Hydrolyzed Proteins in Preterm Infants

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Abstract
Milk proteins are an essential component of the diet of preterm infants who have high requirements. Hydrolyzed proteins (HPs) have been introduced in infants’ formulas (HPFs) to treat gastrointestinal disorders and to prevent allergic diseases. Several studies have evaluated the adequacy of HPs in preterm infants. Protein source significantly influences plasma amino acid concentrations. Protein utilization and efficiency are usually lower with HPFs compared to formulas with intact proteins. When protein intake is similar, a lower weight gain is generally observed with HPFs 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. 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 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, therefore, further high-quality studies are needed.

Introduction
In preterm infants, nutritional support is critical to sustain adequate postnatal growth and development and long-term well-being to a level similar to those of term infants. Their expected growth rate and protein/amino acid (AA) turnover is very high compared to term infants and children, which explains their high nutritional requirements, especially for protein. During the last decades, great
attention was focused on the importance of protein intake in order to reduce nutritional deficits and postnatal growth restriction and to improve short- and long-term outcomes in preterm infants [1, 2]. Unfortunately, the immaturity of these infants exposes them to potentially severe postnatal diseases and many metabolic disorders. Thus, the range of optimal intakes is reduced in preterm infants due to the potential adverse effects of either insufficient or excessive AA concentrations. Therefore, both the quantity and the quality of protein intakes need to be considered when discussing preterm infants’ protein requirements [3].

Human milk (HM) represents the ‘gold standard’ in infant’s nutrition due to its important properties [4]. Recent proteomic evaluation of HM has revealed that it contains many species-specific proteins that are associated with the postnatal maturation and development of the gastrointestinal tract, the immune system and the brain [5–8]. When preterm infants cannot receive HM, they request specific preterm infant formulas (PTFs) produced according to several regulations and directives [9–11]. Many researches have been conducted to improve the protein profile and composition in infant formulas to mimic short- and long-term benefits of HM, in particular for hydrolyzed protein formulas (HPFs) [6, 12].

HPFs are processed by enzymatic hydrolysis of various protein sources, such as bovine casein/whey, soy and rice, followed by further processing, such as heat treatment and/or ultrafiltration, or they are based on AA mixtures [12, 13]. They have been introduced many years ago in preterm infants as HM fortifiers [14, 15] or as elemental diets to treat intractable diarrhea [16]. Nowadays, HPFs are mainly used to treat allergic diseases and to prevent allergic sensitization in infants and toddlers [17–19]. They are also used to improve feeding tolerance and to reduce undesired gastrointestinal manifestations such as regurgitation, colics and/or constipation [19, 20]. However, industrial processes can significantly interfere with the bioavailability of the nitrogen content as well as the nutritional value of HPFs [12, 13, 21]. In addition, accumulating evidence has demonstrated the nutritional importance of the quality of the protein source for the postnatal maturation of the gastrointestinal tract in animal studies [19, 22, 23].

The objective of this review is to evaluate the nutritional adequacy of HPFs and their potential to improve feeding tolerance (gastrointestinal transit) and to reduce allergic diseases in preterm infants.

**Nutritional Adequacy**

The nutritional adequacy of a formula is usually assessed by the evaluation of growth rate (body weight, length and head circumference), metabolic balances (absorption rate, net utilization and efficiency) and biochemical parameters
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(Plasma AA concentrations, blood urea nitrogen and prealbumin, for example) [12]. Several studies have been published on the nutritional adequacy of HPFs in preterm infants. Some of them concerned experimental HPFs given to a small number of infants and only focused on some aspects of nutritional adequacy.

In the early 1990s, Rigo et al. [12] and Rigo and Senterre [24] performed a 3-day metabolic balance trial to evaluate plasma AA profiles with different types of HPFs: 100% whey (n = 7), 78% whey and 22% casein (n = 7), and 78% whey and 22% casein enriched with histidine (n = 5). A control group was fed a standard PTF (sPTF) with intact proteins (n = 39). Both nitrogen absorption and efficacy were significantly decreased with HPFs (83 and 64%, respectively) compared to the control group (90 and 70%, respectively). Mineral absorption was also lower, especially for phosphorus. The plasma AA profile differed significantly from that of infants fed HM or sPTF. It mainly depended on the protein source and was related to the AA content of the formula [12, 24]. Plasma threonine concentration was increased, whereas plasma tyrosine, histidine and tryptophan concentrations were significantly decreased in the HPFs without supplementation [12, 24].

Following these studies, HPFs have been improved by the selection of the protein source (formulas containing various whey:casein ratios and acidic whey instead of sweet whey), by an increased protein content (i.e. protein:energy ratio), by the addition of some individual AAs to correct the AA imbalance and by improved hydrolysis technologies. In addition, mineral retention was also improved by increasing calcium and phosphorus contents in HPFs and by the selection of minerals with higher bioavailability. In 1999, Mihatsch and Pohlandt [25] compared an experimental HPF (60/40 whey/casein ratio) with a sPTF in a 5-day crossover trial (n = 15). This experimental HPF had higher protein (12%), energy (15%), calcium (34%) and phosphorus (46%) contents to counteract the reduced absorption rates. During the short period of study, the growth rate was similar. Only little differences in plasma AA profiles were observed and AA profiles were considered globally similar. Blood urea concentration was higher with the HPF in relation to the increase in nitrogen content [25].

In 2001, Szajewska et al. [26] evaluated extensively HPF (eHF; n = 16) and partially HPF (pHF; n = 15) during a 12-week period. There were two control groups: one fed fortified HM (n = 15) and one fed a sPTF (n = 15). Compared to the sPTF, protein contents were 9 and 14% higher in the eHF and pHF, respectively. Weight gain tended to be lower in infants fed the HPF compared to the sPTF, with a mean difference in weight gain of –4.7 g/day (eHF) and –2.8 g/day (pHF) during the first 4 weeks and –2.0 and –1.3 g/day, respectively, during the 12-week study period. Due to the limited number of infants, these differences were not significant. Nevertheless, considering the higher protein
content in the HPFs, the nutritional efficacy seems in favor of the sPTF and non-inferiority could not be demonstrated for the HPFs. In this study, the plasma AA profile of the HPFs appears to be similar to that of the sPTF, but it differs from that of the fortified HM group.

In a longitudinal study (8 weeks), Picaud et al. [27] evaluated another experimental pHF (skim milk mixture of acid and rennet whey) in very preterm infants (n = 9). The control group was fed a sPTF (n = 7). This study confirmed that nitrogen absorption was significantly lower with the HPF (83 vs. 89%). They also confirmed that average protein content should be 10% higher in HPFs to obtain similar protein absorption than with sPTFs. AA profiles were similar (same protein source) and both were within the normal range. At theoretical term, body weight was significantly lower in the HPF group than in the sPTF group. That difference was the result of a significantly lower weight gain from study inclusion to discharge in infants fed the HPF compared with those fed the sPTF (28.1 ± 5.1 vs. 32.8 ± 2.4 g/day, respectively; p = 0.03).

In 2005, Maggio et al. [28] compared a HPF (100% acid whey, n = 10) to a sPTF (51% sweet whey/49% casein, n = 11) with similar protein content (2.7 g/100 kcal). A slower weight gain (17.4 ± 3.4 vs. 20.5 ± 3.3 g/kg/day, respectively; p = 0.045) was observed with the HPF as well as a lower change in the z-scores for weight (–0.18 ± 0.16 vs. 0.00 ± 0.09; p = 0.009) and head circumference (–0.06 ± 0.13 vs. 0.06 ± 0.13; p = 0.049). In that study, a significantly higher urinary excretion of essential AAs was also reported after 14 days in the HPF group, suggesting a decrease in protein efficiency with the HPF.

In 2006, Cooke et al. [29] evaluated two HPFs with two different protein contents (3.6 and 3.0 g/100 kcal) in a 1-week crossover trial. They reported a nitrogen absorption rate of 83 and 84%, respectively, i.e. a value similar to that generally reported with HPF. Weight gain, blood urea nitrogen, retinol binding protein and plasma AA concentrations were higher in the group receiving the HPF with the high protein content (3.6 g/100 kcal).

In 2009, Florendo et al. [30] evaluated a pHF during a 3-week period (n = 42); the control group received an sPTF (n = 38). Growth (weight, length and head circumference gain) was similar between the two groups that received similar protein and energy intakes. Plasma AA profiles were similar, but several differences were observed in serum biochemistries although all were within the normal range. Blood urea was higher with the HPF while total serum protein and albumin were lower. In addition, blood calcium and phosphorus concentrations were lower with the HPF at the end of the study, but no difference was observed for alkaline phosphatase [30].

More recently, Cooke et al. [31] compared two pHFs containing 3.0 and 3.6 g of protein per 100 kcal. No differences were detected in weight gain, body weight
or weight z-scores, but in both groups z-scores remained in parallel to those in utero. In addition, length z-scores were greater in infants fed the high protein content formula (p < 0.05). As the study progressed, length z-scores did not change in infants fed the high protein content formula, closely paralleling scores in utero, but decreased significantly in infants fed the low protein content formula (p < 0.0001) [31]. This study suggests that a high protein content (high protein/energy ratio, 3.6 g/100 kcal) in pHF promotes linear growth by improving the lean body mass accretion rate. However, this improvement in linear growth and lean body mass accretion in preterm infants requires confirmation.

In summary, most of these studies showed that the use of HPFs instead of sPTF with similar protein content induced a slower weight gain (table 1) due to a lower nitrogen/protein absorption rate. The lower nutritional efficiency of the HPFs implies that an average increase of 10% in protein content within HPFs is necessary to induce protein retention similar to that with sPTF made with intact proteins. Initial studies showed that the protein source of HPFs significantly influenced the plasma AA profile but most AA imbalances have been corrected in recent HPFs. Mineral absorption may also be decreased with HPFs compared to sPTFs, but the quality of the mineral source had a higher

<table>
<thead>
<tr>
<th>First author</th>
<th>HPF</th>
<th>sPTF</th>
<th>p value</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Szajewska [26] (2001)</td>
<td>27.2±8.5</td>
<td>31.90±12.9</td>
<td>n.s.</td>
<td>eHF with 9% higher protein content (n = 16 vs. 15 during 4 weeks)</td>
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<td></td>
<td>32.2±5.7</td>
<td>34.22±4.7</td>
<td>n.s.</td>
<td>eHF with 9% higher protein content (n = 16 vs. 15 during 12 weeks)</td>
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<td></td>
<td>29.1±5.7</td>
<td>31.9±12.9</td>
<td>n.s.</td>
<td>pHF with 5% higher protein content (n = 15 vs. 15 during 4 weeks)</td>
</tr>
<tr>
<td></td>
<td>32.9±5.1</td>
<td>34.22±4.7</td>
<td>n.s.</td>
<td>pHF with 5% higher protein content (n = 15 vs. 15 during 12 weeks)</td>
</tr>
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<td>Picaud [27] (2001)</td>
<td>21.9±2.1</td>
<td>23.0±2.0</td>
<td>n.s.</td>
<td>Similar protein content (n = 9 vs. 7 during 7 days)</td>
</tr>
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<td></td>
<td>28.1±5.1</td>
<td>32.8±2.4</td>
<td>0.03</td>
<td>Similar protein content (n = 9 vs. 7 during 8 weeks)</td>
</tr>
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<td>Maggio [28] (2005)</td>
<td>17.4+3.4</td>
<td>20.5+3.3</td>
<td>0.045</td>
<td>Similar protein content (n = 10 vs. 9 during 4 weeks)</td>
</tr>
<tr>
<td>Florendo [30] (2009)</td>
<td>27.4±8.3</td>
<td>28.1±4.3</td>
<td>n.s.</td>
<td>Similar protein content (n = 38 vs. 34 during 3 weeks)</td>
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1 Weight gain units = g/kg per day.
impact on the absorption rate than the protein source. No data exist concerning the influence of HPFs on trace elements and vitamin absorption. Finally, recent proteomic studies on HM and cow milk proteins as well as the effect of bioactive peptides produced during digestion of HM and formula [5–8] suggest that further research is needed before promoting the use of HPFs in preterm infants.

**Gastrointestinal Transit Time and Feeding Tolerance**

Preterm infants frequently suffer from some kind of feeding intolerance, and necrotizing enterocolitis always represents a dramatic complication in these infants. The definition of feeding intolerance is not clear in preterm infants but many efforts have been made to reduce the incidence and the severity of gastrointestinal symptoms such as gastric residuals, regurgitation/spitting, abdominal distension and constipation [32]. In 2007, Szajewska [33] reviewed the effect of HPFs on feeding tolerance in three studies. In the study by Riezzo et al. [34], no difference was observed between infants fed HPF (n = 18) or sPTF (n = 18) although regurgitation and vomiting were very frequent (64 and 78%, respectively). In addition, no differences were found in terms of gastric electrical activity and gastric emptying time between the two groups [34]. In the study by Mihatsch et al. [35], HPF was well tolerated with similar vomiting/spitting and similar gastric residuals. The number of stools was similar, but soft stools were more frequently reported with HPF. Gastrointestinal transit time was also shorter: 9.8 versus 19 h on average (range 5–20.8 vs. 6–66) [35]. In a separate publication [36], theses authors showed that in infants with very low birth weight fed HPF the time to establish full enteral feeding was significantly decreased compared to those fed sPTF (mean reduction: 2 days, i.e. 10 vs. 12 , range 9–27 vs. 9–28 days; p = 0.0024). In her review, Szajewska [33] concluded that despite some potential clinical advantages, like shorter time to achieve full feeding, it is unclear whether the use of HPFs should be adopted as routine practice because of its limited information regarding nutritional adequacy.

In addition to these studies, Picaud et al. [27] showed that HPF was well tolerated and no difference was observed for the occurrence of vomiting and in the volume of gastric residuals. Gastrointestinal transit time was shorter with HPF in their study (mean: 18 ± 4 vs. 25 ± 10 h) [27]. In the study by Maggio et al. [28], HPF was also well tolerated with similar vomiting/spitting and similar gastric residuals. In the study by Florendo et al. [30], feeding tolerance was excellent and no difference was observed between HPF and sPTF.
Recently, Corvaglia et al. [37] evaluated the effect of eHF on symptoms of both gastroesophageal reflux (frequent regurgitation and/or postprandial desaturation) and feeding intolerance (large gastric residuals, abdominal distension and constipation) using a crossover design (n = 18). They were unable to demonstrate any effect of the HPF on gastrointestinal symptoms and on multi-channel intraluminal impedance monitoring. Besides, they showed a reduction in gastroesophageal reflux episodes detected by pH monitoring [37]. In a letter to the editor, it was suggested that this effect on esophageal pH was mainly due the buffering effect of HPF on gastric pH explaining the absence of an effect observed with impedance monitoring [38].

In summary, HPFs improved feeding tolerance and are usually associated with accelerated gastrointestinal transit time and softer stools. However, HPFs do not seem to reduce feeding intolerance symptoms although time to achieve full enteral feeding was reduced in a study in very preterm infants. This potential benefit needs to be further confirmed by high-quality randomized controlled trials.

Prevention of Allergic Diseases

Preterm infants have increased intestinal permeability and higher food antigen uptake [39]. As it is combined with immature immune functions, it has led some authors to consider preterm infants at higher risk of food allergy [40, 41]. Some studies have suggested that preterm infants may more frequently develop recurrent wheezing and symptoms of asthma [42, 43]. However, there is some debate about considering all wheezing manifestations as allergic diseases in former preterm infants [44, 45]. These symptoms may be more frequently related to persistent bronchial hyperreactivity due to bronchopulmonary dysplasia rather than real allergic diseases [46, 47]. Up to now, there is no evidence demonstrating any difference in the rate of food allergy and other allergic diseases in preterm infants compared to term infants [48–54]. By contrast, some authors even pointed out that prematurity may be associated with less allergic sensitization in children as early exposure to an antigen would favor tolerance and lower the risk of sensitization [55, 56].

In a large randomized controlled trial (n = 777), Lucas et al. [49] did not find any difference in the incidence of allergic reactions after 18 months in preterm infants fed bank donor HM or sPTF. In very preterm infants (n = 62), Kwinta et al. [46] evaluated the effect of eHF on the incidence of atopic diseases and markers of allergy at 5–7 years of age. They were not able to suggest any benefit of a 1-month intervention in their unselected cohort of preterm infants [46].
A variety of factors are known to influence the risk of allergic diseases, such as family history, and allergen and environmental exposure (atmospheric pollution, passive tobacco smoke, house dust and urban residency) [57]. In preterm infants, it has also been confirmed that familial predisposition to allergic diseases significantly increases the risk of atopic dermatitis and recurrent wheezing [49, 54].

One study evaluated whether the use of pHF (n = 32) or eHF (n = 26) until 4–5 months of age may prevent allergic disease in preterm infants with familial allergic predispositions [58]. Two control groups were included in the study, one receiving sPTF (n = 32) and one receiving HM (n = 32). In this study, the overall incidence of allergic diseases at both 4–5 and 12 months of age and prevalence of total IgE and/or cow’s milk protein-specific IgE levels did not differ. Besides, the eHF had a high rate of nonacceptance (23%) leading to a significant proportion of infants fed the eHF who were no longer part of the per-protocol analysis at 4–5 and 12 months of life. Nevertheless, the intention-to-treat analysis showed that the risk of atopic dermatitis by 12 months of age was significantly reduced in the eHF group [58].

In summary, prematurity does not seem to induce a higher risk of developing allergic diseases in later life. As for term infants, a family history of allergic diseases is also the main risk factor for developing allergic diseases in preterm infants. Further studies are needed to evaluate the role of HPFs in the prevention of later allergic diseases in preterm infants, especially in neonates with a family history of allergy.

**Conclusion**

Breastfeeding, own mother milk and banked donor HM remain the best source of nutrients for preterm infants. When HM is not available, sPTFs are required to meet the high requirements of these infants. HPFs are well tolerated by preterm infants and harmful effects of modern HPFs for preterm infants have not been reported. However, most studies have demonstrated that their nutritional value is usually lower than that of intact protein formulas, indicating that a higher protein content is necessary to allow for similar protein retention rates. Similarly, a higher mineral content is also frequently required. Potential clinical benefits on feeding tolerance have not been clearly demonstrated. Further high-quality studies including a large number of infants are necessary to demonstrate the nutritional noninferiority of HPFs compared to modern intact protein formulas in preterm infants. These studies should also evaluate potential consequences on gastrointestinal functions, on the incidence of infections and necrotizing enterocolitis, and on immune, metabolic and hormonal long-term outcomes.
Disclosure Statement

T. Senterre has participated as a speaker for Nestlé, Nutricia and Mead Johnson Nutrition, and J. Rigo as a clinical investigator, advisory board member or speaker for Mead Johnson Nutrition, Danone, Nestlé and Nutricia.

References
