Clinical Outcome of Low Birthweight, Long-Term Consequences


Amino Acid Homeostasis in the Preterm Infant

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Abstract

Functional outcome of preterm infants is highly related to the quality and quantity of nutrients provided during the first few weeks of life. New guidelines, as published by the ESPGHAN in 2010, have provided means to prevent undernutrition in the NICU. Especially proteins and amino acids seem to play a pivotal role, and the optimal regimen has not yet been determined. New data on the intrauterine nutrient supply suggest a high amino acid intake during the fetal period. How these results might translate into improvement of especially neurocognitive outcome needs to be investigated.

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Introduction

Undernourishment during the early phase of life leads to reduced neurocognitive functioning in term infants [1]. Also in low birthweight and preterm infants, observational studies have suggested that inadequate nutrient intakes in the first few days to weeks following birth have adverse effects on long-term neurocognitive function [2–4]. Unfortunately, only a few prospective studies have been conducted in preterm infants. These studies, most originating in the 1980s, have shown that both quantity and quality of the provided nutrients to preterm children and subsequent growth have pronounced effects on neurocognitive function [5], cardiovascular health [6, 7], and metabolic and endocrine status [8, 9].
These effects seem to persist over a long period, well beyond childhood [9–11], and justify all efforts to provide especially preterm infants with adequate nutrients in early life.

**Different Approaches in Determining Requirements for Preterm Infants**

Two approaches can be used in determining adequate requirements for infants following premature birth. Since preterm infants have not yet matured well enough, their organs are still developing and growth is extremely rapid, it follows that the supply to the fetus of similar gestational age can be regarded as suitable. However, some drawbacks to that theory can be made. The fetus thrives in an entirely different environment, despite care for these newborns in warmed and humidified incubators. Furthermore, the fetus hardly uses the lungs or intestines, and the maturation pattern is different due to less stressors from outside, including a potentially hostile microbial environment. Also, most preterm infants are ill, certainly at birth, requiring mechanical ventilation, antibiotic therapy and sometimes vasopressors. It is likely that these conditions influence nutrient requirements. Altogether, it is unlikely that the nutrient supply via the umbilical cord adequately reflects the nutrient requirement of prematurely born infants being treated at the neonatal intensive care unit.

The other approach is based on the composition of human milk and the factorial approach. The factorial approach is a method in which the growth rate and the composition of newly formed tissues of a fetus of a certain gestational age are estimated. The composition of the fetal tissues is derived from carcass analyses of deceased fetuses [12]. These data are rather old, originating from 1890s onwards, with little knowledge on the health status of the fetomaternal dyade. The other factor in this method is the composition of human milk. Own mother’s milk is the optimal source of nutrients for term-born children, but both quantity and content vary considerably. Hindmilk has a different fat content than foremilk, and milk produced in the first week of life has a higher protein content than mature human milk. Mothers with a high fish diet, e.g. from Japan or Greece, have different long-chain polyunsaturated fatty acid concentrations in their milk than mothers from a different region. Consequently, the mean concentration of nutrients and a mean volume intake are just rough estimates of what the actual intake is. Therefore, also the factorial approach and human milk composition are not likely to provide the exact requirements for all nutrients of the prematurely born infant.

So, both methods have their drawbacks in estimating the exact nutrient requirements of prematurely born children who are taken care of in an incubator with all kinds of accompanying illnesses. Knowledge on both approaches is how-
ever pivotal in developing the appropriate nutritional management, which should be based upon estimates using both methods and subsequent well-conducted clinical trials. Since hardly any data were available on the first approach, the fetal nutrient supply, we undertook a few studies to determine these.

### Fetal Nutrient Intake

In a series of studies, we were able to determine the fetal amino acid uptake of fetuses around term gestational age [13–15]. Prior to caesarean section, pregnant women received intravenously administered amino acids that were labeled with stable isotopes. The measurement of flow rate at the umbilicus and the measurement of both arterial and venous umbilical blood directly after birth enabled us to quantitate the actual uptake of the studied amino acids by the fetus just prior to birth.

We were able to demonstrate that the placenta provides the fetus with a great surplus of amino acids. Only 20% of the available phenylalanine is utilized by the fetus, of which a part is hydrolyzed to tyrosine (fig. 1) [13]. That is important information, since tyrosine was considered a conditionally essential amino acid, especially following birth. These data show that even a fetus is capable of hydroxylizing phenylalanine, so that tyrosine can be considered a non-essential amino acid.
Based upon valine and leucine tracers, we estimate that up to half of these amino acids taken up from the umbilical cord are oxidized instead of being used for anabolism [14]. This demonstrates that just as in animal models like sheep [16], amino acids should be regarded as a significant energy source during fetal life. This directly also shows a disadvantage of the factorial approach as only accreted nutrients are counted in, and not those oxidized for energy generation.

**Current Practice**

Undernutrition, with subsequent growth failure, occurs predominantly during the first week of life of premature infants. Recent recommendations for enteral nutrition prevent undernutrition during the enteral phase [17, 18]. However, many neonatologists are reluctant to start with high nutrient intakes directly following birth. They fear that the renal and hepatic systems are not developed well enough to handle high amounts of (par)enterally administered nutrients and their metabolites. That seems logical since in utero, the placenta and the mother have large capacities to handle off possible detrimental metabolites. However, several trials on early amino acid administration showed improvements of nitrogen balances, and thus anabolism, without clinical significant adverse events [19–21]. According to current guidelines, 2–3 g amino acids/kg per day should be started as soon as possible after birth, with increments to a maximum dose of 4 g/kg per day in the next few days [22–24]. Present amino acid mixtures and lipid emulsions are improved when compared to mixtures that were marketed years ago. Marked differences in enteral feeding practices and consequently also in parenteral policies were found in recent surveys, evaluating clinical practices amongst over 100 NICUs around the world [25–29]. Nevertheless, despite newer and possibly beneficial alternatives [30], the lipid emulsion still used most often in NICUs is a 100% soybean emulsion, of which the composition has not changed during almost half a century except from increasing its concentration from 10 to 20%.

**Quality of Amino Acid Mixtures**

The quality of parenteral amino acid mixtures is difficult to judge since we do not know the exact requirements for individual amino acids in parenterally fed preterm infants. For term neonates, some requirements (threonine, methionine, sulfur amino acids) are known [31–33]. Consequently, the composition of current pediatric amino acid solutions differs widely among the different brands. Some brands used plasma amino acid concentrations of healthy, term, breastfed infants as a guiding
reference, while others derived the composition from fetal and neonatal cord blood amino acid concentrations. Overall, amino acid mixtures are low in tyrosine and cysteine due to poor solubility or stability in parenteral nutrition. Future studies should indicate the optimal composition of amino acid mixtures for preterm infants.

**Amino Acid Tolerance**

Specific parameters for amino acid intolerance are lacking. Biochemical parameters, such as acidosis, elevated plasma urea concentrations, increased ammonia concentrations and concentrations of potentially (neuro)toxic amino acids above reference ranges for term infants are often used as a proxy for intolerance. However, these parameters are also influenced by the general clinical status of the neonate. Like in fetuses who use amino acids for both protein synthesis and energy generation (oxidation), elevated urea concentrations in preterm neonates are the result of functional amino acid oxidation and not purely a sign of amino acid intolerance. In addition, correlations between urea concentrations and amino acid intake are inconsistently found. Therefore, elevations in plasma urea concentration should not automatically lead to withholding of amino acids in preterm infants.

**Early Amino Acid Administration**

In a randomized controlled trial, we aimed to find a difference in nitrogen balance, a proxy for anabolism, on day 2 of life by supplying 2.4 g of amino acids per kilogram per day from birth onwards, whereas the control group received dextrose only [20]. The latter group started after 36 h with 1.2 g of amino acids, with an increase to 2.4 g on day 3 of life. Despite very low energy intakes (30–50 kcal/kg per day), the preterm infants (average birthweight of 1 kg) were able to use the provided amino acids and increase whole-body protein synthesis [34] and albumin synthesis [35], and turn nitrogen balance from negative (catabolic state) to positive (anabolic state) [20]. In addition, the synthesis rate of the main intracellular antioxidant, glutathione, was higher as well [36]. We could not detect any negative side effects. So, this study showed that supplementing preterm infants with 2.4 g amino acids per kg per day resulted in a clear beneficial effect in the short term. Studies where amino acid administration was started not directly following birth but within the first few days yielded similar results [37]. Other trials questioned the efficiency and warned of possible detrimental effects [38, 39]. Very recently, long-term follow-up from one of these studies (not powered for long-term follow-up though) became available, showing diminished growth dur-
ing the first 2 years of life and cumulative and single plasma AA concentrations negatively correlating with MDI and postnatal growth [40]. However, these data are derived from only 16 infants in each group who were available at age 18 months (63% of surviving infants). Nevertheless, their data indicate we should be careful infusing the most immature infants (<25 weeks’ gestation) with high-dose amino acids (4 g/kg per day) without further investigations.

Two-year follow-up of our own trial (follow-up n = 111, 97% of those surviving) revealed no detrimental effects. Even the opposite, preterm boys had significantly more often normal outcome (survival without major handicaps) when they were supplemented with high amino acid intakes from birth onwards. No significant effect was noticed in girls [manuscript submitted].

**Fetal Albumin Synthesis Rate**

In the same series on fetal metabolism as described above, we infused several $^{13}$C-labeled amino acids in a staggered manner to pregnant women prior to (preterm or term) caesarean section, allowing for the determination of fetal protein synthesis, such as fetal albumin [41]. Albumin, the major transport protein with antioxidative capacities, is frequently found at low concentrations in premature infants. A low concentration results in low colloid osmotic pressure with subsequent edema. Albumin serves as an indicator of nutritional status, although concentrations are not as sensitive as synthesis rates. The use of stable isotopes enabled us to determine fetal albumin synthesis rates and compare those with rates found following admission to the NICU after different amino acid intakes (fig. 2). The administration of amino acids from birth onwards at a rate of 2.4 g/kg per day improved synthesis rates significantly when compared to the unsupplemented infants, but the rates remained below those obtained in utero. This might be related to neonatal stress or illness following birth, but might also illustrate that nutrient supply in these infants failed to meet the requirement [15]. Nevertheless, and most importantly, it was shown that the liver of a premature fetus is capable of synthesizing protein at very high rates and regarding this aspect, the liver should not be regarded as immature.

**Meeting the Needs Directly following Birth**

Very recently, we finished our second trial on early nutritional support of small infants. One hundred and forty-four infants were included, with different lipid and amino acid intakes from birth onwards. The highest intake group (3.6 g/kg
per day amino acids and 2 g/kg per day lipids) was most anabolic as was shown by classic nitrogen balances. These results need further evaluation but confirm the results obtained by Ibrahim et al. [19].

**Conclusion**

Optimal nutritional strategies for preterm infants are continuously being developed. Suboptimal quality and quantity of nutrients, even during only the first few days of life, are likely to have long-term consequences. Providing high amounts of nutrients form birth onwards should however be carefully evaluated, and randomized trials with ample power should be conducted. Not only short-term proxy outcome variables should be measured, but also long-term follow-up should be included as end points of such trials to evaluate the choices made in feeding this vulnerable population.

**Disclosure Statement**

The authors have no conflict of interest to declare that has a relationship with this paper. The hospital of J.B.v.G. received a fee for the presentation of the results described in this paper at a Nestlé Nutrition Institute workshop held in March 2012, Goa, India.
References


