How Proteins Improve the Development of Preterm Infants

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Abstract

Amino acids and proteins play a pivotal role during growth and development. Besides acting as building blocks during tissue synthesis, amino acids or proteins act specifically by upregulating defense systems or by stimulating key sites in metabolic pathways. Following premature birth, the neonatologist is responsible for delivering the right amount and quality of nutrients to the neonate, while exact requirements are largely unknown. However, nutrition matters, both in quantity as well in quality, especially during the first few weeks and months of life. It is increasingly recognized that proteins and amino acids in the immediate postnatal phase have both short- and long-term influence on later life.

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Introduction

Major innovations in perinatal care, like antenatal steroids and improved respiratory support, have improved the survival rate of preterm infants remarkably. This increase in survival has, however, not been accompanied by a similar decrease in morbidity, although for instance cystic periventricular leukomalacia has almost disappeared [1, 2]. Adverse neurodevelopmental outcome is still substantial [3, 4] and improvements in the field of nutrition do not seem to have resulted in a lower incidence of postnatal growth failure [5–7] or substantial improved outcome [8–9]. As stated recently by the ESPGHAN Committee on Nutrition, the goal of caring for premature infants is obtaining a functional outcome comparable to infants born at term [10]. Thus, it is mandatory to optimize neonatal nutrition in such a way that unhampered
brain growth and development is stimulated. In the field of nutrition, amino acids or proteins have, apart from specific bioactive functions, a central role as they form the matrix of all new tissue and are thus responsible for growth. Therefore, an overview of several aspects of amino acids and proteins in neonatal research will be presented.

**Nutrition for Premature Infants – A Brief History**

Apart from its relevance in pediatrics, long ago the importance of the newly recognized substance protein was immediately acknowledged. The word protein was coined by the Swedish chemist Jöns Jakob Berzelius in a letter sent in 1838 to his Dutch research associate Gerhardus Johannes Mulder who first documented its chemical structure. Berzelius wrote: ‘The name protein that I propose for the organic oxide of fibrin and albumin, I would like to derive from πρωτειος [proteios] (meaning of the first rank or position), because it appears to be the primitive or principal substance of animal nutrition’ [11].

Later, in the early 1900s, Pierre Budin, a French obstetrician, together with his mentor Stéphane Tarnier, the founders of modern perinatal care, provided a clear statement on neonatal nutrition. Budin wrote: ‘The path of pleasure, for adults, is drinking. May it not be the same for weaklings? I increased their absorption of milk with, as you have seen, the happiest of results’ [12]. Thus, in those days premature infants, called weaklings, received high volumes of human milk by tube feeding (up to 200 ml/kg per day) to stimulate rapid growth [13]. However, from the 1940s onwards, concerns about aspiration pneumonias and kidney failure resulted in withholding all fluids for up to 72 h after birth. Up until approximately 1965, very little attention was paid to nutrition. This changed once it was recognized that adverse neurodevelopmental outcome could be attributed to low initial fluid and nutrient intake and early provision of fluids/feedings was again advocated [13]. Since then, several small adaptations to formulae or breast milk fortifiers have resulted in the current methods used to feed babies enterally [14].

Intravenous nutrition also has a long history. The first report on intravenous amino acid administration to young infants in 1939 described many complications [15]. More triumphant was the report that appeared in 1944 where a marasmic suckling received solely total parenteral nutrition for 5 consecutive days [16]. Almost 25 years later, a low birthweight neonate with near-total small bowel atresia received total parenteral nutrition for 44 consecutive days without any enteral feedings; her weight increased by 80% during the study period [17]. Besides stimulating growth, these first solutions containing hydrolyzed amino acid residues also caused significant problems such as hyperammonemia [18]. After the introduction of synthetic crystalline solutions, other undesirable effects such as acidosis became apparent [19].
These findings, together with a report that very high enteral protein intake (6.0–7.2 g/kg per day) in infants born below 1,300 g resulted in lower IQ scores at age 5 [20], still have a profound effect on current nutritional policies. Although it was recognized that withholding amino acids resulted in a catabolic state, they were withheld during early life under the assumption that the preterm infant was ‘intolerant’ to amino acid solutions. We have come to realize that both the method of manufacture and the composition of the amino acid solutions were likely to have caused complications such as hyperammonemia and metabolic acidosis, rather than the amino acids solutions per se. Nevertheless, fear of metabolic derangements is still firmly rooted in clinical practice.

**Fetal Nutrition**

Whether fetal nutrition can serve as a model for neonatal nutrition for premature infants is a difficult question. Certainly, postnatally there is a different physical environment often complicated by disease and medical interventions. Besides, waste products such as ammonia cannot be excreted anymore through placental removal. Yet, for example, metabolite concentrations in fetal plasma provide a safe threshold as to which postnatal values can be referenced. Additionally, fetal enzymatic activity and metabolic rates may indicate metabolic capacities at a certain gestational age which should also pertain to the newborn of similar age. Third, the fetal nutrient deposition during normal growth, provide the minimum amount of nutrients that is also necessary after birth to support a similar growth rate. Studying fetal metabolism can also give good insight into the differences between intrauterine growth restricted infants and those normally grown. Unfortunately, knowledge on fetal nutrition, metabolism, and growth remains scarce, especially in humans [21–23].

From fetal studies, largely performed in sheep, we have come to learn since long that, for example, large amounts of amino acids are actively transported across the placenta towards the growing fetus. These rates well exceed those necessary for tissue deposition or growth [24, 25], and are used as additional fuel source as also demonstrated by large urea formation [26]. These observations, for example, have now contributed to a higher targeted protein intake in premature infants [10].

A different area in fetal research pertains more to developing an effective antenatal therapy for fetuses with intrauterine growth failure, also in humans. Enteral and parenteral nutrient supplementations to the mother have mostly been unsuccessful or even contraproductive [27, 28]. Imbalanced diets together with reciprocal placental transporter inhibition are at least partially causative. More invasive and direct attempts have also been made by intraamniotic infusion of nutrients [29]. Infection risk and potential induction of
preterm labor make repetitive clinical implementation improbable. Recently, however, a case report was published where a port system was implanted subcutaneously to gain permanent access to the umbilical vein [30]. This enabled chronic fetal parenteral nutrient supplementation after which successful fetal growth acceleration was claimed. Apart from being uncontrolled experiments in which fetal growth is hard to follow longitudinally, many lessons from animal research have also been learned. Not only is nutrient status usually compromised during fetal growth failure, optimal transplacental oxygen delivery is for example also crucial for ongoing metabolism. Direct umbilical nutrient infusion can thus result in worsening acidosis with adverse outcome.

**Neonatal Nutrition**

After birth, the nutrient supply through the umbilical cord stops abruptly. Very low birthweight (VLBW) infants are then dependent on externally administered nutrition while their endogenous nutrient stores are very limited. Without adequate nutrient supply, protein breakdown will increase, resulting in a catabolic state. Although it was already stated in 1977 by the American Academy of Pediatrics Committee of Nutrition that a premature’s postnatal growth rate should duplicate fetal growth rate [31], this goal is still not achieved most of the time [5, 6, 32]. Note, however, that initial postnatal weight loss is also due to excreting excess extracellular water and not solely catabolism. Energy and protein deficits develop mainly during the first 2 postnatal weeks when parenteral nutrition is not initiated at target intakes and tolerance of enteral substrates is low [33]. These deficits prove hard to recoup.

Causes of inadequate nutrient intake include fear for intolerance of parenteral and enteral nutrition. Also, fluid intake is restricted to minimize complications such as patent ductus arteriosus and chronic lung disease. Third, acute neonatal illness such as ongoing sepsis or necrotizing enterocolitis (NEC) as well as metabolic derangements such as hypertriacylglycerolemia or severe uremia all result in a reduced nutrient administration. Aside from stagnation of somatic growth, other short- and long-term adverse effects from undernutrition are not encountered instantly. However, growth failure also reflects overall underdevelopment of many organs which has life-long consequences for the functioning of these organs [34], thereby making the individual more prone to diseases such as diabetes and cardiovascular diseases. Short-term consequences of under- or malnutrition can result in increased vulnerability to infectious diseases [35], higher susceptibility to lung injury caused by impaired tissue repair and muscle weakness [36], and decreased maturation of intestine [37, 38] or brain [39].
**Parenteral Nutrition**

During the last decade, several studies have shown the beneficial effects of early parenteral amino acid administration; it reverses a negative nitrogen balance towards anabolism, and it increases plasma amino acid concentrations towards reference ranges found in fetuses or healthy neonates [40–42]. Possible involved pathways, other than providing anabolism, include reduction in oxidative stress by upregulating glutathione synthesis rates [43]. In addition, the increased synthesis rate of e.g. albumin following amino acid administration, may be responsible for an increased transport and binding capacity, with a reduction of potentially toxic levels of free bilirubin or medicines [44]. However, the exact amount and composition of nutrients, required by premature neonates for optimal growth and development, remains unknown [45]. In some neonatal intensive care units (NICUs), amino acids are infused to premature infants from birth onwards, but elsewhere more than 36 h are awaited before commencement of parenteral amino acid administration. Also starting doses vary widely between different NICUs; 0.5 g/kg per day followed by a stepwise increase to 2.5 g/kg per day, but also 3.5 g/kg per day at once. An awaiting attitude results from fluid limitations, risk of hyperglycemia in the case of mixed glucose/amino acid solutions and fear for intolerance, although no firm ground exists for this approach.

Over the years, the quality of intravenous amino acid solutions has improved. Nevertheless, the fear for intolerance in premature infants is still deeply rooted. Safety of amino acid administration is clinically mainly based on biochemical parameters, such as acidosis and plasma concentrations of urea, ammonia, or individual amino acids. However, none of these parameters are specific for amino acid intolerance, and all are influenced by the clinical status of the neonate as well.

Te Braake et al. [40] for example showed that VLBW infants fed glucose and amino acids (2.4 g/kg per day) from birth onwards did not have clinically relevant aberrations in acid-base status. Urea concentrations were, however, significantly raised in the intervention group (9.6 ± 2.8 mM), but no potential side effects of increased urea concentrations at these ranges have been reported [46]. Others did not observe a correlation between amino acid intake and acid-base status [42, 47, 48] or uremia [42, 49] at all. However, Blanco et al. [50] infused extremely premature infants (25.7 ± 2.0 weeks’ gestation) with high-dose amino acids soon after birth (up to 4 g/kg per day on day 3 of life). Whereas the mean peak urea concentration was already very high (19.6 ± 6.8 mM), it even ranged up to 36 mM in some of the most immature infants (≤24 weeks). Ammonia concentrations were also elevated in these infants (~100 mM), where normal values during early life in fasting premature infants are 70 ± 25 mM [51] and a very wide range can be measured in cord blood [52].

Effects on anthropometric measurements are not consistent among studies on early amino acid administration. Valentine et al. [53] observed a greater
weight gain till discharge in premature infants where amino acids were started within 24 h after birth. Poindexter et al. [8] also found in a large observational study a greater weight, length and head circumference at 36 weeks postmenstrual age in those neonates who had achieved an amino acid intake higher than 3 g/kg per day before day 5 of life. At 18 months corrected age, no differences could be detected anymore apart from a larger occipitofrontal circumference in boys only. Also no differences in the mental and psychomotor indexes, as well as the occurrence of handicaps between both groups were observed. Stephens et al. [54] on the other hand, found that in extremely low birthweight infants, after adjusting for confounding variables related to disease, an increase of 1 g/kg per day of protein intake during the first week of life was associated with an 8.2-point increase in mental developmental index. Higher protein intake was also associated with a lower likelihood of length <10th percentile, but not weight or head circumference, at 18 months corrected age. Ehrenkranz et al. [9] showed that a higher growth velocity during NICU admission has a significantly, and possibly independent, effect on growth and neurodevelopment at 18 and 22 months corrected age. The rate of weight gain was significantly and inversely related to the likelihood of bodyweight and length below the 10th percentile around 20 months corrected age, but not related to the likelihood of head circumference below the 10th percentile. Nevertheless, it was shown that with increased weight gain and increased growth of head circumference, the incidence of cerebral palsy, mental and psychomotor developmental index scores <70, and abnormal neurologic examinations fell significantly.

In conclusion, most data provide some evidence for the beneficial effect of rapid initiation of relatively high-dose amino acid administration to the average premature infant.

**Enteral Nutrition**

Nowadays in most NICUs, trophic or minimal enteral feeding (MEF) is initiated directly after birth (e.g. 1.5–3 ml, six times daily). These small volumes of milk are nutritionally insignificant from a nutritional view, but are thought to stimulate maturation of the developing gut. Infants given MEF show enhanced activity of digestive enzymes increased digestive hormone levels and improved gut motility when compared to infants who do not receive MEF. Infants given MEF tolerate full enteral feeding earlier, without increased incidence of NEC [55]. Enteral nutrition can be given in the form of human milk (mother’s own milk or pasteurized human donor milk) or as (preterm) infant formula. Since the first commercially available infant formula in 1915, much research has been devoted towards the development of a formula that resembles the composition of human milk as much as possible and evokes similar physiologic responses in infants. The proteins in formula are most often derived from cow’s milk. An important difference between human milk and cow’s milk protein is the whey-to-casein protein ratio. Bovine milk has a whey
content of approximately 20%, whereas human milk has a whey content of 80% (early lactation) to 50% (late lactation). This has important implications for the amino acid profiles that become available after degradation of milk proteins. For protein synthesis to proceed at optimal rates all essential amino acids must be present in the diet in appropriate amounts. Also, as the brain relies on one single amino acid transporter for all large neutral amino acids to be transported across the blood-brain barrier, imbalances in blood amino acid profiles may also lead to differences in brain amino acid concentrations which could result in altered neurotransmitter concentrations. If and how this would affect the formation of synapses and differentiation of brain cells in the developing brain is not yet elucidated.

Human milk is the optimal nutrition for healthy term born infants, and it is assumed that fortified human milk is also the optimal nutrition for preterm neonates. An additional important difference between human milk and cow’s milk is the lower availability of cysteine and tryptophan in cow’s milk, and therefore these amino acids may become limiting in a diet based on whole cow’s milk. This difference in availability is partly explained by differences in whey-to-casein ratios between human milk and cow’s milk. Specific formulae, such as whey-enriched and α-lactalbumin-enriched formula, have been developed to more closely resemble protein and amino acid profiles of human milk.

Many human milk proteins not only supply the amino acids for protein synthesis but also serve as biologically active components. Human milk proteins can for example stimulate intestinal maturation (growth factors), aid in nutrient absorption (e.g. bile salt-stimulated lipase) and provide protection against pathogens (e.g. immunoglobulins). Some proteins might exert their effect as signaling molecules or might affect through modulating the intestinal flora or immune system. A part of the biologically active components of human milk can be synthesized by e.g. recombinant techniques or gained from cow’s milk and added to formula. Some of them retain their activity and have shown to be of benefit for the preterm infant when added to artificial formula (e.g. lactoferrin, which might reduce the incidence of late-onset sepsis [56]) whereas other constituents (e.g. IGF-I [57]) do not seem to be of any advantage to preterm infants if administered outside the matrix of human milk.

There is debate on the usability of some of the biologically active proteins, as they have been found intact in the stools of breastfed infants, and consequently on the true protein intake of breastfed (preterm) neonates. Lactoferrin and sIgA are quantitatively the most significant (together 0.2 g/100 ml in mature milk) of the relatively indigestible proteins. It has been estimated that 6–10% of lactoferrin escapes digestion by breastfed infants [58]. This would be a potential loss of 0.012–0.02 g of protein per 100 ml, and therefore the effect of the loss of these amino acids on the protein intake of infants is likely to be insignificant. These amounts might be higher in the
premature neonate. Another important difference is the high non-protein nitrogen (NPN) content of human milk (20–25% of total nitrogen) when compared to the NPN content of milk from other species (usually <5%) [58]. The NPN fraction consist of entities like free amino acids, peptides and about 50% is urea [59]. The urea in human milk might be used by colonic bacteria for the synthesis of amino acids that become available for the host. To what extent this contributes to bioavailability of amino acids most likely depends on the colonization pattern of the host, which is severely disrupted in the premature, partly formula-fed, antibiotic-treated infant admitted to the NICU. From adult studies, we know that a significant part of the essential amino acid requirements are provided by intestinal bacteria [60]. Therefore, when thinking about the nutritionally available protein or amino acid content of human milk, the less digestible proteins and the high NPN content should be taken into account.

Human milk itself generally does not contain enough protein (and energy) to meet the high demands of the VLBW infant. Multinutrient supplements are composed of extra protein and carbohydrate, but also vitamins and minerals are added. Recent studies show that infants fed fortified human milk still often receive less protein than they actually need and less than is assumed by their physicians. The reason for this discrepancy is that manufacturers of breast milk fortifiers designed their products to fortify preterm milk with an average protein content of 1.5 g/100 ml. Assumptions that all preterm milk has an average protein content this high is unjustified. Although preterm milk might have a higher protein content during the first weeks of lactation, this declines within a few weeks to the level of term milk, amounting on average to 1.2 g/100 ml [61]. Fortifiers, when prepared according to the manufacturer’s instructions, add on average 0.8 g of protein per 100 ml of milk. To reach the same protein levels as in preterm formula (2.5 g/100 ml), the unfortified milk must thus contain 1.7 g/100 ml, a level that will not be met in most cases. A possible solution to this problem could be titration of extra protein to human milk on the basis of regularly determined biochemical parameters such as serum urea [62]. However, it is debatable what would an appropriate parameter be and what reference values should be used. Another solution, but labor intense, is bedside measurement of protein content by the use of easy to operate human milk analyzers and to adjust the addition of fortifier to the found values. However, weight gain rates using such a personalized approach do not show a great improvement over standard fortification [62].

By adding a fortifier to human milk, however, one must note that cow’s milk protein is introduced into the infant’s diet. Based on the observation that there is a higher incidence of NEC in formula-fed infants, it can be hypothesized that this is not due to protective effects of human milk but rather to a sensitizing or disrupting effect of milk (protein) derived from cows [63]. With relatively simple techniques like ultrafiltration and freeze
drying, it is possible to gain human milk proteins from donor milk [64]. This, although requiring financial investments and additional training of staff, makes production of a human milk-based fortifier possible for human milk banks. It can be hypothesized that this could have beneficial effects such as lower incidence of sepsis and NEC. But also improved neurodevelopment in the long-term can be expected as a consequence of amino acid profiles more suited for the human neonate. However, this needs to be confirmed in large randomized trials. Several studies so far have examined the use of an exclusive human diet. It was shown that addition of a human milk-based fortifier did not result in altered growth or blood parameters of protein status when compared to a bovine fortifier [64]. A recent study suggested a reduction in NEC incidence when the infants were put on an exclusive human diet [63].

**Nutrition and Epigenetics**

Premature infants are born at a time which, in utero, is characterized by rapid brain and body growth. As discussed above, often nutritional and therefore growth targets are not met. Fetal and early postnatal life is characterized by a high plasticity. During this phase, ‘signals’ from the environment may induce changes in the expression of the genome and thereby permanently changing the phenotype of the organism. This capability of adjusting to the environment is probably limited to a critical period in early life and is followed by loss of plasticity and fixed functional capacity. If the resulting phenotype is well adapted to the future environment, this may confer a fitness advantage. However, ‘erroneous’ signals or assaults in early life may give rise to a phenotype that is more prone to disease [65]. Nutrition is an important link between the environment and the (developing) organism, and evidence is accumulating that early nutrition is strongly influencing the risk of adult-onset diseases, such as diabetes and obesity. The hypothesis is that when an organism is malnourished during fetal or early postnatal life, it anticipates to receiving a low nutrient supply in later life by adjusting the setting of hormones and metabolism. The molecular basis for this kind of adaptations to the environment is only partially understood. It is thought to be the result of an altered expression of the genome caused by changes in DNA methylation and covalent modification of histones (‘epigenotype’). Alternations in epigenotype are therefore seen as the molecular basis for the long-term effects of early nutrition. Additionally, as nutrition is a key factor for normal cell growth, poor growth in early life may also directly result in a fewer number of cells in key organs, thereby compromising organ function. Early disrupted growth and formation of pancreatic islets of Langerhans are, for example, an easily conceivable concept to eventually result in diabetic mellitus.
Conclusions

We here presented a broad overview of some aspects of the role of amino acids and proteins in human neonatal nutrition and how these might affect outcome. Although it is well known that proteins and amino acids have a central role in growth and development, large randomized controlled trials remain sparse in order to tailor nutritional intake optimally towards unhampered growth and development. Nevertheless, a lot of progress has been made over the years. More evidence is accumulating that rapid provision of parenteral nutrients in the immediate postnatal phase benefits the prematurely born neonate. However, the long-term outcome of early nutritional interventions with substantial power mainly comes from observational studies. Concerning enteral nutrition, the beneficial role of breast milk is increasingly recognized over that of formula feeding. Its specific protein content with several bioactive proteins is not fully present in formula. An important disadvantage of feeding premature neonates with human milk is the low nutrient content, which is at present only partly solved by the addition of a milk fortifier. However, current available methods for fortification leave room for improvement.

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Discussion

Dr. Stettler: Can you speculate on the mechanism that underlies the difference between boys and girls in response to protein intake in the first 2 days?

Dr. van Goudoever: It's a very good question. What we see in all kinds of nutritional trials in preterm infants, and this also goes for the 6 or 7 trials on post-discharge formula that are out there, is that there is a gender effect. There is a gender effect that boys are sensitive to nutritional change, whereas girls are much less sensitive to changes in nutritional regimen. Also with regard to example given glutathione synthesis rates, we find these differences. So on all different kinds of levels (body composition, weight gain rates or metabolic pathways) we see differences in gender, and they are always directed towards an effect, an improvement in effect in boys, whereas hardly any effect in girls. But again, all the major negative outcomes, such as necrotizing enterocolitis or BPD – they are more frequently observed in boys than in girls.

Dr. Stettler: I am wondering whether understanding better what the mechanism is could lead to therapeutic implications.

Dr. van Goudoever: We may end with different kinds of nutritional interventions for boys and girls because sometimes we have a hint, also in our new study on parenteral intakes, that actually girls are worse off with higher protein intakes than boys. So, if you perform an intervention for the whole group, you might see no effect, whereas on average boys are doing better and girls are doing worse. I think that at present the results our trials should always make a distinction between boys and girls.

Dr. Haschke: A practical question related to amino acid composition of breast milk versus cow's milk or cow's milk-based formula. If premature infants receive milk from their own mother, how will the next generation of human milk fortifier look like? Should it be an amino acid supplement or a supplement based on cow's milk protein fortified with the necessary amino acids?

Dr. van Goudoever: There are two explanations why formula-fed infants have more necrotizing enterocolitis than infants fed mother's milk. One is that there is a protective effect of human milk, the other one is that there is a detrimental effect of intact cow's milk proteins. In this context, does the quality of human milk versus formula milk depend on whether you fortify it with a bovine milk protein, a hydrolyzed bovine milk protein, completely extensively hydrolyzed amino acids, or should you supplement mother's milk with a human milk-based fortifier? The result from the Sullivan study actually suggests that cow's milk protein might be detrimental. Coming back to your original question (regarding the different requirement of the amino acids), we are still far away from knowing each individual amino acid requirement to know exactly what preterm infant needs. The majority of studies performed so far have just been looking at total protein and maybe protein energy to quality ratio, and not so much at the individual quality of proteins.

Dr. Chittal: The data are convincing that only protein intake is good for growth and also the neurodevelopmental outcome, but these early protein intakes are also
causing increased blood pressure even at 7–8 years. So where is the exact balance of protein intake?

**Dr. van Goudoever:** Very good question, and I don't know. The only argument I have is that we have to make a distinction between preterm and term infants. Preterm infants are at risk for having severe neurocognitive impairment, and that should be addressed first before I would bother too much about higher blood pressure or outcomes like that. What I think we are doing in our wards and in our step-down units is actually underfeeding these infants, we are not giving too much. By increasing their protein intake you might be right that this would lead to higher blood pressure rates. However, and this relates back to Dr. Haschke’s question, we have to consider the quality of the proteins as well. You can give lots of protein, but growth rate is limited by the first limiting essential amino acid in your diet. So, if you are low on one specific essential amino acid, you can put in as much other amino acids as you want, the one that is the limiting one is limiting your growth and also your organ growth and also your nephron growth and brain growth, etc.

**Dr. Saluja:** In the NICU, most of the preterm neonates get about 2.5–3 g of proteins as a fortification of their formula. What should be the policy for those preterm neonates who move on to direct human milk or direct breastfeeding in the post-discharge phase? We know that they may not be able to consume enough proteins. If we want to follow their growth velocity in terms of weight, length and head size, what should our targets be? And how should we customize their intakes to match their growth references?

**Dr. van Goudoever:** Again an excellent question, and we are focusing all the time on weight gain rates because that’s easy to do. But Prof. Harding already showed that not only weight gain or rather anything else than weight gain might be important if you are interested in long-term consequences. Still, there is not that much we can measure in daily care, so then probably weight gain measurement is the easiest thing to do. The infants who are on human milk should receive fortifiers. A Danish study has been published where infants fed fortified breast milk only once a day with additional proteins displayed an increase in weight gain rates in the post-discharge phase or in the late hospital phase. I don’t know whether fortifiers are available in India, but supplementing human milk with fortifiers once or twice a day would be an option.

**Dr. Saluja:** We do have a fortifier, but unfortunately the quality and amount of proteins it provides are inappropriate. Is it fair to say that when we follow the infants’ growth, we should continue to follow the same centiles as in the prenatal period, and that if they are growth restricted we don’t allow them to catch up and cross the centiles?

**Dr. van Goudoever:** The catch up is again an important question. In my view, what is happening in the first couple of weeks to months is that we underfeed our infants and they become growth retarded. In my unit in Rotterdam, the average birth z score was –0.8, and at discharge they reached –1.6. The majority of that loss is in the first few weeks. In my view, if we are able to feed them appropriately in those first few weeks, which is really much more than we do now, then the whole question about detrimental catch-up growth is futile. So, I think the key is within the first few weeks of life.

**Dr. Pereira-da-Silva:** I have a practical query. You suggested that a daily intake of 4–5 g per kg of bodyweight of amino acid might be appropriate for the very premature babies. This exceeds the current recommendations, at least for parenterally fed premature infants [1]. Which short-term risks should we expect when administering excessive doses of amino acids to these infants?

**Dr. van Goudoever:** Basically, the newest recommendations are 3.5 g of parenteral amino acid intake and up to 4.5 g of enteral protein intake [2], and the difference
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is of course caused by the utilization of amino acids by the intestine. What are the effects of a high amino acid/protein intake: (1) hyper ammonia, (2) high amino acid levels with detrimental effects, and (3) high urea levels which might cause kidney problems later on. Of course, there have been studies in the 1970s with high, very high (up to 5 or 6 g) levels of amino acids. There have also been studies on parenterals with high amino acid levels, but in that case there were no crystalline solutions, for instance, so they had all kinds of detrimental effects. I think quality is the subject that we should pay a lot of attention to in the next 5 years, and maybe we can even lower total protein intake again by improving the quality, by improving lets say the first 3 or 4 limiting amino acids in the diet. If we can increase those intakes, we might even decrease the total protein intake.

Dr. Pereira-da-Silva: And guided by serum ammonia and urea, is it possible to push the intake over the currently recommended dose?

Dr. van Goudoever: Yes that’s actually what we do nowadays, we measure urea levels. Based on urea levels, we have set the maximum cord blood level at 10 mM, and we basically titrate amino acid intake based on that level.

Dr. van Elburg: You were talking about amino acid quantity and that you might differentiate between the different amino acids that are added to the infant’s diet. Based on your stable isotope studies, which amino acids would you increase and which ones would you decrease?

Dr. van Goudoever: It’s interesting, across all mammals lysine and threonine are the first two limiting amino acids in the diet, so those are the two that are limiting for enteral nutrition. Actually, the pig industry has known that for a long time, they add lysine into pig food and the piglets grow faster. For parenterals, I don’t know exactly. I know that Paul Pencharz in Canada is doing lots of studies on parenteral amino acid intake requirements, but mostly in older children, and those studies need to be done as well.

Dr. Iramain: We know that in preterm newborn a daily intake of 4 g protein/kg can cause renal failure. How can we approach this issue?

Dr. van Goudoever: I agree; that’s why I say let’s monitor some short-term metabolic outcomes like urea levels. But then again, what would be the most important improvement you want to see in preterm infants? I think that the most important improvement we would like to see concerns neurocognitive development, and I think that is something that we should strive for. The amino acid intake through the umbilical cord is about 5 g/kg per day, but maternal metabolism will take away all metabolites.

Dr. Mace: What about the body composition of these infants? Don’t you think that fat mass is also something important to consider?

Dr. van Goudoever: I completely agree. Again, it’s hard to measure fat mass in the NICU. In our new studies, we give doubly labeled water to first measure energy expenditure, but we also get fat mass. So, it needs to be done; it’s exactly what you say, it’s not only weight gain rates but it’s also body composition.

Dr. Fasano: I was intrigued by your concept of the challenge of neonatologists to recreate the in utero nutritional environment for the very low-birthweight infants and the debate cow’s milk versus breast milk versus placental nutrition and so on and so forth; but I would like to know what your thought is about the fact that now you have a different situation because the intestine is very different in utero (sterile) than in an NICU where you have colonization. But I am assuming all these kids are on antibiotic treatment. Does this change the dynamic of the game that you are going to play in terms of nutrient utilization?

Dr. van Goudoever: It certainly did. I was very reluctant to make big steps in trying to improve the nutritional management until I had the data about what the infants
were receiving in utero. Recently, we designed a trial which is currently running with larger steps, looking at long-term outcomes with 240 kids, for instance.

Dr. Were: I was intrigued by your studies telling us that if you delay introducing proteins as early as the first or second day you lose time, and the dose that you build up slowly from 1.1 up to 4.5 has worse outcomes. It would require having in-house facilities for parenteral nutrition to start feeding infants early. What advice would you give to the centers like the one I come from where we don’t have those facilities? How can someone in a little corner of Malawi be able to attempt to get close to giving 2.4 g on day 1 to a 1.2-kg baby?

Dr. van Goudoever: It’s a good question, a question which I have been asked frequently in Southeast Asia as well. The question is where do you put your money? Do you put your money in a new ventilator or do you put your money in getting parenteral nutrition in your unit? It’s all about costs, how much resources you have, to what extent you can convince the director of your hospital that nutrition, although it seems so simple, has such a major effect on long-term outcomes. Of course, it’s not easy to accomplish parenteral nutrition in Malawi for premature infants. But if we can convince people that this is a very important issue, that although nutrition is not very fancy, it really can change late outcome in many lives, we are on the right track. We need to convince key opinion leaders in the different hospitals that early nutrition has a major impact, and I think that the time now is right to do it.

Dr. Agarwal: Why don’t we give pregnant women amino acids in the later part of pregnancy? You give them infusions at the recommended doses before the cesarean section. Maybe this way the preterm delivery would also be prevented.

Dr. van Goudoever: That’s a good question as well. There have been studies in the 1970s of actually providing pregnant women in New York with high levels of protein, but the outcome was worse: higher infant mortality, higher number of premature births, and so basically nobody dares to do those kinds of studies again. Infusion of amino acids in growth-retarded infants didn’t work. So, there are a lot of questions there. It seems simple, and I completely agree, but it doesn’t seem to work.

References