Proteins, Peptides and Amino Acids: Role in Infant Nutrition

Sophie Nutten

Proteins are the main building blocks of the body. They are polymers composed of 30 or more amino acids. Twenty different standard amino acids combine to form the proteins. Some amino acids are essential dietary components, since they are not synthetized by human metabolic processes.

Proteins are present in and vital to every living cell. They are essential for healthy growth and development and also influence major functions of the body (fig. 1). They can influence metabolic parameters (weight gain and adipogenic activity), presence of beneficial bacteria in the gut, risk of developing atopic dermatitis, digestive functions and the renal system.

The infant’s first year is a critical time of rapid growth and development; this rapid growth must be supported by a high rate of protein synthesis. Infant nutrition requirements are primarily satisfied by a single and highly specific food source: breast milk. The composition of breast milk is the gold standard for estimated total protein and essential amino acid requirements during infancy [1]. Both total protein content and concentrations of individual proteins in human milk change throughout the first year of lactation to fulfill the needs of the infant. Milk proteins are also a source of biologically active peptides, released by gastrointestinal digestion, with a positive impact on body functions and ultimately health [2].

Infant formulas have been designed for infants who cannot be breastfed. These infant formulas need to be similar to breast milk in their composition but also in functional outcomes, to insure appropriate growth, optimal development, maturation of the immune system and programming of the metabolic system, for example [3]. They have been evolving throughout decades along with the scientific knowledge. Protein sources and processes have been optimized to provide amino acid profiles very similar to those found in breast milk. More recently, clinical data showed that a lower protein content in infant formula has long-term preventive impacts on body mass index and obesity risk [4].
In addition to guaranteeing healthy growth and development of bottle-fed infants, specific infant formulas have also been designed for specific needs, by modifying their protein component.

As an example, three different types of infant formulas have been designed for the management of cow’s milk allergy (affecting around 5% of infants): partially hydrolyzed, extensively hydrolyzed and amino-acid-based infant formulas (fig. 2). Allergy is triggered by protein components; one way to decrease allergenicity of proteins is to modify their conformation and/or structure responsible for the allergy, by cutting proteins into peptides which are no longer able to trigger an allergic reaction.

Some partially hydrolyzed whey-based infant formulas have been clinically proven to prevent atopic dermatitis in infants [5], and a first-ever FDA claim has been granted for their use in infants at risk of allergy. The specific process leading to partially hydrolyzed infant formulas leads to a reduction in allergenicity of the milk proteins and generates specific immunomodulatory peptides promoting beneficial effects.

Extensively hydrolyzed infant formulas result from a different production process leading to very short peptides, which have almost lost their allergic properties. These infant formulas have been designed for therapeutic applications, offering a solution to reduce symptoms in infants allergic to cow’s milk proteins.
Finally, in case of severe allergic reactions to cow’s milk proteins, only amino-acid-based infant formulas are able to decrease symptoms because they are totally devoid of allergens.

In conclusion, proteins provided via breast milk or infant formula are essential components of the infant’s diet and, therefore, the specific quality, quantity and conformation of these proteins are essential to sustain safe growth and development of infants.

References

1 Dupont C: Protein requirements during the first year of life. Am J Clin Nutr 2003;77(suppl):1544S–1549S.

Fig. 2. Infant formula designed for allergy management (modification of protein conformation and structure).
Hydrolyzed Proteins in Allergy

Silvia Salvatore and Yvan Vandenplas

Hydrolyzed proteins are used worldwide in the therapeutic management of infants with allergic manifestations and have long been proposed as a dietetic measure to prevent allergy in at-risk infants.

Cow’s milk (CM) allergy (CMA) is the most frequent food allergy in early childhood and affects about 0.5–6% of infants, but the disease range varies according to patient selection, type of feeding (breast- or bottle fed) and criteria of diagnosis (symptom based or challenge proven) [1]. CM presents two different fractions of proteins: casein and whey, with both having allergenic properties. Immunoreactive epitopes and peptide fragments of both β-lactoglobulin and casein have been well characterized [2]. Milk allergenicity is reduced by various processes but mainly by hydrolysis. Hydrolyzed formulas (HFs) differ according to the methods of hydrolysis (such as enzymatic hydrolysis, ultraheating, ultrafiltration, pressure and glycation), the timing of hydrolysis, the degree of hydrolysis (i.e. intact protein molecules are broken down into peptides of various molecular weights), the protein source (casein, whey, rice or soy) and other nonprotein components. Based on all the above-mentioned characteristics, the in vitro results and the clinical effects of one formula cannot be transferred to another one, and commercial old and new formulas, even if from the same manufacturer, are not always comparable. There is no general agreement on unique standards to specifically define partially (pHFs) or extensively hydrolyzed formulas (eHFs), but the distinction is generally made by the molecular weight and percentage of the peptide fragments. A pHF contains peptides with a molecular weight generally <6 kDa, ranging from 3 to 10 kDa, with some commercial pHFs containing 18% of peptides >6 kDa; an eHF usually has more than 90% of peptides <3 kDa, with 1–5% of peptides >3.5 kDa [3]. In contrast, the molecular weight of whole CM protein ranges from 14 kDa (α-lactalbumin) to 24 kDa (casein) up to 67 kDa (bovine serum albumin) [2]. The weight of peptides has immune and clinical relevance because the ‘bigger’ the peptide the ‘more allergenic’ it can be. Peptides >6 kDa, and predominantly those >10 kDa, frequently act as allergens [4], but already those in the
range between 0.97 and 1.4 kDa are able to bind IgE in vitro, those >1.4 kDa can produce skin reactivity and those >3 kDa can cause a type I reaction in sensitized patients [5]. pHFs have been developed with the aim of minimizing the number of sensitizing epitopes within CM proteins, while at the same time retaining peptides with sufficient size and immunogenicity to possibly stimulate the induction of oral tolerance. Two different meta-analyses [6, 7] showed that a specific whey-based pHF offers a valid option for primary allergy prevention, mainly for atopic dermatitis, in high-risk infants who are not exclusively breastfed. In one meta-analysis, it significantly halves the incidence of atopic dermatitis [11 trials; summary relative risk (RR) estimate 0.56, 95% CI 0.4–0.77] up to 3 years of life compared to a standard CM formula [6]. In the other meta-analysis involving 3,284 participants (1,027 in pHF and 2,257 in control groups), a reduction in all allergic diseases of 52% [5 randomized controlled trials (RCTs); RR 0.48, 95% CI 0.23–1.00] was found at 3 and 6 months of age, 38% at 12 months (4 RCTs; RR 0.62, 95% CI 0.45–0.85; number needed to treat 12) and 58% at 30–36 months (1 RCT; RR 0.42, 95% CI 0.19–0.90) compared to a standard formula [7]. For atopic dermatitis or atopic eczema (8 RCTs), using a random-effect model, the use of pHF compared with standard formula statistically significantly reduced the incidence of eczema at 1 year (4 RCTs; RR 0.68, 95% CI 0.48–0.98; I² ¼0%), but not at 4–6 months (5 RCTs), 2 years (3 RCTs) or 30–36 months (2 RCTs) [7].

In the (so far) largest dietary intervention study (GINI) that prospectively investigated in a randomized and double-blind design the allergy-preventive effect of three different HFs compared with a standard (CM) formula in a cohort of 2,252 infants with at least one first-degree allergic relative, a significant reduction in eczema was noted at all study points (1, 3, 6 and 10 years) when using pHF [odds ratio (OR) 0.56; 95% CI, 0.32–0.99] or a casein-based eHF (OR 0.42, 95% CI, 0.22–0.79) but not a whey-based eHF or a standard CM formula [8]. More studies are needed to determine the nutritional effect and the real benefit of pHFs in the prevention of CMA in both high- and low-risk infants.

Because pHFs contain large CM peptides that can cause severe reactions in CMA patients, pHFs are not recommended for treatment of CMA [1, 9], and CM protein-based eHF is the preferred option in CMA infants who are not breastfed [1, 9–11]. eHFs have been extensively hydrolyzed in order to destroy allergenic epitopes. However, the molecular weight profile only enables to differentiate protein characteristics of formulas, but does not clearly determine the allergenic formula properties and clinical response that should be tested in vivo. Although lower than in pHF, residual allergenicity is present even in eHF whilst the only anallergic formulas are the elemental ones based on free amino acids that cannot determine an immune stimulation [1, 9]. Amino-acid-based formulas (AAFs)
are recommended in infants who refuse or do not tolerate eHFs or in the most severe cases of CMA [1, 9, 11]. Compared to eHFs, AAFs have, in most countries, higher costs, different taste and possibly different long-term nutritional effects [9, 10].

There is limited evidence that the addition of probiotics (e.g. *Lactobacillus rhamnosus* GG or *Bifidobacterium breve*) to an eHF offers additional benefit [12, 13]. In a recent prospective trial in 38 infants with CMA (confirmed by food challenge) fed for 6 months a new rice protein-based eHF (with more than 95% rice peptides <3 kDa) without lactose but enriched with pectin, lysin and tryptophan, clinical tolerance and normal growth were noted in all patients [14].

The maintenance of a decreased well-balanced diet is not easy, especially in more severe cases of CMA, but is mandatory for each child. The choice of the eHF should be based on scientific evidence of efficacy, tolerance and nutritional adequacy [10].

References


Infant Formula with Partially Hydrolyzed Proteins in Functional Gastrointestinal Disorders

Yvan Vandenplas and Silvia Salvatore

Partially hydrolyzed protein formulas (pHFs) are increasingly used in the prevention of atopic disease and in the management of infants with functional gastrointestinal (GI) manifestations. Nowadays, pHFs are more likely to be used by primiparous women and those breastfeeding longer, and in infants with a family history of allergy. In a Cochrane review, no serious adverse events associated with pHF were reported. Adverse events were mentioned in three studies, but none was attributed to pHF. There may be a theoretical concern that both absorption and metabolism of pHF is faster than of intact protein formulas. Whether this has any impact on health outcome is not known. Long-term safety data are nonexistent.

A prospective double-blind, randomized crossover trial in 115 regurgitating infants showed a significant decrease in the mean number and volume of regurgitations with two thickened formulas, with statistically better results for pHFs. No difference was reported in stool frequency and consistency between the two groups.

Data suggest that pHFs may reduce infant colics. However, dietary changes also often include a reduction in lactose and supplementation with prebiotic oligosaccharides and structured lipids with a higher proportion of sn-2-position β-palmitate, decreasing the formation of calcium soaps. No randomized clinical trials have been performed demonstrating the efficacy of partially hydrolyzed protein as single change in the formula in infantile colic. Experience has shown that pHF can be a useful option when cow’s milk protein allergy is not a potential cause of the colic. In fact, various randomized controlled trials demonstrating the efficacy of pHFs have been published. However, the role of (reduced) lactose can be questioned, as soy formula was not associated with a decrease in infantile colic. There are insufficient data to recommend pHF as single dietary intervention in colicky infants as most studies included other dietary changes as well.
Constipation is more frequent in casein- than in whey-predominant formulas. pHFs result in more frequent and softer stools in nonconstipated infants. Significantly more stools were passed by breastfed infants and infants fed extensively hydrolyzed formula versus those receiving standard or soy-based formulas. Infants receiving breast milk or an extensively hydrolyzed formula had twice as many stools as the other formula groups. GI transit time is shorter in preterm infants fed pHF than in those fed standard formula. pHFs had a markedly shorter GI transit time (9.8 h) than standard infant formula (19 h). pHFs, fortified with pre- and/or probiotics, with high sn-2 palmitate in the fat blend or without palm oil as the main source of fat in the oil blend, have been tested lately and seem to offer a good alternative for managing functional constipation in infancy. There are no studies evaluating the efficacy of pHF as single intervention in constipated infants.

In infants with minor GI problems such as infantile colic, regurgitation and/or constipation who were fed for 14 days with a formula containing a mixture of oligosaccharides, partially hydrolyzed whey protein and low levels of lactose and palmitic acid in the β-position, a reduction in the frequency of colics and regurgitation was reported in 79 and 70% of infants, respectively, whereas an increase in defecation was noticed. Testing the same formula in 267 infants with infantile colic, the authors demonstrated a statistically significant decrease in colic episodes after 1 and 2 weeks compared to standard formula and simethicone.

In conclusion, based on the limited available literature, pHFs tend to have some beneficial effect on functional GI manifestations such as regurgitation and constipation, although the evidence is insufficient to formulate a recommendation.

**Suggested Reading**


Hydrolyzed Proteins in Preterm Infants

Thibault Senterre

Prematurity occurs during a critical period of development with the most rapid rate of growth in lifespan. The adequacy of nutritional support, in particular protein intakes, plays an important role in many short- and long-term outcomes. Proteins are the major driving force of growth and represent the major functional and structural components of the human body. Their properties and functions depend on the structure of their amino acid (AA) polypeptide chains. Body proteins are constantly degraded and synthesized to and from AAs and protein turnover is very high in preterm infants compared to older infants, children and adults. It implies that the AA pool of the body is in constant equilibrium with potential instabilities and adverse effects of either insufficient or excessive AA concentrations.

Postnatal enteral nutrition is essential to enhance gastrointestinal maturation and postnatal development, but feeding problems are very frequent in preterm infants. These infants frequently suffer from postnatal feeding intolerance and sometimes develop severe gastrointestinal diseases such as necrotizing enterocolitis. Human milk is considered the preferred source of nutrients for preterm infants but is not always available. Thus, the industry has developed specially designed formulas for preterm infants (PTFs, preterm infant formulas).

Most infant formulas are developed from cow’s milk after several adaptations to meet the infants’ requirements. Most of them contain intact cow’s milk proteins. Extensively and partially hydrolyzed protein formulas (HPFs) have been developed to treat cow’s milk protein allergy or to prevent allergic sensitization. These formulas are also proposed and used when facing several digestive and behavioral problems in infants. Thus, for different kinds of reasons, term infants’ HPFs have also been used in preterm infants, and the industry has also developed specific PTFs with hydrolyzed proteins (HPs).

Few studies have been published evaluating the use of HPs in preterm infants. Most studies included varying sources of protein, varying degrees of protein hydrolysis and varying nutrient contents.
These studies demonstrated that the protein source plays an important role in nutritional adequacy and that adequate sources need to be used in PTFs. Protein utilization and efficiency is generally lower for HPs. When protein intake is similar, a lower weight gain is generally observed with PTFs and a 10% increase in protein content is usually necessary to compensate for this reduction in protein utilization. Mineral absorption may also be reduced, and no data exist for trace elements and vitamins.

HPFs have also been proposed in preterm infants in order to improve their feeding tolerance. Most HPFs are associated with accelerated gastrointestinal transit time and softer stools, but without clear benefit on feeding tolerance. Preterm infants seem to be at similar risk of allergic diseases than term infants, but the preventive effect of HPFs has not been sufficiently explored especially in preterm infants.

In conclusion, the quantity and the quality of protein intakes play a major role in preterm infants. Most modern HPFs designed for preterm infants are well tolerated and have adapted their nutrient content to improve nutrient absorption and retention. However, their benefits and safety have not been demonstrated and further high-quality studies are needed.
Hydrolyzed Formula for Every Infant?

David M. Fleischer, Carina Venter and Yvan Vandenplas

Hydrolyzed formulas (HFs) are presently used primarily in infants that cannot be exclusively breastfed and those with documented cow’s milk (CM) allergy, and for primary prevention of allergic disease. HFs are increasingly being used worldwide (table 1), begging the question if HFs may be recommended as the optimal choice for all standard-risk, full-term infants who are not exclusively breastfed.

From the regulatory standpoint, all extensively HFs (eHFs) in the United States and Canada are approved for use only under physician supervision, thus not approved or commercialized for routine use in healthy infants. Only one of the partially HFs (pHFs) which contains 100% whey partially hydrolyzed with trypsin is approved, marketed and commercialized as a routine-use infant formula for healthy term infants. In Europe, eHFs fall under the category of food for special medical purposes, also meant for use under medical supervision, but pHFs in Europe have been commercialized for routine use for a number of years. However, the most recent directive from the European Food Safety Authority states that only pHFs containing 100% whey using a specific hydrolysis process are appropriate for use as routine formulas for healthy term infants.

Data regarding the nutritional adequacy of modern-day HFs are scarce and lack long-term data suggesting that growth in infants fed HF versus intact protein formula (IPF) is different. There may be theoretical concern that partially hydrolyzed protein is both absorbed and metabolized faster than intact protein; whether this has any impact on the health outcomes of infants is unknown, but available data from eHFs are reassuring.

While human breast milk is the optimal source of nutrition for multiple reasons, a 2006 systematic review determined there were no comparable long-term studies regarding the prolonged use of HFs versus breastfeeding [1]. There are studies, however, that have examined the use of various formulas as a primary source or supplement to reduce the risk of atopic disease. Meta-analyses of formula consumption and the risk of atopic dermatitis (AD) have found that infants fed pHF compared to
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<td>pHFs</td>
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<td>USA</td>
<td>pHF-W: Gerber® Good Start® Gentle/Soothe/ Gentle for Supplementing</td>
<td>eHF-W: Gerber® Extensive HA™</td>
<td>pHF-WC:Similac® Total Comfort™</td>
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<td>pHF-WC: Enfamil® Gentlease™/ Reguline™</td>
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<td>Similac total Comfort</td>
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**Table 2. Should HFs be considered for routine use in healthy, term infants who cannot be breastfed?**

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<td><strong>Regulatory status</strong></td>
<td>Per US, Canadian and European regulatory agencies, pHF-W meets requirements for routine use Other HFs do not</td>
<td>Data for many HFs are outdated, and hydrolyzation methods have changed, resulting in different HF compositions; therefore, data on modern-day HFs as a category are lacking</td>
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<td><strong>Allergenicity</strong></td>
<td>Europe: pHFs and eHFs considered ‘hypoallergenic’ North America: pHF-W may claim allergy risk reduction</td>
<td>Europe and North America: eHFs not for routine use even if demonstrated efficacy for allergy risk reduction or management of CM allergy Always for use under medical supervision</td>
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<td><strong>Metabolism</strong></td>
<td>The metabolic consequences of different peptide formations in HFs from industrial hydrolysis are unknown, but based on published data, there is insufficient evidence to consider HFs as potentially harmful to term infants</td>
<td>New peptides with unknown functions are formed during industrial hydrolysis, and peptides normally formed by digestion of milk proteins may be absent, which could result in different bioactive peptides with different effects</td>
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<td><strong>Absorption</strong></td>
<td>Adequate short-term growth has been demonstrated for all or most current HFs Select HFs have well-documented growth studies and health follow-up for up to 10 years No adverse safety issues identified by regulatory agencies</td>
<td>Long-term data are insufficient regarding absorption, blood metabolites and hormonal responses of various HFs vs. breastfeeding In preterm infants, weight gain rates were lower with older HFs</td>
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### Table 2. (continued)

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| **Gastric emptying**  | In term infants, some HFs induce gastric emptying closer to breast milk than IPFs  
                        | In preterm infants, decreased transit time promoted feeding tolerance | Some HFs likely result in faster gastric emptying based on several small studies, which may negatively affect gastric digestion |
| **Gut maturation**    | There are insufficient data to conclude that HFs delay gut maturation compared to IPFs | There are insufficient data to conclude that HFs accelerate gut maturation compared to IPFs |
| **Allergy prevention** | If breastfeeding is insufficient or not possible for the first 4–6 months of life, the use of certain HFs has been shown to reduce the risk of developing AD in high-risk infants | While some studies have not shown a beneficial effect with the use of certain HFs for allergy prevention, systematic review and meta-analysis generally support their use |
| **Cost-effectiveness** | There are sufficient data to suggest that pHF-W given to formula-fed infants at high risk for AD is cost-effective for the prevention of AD in several countries | Studies not done for most HFs  
The costs of formulas vary significantly among countries, making it difficult to incorporate this into a global decision-making process |
IPF had a lower risk of AD [2, 3], but there are significant limitations to these studies, making conclusions about the general use of HFs problematic. Some of the strongest evidence for use of HFs for allergy prevention comes from the German Infant Nutritional Intervventional (GINI) study, which followed 945 high-risk newborn infants in a randomized trial investigating the effects of breastfeeding supplemented with one of four formulas, CM, whey-based pHF (pHF-W), whey-based eHF (eHF-W) or casein-based eHF (eHF-C), in the first 4 months of life [4]. Feeding with either pHF-W or eHF-C had a preventive effect on the cumulative incidence of AD in high-risk children that lasted until 10 years, the current point of published data; however, it should be noted that the primary preventive effect on AD by using either pHF-W or eHF-C was seen within the first 2 years of life, with no significant change in effect in the remaining 8 years. Additional trials are needed in high-risk infants to confirm these findings.

Costs should be considered in decision-making regarding the choice of the formula, but global comparison of this is difficult given large cost differences in different countries. Data suggest that pHF given to infants who are not exclusively breastfed is a cost-effective intervention for the prevention of atopic disorders, such as AD [5], though the question has been raised that the impact of allergy prevention in studies using HFs is limited to AD prevention, which implies that these preventive effects cannot be generalized to other allergic diseases in the atopic march, such as asthma and allergic rhinitis.

Despite the issues raised here (table 2), the desire to provide concrete recommendations of widespread HF use needs to be assessed carefully so as not to overstate claims of benefit. Long-term studies are needed to investigate the feasibility of HF as a routine feeding option for healthy, standard-risk infants. Because of the paucity of data for pHFs or eHFs, the routine use of HFs for every infant as an equivalent option to breastfeeding or IPF cannot be supported at present based on available scientific evidence.

References

The Benefits of Breast Feeding

Raanan Shamir

Human milk is considered as the gold standard for infant feeding [1, 2]. The advantages of breast feeding for infants are summarized in table 1. The multitude of studies and accumulated evidence for the benefits of breastfeeding are largely based on observational studies, and only one prospective study, PROBIT (Promotion of Breastfeeding Intervention Trial), was performed using a cluster randomized design comparing populations where promotion of breastfeeding was exercised. In this review, the current knowledge on some of the effects of breastfeeding on the health outcome of breastfed full-term infants will be discussed briefly.

Infections

Breastfeeding reduced the risk of gastroenteritis by 64% and of otitis media by 23% [2]. In a recent Cochrane review, based on PROBIT, comparing exclusive breastfeeding for 6–7 versus 3–4 months, there was a risk ratio of 0.67 for having an episode of acute gastroenteritis at 12 months of age [3]. Also, there was a reduced risk of hospitalization due to respiratory illness (risk ratio 0.75).

Neurodevelopmental Outcome

Many publications (usually based on observational studies) demonstrate better neurodevelopment of breastfed infants compared to formula-fed infants [1, 2, 4].

In the only prospective randomized study (PROBIT) in infants at the age of 6.5 years, the intervention group had significantly higher adjusted outcomes of intelligence scores and teacher’s ratings. However, further observational studies provide contradictory results that could be explained either by confounders or by genetic predisposition to the effects of breastfeeding.
Allergy

Observations with respect to the benefit of breastfeeding in reducing the risk of atopic diseases are available already from a study published in 1936, where a 9-month follow-up of more than 20,000 children found a 7-fold reduction in the incidence of eczema in breastfed infants [1]. Ever since, studies have provided conflicting results, including studies demonstrating a protective effect, no effect and even an increased risk of...
allergic disorders in breastfed infants [1, 5]. These contradictory results could be explained by confounding factors, including, among many others, the inability to control for maternal diet, partial breastfeeding, introduction of solids, inconsistent diagnostic criteria for allergic diseases as well as reverse causality (mothers to high-risk infants may tend to breastfeed) [1].

**Celiac Disease as a Model for Autoimmune Disorders**

Since 2012, a plethora of evidence arising from observational and intervention studies was published, including 2 intervention trials. Based on those data, there is no significant effect of breastfeeding, or breastfeeding at the time of gluten introduction, on the risk of developing celiac disease.

**Closing Remarks**

One should be cautious in interpreting the evidence that is based on observational cohorts and intervention studies where breastfeeding effects were not the primary outcome. Furthermore, the only intervention study (PROBIT) with breastfeeding effects as the primary outcome measure was unable to show significant positive effects on many chronic diseases due to the lack of power to detect differences. These include the effects of breastfeeding on autoimmune disorders as well as other diseases where observational studies demonstrated an effect, such as cancer, sudden infant death syndrome as well as other diseases (listed in table 1) not mentioned in this review.

Human milk is the preferred food and breastfeeding is the preferred feeding method for infants, with added value in premature infants. Thus, one should look at the whole spectrum of benefits, proven and unproven health outcome measures as well as other advantages (for example psychological ones), recognize the limitations of research on breastfeeding, and continue to protect, promote and support breastfeeding [1].

**References**


Given the documented short- and long-term advantages of breastfeeding, human milk as a sole source of nutrition for the first few months of life is considered a normative standard. Each macro constituent of human milk plays a crucial role in growth and development of the baby. Lipids are largely responsible for the provision of more than 50% of the energy as well as for essential fatty acids and minor lipids that are integral to all cell membranes. Carbohydrates can be broadly divided into lactose and oligosaccharides, which are readily digestible sources of glucose and indigestible nonnutritive components, respectively. Proteins in human milk provide essential amino acids, which are indispensable for the growth of infants. What is more interesting is that protein concentration profoundly changes from colostrum to mature milk. In this report, we share data from an observational, single-center, longitudinal trial assessing the constituents of human milk collected 30, 60 and 120 days postpartum from 50 mothers (singleton deliveries of 25 male and 25 female infants). Human breast milk is highly dynamic [1–3] and has been shown to vary with the timing of expression, the side of the breast and the phase of the lactation cycle. These confounders were reduced in the study carried out by having early morning expression from the same side for each study subject. The breast milk was analyzed with the Miris milk analyzer for its energy, fat, carbohydrate as well as protein contents, and results were also confirmed using appropriate methods. The protein content decreased with evolving stages of lactation from an average of 1.45 to 1.38 g/100 ml (fig. 1). In contrast to our previous study [4] showing gender differences for lipid content 120 days postpartum, we did not reveal any gender differences in this trial. This finding was consistent with the previous literature on protein evolution of human milk during the first year of lactation. Despite the low protein content in breast milk, both groups of girls and boys managed to achieve normal growth during the study period regarding weight, height and also head circumference as per the World Health Organization Child Growth Standards [5, 6]. Further work will
be performed to analyze the dietary intake of the lactating mothers and to assess the macronutrients of breast milk as well as the other breast milk components.

References

Metabolic Programming: Effects of Early Nutrition on Growth, Metabolism and Body Composition

Ferdinand Haschke

Effects on Growth

Accelerated weight gain during infancy and early childhood is a strong predictor of childhood and adult obesity. Meta-analyses indicate that rapid weight gain in infancy explains 20–30% of the obesity risk in the adult population [1, 2]. Breastfeeding, in particular exclusive breastfeeding during the first 4–6 months of life and continuation of breastfeeding during the second half of infancy, seems to protect from obesity in child- and adulthood [3]. The WHO has published global growth standards [4] which are based on longitudinal data of predominantly breastfed (>6 months) children whose mothers were not malnourished (i.e. BMI 18–25). Weight from 4 months to 2 years is lower in the WHO standards than in international growth references, which are based on data from both formula- and breastfed children. The WHO growth standards are now used in most countries of the world. Longitudinal randomized clinical trials indicate that children who are fed infant and follow-up formulas with protein concentrations >2.25 g/100 kcal (high protein formulas) during the first year of life grow faster than indicated by the WHO standards [5–8]. How can we slow accelerated growth in formula-fed infants? The best way is to promote breastfeeding. If breastfeeding is not possible, a meta-analysis [5] now indicates that infants fed a formula with protein of 1.8 g/100 kcal (modified whey) during the first 4 months can grow according to the WHO standards – i.e. like breastfed infants. Two longitudinal randomized trials show that infants receiving low protein formulas with modified whey protein (1.6–1.8 g/100 kcal) between 3 and 12 months have lower weight for age and slower weight gain than infants fed high protein formulas [7, 8].
Effects on Biomarkers

Biomarkers which are indicators of growth, such as IGF-1, insulin, C-peptide and branched-chained amino acids, are higher in infants receiving high protein formulas than in breastfed infants or infants fed low protein formulas [7, 9]. The IGF axis regulates early growth and influences adipose tissue differentiation and early adipogenesis. The branched-chained amino acids leucine, isoleucine and valine are physiologic stimulators of insulin secretion. High protein intake of infants fed formula stimulates the IGF axis and insulin release, which is associated with a higher weight-for-length and BMI at the age of 2 years. Recently, it has been discussed that lower β-oxidation of fatty acids in infants who are fed high protein formulas might result in higher early weight gain and increased body fat deposition.

Effects on Body Composition

Estimation of fat mass (FM) and fat-free mass (FFM) allows more detailed insights into both quantitative and qualitative weight gain during or after feeding high or low protein formulas. A randomized controlled trial in infants who were fed high or low protein formulas from birth indicates that FM at 6 months (isotope dilution) correlates with BMI and weight gain velocity [10]. In infants of overweight and obese mothers who were fed high or low protein formulas, weight gain between 3 and 12 months and weight at 12 months were significantly higher in the group fed the high protein formula. Percent FM and FFM were similar at 12 months (dual energy X-ray absorptiometry) [7]. One randomized prospective study in an unselected US population has longitudinal data on infants fed high or low protein formulas. Children were followed until 60 months of age. Weight gain and composition of weight gain (FM and FFM in grams; Pea Pod) from 3 to 6 months were similar when the infants were exclusively fed the formulas. During follow-up, children who had the high protein formula gained significantly more fat from 6 to 36 months and from 6 to 60 months (dual energy X-ray absorptiometry) [8; unpubl. data].

Conclusions

Quantitative and qualitative growth indicators are among the most sensitive biomarkers to monitor long-term effects of early nutrition on child health. Several studies now indicate that the growth of children can be influenced by early nutrition. Breastfeeding and the use of low protein formulas in those infants who cannot be breastfed can help to prevent
accelerated growth during infancy and early childhood. In addition, fat gain until 5 years is lower in children who had been breastfed or fed a low protein formula. It is most important that the new low protein formulas are safe and adequate for the whole infant population. Based on new protein technologies, their essential and branched-chained amino acids are now closer to breast milk.

References
Human Milk: Bioactive Proteins/Peptides and Functional Properties

Bo Lönnerdal

Breastfeeding has been associated with many benefits both in the short term and in the long term. Infants being breastfed generally have less illness and have better cognitive development at 1 year of age than formula-fed infants. Later in life, they have a lower risk of obesity, diabetes and cardiovascular disease. Several components in breast milk may be responsible for these different outcomes, but bioactive proteins/peptides likely play a major role (table 1). Some proteins in breast milk are comparatively resistant towards digestion and may therefore exert their functions in the gastrointestinal tract in intact form or as larger fragments. Other milk proteins may be partially digested in the upper small intestine, and the resulting peptides may exert functions in the lower small intestine.

Lactoferrin, lysozyme and secretory IgA have been found intact in the stool of breastfed infants [1] and are therefore examples of proteins that are resistant against proteolytic degradation in the gut. Lactoferrin is the major iron-binding protein in breast milk and has been shown to bind to a specific lactoferrin receptor in the small intestine [2]. In the gut lumen, lactoferrin has bacteriostatic and bactericidal activities, and the lactoferrin receptor will facilitate the uptake of both lactoferrin

<table>
<thead>
<tr>
<th>Table 1. Bioactive proteins in breast milk</th>
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<tr>
<td>Lactoferrin</td>
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<tr>
<td>Lysozyme</td>
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<tr>
<td>Secretory IgA</td>
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<tr>
<td>Bile salt-stimulated lipase</td>
</tr>
<tr>
<td>Milk fat globule membrane proteins</td>
</tr>
<tr>
<td>α-Lactalbumin</td>
</tr>
<tr>
<td>β-Casein</td>
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<tr>
<td>κ-Casein</td>
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<tr>
<td>Osteopontin</td>
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and iron into the intestinal cell. Internalized lactoferrin can bind to the nucleus and affect the expression of genes involved in cell growth and proliferation as well as in immune function. Lysozyme can kill Gram-positive bacteria but also, together with lactoferrin in a synergistic fashion, Gram-negative bacteria [3]. Secretory IgA confers maternal immunity to her breastfed infant by providing antibodies towards bacteria and viruses she has been exposed to. Together, these proteins serve protective roles against infection and support immune function in the immature infant.

Bile salt-stimulated lipase is an active enzyme involved in lipid digestion in the upper alimentary tract [4]. This protein, however, is sensitive to proteolytic digestion and likely broken down in the mid- to lower small intestine as no peptides related to bile salt-stimulated lipase are found in the digesta. Milk fat globule membrane proteins have been shown to have antibacterial and antiviral activities. Little is known about the digestive fate of these proteins, but they are likely more or less completely digested by the time they reach the lower small intestine.

Fig. 1. Schematic picture of the digestion of breast milk (BM) proteins and peptides. Lf = Lactoferrin; Lyz = lysozyme; slgA = secretory IgA.
α-Lactalbumin, β-casein, κ-casein and osteopontin are examples of proteins that are partially digested in the upper part of the small intestine, and the resulting peptides provide functions in the gut. Such functions include prebiotic effects, stimulation of immune function, mineral and trace element absorption and defense against infection. Eventually, however, these peptides will be digested and provide amino acids for the rapidly growing infant.

Cow’s milk proteins in infant formula will also be digested and some peptides formed have been shown to have bioactivities. In fact, due to similarities between some proteins in human and cow’s milk, several of these peptides are identical to or very similar to human milk bioactive peptides [5]. Other peptides, however, display different bioactivities. Hydrolysate formulas have already been industrially treated with proteolytic enzymes, resulting in a mixture of peptides which are relatively large in partially hydrolyzed infant formulas and smaller in extensively hydrolyzed formulas. When such formulas are ingested and digested by the infant, many of the biologically active peptides will be hydrolyzed to amino acids or to novel small peptides (fig. 1). The activities and metabolic fate of such peptides are less known.

References

Human Milk for Preterm Infants and Fortification

Jatinder Bhatia

The World Health Organization, the American Academy of Pediatrics, European Society for Pediatric Gastroenterology, Hepatology and Nutrition and others, all support the feeding of human milk for all infants, including preterm infants. The benefits of human milk include immunologic, nutritional, developmental, psychological, social and economic advantages. In preterm infants, feeding of human milk is associated with a reduction in necrotizing enterocolitis and sepsis. In the long term, premature infants also demonstrate advantages in neurocognitive development.

However, there are several challenges in providing exclusive human milk feedings to meet the nutritional needs of infants with very low birth weight. These include inadequate milk supply, the variability in nutrient composition of human milk and the limitation of human milk itself. Human milk varies in volume with the method of milk expression, time of day, type of milk (fore- or hind milk) and stage of lactation. Reasons for low volumes include stress, lack of family or medical support, maternal illness, lack of a breast pump and difficulties in storing and transporting milk.

Preterm infants have higher requirements than term infants, and after the milk transitions to mature milk in 2–3 weeks, the protein content is usually insufficient to meet the nutritional demands of a rapidly growing infant. Similarly, human milk does not have sufficient quantities of calcium, phosphorus and vitamin D to support bone health. Energy density of human milk also declines over time. When mother’s own milk is not available, it is recommended that donor milk be fed to infants. In the first place, donor milk is usually obtained from mothers who have been breastfeeding for several months and, therefore, protein and energy content are low. In addition, pasteurization alters the concentrations of some water-soluble vitamins as well as the activity of several bioactive components of human milk.

Poor growth is thus seen both with the use of unfortified mother’s own milk as well as donor milk, especially in infants with very low birth
weight, making fortification of human milk a priority. In this discussion, macronutrient requirements of preterm infants and the composition of mother’s own milk and donor milk will be reviewed. Fortifiers, based on bovine and human milk, fortification strategies and duration of fortification will also be discussed. Thus, using appropriate fortification methods will assist in meeting the nutrient requirements of these infants, while protecting the beneficial effects of human milk itself.
Protein Needs of Preterm Infants: Why Are They So Difficult to Meet?

Ekhard E. Ziegler

Because of their exceedingly high rate of growth, premature infants have very high needs for all nutrients. But because protein is limiting for growth, requirements for protein are of particular importance. Requirements have been estimated by the factorial method based on the body composition of the fetus. In spite of using a somewhat different data and different methods of data analysis, Forbes [1] and Ziegler et al. [2] arrive at very similar estimates of protein needs for growth of the premature infant. Table 1 summarizes our estimates.

Table 1 summarizes our estimates.

Meeting these intakes is not particularly difficult if nutrients are provided by formulas. But human milk is the preferred feeding for premature infants because of its protective effects. When human milk is fed it must be fortified with nutrients, and then the variability in the composition of expressed milk seems to pose a problem. On the basis of studies carried out several decades ago using very high protein intakes, it is believed that

<table>
<thead>
<tr>
<th>Body weight</th>
<th>500 to 700 g</th>
<th>700 to 900 g</th>
<th>900 to 1,200 g</th>
<th>1,200 to 1,500 g</th>
<th>1,500 to 1,800 g</th>
<th>1,800 to 2,200 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain per day</td>
<td>13</td>
<td>16</td>
<td>20</td>
<td>24</td>
<td>26</td>
<td>29</td>
</tr>
<tr>
<td>g/kg</td>
<td>21</td>
<td>20</td>
<td>19</td>
<td>18</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>Protein requirements, g/kg per day</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.4</td>
<td>3.2</td>
<td>3.0</td>
</tr>
<tr>
<td>Parenteral</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>3.9</td>
<td>3.6</td>
<td>3.4</td>
</tr>
<tr>
<td>Enteral</td>
<td>89</td>
<td>92</td>
<td>101</td>
<td>108</td>
<td>109</td>
<td>111</td>
</tr>
<tr>
<td>Energy requirements, kcal/kg per day</td>
<td>105</td>
<td>108</td>
<td>119</td>
<td>127</td>
<td>128</td>
<td>131</td>
</tr>
<tr>
<td>Parenteral</td>
<td>3.8</td>
<td>3.7</td>
<td>3.4</td>
<td>2.8</td>
<td>2.6</td>
<td>2.6</td>
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high intakes of protein may be dangerous for premature babies [3]. To prevent protein intakes from being too high when the protein content of expressed milk should be high, the protein content of fortifiers has been kept low. This has had the result that protein intakes of preterm infants have been too low most of the time.

References

The idea that protein intake in the preterm infant may influence, or program, the long-term health of the infant born preterm has been strongly supported by several decades of research starting from the early 1980s. At the time, it was recognized that a high protein intake was required in preterm infants to achieve a postnatal growth rate closer to the intrauterine rate of growth of a normal fetus of the same postconceptual age, a goal regarded optimal for short- and long-term health. Subsequently, long-term follow-up of preterm infants randomized to a high protein formula (for an average of only 4 weeks after birth) demonstrated beneficial effects up to 16 years later on brain structure and function, including 10% greater volume of the caudate nucleus, higher IQ and practical benefits for cognitive function (e.g., mathematical reasoning, numerical operations and reading comprehension) [1]. Since this early research, numerous observational studies have demonstrated an association between suboptimal nutrition in the early postnatal period (as measured by faltering growth, poor growth in head circumference and inadequate protein intake [2]) and impaired long-term neurocognitive development. Consequently, international recommendations for protein intake in infants born prematurely have increased progressively.

Nonetheless, despite the extensive observational evidence, the role of early protein intake in preterm infants for later neurodevelopment remains unclear. For instance, Cochrane reviews of randomized trials have not shown evidence supporting early amino acid administration [3] or higher versus lower protein intake in formula-fed preterm infants for improving later neurodevelopment [4]. Therefore, although there are strong associations between early postnatal protein intake and neonatal growth, and between growth faltering and impaired later neurodevelopment, whether high protein supplementation can improve cognitive function in preterm infants remains controversial.

In contrast to the benefits for neurodevelopment, a longer-term follow-up of the same infants in the preterm nutritional trials mentioned
above have suggested that faster postnatal weight gain increased later risk factors for cardiovascular disease. Infants randomized to a higher protein formula for the first 4 weeks were shown to have increased adiposity, insulin resistance, dyslipidemia, levels of inflammatory markers and vascular endothelial dysfunction up to 16 years later. These programming effects of early growth, termed the growth acceleration hypothesis, have now been demonstrated in randomized and observational studies in several preterm populations, as well as in infants born at term with both low and appropriate weight for gestation [5]. Therefore, as is common in biological systems, faster infant weight gain appears to have both benefits and costs on long-term health outcomes.

Current nutritional policy for preterm infants is based on the widely accepted consensus that supporting optimal neurodevelopment is the neonatologist’s highest priority. Therefore, on balance, this policy favors early administration of a higher protein intake in order to improve later cognitive function, irrespective of any increase in cardiovascular risk. However, this consensus is largely based on research that has focused on infants <31 weeks of gestation and it is uncertain whether the risk-benefit ratio of faster weight gain differs for the larger, more mature, healthy preterm infants than those with extreme prematurity. Furthermore, the critical window for these effects is unknown and whether the same nutritional policy should apply after discharge is controversial. For instance, a randomized trial of formulas fed after discharge showed that a higher protein intake after hospital discharge, although increasing the rate of growth, did not have adverse effects on later body composition or cardiovascular risk factors.

This presentation will consider the role of protein intake on long-term health outcomes in infants born preterm, focusing on the risk-benefit ratio for accelerated growth and emphasizing the need for further research.

References

Amino acids and proteins form 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 onwards and in larger quantities. Studies showed positive results on efficacy, which was usually measured in terms of nitrogen balance (fig. 1) or stable isotope studies. Short-term safety has been questioned as parameters are difficult to interpret, while long-term effects are not frequently assessed. Besides, it is unlikely that we have achieved the optimal therapy with regard to protein supplementation for premature neonates yet.

It is therefore also important to gain insight into how the developing fetus is able to metabolize amino acids and proteins, and to translate this to preterm infants of similar gestational age. Exploring fetal metabolism and growth could enhance our understanding of the challenges of postnatal development, even though the placenta can no longer adjust and filter metabolites postnatally. Unfortunately, very little is known about fetal protein metabolism, which is also due to ethical and technical reasons. However, we hardly know how much nutrients the human fetus actually receives. Only a few studies have been performed using stable isotope techniques, which give us an indication of the uptake and metabolism of amino acids in human fetuses. These studies show that a relatively large proportion of amino acids taken up are utilized for oxidation rather than solely being used for protein synthesis [1, 2]. Extrapolation of the individual amino acid uptakes to total amino acid intakes (that could serve as a basis to determine total amino acid requirements of preterm infants of similar age) are hampered by the different metabolic fates of the individual amino acids. In addition, these studies do show that the
fetal liver is capable of synthesizing large quantities of albumin, possibly even higher than is currently seen in premature infants fed current recommended intakes [3]. Theoretically, it would thus seem to be possible to improve certain aspects of postnatal metabolism as well, as the metabolic apparatus of a premature infant should ontogenetically be able to achieve a high hepatic rate of protein synthesis under optimal circumstances (as in utero). Nevertheless, we must acknowledge the complex interplay between the placenta and the fetus [4].

Fig. 1. Correlation plot of results in different trials investigating different amino acid intakes and nitrogen balances during the first few days of life of preterm infants. The size of the symbols resembles the number of infants included in the clinical trial.
During the last decades, several studies have been performed in premature neonates on amino acid metabolism, mostly comparing different nutritional regimens in terms of protein content. Protein metabolism is, however, influenced by many other factors. For example, the quality (individual amino acid composition) of the intravenous solution or enteral formula or concomitant energy intake could influence the efficacy of protein handling and, therefore, the total requirements as well. Individual amino acid requirements during different stages of the postnatal course are, therefore, to be determined, of which only a start has been made. Besides, nonnutritional factors will influence the requirements and tolerability of amino acids, although these are hardly ever studied. Efforts should be undertaken to study the effects of intrauterine growth restriction, or needs during and following additional critical illnesses (besides prematurity itself) [5].

Thus, although we might attempt to determine amino acid requirements for the stable preterm infant based upon improved knowledge on both fetal and neonatal physiology, we are far from being able to predict the requirements for specific subgroups, such as being small for gestational age or stressed infants with additional diseases. Unfortunately, only few of these factors have been unraveled. Only 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.

References

A large proportion of extremely low-birth-weight infants require parenteral nutrition for variable lengths of time. Amino acids are the key ingredient of parenteral nutrition. The aim of appropriate amino acid administration is to promote anabolism and optimal cellular development with the final goal of reducing postnatal growth restriction, which is associated with neurodevelopmental delays. The benefits of early amino acid commencement is compelling, especially on nitrogen balance, while long-term outcome studies are lacking. An intake of 2.5 g/kg per day of amino acids is to be preferred to lower amounts. Benefits of amino acid intakes >2.5 g/kg per day without extra energy remain controversial. Two randomized controlled trials do not show benefits on short-term growth and neurodevelopment at the 2-year follow-up. Studies with amino acid intake >2.5 g/kg per day with extra energy are warranted.
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