Protein Quality and Quantity in Cow’s Milk-Based Formula for Healthy Term Infants: Past, Present and Future

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Abstract

The development of infant formula with optimized protein quality and quantity has been, and still is, the subject of intense investigation. A better understanding of the protein composition of breast milk and infant needs in association with technological breakthroughs in cow’s milk fractionation, has led to the development of infant formulas with a protein content that is closer to that of human milk. Today, infant formulas with a protein/energy ratio of 1.8 g/100 kcal are commercially available. These formulas have been shown to be safe and nutritionally adequate for term infants. However, the short-term and potentially long-term metabolic benefits of formulas with reduced protein content have still to be elucidated and are currently under investigation. In addition to providing amino acids as building blocks for growth, milk is the source of numerous bioactive factors/hormones which are involved in multiple physiological processes. Continuous efforts are being made to identify new bioactive compounds in human milk. However, a better understanding of their biological functions in suckling infants as well as a comparison with their bovine counterparts are needed. Technological processes, which preserve some bioactive factors in cow’s milk already exist. These processes could be applied to infant formulas.

Introduction

Since the first commercially prepared infant formulas were available as powders in the late 1800s, constant improvement in the formulation has been done to match the composition of human milk more closely and, most importantly, to mimic the functional outcome of breast-fed infants (i.e., appropriate growth, development and health). In this context, the development of infant formula
with optimized protein quality and quantity has been, and still is, the subject of intense investigation.

From Casein-, Whey-Predominant to Modified Whey Infant Formula

In 1919, Gerstenberger and Ruh [1] developed the first commercially available formula with cow’s milk as the exclusive source of proteins. The original protein content of the manufactured formula was 1.8 g/100 kcal, but was increased to 2.2 g/100 kcal in 1945 [2]. In the middle of the 20th century, it was considered that formula-fed (FF) infants require a considerably greater intake of protein than breast-fed (BF) infants. This recommendation was based on an overestimation of both the nutritionally available protein content of human milk and the protein intake requirements of the infants [3, 4]. Furthermore, cow’s milk protein quality and digestibility was considered far inferior to that of human milk for satisfying the amino acid needs of infants. In the 1960s, the protein content of a number of widely used formulas ranged from 3.3 to 4.0 g/100 kcal and some formulas, designed for managing diarrhea, even provided up to 6.7 g/100 kcal [5]. Subsequently, national health institutes and pediatric associations defined standards for protein content in infant formulas (table 1). While there is now a consensus for the minimum required values (1.8 g protein/100 kcal), a broader range of recommended maximum values can be found (2.8–4.5 g protein/100 kcal). In 1991, a European Directive set the maximum protein content to 3.0 g/100 kcal. However, the Food and Drug Administration still recommended a maximum required intake level of 4.5 g/100 kcal, in spite of the revisions proposed by a task force of the American Academy of Pediatrics [5]. A current revision of the Codex

Table 1. Standards for protein content in infant formulas

<table>
<thead>
<tr>
<th>Institutions/associations</th>
<th>g protein/100 kcal</th>
<th>Year of issue</th>
</tr>
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<tbody>
<tr>
<td>Food and Drug Administration [54]</td>
<td>1.8–4.5</td>
<td>1985</td>
</tr>
<tr>
<td>Codex [55]</td>
<td>1.8–4.0a</td>
<td>1981</td>
</tr>
<tr>
<td></td>
<td>1.8–3.0b</td>
<td>under revision</td>
</tr>
<tr>
<td>European Economy Community [56]</td>
<td>1.8–3.0</td>
<td>1991</td>
</tr>
<tr>
<td>Committee on Nutrition of the American Academy of Pediatrics [57]</td>
<td>1.8–4.5</td>
<td>1976</td>
</tr>
<tr>
<td>European Society for Pediatric Gastroenterology and Nutrition [58, 59]</td>
<td>1.8–2.8</td>
<td>1977</td>
</tr>
<tr>
<td></td>
<td>1.8–3.0b</td>
<td>2005</td>
</tr>
</tbody>
</table>

aFollow-on formula (1989): 3.0–5.5 g protein/100 kcal.
bStarter and follow-on formulas.
proposes a maximum protein content of 3.0 g/100 kcal, for both starter and follow-on formulas, based on new recommendations from the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (table 1).

The intrinsic superiority of human milk protein over that of cow's milk is due to its higher whey to casein protein ratio (approximately 60:40 in human milk and 20:80 in bovine milk). In 1961, a major technological breakthrough (i.e. demineralization of whey protein through an electrodialysis process) allowed the addition of equal parts of casein and whey in formula, and by the mid 1990s, whey-predominant formulas prevailed in the US and Europe. Nevertheless, both the whey and casein fractions of cow's milk are quite different from those of human milk (fig. 1). Consequently, the amount of amino acids delivered by breast milk and formula differ. Compared to breast milk, the levels of most of the essential amino acids are, per gram of protein (or nitrogen), lower in the casein-predominant formulas, while leucine, phenylalanine, tryptophan, and to a lesser extent valine, are limited in whey-predominant formulas (fig. 2). In order to compensate for these quantitative differences, the amount of proteins per energy content must be higher in formula than in human milk. A casein- or a whey-predominant formula containing 2.5 g protein/100 kcal, provides, an excess of most of the essential amino acids found in breast milk. Nevertheless, tryptophan and the conditionally essential amino acid cystine are limiting factors for further reducing the protein quantity in cow's milk formula (fig. 3). Indeed, Janas et al. [6] demonstrated that term infants fed formulas with reduced protein content (1.8 g/100 kcal) and various whey/casein ratios had normal plasma cystine levels but depressed levels of tryptophan when compared to those fed human milk. New approaches/processes were therefore needed to adjust the protein/energy ratio to the minimal recommended value (1.8 g/100 kcal) and, thereafter, to that more closely resembling human milk.

In principle, the easiest way to avoid lower plasma levels of tryptophan in FF infants is to supplement the formula with free tryptophan. In 1992, studies demonstrated that term infants, fed a casein-predominant formula, reduced in protein (1.9–2.0 g/100 kcal) but fortified in free tryptophan, had similar plasma tryptophan levels to BF infants [7, 8]. However, taking into account the absorption kinetics of free and protein-bound tryptophan, as well as toxicological and economical considerations, supplementing with free tryptophan is not the most favorable option. Addition of α-lactalbumin (αLA) may provide a promising alternative [9]. The αLA fraction is rich in tryptophan (5.9%) but the proportion of αLA in bovine milk (4%) is considerably lower than in human milk (28%; fig. 1). Whey protein concentrates, or isolates enriched in αLA and produced by either ion exchange or membrane fractionation, became commercially available in the late 1990s [10]. The effect of αLA enrichment on tryptophan supply has been studied in healthy term infants fed a whey-predominant formula containing 2.0 g protein/100 kcal and 2.2 g tryptophan/16 g N over a 2-week period [11]. It was demonstrated that the
Fig. 1. Protein composition of human and cow’s milk. Adapted from Heine et al. [9].
serum tryptophan levels of the FF infants did not differ significantly from that of an exclusively BF group [11]. A growth and safety study was recently performed in term infants fed a whey-predominant control formula or a reduced protein experimental formula with added bovine αLA [12]. The αLA/βLA ratio was 0.4 in the control formula and 1.6 in the αLA-enriched formula. Growth and serum albumin were comparable in both groups of infants for the first 12 weeks of life, suggesting adequate protein delivery using the reduced protein formula with added bovine αLA. However, as pointed out by Raiha [13], the difference in protein content between the control (15.1 g/l) and experimental formula (14.4 g/l) was in fact rather small (2.25 and 2.15 g protein/100 kcal, respectively, assuming an energy density of 67 kcal/100 ml). Additional studies are still required to address the safety of formulas with an even lower protein content (e.g. 1.8 g/100 kcal).

In the meantime, other approaches have been explored. Caseinoglycomacropeptide is released from casein during the enzymatic precipitation of κ-casein and remains in the sweet whey fraction commonly used for the production of whey-predominant infant formulas. An original process of whey

**Fig. 2.** Amino acid composition (g/16 g N) of human milk (□), casein- (■) and whey-predominant (■) formulas. ◊Essential amino acids.
fractionation was developed to first of all remove the caseinoglycomacropeptide fraction, which is rich in threonine but poor in tryptophan, and then to increase the proportion of the αLA fraction which is rich in tryptophan (patent WO 01/11990). The resulting modified sweet whey (MSW) allowed the development of an infant formula with an essential amino acid profile that was closer to human milk (table 2; g amino acids/16 g N). In theory, this new formula allows the reduction of the protein/energy ratio to the minimum recommended value (1.8 g/100 kcal), without a deficit in essential and conditionally essential amino acids (table 2; mg amino acids/100 kcal). The protein adequacy of the MSW formula (1.83 g/100 kcal) was established with metabolic balance studies. The results showed that, despite the lower protein content, infants had similar nitrogen retention to those infants fed a formula with higher protein content (2.24 g/100 kcal) [14]. The nutritional adequacy of long-term feeding with a MSW formula (whey/casein ratio 70/30, protein content 1.8 g/100 kcal) was tested in healthy, term infants from birth to 4 months and compared to a conventional whey-predominant formula (whey/casein ratio 60/40, protein content 2.2 g/100 kcal) and to breast milk [15]. No differences were found among the 3 feeding groups for weight or length gains or for body mass indices. Protein intakes were lower in the infants fed the MSW

Fig. 3. Amino acid composition (mg/100 kcal) of human milk (□), casein- (■) and whey-predominant (■) formulas. aEssential amino acids.
Table 2. Amino acid composition of human milk (HM), whey-predominant formula (WPF) and modified sweet whey formula (MSWF)

<table>
<thead>
<tr>
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<th>g/16 g N</th>
<th>mg/100 kcal</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>HM</td>
<td>WPF</td>
</tr>
<tr>
<td>Isoleucine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.4</td>
<td>6.4</td>
</tr>
<tr>
<td>Leucine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.5</td>
<td>9.8</td>
</tr>
<tr>
<td>Lysine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.9</td>
<td>8.7</td>
</tr>
<tr>
<td>Methionine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7</td>
<td>2.4</td>
</tr>
<tr>
<td>Phenylalanine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.6</td>
<td>3.9</td>
</tr>
<tr>
<td>Threonine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.6</td>
<td>6.0</td>
</tr>
<tr>
<td>Tryptophan&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Valine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.8</td>
<td>6.4</td>
</tr>
<tr>
<td>Cystine</td>
<td>2.3</td>
<td>1.9</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>4.7</td>
<td>4.5</td>
</tr>
<tr>
<td>Arginine</td>
<td>4.2</td>
<td>2.8</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.8</td>
<td>2.1</td>
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</table>

Protein content: HM, 1.5 g/100 kcal; WPF, 2.46 g/100 kcal; MSWF, 1.83 g/100 kcal.
<sup>a</sup>Essential amino acids.

Table 3. Plasma concentrations (μmol/l) of amino acids of 120-day-old infants breast-fed or fed either a whey-predominant formula (WPF) or a modified sweet whey formula (MSWF)

<table>
<thead>
<tr>
<th></th>
<th>BF</th>
<th>WPF 2.2</th>
<th>MSWF 1.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoleucine&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>47.8 ± 12</td>
<td>73.1 ± 13.6</td>
<td>55.6 ± 10.1</td>
</tr>
<tr>
<td>Leucine&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>87.8 ± 24.3</td>
<td>121.7 ± 22.6</td>
<td>106.2 ± 18.6</td>
</tr>
<tr>
<td>Lysine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>132.9 ± 35.4</td>
<td>194.2 ± 42.9</td>
<td>170 ± 35</td>
</tr>
<tr>
<td>Methionine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.2 ± 4.2</td>
<td>27.9 ± 5.2</td>
<td>27.1 ± 5.7</td>
</tr>
<tr>
<td>Phenylalanine&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>38.9 ± 7.7</td>
<td>51.5 ± 8.8</td>
<td>47.9 ± 10.2</td>
</tr>
<tr>
<td>Threonine&lt;sup&gt;a&lt;/sup&gt;</td>
<td>103.8 ± 23.6</td>
<td>164.2 ± 44.8</td>
<td>132.8 ± 27.4</td>
</tr>
<tr>
<td>Tryptophan&lt;sup&gt;a&lt;/sup&gt;</td>
<td>66.5 ± 12.5</td>
<td>63.3 ± 11.6</td>
<td>75 ± 11.5</td>
</tr>
<tr>
<td>Valine&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>139.2 ± 31.2</td>
<td>206.8 ± 29.3</td>
<td>151.6 ± 19.6</td>
</tr>
<tr>
<td>Arginine&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.6 ± 20</td>
<td>86.3 ± 22.1</td>
<td>110.6 ± 26.6</td>
</tr>
</tbody>
</table>

Protein/energy ratio of 2.2 and 1.8 g protein/100 kcal for WPF and MSWF, respectively. Values are given as mean ± SD.
<sup>a</sup>Essential amino acids. <sup>b</sup>Insulin-secretagogue amino acids.

Formulas than in those fed the classical whey formula, however the energy intakes were identical in the 2 groups. Finally, both urea [15] and plasma amino acid levels (table 3) of MSW FF infants were closer to those found in BF infants. The adequacy and safety of a similar formula developed with partially hydrolyzed proteins have also been demonstrated [16].
Metabolic Advantage of Reduced Protein Formula

Infants fed ‘classical’ casein- or whey-predominant formulas have higher levels of some amino acids and consistently elevated urea levels in their blood when compared to BF infants [17]. The short-term effects of these differences on infant growth and health have been carefully assessed and considered as safe. Nevertheless, the impact of both the quantity and quality of protein intake during early life on the incidence of disease later in life is, as yet, not known.

Dehydration and Kidney Functions

Potential renal solute load refers to solutes of dietary origin that would need to be excreted in the urine if not utilized by the body. It represents the sum of dietary nitrogen, sodium, potassium, chloride and phosphorus [18] and is a suitable parameter to measure the risk of dehydration illness. When ingested in excess, proteins constitute a considerable part of the solutes that must be excreted by the kidneys. Taking this into consideration, Ziegler and Fomon [18] recommended reducing the maximum protein content of infant formula from the level of 4.5 g/100 kcal, specified by the Food and Drug Administration, to 3.2 g/100 kcal. As an adaptive response to a high solute load, glomerular filtration rate and kidney size increase. While the adverse effects of a high protein intake in patients with kidney disease have been documented, there is to date no clear evidence of such detrimental effects in healthy individuals. The effect of formula- vs. breastfeeding on kidney growth was recently investigated in a cohort study of 631 healthy children examined at 3 and 18 months of age [19]. The results showed that kidney size and serum urea nitrogen were greater in the FF infants. Nevertheless, the differences in relative kidney size were temporary, as they were no longer apparent at 18 months of age [19]. The consequences of such increased kidney growth on kidney functions later in life are unknown.

Growth and Adiposity

Growth velocity seems to differ between BF and FF infants, especially when infants are breast fed for more than 6 months. Exclusively BF infants tend to grow more rapidly in the first 2–3 months of life, but from 6 to 12 months have a body weight, body length or body weight for length that are slightly lower than FF infants [20–23]. A significantly higher protein and/or energy intake [24–26] is associated with the faster growth rate observed in FF infants, but conflicting results exist [27, 28]. To date, the long-term impact of this moderate difference in growth on obesity risk later in life, is unknown. An association between high dietary protein intake during early childhood and subsequent adiposity has also been proposed [29]. In a longitudinal study a positive correlation was found between protein intake at the age of 2 years, but not at 10 months of age, and the body mass index and subcapular skin-fold at 8 years of
age [29]. Although some studies [30, 31] have described an association between protein intake at ≥9 months of age and adiposity in later childhood, other studies have reported an association with body size but not with body fat mass [32]. Interestingly, the influence of excess protein intake during the first year of life on weight gain in infancy and the risk of obesity later in life is now being studied in a large European Childhood Obesity Project [33].

A high protein intake is likely to have endocrine effects. It has been suggested that the higher growth velocity reported in FF vs. BF infants is due to a high protein intake early in life that promotes secretion of insulin-like growth factor-1 (IGF-1), a trophic hormone involved in longitudinal growth as well as muscle and fat mass development. Although there is limited knowledge about neonatal endocrine responses to milk feeding, there is increasing evidence that at 2 [34] and 6 months of age [35] FF infants have greater serum IGF-1 levels than BF infants. In a recent study, Savino et al. [34] observed that plasma IGF-1 levels are directly correlated with the Z score for weight, body mass index and tricipital skin-fold thickness in 2-month-old infants. Interestingly, we have observed that, during the first 4 months of life, infants fed a MSW formula (1.9 g protein/100 kcal) have a different evolution of their IGF-1 plasma levels to infants fed a normal sweet whey formula (2.4 g protein/100 kcal). IGF-1 levels decrease significantly (p = 0.013) between 28 and 112 days in infants fed formulas with a reduced level of protein (fig. 4).

Fig. 4. IGF-1 levels (µg/l) in infants fed a modified sweet whey protein formula with a reduced protein content of 1.9 g protein/100 kcal (▲) or a normal sweet whey formula containing 2.4 g protein/100 kcal (■).
IGF-1 levels average 79.3 ± 34.0 ng/ml (mean ± SD, n = 35) at 28 days and 58.9 ± 37.8 ng/ml (n = 41) at 112 days of age. On the other hand, in infants fed control formula IGF-1 levels did not decrease. IGF-1 levels average 77.5 ± 31.0 ng/ml (n = 21) at 28 days and 80.8 ± 37.8 ng/ml (n = 27) at 112 days of age (fig. 4). This confirms the strong influence of protein intake on IGF-1 levels. Interestingly, IGF-1 levels with a reduced protein formula are similar to those reported for BF infants [36].

Insulin acts as a growth factor during development and is considered as an anabolic factor of lean and fat mass. Moreover, protein intake is known to potentiate glucose-stimulated insulin secretion [37]. The amino acid profile of the ingested protein itself plays a role in the insulin response to feeding. In this respect, lysine, leucine, phenylalanine, valine and arginine are the amino acids which are the most potent insulin secretagogues [38]. Interestingly, FF newborns show higher postprandial plasma insulin levels than their BF counterparts [39]. Up to 6 months of age, urinary C-peptide (a marker of insulin secretion) correlates with plasma valine levels and is 2 to 3 times greater in FF infants than in BF infants [40]. Similarly, infants fed a formula containing 2.5 g protein/100 kcal between 4 and 6 months of age have higher levels of urinary C-peptide than BF infants or infants fed a formula containing 1.8 g protein/100 kcal [24]. In this study, weight gain is reported to correlate with C-peptide excretion, protein intake and plasma concentrations of the branched-chain amino acids, valine, leucine and isoleucine [24]. Interestingly, infants fed a modified sweet whey formula (1.8 g protein/100 kcal) vs. a classical whey-predominant formula (2.2 g protein/100 kcal) displayed significantly lower plasma levels of a number of amino acids, such as valine, leucine and isoleucine (table 3), which are considered to be insulin secretagogues. While the arginine levels were higher with the modified formula, the phenylalanine levels were unchanged (table 3). It would be of interest to investigate whether these differences are associated with decreased insulin levels in the plasma and/or C-peptide excretion. Additional studies are also needed to determine the short- and long-term metabolic consequences of these differences.

**Future Development for Improving the Protein Quality in Infant Formula**

New technological processes have allowed the development of nutritionally adequate and safe infant formulas with a protein content closer to breast milk. The benefits of further decreasing the protein/energy ratio of infant formulas to <1.8 g/100 kcal are probably limited. However, providing the required protein and energy intake is not the only means to prevent malnutrition or disease. In addition to its nutritional qualities, human milk provides a diversity of specific bioactive proteins such as hormones, cytokines, and growth factors
which have physiological relevance beyond purely nutritional properties [41].
These factors are implicated in growth and development, protective defense mechanisms, energy metabolism and immune homeostasis in multiple tissues. Despite the improvement in infant formula development, differences in response to infection and the development of allergy and atopic disease have been reported for FF and BF infants [42]. Although some of these bioactive proteins are also present in bovine milk [41], it has generally been assumed that they do not survive the technological processing used in the manufacture of infant formulas. As such, infant formulas are considered to lack some of the protective factors which human milk inherently provides.

With the advent of new methodology, the identification of milk proteins and their biological activity has received more attention. Indeed, we have recently reported that human milk contains proteins such as transforming growth factor (TGF)-β [43], osteoprotegerin [44] and soluble CD14 [45], which are involved in immune homeostasis, innate responses to bacterial components and bone metabolism. TGF-β is a multifunctional polypeptide. It acts upon a variety of different cells to regulate their growth, differentiation and survival, and plays a crucial immunoregulatory function in mechanisms of tolerance and the prevention of disease and autoimmunity [46]. TGF-β is present in milk and may be activated by acidification or mild enzymatic treatment [47], thus it is feasible that it is activated during intestinal transit to exert a biological effect in the intestinal epithelium of the host. Furthermore, since TGF-β is present in bovine milk, we considered that it may remain biologically active after exposure to some manufacturing processes. To address this possibility, we analyzed a range of milk-based preparations and raw materials for their TGF-β content. As expected, its presence is dependent on the milk protein source and the processing conditions [43]. Interestingly, we showed that a casein-based formulation, with acceptable preserved levels of TGF-β, prevents diarrhea in animal models of inflammation [48, 49] and induces remission and mucosal healing in children with small bowel Crohn’s disease [50, 51]. It is acknowledged that the technological process used to make the TGF-β-containing casein may also have preserved some other bioactive molecules. Nevertheless, this work on TGF-β casein, although carried out for a different application, is one illustration of how further improvement in infant formula products may be possible. It remains to better characterize the bioactive molecules in human milk and more importantly to demonstrate their biological activities and their benefits for infant growth and development.

To mediate optimal biological activity some factors will require binding to specific host receptors. In this respect, supplementation with recombinant human proteins may be advantageous. Indeed, the potential benefits of using recombinant human lactoferrin expressed in plants [52] or transgenic cows [53] is currently being investigated. However, such approaches have legal and regulatory issues, which are unlikely to be resolved in the immediate future.
In the interim, new biotechnology is preferred which enables the use of naturally occurring molecules in physiological doses and in an environment which is more likely to exert optimal biological activity.

**Conclusions**

Human milk composition is unique and breastfeeding provides components that nourish, protect and develop infants in the best way. Since the beginning of infant formula development, much effort has been made to try to mimic the characteristics of human milk. A better understanding of the protein composition of breast milk in association with technological breakthroughs in cow’s milk fractionation, have led to the development of infant formulas with a protein content that is closer to that of human milk. Today, infant formulas with a protein/energy ratio of 1.8 g/100 kcal are commercially available. These formulas have been shown to be safe and nutritionally adequate for term infants. However, the short-term and potentially long-term metabolic benefits of formulas with reduced protein content have still to be elucidated and are currently under investigation. Further improvement in the protein quantity in infant formula and the health benefits for the infants will be difficult. However, in addition to providing amino acids as building blocks for growth, milk is a source of numerous bioactive factors which are involved in multiple physiological processes. Continuous efforts are being made to identify new bioactive compounds in human milk. However, a better understanding of their biological functions in suckling infants as well as a comparison with their bovine counterparts are needed. Technological processes which preserve some bioactive factors in cow’s milk already exist. These processes could certainly be applied to infant formulas. Finally, the use of recombinant proteins is an interesting option for the development of infant formulas, which provide functions that resemble even more those provided by human milk.

**References**


Protein Quality and Quantity in Cow’s Milk-Based Formula

57 American Academy of Pediatrics, Committee on Nutrition: Commentary on breastfeeding and infant formulas, including proposed standards for formulas. Pediatrics 1976;57:278.

Discussion

Dr. van Goudoever: Does your study mean that other factors are more rate-limiting than tryptophan or what explanation do you have, although you find a better metabolic tolerance by lowering the nitrogen excretion rate and the urea levels?

Dr. Macé: Perhaps I misunderstood but there is no deficit in tryptophan today in infant formula I believe there are casein-predominant formula or whey-predominant formula or this modified sweet whey. They are all going to supply an adequate amount of tryptophan. But if you want to reduce the whey-predominant formula to 1.8 g protein/100 kcal, here you will have an issue. But today there is no whey-predominant formula on the market at this level for this reason. With the modified sweet whey protein formula you can lower the protein to 1.8 and there will be no deficit in tryptophan or essential amino acids.

Dr. van Goudoever: Let me rephrase the question then. Do you think by giving this specific formula, you said 1.8 g/100 kcal, that by giving 2.0 g/100 kcal, you will improve the weight gain rates?

Dr. Macé: I don’t know because we have not tested this. We have tested 1.9 g/100 kcal with partially hydrolyzed formula, but we didn’t make a comparison between growth in these two studies.

Dr. Haschke: May I comment on this? We are fully satisfied with the weight gain rates because they correspond to those of breastfed infants. So there is no reason to make them fatter.

Dr. Pencharz: What you are basically doing is producing a better quality breast milk substitute protein source. I just have a comment and then a question. In presenting your nitrogen balance data I would suggest that you give the nitrogen balance as nitrogen percent or as a percent of nitrogen balance divided by nitrogen intake because that would show you have a better nitrogen percent utilization in this new product, the sweet whey protein-enhanced product. Now the question I have is in regard to amino acid balance. Threonine is a chronic problem, and it wasn’t clear to me from your star graphs whether the threonine was still high or whether you brought it down. As we have shown the utilization of threonine from formula is different than human milk, the oxidation is not as good; not that hyperthreonemia is pathological but we do see that with whey protein. What did you see with this new product in terms of plasma threonine levels? Finally the other amino acid of interest to me is cysteine; have you looked at glutathione levels? We are not just talking about protein, we are also talking about other physiological issues like antioxidants in terms of glutathione; so threonine and cysteine and the various points I raised.

Dr. Macé: I will follow your advice for the metabolic study. The threonine levels are quite reduced in this formula. I am not sure about the cysteine level. I will have to check that.
Dr. Butte: It was fascinating to see that you had equal energy intake, equal weight gain, with the new formula vs. the breastfed group. So of course it would be very nice to measure the body composition in these children. Have you thought about measuring the sleeping metabolic rate? Another study we did a long time ago was sleep monitoring and in those studies we found that breastfed infants had higher rates of rapid eye movement vs. non-rapid eye movement compared to the formula-fed infants. It is also intriguing that you are now matching the plasma tryptophan levels to see the effects on the sleeping metabolic rate and doing sleep studies.

Dr. Dewey: I want to follow up on Dr. Butte’s question because you mentioned the growth data but you didn’t show them. I am very interested to know what the sample size was in your groups; whether the birth weights were similar between the breastfed and the modified formula group; if it was sex balanced; was the breastfed group exclusively breastfed, and lastly have you looked separately at birth to 4 months, or was it 1–4 months?

Dr. Haschke: May I clarify this? All infants were enrolled during the first week of life and were measured for the first time at 7 days of age. The data that were shown here were the weight gains between 30 and 120 days. All other questions can be answered by looking at the publication. Prof. Räihä was the first author and it was published 2 years ago in the Journal of Pediatric Gastroenterology and Nutrition [1]. The trial was done according to the FDA rules, and was FDA approved. All the questions you have related to sex, birth weight and breastfeeding are explained in that publication. We followed the rules.

Dr. Macé: There is no statistical difference in length gain but the size of the cohort was limited: 28 per group.

Dr. Rigo: Can you speculate on the results of the study on α-lactalbumin where it was shown that there is a reduction in blood urea nitrogen in boys only [2]? In your study you have two levels of protein intake so it is more difficult to see if there was an effect on blood urea nitrogen.

Dr. Macé: I have to admit that I put the two formulas in protein density which was not mentioned in the paper; it was grams per liter. The calorie density of the formulas was not also mentioned. In fact we don’t know what the protein density of these formulas was, but at least with the information we got the difference was not so high and it is surprising that they saw a difference in urea excretion. But I am not a pediatrician; perhaps someone can answer that.

Dr. Haschke: The energy density of the two formulas was 67 kcal/100 ml.

Dr. Rigo: What is the bioactive role of glycomacropeptide?

Dr. Macé: This morning Dr. Butte also asked if cyclic guanosine monophosphate (cGMP) is released in human milk. I cannot answer this question; I don’t know if anyone else has the answer. The k-casein content of human milk is only 5% so I doubt that this small amount of cGMP, even if it is released during the ingestion of human milk, is going to have a biological impact on satiety for example. What has also been shown with cGMP, but again with high levels of cGMP, is that it increases the glucagon-like peptide-1 levels and cholecystokinin. So it seems that it can trigger the secretion of some of the ingredients involved in food intake, but this has been done in rats or even in vitro. Unfortunately I can’t answer your question; it is not clear what the role of cGMP is in human milk.

Dr. Haschke: There is a very interesting effect of cGMP, in that it protects against caries. We discovered that approximately 10 years ago when we began to modify the formula protein level, and the cGMP fraction became available. Now the Colgate Company has bought our patent and they add it to toothpaste.

Dr. Lönnerdal: To follow up the question about cGMP; I think that there are two possible physiological effects and we should be a bit concerned about the concentration
dependency. Some of these bioactive components can be quite potent at low concentrations, but I will come back to that in my talk. cGMP provides oligosaccharides in bound form and oligosaccharides have prebiotic effects and also other potential antibacterial effects with regard to attachment, and potentially the cGMP-bound carbohydrates could be somewhat more stable in the gut. Further, cGMP is heavily sialylated so it is negatively charged and when we used a formula enriched in cGMP in infant rhesus monkeys, we found a positive effect on both iron and zinc absorption [3]. The concentration of cGMP may be too low in breast milk to have any significant effect but in formulas it potentially could have some significance with regard to absorption of these cations.

Dr. Giovannini: Do you have any data about the neural behavior of children with this formula? We studied long-chain polyunsaturated fatty acids and behavior some years ago [4]. Because tryptophan is the basis for serotonin metabolism, it is interesting to see how the children cry and sleep. I think these are very important parameters when there is more tryptophan in a formula with less protein vs. a traditional standard formula. It is important to recognize this problem because when we use different amino acids and different diet systems we may see differences in crying in the first period of life and also later in sleep.

Dr. Macé: I fully agree that tryptophan is important for the sleep pattern and things like that because of serotonin, but we haven’t found a deficit, it is like in human milk. So to answer your question, no we didn’t measure behavior in infants on these formulas. But I would not expect to see a difference with breastfed infants because the amino acids or the tryptophan levels are not higher or lower, they are close to human milk.

References
