REVIEW

Protein metabolism in preterm infants with particular reference to intrauterine growth restriction

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There is growing evidence that neonatal and long-term morbidity in preterm infants, particularly those born before 32 weeks' gestation, can be modified by attained growth rate in the neonatal period. Guidelines for optimal growth and the nutritional intakes, particular of protein, required to achieve this are not well defined. Due to delays in postnatal feeding and a lack of energy stores developed in the last trimester of pregnancy, preterm infants often suffer early postnatal catabolism until feeding is established. There are indications that infants born with intrauterine growth restriction have perturbations in protein metabolism. Therefore, they may have different protein requirements than appropriate for gestational age infants. This review summarises what is known about protein requirements and metabolism in the fetus and preterm infant, with particular emphasis on the distinct requirements of the growth-restricted infant.

percentile for gestational age. A more appropriate concept describing a pathological cause of low birth weight is intrauterine growth restriction (IUGR), which has been defined as "a pathologic decrease in the rate of fetal growth, [resulting] in a fetus that does not achieve its inherent growth potential".⁶ The difficulty with this term is that it indicates an impairment in the fetal growth trajectory that cannot be defined by birth weight alone.

There is growing evidence that both neonatal and long-term morbidity can be modified by attained growth rate in the neonatal period.⁷ However, guidelines for optimal neonatal growth, and the nutritional intakes required to achieve this are not well defined for preterm infants and are a continuing field of investigation. In particular, much research has focused on protein requirements and metabolism, as protein is essential for the growth of lean body mass. At present, all preterm infants are commonly fed the same high-protein, high-energy diets. However, IUGR caused by intrauterine undernutrition may be associated with altered metabolism in utero.⁸ It is therefore possible that the nutritional needs of IUGR infants are different from those of normally grown preterm infants.

This review outlines what is known about protein requirements and metabolism in the fetus and preterm infant, with particular emphasis on the distinct requirements of the infant with IUGR.

FETAL GROWTH AND METABOLISM

Fetal amino acid supply

The fetus relies on the placenta for provision of nutrients such as glucose and amino acids for its growth and development. Plasma amino acid concentrations have been found to be higher in the fetus than in the mother, indicating active transport across the placenta against a concentration gradient.⁹ To date, approximately 15 different amino acid transport systems have been identified in the human placenta.¹⁰ The placenta does not simply transport amino acids from the maternal to the fetal circulation, rather it is a metabolically active organ. Substantial placental synthesis, interconversion and utilisation of amino acids has been shown in sheep,¹¹ and similar complexity is likely in the human placenta. So it is more appropriate in terms of amino acid economy to consider the feto-placental unit as a whole.

Abbreviations: AGA, appropriate for gestational age; IUGR, intrauterine growth restriction; SGA, small for gestational age

Preterm birth, defined as the delivery of a baby between 20 and 37 weeks' gestation, occurs in 5–11% of all pregnancies in industrialised countries, and it remains a leading cause of perinatal morbidity and mortality.¹ The risk for adverse outcome increases considerably with decreasing gestational age, with the highest risk occurring in infants born at less than 32 weeks' gestation.² The risk of neonatal mortality is further compounded by birth weight; infants with lower birth weights have higher risk of mortality.³ The effects of birth weight continue throughout life: relationships have been found between birth weight and cognitive function during childhood⁴ and the risk for adult-onset diseases, such as hypertension and type 2 diabetes.⁵

Studies assessing the effects of birth weight on clinical outcomes have been difficult to interpret due to inconsistencies in the terminology used. Many studies focus simply on birth weight itself, using the term low birth weight for the population of infants born with a birth weight of less than 2500 g. However, this term does not distinguish between term and preterm infants or give any indication of quality of growth; many low birth-weight infants are born at a birth weight that is appropriate for their gestational age (AGA). Therefore, another often-used term is small for gestational age (SGA), but this signifies an arbitrary statistical cut-off point rather than a physiological condition. SGA is most commonly defined as a birth weight below the tenth

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Plasma amino acid profiles are well described in both AGA and IUGR human fetuses.^{9–12} Concentrations of many fetal amino acids and total fetal α -amino nitrogen are lower in the IUGR fetus than in the AGA fetus during the second and third trimesters.^{9–12} It is mainly the concentrations of essential amino acids that are reduced, in particular the branched-chain amino acids, leucine, valine and isoleucine.^{12–13} Furthermore, the normal pregnancy-related decrease in maternal plasma amino acid concentrations does not occur in IUGR pregnancies, resulting in a decrease in the fetal–maternal amino acid concentration difference for most amino acids.⁹ The lack of change in maternal amino acid profiles is often detectable before the onset of IUGR. It is thought that this indicates impaired uteroplacental adaptation to pregnancy, which may be an early step in the development of the condition.¹⁴

The lower fetal amino acid concentrations in IUGR pregnancies are most probably the result of reduced transplacental transport. Several studies in both humans^{15–16} and sheep^{17–18} have shown lower in vivo placental transport of labelled amino acids in IUGR pregnancies than in AGA pregnancies. Furthermore, there is reduced activity of several placental amino acid transporters^{19–20} in syncytiotrophoblast membrane vesicles of human IUGR placentas.

Fetal protein requirements

At any age, the rate of protein synthesis is several-fold higher than the protein intake, which means that body proteins are constantly being broken down and resynthesised.²¹ This is generally referred to as protein turnover. To increase lean body mass, the rate of protein synthesis must exceed that of protein breakdown, resulting in net protein accretion. Little is known about the protein requirements or metabolism in the human fetus, as these are generally determined using invasive kinetic methods. The currently available standards for intrauterine protein accretion were acquired from the body composition analysis of stillborn preterm infants. These suggest that the third trimester human fetus grows at approximately 15 g/kg a day and has a protein accretion of around 2 g/kg a day, decreasing slightly towards term.²²

Most information about fetal protein requirements and metabolism comes from studies in experimental animals, particularly fetal sheep. However such studies must be interpreted with caution, given the substantial differences between species in critical aspects such as fetal growth rates, placental structure and body composition, all of which may have potentially important implications for feto-placental metabolic requirements. In pregnant sheep, amino acids are taken up by the umbilical circulation in excess of what the fetus requires for protein accretion, with much of the excess used for oxidative metabolism.²³ Furthermore, supplementing the fetus with additional intravenous amino acids can increase fetal protein accretion.^{24–25} This increase is achieved by an increase in protein synthesis, with protein catabolism either marginally suppressed²⁴ or unchanged.²⁵

Only a few studies have investigated protein metabolism in the IUGR fetal sheep. In one study where IUGR was induced by heat-stress, utilisation of uteroplacental leucine was found to be reduced. Most of the reduced transplacental leucine flux was redirected towards fetal metabolism.¹⁷ Placental leucine disposal and rate of oxidation were reduced, suggesting a compensatory mechanism that ensures sufficient substrate availability to maintain normal fetal protein metabolism per kilogram body weight. A more recent study using placental embolisation to induce IUGR also found no differences in protein metabolism between embolised and control fetuses.²⁴ However, in this study the growth restriction was only moderate; growth patterns were altered in the embolised animals but fetal weights at post mortem were not different between groups.

PREVENTION OF EARLY POSTNATAL CATABOLISM

Birth represents a period of major metabolic and endocrine change for the fetus. The umbilical supply of intravenous nutrients ceases and the neonate requires alternative sources of nutrients before anabolism can be re-established. The surge of catabolic hormones associated with parturition causes initial postnatal catabolism and gluconeogenesis, providing the neonate with energy substrates until feeding is established.²⁶ The neonate also relies on lipolysis to meet the additional energy requirements of postnatal life, which now include regular breathing and thermoregulation.²⁶ As most of the deposition of adipose tissue occurs in the final weeks of pregnancy, this poses another difficulty for the preterm infant, who will not have developed the required energy stores.

As immaturity of the gastrointestinal tract commonly delays the onset of full enteral feeding, most preterm and IUGR infants initially receive nutrients by the intravenous route. Infants who receive only intravenous glucose may lose protein stores at a rate of approximately 0.6–1.0 g/kg a day.^{27–29} Early reports suggested that parenteral amino acid supplementation is associated with metabolic acidosis³⁰ and hyperammonaemia³¹ in the preterm infant. This resulted in an understandable caution in the use of intravenous amino acids, so that such neonates were commonly not given total parenteral nutrition containing amino acids until several days after birth, resulting in further catabolism.

More recently, several studies have shown that, using modern amino acid solutions, parenteral amino acid intakes of 1.0–1.5 g/kg a day are enough to prevent the initial protein catabolism in most neonates.^{28–29–32} More “aggressive” parenteral amino acid supplementation strategies, using higher intakes and starting on the first day of life, have resulted in positive nitrogen balance without adverse side effects. Thureen *et al* showed that parenteral amino acid intakes of 2.65 g/kg a day within 24 hours after birth in infants with a birth weight less than 1300 g, resulted in protein accretion rates of about 1.0–1.6 g/kg a day without any signs of toxicity.³³ Furthermore, plasma amino acid concentrations in these infants were comparable with those of normally growing second and third trimester fetuses. Ibrahim *et al* administered parenteral amino acids to preterm infants at 3.5 g/kg a day, starting as early as one hour after birth. This resulted in a positive nitrogen balance without any signs of metabolic acidosis and no significant changes in blood urea nitrogen (BUN).³⁴ te Braake *et al* gave neonates with a birth weight below 1500 g supplemental parenteral amino acids at 2.4 g/kg a day immediately after birth. They did find higher BUN values compared with those in neonates who received gradually increasing parenteral amino acid intakes from day 2 onwards.³⁵ However, the higher BUN values were accompanied by higher urinary nitrogen excretion, suggesting increased amino acid oxidation rather than amino acid toxicity. It can therefore be concluded that, using modern amino acid solutions, parenteral amino acid supplementation can be safely started immediately after birth even in extremely preterm infants.

Some studies on the effects of parenteral amino acid supplementation on protein turnover in preterm infants have indicated that the anabolic effect of amino acids is caused by an increase in protein synthesis rather than decreased protein breakdown.^{27–36–37} Several other studies have shown inhibition of protein breakdown and urea production in preterm infants after an acute (up to 5 h) increase in parenteral amino acid supplementation in the first week of life.^{38–39} When the increased supplementation is prolonged, the rate of protein breakdown returns to baseline and urea production increases.^{38–39} This suggests that the increase in amino acid availability is initially directed towards accretion but is subsequently redirected towards oxidation. However, other studies have not shown acute suppression of protein breakdown after increased amino acid

supplementation.³⁶ Furthermore, protein breakdown can be suppressed in preterm infants by supplementing the parenteral amino acid mixture with glutamine alone.⁴⁰

PROTEIN AND ENERGY REQUIREMENTS FOR GROWTH

It is generally assumed that breast feeding can meet the protein needs of healthy, term infants.^{41–42} Therefore, the average growth rates and protein intakes of breastfed infants are considered to be the “gold standard” for healthy term infants. No such standard is available for the preterm infant, in particular when born before 32 weeks’ gestation. It is generally assumed that AGA preterm infants should achieve a postnatal growth rate equivalent to the growth rate of a fetus of the same gestational age.⁴³

Ehrenkranz *et al* showed that AGA infants born before 29 weeks’ gestation generally reached a body weight of 2000 g four to five weeks later than the normally growing fetus of the same gestational age.⁴⁴ These infants, therefore, need to achieve growth rates equal to those of the fetus, and need “catch-up” growth to recover from their early neonatal growth restriction. Heird⁴⁵ postulated that a 27-week-old fetus of 1000 g would need 35 days to reach a body weight of 2200 g. A preterm infant of the same gestational age and weight at birth who, after the initial postnatal weight loss, regains its birth weight at 2 weeks of age, will have only 21 days to complete catch-up growth to 2200 g. This requires a weight gain of 36 g/kg a day, which could theoretically be achieved at protein and energy intakes of 6.5 g/kg a day.⁴⁵ Protein intakes of this magnitude are near impossible to achieve and could result in protein toxicity.

METABOLIC CHALLENGES OF IUGR INFANTS

IUGR infants usually require additional nutrients for catch-up growth, regardless of the initial postnatal weight loss. However, providing these infants with sufficient protein for catch-up growth may be even more challenging than it is for AGA infants, due to possible metabolic immaturity in the IUGR group.

Definitions used for IUGR in the existing literature can be confusing: IUGR is often used interchangeably with SGA and many studies simply focus on low birth weight. To our knowledge, no studies have been done on nitrogen metabolism in infants with clinically diagnosed IUGR. A few studies have focused on nitrogen metabolism in SGA neonates, using the definition of a birth weight below the tenth percentile.

Several studies have investigated the urea cycle activity of SGA neonates. Excess nitrogen derived from protein metabolism enters the circulation in the form of ammonia, a neurotoxin. The liver detoxifies ammonia by transforming it into urea via a series of enzymatic steps collectively called the urea cycle. It has been suggested that urea cycle function is impaired in SGA neonates, potentially resulting in toxic hyperammonaemia. This was most obvious in the early studies of Boehm *et al*, who showed that in preterm SGA infants plasma and urinary concentrations of α -amino nitrogen and ammonia were elevated,^{46–48} whereas urinary urea excretion was reduced,⁴⁷ in comparison with AGA infants of the same birth weight. The differences between the two groups became more pronounced with increasing protein intake⁴⁶ and were related to the degree of growth restriction.⁴⁹ Furthermore, postnatal maturation of the urea cycle, which is associated with increased urea excretion and reduced urinary and plasma α -amino nitrogen concentrations, is delayed in preterm SGA infants.⁴⁸

Another interesting observation was made by Van Goudoever *et al*, who administered [¹⁵N]glycine intragastrically to preterm AGA and SGA infants to achieve ¹⁵N enrichment in urinary ammonia and urea.⁵⁰ In all AGA infants plateau enrichments in urinary urea were eventually reached, but in most of the SGA infants there was no significant enrichment of [¹⁵N]urea.

Similar observations were previously made by Jackson *et al*, who failed to find enrichment in urinary urea in SGA infants after oral [¹⁵N]glycine administration.⁵¹ The [¹⁵N]ammonia enrichment curve in the SGA infants was normal, suggesting that the ammonia derived from glycine should be available for urea production. These observations strongly suggest that urea production is impaired in SGA infants.

Impaired urea production associated with IUGR probably develops in utero. It has been shown that the activity of the hepatic urea cycle enzymes significantly reduced in fetal guinea-pigs made IUGR by unilateral uterine artery ligation.⁵² This was associated with significantly lower urea production in the IUGR fetuses, whereas the ammonia concentration in liver slices was nearly 16-fold higher than in controls. Furthermore, we have recently demonstrated a progressive decrease in urea production in the IUGR ovine fetus in late gestation.⁵³

After birth, a substantial amount of urea is hydrolysed by the microbial flora in the gastrointestinal tract of most mammals, regenerating ammonia as a potential nitrogen source.⁵⁴ Through this recycling of urea the body is able to maintain a constant source of nitrogen even if intake is limited.^{55–56} Regulation of urea production and excretion is thus a means of nitrogen conservation.

It has been suggested that urea recycling in SGA infants is increased, which means that protein usage is more efficient in these infants.⁵⁰ This is supported by some studies on whole-body protein kinetics. Van Goudoever and colleagues found that preterm infants with low urea production rates had an increased ratio of leucine accretion to leucine turnover, suggesting a higher efficiency of protein accretion.⁵⁰ Another study showed that preterm SGA infants had normal protein accretion rates at lower rates of protein synthesis compared with preterm AGA infants. This also suggests greater efficiency of protein metabolism in SGA infants.⁵⁷ However, not all protein turnover studies in SGA infants support this hypothesis. Pencharz *et al* reported higher protein turnover, synthesis and breakdown rates in SGA infants than in AGA infants.⁵⁸ Van Goudoever also found higher synthesis to accretion rates in SGA infants when using orally administered glycine as a tracer instead of intravenous leucine.⁵⁰ These results suggest a lower efficiency of protein accretion in SGA infants. In addition, the higher total nitrogen excretion by SGA infants reported above does not suggest a nitrogen-conserving strategy but rather a decreased capacity to use or to metabolise protein.

As ammonia is a neurotoxin and hyperammonaemia can cause lethargy and coma,⁵⁹ feeding the SGA infant may require a constant trade-off between keeping protein intake low enough to prevent ammonia intoxication, but high enough to facilitate adequate growth. A recent study investigated the effects on protein metabolism and rate of production of urea of the two main dietary options for preterm SGA infants: their own mother’s milk fortified with protein and minerals, and a preterm formula.⁶⁰ Despite the protein fortification of the human milk, protein intakes were significantly higher in the formula-fed infants (median 3.6 g/kg a day, range 3.1–3.8 (~3.0 g/100 kcal) v 2.9 g/kg a day, range 2.5–3.5 (~2.6 g/100 kcal)). This resulted in protein accretion rates that were 70–80% higher in the formula-fed infants. However, there was no difference in the rates of production of urea between the two groups, suggesting that even the higher intakes were still metabolically safe.

CONCLUSION

Providing the preterm infant with sufficient nutrition for adequate growth and development remains a challenge in modern neonatal care. In particular, establishing adequate protein intakes to promote optimal growth of lean body mass in the immediate neonatal period is difficult. As a result, preterm

infants often lose protein stores soon after birth, resulting in acquired postnatal growth restriction and hence additional nutrient demands for catch-up growth. Recent studies have shown that parenteral amino acid intakes as high as 3.5 g/kg a day, started immediately after birth, can result in a positive nitrogen balance in even the most extremely preterm infants, without signs of toxicity or metabolic acidosis.

Preterm SGA infants are even more vulnerable in the early neonatal period, as they do not have the large energy deposits to draw on until postnatal feeding is established. Furthermore, several studies have found evidence of perturbations in protein and urea metabolism in the SGA neonate, indicating that these infants may tolerate additional protein supplementation less well, and have specific nutritional needs. However, these perturbations are not well understood, and most studies have not distinguished between low birth weight, SGA and IUGR infants. More detailed studies are needed to clarify the metabolic and nutritional needs of the IUGR infant to optimise long-term outcomes.

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IMAGES IN NEONATAL MEDICINE.....

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Massive bacterial cell invasion and reactive type II pneumocytes in bronchoalveolar lavage fluid from a preterm neonate

Bronchoalveolar lavage fluid was collected from a ventilated preterm neonate (gestational age 28 weeks; birth weight 490 g) on day 5, 7 and 11 as part of a study protocol. She

Figure 1 Day 5 bronchoalveolar lavage cytocentrifuge preparation (May–Grünwald–Giemsa staining; magnification 400×). There is extensive bacterial cell invasion of both neutrophils and macrophages.

Figure 2 Day 5 bronchoalveolar lavage cytocentrifuge preparation (May–Grünwald–Giemsa staining; magnification 400×). Reactive type II pneumocytes are seen, typically clusters of large vacuolated cells with a low nuclear to cytoplasmic ratio and peripheral nuclei.

was unexpectedly diagnosed as having pneumonia, despite treatment with amoxicillin and gentamicin. The first cyto-centrifuge preparation revealed an extremely high number of bacteria and extensive cellular invasion (fig 1). The total cell count was 4.2×10^6 cells/ml, 70.3% of which contained intracellular bacteria (67.7% neutrophils; 2.6% macrophages). Coagulase-negative staphylococci (CONS) were cultured from the fluid, and blood cultures taken before starting the antibiotic treatment remained negative. Treatment was switched to vancomycin. Following this both total cell count and the percentage of cells containing intracellular bacteria decreased over time (day 7: 1.6×10^6 cells/ml, 60.8% of cells containing bacteria; day 11: 0.7×10^6 cells/ml, 15.4% of cells containing bacteria). Numerous ghost cells were seen at day 7 (not shown). At both day 5 and 11 reactive type II pneumocytes were noted (fig 2). Despite adequate treatment the baby girl remained dependent on the ventilator, developed severe bronchopulmonary dysplasia, and died at age 4 months. To our knowledge this is the first report of intracellular bacteria and reactive pneumocytes in bronchoalveolar lavage fluid from a preterm.

CONS are the most common cause of septicaemia in preterms. Our patient most probably did not have prior septicaemia, as blood cultures remained negative, and the pneumonia was possibly ventilator associated. CONS pneumonia is rare in preterms, therefore an underlying immune deficiency may have been present. Prior to the infection the baby had marked leucopenia, which may have increased her susceptibility to the infection. Yet while in hospital she had several episodes of infection, and she may have been susceptible to infection as a result of prematurity and extreme intrauterine growth retardation. However, an underlying immune deficiency cannot be ruled out.

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