Effects of High Protein Intakes

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Abstract
Among other nutrients of breast milk, the amino acid pattern is considered normative throughout infancy. Exclusive breastfeeding by a healthy mother should be the standard from birth to 6 months. During the breastfeeding period the protein intake is low in the human being compared too many other animals. The protein content in breast milk is about 1 g/100 ml and the daily protein intake approximately 1 g/kg/day. When other foods are introduced during the weaning period the protein intake increases remarkably to 3–4 g/kg/day in spite of the fact that the protein requirement is decreasing. The long-term consequences of this phenomenon are obscure. A high protein intake may have both endocrine, metabolic, physiologic effects and may increase the risk of obesity. Studies in humans are still surrounded by a number of uncertainties. Few studies have addressed the nutritional needs of infants at the time of weaning and the long-term consequences of the changes in the diet.
Here the methods by which the international recommendations for protein intake have been determined will be discussed. Furthermore the metabolic, endocrine and physiologic effects of a high protein intake will be considered.

**Requirement and Recommended Protein Intake of Term Infants**

Protein requirement during the first 6 months of life has been estimated using the healthy breast-fed infant as a model [1, 2]. Another approach to estimate the protein requirement of infants is the theoretical calculations used in the ‘factorial method’ [2]. This method for calculation of protein requirements is used especially in infants between 6 and 12 months of age who are not normally exclusively breast-fed. Different authorities give different recommendations depending on the method used for calculating the requirement. The factorial method consists of two parts, the requirement for maintenance and the requirement for growth.

The protein requirement for maintenance is that needed to replace losses through urine, feces and the skin. The factorial method uses many assumptions such as the adequacy of nitrogen balance data for calculating maintenance requirements, the method used for estimation of the needs for growth, the degree of intra-individual variation of growth and the efficiency of converting dietary protein to body protein. According to Dewey et al. [3] the protein requirement has been overestimated using this method. Revised estimates for the mean requirement and safe level of protein intake by Dewey et al. [3] are given in table 1.

In addition to the methods mentioned above, clinical investigations are useful in which protein intakes are accurately determined and measurements of growth and protein nutritional status are carried out.

**Calculation of Protein Content in Formulas**

Authors refer to protein content in infant formula in different ways. There are three possible methods to determine the protein content: calculation of nitrogen content, protein determination, and protein as a sum of amino acids.

Crude protein includes all non-protein nitrogen (NPN)-containing substances. In cow’s milk this NPN amounts to 25–30 mg/100 ml consisting of urea nitrogen and free amino acids. True protein has been defined most often as total nitrogen minus NPN multiplied by the appropriate conversion factor. This calculation excludes nitrogen that is partially metabolically available to the body. Levels of NPN and urea nitrogen in formulas are dependent on the
The Scientific Committee for Food proposes to determine the crude protein content of all types of infant formula and follow-on formula as total nitrogen $\times 6.25$. In addition, the NPN content must not be higher than 15% of the total nitrogen content in formula based on intact proteins. The recommendation given by the Scientific Committee of Food for protein content in formula is 1.8 g/100 kcal (minimal protein content) for formula based on intact cow’s milk protein. The maximum value is 3.0 g/100 kcal based on all types of protein sources. The same values are proposed for follow-on formula.

**Metabolic Consequences of Different Protein Intakes**

Most metabolic experimental studies of infants have been on formula-fed infants during the first 3 months of life. Several of these studies have shown that infants fed formula with a true protein level of 15 g/l or higher have shown significantly higher plasma levels of several amino acids and urea nitrogen than those found in breast-fed infants [6–12].

A true protein level of 13 g/l has been shown to result in a plasma amino acid pattern similar to that of breast-fed infants [13]. It should be noted that bovine casein and whey are quite different from human casein and whey. A casein-predominant (18:82) formula gives higher concentrations of plasma tyrosine and phenylalanine and lower values of threonine compared to a formula with a whey:casein ratio of 60:40 [14]. Picone et al. [13] showed that formula with a whey:casein ratio of 50:50 provided an amino acid pattern more

### Table 1. Revised estimates for mean requirement and safe level of protein intake for infants

<table>
<thead>
<tr>
<th>Age months</th>
<th>Protein requirement of intake g/kg/day</th>
<th>Safe level g/kg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1</td>
<td>1.99</td>
<td>2.69</td>
</tr>
<tr>
<td>1–2</td>
<td>1.54</td>
<td>2.04</td>
</tr>
<tr>
<td>2–3</td>
<td>1.19</td>
<td>1.53</td>
</tr>
<tr>
<td>3–4</td>
<td>1.06</td>
<td>1.37</td>
</tr>
<tr>
<td>4–5</td>
<td>0.98</td>
<td>1.25</td>
</tr>
<tr>
<td>5–6</td>
<td>0.92</td>
<td>1.19</td>
</tr>
<tr>
<td>6–9</td>
<td>0.85</td>
<td>1.09</td>
</tr>
<tr>
<td>9–12</td>
<td>0.78</td>
<td>1.02</td>
</tr>
</tbody>
</table>

From: Dewey et al. [3] with permission.
characteristic of that of human milk than any of the widely used infant formulas with whey:casein ratios of 60:40 or 20:80.

In a series of studies, a low protein formula (13 g/l or 1.8 g/100 kcal) given to infants from 3 to 12 months resulted in serum urea and plasma amino acid concentrations similar to that found in breast-fed infants [13, 15–17].

In a recent study by Räiha et al. [14, 18] the plasma concentrations of valine were 132 (breast-fed), 149 (experimental whey-modified) and 210 (standard formula) \( \mu \text{mol/l} \), respectively. For threonine the figures were 124, 147 and 201 \( \mu \text{mol/l} \), respectively. Even for cysteine the plasma concentrations in the infants fed whey-modified low protein were closer to that found in breast-fed infants [14] (formula closer to the reference Nestlé Workshop, vol 47, suppl, Bachman [14]). Thus an improvement in the amino acid metabolism was found for several amino acids in this study.

**Protein Intake and Growth**

Since the theoretical calculations regarding protein requirements are somewhat insufficient as pointed out above, clinical experiments are necessary as a supplement.

Räihä et al. [9, 10] published studies in which normal term infants were fed either human milk, a standard formula (protein 15 g/l, 2.2 g/100 kcal), or a formula with reduced protein content of 12 g/l (18 g/100 kcal). Growth was adequate in all feeding groups from birth to 3 months of age. At 8 and 12 weeks the protein intake was significantly higher in infants fed standard formula when compared to breast-fed infants or the infants fed the reduced protein formula: 2.7 vs. 1.6 and 1.7 g/kg/day, respectively. Other studies confirm that formulas with protein concentrations of 11–12 g/l or 1.6–1.8 g/100 kcal result in adequate growth rates [13, 15, 19].

Between 4 and 8 months, the mean protein intake in exclusively breast-fed infants varies between 0.9–1 and 2 g/kg/day [20–22] and in partly breast-fed infants between 0.85 and 1.5 g/kg/day [16, 20, 23]. In this age group both formula feeding and cow’s milk feeding increased the mean protein to 2.74 and 4.75 g/kg/day, respectively [15, 19, 24–28]. After 9 months the corresponding protein intakes were 3.1 and 4.35 g/kg/day in formula and cow’s milk-fed infants [24, 26–28]. Thus, during the weaning period the protein intake increases remarkably although the requirement decreases.

Infants who were exclusively breast-fed, with a protein intake of 0.9 g/kg/day at 9 months [21], had a slower growth rate than formula-fed infants. Even if supplementary foods were given to breast-fed infants after 4 months of age, the weight gain was significantly lower in breast-fed compared to formula-fed infants between 4 and 18 months [29, 30]. Weight for length and skin-fold thickness were also found to be higher in formula-fed infants.
than in breast-fed infants during this age period [31]. The explanations for the difference in weight gain may be differences in protein intake together with differences in energy intake [24, 29]. If a formula with a protein content of 15 g/l is given together with supplementary foods during the first year of life, no deviation from current growth curves has been noticed in the studies that are currently available [9, 12, 15, 19, 23, 30]. In one published study, the growth pattern of infants who were gradually weaned between 3 and 12 months of age to a formula with a protein content of 13 g/l did not deviate from the standard growth curves [23].

**Hormonal Effects and Relation to Growth**

The effects of different protein intakes on weight gain, insulin secretion, and plasma concentrations of amino acids have been evaluated in a prospective study involving term infants fed breast milk, low protein formula, or high protein formula. The urinary C-peptide excretion in the infants fed the high protein formula was significantly higher than that in the infants fed the low protein formula or the breast-fed infants. Weight gain correlated with urinary C-peptide excretion and with protein intake [32].

These results were confirmed in another study [33]. Between 3 and 12 months, 71 healthy infants were breast-fed (BM group) or fed formulas with 13, 15 or 18 g/l of protein (F13, F15, F18 groups, respectively) and given the same weaning foods. Plasma BCAA (isoleucine, leucine, valine) and plasma C-peptide and urinary C-peptide were analyzed, and weight and length were measured at 6 and 12 months.

At 6 months, plasma BCAA was higher in F18 (p < 0.0001), F15 (p < 0.0001) and F13 (p = 0.022) than in BM and slightly higher in F18 than in F13 (p = 0.053; fig. 1). At 12 months, plasma BCAA was higher in F18 than in BM (p = 0.015). At 6 months, urinary C-peptide was higher (p = 0.017), and at 12 months slightly higher (p = 0.09) in F18 than in BM (fig. 1). Plasma C-peptide did not differ significantly between the groups at 6 or 12 months. At 6 months, protein intake in formula-fed infants was found to correlate with plasma BCAA (rs = 0.50; p = 0.016) and urinary C-peptide (rs = 0.48; p = 0.017). Plasma BCAA at 6 months in formula-fed infants was found to correlate with weight gain between 6 and 12 months (rs = 0.55; p = 0.0084).

In conclusion this study indicates that a higher protein formula induces higher plasma BCAA and higher insulin release at 6 months than if breast milk or lower protein formula is given. The effects of breast milk on plasma BCAA and insulin release persist, though attenuated throughout infancy. Positive correlations between plasma BCAA at 6 months and weight gain between 6 and 12 months may indicate that protein intake influences growth.

Furthermore in the same study we evaluated the effects of protein intake on insulin-like growth factor (IGF)-1 and IGF-binding protein (IGFBP)-1 in
3- to 12-month-old infants fed breast milk or formulas with different protein concentrations [34].

During the period 3–6 months, plasma IGF-1 decreased (median 43.0 (range 60.0) vs. 37.0 (63.0) μg/l, respectively; \( p = 0.02 \)) whereas plasma IGFBP-1 was found to increase (66.0 (164) vs. 90.0 (206) μg/l, respectively; \( p = 0.012 \)) in breast-fed infants. Plasma IGF-1 and plasma IGFBP-1 then remained unchanged between 6 and 12 months in breast-fed infants. At 6 months, plasma IGF-1 tended to be lower in infants fed formula with 13 g/l protein than in those fed formula with 18 g/l protein (39.0 (8.0) vs. 43.0 (23.0) μg/l, respectively; \( p = 0.073 \)). At 2 months, plasma IGF-1 was significantly lower in infants fed formula with 13 g/l of protein than in those fed formula with 18 g/l (34.5 (46.0) vs. 46.0 (59.0) μg/l, respectively; \( p = 0.009 \); fig. 2). Plasma IGFBP-1 was similar in breast-fed and formula-fed groups at 6 and 12 months.

Protein intake seems to influence plasma IGF-1 during infancy. Despite an increasing intake of weaning foods, the influence of breastfeeding on plasma IGF-1 remains during the second half of infancy.
Conclusions

Diets high in protein offer no benefits and can theoretically have a number of adverse effects. High circulating blood levels of amino acids may exceed the capacity of the hepatic and renal systems to metabolize and excrete the excess nitrogen. This may lead to acidosis, diarrhea and elevated levels of blood ammonia and urea. The high potential renal solute load associated with diets rich in protein reduces the margin of safety related to the maintenance of water balance. Consequently, during periods of illness with associated dehydration, the reduced capacity to excrete waste products increases the risk of hypernatremia.

In addition to the risk that high protein intakes can compromise fluid balance, excess protein intake has also been linked to obesity in later life which can be related to the hormonal effects.

References

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Discussion

Dr. Haschke: I have a short comment on the formula with lower protein content. The formulas that you are addressing, with 1.8 g of protein/100 kcal are now on the market, mainly due to the pioneering work of your group and Prof. Räihä. They are now available for infants who need formula feeding. Our company for example has introduced the 1.8 g formula/100 kcal starter formula in 68 countries around the world already, and in Viet Nam it will come during the next few weeks. The problem remaining is the regulatory framework for follow-up formulas. The protein level of follow-up formulas should go down as well as we all know, and you have demonstrated that. However, our regulatory framework, at least in the European Union and Codex Alimentarius, at present does not allow a reduction in the protein level.

Dr. Axelsson: But the new data from the Scientific Committee for Food say that you can use the same protein content in a standard infant formula as in a follow-on formula.

Dr. Haschke: Yes, but this is an expressed opinion; it has not been translated in European law so far.

Dr. Axelsson: I think there are still problems with the follow-on formulas in the United States.

Dr. Haschke: I am not so sure about the follow-on formulas because in the United States they play a minor role. It is clear that you can have formulas above 1.8 g/100 kcal as far as I am aware.

Dr. Ziegler: May I comment on this? The regulations in the United States are that every formula has to be suitable for the entire first year of life. So with a follow-on formula you still have to be able to meet the nutrient requirements of very young infants. That is the current rule.

Dr. Axelsson: I think that is good. But don’t you use follow-on formulas later?

Dr. Ziegler: In the United States we do not have any follow-on formulas.

Dr. Haschke: I think there are soy formulas that have stage 1 and stage 2. Good Start has just one stage. Maybe one more comment which is also important: in Europe there is the regulation that partially hydrolyzed protein formulas should have a higher protein concentration than regular formulas, and we are also working towards bringing down the protein content in partially hydrolyzed formulas. We feel that the high protein intake with partially hydrolyzed formulas is unnecessary and once the safety of reduced protein partially hydrolyzed formulas can be shown, they should also come onto the market with a protein level of 1.8 g/100 kcal.

Dr. Giovannini: Could you speculate on the possible association between higher protein intakes in older formulas in the later development of type-1 or type-2 diabetes and the possible link with reduced glucose tolerance? Because everybody speaks about obesity but glucose intolerance may be interesting.
**Dr. Axelsson:** I think it is very difficult to speculate. We have a very high frequency of obesity, even in Sweden. In 4-year-old children we find overweight and obesity in about 20%, and we have a very high frequency of breastfeeding. I don't know if a high protein intake would increase the risk of diabetes. There is so much speculation about that, and in the studies by Åkerblom et al. [1] from Finland they used Nutramigen hydrolysate of casein in infants who have diabetes in the family.

**Dr. Giovannini:** In the future young investigators must study glucose tolerance in the older people in our population, especially people born in the 1950s when human milk was not used very much. There are a lot of people who receive hypoglycemic drugs nowadays, and the clinicians forget to ask whether they were breastfed or not. So we need more data, and for this reason we need young investigators to check this in the future. Years ago there was a study on protein intake at 1 year of age in Italy where supposedly the Mediterranean diet is tradition [2]. But the Mediterranean diet exists only on paper because in the north of Italy the diet is similar to the middle European diet. In this study we found a protein intake 1.5 times higher than the allowance. Now in every part of the world you see mothers who think that giving more protein to children is healthy. This is a problem, it is important not only to think of weight but also of all the metabolic parameters.

**Dr. Pencharz:** I just wanted to touch on Dr. Haschke's question. In the report that Koletzko et al. [3] put together the recommendation to the Codex Alimentarius Commission is to basically not have a different protein intake, but it has not yet been agreed upon. First of all it has to be accepted by the Codex and ultimately in terms of European regulations, but it is moving in that direction.

**Dr. Bozo:** I have a comment and a question about hypernatremic dehydration and its relation to sodium in infant formula. To make this relation I think it is very important also to note the sodium concentration in the oral rehydration solution utilized during acute gastroenteritis. There are some recommendations to have a lower sodium concentration in the oral rehydration solution. Was this mentioned in the study you presented?

**Dr. Axelsson:** The sodium content in this formula was also high; both sodium and protein.

**Dr. Bozo:** What was the sodium concentration in the oral rehydration solution used during acute diarrhea? Was it high or 60 or 90?

**Dr. Axelsson:** It was 60, and the baby was given a very small amount, about 25 ml.

**Dr. Gomes-Pedro:** Kidney growth seems to be related to increased IGF-1 production with high protein diet. Have you got some data on urinary IGF-1 and kidney growth?

**Dr. Axelsson:** No, I have not.

**Dr. Yates:** When you were talking about complementary feeding, you mentioned that the high protein intake in the young child was perhaps too high because of the types of foods that were being given to the children or the very young child. What types of food would you suggest as a better source of weaning foods?

**Dr. Axelsson:** I can speculate. I think it is very difficult to come down to the requirement and the recommendation. You have to change a lot in the diet. Cow's milk is good for calcium and perhaps we should fortify it. You need meat for iron. It is difficult; it is a paradox.

**Dr. Shiuh-Bin Fang:** I am quite interested in what you said about the changes in kidney size between 3- and 18-month-old infants [4]. If there is an adaptation of kidney size, is it possible to find any functional changes or long-term defects after a transient renal adaptation? If it is an anatomic phenomenon, do you perform any functional evaluation on those babies who are formula-fed or partially formula-fed, such as hormonal tests or their long-term outcome?
Dr. Axelsson: In studies in rats for example, more has been shown about the function and the long-term effects. I can't answer regarding the long-term effects in children.

Dr. Shiuh-Bin Fang: Some changes in the kidney size are seen, however we don't know whether or not there are any long-term effects such as a hormone defect; hypertension may even develop in these babies.

Dr. Axelsson: Changes in the glomerular infiltration rate have been shown in rats for example, and also we have shown higher values of creatinine in urine. But I can't say more about that.

Dr. Turck: The Danish group of Hoppe et al. [5] showed that there was a strong relationship between protein intake in infancy and final height. On the other hand, even if the literature is contradictory, it has been suggested that there is a relation between growth rate, meaning length not weight, and an increased risk of cancer. Do you think that there might be a bridge between high protein intake and infancy and risk of cancer in adulthood?

Dr. Axelsson: I forgot to mention the study from Denmark. We are looking at these children who are now 7 years old, but I can't answer your question.

Dr. Ziegler: Could I ask you a sort of philosophical question? Why is there such a strong tendency in most cultures to provide older infants and toddlers with unduly high protein intakes? Is it that the mothers think it is good, or why is it?

Dr. Axelsson: I think it is tradition.

Dr. Telmesani: A question for Dr. Haschke. In the formulas that are partially hydrolyzed, why do we need to go higher on the proteins? Are we losing some of the proteins in the process of hydrolyzation?

Dr. Haschke: The EU initially recognized that measurements such as nitrogen balance in animals indicated lower protein retention from hydrolyzed formulas. However this was the first generation of hydrolyzed formulas. In the meantime, considerable progress has been made in the process of protein hydrolysis. Indeed, Dr. Ziegler has done a balance study with such a new formula showing that with 1.8 or 1.9 g/100 kcal, protein nutrition is perfectly adequate. So it was an improvement in technology that now allows us to decrease the protein content in hydrolyzed formulas.

References


