Protein Requirement of the Extremely Low-Birthweight Preterm Infant

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CLINICAL OBJECTIVES: WHAT ARE THE OUTCOME PRIORITIES?

In most neonatal units, the number of extremely immature infants has increased considerably over the last 15 years. Their mortality and morbidity have decreased, and more attention has been paid to their nutritional needs.

There is continuity between intrauterine and postnatal life for all processes related to growth, including protein metabolism. During the last two decades, the development of bedside investigative methods has provided new insight into the processes of whole-body protein accretion in the mother during pregnancy, in the fetus, and in the preterm infant. However, the major difficulty in evaluating the protein needs of the extremely low-birthweight (ELBW; < 1,000 g birthweight) preterm infant is the lack of a generally accepted goal for feeding such infants.

For practical purposes the goals chosen here for the most appropriate protein supply for the ELBW infant are:

1. to achieve a protein gain that approximates the in utero protein gain of a normal fetus of the same postconceptional age (Fig. 1); and
2. to achieve long-term linear growth and psychomotor development close to the physiological ranges for normal term infants of the same corrected (postconceptional) age.

By combining the information given by the factorial, metabolic, and neurodevelopmental approaches, a range of advisable enteral and parenteral intakes can be defined. During the transitional early postnatal period, when a combination of enteral and parenteral nutrition is needed. Protein is increased in daily increments from 0.5 to 3.6 to 3.8 g/kg-d during the first 14 days. Similarly, the energy supply increases from 35 to 130 kcal/kg-d over the same period (Fig. 2). The time course of the enteral protein energy supply often has to be lengthened according to the clinical situation. Once full enteral feeding is achieved, most ELBW infants have growth rates similar to the intrauterine values.
PROTEIN REQUIREMENT OF PRETERM INFANT

FIG. 1. Overview of weight gain of the fetus and placenta during gestation. The protein gain of the fetus (shaded area) is compared with the total rate of protein gain (mother + fetus + placenta). The ratio of protein synthesis to gain decreases during gestation, reflecting the fact that less protein needs to be synthesized for every gram of protein gained. Thus protein turnover seems to cycle on a less futile mode at the end of gestation. (Adapted from refs. 31, 38, and 39.)

INTRAUTERINE AND POSTNATAL GROWTH

We have no better model for growing ELBW and VLBW (very low birthweight; <1,500 g birthweight) preterm infants than the growing fetus of the same gestational age. Thus it is important to look at fetal growth curves and at the evolution of fetal whole-body composition during gestation before going into more detail about the physiological aspects of amino acid and protein metabolism. Data on the “average” fetus, obtained previously from infants born at different gestational ages, have been greatly improved over the last 15 years by serial ultrasonographic measurements during normal pregnancies. More recently, data on fetal and postnatal protein content and accretion have reached a sufficient degree of reliability and can be used at the bedside to plan the nutritional management of an extremely low-birthweight preterm infant.
Figure 3 shows the continuity between intrauterine and extrauterine growth for body length, body weight, and body content of lean mass and fat. It is apparent that for the period between 24 and 28 weeks, growth rates are extremely high (~2.5% and 1.5% per day, for weight and length gain, respectively). This growth reflects the increase in lean mass, with daily protein gains of 2.1 to 2.2 g/kg·d. The corresponding transplacental amino acid and glucose uptakes are, respectively, 3 to 3.5 g/kg·d and 7 to 10 g/kg·d.

Thus by giving intravenous amino acids as soon as possible after birth to ELBW infants, an attempt is made to minimize the effects of an acute withdrawal of amino acid supply at the postconceptional age of maximum protein gain.

BODY COMPOSITION

Measurements of body composition in preterm infants are of major importance and represent the basis for the estimation of protein needs. The total protein content of a 26-week-old fetus weighing 900 g must increase by about fivefold to reach the values observed in a term infant of 3,500 g. There is a concomitant increase in serum protein concentration and a decrease in the hydration of lean tissue from 86% at 26 weeks to 80% at term (1–3).

FIG. 2. Intakes of fluid, energy, and protein + amino acids during the transitional phase of extrauterine life. This figure applies to “healthy” extremely low-birthweight (ELBW) infants. (Adapted from refs. 40 and 41.)
PROTEIN REQUIREMENT OF PRETERM INFANT

- Body length
- Weight
- Fat
- Lean mass

Rate of growth (%/day)

Protein gain (g/kgxday)

Metabolizable protein intake

Age: 24-40 weeks, 2-6 months
Measurements of the fetal body composition at sequential gestational ages have been published by Widdowson (1,2) and by Ziegler (3), whereas Fomon et al. (4) have compiled normative data on body composition for the term baby, from birth up to 10 years. Figure 3 shows the pattern of change in lean body mass and fat during growth. Lean mass is a heterogeneous compartment, composed essentially of water, protein, glycogen, and minerals. The protein content of the lean body mass increases with gestational age (1,3). It is apparent that there is a rapid growth of fat-free weight and fat weight during the intrauterine period. At 22 weeks the fetus is approximately 99% lean mass and 1% fat, compared with 86% and 14% at 40 weeks, respectively (see Fig. 3).

PHYSIOLOGY

Protein Gain to Protein Intake Relation

The simplest physiological issue related to protein metabolism and growth is the proportion of the daily protein intake incorporated into the lean tissues (protein gain). This can be determined by the nitrogen balance technique. Nitrogen balance is the difference between nitrogen intake and nitrogen excretion. In spite of several potential sources of variability (5–7), the results of several studies are in good agreement (see Fig. 3). This method is the one most extensively used for studying protein metabolism in vivo, even in very preterm infants (5–13).

Once nitrogen balance has been calculated \(N\), the protein gain can be obtained using the factor of gram \(N \times 6.25 = \text{grams of protein}\).

The mean values of various neonatal studies relating protein gain to intake are shown in Fig. 3. The daily protein gain increases linearly with protein intake in the range between ~2 and ~4 g protein/kg-day. Above this level it appears that the effect on protein gain is diminished. It should be noted, however, that newborn infants receiving the highest protein intakes had altered plasma aminograms (10,11). Thus even if it were possible to achieve further protein gain with a diet providing more than 4 g protein/kg-day, this would be undesirable because of the deleterious metabolic consequences.

Coefficient of Protein Utilization

The relation between protein intake and protein gain gives an index of the efficiency whereby metabolizable protein intake can be channeled to tissue growth. This is sometimes called the coefficient of protein utilization or the efficiency of protein gain.
(ratio of protein gain to intake). Many factors are known to affect protein utilization, and these can be divided as follows:

- **Nutritional factors**—that is, the biological value of protein ingested, the energy-to-protein ratio, and the nutritional status (6,7).
- **Physiological factors**—for example, individual variations, catch-up growth in small-for-gestational-age infants (14–16).
- **Endocrine factors**, including insulin-like growth factors and others.
- **Pathological factors**—for example, sepsis and other disease states.

In "healthy" preterm infants, the efficiency of protein gain has been fairly well established at a mean value of 0.7 (70%). The mean (±SD) of the published values is 0.72 ± 0.08 for 331 infants ranging between 26 and 35 weeks of gestational age. This means that 70% of the absorbed amino acids are laid down as protein in the tissues and that the remaining 30% are oxidized and excreted. It is of interest that, according to these studies, the coefficient seems to be independent of gestational age.

Since the synthesis of protein molecules entails a considerable amount of energy, the protein–energy interaction and the in vivo contribution of protein gain to the energy expenditure during rapid growth both need brief discussion.

**PROTEIN–ENERGY INTERACTION**

Protein gain is related not only to protein intake, but also to energy intake (6,7,12,13,17–19). Therefore rapid growth is a situation in which protein-energy interrelations are of special relevance. One cannot consider the effects of protein intake and energy intake on protein gain independently of each other. Protein and energy are supplied concurrently, and it is likely that there is an optimal range of protein and energy intakes for each newborn infant. If this optimum is not achieved, the following consequences are observed:

- When energy intake is deficient, endogenous protein is used as an energy source and the nitrogen balance becomes negative.
- When energy intake reaches a suboptimal level (metabolizable energy ~50 to 90 kcal/kg·d), the newborn infant is in a very sensitive range of protein energy interaction. An increase in either the energy intake or the protein intake will result in an increase in nitrogen retention (Fig. 4). Similarly, if protein intake is suboptimal, then increasing the energy intake will spare protein for lean tissue gain. Situations of suboptimal protein energy supply are frequently met, both in ELBW infants during the postnatal transitional period and in other neonatal intensive-care situations, and have been repeatedly investigated (6,7).
- If there is a surfeit of energy for a given protein intake, the protein gain plateaus and there is no further positive effect of increasing energy intake.

By combining parenteral and enteral feeding, it is possible to maintain a positive protein gain during the transitional phase of extrauterine adaptation.
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FIG. 4. Effect of increasing energy intake on protein gain at different protein intakes grouped into five categories: 2.0 (○), 2.5 (□), 3 (■), 3.5 (●), and 4 (●) g/kg-d. Each symbol represents the mean value of a group of newborns. In the range of “suboptimal metabolizable energy supply” (50 to 90 kcal/kg-d), protein gain can be improved by increasing energy intakes, whereas above 100 kcal/kg-d there is no further positive effect on protein gain. (Adapted from refs. 7, 8, 12, 14, 18, 19, 21 25, 38, and 40.)

Metabolic Cost of Protein Gain

The energy required for protein deposition can be partitioned into the “energy content” of the gained protein (also called retained or stored energy; 4 kcal/g protein) and the extra “energy expenditure” (20) associated with the formation of new protein (called the metabolic cost of protein gain). The latter can be estimated either at the bedside, by correlating the results of nitrogen balance to measurements of energy expenditure, or theoretically on the basis of the energy equivalent of ATP.

In vivo studies have shown a linear relation between energy expenditure and protein gain. From the slope of the regression line one can infer that the metabolic cost of protein gain is approximately 10 kcal/g gain (20–23). A theoretical approach can also be used to estimate the cost of protein gain in a growing baby (20). The incorporation of 1 mole of amino acids into protein requires 6 mole of ATP. If one takes an average value of 18 kcal energy released per mole ATP, 108 kcal (6 × 18) are needed to incorporate 1 mole of amino acids into protein. Assuming that 1 mole of an average amino acid is about 110 g, the theoretical cost of protein gain is approximately 1 kcal/g.
The discrepancy between this result (1 kcal/g) and the in vivo data obtained at the bedside (∼10 kcal/g) is striking and prompts us to re-examine the theoretical cost of protein gain. The theoretical approach makes the incorrect assumption that protein gain during growth is equal to the amount of protein synthesized. It is therefore called a static approach. The difference between the in vivo measurements and the theoretical calculations can be explained by the dynamics of protein turnover, where protein gain results from the difference between protein synthesis and breakdown, and each gram of protein gain needs five to six times more protein to be synthesized.

PROTEIN TURNOVER AND ITS CLINICAL RELEVANCE

Stable Isotope Techniques and Protein Turnover

The dynamic aspects of protein metabolism have been studied by using nonradioactive, stable 15-N or 13-C labeled amino acids as biological markers (5,8,14,24). Whole-body protein synthesis and protein breakdown in very low-birthweight preterm infants reared on human milk, reared on milk formulas, or given total parenteral nutrition were found to be increased when compared with older infants (8,20). Since the formation of peptide bonds has a high energy cost, it is of interest to determine the rate of protein synthesis and the rate of energy expenditure simultaneously. The validity of the methods used metabolism of stable isotopes, and indirect calorimetry is generally well accepted (5,20). A stable isotope enrichment technique for the in vivo assessment of protein turnover can be summarized in the following example.

Constant doses of 15-N glycine are added to each feed given to a preterm infant. Since this technique requires repeated feeding at short, regular intervals, preterm infants who are typically fed every 3 hours by intragastric tube represent an ideal situation for such measurements. Soon after the beginning of the experiment, the infant’s urinary excretion of 15-N urea increases. Within 48 to 60 hours the enrichment of 15-N in urinary urea appears to have reached a plateau. This plateau allows the computation of the nitrogen flux through the amino acid pool as well as the rates of protein synthesis and protein breakdown (24).

Ratio of Protein Synthesis to Protein Gain

The preterm baby has a rate of protein synthesis that greatly exceeds that necessary for net protein gain (10 g protein/kg·d for synthesis versus 2 g protein/kg·d for gain). Energetically, the excess protein synthesis would appear to be a wasteful mechanism. However, it has been suggested that this “futile cycling” can also be considered from the evolutionary point of view as a positive adaptation. For example, in cases of suboptimal supply of protein and energy, the protein gain decreases while protein synthesis and breakdown decrease in parallel, with a concomitant reduction in energy needs (20). This dynamic mechanism of protein turnover may also be viewed as a physiological phenomenon allowing fast remodeling of body protein during rapid growth.
Cellular and Molecular Aspects

All in vivo tracer studies of whole protein metabolism support the conclusion that the more rapid the expected growth and expected protein gain, the higher the rates of protein synthesis and breakdown. This means that the net intracellular production of protein must be adjusted with a high sensitivity through regulated changes in the rates of protein synthesis and breakdown.

The key genes regulating protein breakdown have been cloned. Thus there is hope that information will be provided relatively soon at the molecular level to answer a series of clinical questions regarding situations of growth and nongrowth, as well as metabolic responses of “sick” neonates to stress (8,14,24–26).

Endocrinological Aspects

Insulin and insulin-like growth factors appear to be the major hormones regulating fetal growth. During intrauterine life and during early postnatal life in very preterm infants, the secretion of insulin depends on the plasma concentration of certain amino acids (e.g., arginine and leucine) as much as, or perhaps more than, on the concentration of glucose (27). Thus a shortage of supply in these and perhaps other amino acids not only limits protein metabolism but also, through a reduction in insulin and insulin-like growth factors, directly limits glucose transport and energy metabolism.

A particularly interesting group of proteins with a high turnover rate is the family of glucose transporters. Their molecular physiology and regulation have recently been fairly well established. Briefly, a shortage in amino acid can rapidly have the following effects:

- Reduce insulin and insulin-like growth factors.
- Downregulate glucose transporters at the cell membrane level.
- Result in intracellular energy failure.

By giving intravenous amino acids as soon as possible after birth a step is taken toward preventing downregulation of glucose transporters, hyperglycemia, hyperkalemia, and intracellular energy failure.

FACTORS AFFECTING PROTEIN TURNOVER

Postconceptional Age

A significant inverse relation was observed between postconceptional age and the ratio of protein synthesis to gain; thus the more immature the infant, the higher the rate of protein turnover (14). This relation is also apparent in the interstudy comparison effect of gestational age (Fig. 5). Similar studies of animal fetuses show that the rate of protein synthesis per unit body weight decreases throughout gestation. It appears that besides energy and protein intakes, postconceptional (or postmenstrual) age plays a major role in the regulation of whole-body protein metabolism. This age-
FIG. 5. Each symbol represents the mean results of protein-synthesis-to-protein-gain ratio in appropriate-for-gestation (AGA) premature infants, obtained by using 15-N glycine as tracer and 15-N urea as end product. The ratio of protein synthesis to protein gain decreases with postconceptional age. As the same trend can be observed in utero (see Fig. 1), it is reasonable to assume that very immature infants (<28 weeks) have very high protein turnover rates, need to synthesize (and break down) much more protein than needed for their gain, and thus have a high metabolic cost of growth. (Adapted from refs. 38–40.)

related effect may influence the optimal percentage of protein energy to be given to very immature infants below 27 weeks of gestation.

Nutritional Status

It has been observed that the protein turnover, synthesis, and breakdown rates are higher during catch-up growth in infants born small for gestational age than in normally grown infants of the same gestation. These observations are consistent with the studies on the rate of protein turnover in malnourished infants during and after the completion of refeeding.

On the other hand, when the protein turnover of a group of small-for-gestational-age infants was compared with that of a group of identically fed preterm infants of the same birthweight, the former had the lower values and the better protein-synthesis-to-gain ratio. This may explain why small-for-gestational-age infants have a faster rate of postnatal growth than identically fed, normally grown preterm infants (14,25).
Route of Feeding

Amniotic fluid is swallowed throughout most of gestation. It is therefore unphysiological to completely deprive an ELBW preterm infant of enteral feeding, since this deprivation would never normally occur in utero. The problems linked to enteral feeding in very immature infants have been thoroughly reviewed (28–37). Large gastric aspirates and abdominal distention are common, leading to frequent interruptions or decreases in enteral feeds. Thus several days may pass during which the nutrient intake is grossly deficient, with the risk of a further reduction in gut function.

It has been well established in experimental animals that the growth of the intestinal tract in the neonate is dependent on enteral feeding. Infants who are fed intravenously have changes in their gut hormones and significantly lower protein turnover rates in the intestinal tract. It appears that this lower turnover is probably linked to differences in visceral protein metabolism and gut hormones (38–40). For these reasons it makes biological sense to try to avoid a complete interruption in enteral feeding and to give small, subnutritional feeds, since these should stimulate gut development. The concept of “minimal enteral feeding” has been coined by Lucas (41). Indeed, it has been proven successful, because it dissociates the nutritional from the nonnutritional or subnutritional effects of enteral feeding. Minimal enteral feeding may stimulate insulin release and decrease glucose intolerance, which is a significant problem in extremely low-birthweight preterm infants.

The primary purpose of making an early start to enteral feeding would not be to achieve complete enteral feeding as soon as possible, but to initiate the postnatal adaptive events in the gut and intermediary metabolism at an “unphysiological” time of development (41).

CLINICAL ASPECTS

Hyperglycemia

Hyperglycemia is a common problem in very low-birthweight preterm infants with reported incidences as high as 20% to 86%, particularly during the first few days of life. It is often associated with nonoliguric hyperkalemia. The risks linked to this problem are partly the result of hyperosmolality, glucosuria, and dehydration; the major risks, however, are intracellular energy failure and Na⁺/K⁺-ATPase deficiency (41,42).

Classically, high glucose concentrations in neonates are attributed to both increased secretion of hormones with counterregulatory effects on hypoglycemia (catecholamines, glucagon, and, longer term, cortisol) and diminished end organ sensitivity to insulin. This does not apply to very preterm infants, as they have low catecholamine secretion rates and respond normally to exogenous insulin (41).

The cause appears to be reduced insulin secretion in response to glucose, characteristic of extreme immaturity, and reduced insulin secretion in response to a fall in the plasma concentration of those circulating amino acids responsible for stimulating insulin (arginine and leucine, for example). Giving amino acids as soon as possible...
after birth stimulates endogenous insulin secretion and can prevent to a certain extent the need for intravenous insulin infusions.

**Potential Toxicity of Protein and Amino Acid Linked to Biochemical Immaturity of the Preterm Infant**

Premature infants have incomplete development of several amino acid metabolic pathways. This biochemical immaturity substantially narrows the margin between an adequate protein intake and the possible adverse effects of deficiency or excess. Many of the amino acids previously thought to be nonessential may be essential for the immature organism and must be supplied by the diet (e.g., taurine and glycine). Incomplete amino acid catabolism, in case of an excessive protein intake, may result in increased concentrations of amino acids, hydrogen ion, and ammonia (12). It is still a matter of discussion whether these metabolic changes are harmful or benign; indeed, similar values have been found in fetal blood at fetoscopy and from the umbilical cord at birth (26,31,38,40).

At least three different gold standards have been proposed for assessing plasma amino acid responses to feeding in the prematurely born infant: the amino acid concentrations of mid-trimester fetal blood, the concentrations in rapidly growing preterm infants receiving their own mother’s milk, and the concentrations in healthy breastfed term infants. In general, the blood amino acid levels of preterm infants fed either preterm milk or protein-enriched human milk fall within the broad range of plasma amino acids reported for breastfed term infants (26,38,40).

**METHODS FOR DETERMINING IF THE PROTEIN NEEDS OF ELBW INFANTS ARE MET**

Weight gain is a fundamental expression of growth, and although it is nonspecific and provides no information on changes in body composition, it remains the cornerstone of growth assessment. Length gain is less susceptible to the confounding effects of changing body composition and should be a better estimate of gain in lean body mass. However, the practical limitation is that measurements of length, unless they are taken over long observation periods, are difficult to perform reproducibly (40).

More specific indicators of the adequacy of protein intake include serum concentrations of albumin, total protein, prealbumin, and retinol binding protein (5). The usefulness of these serum biochemical indices is severely limited by the difficulties in interpretation. Severe deficiencies are easily classified as abnormal, but borderline values are not (40). Serum and urine urea concentrations, however, have proved to be of real clinical interest. Inadequate or excessive protein intakes can be detected by measurement of urea concentrations in serum or urine. The latter obviates blood sampling.

The physiological approach appears to be the most logical at present: an adequate protein intake results in rates of weight gain and protein gain approximating *in utero* growth. Protein intake must be considered in conjunction with energy intake since
the energetic cost of growth is mainly dependent upon protein metabolism. To illustrate this point, 10 metabolic studies were selected on the basis that the rates of protein gain measured in these studies were very similar to intrauterine values (38,40). Interestingly, this effect was obtained in a relatively narrow range of protein and energy intakes, and served as a basis for guidelines (38,40).

However, the weight gains of the infants studied, as well as the composition of their weight gains, were different, as evidenced by various energy densities (retained energy/unit weight). The highest energy and the lowest protein intakes led to the greatest fat deposition. The highest protein and lowest energy intakes had the opposite effect (38,40).

A comparison between the results of the previously mentioned studies (41–43) and the estimated values of the American Academy of Pediatrics (44) strongly emphasizes the fact that “healthy” low-birthweight infants can accommodate various levels of protein intake, provided the energy intake is not limiting.

The amino acid profile in plasma and the coefficient of protein utilization can help assess the type of protein most suitable for growth. Premature infants fed whey/casein protein ratios of 60/40 (similar to that of breast milk) have reasonably well-balanced plasma amino acids (40) and have a coefficient of protein utilization (protein gain/intake) of 0.7, as shown in the median part of Fig. 3. Higher proportions of casein cannot be handled with the same efficiency, as evidenced by the development of metabolic acidosis and higher plasma tyrosine and phenylalanine concentrations reported with 18/82 whey/casein ratio formulas.

The major proteins supplied in enteral nutrition to preterm infants are derived either from human milk (70% human whey, 30% human casein) or from bovine milk (60% or 18% bovine whey and 40% or 82% bovine casein). Bovine casein proteins are particularly rich in phenylalanine and tyrosine, so infants fed formulas in which bovine casein predominates typically have higher concentrations of plasma phenylalanine and tyrosine than infants fed formulas with a predominance of bovine whey or infants fed human milk. Bovine whey proteins are very rich in threonine, in contrast to bovine casein proteins or human milk whey proteins. Infants fed bovine whey protein–predominant formulas have increased plasma threonine concentrations over those fed either bovine casein protein–predominant formulas or human milk (28,31,38). At present, it is difficult to know the implications of this for protein metabolism in preterm infants, particularly ELBW infants.

AVAILABLE FOODS AND RECOMMENDATION FOR THE STABLE GROWING PERIOD

Recommendations for intakes during the stable growth period are given in Tables 1 and 2.

**Human Milk**

The protein content of mature human milk is about 1.2 g/dl (1.8 g/100 kcal) when expressed as total nitrogen × 6.25; about 25% of the total nitrogen is nonprotein nitro-
TABLE 1. Recommendations for the stable growing period

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<td>Body weight (g)</td>
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<td>Advisable intake</td>
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<tr>
<td>Body weight (g)</td>
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<td>1001–1800</td>
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Not all human milk proteins are fully absorbed from the gut, and thus the nutritionally available protein may be less than expected (11,37,42). Colostrum (milk produced in the first 5 days postpartum) contains 2.3 g protein/dl, and transitional human milk (6 to 10 days postpartum) contains 1.6 g protein/dl. It has been reported that the milk of women delivering prematurely contains approximately 20% more nitrogen than the milk of mothers delivering at term. However, the higher nitrogen content decreases rapidly postnatally, and after the first 14 days there is no, or only a minimal, difference between preterm and term milk. The whey proteins represent more than 70% of the total proteins in human milk (28). Unfortified human milk from milk banks is usually inadequate in protein content for VLBW preterm infants, and its use may result in growth failure and other long-term handicaps (29). Banked milk can be used successfully when enriched with human milk protein or with bovine milk preparations (38,40,41).

Cow’s Milk–Based Formula

Formulas designed for VLBW infants have a higher protein content (1.8 to 2.4 g/dl) and a higher energy density (75 to 85 kcal/dl) than the formulas generally used for term infants. They provide about 2.8 to 3.2 g protein/100 kcal.

Own Mother’s Milk

A daily intake of about 250 ml/kg of banked human milk (containing 1.2 g protein/dl) would be necessary to provide a protein intake of 3.0 g/kg·d in a preterm infant, but such high intakes are not commonly used. Intakes of 185 to 200 ml/kg·day of own mother’s fresh milk are not unusual in moderately low-birthweight preterm infants (around 1.500 g) but are very unusual in ELBW infants. They may be considered an
TABLE 2. Practical application: protein and fluid supply during the transitional period of the first postnatal days. ELBW <1000 g, <27 weeks.

All these figures have to be modified according to individual needs.

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<td>60</td>
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<td>Amino acid (g/kg)</td>
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<td>0.75</td>
<td>1.0</td>
<td>1.25</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
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<td>1.5</td>
<td>1.2</td>
<td>1.0</td>
<td>0.5</td>
<td>—</td>
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<tr>
<td>Total protein + a. acid enteral + parenteral (g/kg)</td>
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<td>1.0</td>
<td>1.5</td>
<td>2.0</td>
<td>2.5</td>
<td>2.7</td>
<td>2.9</td>
<td>3.2</td>
<td>3.4</td>
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<td>3.8</td>
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Modify intakes if:
- Unexplained metabolic acidosis (check ammonia, reduce protein intake, investigate metabolism)
- Weight loss >10% of birthweight: increase fluids, watch glucosuria.
- Infant on a ventilator (fluid reduction around 20 ml/kg)
- Weight gain during the first 3–4 postnatal days (fluid reduction around 20 ml/kg)
- As long as body weight < birthweight take the latter as reference for calculations

Preterm formula 2.4 g protein/dl; 85 kcal/dl; 2.8 g protein/100 kcal
Enriched mother's milk 2.1 g protein/dl; 85 kcal/dl; 2.45 g protein 100 kcal (approximative values)
"ideal" intake. The amino acid profile of such infants has been proposed as the gold standard. However, when own mother's milk is not available in such large quantities or if the mother's milk is heat-treated, this ideal situation ceases to hold (40).

In early life, when the protective factors of maternal milk may be of greatest value, it is an advantage to provide the mother's own fresh preterm milk, especially for very immature ELBW infants (41). Soon after this critical period for survival is over, the metabolic needs of growth and development have to be met. For such matching to occur, the mother's milk needs to be enriched or a preterm formula needs to be provided. Owing to the many advantages of nonnutritional components in human milk, fortified mother's milk or fortified donor milk (if available) should be the diet of first choice. However, if human milk is not available, special preterm formulas can be used.

Parenteral Amino Acids

The optimum concentration and composition of parenteral amino acids for preterm infants are unknown. However, the current amino acid formulations come close to meeting their needs, as far as can be observed on the basis of growth, nitrogen retention, plasma amino acid profile, and acid/base status. From recent reviews of the subject (40) one can conclude that preterm infants receiving total parenteral nutrition require more than 70 kcal/kg of nonprotein energy daily and more than 2.5 g/kg of amino acids to achieve acceptable (albeit not optimal) protein gain. Parenteral amino acids are used not only for total parenteral nutrition in preterm infants who cannot be fed enterally, but also as an obligatory adjunct to enteral feeding during the first postnatal days in ELBW infants.

CLINICAL CONDITION AFFECTING THE PROTEIN REQUIREMENT

Protein Requirements of "Sick," Nongrowing, Very Low-Birthweight Infants

Very commonly, the clinician has to deal with life-threatening illnesses that severely stress the infant, so that growth does not occur even when all nutrients are provided. The goal of nutritional management is limited to a nitrogen balance close to equilibrium. A pragmatic approach suggests that about 1.0 to 1.5 g/kg·d of protein, given as intravenous amino acids, would be a reasonable estimate. These figures may even be too high in some critically ill ELBW infants. Another approach stems from physiological studies in stable, growing preterm infants. The idea is to define "minimum intakes" of protein and energy that would prevent net protein loss by keeping protein synthesis and breakdown in balance. An extrapolation of the regression lines of protein gain versus intake (20,38,40) shows that a protein intake of 0.5 g/kg·d is associated with zero net protein gain. However, the relevance of these findings to the situation of the unstable, nongrowing ELBW infant is unknown.

A reasonable way of solving the issue is to start with a "minimum protein intake" of 0.5 g/kg·d and to increase this gradually if the sick neonate seems free of adverse effects. So long as the infant is critically ill, the intakes should not go beyond the "pragmatic limit" of 1.0 to 1.5 g/kg·d. An intake of this order may be expected to
maintain the infant at zero protein gain. In this situation net synthesis of protein equals protein breakdown.

**Protein Requirements of Small-for-Gestational-Age, Extremely Low-Birthweight Infants**

Neither the American nor the European recommendations (28,40,44) differentiate small-for-gestational-age (SGA) from appropriate-for-gestational-age (AGA) ELBW infants. In fact, in many neonatal units SGA infants receive higher protein and energy intakes than AGA infants to facilitate their catch-up growth. Published studies comparing the protein metabolism of a group of SGA infants with a group of AGA infants (13,16) used feeding schedules that were not the same in the two groups and thus did not answer the clinical question of whether SGA infants should be fed differently from AGA infants. A study comparing a group of AGA infants with a group of SGA infants under the same feeding conditions showed that the latter have a slower rate of protein turnover (20). This means that for the same gain of protein, the rates of synthesis and breakdown are smaller in SGA infants than in AGA infants (13,38,40).

Thus, being more mature in various physiological and biochemical systems, SGA infants have a lower protein-synthesis-to-gain ratio than AGA infants of the same birthweight and are consequently better able to handle protein efficiently. They may be able to tolerate higher protein intakes without adverse effects. However, even during catch-up growth, there is no conclusive evidence that protein intakes beyond the recommended values should be beneficial.

**EARLY DIET AND LONG-TERM OUTCOMES OF EXTREMELY LOW-BIRTHWEIGHT PRETERM INFANTS**

**Growth**

In “healthy” ELBW (without major problems during the first postnatal months), growth rates are in the lower range of normal values during the first 3 years of life (41). Although genetic and parenting factors may explain these findings, there is evidence that the reduced growth rates are linked with an inadequate protein energy supply during the early postnatal period (42).

**Neurodevelopment**

In parallel with the successful regionalization of perinatal care, various neonatal centers have reported a decline in mortality and major neurological impairment in ELBW infants (45–47). The results shown in Fig. 6 illustrate this trend.

**Major Impairments**

Major impairments include cerebral palsy, mental retardation, severe visual and auditory disorders, epilepsy, and multiple handicap. Because of the many neurological
FIG. 6. The results of our reference center for a geographically well-defined population of 1 million residents (9,000 to 10,000 deliveries/year) are shown. The data cover a period of 22 years. Since the mid-1970s, nearly all preterm infants <32 weeks and very low-birthweight infants have been born in the university hospital. **Upper panel:** In parallel with the steady decline in mortality, there has been a decrease in major handicap. **Lower panel:** The data have been divided into two periods (1972-1982 and 1982-1994). The year 1982 was chosen as a break point because several advances (improved perinatal care, improved neonatal intensive-care technology, and cerebral ultrasound) were taking place around that time. The comparison between the two periods shows that overall there has been a decrease in mortality and a lower incidence of major and minor handicaps among survivors. The possibility that early diet, at a potentially vulnerable period of brain development, could have an impact on long-term developmental outcome and prevent minor handicaps, the so-called new morbidities, is a key question with important implications for practice.

**Minor Impairments**

Roughly one in five surviving ELBW infants has “minor” neurodevelopmental impairment (see Fig. 6). These include moderate visual/auditory disorders, mild neuromotor signs, and language, learning, and behavior problems. The possibility that an improvement in early postnatal diet, including substances that influence neurodevel-
opment (e.g., amino acids, long-chain lipids, hormones, and growth factors), might prevent these handicaps requires further investigation.

REFERENCES


47. Monset-Couchard M, de Bethmann O, Kastler B. Mid- and long-term outcome of 89 premature infants weighing less than 1000 g at birth—all appropriate for gestational age. *Biol Neonate* 1996;70:328–338.

DISCUSSION

Dr. Putet: You said that 63% of your infants weighing less than 1,000 g were fed according to the guidelines. Have you done any follow-up on them? Did they grow better than the others?

Dr. Micheli: Babies fed according to the guidelines do better, but they are still below the normal centile range.

Prof. Ziegler: You said that 63% of the babies were fed according to guidelines. How did you define this? Do you have energy guidelines, protein guidelines, guidelines for other nutrients? And how did you define that the baby was in compliance? Was it compliance on all days or on certain days? Could you explain this a bit more?

Dr. Micheli: It was a very pragmatic approach. Every day on rounds, we would check if the energy and protein intakes fell within the acceptable zone. If not, that was one day on which the guidelines were not met.

Prof. Ziegler: I think that the percentage meeting such guidelines in our nursery would be much lower than in yours. Why do you think that authoritative guidelines are so widely ignored?

Dr. Micheli: In general, I think the guidelines are not followed because the infants are perceived to be intolerant of enteral feeding, which may not always be the case. The fear of necrotizing enterocolitis also plays a major role.

Prof. Heird: I was interested that although 63% of the babies met the guideline, at discharge they were still well below the normal centile range. Does this mean the guidelines are not optimal, or does it mean that it is futile to attempt to achieve catch-up growth during this period?

Dr. Micheli: The guidelines are designed for stable growing periods. There is a gray area covering the periods of intermediate feeding, where you combine intravenous and enteral feeding during the first 12 postnatal weeks. I often wonder why we still keep depriving these fetuses of nutrients, because they are still fetuses—if they had stayed in utero, they would be receiving their full amino acid load every day. But we, on the other hand, suddenly decrease it to a fraction of what they should be getting, which constitutes acute deprivation of amino acids. Certainly this has consequences.

Prof. Heird: As I read them, the guidelines don’t really allow for catch-up growth; the magnitude of nutrient losses during this early period can be pretty staggering.

Dr. Guesry: As I understand it, the important fact is not that 63% of the babies received the guidelines but that during 2,700 days of hospital treatment of 79 babies they were fed only during 1,800 days. This means that as a group the babies received only two-thirds of the guidelines. There is no question that they were underfed. If you give only 2 g/kg of protein instead of 3 g/kg, they will be short of protein!

Dr. Micheli: I agree with you. I think it was clear that I was referring to days of care and not babies. Certainly there is a strong correlation between intake and growth. But other factors may be important in this kind of situation too. For example, why do ELBW babies so often have glucose intolerance? They get less glucose than they would receive in utero and fewer amino acids. One hypothesis from animal experiments concerns glucose transporters and the idea that these could be linked more to certain of the amino acids than to glucose itself. Insulin-like growth factor may also play an important role. Certainly it is no wonder that these infants grow less, but there are factors other than purely protein.

Prof. Moro: You mentioned that you had nine cases of NEC, or a rate of approximately 10% to 12%. Did the NEC occur before or after enteral feeds were begun, and do you use a regimen of minimal enteral feeding with human milk?
Dr. Micheli: All the cases occurred after the start of enteral feeding, and we begin minimal enteral feeding on day 2.

Prof. Haschke: You showed that you had an 18% incidence of minor handicap. Is this related to inadequate feeding? Do you have data that there is an interaction between not meeting the guidelines and developing handicap?

Dr. Micheli: No. The only thing I can say is that in half of these minor handicaps there was no documented hypoxic/hypotensive episodes and the cerebral ultrasonography was perfectly normal. We have to look for something else, and of course the first thing you think of is the impact of suboptimal nutrition.

Dr. Debauche: You told us that the babies stayed in the unit for about 4 weeks. Did you find any difference in compliance with the guidelines during the four separate weeks?

Dr. Micheli: Yes, of course, the number of episodes where the nurses stopped feeding was greater early on. But we still had surprises—babies who suddenly start having large gastric residuals at 15 days or 17 days. But most of the problems were in the first week.

Dr. Debauche: What are good guidelines for the first week?

Dr. Micheli: At present the guidelines don’t fix rules for the first postnatal days.

Prof. Lucas: I imagine that it was the smallest, sickest infants who were most likely to fail to meet the guidelines, because they were the ones causing most concern. Since those infants have the worst outcome for other reasons, it’s very difficult to disentangle whether failing to meet the guidelines does have adverse consequences or not. This is another reason for doing aggressive intervention trials on a randomized basis.

Dr. Micheli: I agree. Interventional studies need to be done to settle this point. We also need to know more about the growth of the brain, particularly the hippocampus, in relation to nutrition.

Dr. Lauterbach: What is the minimum weight of your babies when you decide to discharge them from hospital?

Dr. Micheli: We have no weight limit for discharge. We discharge in peripheral hospitals once the infants are stable and feeding well, with no apneic episodes or supplemental oxygen requirement. Some of them leave our unit at quite low weights compared with 10 or 15 years ago, when we discharged them once they reached 2 kg. Now we sometimes discharge infants as small as 1.2 kg.

Dr. Lightdale: Are you using indomethacin prophylaxis and is there a problem with persistent ductus arteriosus (PDA)?

Dr. Micheli: We use indomethacin early when there are clinical and/or echographic signs of PDA. This occurs in the majority of our ELBW infants, but we don’t use it prophylactically.

Prof. Ziegler: The main reason that it’s difficult to meet guidelines, or a reasonable protein intake, is that preterm formulas have too low a protein content for our very small premature babies, and the same is true for human milk fortifiers. They are too low in protein to meet a reasonable protein intake for the very small premature baby. I don’t know how you managed to achieve an intake of 3.6 or 3.8 g/kg-d, but in our nursery it would be very unusual to achieve such an intake. We have volume restrictions in our nursery that are imposed by other factors and considerations, and this means that we cannot achieve the volumes necessary to achieve a protein intake of 3.6 or 3.8 g/kg. So it’s not just that the resident withholds the feeding during the night because the abdomen gets distended; the other reason is that our feeding regimens across the board are too low in protein.

Prof. Lucas: The only preparations we have for increasing nutrient density, other than fortifiers that contain everything, are energy sources. We don’t in general add extra protein to the diet when volume is limited. That might be a positive suggestion for industry—it would be
really useful to have a commercially available protein supplement that could be added to either formula or human milk as protein alone. You would need to use it in various combinations with energy supplements, of course.

Prof. Heird: I think there is a larger problem, and that’s the rather general impression that the most important thing is the energy intake. When you’re dealing with a ready-made formula, that emphasis is fine, because the formula provides sufficient protein and other nutrients, and if you judge the intake by the calories, it will probably be reasonably adequate in other respects. But the automatic impression that you should do something to increase the energy intake completely ignores the important concept of the linkage between protein and energy that took years to work out. Maybe an even better suggestion would be to re-educate people in the equal importance of both energy and protein.

Dr. Schanler: There are at least two protein supplements available in the United States, and probably elsewhere around the world—a casein and a whey protein supplement. We would recommend using those in our nursery when we are concerned about poor growth.