table 5].

Discussion

This study describes the splanchnic threonine kinetics in preterm infants during early postnatal life. The role of the splanchnic tissues is essential in the delivery of dietary amino acids to peripheral tissues. The splanchnic utilization of a given amino acid is a result of adequate transport and intracellular metabolism. Since threonine is an essential amino acid, a high splanchnic utilization may result in the depletion of the systemic plasma pool of threonine.

Therefore, the most striking observation to emerge from this study was that the fractional first-pass threonine uptake by the intestine was very high in the first week of life in preterm infants during a restricted enteral intake. This observation indicates a high obligatory visceral need for threonine in neonates. Although the gastrointestinal tissues represent only 5% of body weight, they account for 15% to 35% of whole-body oxygen consumption and protein turnover due to their high rates of metabolism. [20-22] Of the many factors that affect neonatal gut growth and adaptation, probably the most physiologically significant stimulus is enteral nutrition. [23-27] Enteral feeding acts directly by supplying nutrients for growth and mucosal metabolism of epithelial cells.

The route of administration of adequate nutrition is a major issue in the clinical care of preterm infants, because of enteral feeding intolerance and associated morbidity. [28-30] To reduce the complications of total parenteral nutrition, and to accelerate the adaptation to full enteral feeding, many neonatologists often provide small volumes of enteral nutrition, e.g., minimal enteral feeding, in combination with TPN to preterm infants in their first weeks of life. [31] However, recent studies in neonatal piglets have shown that an enteral intake of 20% is necessary to prevent gut protein loss, whereas an intake of at least 40% is needed to maintain normal growth. [32,33] In our study, preterm

infants were fed 40% enterally of the total nutrient intake during period A, and the results show the fractional first-pass threonine requirements were significantly higher (82% of dietary intake) than those of fully-fed infants (70% of dietary intake).

The high enteral threonine uptake observed during both partial and full enteral feeding reflects the use of absorbed threonine for the synthesis of secretory (glyco-)proteins, for the synthesis of mucosal cellular proteins, or for oxidative purposes. [2-5] We did not observe any first-pass threonine oxidation during either feeding period, indicating the use of enterally absorbed threonine for the other two metabolic pathways. Although the intestinal mucosa is a highly secretory and proliferatory tissue, dietary threonine is not incorporated into constitutive mucosal proteins to a great extent. [4] However, the intestinal mucosa is protected by a complex network of (glyco-) proteins, and the core-proteins of the highly glycosylated domains of small intestinal mucins contain large amounts of threonine. [34] Probably a significant proportion of the utilized threonine is channeled towards mucin production. These secretory mucins play a key role in the defense of the mucosa. In fact, there is evidence that mucin production is impaired in piglets fed threonine-deficient diets and in addition supplying threonine parenterally can not restore normal mucin production. [9,35]

An important issue in this respect is the critical gut barrier function in preterm neonates, especially with regard to the immaturity of the innate and specific immune defense mechanisms. As a consequence, preterm infants are susceptible to infections in their first weeks of life, particularly TPN-fed infants, with sepsis being a common complication of TPN. The high first-pass threonine uptake observed in the first weeks of life is probably necessary to support a normal homeostatic environment and balance among luminal microbes, epithelial cells and the immune system. Dietary threonine may, therefore play a specific role in the defense capacity of neonates.

Recently, we have shown that intestinal recycling of secreted (glyco-)proteins contributes significantly to the systemic availability of

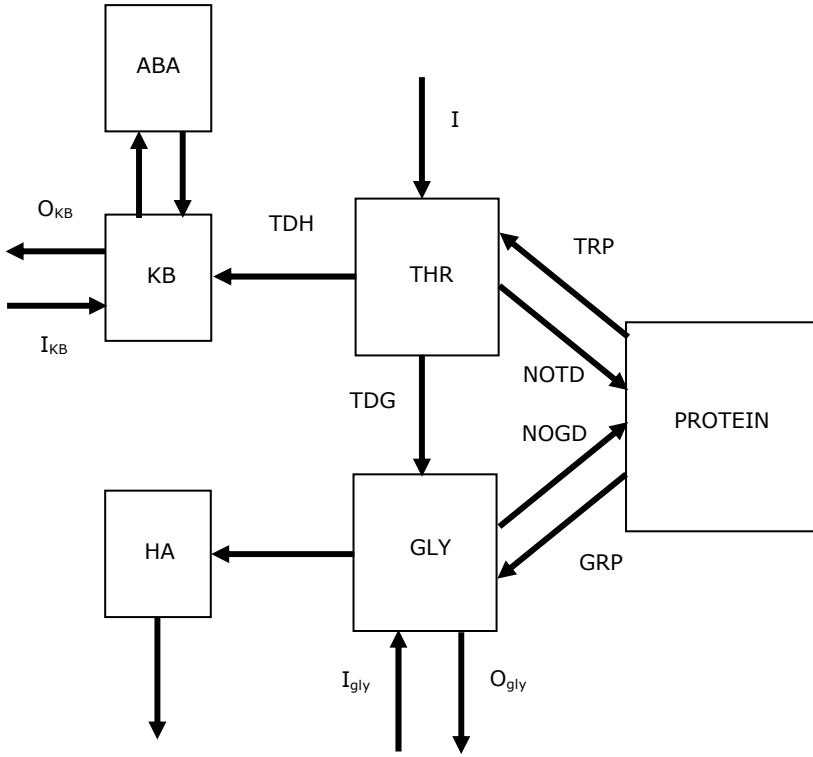
amino acids. [36] Therefore, we postulate that a large portion of utilized threonine is incorporated into (glyco-)proteins, which are subsequently reabsorbed and made available to the systemic circulation. However, our study protocol did not allow us to quantitate the amount of recycled threonine that appears in the systemic circulation.

A second aim of this study was to determine whole-body threonine kinetics in neonates under two different feeding circumstances. Two pathways are known for the enzymatic degradation of threonine (Figure 3): *1)* conversion of threonine to a-ketobutyrate, and subsequently propionate via threonine dehydratase (TDH); *2)* cleavage of threonine to glycine and acetaldehyde depending on threonine aldolase and on threonine-3 dehydrogenase (TDG). [37-39] Darling et al. estimated that the conversion rate to glycine (TDG pathway) in preterm infants accounted for 44% of the total threonine degradation, whereas degradation via the TDH pathway accounted for 56% of threonine disposal. [40]

We were unable to detect significant enrichments of plasma glycine during the infusion of labeled threonine, suggesting that hardly any threonine was catabolized through the threonine dehydrogenase pathway. During both partial and full enteral feeding, whole-body threonine oxidation accounted for approximately 10% of the threonine flux, which is comparable to, but slightly higher than the fractional oxidation rates found by Darling et al in breast milk-fed infants. [41]

In addition, we could not detect any first-pass threonine oxidation by the splanchnic tissues in either feeding periods. Several studies in animal species have shown that the catabolic enzymes for threonine oxidation are located mainly in the liver. [37,41,42] As the splanchnic tissues include the intestine and liver, we speculate that threonine metabolism is compartmentalized in these tissues. It is possible that the hepatocytes are capable of only oxidizing arterially delivered threonine. This idea is supported by our observations of an absence of first-pass threonine oxidation, and by other studies showing channeling of amino acids to specific endproducts depending of the site of entrance, i.e. via the diet or via the systemic circulation. [4, 43]

Figure 3. Schematic model of threonine metabolism



ABA, 2-aminobutyrate acid; I, threonine intake; I_{gly}, glycine input from synthesis and diet; I_{KB}, 2-ketobutyric acid input from synthesis; GLY, glycine; GRP, glycine release of proteins; HA, hippuric acid; KB, 2-ketobutyric acid; O_{gly}, glycine oxidation; O_{KB}, 2-ketobutyric acid oxidation; NOGD, non-oxidative glycine disposal; NOTD, non-oxidative threonine disposal; TDG, threonine dehydrogenase pathway; TDH, threonine dehydratase pathway; THR, threonine; TRP, threonine release of protein.

In conclusion, this study demonstrated that the splanchnic tissues of preterm infants utilize the dietary threonine intake to a substantial degree, i.e. more than three-quarters, irrespective of the amount of enteral threonine delivery. Surprisingly, threonine taken up by the

intestine apparently is not oxidized during either partial enteral feeding, or full enteral feeding. Furthermore, our data demonstrate that approximately 10% of the threonine flux is oxidized and the route of feeding does not affect this whole-body threonine oxidation. Overall, we suggest that the major metabolic fate of intestinal utilized threonine is mucosal (glyco-)protein synthesis, which is most likely reabsorbed distally in the intestine to enrich the whole-body pool of threonine.

References

1. Ehrenkranz RA, Younes N, Lemons JA, Fanaroff AA, Donovan EF, Wright LL, Katsikiotis V, Tyson JE, Oh W, Shankaran S, Bauer CR, Korones SB, Stoll BJ, Stevenson DK, Papile LA. Longitudinal growth of hospitalized very low birth weight infants. Pediatrics. 1999;104:280-289.
2. Stoll B, Henry JF, Reeds PJ, Yu H, Yahoor F, Burrin DG. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein fed piglets. J. Nutr. 1998;128:606-614.
3. Van Goudoever JB, Stoll B, Henry JF, Burrin DG, Reeds PJ. Adaptive regulation of intestinal lysine metabolism. Proc. Nat. Acad. Sci. 2000;97:11620-11625.
4. Van der Schoor SRD, Van Goudoever JB, Stoll B, Henry JF, Rosenberger JR, Burrin DG, Reeds PJ. The pattern of intestinal substrate oxidation is altered by protein restriction in pigs. Gastroenterology. 2001;121:1167-1175.
5. Stoll B, Burrin DG, Henry J, Yu H, Jahoor F, Reeds PJ. Dietary amino acids are the preferential source of hepatic protein synthesis in piglets. J. Nutr. 1998;128:1517-1524.
6. Benevenga NJ, Radcliffe BC, Egan AR. Tissue metabolism of methionine in sheep. Aust. J. Biol. Sci. 1983;36:475-485.
7. Roberton AM, Rabel B, Harding CA, Tasman-Jones C, Harris PJ, Lee SP. Use of the ileal conduit as a model for studying human small intestinal mucus glycoprotein secretion. Am. J. Physiol. 1991;261:G728-G734.
8. Bertolo RF, Chen CZ, Law G, Pencharz PB, Ball RO. Threonine requirement of neonatal piglets receiving total parenteral nutrition is considerably lower than that of piglets receiving an identical diet intragastrically. J. Nutr. 1998;128:1752-1759.
9. Iiboshi Y, Nezu R, Kennedy M, Fujii M, Wasa M, Fukuzawa M, Kamata S, Takagi Y, Okada A. Total parenteral nutrition decreases luminal mucous gel

and increases permeability of small intestine. J. Parenter. Enteral. Nutr. 1994;18:346-350.
10. Usher RH, Mclean F. Intrauterine growth of live-born caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J. Pediatr. 1969;74:901-910.
11. Rautonen J, Makela A, Boyd H, Apajasalo M, Pohjavuori M. CRIB and SNAP: assessing the risk of death for preterm neonates. Lancet. 1994; 343:1272-1273.
12. Perman JA, Barr RG, Watkins JB. Sucrose malabsorption in children; a noninvasive diagnosis by interval breath hydrogen determination. J. Pediatr. 1978;93:17-22.
13. Veereman-Wauters G, Ghoos Y, Van der Schoor S, Maes B, Hebbalkar N, Devlieger H, Eggermont E. The ^{13}C-octanoic acid breath test: a noninvasive technique to assess gastric emptying in preterm infants. J. Pediatr. Gastroenterol. Nutr. 1996;23:111-117.
14. Van Dijk-van Aalst K, Van den Driessche M, Van der Schoor S, Schiffelers S, Van't Westeinde T, Ghoos Y, Veereman-Wauters G. ^{13}C Mixed triglyceride breath test: a noninvasive method to assess lipase activity in children. J. Pediatr. Gastroenterol. Nutr. 2001;32:579-585.
15. Chaves Das Neves HJ, Vasconcelos AM. Capillary gas chromatography of amino acids, including asparagine and glutamine: sensitive gas chromatographic-mass spectrometric and selected ion monitoring gas chromatographic-mass spectrometric detection of the N,O(S)-tert.-butyldimethylsilyl derivatives. J. Chromatogr. 1987;392:249-258.
16. Zuijdgeest-van Leeuwen SD, Van den Berg JW, Wattimena JL, Van der Gaast A, Swart GR, Wilson JH, Dagnelie PC. Lipolysis and lipid oxidation in weight-losing cancer patients and healthy subjects. Metabolism. 2000;49:931-936.
17. Steele R. Influence of glucose loading and of injected insulin on hepatic glucose output. Proc. NY. Acad. Sci. 1959;82:420-430.
18. Tserng K, Hay WJ. Calculation of substrate turnover rate in stable isotope tracer studies. Am. J. Physiol. Endocrinol. Metab. 1983;245:E308-11.
19. Van Goudoever JB, Sulkers EJ, Chapman TE, Carnielli VP, Efstatopoulos T, Degenhart HJ, Sauer PJ. Glucose kinetics and glucoregulatory hormone levels in ventilated, preterm infants on the first day of life. Pediatr. Res. 1993;33:553-559.

20. Edelstone DI, Holzman IR. Oxygen consumption by the gastrointestinal tract and liver in conscious newborn lambs. Am. J. Physiol. 1981;241:G289-293.
21. Ebner S, Schoknecht P, Reeds P, Burrin D. Growth and metabolism of gastrointestinal and skeletal muscle tissues in protein-malnourished neonatal pigs. Am. J. Physiol. 1994;266:R1736-R1743.
22. McNurlan MA, Garlick PJ. Contribution of rat liver and gastrointestinal tract to whole-body protein synthesis in the rat. Biochem. J. 1980;186:381-383.
23. Hughes CA, Dowling RH. Speed of onset of adaptive mucosal hypoplasia and hypofunction in the intestine of parenterally fed rats. Clin. Sci. 1980;59:317-327.
24. Widdowson EM. Development of the digestive system: comparative animal studies. Am. J. Clin. Nutr. 1985;41:384-390.
25. Berseth CL, Nordyke C. Enteral nutrients promote postnatal maturation of intestinal motor activity in preterm infants. Am. J. Physiol. 1993;264:G1046-G1051.
26. Rojahn A, Lindgren CG. Enteral feeding in infants <1250 g starting within 24 h post-partum. Eur. J. Pediatr. 2001;160:629-632.
27. Hughes CA, Prince A, Dowling RH. Speed of change in pancreatic mass and in intestinal bacteriology of parenterally fed rats. Clin. Sci. 1980;59:329-336.
28. Berseth CL. Gut motility and the pathogenesis of necrotizing enterocolitis. Clin. Perinatol. 1994;21:263-270.
29. Berseth CL, McCoy HH. Birth asphyxia alters neonatal intestinal motility in term neonates. Pediatrics. 1992;90:669-673.
30. Schanler RJ, Shulman RJ, Lau C, Smith EO, Heitkemper MM. Feeding strategies for premature infants: randomized trial of gastrointestinal priming and tube-feeding method. Pediatrics. 1999;103:434-439.
31. Thureen PJ, Hay WW Jr. Early aggressive nutrition in preterm infants. Semin. Neonatol. 2001;6:403-415.
32. Stoll B, Chang X, Fan MZ, Reeds PJ, Burrin DG. Enteral nutrient intake level determines intestinal protein synthesis and accretion rates in neonatal pigs. Am. J. Physiol. Gastrointest. Liver. Physiol. 2000;279:G288-294.
33. Burrin DG, Stoll B, Jiang R, Chang X, Hartmann B, Holst JJ, Greely Jr GH, Reeds PJ. Minimal enteral nutrient requirements for intestinal growth in neonatal piglets: how much is enough? Am. J. Clin. Nutr. 2000;71:1603-1610.
34. Bengmark S, Jeppsson B. Gastrointestinal surface protection and mucosa reconditioning. J. Parenter. Enteral. Nutr. 1995;19:410-415.

35. Ball RO, Law G, Bertolo RFP et al. Adequate oral threonine is critical for mucin production and mucosal growth by the neonatal piglet,. In VIIIth International Symposium on Protein Metabolism and Nutrition: Wageningen, The Netherlands, Wageningen Pers, 1999:31.
36. Van der Schoor SRD, Reeds PJ, Stoll B, Henry JF, Rosenberger JR, Burrin DG, Van Goudoever JB. The high metabolic cost of a functional gut. Gastroenterology. 2002;123:1931-1940.
37. Ballèvre O, Cadenhead A, Calder AG, Rees WD, Lobley GE, Fuller MF, Garlick PJ. Quantitative partition of threonine oxidation in pigs: effect of dietary threonine. Am. J. Physiol. 1990;259:E483-E491.
38. Ballèvre O, Buchan V, Rees D, Fuller MF, Garlick PJ. Sarcosine kinetics in pigs by infusion of [1-^{14}C]sarcosine: use for refining estimates of glycine and threonine kinetics. Am. J. Physiol. 1991;260:E662-E668.
39. Davis AJ, Austic RE. Dietary threonine imbalance alters threonine dehydrogenase activity in isolated hepatic mitochondria of chicks and rats. J. Nutr. 1994;124:1667-1677.
40. Darling PB, Dunn M, Sarwar G, Brookes S, Ball RO, Pencharz PB. Threonine kinetics in preterm infants fed their mothers' milk or formula with various ratios of whey to casein. Am. J. Clin. Nutr. 1999;69:105-114.
41. Green ML, Elliot WH. The enzymatic formation of aminoacetone from threonine and ist further metabolism. Biochem. J. 1964;92:537-549.
42. Le Floc'h N, Thibault JN, Sève B. Tissue localization of threonine oxidation in pigs. Br. J. Nutr. 1996;77:593-603.
43. Reeds PJ, Burrin DG, Stoll B, Jahoor F, Wykes L, Henry J, Frazer ME. Enteral glutamate is the preferential source for mucosal glutathione synthesis in fed piglets. Am. J. Physiol. 1997;273:E408-E415.

Chapter 8

SPLANCHNIC BED GLUCOSE METABOLISM IN THE PRETERM NEONATE

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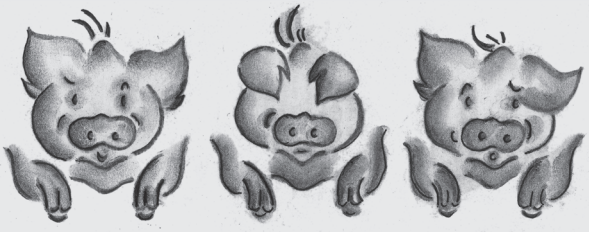

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Background & Aims: Glucose is a major oxidative substrate for intestinal energy generation in neonatal animals, while data in preterm infants are hardly available. However, early administration of enteral nutrition, including glucose, may offer an effective strategy to support the intestinal adaptation to extra-uterine life in preterm neonates. The purpose of the present study was to obtain data in human neonates by quantifying the first-pass uptake and oxidation of glucose by the splanchnic tissues in newborn infants. Methods: Eight preterm infants (birth weight: 1.19 ± 0.22 kg, gestational age: 29 ± 1 wk) were studied during two different enteral intakes [A: 40% enteral 60% parenteral, total glucose intake: 7.5 ± 0.5 mg/(kg·min), and B: 100% enteral, total glucose intake: 7.8 ± 0.4 mg/(kg·min)]. Splanchnic and whole-body glucose kinetics were measured by dual tracer techniques. Results: During both feeding periods, approximately one-third of the dietary glucose intake was utilized in first-pass by the intestine. More than three-quarters of this utilized glucose was oxidized in both periods [A: 79% ± 36 vs. B: 84% ± 45]. The whole-body glucose oxidation was substantial under both circumstances, 72% ± 5 and 77% ± 6 of the glucose flux was oxidized during partial and full enteral feeding. Conclusions: We conclude that approximately one-third of the dietary glucose is utilized in first-pass, irrespective of the dietary intake. The majority of the utilized glucose is used for energy generation.

In utero, the fetus continuously receives nutrients via the umbilical vein. After birth, newborns undergo a number of physiological adaptations to adjust to their extra-uterine environment. In particular, they must adapt to a shift from glucose as the major energy source to a carbohydrate-fat mixture as the energy source. [1,2] Therefore, the neonate has to mobilize stores of carbohydrates, proteins, and lipids in adequate amounts to meet its substantial metabolic needs. Energy expenditure in neonates is approximately three times higher per unit body mass than in adults. [3]

Studies in a number of mammalian species have shown that the portal-drained viscera (PDV; the intestines, pancreas, spleen and stomach) account for 35% of whole-body energy expenditure, whereas they only contribute less than 5% of the whole-body weight. [4-6] Matthews et al. showed in adults that the gut is the key organ in the process of sequestration of glutamine and glutamate for the purpose of oxidation, and they are key fuels for the splanchnic tissues. [7-9] In contrast, no studies in infants have been performed to quantify the substrates that are used by the intestine as fuel sources. This information is critical, especially in situations in which the intestine is damaged, e.g. by infection, chemotherapy or following ischemia due to a decreased splanchnic blood flow such as in case of necrotizing enterocolitis (NEC) or asphyxia. Moreover, identifying the substrates used to favor this high intestinal oxidative activity is also important for the atrophied intestine after periods of prolonged total parenteral nutrition or directly postpartum in preterm neonates. Knowing the required oxidative substrates can help in re-establishing a functional gut by supplying the appropriate substrates which might result in a fast recovery of the gut function.

The extent to which orally administered nutrients are absorbed by the intestine, and are metabolized within the intestinal tissues or transported to the systemic circulation, can be determined by dual stable isotope tracer methodology. Recently, using this technique in neonatal pigs, we showed that under normal feeding conditions, the PDV extracted 6% of the dietary glucose intake. [10] Studies in healthy

adult volunteers showed that a similar fraction of the oral glucose load (10%) was utilized by the splanchnic tissues, and 90% of the dietary glucose was available for the peripheral tissues. [11] In preterm neonates, however, little is known about the first-pass glucose uptake by the splanchnic tissues.

Moreover, we found in neonatal pigs that the intestinal energy production during a normal protein intake is largely derived from the oxidation of glucose and amino acids. [10,12,13] During protein restriction, the splanchnic tissues maintain their high rate of energy metabolism, although amino acid oxidation was significantly reduced whereas intestinal glucose oxidation became more important.

In an oxidation study in parenterally fed preterm infants during the first weeks of life, we also showed that on whole-body level, glucose is an important fuel source accounting for 30 to 40%, whereas protein oxidation accounted for 10 to 15%. The remaining 40 to 50% was probably due to lipid oxidation. [14,15] Whether glucose is catabolized by the splanchnic tissues in neonates has not been studied yet.

In the present study, the dual stable isotope tracer technique was employed to examine splanchnic and whole-body glucose kinetics in preterm infants. Specifically, the study was designed to determine the first-pass glucose uptake by the intestine during two different enteral protein intakes. The quantification of the intestinal and whole-body glucose oxidation both during partial and full enteral feeding was the second objective of our study. To investigate the influence of postnatal age on splanchnic uptake and oxidation of glucose, these variables were studied during different postnatal ages.

Methods

Subjects

Patients eligible for this study were premature infants with a birth weight ranging from 750 to 1500 g who were appropriate for the gestational age according to the charts of Usher and McLean. [16] Exclusion criteria were: congenital anomalies, gastrointestinal or liver diseases, and maternal diabetes. The study protocol was approved by

the Erasmus University Institutional Review Board. Written, informed consent was obtained from the parents.

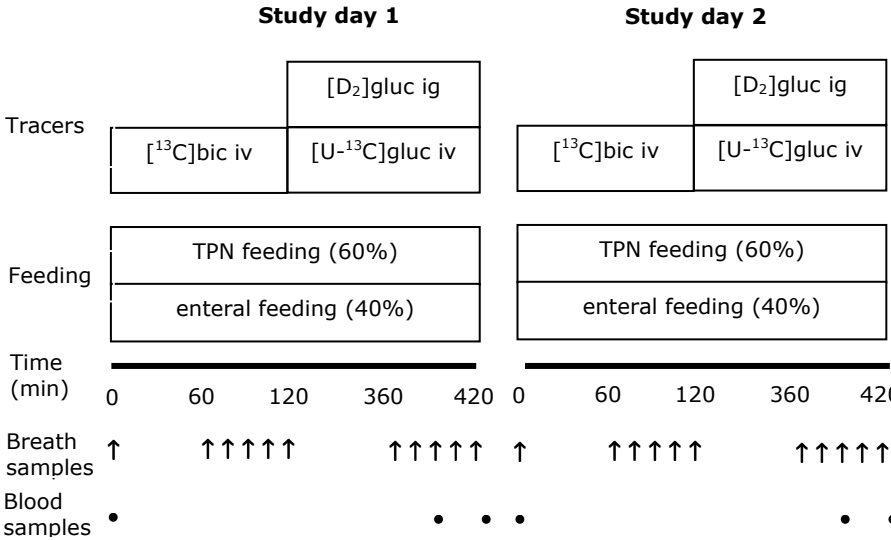
Eight preterm infants were included in the study (Table 1). CRIB-scores (indication of illness, range: 0-23) on the first day of life were all below two. [17] The infants received a standard nutrient regimen according to our neonatal intensive care feeding protocol; a combination of breast or formula feeding (Nenatal®, Nutricia, Zoetermeer, The Netherlands; 0.024 g/ml protein, 0.077 g/ml carbohydrate, 0.044 g/ml fat) and parenteral nutrition containing glucose (glucose 10%), amino acids (Primene® 10%, Clintec Benelux NV, Brussels, Belgium), and lipids (Intralipid® 20%, Fresensius 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

To investigate the influence of enteral intake and postnatal age on splanchnic glucose uptake, the study was implemented on two periods of two consecutive study days; during period A the infants received 40% enteral feeding and 60% parenteral feeding and they were 6 ± 1 d old, while during period B they received full enteral feeding and were 13 ± 2 d old (Figures 1 and 2). During period A the infants had both an arterial and an intravenous catheter for the withdrawing of blood samples and the infusion of tracers, while during period B a peripheral intravenous catheter was available for the infusion of tracers. During period B blood samples were collected by heelstick. To collect breath samples from these small infants, we employed the method described by Perman et al., who used a nasal tube. [18] This method has been validated and used for the non-invasive diagnosis of sucrose malabsorption by H_2 breath tests in children. [18] We validated this method for [^{13}C]substrate studies (Van der Schoor, unpublished data), although this method already has been used in [^{13}C]labeled substrate studies before. [19,20] Briefly, a 6 Fr gastric tube (6 Ch Argyle; Sherwood Medical, Tullamore, Ireland) was placed for 1 to 1.5 cm into the

nasopharynx, and 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 later analysis.

Figure 1. Schematic overview of period A during study days 1 and 2

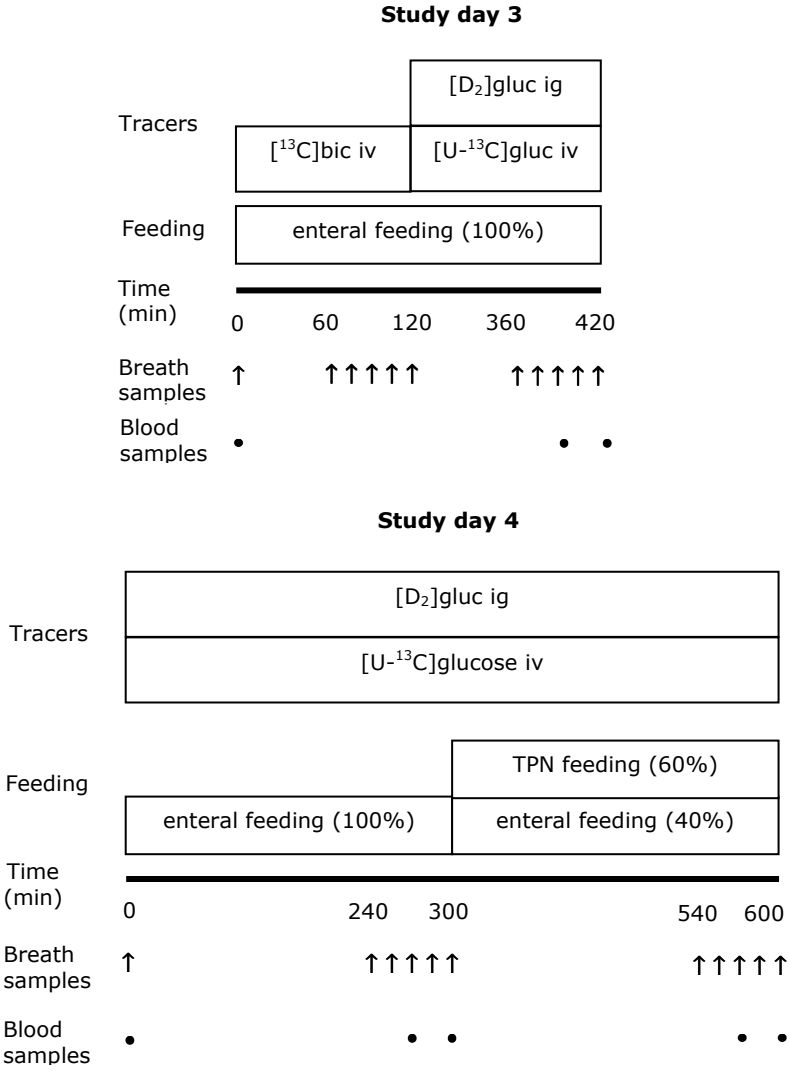


[^{13}C]bic, [^{13}C]sodiumbicarbonate; [D_2]gluc, [D_2]glucose; [U-^{13}C]gluc, [U-^{13}C]glucose.

Isotope infusion

^{13}C-labeled sodium bicarbonate ($NaH^{13}CO_3$, 99 mol% ^{13}C; Cambridge Isotopes, Woburn, MA), uniformly labeled glucose ($^{13}C_6H_{12}O_6$, 99 mol% $^{13}C_6$; Cambridge Isotopes, Woburn, MA), and deuterium labeled glucose ($C_6H_{10}D_2O_6$, 98 mol% 6,6-D_2; Cambridge Isotopes, Woburn, MA) were diluted in 0.9% NaCl by the hospital pharmacy and filtered through a 0.22-µm Millipore filter into sterile vials.

Figure 2. Schematic overview of period B during study days 3 and 4



[^{13}C]bic, [^{13}C]sodiumbicarbonate; [D_2]gluc, [D_2]glucose; [U-^{13}C]gluc, [U-^{13}C]glucose.

Three different stable isotope infusions were performed during each study day in both periods. First, a primed, continuous 2-h infusion (10.02 μmol/kg and 10.02 μmol/(kg·h)) of $NaH^{13}CO_3$ was administered at a constant rate. The ^{13}C-labeled bicarbonate infusion was immediately followed by two primed, continuous 5-h infusions (10.00 μmol/kg and 5.00 μmol/(kg·h)) of [U-^{13}C]glucose and [D_2]glucose designed to assess whole-body and splanchnic glucose kinetics. The route of tracer administration on the different study days is shown in Figures 1 and 2. In order to quantify the effect of postnatal age on whole-body and splanchnic glucose kinetics, we performed a separate study on the fourth study day (Figure 2). The first 5-h the infants received full enteral feeding simultaneously with two labeled glucose tracers intravenously and intragastrically; the next 5-h the infants received 40% enteral and 60% parenteral feeding simultaneously with two labeled glucose tracers intravenously and intragastrically. Blood samples were drawn before the infusion of the stable isotopes, at 30 min before the end, and at the end of the labeled glucose infusions. The total amount of blood withdrawn during a study day was 1.2 mL, which is less than 2% of blood volume in a 1000-g infant. Blood was centrifuged immediately and stored at −70°C for further analysis.

At time zero two duplicate baseline breath samples were collected. During the last hour of each tracer infusion, breath samples were collected at 15-min intervals (T 60, 75, 90, 105, 120, 360, 375, 390, 405, 420 min).

Analytical methods

Blood samples were prepared for mass spectrometry as described previously. [10] Gas chromatography-mass spectrometry was performed with the pentacetate derivative of glucose. The analyses were performed with a 6890 series gas chromatograph linked to a model 5973 quadrupole mass spectrometer (Hewlett Packard, Palo Alto, CA). Breath samples were analyzed for enrichment of $^{13}CO_2$ on an isotope ratio mass spectrometer (ABCA; Europa Scientific, Van Loenen Instruments, Leiden, The Netherlands). [21]

Calculations

Plasma enrichments of glucose were used to calculate the rate of glucose turnover. Glucose turnover was calculated by dividing the tracer infusion by the mean of two plateau measurements at plateau of the stable isotope tracer enrichments (Appendix, eq.**1**). The first-pass glucose uptake was calculated by dividing both fluxes of glucose tracers multiplying by the enteral glucose intake (Appendix, eq.**2**). The glucose production rate (GPR) was calculated by subtracting the rate of glucose infusion and the first-pass glucose uptake from the glucose flux (Appendix, eq.**3**).

The splanchnic and whole-body glucose oxidation (Appendix, eq.**4-10**) were determined assuming that the CO_2 production during the $NaH^{13}CO_3$ infusion was equal to the CO_2 production during the [U-^{13}C]glucose infusion. By determining the increase in $^{13}CO_2$ enrichment of each individual infant during both the $NaH^{13}CO_3$ infusion and the [U-^{13}C]glucose infusion, variance can be diminished and there is no need for correction factors that account for [^{13}C]bicarbonate sequestration in the whole-body. [14,22]

Statistics

The data are expressed as the mean values obtained from samples taken over the last hour of each tracer infusion ± SEM. Statistical comparisons were performed with a paired Student *t* test, and a Mann-Whitney test whenever the results were not normally distributed. A value of $P < 0.05$ was taken as statistically significant.

Results

All infants were appropriate for gestational age because of the inclusion criteria (mean gestational age 29 ± 1 wk, Table 1). Two infants were mechanically ventilated during both periods, and six infants received supplemental oxygen by a nasal prong. All infants received caffeine with therapeutic plasma levels within the normal range, and were clinically stable at the time of the study. The infants did not receive any vasopressic drugs that could influence the splanchnic blood flow. Seven

infants had received antenatal steroids. Routine blood chemistry and hematology (electrolytes, calcium, glucose, acid base, hematocrit, thrombocyte count and white blood cell count) were all within normal limits for preterm infants, and there were no significant changes in these parameters for 24 h before and after the study. Intakes of glucose, and energy are shown in Table 2. By study design, no differences were found in protein and carbohydrate intakes during both feeding periods [protein: A: 3.4 ± 0.1 vs. B: 3.2 ± 0.2 g/(kg·d), and glucose: A: 7.5 ± 0.5 vs. B: 7.8 ± 0.4 mg/(kg·min)]. The fat intake was significantly higher during full enteral feeding [A: 4.7 ± 0.3 vs. B: 5.8 ± 0.3 g/(kg.d), $P < 0.05$)], which resulted in a slightly, but significantly higher energy intake (+11%) during period B.

Table 1. Subject characteristics

P	*GA*	*BW*	*CRIB Score*	*PA A*	*PA B*	*SW A*	*SW B*
	wk	kg		d	d	Kg	kg
1	27	0.98	2	7	14	0.92	0.97
2	29	1.38	1	8	13	1.34	1.32
3	29	1.38	2	7	13	1.23	1.27
4	29	1.11	1	5	11	1.01	1.10
5	29	1.51	0	5	11	1.33	1.53
6	29	1.06	2	6	16	1.01	1.05
7	28	0.88	2	7	11	0.86	0.90
8	30	1.25	2	8	11	1.18	1.30
	29 ± 1	1.2 ± 0.2	1 ± 1	6 ± 1	13 ± 2	1.1 ± 0.2	1.2 ± 0.2

Values are means ± SD; P, patients; GA, gestational age; BW, birth weight; PA, postnatal age; SW, study weight; A, Period A; and B, Period B.

Isotopic plateau

Glucose kinetics were calculated from the plateau enrichment values in breath and plasma. Details of the isotopic enrichments of plasma glucose at baseline and plateau during periods A and B are given in Table 3. The background (baseline) recovery of the [^{13}C]label in

expiratory air was not significantly different between both periods [A: 1.0918 ± 0.0007 vs. B 1.0883 ± 0.0018 APE].

Table 2. Glucose intake (mg/(kg·min)), and energy intake (kcal/(kg·d)) during period A (partial enteral feeding) and period B (full enteral feeding)

	Partial enteral feeding (A)		*Full enteral feeding (B)*	
Total glucose intake	7.5 ± 0.5		7.8 ± 0.4	
enteral		2.7 ± 0.3		7.1 ± 0.3
parenteral		5.0 ± 0.4		0.7 ± 0.1
Total energy intake	100 ± 4 *		111 ± 5	
enteral		41 ± 5		107 ± 5
parenteral		58 ± 6		4 ± 1

*Means ± SD, n = 8, and * indicates significant difference at P < 0.05.*

Table 3. Baseline and steady-state plasma isotopic enrichments on study days 1 and 2 during period A (partial enteral feeding) and on study days 3 and 4 during period B (full enteral feeding)

D	*Feeding*	*[U-^{13}C]glucose*		*[D_2]glucose*	
		baseline	plateau	baseline	plateau
		mol %	mol %	mol %	mol %
1	Partial enteral	0.0192 ± 0.0043	0.1308 ± 0.0610	2.7115 ± 0.0519	2.7729 ± 0.0702
2	Partial enteral	0.0199 ± 0.0039	0.1135 ± 0.0629	2.7347 ± 0.0720	2.7883 ± 0.0715
3	Full enteral	0.0199 ± 0.0073	0.1309 ± 0.0644	2.6702 ± 0.0735	2.8245 ± 0.0955
4	Partial enteral	0.0166 ± 0.0032	0.1739 ± 0.0262	2.6725 ± 0.0258	2.7780 ± 0.0430
4	Full enteral		0.1895 ± 0.0497		2.7621 ± 0.0604

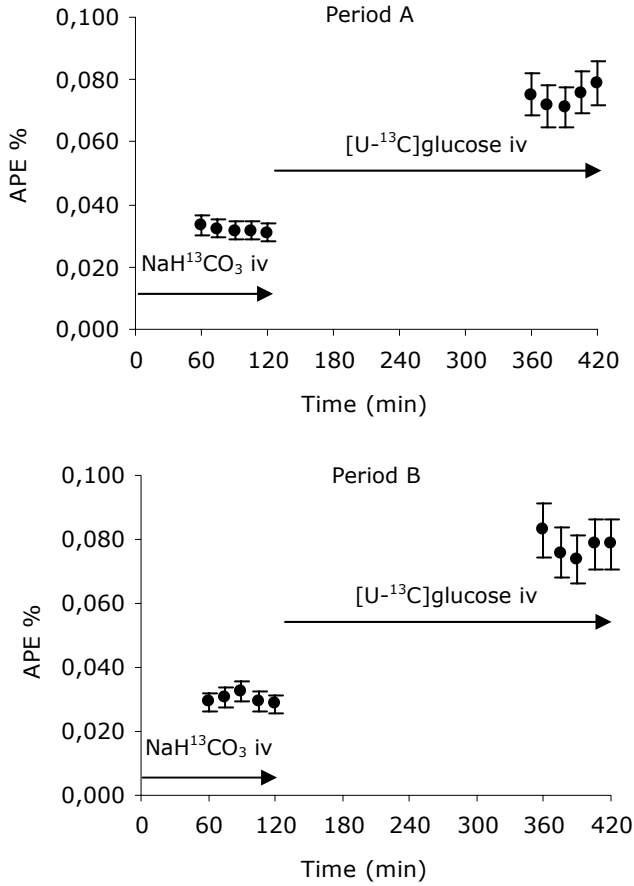
Values are means ± SD; D. study day; n = 8. On study days 1 and 4 [U-^{13}C]glucose was administered intravenously and [D_2]glucose intragastrically; on study days 2 and 3 [U-^{13}C]glucose was given intragastrically and [D_2]glucose intravenously.

Figure 3 shows the mean increase in $^{13}CO_2$ enrichment in expired air at both enteral intakes. The $^{13}CO_2$ enrichment in breath during [^{13}C]sodium bicarbonate infusion rose rapidly in the first hour of infusion in both periods, to become constant in all infants by 120 min, with <3% variation in plateau values [A: 1.6 ± 0.5% vs. B: 2.0 ± 1.1%]. The coefficient of variation (mean ± SD) of breath [^{13}C]glucose enrichment above baseline at plateau was 1.2 ± 0.8% during period A and 1.9 ± 1.0% during period B.

Glucose kinetics

The glucose fluxes were higher for the oral than for the intravenous tracer group during the two periods [A: flux ig 5,537 ± 1,328 vs. flux iv 3,405 ± 220 μmol/(kg·h); B: flux ig 4,190 ± 614 vs. flux iv 2,885 ± 233 μmol/(kg·h)]. This is due to the first-pass uptake of the intragastrically administered glucose tracer within the splanchnic region. Table 4 summarizes the first-pass kinetics of glucose by the intestine during partial and full enteral feeding. The first-pass glucose uptake, expressed as a percentage of dietary intake, was not significantly different between both feeding periods. More than one-third of the dietary intake was utilized by the splanchnic tissues during both partial and full enteral feeding. The postnatal age did not influence the first-pass glucose uptake, at a postnatal age of 13 days and a restricted enteral intake, still 39 ± 8% of the dietary intake was utilized. During a restricted enteral intake, the intestine oxidized dietary glucose at a rate of 323 ± 136 μmol/(kg·h). During full enteral feeding, glucose oxidation by the splanchnic tissues was 573 ± 273 μmol/(kg·h). Taken together, the first-pass glucose oxidation was not significantly different between both feeding periods, and accounted for more than three-quarters of the utilized glucose.

Figure 3. Time course of $^{13}CO_2$ enrichment in breath after a primed constant $NaH^{13}CO_3$ and [U-^{13}C]glucose intravenous infusion during period A and period B in 8 patients



Values are presented as mean ± SD.

Table 4. First-pass glucose uptake and oxidation during period A (partial enteral feeding) and period B (full enteral feeding)

	Partial enteral feeding (A)	*Full enteral feeding (B)*
FP uptake (μmol/(kg·h)	398 ± 65	825 ± 253
Fractional FP uptake (%)	44 ± 8	32 ± 9
FP oxidation (μmol/(kg·h)	323 ± 136	573 ± 273
Fractional FP oxidation of FP uptake (%)	79 ± 36	84 ± 45

Means ± SEM, n = 8.

Whole-body glucose oxidation, and glucose production rates are shown in Table 5. There was no significant difference in the amount of glucose oxidized by the whole-body between the two feeding periods. During both intakes, whole-body glucose oxidation accounted for approximately 75% of glucose flux [A: 72 ± 5% vs. B: 77 ± 6%]. Postnatal age did not influence the whole-body glucose oxidation, at a postnatal age of 13 days and a restricted enteral intake, 69 ± 5% of the glucose flux was oxidized. The GPR was approximately 1,500 μmol/(kg·h) in both periods, and accounted for 40 ± 3 and 62 ± 6% of the glucose flux, respectively.

Discussion

To our knowledge, this is the first study that provides insight into the splanchnic glucose uptake as well as the splanchnic oxidative capacity in preterm infants. Our data indicate that the splanchnic tissues utilize one-third of the oral glucose intake, irrespective of the level of enteral feeding. This utilized glucose is oxidized for approximately 80%, presumably to sustain the high rate of visceral metabolism. The whole-body glucose oxidation accounted for three-quarters of the glucose turnover. Given the finding that splanchnic glucose oxidation was substantial in the early weeks of life of preterm infants, early administration of enteral nutrition, including glucose, may offer an

effective strategy to support the intestinal adaptation to extra-uterine life in preterm neonates. These data demonstrate that the splanchnic tissues extract a disproportionate amount of dietary glucose to serve mainly as fuel source in the early life of preterm neonates.

Table 5. Whole-body glucose kinetics in preterm infants during period A (partial enteral feeding) and period B (full enteral feeding)

	Partial enteral Feeding (A)		*Full enteral feeding (B)*
	6 days old	*13 days old*	*13 days old*
	μmol/(kg·h)	*μmol/(kg·h)*	*μmol/(kg·h)*
Flux iv	3,405 ± 220	3,108 ± 295	2,885 ± 233
Flux ig	5,537 ± 1,328	4,854 ± 843	4,190 ± 614
WB oxidation	2,522 ± 331	2,305 ± 163	2,161 ± 101
WB oxidation of flux (%)	72 ± 5	69 ± 5	77 ± 6
GPR	1,433 ± 255	1,343 ± 146	1,781 ± 221

Means ± SEM, n = 8. GPR, glucose production rate; WB oxidation, whole-body oxidation of glucose.

The neonate depends on the gastrointestinal tract for the acquisition of nutrients through the processes of propulsion, digestion and absorption of ingested food. Studies in adults demonstrated less than 0.5 mg of the residual glucose in gastric washings, obtained three hours after ingestion of 100 g oral glucose. [23] Furthermore, at least 98% of glucose is being removed from the intestinal lumen before it reaches the ileum. [24] However, glucose was not the sole source of carbohydrates; the formula contained also lactose, maltose and glucose polymers. Others have shown that glucose polymers are well absorbed by young infants. [25,26] Kien et al. showed that lactose digestion from formula feeding was approximately 79%, [27] although there was a significant linear correlation between lactose digestion and post-conceptional age. Thus, most of the dietary milk carbohydrates are absorbed by the intestinal mucosa at a postnatal age of one week. And therefore, glucose that does not appear in the systemic circulation,

is utilized by the splanchnic tissues and is not lost via the faeces before being used for synthetic purposes.

The substantial dietary glucose uptake observed during both partial and full enteral feeding reflects the use of absorbed glucose for different splanchnic metabolic fates, at least under clinically stable conditions and in the absence of significant intestinal diseases. Splanchnic glucose extraction equals the sum of intestinal and hepatic glucose extraction. Any combination of the following four possibilities could explain the lower systemic availability of the dietary glucose: *1)* glucose is used by mucosal tissue as a energy-providing substrate, *2)* glucose is used for the synthesis of intestinal glyco-proteins, *3)* in the process of absorption some glucose is converted into three-carbon compounds, which are then released into the portal vein, and *4)* glucose is taken up by the liver. It should be emphasized quit clearly that catheterization of the portal vein was not ethically feasible in the small infants in our study. Consequently, it was not possible to quantify the net hepatic uptake of dietary glucose and the relative contributions of intestinal and hepatic tissues to the splanchnic glucose oxidation.

By using dual stable isotope methodology, we were able to measure the first-pass glucose oxidation by the intestine, and we found that more than three-quarters of the intestinally absorbed glucose was oxidized. Thus, it seems that dietary glucose is an important energy source for the intestinal mucosa. The present data with regards to intestinal glucose oxidation, are in line with our previous observations in protein-restricted piglets, in which we found that intestinal glucose oxidation accounted for approximately 50% of the total visceral CO_2 production. [10] Similarly, in vitro studies with human enterocytes reported that the respiratory fuels of human enterocytes include glucose, glutamine, and to a lesser extent, ketone bodies as indicated in the proportion of oxygen consumption attributed to these fuels. [28]

A second metabolic fate of first-pass utilized glucose might be mucin synthesis. Intestinal mucins are key components of the first line of host defense against intestinal pathogens. These large glyco-proteins secreted by specialized goblet cells form viscous gels that trap

microorganisms and limit their diffusion to the intestinal epithelium. Isolated mucin has high concentrations of carbohydrates, representing 65% of the mucin by weight. [29,30] Recently, we found in preterm infants that more than 80% of the dietary threonine intake was utilized in first-pass, which was not used for oxidative purposes but for synthetic purposes. (Van der Schoor SRD, unpublished data) Therefore, we speculate that preterm neonates have a high visceral need of threonine presumably for the synthesis of glyco-proteins, and consequently, neonates may have a high need for glucose as well.

After the splanchnic release into the systemic circulation, glucose can be oxidized in the peripheral tissues, metabolized to lactate and recycled to glucose via gluconeogenesis, stored as glycogen, or used in various synthetic processes. Because glucose represents the main source of energy for the preterm infant during most of the neonatal period, it is important to determine the extent of whole-body glucose oxidation and its contribution to the total glucose use. This knowledge assists in the clinical determination of the optimal rate of glucose infusion for the preterm infant, which is the rate that is appropriate to the neonate's capacity to oxidize glucose. In the present study we quantified the whole-body oxidation of glucose and the glucose production rate in relation to an increasing enteral intake. Our results show that more than 70% of the glucose turnover is oxidized, and these observations are similar to other studies, except for the study done by Cowett et al. [31] In preterm infants, we and others previously showed 50-65% of the whole-body glucose turnover was oxidized. [13,32-35] Cowett et al. studied glucose oxidation in preterm infants with different postnatal ages and found that the fraction of glucose oxidized decreased with postnatal age. [31] We did not find a significant different effect of postnatal age on whole-body glucose oxidation, which might be due to a difference of only 1 week. However, the purpose of this part of the study was to show that there was no difference in glucose metabolism caused by an increasing postnatal age or feeding pattern.

In conclusion, we have shown that the splanchnic tissues extract one-third of the dietary glucose intake in preterm infants in the first

weeks of life. A significant amount of this utilized glucose is used for oxidative purposes. The results also show that neither the amount of enteral feeding nor the postnatal age significantly affects the splanchnic glucose uptake and oxidation. Taken together, the results highlight the critical importance of the splanchnic tissues in actively regulating the glucose flow to the peripheral tissues, already in the first weeks of life in preterm neonates.

Appendix

The rate of glucose appearance (Q = flux) is calculated as follows:

$$Q = I_G \times [(IE_i / IE_p) - 1] \quad \textbf{[1]}$$

where Q is the rate of appearance, I_G is the glucose tracer infusion rate in µmol/(kg·min), IE_i is the isotopic enrichment of the infusate, and IE_p is the isotopic enrichment of [U-^{13}C or D_2]glucose (m^{+6}, m^{+2}) of the plasma.

The first-pass glucose uptake was calculated according to the expression:

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

where U is the first-pass glucose uptake in µmol/(kg·h), Q_{ig} is the flux of the intragastric glucose, Q_{iv} is the flux of the intravenous glucose, and I is the enteral glucose intake.

Under steady state conditions, the rate of glucose appearance is equal to the rate of glucose disappearance. Glucose can enter the glucose pool by intravenous or enteral glucose infusion and by glucose production from other substrates, e.g. glycerol and some amino acids. Glucose can leave the pool by oxidation or non-oxidative disposal.

$$GPR = Ra - (GI - U) \quad \textbf{[3]}$$

where GPR is glucose production rate µmol/(kg·h), and GI is the total glucose intake (enteral and intravenous) in µmol/(kg·h).

To measure the splanchnic and whole-body glucose oxidation rate with the use of a continuous infusion of [U-^{13}C]glucose, usually a correction factor is used to compensate for the losses of labeled carbon within the bicarbonate pool. This correction factor can be determined

beforehand in a separate study or calculated from the energy intake. [32] The equation used for the oxidation is:

$$GO = [(V_{CO2}(G) \times IE_g) / (I_G \times c)] \times Ra \quad \textbf{[4]}$$

where GO is the glucose oxidation in μmol/(kg·h), $V_{CO2}(G)$ is the rate of CO_2 excretion in μmol/(kg·h) during the [U-^{13}C]glucose infusion, IE_g is the isotopic enrichment of expired CO_2 at plateau during the [U-^{13}C]glucose infusion, I_G is the glucose infusion rate in ^{13}C/(kg·h), and c is the correction factor.

A more accurate method is to measure the bicarbonate retention for each infant individually. The correction factor is determined using the following equation:

$$c = [(V_{CO2}(B) \times IE_n)] / I_N \quad \textbf{[5]}$$

where $V_{CO2}(B)$ is the rate of CO_2 excretion in μmol/(kg·h) during the $NaH^{13}CO_3$ infusion, IE_n is the isotopic enrichment of expired air at plateau during the $NaH^{13}CO_3$ infusion, and I_N is the $NaH^{13}CO_3$ infusion rate in μmol/(kg·h).

Combining eq.**4** and **5** leads to:

$$GO = [(V_{CO2}(G) \times IE_g)/((I_G) \times ((V_{CO2}(B) \times IE_n))/I_N] \times Ra \quad \textbf{[6]}$$

Since the $NaH^{13}CO_3$ infusion is immediately followed by the [U-^{13}C]infusion and no changes are made in the intake of the infants, we can assume that $V_{CO2}(G) = V_{CO2}(B)$. Removing the term V_{CO2} from both the numerator and the denominator, the following equation can be derived:

$$GO = [(IE_g \times I_N) / (I_G \times IE_n)] \times Ra \quad \textbf{[7]}$$

Eq.**7** shows that, in case of a constant CO_2 production over a few hours, it is not necessary to measure V_{CO2}.

The calculation of the metabolism of enterally administered [U-^{13}C]glucose to CO_2 is complicated by the fact that some of the glucose tracer is absorbed and thereby labels the arterial pool. Thus, the non-first pass oxidation of [^{13}C]labeled glucose that was administered enterally, but was absorbed and entered the body at study days 2 or 3 can be calculated as follows:

Non-first pass oxidation of the enteral administered

$$[U\text{-}^{13}C]glucose = (IE_{13C\ ig} / IE_{13C\ iv}) \times GO \quad \textbf{[8]}$$

where $IE_{13C\ ig}$ is the ^{13}C enrichment in plasma during the intragastric [^{13}C]glucose infusion at study days 2 or 3, and $IE_{13C\ iv}$ is the ^{13}C enrichment in plasma during the intravenous [^{13}C]glucose infusion at study days 1 or 4.

The glucose oxidation at study days 2 or 3 is calculated as described before:

$$\text{Total glucose oxidation} = [(IE_G \times I_N) / (IE_B \times I_G \times 6)] \times Q_{iv} \quad \textbf{[9]}$$

where Q_{iv} is the flux of the intravenous [D_2]glucose tracer in μmol/(kg·h).

The first-pass glucose oxidation can thus be calculated with the following equation:

$$\text{First-pass glucose oxidation} = \text{Eq.}\mathbf{9} - \text{Eq.}\mathbf{8} \quad \textbf{[10]}$$

References

1. Morriss FH, Makowski EL, Meschia G, Battaglia FC. The glucose/oxygen quotient of the term human fetus. Biol. Neonate. 1975;25:44-52.
2. Senterre J, Karlberg P. Respiratory quotient and metabolic rate in normal full-term and small for date newborn infants. Acta. Pediatr. Scan. 1970;59:653-658.
3. Van Goudoever JB, Sulkers EJ, Lafeber HN, Sauer PJ. Short-term growth and substrate use in very-low-birth-weight infants fed formulas with different energy contents. Am. J. Clin. Nutr. 2000;71:816-821.
4. Burrin DG, Ferrell CL, Britton RA, Bauer M. Level of nutrition and visceral organ size and metabolic activity in sheep. Br. J. Nutr. 1990;64:439-448.
5. Brundin T, Wahren J. Influence of a mixed meal on splanchnic and intracapsular energy expenditure in man. Am. J. Physiol. 1991;260:E232-E237.
6. Brundin T, Thörne A, Wahren, J. Heat leakage across the abdominal wall and meal-induced thermogenesis in normal weight and obese subjects. Metab. Clin. Exp. 1992;41:49-55.
7. Matthews DE, Marano MA, Campbell RG. Splanchnic bed utilization of glutamine and glutamic acid in humans. Am. J. Physiol. 1993;264:E848-E854.

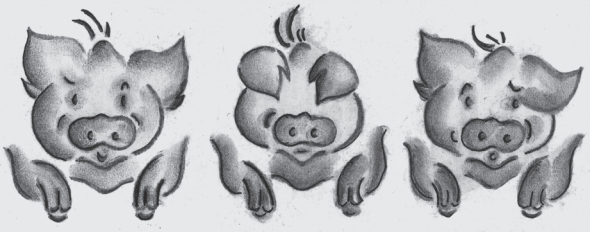
8. Battezzati A, Brillon DJ, Matthews DE. Oxidation of glutamic acid by the splanchnic bed in humans. Am. J. Physiol. 1995;269:E269-E276.
9. Haisch M, Fukagawa NK, Matthews DE. Oxidation of glutamine by the splanchnic bed in humans. Am. J. Physiol. Endocrinol. Metab. 2000;278:E593-E602.
10. Van der Schoor SRD, Van Goudoever JB, Stoll B, Henry JF, Rosenberger JR, Burrin DG, Reeds PJ. The pattern of intestinal substrate oxidation is altered by protein restriction in pigs. Gastroenterology. 2001;121:1167-1175.
11. Radziuk J, McDonald TJ, Rubenstein D, Dupre J. Initial splanchnic extraction of ingested glucose in normal man. Metabolism. 1978;27:657-669.
12. Stoll B, Burrin DG, Henry JF, Yu H, Jahoor F, Reeds PJ. Substrate oxidation by the portal drained viscera of fed piglets. Am. J. Physiol. 1999;277:E168-E175.
13. Van Goudoever JB, Stoll B, Henry JF, Burrin DG, Reeds PJ. Adaptive regulation of intestinal lysine metabolism. Proc. Nat. Acad. Sci. 2000;97:11620-11625.
14. Van Goudoever JB, Sulkers EJ, Chapman TE, Carnielli VP, Efstatopoulos T, Degenhart HJ, Sauer PJJ. Glucose kinetics and glucoregulatory hormone levels in ventilated preterm infants on the first day of life. Pediatr. Res. 1993;33:583-589.
15. Sauer PJJ, Carnielli VP, Sulkers EJ, Van Goudoever JB. Substrate utilization during the first weeks of life. Acta. Paediatr. Suppl. 1994;405:49-53.
16. Usher RH, Mclean F. Intrauterine growth of live-born caucasian infants at sea level: standards obtained from measurements in 7 dimensions of infants born between 25 and 44 weeks of gestation. J. Pediatr. 1969;74:901-910.
17. Rautonen J, Makela A, Boyd H, Apajasalo M, Pohjavuori M. CRIB and SNAP: assessing the risk of death for preterm neonates. Lancet. 1994;343:1272-73.
18. Perman JA, Barr RG, Watkins JB. Sucrose malabsorption in children; a noninvasive diagnosis by interval breath hydrogen determination. J. Pediatr. 1978;93:17-22.
19. Veereman-Wauters G, Ghoos Y, Van der Schoor S, Maes B, Hebbalkar N, Devlieger H, Eggermont E. The ^{13}C-octanoic acid breath test: a noninvasive technique to assess gastric emptying in preterm infants. J. Pediatr. Gastroenterol. Nutr. 1996;23:111-117.
20. Van Dijk-Van Aalst K, Van den Driessche M, Van der Schoor S, Schiffelers S, Van't Westeinde T, Ghoos Y, Veereman-Wauters G. ^{13}C mixed triglyceride

breath test: a noninvasive method to assess lipase activity in children. J. Pediatr. Gastroenterol. Nutr. 2001;32:579-585.
21. Zuijdgeest-Van Leeuwen SD, Van den Berg JW, Wattimena JL, Van der Gaast A, Swart GR, Wilson JH, Dagnelie PC. Lipolysis and lipid oxidation in weight-losing cancer patients and healthy subjects. Metabolism. 2000;49:931-936.
22. Sulkers EJ, Van Goudoever JB, Leunisse C, Degenhart HJ, Lafeber HN, Sauer PJJ. Determination of carbon-labelled substrate oxidation rates without measuring VCO_2. In: Lafeber HN, ed. Fetal and Neonatal Physiological Measurements. New York: Elsevier, 1991: 297-304.
23. Perley MJ, Kipnis DM. Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic subjects. J. Clin. Invest. 1967;46:1954-1962.
24. Kien CL, McClead RE, Kepner J, Grotjohn K. Comparison of methods for estimating fecal carbohydrate excretion in premature infants. J. Pediatr. Gastroenterol. Nutr. 1993;17:276-282.
25. Cicco R, Holzman IR, Brown DR, Becker DJ. Glucose polymer tolerance in premature infants. Pediatrics. 1981;67:498-502.
26. Lebenthal E, Heitlinger L, Lee PC, Nord KS, Hodge C, Brooks SP, George D. Corn syrup sugars: in vitro and in vivo digestability and clinical tolerance in acute diarrhea of infancy. J. Pediatr. 1983;103:29-34.
27. Kien CL, McClead RE, Cordero LJr. In vivo lactose digestion in preterm infants. Am J Clin. Nutr. 1996;64:700-705.
28. Ashy AA, Salleh M, Ardawi M. Glucose, glutamine, and ketone-body metabolism in human enterocytes. Metabolism. 1988;37:602-609.
29. Allen A. Structure and function of gastrointestinal mucus. In: Johnson LR, ed. Physiology of the Gastrointestinal Tract. New York: Raven Press, 1981:617-639.
30. Montagne L, Toullec R, Lalles JP. Calf intestinal mucin : isolation, partial characterization, and measurement in ileal digesta with an enzyme-linked immunosorbent assay. J. Dairy. Sci. 2000;83:507-517.
31. Cowett RM, Carpenter MW, Carr S, Kalhan S, Maguire C, Sady M, Haydon B, Sady S, Dorcus B. Glucose and lactate kinetics during a short exercise bout in pregnancy. Metabolism. 1996;45:753-758.
32. Sauer PJ, Van Aerde JE, Pencharz PB, Smith JM, Swyer PR. Glucose oxidation rates in newborn infants measured with indirect calorimetry and [U-^{13}C]glucose. Clin. Sci. (Lond) 1986;70:587-593.

33. Lafeber HN, Sulkers EJ, Chapman TE, Sauer PJ. Glucose production and oxidation in preterm infants during total parenteral nutrition. Pediatr. Res. 1990;28:153-157.
34. Hoshi J, Nishida H, Yasui M, Ohishi M, Takahashi M. [^{13}C] breath test of medium-chain triglycerides and oligosaccharides in neonates. Acta. Paediatr. Jpn. 1992;34:674-677.
35. Van Aerde JE, Sauer PJ, Pencharz PB, Canagarayar U, Beesley J, Smith JM, Swyer PR. The effect of energy intake and expenditure on the recovery of $^{13}CO_2$ in the parenterally fed neonate during a 4-hour primed constant infusion of $NaH^{13}CO_3$. Pediatr. Res. 1995;19:806-810.

Chapter 9

DISCUSSION



Neonatologists dealing with the preterm infant are privileged to watch ontogeny occurring, while simultaneously they have to struggle with the problems of immaturity.

In the context of improvements in survival for the VLBW infant, the importance of nutrition has become notable. Preterm infants as compared with term infants are more at risk for nutrient deficiencies and/or toxicity for several reasons. These factors include: *1)* an early birth limits the infant's nutrition sources, *2)* rapid growth quickly depletes the infant's nutrition sources and creates the urge for nutrition to meet the demands of growth, *3)* physiologic immaturity, in specific the gastrointestinal tract interferes with the advancement and tolerance of enteral nutrition and *4)* illness is frequently present and changes nutrient demands and feeding tolerance. Because of these factors, the preterm infant's nutrient needs are greater than those found in the healthy term neonate. To be adequate, nutrient intakes must meet needs for accretion and replace obligatory losses. Accretion includes the amounts actually laid down in new tissues plus the cost of accretion. Therefore, the VLBW infant presents a nutritional emergency: even with the administration of parenteral nutrition from day 1, weight loss exceeds 10% in this group and is partly related to their high body water composition and caloric deficit, and birth weight is regained at a mean of 11 days. [1] The short term consequences of inadequate nutrition in this group of infants are easily recognized; e.g. increased susceptibility for infection, and delayed recovery. Accumulating evidence points strongly to the fact that the clinical management of nutrition has long term consequences and susceptibility to chronic diseases in adulthood. [2-4] And, perinatal nutrition is recognized to have a profound and persistent influence on neurologic development and cognitive function. [5,6]

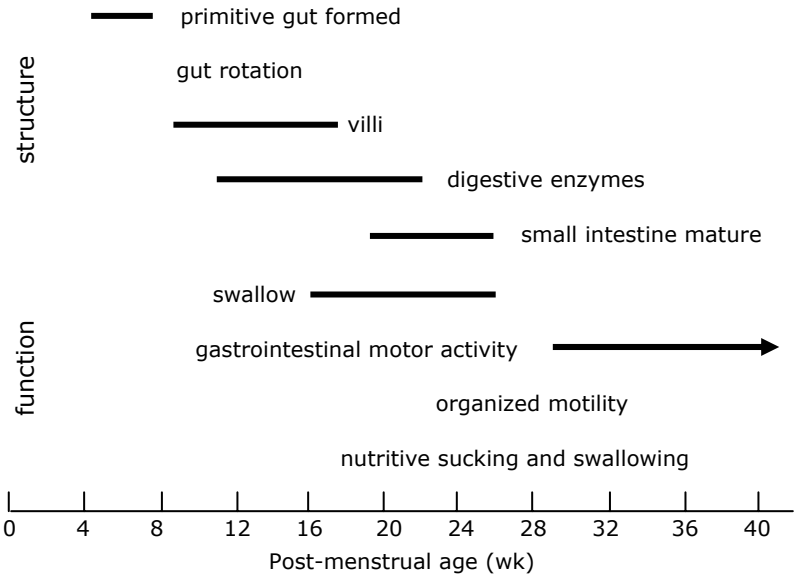
The current variation in clinical practice between neonatal intensive care centers highlights the absence of an adequate evidence rationale based on which today's practice can be found. [7] A growing body of data is available in gastrointestinal ontogeny, highlighting the fetal and postnatal events that influence gut development and allowing

an increasing rational approach to the use of enteral nutrition in VLBW infants.

Gut development and digestion

The gastrointestinal tract is one of the first structures discernable in the developing embryo. During fetal life, increase in gut length is particularly fast during the second trimester and, intestinal length continues to increase more rapid than body length for 3 to 4 years of life. [8] The ontogenetic timetable is complex (Figure 1). In the intestinal mucosa, cellular differentiation and villus development occur early in the second trimester. At the beginning of the third trimester, lactase activity is around 5% of adult levels. This intestinal growth and maturation before birth prepare neonates for the abrupt transition from receiving nutrients via the placenta and umbilical vein and effectively bypassing the intestine, to complete dependence on the intestine for processing and absorbing nutrients.

Figure 1. The ontogenic timetable showing structural and functional gastrointestinal development. [52]



As the ontogenetic timetable suggests (Figure 1), problems with motility are likely to be critical in determining an VLBW infant's ability to tolerate enteral nutrition. Thereby, prematurity is the principal risk factor for problems with enteral feeding relating to gastrointestinal disorder or necrotizing enterocolitis (NEC). Susceptibility to NEC may thus be closely related to poor gut motility. A multifactorial pathogenesis has been proposed, but the pathogenesis remains obscure. Because NEC seldom occurs in infants without enteral feeding, enteral nutrition have come to be seen as risk factor of NEC. The association between enteral nutrition and NEC is likely to be explained by the fact that feeding introduces bacterial or viral pathogens or toxins, which are more likely to survive the gastric barrier because of low acidity and against which the immature intestine is poorly able to defend itself. On the other hand, preterm infants fed human milk showed a lower incidence of NEC. [9] Efforts aimed at minimizing the risk of NEC have focused on the time of introduction of feedings, on volumes, and on the rate of feeding volume increments.

In case of intrauterine growth retardation (IUGR) due to inadequate placenta function, poor fetal oxygenation and nutrition occur. The changes in the fetus are similar to the diving mammal, in which a diversion of blood flow from the splanchnic circulation and redistribution toward cerebral blood flow occurs. [10,11] The consequence is a negative effect upon intestinal and pancreatic growth and an increased susceptibility towards dysmotility and NEC. Awareness of this problem and the common observation of difficulties with the introduction of enteral nutrition in the IUGR infant leads, in many centers, to the deliberately withholding of enteral nutrition for the first 24 to 48h in VLBW infants who have evidence of IUGR. However, no substantive data are available to support the view that this prevents NEC. And, although parenteral nutrition has been used widely in the management of ill VLBW infants, a smooth transition to the enteral route is most desirable.

Animal studies have shown that total parenteral nutrition is associated with mucosal atrophy and reduction of enzymatic activity. [12-14] Therefore, an important advance in nutrition field is minimal enteral feeding (MEF) which follows the recognition that small volumes of milk promote maturation of gut function while avoiding the possible disadvantages or difficulties of full enteral feeding. MEF is the administration of nutritionally inconsequential quantities of feed while the main route for nutrition is parenteral. Typical volumes used in MEF are 12 to 24 ml/(kg·d). Infants who have received MEF show more normal patterns of gastroduodenal motility, showing maturation of the fed response, an effect that is not observed in case of enteral water administration. [15-17] Other clinical benefits of MEF include a reduction in hospital stay without a concomitant increase in the risk of NEC, and indeed MEF may protect against this condition. [18] Summarizing, MEF allows the preterm infant to avoid the effects of enteral starvation and to benefit from the trophic properties of milk feeding.

To put the limited existing data in context, and to provide a basis for the appropriate design of future studies, a detailed discussion of the factors affecting intestinal protein metabolism is required. It follows that the understanding of fetal nutrition may be helpful in designing nutritional strategies in children with a compromised gut function.

Intestinal metabolism

Besides being primarily an agent of digestion, the intestine also has an endocrine function, and participates in inter-organ nitrogen homeostasis. These functions emphasize that metabolism within the intestinal tissues is important due to the fact that a substantial part of the dietary nutrients being absorbed are used locally to meet the needs of the gut. The present thesis has focused primarily on the first-pass uptake of dietary nutrients, and the metabolism of nutrients in tissues of the visceral region. Several studies in animals concluded that the PDV (portal-drained viscera; intestine, pancreas, spleen, and stomach) accounted for about one-quarter of whole-body energy metabolism, [19-21] which is a disproportionate amount of energy for the size of the

tissue (about 6% of the whole-body weight). Indeed, the intestine, as an organ with a dynamic activity, has one of the highest rates of protein synthesis of any tissue in the body. [22] This is to be expected given the large number of rapidly dividing cells, especially those of the mucosa and the gut-associated lymphoid tissue and its high secretory activity. To favor this high rate of intestinal protein synthesis, the villous enterocytes are unique among body cells and are presented with substantial quantities of substrates from both the diet and the mesenteric circulation. Research on nutrient metabolism in the small intestine is limited in specific regarding the site and importance of absorption of substrates. Already in the early seventies, Alpers reported that intestinal crypt cells use arterial amino acids for intestinal metabolism, and villus cells use luminal amino acids for protein synthesis. [23] Do these results suggest that arterial and luminal nutrients are metabolized differently?

As described in part one of this thesis, we were able to distinguish between luminal and arterial substrate absorption by the mucosal cells under various feeding circumstances with our arterial-portal catheterized piglet model in combination with the use of stable isotopically labeled substrates and measurements of portal tracee and tracer uptake. We showed a substantial first-pass uptake of dietary amino acids (more than 50%) by the intestine in both species. We wondered whether these rates of amino acid utilization represented the minimum level of nutrients that must be provided enterally to maintain normal intestinal metabolism.

The assignment of the small intestine is to efficiently absorb as much as of the dietary nutrient input as possible, in particular during a limited nutrient supply. Our study design included a normal dietary protein intake and a restricted dietary protein intake (100% vs. 40% dietary protein). At a level of 40% dietary protein intake, the net intestinal protein utilization was substantial, resulting in a diminished systemic availability of amino acids initially. These results are consistent with previous findings in neonatal piglets under conditions of various levels of enteral nutrition. [14] Furthermore, Stoll et al. found

regional differences in the intestinal response to the input of luminal nutrients, and speculated that the distal small intestine is less dependent on enteral intake for maintenance of protein balance. It is conceivable that the distal part of the intestine is more reliable on the arterial supply of amino acids. However, from a nutritional perspective, the current results from our studies in both preterm neonates and piglets support the idea that enteral nutrition is required for the maintenance of intestinal protein balance, and the gut fulfills its own amino acid needs first.

In specific, a notable observation was the disproportionate high utilization of threonine by the intestinal tissues in both preterm neonates and piglets (Chapter 2 and 7). During protein restriction, the equivalent of virtually the total dietary threonine intake was extracted by the mucosal cells. Obviously, the intestine has a very high obligatory need of dietary threonine, presumably to support the substantial (glyco-)protein synthesis. Mucin is a (glyco-)protein with a high molecular mass and regions rich in threonine, and it is the main constituent of the gastrointestinal mucus which protects against bacteria, self-digestion and toxins. Considering the relatively large mass of the small intestine and its high secretory and proliferatory activity, the mucus layer is in a permanent state of renewal. It probably represents one of the most significant contributions to the flow of endogenous protein out of the small intestine. [24] Intestinal reabsorption of endogenous protein was estimated to be equivalent to at least 86% of the jejunum flow, [25] demonstrating the importance of mucins as a source of endogenous nitrogen. In line with our observed high intestinal threonine utilization irrespective of the dietary protein intake (Chapter 2 and 7), Fan et al. showed a strong relationship in pigs between protein intake and the apparent ileal digestibility of amino acids. [26,27] The strength of this relationship suggests that variations in the level of protein intake have little impact on the amino acid compositions of the endogenously secreted (glyco-)proteins. [28] Therefore, the intestinal utilization of amino acids for protein synthesis does not necessarily involve a net loss from the body, as the amino

acids subsequently released from protein breakdown are available for reutilization. Moreover, we showed that intestinal recycling of amino acids made a significant contribution to the systemic availability of amino acids. Over a 24-h period including a fed and a fasted state, 26% of the dietary protein intake reappeared in the portal vein by way of recycling of previously secreted (glyco-)proteins (Chapter 3).

On the other hand, the utilization of amino acids by the gut tissue for purposes other than protein synthesis (i.e. oxidation) has important effects on whole-body amino acid economy (Chapter 4). In the neonatal pigs, we found that substrate metabolism, including catabolism, is compartmentalized in the intestinal tissues, in accordance with previous reports of intestinal channeling of dietary and arterial amino acids. [29,30] Using simultaneous intragastric and intravenous threonine tracers, we measured an equal utilization by the PDV of both dietary and systemic threonine (Chapter 2). Although both tracers were utilized by the intestinal tissues, the amount of dietary threonine being used for oxidative purposes was higher than threonine from the arterial site during protein restriction. In other words during a limited enteral protein supply, there is substantial greater chance of a threonine molecule that enters the enterocytes via the apical membrane being oxidized than one that enters the cell from the arterial site. Nevertheless, only 10% of the total threonine utilization by the PDV was used for oxidative processes under both feeding conditions, implying not a major metabolic fate. Furthermore, it is important to note that when account is taken of the relative molar uptake of amino acids from the diet and the mesenteric artery, dietary threonine becomes even more important for the intestinal tissues for synthetic purposes.

Other potential fuels for the PDV are glucose and amino acids in general, but the relative importance of these substrates and the changes that occur with lowering the level of protein are poorly established in vivo until recently. Furthermore, the relative importance of these fuels may differ among species. Chapter 3 deals with the substrates for intestinal energy generation in neonatal pigs. We found

in normally-fed pigs that arterial glucose and enteral glutamate were the most important sources of energy for the intestinal tissues, with leucine and threonine being less important energy substrates. In fully-fed human neonates, we could not demonstrate any lysine or threonine oxidation by the intestine (Chapter 6 and 7). Glucose, similar as in pigs, was a major important intestinal fuel source (Chapter 8). The results in preterm infants show that essential amino acids, i.e. lysine and threonine, are utilized by the intestine for other metabolic fates, implying nutritional efficacy early in postnatal life.

In both species, we investigated the effect of lowering the level of dietary protein on intestinal substrate oxidation. In neonatal pigs fed a low protein diet, the intestinal tissues maintain a high rate of energy expenditure by increasing the oxidation rate of dietary glucose, and reducing the amino acid oxidation (Chapter 4). Presumably, fatty acids and ketone bodies become more important for intestinal energy generation in order to favor the availability of dietary amino acids for whole-body growth. Results from our studies in human preterm infants showed that dietary essential amino acids are not used for oxidative purposes when dietary protein is limiting, like in normal feeding circumstances, whereas dietary glucose is a major important fuel source during both a high and a low protein intake. These results are consistent with the idea that dietary glucose acts as a fuel that is used up first by the intestine, whereas dietary amino acids are used for other metabolic pathways in preterm infants.

As shown by these results, there is a substantial variation in intestinal metabolism among amino acids and glucose, reflecting the fact that whereas some are catabolized within the intestinal tissues, others are synthesized. From other studies we know that the dietary leucine oxidation does not reflect the oxidation of all the amino acids, as amino acids exhibit different regulatory levels with regard to oxidative pathways. In healthy adults receiving increasing levels of dietary protein, leucine oxidation was significantly decreased, whereas lysine catabolism remained constant. [31] In other words, essential amino acids respond differently to identical metabolic situations. And

therefore, estimates using single amino acid kinetics may not adequately represent the dynamic aspects of whole-body protein metabolism.

Methodological critical points

Amino acid kinetics studies during feeding raise several methodological issues. The amino acid composition, protein quantity and protein digestion rate of dietary proteins are major factors affecting whole-body protein accretion. The speed of absorption of dietary amino acids depends on gastric and intestinal motility, luminal digestion and on mucosal absorption. Moreover, nitrogen can be ingested as either whole-body proteins, free amino acids, or peptides of various chain lengths, the two latter forms being used in enteral nutrition when digestive capacities are compromised. Given the different transporters for free amino acids and peptides in the intestinal mucosa, different absorption times are reported for these three substrates. [32] Dipeptides can be absorbed directly by the enterocyte faster than a free amino acid mixture. However, the absorption of a peptide based diet closely depends on the peptide profile (e.g. chain length). Whether or not a faster absorption renders into a better nitrogen utilization is controversial. [33,34] Boirie et al. proposed the concept of "slow and fast proteins". [35] They found that the plasma appearance of dietary amino acids is high, fast and transient after whey protein (WP) ingestion. In contrast, they found that the plasma appearance of dietary amino acids after casein protein (CAS) ingestion is lower and slower. However, the significance of these findings for the interpretation of the intestinal and whole-body requirements of dietary proteins in neonates, is unclear, as a single meal of protein alone was given. Nenatal®, the standard milk feeding of preterm neonates besides human milk, consists of both WP and CAS protein, carbohydrates and fat. Therefore, we speculate that the concept of slow and fast absorption is blunted during more complex meals as Nenatal®. Moreover, in the studies presented in this thesis, measurements regarding amino acids kinetics are done 4-5h after initiation of continuous feeding, once isotopic and substrate steady

state is achieved. Under these conditions, any difference related to the speed of dietary amino acid absorption is faded away.

Another confounding factor in studying amino acid kinetics, might be the different postprandial metabolic fate of labeled tracers when they are ingested as a component of mixed meals (intrinsically labeled) or together with a mixed meal (extrinsically labeled). In our studies we used extrinsically labeled tracers. However, a potential error at this level was not found by Metges et al. In steady state conditions, free [^{13}C]leucine added to protein induced identical enrichments and leucine fluxes to those observed with an intrinsically [^{13}C]leucine-labeled protein. [36] Therefore, it is likely that free tracers added to Nenatal®/Litterlife® solutions does represent the digestion rate of the meal, at least during the steady state period.

Finally, recycling of an amino acid tracer can influence amino acid kinetics. A labeled amino acid can be incorporated into and subsequently be released from protein over the course of an isotope infusion, called recycling, and can result in significant errors in kinetic parameters. The occurrence of recycling of an amino acid label has been suggested on the basis of studies employing isotope infusions for up to 18 h in humans. [37] Studies carried out in a limited number of subjects demonstrated delayed decline of the isotope from the plasma after 8h of isotope infusion compared with 4h of isotope infusion. [38] We performed 5h tracer infusions, and our experimental design did not permit us to determine when significant recycling occurred. Therefore, we presume that the re-entry of the label was very small.

Future perspectives

There has been limited research regarding the nutritional requirements of ill and physiologically unstable preterm infants. The rate of administration of protein to septic neonates is currently based on nutrition studies performed in healthy term and preterm infants. [39,40] The available literature suggests that septic neonates have increased muscle proteolysis, decreased protein synthesis, and a negative nitrogen balance during illness. Therefore, the impact of a catabolic

illness in the preterm neonate might be devastating, because growth will be discontinued, leaving the infant with a second "disease": lost growth.

Alterations in whole-body protein turnover in septic infants may be accompanied by more subtle changes in the metabolism of individual groups of amino acids. Healthy neonates have immature pathways of synthesis of some amino acids. Sepsis, with increased protein demands, and increased demands of specific amino acids, might increase these physiological immaturities. For example, there are alterations in phenylalanine metabolism in septic infants, and cysteine, tyrosine and other amino acids may become conditionally essential. [41-43] Furthermore, Becker et al. showed that infants with NEC have certain plasma amino acids deficiencies, i.e. arginine and glutamine. [44] It is likely that these deficiencies are a result of consumption of these amino acids at critical locations during intestinal inflammation.

Another nutritional important amino acid is arginine, which has been shown to be essential in piglets. [45] The importance of arginine to the neonatal intestine is related to its role as nitrogen donor for nitric oxide synthesis, and might be critical for maintenance of the immune function. [46-48] Nitric oxide plays an important role in regulating intestinal blood flow, integrity, secretion and epithelial cell migration. [49] Given the extensive gut atrophy observed in parenterally fed piglets, arginine synthesis may therefore be inadequate to meet whole-body requirements. [50] In human neonates, limited information is known regarding the inter-organ arginine metabolism and the arginine requirements. In addition, because arginine deficiency, which cause life-threatening hyperammonemia, occurs in preterm infants, [51] it is important to elucidate the mechanisms responsible for arginine synthesis during fetal and postnatal development. A better understanding of the arginine requirement by the gut tissues in preterm infants may allow neonatologists to provide adequate arginine for growth of all body tissues. Therefore, this interesting area of amino acid metabolism merits further investigation.

No studies have investigated substrate metabolism in critically ill neonates, and no studies in infants have addressed the possibly beneficial impact of enteral nutrition on whole-body protein turnover during sepsis. We found high intestinal amino acid utilization rates in healthy VLBW infants, even during partial enteral feeding. It is conceivable that preterm infants with a compromised gut function, or septic preterm infants, have a substantial higher intestinal need for certain amino acids. In specific, threonine could play an important role in the maintenance of the gut integrity. By using the dual stable isotope approach in ill preterm infants, the intestinal needs of particularly important amino acids, e.g. threonine, arginine, and glutamate, can be determined. Knowing the requirements, can help in novel nutritional interventions in the ill preterm neonate. Supplying the intestine with enough energy substrates and amino acids for intestinal protein synthesis, will benefit the ill preterm infant by reducing a major complication; lost growth. Thus, future studies are merited to evaluate the need for specific amino acids in septic neonates and neonates with a compromised gut function.

Concluding remarks

Today, we have the opportunity to use stable isotopically labeled amino acid tracers to accurately determine amino acid kinetics at the level of individual and whole-body tissues. Moreover, the studies in this presented thesis show, once again, the applicability of the neonatal piglet model to the human neonate. These data are useful in increasing the precision of the diet formulation and growth modeling in neonates. The tracer method enables measurements of substrate requirements in preterm infants during periods of rapid change, such as postnatal growth. Our current knowledge of intestinal amino acid metabolism helps to explain why parenteral nutrition decreases protein synthesis in the intestinal mucosa, and further supports the concept that dietary amino acids are obligatory for maintaining intestinal mucosal mass and integrity. One theme that has emerged from this thesis is that intestinal amino acid metabolism plays an major role in modulating the

entry of absorbed dietary amino acids into the systemic circulation. Thus, the pattern of amino acids in the diet differs considerably from that in the portal circulation and does not reflect their availability to the peripheral tissues. This concept has important implications for protein and amino acid nutrition. First, the intestinal tissues can be seen as a amino acid pool, in which recycling plays an important role to favor the whole-body amino acid requirements. Second, catabolism of dietary essential amino acids in first-pass by the splanchnic tissues results in a decreased nutritional efficiency. Third, because of developmental changes and disease associated alterations in intestinal amino acid metabolism, these factors should be taken into consideration in recommending dietary amino acid requirements.

References

1. Pauls J, Bauer K, Versmold H. Postnatal body weight curves for infants below 1000 g birth weight receiving early enteral and parenteral nutrition. Eur. J. Pediatr. 1998;157:416-421.
2. Fall C, Hindmarsh P, Dennison E, Kellingray S, Barker D, Cooper C. Programming of growth hormone secretion and bone mineral density in elderly men: a hypothesis. J. Clin. Endocrinol. Metab. 1998;83:135-139.
3. Lucas A. Programming by early nutrition: an experimental approach. J. Nutr. 1998;128:401S-406S.
4. Waterland RA, Garza C. Potential mechanisms of metabolic imprinting that lead to chronic disease. Am. J. Clin. Nutr. 1999;69:179-197.
5. Dobbing JJ. Vulnerable periods in the developing brain. In: Brain, behaviour and iron in the infant diet, Dobbing JJ (ed) London, Springer-Verlag 1989:1-26.
6. Lucas A. Influence of neonatal nutrition on long term outcome. In: Salle BL, Swyer PR (ed) Nutrition of the low birthweight infant, Nestle Workshop series, New York, Vevey/Raven Press 1993;32:183-196.
7. McClure RJ, Chatrath MK, Newell SJ. Changing trends in feeding policies for ventilated preterm infants. Acta. Paediatr. 1996;85:1123-1125.
8. Weaver LT, Austin S, Cole TJ. Small intestinal length: a factor essential for gut adaptation. Gut. 1991;32:1321-1323.
9. Lucas A, Cole TJ. Breats milk and neonatal necrotizing enterocolitis. Lancet 1990;336:1519-1523.
10. Scherjon SA, Smolders-DeHaas H, Kok JH, Zondervan HA. The "brain-sparing" effect: antenatal cerebral Doppler findings in relation to neurologic outcome in very preterm infants. Am. J. Obstet. Gynecol. 1993;169:169-175.
11. Wladimiroff JW, Noordam MJ, van den Wijngaard JA, Hop WC. Fetal internal carotid and umbilical artery blood flow velocity waveforms as a measure of fetal well-being in intrauterine growth retardation. Pediatr. Res. 1988;24:609-612.
12. Goldstein RM, Hebiguchi GD, Luk F, Taqi TR, Guilarte FA, Franklin PW Jr, Niemiec DL, Dudgeon DL. The effects of total parenteral nutrition on gastrointestinal growth and development. J. Pediatr. Surg. 1985;20:785-791.
13. Morgan W III, Yardley J, Luk G, Niemiec P, Dudgeon D. Total parenteral nutrition and intestinal development: a neonatal model. J. Pediatr. Surg. 1987;22:541-545.

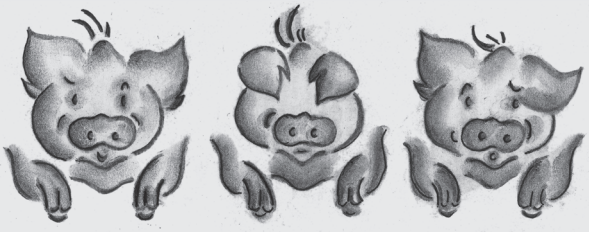
14. Stoll B, Chang X, Fan MZ, Reeds PJ, Burrin DG. Enteral nutrient intake level determines intestinal protein synthesis and accretion rates in neonatal pigs. Am. J. Physiol. 2000;279:G288-G294.
15. Berseth CL, Nordyke C. Enteral nutrients promote postnatal maturation of intestinal motor activity in preterm infants. Am. J. Physiol. 1993;264:G1046-G1051.
16. Berseth CL. Gastrointestinal motility in the neonate. Clin. Perinatol. 1996;23:179-190.
17. Al Tawil Y, Berseth CL. Gestational and postnatal maturation of duodenal motor responses to intragastric feeding. J. Pediatr. 1996;129:374-381.
18. La Gamma EF, Browne LE. Feeding practices for infants weighing less than 1500 G at birth and the pathogenesis of necrotizing enterocolitis. Clin. Perinatol. 1994;21:271-306.
19. Burrin DG, Ferrell CL, Britton RA, Bauer M. Level of nutrition and visceral organ size and metabolic activity in sheep. Br. J. Nutr. 1990;64:439-448.
20. Johnson DE, Johnson KA, Baldwin RL. Changes in liver and gastrointestinal tract energy demands in response to physiological workload in ruminants. J. Nutr. 1990;120:649-655.
21. Reynolds CK, Tyrrell HF, Reynolds PJ. Effects of diet forage-to-concentrate ratio and intake on energy metabolism in growing beef heifers: whole body energy and nitrogen balance and visceral heat production. J. Nutr. 1991;121:994-1003.
22. Waterlow JC, Garlick PJ, Millward DJ. 1978. Protein turnover in mammalian tissues and in the whole-body. Amsterdam: Elsevier/North-Holland Biomed.
23. Alpers DH. Uptake and fate of absorbed amino acids and peptides in the mammalian intestine. Fed. Proc. 1986;45:2261-2267.
24. Fuller MF, Cadenhead A. Effect of the amount and composition of the diet on galactosamine flow from the small intestine. In: Proc Vth Symposium on Digestive Physiology in Pigs, Verstegen MWA, Huisman J, Den Hartog LA (eds) EAAP Publication 54, Wageningen, The Netherlands 1991;330-333.
25. Montagne L, Toullec R, Lalles JP. Intestinal digestion of dietary and endogenous proteins along the small intestine of calves fed soybean or potato. J. Anim. Sci. 2001;79:2719-30.
26. Fan MZ, Sauer WC, Hardin RT, Lien KA. Determination of apparent ileal amino acid digestibility in pigs: effect of dietary amino acid level. J. Anim. Sci. 1994;72:2851-2859.
27. Fan MZ, Sauer WC. Determination of true ileal amino acid digestibility in feedstuffs for pigs with linear relationships between ileal outputs and dietary inputs of amino acids. J. Sci. Food. Agric. 1997;73:189-199.

28. Montagne L, Toullec R, Formal M, Lalles JP. Influence of dietary protein level and origin on the flow of mucin along the small intestine of the preruminant calf. J. Dairy. Sci. 2000;83:2820-2828.
29. Stoll B, Henry J, Reeds PJ, Yu H, Jahoor F, Burrin DG. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. J. Nutr. 1998;128:606-614.
30. Van Goudoever JB, Stoll B, Henry JF, Burrin DG, Reeds PJ. Adaptive regulation of intestinal lysine metabolism. Proc. Nat. Acad. Sci. 2000;97:11620-11625.
31. Zello GA, Telch J, Clarck J, Ball RO, Pencharz BP. Reexamination of protein requirements in adult male humans by end product measurements of leucine and lysine. J. Nutr. 1992;122:1000-1008.
32. Matthews DE, Adibi SA. Peptide absorption. Gastroenterology. 1976;71:151-161.
33. Moriarty KJ, Hegarty JE, Fairclough PD, Kelly MJ, Clark ML, Dawson AM. Relative nutritional value of whole-body protein, hydrolysed protein and free amino acids in man. Gut. 1985;26:694-699.
34. Silk DBA, Grimble G. Relevance of physiology of nutrition absorption to formulation of enteral diets. Nutrition. 1992;8:1-12.
35. Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL, Beaufrère B. Slow and fast dietary proteins differently modulate postprandial protein accretion. Proc. Nat. Acad. Sci. 1997;94:14930-14935.
36. Metges CC, El-Khoury AE, Selvaraj AB, Tsay RH, Atkinson A, Regan MM, Bequette BJ, Young VJ. Kinetics of L-[1-^{13}C]leucine when ingested with free amino acids, unlabeled or intrinsically labeled casein. Am. J. Physiol. 2000;278:E1000-E1009.
37. Tsalikian E, Howard C, Gerich JE, Haymond MW. Increase leucine flux in short-term fasted human subjects: evidence for increased proteolysis. Am. J. Phyiol. 1984;10:E323-E327.
38. Abumrad NN, McRae JR, Lacy WW. Inadequacy of leucine isotopic methods for measurements of whole-body protein turnover in vivo. Clin. Res. 1984;32:387A.
39. Motil KJ. Protein needs for term and preterm infants. In: Tsang RC, Nichols BL (ed) Nutrition during infancy St Louis CV Mosby 1988:100-121.
40. Waterlow JC. In: Tsang RC, Nichols BL (ed) Nutrition during infancy St Louis CV Mosby 1988:1-19.
41. Castillo L, Yu YM, Marchini JS, Chapman TE, Sanchez M, Young VR, Burke JF. Phenylalanine and tyrosine kinetics in critically ill children with sepsis. Pediatr. Res. 1994;35:580-588.

42. Kilani RA. Phenylalanine hydroxylase activity in preterm infants: is tyrosine a conditionally essential amino acid? Am. J. Clin. Nutr. 1995;61:1218-1223.
43. Heird WC, Denne SC. Maximizing protein nutrition, limits of protein utilization, and interaction between energy intake and protein utilization. Seminars in Neonatal Nutrition and Metabolism. 1998;5:3-4.
44. Becker RM, Wu G, Galanko JA, Chen W, Maynor AR, Bose CL, Rhoads JM. Reduced serum amino acid concentrations in infants with necrotizing enterocolitis. J. Pediatr. 2000;137:785-793.
45. Ball RO, Atkinson JL, Bayley HS. Proline as an essential amino acid for the young pig. Br. J. Nutr. 1986;55:659-668.
46. Blachier F, Selamnia M, Robert V, M'Rabet-Touil H, Duee PH. Metabolism of L-arginine through polyamine and nitric oxide synthase pathways in proliferative or differentiated human colon carcinoma cells. Biochim. Biophys. Acta. 1995;1268:255-262.
47. Wu G, Morris SM Jr. Arginine metabolism: nitric oxide and beyond. Biochem. J. 1998;336:1-17.
48. Wallace JL, Miller MJ. Nitric oxide in mucosal defense: a little goes a long way. Gastroenterology. 2000;119:512-520.
49. Alican I, Kubes P. A critical role for nitric oxide in intestinal barrier function and dysfunction. Am. J. Physiol. 1996;270:G225-37.
50. Bertolo RF, Chen CZ, Pencharz PB, Ball RO. Intestinal atrophy has a greater impact on nitrogen metabolism than liver by-pass in piglets fed identical diets via gastric, central venous or portal venous routes. J. Nutr. 1999;129:1045-1052.
51. Batshaw ML, Wachtel RC, Thomas GH, Starrett A, Brusilow SW. Arginine-responsive asymptomatic hyperammonemia in the premature infant. J. Pediatr. 1984;105:86-91.
52. Newell SJ. Gastrointestinal function and its ontogeny: How should we feed the preterm infant? In: Ryan S (ed) Seminars in Neonatology, London, WB Saunders Compan Ltd 1996:59-66.

Chapter 10

SUMMARY (SAMENVATTING)



Between 26 and 36 weeks of gestation the weight gain of the fetus is higher than at any other period thereafter in life. This in utero phenomenon is potentially lost to the infant born preterm. Adequate nutrition should be provided to obtain similar growth rates as in utero, with protein being an important factor for whole-body growth in neonates. Given the key role of the gut in digesting and absorbing nutrients and its effect on whole-body growth, there has been considerable interest in the first-pass utilization and metabolism of amino acids by the intestine. This thesis describes the splanchnic amino acid metabolism in neonates in their first weeks of life. In the first part of this thesis, the intestinal amino acid metabolism is studied in neonatal piglets under different feeding regimens, including a fasting state. In the second part of this thesis, we investigated the first-pass and whole-body amino acid kinetics in preterm infants during partial enteral and full enteral feeding.

In **Chapter 1** we describe the background and aims of the studies presented in this thesis. We provide an introduction on the use of stable isotopes to assess splanchnic and whole-body protein kinetics in clinical studies. The available literature on protein metabolism in neonates is reviewed.

In **Chapter 2** we show the first-pass uptake and metabolism of threonine during a high and a low protein intake in neonatal piglets. Firstly, it appears that protein restriction in the diet has a substantial negative effect on total body weight. First-pass threonine utilization by the intestine is not significantly affected by a lower protein intake, but the dietary utilization is much lower. Notably, the net utilization of threonine by the PDV during low protein feeding is not different from the intake. Almost 98% of the total threonine utilization are derived from the diet, which is in contrast to the almost equimolar amounts of utilized systemic and dietary threonine during high protein feeding. Regarding the metabolic fate of the utilized threonine, the PDV oxidized both dietary and systemic threonine in almost equimolar amounts during both feeding intakes. However, threonine catabolism was not a major

metabolic fate, the threonine oxidation by the PDV represented approximately 10% of the total threonine utilization by the PDV.

In **Chapter 3** we present a study in neonatal piglets during a 12-h fed and a 12-h fasted state. The recycling of (glyco-)proteins by the intestine is measured using different stable isotopically labeled lysine and threonine tracers and by quantifying the portal mass balances of all amino acids and glucose during a 24-h period. During a prolonged continuous feeding period (>6h), the gut utilizes a disproportionately large amount of the dietary protein intake, more than 50%. Moreover, during a 24-h period including a fed and a fasted state, 26% of the dietary protein intake reappears in the portal vein by way of recycling of previously secreted (glyco-)proteins released luminally by the intestine. In case of the essential amino acid lysine, after 6 h of feeding even 46% of lysine appearance in the portal vein was derived from secreted protein recycling. With the recycling we mean the following process: the majority of the utilized amino acids are incorporated into (glyco-)proteins that are secreted into the intestinal lumen. After secretion into the lumen, the synthesized proteins are either digested and reabsorbed, or they are passed to the colon, where they are degraded, fermented and lost from the body. When the previously secreted amino acids are digested and absorbed, they will finally appear in the portal circulation to become available for whole-body growth.

Previous animal studies indicate that amino acids and glucose are the major oxidative substrates for intestinal energy generation. In **Chapter 4**, the intestinal substrate oxidation is quantified in neonatal piglets under high protein (0.9 g/(kg·h)) and low protein (0.4 g/(kg·h)) feeding conditions. During a high protein diet arterial glucose and enteral glutamate were the most important sources of energy (glucose iv 39% and glutamate id 32%) for the intestine. Visceral amino acid oxidation was substantially suppressed during a low protein intake, whereas glucose oxidation increased to 50% of the total visceral CO_2 production. However, the oxidation of glucose and amino acids does not provide enough substrate for the energy requirements of the portal-drained viscera (PDV) during protein restriction. Suggesting that, the gut has to

use other substrates like fatty acids or ketone bodies to maintain the high mucosal metabolism.

After the results obtained in neonatal piglets, we performed similar stable isotope studies in preterm infants to quantify the first-pass and whole-body kinetics of amino acids and to confirm the applicability of the neonatal piglet model, as presented in the second part of this thesis.

In clinical studies, the oxidation of [^{13}C]labeled substrates to $^{13}CO_2$ and the measurement of the appearance $^{13}CO_2$ in expired air has progressed to an increasingly common method to study substrate oxidation. The collection of respiratory CO_2 currently used, occurs via trapping of CO_2 in sodium hydroxide (trapping method) sometimes in conjunction with indirect calorimetry. To collect breath samples from preterm infants in a suitable manner, we applied the direct naso-pharyngeal sampling method. This technique collects expiratory CO_2 directly from the patient with a gastric tube placed for 1 to 1.5 cm in the nasopharynx. In **Chapter 5**, we validate our direct sampling method for the collection of breath samples in preterm infants with the trapping method. Isotopic breath enrichments obtained by the direct sampling technique are precisely related to isotopic breath enrichments garnered by the dynamic trapping method. Our results therefore imply that direct sampling method for the collection of expired air is a suitable method for the use in substrate oxidation studies in preterm infants.

In **Chapter 6** we describe the relationship between the level of enteral intake and the splanchnic and whole-body lysine kinetics in preterm infants. The infants were studied on average postnatal day 7 and 8 with 40% enteral and 60% parenteral feeding and on average postnatal day 21 and 22 with full enteral feeding. The effects on first-pass intestinal lysine uptake and metabolism are measured using dual stable isotope lysine tracer techniques. Approximately one-third of the dietary lysine is utilized in first-pass by the intestine during partial enteral feeding, compared to a lower fraction (18%) during full enteral feeding. None of this utilized lysine is oxidized in first-pass, neither during partial enteral feeding nor during full enteral feeding. Furthermore, the administration of full enteral feeding in preterm infants

results in a lower whole-body lysine oxidation and higher lysine retention than a low enteral nutrient supply.

In **Chapter 7**, the first-pass threonine uptake by the intestine in preterm infants at different postnatal ages is investigated. We quantified threonine kinetics during a low enteral and a high enteral intake. The most striking observation to appear from this study is that the fractional first-pass threonine uptake by the intestine is very high in the first week of life in preterm infants during a restricted enteral intake. More then three-quarter of the enteral intake is utilized, which indicates a high obligatory visceral need for threonine in neonates, even in early life. As we did not find any first-pass threonine oxidation, we suggest that the major metabolic fate of intestinal utilized threonine is mucosal (glyco-)protein synthesis. In addition, full enteral feeding does not affect the whole-body oxidation of threonine.

Finally, in line with our results obtained in neonatal piglets on intestinal substrate oxidation, **Chapter 8** describes the role of dietary glucose as an oxidative substrate for the intestine and the whole-body in preterm infants in their first weeks of life. Furthermore, the first-pass glucose uptake during both a low and high enteral intake is also quantified. Approximately one-third of the dietary glucose intake was utilized in first-pass by the intestine, irrespective of the dietary intake. In absolute amounts, the first-pass glucose uptake was significantly higher during full enteral feeding, due to a significant higher enteral intake. More than three-quarters of this utilized glucose was oxidized in both periods. In other words, dietary glucose is major important fuel source for the generation of intestinal energy in the first weeks of life in preterm neonates.

In this thesis, it is shown that approximately 50% of the dietary amino acid intake are utilized by the portal-drained viscera (PDV) during a 24-h period including 12-h of feeding and 12-h of fasting. To sustain the high rate of protein turnover and energy expenditure, amino acids more than glucose, are major substrates for intestinal energy generation in neonatal piglets. However, during protein restriction glucose becomes

a more important fuel source, probably together with fatty acids and ketone bodies. To favor whole-body growth, intestinal recycling of amino acids contributes significantly to their systemic availability and may be a critical factor in the amino acid nutrition.

Comparable with neonatal pigs, the first-pass utilization of amino acids by the intestine is substantial in preterm infants, even in their first week of life during a low enteral intake. For threonine, the first-pass uptake by the gut is as much as three-quarter of the enteral intake during partial enteral feeding, implying its particularly importance for the neonatal gut growth and function. In contrast to piglets, lysine and threonine are not used as a fuel source by the mucosal cells for their high intestinal metabolism. Glucose, on the other hand, is a major important oxidative substrate for the neonatal gut during both a low and a high enteral intake.

Furthermore, it is shown that full enteral feeding lowers whole-body lysine oxidation and thereby improves whole-body lysine balance in comparison with partial enteral and parenteral feeding. Regarding threonine, the route of feeding does not effect whole-body threonine retention.

The collection of expired air currently used for the measurement of excess ^{13}C after an infusion of [^{13}C]labeled substrates, is not well suited for use in a clinical situation. We have developed an alternative method for the collection of expired air enabling the calculation of substrate oxidation in preterm infants. This direct naso-pharyngeal sampling technique for the collection of breath samples, is a fast, non-invasive, suitable method in preterm infants for determination of substrate oxidation in combination with a [^{13}C]bicarbonate infusion without the use of a correction factor.

Overall, the results demonstrate the applicability of the results obtained in our animal model/neonatal piglets to human neonates. Furthermore, this thesis shows that specific amino acid requirement of the intestine is very high and that this requirement has a major impact on whole-body growth in the very vulnerable patient group of neonates.

The intestine acts selfishly during dietary protein restriction, resulting in a diminished systemic availability of dietary proteins. Presumably, to preserve a healthy and normal gastrointestinal function, the intestine has a high metabolic cost.

Vergeleken met de intra-uteriene gewichtstoename van de foetus tussen de 26ste en 36ste zwangerschapsweek is de groei bij prematuren vaak lager.
Dit "in utero" effect vindt namelijk niet plaats bij neonaten na een premature geboorte. Juiste en gebalanceerde voeding is nodig om een vergelijkbare groeisnelheid als in utero te verkrijgen. Een belangrijke factor voor groei bij neonaten is de opslag van eiwit. Gezien de essentiële rol van de darm bij de vertering en opname van voedingsstoffen en mede daardoor zijn invloed op de lichaamsgroei, is het niet verwonderlijk dat er veel interesse bestaat in het darmmetabolisme van aminozuren. Het eerste deel van dit proefschrift bestaat uit een serie studies op het gebied van intestinaal aminozuur metabolisme in neonatale biggen ten tijde van verschillende voedingsschema's. Het tweede deel beschrijft een aantal studies in prematuren met betrekking tot het aminozuurmetabolisme in first-pass door de darm en in het totale lichaam gedurende een lage en een hoge enterale inname.

In **Hoofdstuk 1** worden de achtergronden en doelstellingen beschreven van de studies, die in dit proefschrift gepresenteerd worden. Tevens wordt een inleiding gegeven over het gebruik van stabiele isotopen voor de bestudering van het eiwitmetabolisme.

In **Hoofdstuk 2** wordt een studie beschreven betreffende het threonine metabolisme in de darm bij neonatale biggen onder omstandigheden van een normale en beperkte eiwitinname. Allereerst blijkt dat het verlagen van de eiwitinname een duidelijk negatief effect heeft op de gewichtstoename. Een lage enterale eiwit inname heeft geen effect op het first-pass threonine verbruik, al het enteraal aangeleverde threonine wordt verbruikt door de PDV. Dit betekent dat er geen threonine systemisch beschikbaar komt, zodat, gezien de essentialiteit van threonine, er ook geen netto synthese van lichaamseiwitten kan plaatsvinden en dus ook geen groei. Ondanks het hoge first-pass verbruik van threonine door de darm, is threonine niet de belangrijkste brandstof voor de darm, slechts 10% van de opgenomen threonine wordt verbrand zowel tijdens een hoge als een lage eiwitinname.

In **Hoofdstuk 3** wordt een studie beschreven in neonatale biggen onder specifieke omstandigheden van voeden en vasten. Om de recycling van (glyco-)proteïnen door de darm te kwantificeren gedurende een 24-uurs periode, hebben we gebruik gemaakt van verscheidene stabiel isotoop gelabelde lysine en threonine tracers. Tevens werden ieder uur arteriële en portale bloedmonsters afgenomen om de netto balans van glucose en aminozuren door de portaal gedraineerde viscera (PDV) te berekenen. Met behulp van masspectrometrie werd vervolgens de verrijking van lysine en threonine in het arteriële en portale bloed gemeten. Gedurende de eerste 6 uur van de continue voedingsperiode wordt netto 66% van de voedingseiwitten door de darm verbruikt. In de tweede helft van de voedingsperiode wordt minder verbruikt, hoewel nog steeds een substantieel deel van de inname (48%). Over de gehele voedingsperiode komt uiteindelijk 26% van de enterale eiwitinname systemisch beschikbaar als gevolg van recycling van intestinaal geproduceerde (glyco-)proteïnen. Voor lysine geldt zelfs dat 46% van de inname beschikbaar komt voor het lichaam als gevolg van recycling. Met recycling wordt het volgende proces bedoeld: een deel van de door de darm verbruikte aminozuren wordt gebruikt voor synthese van (glyco-) proteïnen. Deze (glyco-)proteïnen worden in het darmlumen uitgescheiden. Vervolgens worden deze intestinale eiwitten weer verteerd en geabsorbeerd door de darm, zodat dit deel van de verbruikte aminozuren uiteindelijk systemisch beschikbaar komt voor het lichaam.

In **Hoofdstuk 4** worden de resultaten van een studie in neonatale biggen gepresenteerd waarin we onderzochten of aminozuren ook onder omstandigheden van beperkte eiwitinname als de belangrijkste energiebron voor de darm zouden fungeren. De oxidatie van aminozuren (glutamaat en leucine) door de PDV draagt, onder normale voedingscondities, voor tenminste 44% bij aan de energiebehoefte, terwijl de oxidatie van glucose een bijdrage levert van 39%. Door het toedienen van eiwitbeperkte voeding wordt de verdeling in brandstoffen substantieel anders. De bijdrage van aminozuren als verbrandings-substraat daalt tot slechts 10%. Daarentegen wordt glucose een

belangrijkere bron en de glucose oxidatie bedraagt voor 52% bij aan het totale energieverbruik. Deze relatieve toename in oxidatie van glucose kan echter niet het verlies van aminozuren als energiebron compenseren. Waarschijnlijk nemen korte-keten vetzuren of ketonlichamen een deel voor hun rekening.

Na de resultaten verkregen bij neonatale biggen, hebben we vergelijkbare studies gedaan in prematuren om meer inzicht te verkrijgen in het metabolisme van aminozuren en glucose door de darm, en om te kijken of de resultaten verkregen met het biggenmodel toepasbaar zijn op prematuren kinderen. Het tweede deel van dit proefschrift beschrijft zodoende verschillende studies in prematuren met stabiel isotoop gelabelde tracers.

In **Hoofdstuk 5** worden twee methoden vergeleken, die gebruikt worden bij prematuren om de CO_2 productie van het lichaam te schatten. Onze directe methode voor het afnemen van uitademinglucht bij prematuren is niet-tijdrovend en makkelijk uitvoerbaar in vergelijking met de natronloog techniek. De ^{13}C-verrijking in de uitademinglucht, gemeten met beide methoden, komt goed overeen. Het direct opvangen van uitademinglucht in combinatie met de infusie van [^{13}C]bicarbonaat is dan ook een geschikte methode om bij prematuren de verbranding van substraten te meten.

In **Hoofdstuk 6** wordt de relatie beschreven tussen de enterale eiwit inname en het splanchnisch dan wel het totale lichaamsmetabolisme van lysine. De neonaten werden bestudeerd tijdens twee verschillende perioden; periode A: 40% enterale en 60% parenterale voeding en periode B: volledig enterale voeding. Met behulp van verschillende stabiel isotoop gelabelde lysine tracers intraveneus of intragastrisch toegediend, was het mogelijk om de netto absorptie en oxidatie van lysine uit de zuigelingenvoeding te meten. Circa eenderde van de enterale lysine inname wordt verbruikt in first-pass door de neonatale darm, in vergelijking met een lagere fractie (18%) tijdens volledig enterale voeding. Er wordt geen enteraal lysine geoxideerd door de premature darm, in tegenstelling tot de resultaten gevonden bij neonatale biggen. Daarnaast leidt het toedienen van volledig enterale

voeding tot een hogere lysine retentie in het lichaam als gevolg van een lagere oxidatie van lysine in vergelijking met de toediening van een combinatie van parenterale en enterale voeding.

In vervolg op **Hoofdstuk 4** wordt in **Hoofdstuk 7** een studie beschreven bij premature kinderen betreffende het intestinale threonine metabolisme onder omstandigheden van een beperkte en normale enterale eiwitinname. Het relatieve first-pass verbruik van threonine is zeer hoog met de enterale inname, driekwart van de threonine inname wordt verbruikt door de neonatale darm tijdens de eerste levensweek. Met andere woorden, de threonine behoefte van de darm is zeer hoog en onafhankelijk van de threonine inname. Er vindt geen oxidatie van threonine plaats in de darm, suggererend op een andere metabole "pathway" voor threonine, namelijk de synthese van (glyco-)proteïnen. Verder heeft het toedienen van volledig enterale voeding geen significant effect op de lichaamsoxidatie en balans van threonine in vergelijking tot gedeeltelijk parenterale en enterale voeding.

Tot slot, in navolging van het onderzoek bij neonatale biggen beschreven in hoofdstuk 3, werd de rol van glucose als brandstof voor de darm in detail bestudeerd bij prematuren tijdens twee verschillende enterale innamen. **Hoofdstuk 8** beschrijft dit onderzoek, waarin tevens het first-pass verbruik van glucose tijdens gedeeltelijke enteraal/parenteraal en volledig enterale voeding wordt onderzocht. Uit deze studie blijkt dat de darm ongeveer eenderde van de glucose uit de voeding verbruikt, ongeacht de hoeveelheid aangeboden voeding. Het voornaamste doel van dit hoge percentage opgenomen glucose door de darm is oxidatie. Zo'n 80% van de opgenomen glucose wordt gebruikt voor oxidatieve processen in de darm. Met andere woorden, glucose uit de voeding is een belangrijke brandstof voor de premature darm in de eerste levensweken.

Uit dit proefschrift blijkt dat ongeveer de helft van de eiwitinname wordt verbruikt door de portaal gedraineerde viscera (PDV) en in eerste instantie dus niet systemisch beschikbaar komt. Het hergebruik van door de darm geproduceerde (glyco-)proteïnen vormt uiteindelijk een

belangrijke bijdrage aan de totale systemische beschikbaarheid van aminozuren, met name tijdens vasten. Om het hoge eiwitmetabolisme en energieverbruik te handhaven, verbruikt de PDV verschillende voedingsstoffen. Uit onze studies met neonatale biggen onder normale voedingscondities blijkt dat aminozuren, en niet glucose, als de belangrijkste brandstof voor de darm dienen. Daarentegen dienen aminozuren tijdens eiwitbeperking nauwelijks meer als brandstof voor de portaal gedraineerde viscera.

In vergelijking met neonatale biggen vindt er ook een hoog verbruik van aminozuren door de darm plaats bij premature kinderen, zelfs tijdens een lage enterale inname. In het geval van threonine geldt zelfs een first-pass verbruik van meer dan driekwart van de enterale inname, duidend op een enorme behoefte aan threonine voor groei en andere mucosale functies. In tegenstelling tot de oxidatie van aminozuren bij biggen, worden lysine en threonine niet gebruikt als brandstof door de premature darm. Glucose daarentegen, speelt wel een cruciale rol in de energievoorziening van de darm, zowel tijdens een lage als een hoge enterale eiwitinname. Vervolgens blijkt uit dit proefschrift dat parenteraal toegediend lysine niet zo effectief als is enteraal toegediend lysine in het bevorderen van eiwitdepositie.

Verschillende technieken kunnen worden gebruikt bij prematuren om uitademinglucht op te vangen om zodoende de CO_2 productie van het lichaam te schatten. Wij hebben een alternatieve methode gevalideerd, waarbij direct verrijkte uitademinglucht wordt opgevangen en kan worden gemeten met behulp van massa-spectrometrie. Deze techniek is snel en makkelijk uitvoerbaar en kan goed worden gebruikt in oxidatie studies bij kinderen in de toekomst.

Zowel de resultaten in het proefdiermodel als in de prematuren geven aan dat het darmmetabolisme een enorme weerslag heeft op de systemische beschikbaarheid van voeding. Het blijkt dat een gezonde darm in de eerste levensfase meer dan de helft van de aminozuren uit de voeding consumeert. Voor threonine geldt zelfs een zeer hoog verbruik door darm, tot 82% van de enterale inname. Tevens is het duidelijk dat

de darm zich egoïstisch gedraagt wanneer de enterale voedselinname vermindert, zodat er systemisch nog minder beschikbaar komt van de voeding. Blijkbaar kost het instandhouden van een gezonde darmfunctie in de eerste levensfase veel eiwit en glucose.

List of publications

1. Veereman-Wauters G, Ghoos Y, **Van der Schoor SRD**, Maes B, Hebbalkar N, Devlieger H, Eggermont E. The ^{13}C-octanoic acid breath test: a noninvasive technique to assess gastric emptying in preterm infants. J. Pediatr. Gastroenterol. Nutr. 1996;23:111-117.
2. **Van der Schoor SRD**, M Offringa. Magnetische encephalografie (MEC) bij pasgeboren baby's. Kleine Maatjes; nieuwsbulletin vereniging van ouders van couveusekinderen. 1998;3:32.
3. Van den Driessche M, **Van der Schoor SRD**, Ghoos Y, Veereman-Wauters G. Ademtests met stabiel isotopen; toepassingen in de kindergastroenterologie. Tijdschrift voor Kindergeneeskunde. 2000;68:22-27.
4. Van Dijk-Van Aalst K, Van den Driessche M, **Van der Schoor SRD**, Schiffelers S, Van't Westeinde T, Ghoos Y, Veereman-Wauters G. ^{13}C mixed triglyceride breath test: a noninvasive method to assess lipase activity in children. J. Pediatr. Gastroenterol. Nutr. 2001;32:579-585.
5. Van Goudoever JB, **Van der Schoor SRD**, Reeds PJ. De darm; een egoïstisch orgaan. Tijdschrift voor Kindergeneeskunde. 2001;69:193-2001.
6. **Van der Schoor SRD**, Van Goudoever JB, Stoll B, Henry JF, Rosenberger JR, Burrin DG, Reeds PJ. The pattern of substrate oxidation is altered by protein restriction in pigs. Gastroenterology. 2001;121:1167-1175.
7. **Van der Schoor SRD**, Reeds PJ, Stoll B, Henry JF, Rosenberger JR, Burrin DG, Van Goudoever JB. The high metabolic cost of a functional gut. Gastroenterology. 2002;123:1931-1940.
8. **Van der Schoor SRD**, Reeds PJ, Stoll B, Henry JF, Rosenberger JR, Burrin DG, Van Goudoever JB. The portal-drained viscera are of crucial importance to threonine metabolism in piglets. Under review by Am. J. Physiol.
9. **Van der Schoor SRD**, de Koning BAE, Wattimena JDL, D Tibboel, Van Goudoever JB. Validation of the direct naso-pharyngeal sampling

method for collection of expired air after a [^{13}C]bicarbonate infusion in preterm neonates. Accepted for publication by Pediatr. Res.

10. **Van der Schoor SRD**, Reeds PJ, Stellaard F, Wattimena JDL, Sauer PJJ, Büller HA, Van Goudoever JB. Lysine kinetics in preterm infants; The importance of enteral feeding. Accepted for publication by Gut.
11. **Van der Schoor SRD**, Wattimena JDL, Reeds PJ, Büller HA and and Goudoever JB. Threonine metabolism in preterm infants; The gut takes nearly all. Under review by Am. J. Clin. Nutr.
12. **Van der Schoor SRD**, Stoll B, Wattimena JDL, Büller HA, Burrin DG, Van Goudoever JB. Splanchnic bed metabolism of glucose in the preterm neonate. Under review by Am. J. Clin. Nutr.

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