Defining Protein Requirements of Preterm Infants by Using Metabolic Studies in Fetuses and Preterm Infants

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Abstract
Amino acids form one of the main building blocks for fetal and neonatal growth. Despite improvements in neonatal care, including postnatal nutrition, growth faltering and suboptimal outcome after premature birth are still frequently encountered. Nutrition can partly be held responsible. Over the years, there has been a trend in delivering amino acids earlier from birth on and in larger quantities. Unfortunately, little is known about the specific metabolism of proteins, especially during fetal life or during disease. This review gives an overview of different methods of studying metabolism during early life and what we have come to learn so far. Different examples are given on the complex interplay between the placenta and the fetus. From both ovine and human studies, we know that amino acids are not only used for protein synthesis in the fetus, they are also oxidized to a large extent. Postnataally, we have succeeded in improving the nitrogen balance in preterm infants, but the preconditions need also to be improved before concluding that today’s policy is optimal. Only by gaining more knowledge on both fetal and neonatal physiology and disease will we be able to further optimize growth and functional outcome in premature infants.

Introduction
By definition, being born prematurely will never represent a physiological situation. Therefore, the short-term normative aims to strive for while being cared for in a neonatal intensive care unit (NICU) are difficult to pose. However, the
The goal of caring for a premature infant is to create an environment leading to similar long-term outcomes as infants who are born at term, i.e., firstly, to achieve normal growth and development resulting in healthy life at term-corrected age and beyond. Although it is a true oversimplification, it requires – besides tender love and care – the right amount of nutrients, oxygen and growth-stimulating factors like hormones to support ‘normal’ metabolism. Gaining knowledge on fetal as well as neonatal metabolism during various stages of gestation and diseases could set the standard to help achieve these goals. A broader understanding of both situations will guide us in designing the optimal nutritional management for these infants who are born prematurely. One must of course acknowledge that a premature infant is not just an extrauterine fetus. Intrauterine life is completely different when compared to the life at an NICU with different thermoregulation, fluid balance, exposure to pathogens and redox state. Besides regulating the fluid balance, the placenta helps excreting waste or toxic products of metabolism. In addition, organ functioning, especially of the lungs, bowel and kidney, but also muscle activity is not comparable to postnatal life. Then, it is known that the fetus and amniotic fluid are not completely sterile, but the intrauterine bacterial load and genera are different than those encountered postnatally. Yet, exploring fetal metabolism and growth could enhance our understanding of the (in)capabilities of postnatal development. In this review, we will describe various aspects of fetal and neonatal protein and amino acid metabolism.

Amino acid metabolism can be studied by various methods. Nitrogen balance studies are valuable and relatively easy to perform in larger groups of patients, but will only provide information on the amount of nitrogen retained. It does not specify intermediate metabolism or synthesis rates of certain proteins, whilst this could be informative. Important safe tools in doing so are stable isotope studies [1, 2]. After administration of a labeled amino acid, one can infer from its measured concentration the rate of dilution, reflecting the rate of appearance of its naturally occurring isotopomer which would equal the sum of intake, endogenous synthesis and release from proteolysis. If studied at steady state, the rate of appearance equals the rate of disappearance consisting of its routes of disposal. These usually include use for protein synthesis and oxidation or hydroxylation, for example. Following and quantifying the label in one of these disposal products helps one to infer the other disappearance rates. Besides studying metabolism on a whole body level, it is also possible to quantify synthesis of certain specific proteins, like albumin for example, or from certain tissues if one could sample that organ.
Placental and Fetal Metabolism

Studies on fetal metabolism are relatively scarce, not only in humans, but also in other species. Without a doubt this is to a large extent due to technical and ethical difficulties. With regard to animal studies, one must acknowledge that there exist large interspecies differences, and thus study results in animal data must be interpreted with caution when translating them to the human situation. This is nicely illustrated when looking at the body composition of various mammals. Whereas humans have accreted up to 16% of body size as fat at term birth, in most animals it is limited to 1–4% (table 1). The fetal protein content is, however, quite similar across species, approximating 12% at term. Yet, the interspecies differences in protein handling can for example be illustrated by the fact that in the ovine fetus (which is frequently studied) ammonia is transported from placenta to fetus [3], whereas net transport in humans is the other way around [4]. The ammonia taken up could be a means to provide extra nitrogen to support the very high growth rate of the ovine fetus.

Studying fetal metabolism must include the placenta. Regarding the placenta as a simple conduit is too simplistic. In fact, the placenta itself also has a very high metabolic rate. Of all oxygen taken up by the total conceptus (fetus, placenta and uterus) from the maternal circulation, 40% is retained within the placenta, with the other 60% being transported to the fetus [5]. Glucose consumption by the placenta accounts even up to 60% of the total glucose uptake by the uterine tissues, although this has only been measured in sheep [6]. Under prolonged maternal hypoglycemia, it even increases further to maintain normal placental metabolism, but at the expense of glucose available for fetal transfer [6]. For amino

Table 1. Lipid content in different species at term birth [31–35]

<table>
<thead>
<tr>
<th>Species</th>
<th>Fat, %</th>
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<tbody>
<tr>
<td>Black bear</td>
<td>0.9</td>
</tr>
<tr>
<td>Pig</td>
<td>1.1</td>
</tr>
<tr>
<td>Rat</td>
<td>1.1</td>
</tr>
<tr>
<td>Cat</td>
<td>1.8</td>
</tr>
<tr>
<td>Mouse</td>
<td>2.1</td>
</tr>
<tr>
<td>Horse</td>
<td>2.6</td>
</tr>
<tr>
<td>Baboon</td>
<td>3.0</td>
</tr>
<tr>
<td>Sheep</td>
<td>3.3</td>
</tr>
<tr>
<td>Rabbit</td>
<td>3.9</td>
</tr>
<tr>
<td>Seal</td>
<td>4.0–9.0</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>10.0</td>
</tr>
<tr>
<td>Human</td>
<td>16.0</td>
</tr>
</tbody>
</table>
acids, the difference between uterine and umbilical uptakes indicates that 25% is retained within the placenta and used for placental growth and metabolism [7].

The high metabolic rate of the placenta is necessary not only to maintain its own integrity, but also to facilitate the energy-demanding transport of amino acids towards the fetus. Amino acid concentrations are 1.5–2 times higher in fetal than in maternal plasma, but are known to be highest in the trophoblastic cytoplasm. Amino acids are transported by using more than 20 different amino acid transporters with distinct yet overlapping specificity [8]. The transporters can be divided into three categories. There are accumulative transporters which transport an amino acid into the trophoblast (either from the maternal or fetal side) together with a sodium ion which drives the gradient for transport into the cell. Na⁺-K⁺-ATPase pumps are then responsible for restoring the ion gradient at the expense of ATP. Exchange transporters allow an amino acid to enter the trophoblast against the gradient current in exchange for an amino acid to leave the cell. Finally, facilitative efflux transporters, which are mainly facing the fetal side of the trophoblast, will help amino acids to exit the cell. As only the latter type of transporter allows net fetal uptake, but only has affinity for few amino acids, all three categories of transporters cooperate ensuring balanced net maternal-fetal transport.

True fetal nutrient uptakes in humans are currently not known. In fetal sheep, Chung et al. [7] calculated from umbilical veno-arterial concentration differences and umbilical blood flow (Fick principle) the net uptake of all amino acids. The total uptake amounted approximately to 7.5 g/kg per day, which should not be compared to the human situation as sheep grow much faster (35 vs. 15 g/kg per day). They, however, also showed that the total sum of net nitrogen uptake well exceeded demands necessary for anabolism by on average 40%, with the largest differences between uptake and accretion for the essential amino acids. This implicates that despite the energy-demanding process of placental transport, the large part of transported amino acids is converted to nonnitrogenous metabolites or oxidized in the fetus to yield energy. Also, in humans there are indications that amino acids are oxidized as reflected by higher ammonia and urea concentrations in the umbilical artery than in the vein [4, 9]. Chien et al. [10] were the first to quantify oxidation rates of a single amino acid in the human fetus at term with stable isotope techniques. They showed that one third of all transported leucine was oxidized by the fetus to yield energy. By combining fetal phenylalanine kinetics with our data from fetal leucine and valine metabolism, we theorized that oxidation rates of these branched-chain amino acids were probably even higher, approximating 40 and 60% of total uptake, respectively [11]. Caution must be applied when extrapolating these results to general guidelines of how to feed a preterm infant. First, these human fetal studies are confined to the moments just before elective cesarean section around term
gestation and, second, not all amino acids were studied, which precludes direct calculations of total fetal amino acid uptake. However, despite these limitations, based on calculations from a factorial approach on changing body composition and accretion [12] combined with estimates of oxidation, human net fetal uptake probably amounts at least to 3.0–3.5 g amino acids/kg per day in the second half of pregnancy. The presumed fetal uptakes and neonatal intakes of amino acids and lipids are outlined in figure 1.

The metabolic studies also help us to gather information on human fetal liver protein synthesis. By using a staggered infusion protocol of different labeled amino acids in the hours prior to cesarean section, we showed that the fetal liver was able to synthesize large quantities of albumin, particularly during early gestation and generally more so than is seen postnatally in preterm infants [13]. Thus, our interpretation is that the conditions in which a premature infant is cared for in our NICU need improvement. One possibility is that postnatal protein delivery could be optimized further to achieve synthesis rates comparable to those in intrauterine life.

**Neonatal Studies**

Many studies in premature neonates have resulted in a shift to earlier delivery of intravenous amino acids in higher doses in the early postnatal phase to reduce postnatal growth faltering. These days, it is generally recommended to start

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**Fig. 1.** Stacked bar graph of the total estimated amino acid (a) and lipid (b) uptake in a fetus at 26 and 38 weeks gestation and intakes in a term born healthy infant fed breast milk at 2 months of age. Bars indicate whether protein or lipids will be used for replacement of water by tissue, net tissue accretion or energy generation (oxidation).
providing amino acids immediately after birth, along with glucose. Rapid introduction of concomitant parenteral lipids is advocated, but frequently seems to be delayed or administered only in low amounts during the first 24–48 h after birth. This policy automatically results in relatively low nonprotein energy intakes during this period but might also influence how amino acids are tolerated. Without enough calories to support energy-demanding protein synthesis, amino acids are theoretically more likely to be either stacked, leading to increased concentrations in plasma or intracellularly, or oxidized, yielding energy but also leading to waste products like ammonia that is to be converted into urea [14]. While amino acid oxidation during early life is ontogenetically probably physiological as it is also measured in fetuses [7, 10, 11], its waste products are not absorbed by the placenta anymore after premature birth. High ammonia concentrations are well known to be toxic, but these are not routinely measured. Urea concentrations are more frequently measured although interpretation is difficult as it is unknown whether the upper levels frequently seen are harmful or not [15]. Plasma amino acid concentrations are not measured clinically on daily routine. However, there are many reports showing increased concentrations after early increased amino acid intake [16, 17]. These relatively higher concentrations are also difficult to relate as we do not have a physiological reference group to which we should compare. Should we compare with the intranuclear situation or to a healthy term-born breastfed infant? Comparison with a stably growing premature infant of the same gestation does also not always seem ideal as those results are the result of our handling which is per definition not physiological. Notably, when compared to term infants, especially the most premature infants and those in the first week of life have significant deviations in their amino acid concentrations [18].

In order to improve utilization of amino acids in the direction of anabolism, we performed a randomized controlled trial in which premature neonates received besides amino acids (2.4 g/kg per day) only glucose or besides glucose also lipids during the first 2 days after birth [17]. Adding lipids indeed increased the nitrogen balance and lowered plasma urea concentrations, and decreased several amino acid concentrations better resembling those of term breastfed neonates. However, urea synthesis rates and phenylalanine kinetics, measured with stable isotopes, did not change [17, 19]. Although the beneficial effect of providing more energy and thus lowering the protein/energy ratio during early neonatal premature life on metabolism is to be determined exactly, physiologically, it seems wise to provide sufficient calories along with the amino acids to support anabolism.

When aiming to quantify the absolute amount of protein an infant requires, the quality (i.e. specific amino acid composition) of the solution or formula is
very important. The different intravenous solutions have their composition based to theoretically match amino acid concentrations that can be found either in umbilical-cord blood or in term breastfed neonates. However, this does not necessarily reflect the needs. The specific roles and metabolism of individual amino acids is described elsewhere [20], but especially leucine seems to stimulate protein synthesis the most [21]. However, if a certain (conditionally) essential amino acid is relatively lacking compared to the needs, all other amino acids cannot be used for protein synthesis and thus will be oxidized in order to prevent a rise in concentration. Therefore, to improve the nutritional quality, the specific needs of all (essential) amino acids are to be determined. One can do so by measuring the oxidation rate of a labeled indicator amino acid over an intake range of another amino acid that is to be tested [22]. The indicator oxidation rate will theoretically decline until the needs of the test amino acid have been fulfilled. While for term enterally fed neonates and postsurgically parenterally fed neonates, these requirements have been measured for most essential amino acids [23, 24], this has only been done for cysteine in premature neonates after several weeks of life while fully enterally fed [25]. Once the individual amino acid requirements are better known for several distinct groups of neonates, this could lead to the development of improved solutions or formulae by manufacturers.

Besides all the above-mentioned factors that could influence the need of proteins, hardly anything is known of the impact of several clinical conditions on requirements. There has been very little attention paid to how extreme versus milder prematurity, small versus appropriate for gestational age, or additional critical illnesses and medication affect metabolism. Neonatal nutritional research during the last few decades focused mainly on either the first days or week of life, or the stable phase when fully enterally fed. In the adult literature, there is large debate suggesting that early parenteral nutrition during critical illness might not be beneficial [26]. Increased rates of infection and delayed recovery from organ failure after early parenteral nutrition may be explained by a suppression of autophagy, with inadequate clearance of cell damage and microorganisms [27]. Results of pediatric studies are to follow soon. How the theories behind the adult studies should fit with the neonatal population who could be defined as critically ill during large part of their NICU stay is currently not known. A small study in near-term neonates after abdominal surgery showed no signs of intolerance after early parenteral nutrition and even showed positive nitrogen and leucine balances measured with stable isotopes [28].

Despite all the above, various trials investigating different intakes of parenterally administered amino acids in preterm infants all fit in a linear
relationship with nitrogen balance, as shown in figure 2. Nevertheless, with more focus on stimulating anabolism in the immediate postnatal phase, more frequent reports appear showing electrolyte disturbances, as also seen upon refeeding following starvation in other patient groups [29, 30]. Potassium and phosphate concentrations were shown to be too low during the first days of life with newer feeding regimens (higher amino acid intake), leading to immediate clinical consequences. The micronutrients also need to be revisited when pushing metabolism towards anabolism in the early postnatal phase.
Conclusion

Amino acids or proteins are the most important components of achieving neonatal growth. The amount to be given is, however, difficult to generalize as many factors will influence how administered amino acids will be used for anabolism. Therefore, their metabolism cannot be regarded separately but has to be interpreted together with the other macro- and micronutrients, and with other clinical conditions. Unfortunately, only few of these factors have been unraveled. By gaining more knowledge on both fetal and neonatal physiology and disease, we should be able to optimize growth and functional outcome in premature infants further.

Disclosure Statement

No conflict of interest to report.

References


