Nutritional evaluation of protein hydrolysate formulas in healthy term infants: plasma amino acids, hematology, and trace elements[1–3]

Olle Hernell and Bo Lönnerdal

ABSTRACT
Background: Protein hydrolysate formulas are used for infants with food allergy. Most studies of such formulas focus on their effect on allergy and rarely evaluate their capacity to provide normal nutritional status.
Objective: We compared plasma aminograms, serum urea nitrogen, and trace element status in breastfed infants, infants fed hydrolysate formulas, and infants fed milk formula.
Design: From 6 wk to 6 mo of age, infants were breastfed or fed regular milk formula (RF), 1 of 2 casein-hydrolysate formulas (CH-1 or CH-2), or whey-hydrolysate formula (WH). Anthropometric measures were taken monthly, and blood samples were collected at 6 wk and 6 mo. Plasma amino acids, serum urea nitrogen, hematologic indexes, plasma zinc, and plasma copper were analyzed.
Results: There were no significant differences in hemoglobin, serum transferrin receptor, copper, or zinc among groups. Serum ferritin was significantly lower in infants fed the CH formulas than in the other groups. Infants fed CH-2 had significantly higher serum urea nitrogen than did all other groups. Plasma threonine, valine, phenylalanine, methionine, and tryptophan were significantly higher in the hydrolysate formula groups than in the breastfed group. Plasma tyrosine was significantly lower in infants fed the CH formulas than in the breastfed group, whereas arginine was significantly higher in the WH group than in all other groups. Plasma proline was lower, whereas threonine and tryptophan were higher, in the WH group than in the CH groups.
Conclusions: The iron status of infants fed CH formula was lower than that of all other groups. The amounts of amino acids provided by hydrolysate formulas appear excessive compared with regular formula, which is reflected by high serum urea nitrogen (CH-2) and high plasma amino acid concentrations. A reduced and more balanced amino acid content of hydrolysate formulas may be beneficial. *Am J Clin Nutr* 2003;78:296–301.

KEY WORDS Protein hydrolysate formula, extensively hydrolyzed protein, plasma amino acids, infant nutrition, nutritional evaluation, trace elements, iron

INTRODUCTION

Hydrolysate formulas are frequently recommended for infants with cow milk protein allergy. Recently, it has become increasingly popular to use these formulas to treat infants with general, nonspecific gastrointestinal problems and also as a prophylaxis to avoid milk protein allergy when there is a history of allergy in the family (1). The use of extensively or partially hydrolyzed protein formula is estimated to have increased to 10–50% among formula-fed infants in countries such as the United States and France. Although some hydrolysate formulas can no doubt be used effectively to treat infants with cow milk allergy, the incidence of such allergy is low during infancy (2, 3). The efficacy of extensively or partially hydrolyzed formulas in the prevention of allergy in healthy infants is controversial (4), although several studies have shown such an effect of at least extensively hydrolyzed formulas in high-risk infants with a family history of allergy (5–8).

It is evident that apparently healthy infants are fed protein hydrolysate formulas, in many cases for long time periods. It is particularly important that such formulas provide safe and adequate amounts of all nutrients and not only be designed with their capacity to treat or prevent allergy as a primary goal. Considerable efforts have been made to optimize the protein content of regular infant formulas (9–11). With the use of the fasting plasma amino acid concentrations of breastfed infants as the standard, it has been found that high protein concentrations in formula result in very high concentrations of some amino acids in plasma (9). Such deviations may affect hormonal responses in the infant (12) and possibly also alter the transport of some amino acids through the blood-brain barrier (13). On the other hand, very low protein concentrations in formula may result in plasma amino acid concentrations considerably lower than those of breastfed infants (14). Although there is little evidence for harmful effects of plasma amino acid concentrations that differ from those of breastfed infants, it has been considered prudent to attempt to keep them as similar as possible (15). Few studies have evaluated the amino acid pattern of infants fed hydrolysate formulas for extended periods.

[1] From the Department of Clinical Sciences, Pediatrics, Umeå University, Umeå, Sweden (OH), and the Department of Nutrition, University of California, Davis (BL).

[2] Supported in part by Semper AB, Stockholm; Valio, Helsinki; and Mead Johnson, Stockholm.

[3] Reprints not available. Address correspondence to B Lönnerdal, Department of Nutrition, University of California, Davis, CA 95616. E-mail: bllonnerdal@ucdavis.edu.

Received June 20, 2002.
Accepted for publication March 5, 2003.

TABLE 1
Composition of the study formulas

	Casein-hydrolysate formulas		Whey-hydrolysate	Regular milk
	CH-1	CH-2	formula (WH)	formula (RF)
Energy (kcal/L)	670	700	670	660
Protein (g/L)	19	19	16	13
Fat (g/L)	26	27	35	35
Carbohydrate (g/L)	91	95	73	74
Calcium (mg/L)	630	600	510	460
Iron (mg/L)	13	9	8	4
Zinc (mg/L)	4	4	4	4
Copper (mg/L)	0.6	0.5	0.5	0.4

Trace elements are essential for the normal growth and development of infants (16). These nutrients are often bound to proteins and then gradually released during digestion to be absorbed in the small intestine. Many studies have explored the effect of various protein sources on mineral and trace element absorption (17). However, although it is known that amino acids and small peptides can affect trace element absorption quite differently from intact proteins, few studies have been conducted on the effect of feeding hydrolysate formula on the trace element status of infants. In this study, we investigated the effects of feeding 3 different hydrolysate formulas on plasma amino acids, serum urea nitrogen, and the iron, zinc, and copper status of infants from 6 wk to 6 mo of age. The results are compared with those from infants exclusively breastfed or fed regular cow milk formula.

SUBJECTS AND METHODS

Healthy term (≥37 gestational weeks) infants with normal birth weights (>2500 g) were recruited from 3 well-baby clinics in Umeå, Sweden. Home visits were made monthly by a research nurse. Infants were either exclusively breastfed or fed infant formula from 6 ± 2 wk of age until the end of the study at 6 mo of age; they had primarily been breastfed until the start of the study. The protocol for the study was approved by the Ethics Committee on Research Involving Human Subjects of the Faculty of Medicine and Odontology, Umeå University, and informed consent was obtained from the infants' parents.

Diets

The tested formulas included 2 casein-hydrolysate (CH) formulas [Nutramigen (CH-1; Bristol-Meyers, Evansville, IN) and the experimental product MA-1 (CH-2; Morinaga Milk Company, Japan)], 1 whey protein hydrolysate formula (WH; PeptidiTutteli; Valio, Helsinki), and a powdered whey-predominant (60:40) regular milk-based formula (RF; Baby-Semp 2, Semper AB, Stockholm). All protein hydrolysates were extensively hydrolyzed and in liquid form. The powdered formula was diluted with tap water according to the manufacturer's instructions. No iron drops or solid foods were allowed. However, limited quantities (15 g/d, or 1 tablespoon/d) of fruit purées (without iron) were allowed at 4–6 mo of age. These were provided by the investigators and chosen to minimize interference with trace element status. Infants were randomly assigned to a hydrolysate formula group by the nurse in a single-blind design (samples were coded and analyses were performed by staff who were blinded with regard to treatment group). Each group consisted of ≥10 infants: CH-1, $n = 15$; CH-2, $n = 10$; WH, $n = 20$; and RF, $n = 10$. A group of exclusively breastfed infants was also included ($n = 10$).

The gross nutrient composition of the formulas is given in **Table 1**. Because hydrolysate formulas contain no or very little protein, the "protein" concentration of such products is best expressed on an α-amino nitrogen basis. One of the hydrolysate formulas contained 13 mg Fe/L as ferrous sulfate (CH-1), whereas the other hydrolysates contained 9 (CH-2) and 8 (WH) mg Fe/L, respectively. The powdered formula (RF) contained 4 mg Fe/L as ferrous sulfate. The amino acid composition of the formulas is shown in **Table 2**.

Biochemical and hematologic indexes

Serum urea nitrogen was measured with a commercial kit that used urease (Sigma, St Louis). Proteins were separated from plasma by sulfosalicylic acid precipitation (6%), and free amino acids were analyzed in the supernatant fluid on a Beckman 6300 amino acid analyzer (Mountain View, CA). Hemoglobin was analyzed by the cyanomethemoglobin method, mean corpuscular volume by automated blood counter (SE 9000; Sysmex, Tillquist, Sweden), serum iron and transferrin saturation by commercial kits (Boehringer Mannheim, Indianapolis), serum ferritin by radioimmunoassay (Diagnostic Products Corporation, San Diego), and

TABLE 2
Amino acid composition of breast milk and of the study formulas[1]

	Breast milk	CH-1	CH-2	WH	RF
			μmol/L		
Arginine	2700	4368	4448	2644	2184
Histidine	1920	3677	3503	2129	2000
Isoleucine	4670	8702	8420	6031	6031
Leucine	9500	14 809	13 916	11 145	9924
Lysine	6050	11 164	11 137	9315	7877
Methionine	1090	3960	3523	1812	1946
Cysteine	1780	2479	1901	2727	1653
Phenylalanine	2990	5515	5127	2727	3091
Tyrosine	2970	2320	2215	2376	2707
Threonine	4850	7815	6697	8908	6303
Tryptophan	994	1471	1716	980	931
Valine	6020	12 222	11 222	7436	7179
Alanine	5770	7640	7382	8427	6067
Aspartic acid	9320	11 429	9835	12 932	9248
Glutamic acid	15 650	29 932	21 816	20 816	17 891
Glycine	4360	6133	4760	3733	3200
Proline	8850	18 261	18 461	5217	8783
Serine	5590	11 238	8790	7143	6762
Taurine	574	320	240	400	360

[1] Data for breast milk are from reference 18. The study formulas were analyzed by each manufacturer (acid hydrolysis, ion-exchange chromatography). CH-1 and CH-2, casein-hydrolysate formula; WH, whey-hydrolysate formula; RF, regular milk formula.

TABLE 3
Weight and height of infants at birth and at 6 mo of age[1]

	BF (n = 10)	CH-1 (n = 15)	CH-2 (n = 10)	WH (n = 20)	RF (n = 10)
Weight (g)					
Birth	3623 ± 463	3574 ± 315	3571 ± 398	3501 ± 856	3263 ± 393
6 mo	8004 ± 1063	8190 ± 1114	8126 ± 863	7443 ± 1110	7733 ± 1175
Height (cm)					
Birth	50.5 ± 2.0	50.2 ± 1.8	52.4 ± 1.8	50.0 ± 4.0	49.8 ± 1.6
6 mo	68.0 ± 2.5	68.2 ± 2.1	68.5 ± 2.3	67.0 ± 3.9	67.2 ± 3.6

[1] $\bar{x}$ ± SD. BF, breastfed; CH-1 and CH-2, casein-hydrolysate formula; WH, whey-hydrolysate formula; RF, regular milk formula. There were no significant differences among the groups by ANOVA.

serum transferrin receptor by enzyme-linked immunosorbent assay (Ramco, Houston). Serum zinc and copper were determined by atomic absorption spectrophotometry (19).

Statistical analysis

Statistical analyses were performed by using repeated-measures analysis of variance (ANOVA) with control for baseline values of the variables analyzed. Skewed variables, eg, serum ferritin, were log transformed. When the ANOVA indicated significant group differences (P < 0.05), multiple comparisons were performed on adjusted means by using Tukey's method to identify which groups differed (P < 0.05). Values in the tables and figures are given as means ± SDs. Analyses were carried out with SAS for WINDOWS (version 6.12; SAS Institute Inc, Cary, NC).

RESULTS

All formulas studied were well tolerated by the infants, although some problems with constipation were noted in the CH-2 group and a tendency toward looser stools was noted in the WH group. No significant differences in weight, length, or weight or height gain were found between the groups when adjusted means were used, but the study was not designed as a growth study and did not have adequate statistical power to detect such differences (**Table 3**).

Serum urea nitrogen concentrations in infants fed CH-2 were significantly higher than in all other groups (**Table 4**), whereas infants fed CH-1 had significantly higher serum urea nitrogen values than did breastfed infants. Among the 3 hydrolysate formulas studied, CH-1 and CH-2 had a higher protein concentration than did WH and RF (Table 1).

The essential amino acids threonine (except for the CH-1 group), valine, phenylalanine, methionine, and tryptophan and total branched-chain amino acids were significantly higher in the hydrolysate formula groups than in the breastfed group (**Figure 1**A). Compared with the RF group, only tryptophan was higher in the hydrolysate formula groups, although the difference was not significant for the CH-1 group. Among the nonessential amino acids, only glycine was higher in the hydrolysate formula groups than in the breastfed group, whereas no significant difference was found compared with the RF group (Figure 1B). Tyrosine concentrations were significantly lower in infants fed CH-1 and CH-2 than in infants fed WH or RF. However, concentrations of arginine were significantly higher in infants fed WH than in breastfed infants and infants fed CH-1 and CH-2, and threonine was significantly higher in the group fed WH than in all other groups. Concentrations of proline were higher in infants fed CH-2 than in all other groups. Tryptophan concentrations were significantly higher in the group fed WH than in infants fed CH-1, but the difference was not significant for the group fed CH-2.

There were no significant differences in hemoglobin concentrations among the groups (Table 4). Serum ferritin concentrations, however, were significantly lower (P < 0.05) in infants fed CH-1 or CH-2 than in breastfed infants and infants fed WH or RF, but there was no significant difference between infants fed WH or RF. There were no significant differences in serum transferrin receptor concentrations among groups and no significant differences in serum zinc and copper concentrations.

DISCUSSION

The hydrolysate formulas studied were well tolerated at a young age and resulted in satisfactory growth of healthy, term

TABLE 4
Biochemical and hematologic indexes of study groups at 6 mo of age[1]

	BF (n = 10)	CH-1 (n = 15)	CH-2 (n = 10)	WH (n = 20)	RF (n = 10)
Serum urea nitrogen (μmol/L)	11.2 ± 2.2[a]	18.0 ± 2.7[b]	25.5 ± 2.8[c]	16.7 ± 3.7[a,b]	16.0 ± 3.2[a,b]
Hemoglobin (g/L)	114 ± 8	116 ± 8	116 ± 9	114 ± 10	115 ± 8
Mean corpuscular volume	77.6 ± 3.5	82.0 ± 3.5	81.4 ± 2.0	82.1 ± 3.5	78.4 ± 2.2
Serum iron (μmol/L)	9.9 ± 6.4	8.6 ± 3.5	10.4 ± 1.6	10.4 ± 3.8	13.7 ± 3.8
Total-iron-binding capacity	47.6 ± 6.2	51.6 ± 5.1	52.8 ± 9.8	51.4 ± 7.9	48.5 ± 9.1
Serum ferritin (μg/L)	59 ± 25[a]	35 ± 16[b]	32 ± 13[b]	67 ± 50[a]	46 ± 18[a]
Serum transferrin receptor (mg/L)	7.3 ± 1.6	6.3 ± 2.4	5.3 ± 1.3	7.0 ± 3.8	6.4 ± 1.2
log Transferrin receptor/ferritin	2.1 ± 0.3	2.2 ± 0.7	2.3 ± 0.3	2.1 ± 0.4	2.3 ± 0.3
Serum zinc (mg/L)	0.78 ± 0.18	0.76 ± 0.37	0.83 ± 0.10	0.89 ± 0.28	0.88 ± 0.09
Serum copper (mg/L)	1.15 ± 0.29	0.89 ± 0.24	0.94 ± 0.10	0.96 ± 0.28	1.01 ± 0.17

[1] $\bar{x}$ ± SD. BF, breastfed; CH-1 and CH-2, casein-hydrolysate formula; WH, whey-hydrolysate formula; RF, regular milk formula. Means in a row with different superscript letters are significantly different, P < 0.05 (ANOVA and Tukey's test).

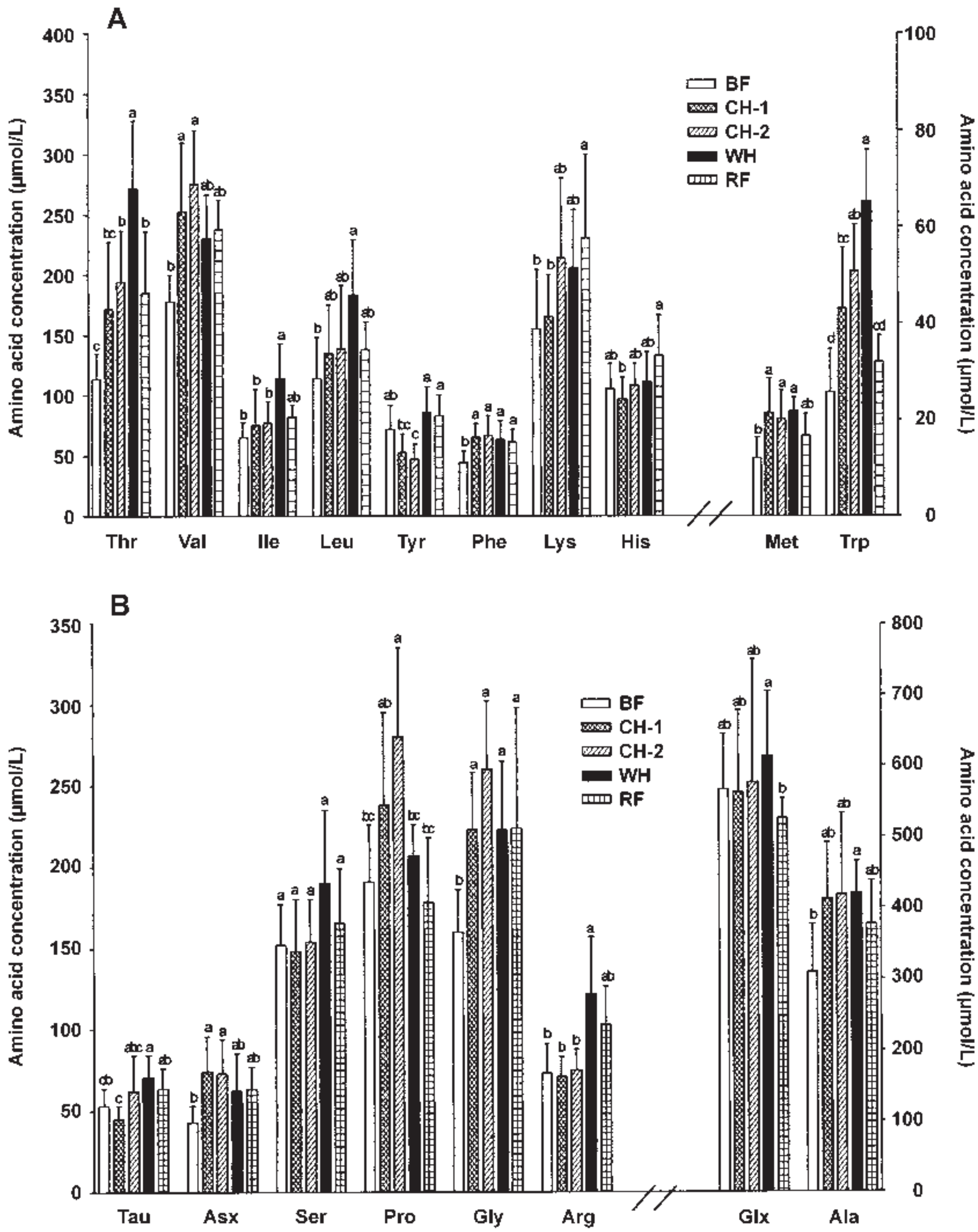

FIGURE 1. Mean (± SD) plasma essential (A) and nonessential (B) amino acid concentrations at 6 mo of age in infants fed breast milk (BF; $n = 10$), regular milk formula (RF; $n = 10$), 1 of 2 casein-hydrolysate formulas [CH-1 ($n = 15$) or CH-2 ($n = 10$)], or whey-hydrolysate formula (WH; $n = 20$) from 6 wk to 6 mo of age. Glx, sum of glutamine and glutamic acid; Asx, sum of asparagine and aspartic acid. The break in the axis denotes a change in scale (from left y axis to right y axis). Columns with different letters are significantly different, $P < 0.05$ (ANOVA and Tukey's test).

infants, although this study was not designed to assess growth as a major outcome. Isolauri et al (20) reported growth to be slower in infants fed whey-hydrolysate formula than in infants fed an amino acid–based formula; however, the infants in that study had multiple allergies and their formula intake may have been suboptimal because of a restricted diet. Infants with a family history of atopic disease who were partially breastfed and partially fed casein-hydrolysate formula from birth had lower body mass indexes than did breastfed infants at 3 mo of age (21), but there were no significant differences in anthropometric indexes. Healthy term infants fed a formula based on a mixture of whey-hydrolysate and casein-hydrolysate (60:40) for the first 2 mo of life were shown to have weight and length gains similar to those of infants fed regular milk formula (22). A study by Vandenplas et al (23) showed normal weight and length gain in infants fed a whey-hydrolysate formula from birth to 3 mo of age, although somewhat in contradiction, the infants' mean daily intake of formula was lower than that of infants fed a regular whey-predominant formula. Rigo et al (24), however, showed that newborn infants fed some hydrolysate formula (based on soy-collagen, whey-casein, or whey) during the first month of life grew slower than did infants fed regular formula, whereas the growth of infants fed other types (brands) of whey-hydrolysate formulas was similar to that of infants fed regular formula. However, the formulas also differed in fatty acid composition, which may have affected energy balance and growth. In our study, the infants were not fed hydrolysate formula for the first 6 wk of life, mainly because few infants in Sweden are fed formula from birth, but partially also because allergic manifestations are rare at this early age. The consequences of feeding hydrolysate formula may differ with postnatal age and maturation.

The significantly higher serum urea nitrogen concentrations observed in the present study in the groups fed CH-1 and CH-2 than in the breastfed infants were most likely due to the high amino acid concentrations of these products. It was shown previously that infants fed formulas with high protein concentrations have high serum urea nitrogen concentrations (25), which result from hepatic catabolism of excess amino acids in plasma. A study by Giovannini et al (21) also showed high serum urea nitrogen concentrations in infants fed casein-hydrolysate formula. The reason for the high amino acid concentrations of the casein-hydrolysate formulas is not entirely clear, but it is possible that in previous studies virtually all infants fed such products had severe atopic disease and had already lost weight or gained less weight and therefore were in need of catch-up growth. Another possibility is that some amino acids in the protein hydrolysates used were low in concentration compared with breast milk; thus, a higher hydrolysate concentration may have resulted in amino acid concentrations more similar to those in breast milk. It is interesting to note that Vandenplas et al (23) found higher serum urea nitrogen concentrations in infants fed a whey-hydrolysate formula than in infants fed a whey-predominant formula, although the protein content of the formulas was similar. This raises the possibility that amino acid utilization may be lower from hydrolysate formulas, as was shown in adults fed an elemental diet (26). Different protein utilization in infants fed hydrolysate formula was also suggested in a study by Decsi et al (22), who found lower total serum protein concentrations in infants fed hydrolysate formula than in those fed conventional infant formula.

The very high concentrations of some plasma amino acids found in the infants fed the hydrolysate formulas are most likely explained by the high protein concentration (ie, amino acid concentration) of these formulas. The long-term physiologic consequences of these high concentrations are difficult to evaluate. However, note that there has been some concern about plasma tryptophan concentrations being too low in infants fed formulas with lower-than-normal protein concentrations (27). Tryptophan is involved in neurotransmitter (serotonin) metabolism, and differences in behavior between breastfed and formula-fed infants, such as sleeping patterns, may be explained by differences in circulating tryptophan (27, 28). A similar argument may be raised about the high plasma tryptophan concentrations found in our study. Furthermore, it has been suggested that the high concentrations of plasma branched-chain amino acids observed in infants fed formula with a high protein content may affect insulin metabolism and, consequently, carbohydrate metabolism, weight gain, and, eventually and hypothetically, diabetes development (25, 29). Although we have no evidence for such effects, it appears prudent to adjust the concentrations of tryptophan and branched-chain amino acids in hydrolysate formula so that plasma amino acid concentrations become more similar to those of breastfed infants.

The higher concentrations of plasma tryptophan, threonine, and arginine found in the infants fed the WH formula than in those fed the CH formulas may reflect the somewhat higher proportions of these amino acids in whey protein than in casein. Note that the concentrations of these amino acids were higher in the CH formulas (Table 2), but this was not reflected in the plasma amino acid pattern (Figure 2). The high concentrations of proline in the groups fed the CH formulas, however, may reflect both the higher proportion of this amino acid in casein than in whey protein and the much higher concentration in the CH formulas. However, Hauser et al (30) studied infants fed whey-hydrolysate formula or regular whey-predominant formula (at similar protein contents) and observed that differences in amino acid composition between the formulas did not explain the differences in plasma amino acid patterns. It is not known whether the differences are due to different rates of absorption of amino acids or peptides or to varying effects on amino acid metabolism. Considering these inherent differences in amino acid composition between casein and whey protein and the response in plasma amino acids, it appears that a combination of these 2 hydrolysates may result in a more physiologic aminogram, ie, one that is more similar to that of breastfed infants. This may be preferred over the addition of pure amino acids, which often have an unpleasant taste.

Although we found no significant differences in hemoglobin concentrations among the groups, the lower serum ferritin concentrations of the infants fed the CH formulas is of some concern, even if the concentrations did not indicate iron deficiency. We do not yet know the cause of these lower ferritin values, but it is known that casein can have a negative effect on iron absorption (31) and that this is due to negatively charged casein phosphopeptides, which may impair iron utilization. It is possible that small casein phosphopeptides formed during the hydrolysis process have a negative influence on iron status. It is therefore possible, but not yet proven, that iron absorption from casein-hydrolysate formula is lower than from whey-hydrolysate formula and that not even a high level of iron fortification (as in CH-1, 13 mg/L) could result in iron stores similar to those of the infants in the other groups. Further studies are needed to clarify this issue.

We found no significant differences in plasma zinc and copper between the formula groups or between the formula groups and the breastfed group. In a previous study in infant rhesus monkeys, we found no significant difference in zinc absorption between formulas based on casein-hydrolysate or whey-hydrolysate (32), and zinc absorption was similar to that from regular infant formula. Krebs et al (33) also measured fractional zinc absorption from casein-hydrolysate formula and found it to be higher than from regular formula, but other differences in composition of the formulas existed. Although casein, or casein phosphopeptides, can have a negative effect on zinc absorption (34) similar to that discussed above for iron, the free amino acids and small peptides present in protein hydrolysate formulas are known to have a positive effect on zinc absorption (35), which possibly compensates for the slight negative effect exerted by the phosphopeptides.

In conclusion, these results show that the total amino acid concentration of several hydrolysate formulas is unnecessarily high and that pronounced differences exist in plasma amino acid concentrations between breastfed infants and infants fed casein-hydrolysate formula or whey-hydrolysate formula. It is possible that the use of combinations of these protein hydrolysates, rather than the exclusive use of any one, may decrease these differences. This would likely also decrease serum urea nitrogen concentrations in infants fed such products and modulate the potential negative effect of casein-hydrolysate on iron status.

We thank Margareta Henriksson and Shannon Kelleher for their superb management of the clinical study and the analyses, respectively, and Janet M Peerson for statistical advice.

Both authors were responsible for the study design, data collection, data analysis, and writing of the manuscript. Both authors are members of the Scientific Advisory Board of Valio, and OH is a member of the Scientific Advisory Board of Semper.

REFERENCES

1. Committee on Nutrition. American Academy of Pediatrics. Hypoallergenic infant formulas. Pediatrics 1989;83:1068–9.
2. ESPGAN Committee on Nutrition. Committee report: Comment on antigen-reduced infant formulae. Acta Paediatr 1993;82:314–9.
3. Høst A. Cow's milk protein allergy and intolerance in infancy. Some clinical, epidemiological and immunologic aspects. Pediatr Allergy Immunol 1994;5(suppl):1–36.
4. Høst A, Koletzko B, Dreborg S, et al. Dietary products used in infants for treatment and prevention of food allergy. Arch Dis Child 1999; 81:80–4.
5. Vandenplas Y, Hauser B, van den Borre C, et al. The long-term effect of a partial whey hydrolysate formula on the prophylaxis of atopic disease. Eur J Pediatr 1995;154:488–94.
6. Chandra RK. Five-year follow-up of high-risk infants with family history of allergy who were exclusively breast-fed or fed partial whey hydrolysate, soy, and conventional cow's milk formulas. J Pediatr Gastroenterol Nutr 1997;24:380–8.
7. Oldaeus G, Anjou K, Björksten B, Moran JR, Kjellman NI. Extensively and partially hydrolysed infant formulas for allergy prophylaxis. Arch Dis Child 1997;77:4–10.
8. Halken S, Hansen LG, Skamstrup K, et al. Comparison of a partially hydrolysed infant formula with two extensively hydrolysed formulas for allergy prevention: a prospective randomised study. Pediatr Allergy Immunol 2000;11:149–61.
9. Karlsland Åkesson PM, Axelsson IEM, Räihä NCR. Protein and amino acid metabolism in three- to twelve-month-old infants fed human milk or formulas with varying protein concentrations. J Pediatr Gastroenterol Nutr 1998;26:297–304.
10. Lönnerdal B, Zetterström R. Protein content of infant formula—how much and from what age? Acta Paediatr Scand 1988;77:321–5.
11. Fomon SJ, Ziegler EE, Nelson SE, Frantz JA. What is the safe protein-energy ratio for infant formula? Am J Clin Nutr 1995;62:358–63.
12. Axelsson IEM, Ivarsson SA, Räihä NCR. Protein intake in early infancy: effects on plasma amino acid concentrations, insulin metabolism, and growth. Pediatr Res 1989;26:614–7.
13. Rassin DK. Essential and non-essential amino acids in neonatal nutrition. In: Räihä NCR, ed. Protein metabolism during infancy. New York: Nestle/Raven Press, 1994:183–92.
14. Järvenpää AL, Rassin DK, Räihä NC, Gaull GE. Milk protein quantity and quality in the term infant. II. Effects on acidic and neutral amino acids. Pediatrics 1982;70:221–30.
15. Heird WC. Interpretation of the plasma amino acid pattern in low birth weight infants. Nutrition 1989;5:145–6.
16. Lönnerdal B. Trace element nutrition in infants. Annu Rev Nutr 1989; 9:109–25.
17. Lönnerdal B. The effects of milk and milk components on calcium, magnesium and trace element absorption during infancy. Physiol Rev 1997;77:634–99.
18. Hanning RM, Paes B, Atkinson SA. Protein metabolism and growth of term infants in response to a reduced-protein, 40:60 whey:casein formula with added tryptophan. Am J Clin Nutr 1992;56:1004–11.
19. Clegg MS, Keen CL, Lönnerdal B, Hurley LS. Influence of ashing techniques on the analysis of trace elements in animal tissue. I. Wet ashing. Biol Trace Elem Res 1981;3:107–15.
20. Isolauri E, Sütas Y, Mäkinen-Kiljunen S, Oja SS, Isosomppi R, Turjanmaa K. Efficacy and safety of hydrolyzed cow milk and amino acid-derived formulas in infants with cow milk allergy. J Pediatr 1995;127:550–7.
21. Giovannini M, Agostoni C, Fiocchi A, Bellu R, Trojan S, Riva E. Antigen-reduced infant formulas versus human milk: growth and metabolic parameters in the first 6 months of life. Am Coll Nutr 1994; 13:357–63.
22. Decsi T, Veitl V, Szasz M, Pinter Z, Mehes K. Plasma amino acids in healthy, full-term infants fed hydrolysate infant formula. J Pediatr Gastroenterol Nutr 1996;22:62–7.
23. Vandenplas Y, Hauser B, Blecker U, et al. The nutritional value of a whey hydrolysate formula compared with a whey-predominant formula in healthy infants. J Pediatr Gastroenterol Nutr 1993;17:92–6.
24. Rigo J, Salle BL, Putet G, Senterre J. Nutritional evaluation of various protein hydrolysate formulae in term infants during the first month of life. Acta Paediatr 1994;402(suppl):100–4.
25. Järvenpää AL, Räihä NCR, Rassin DK, Gaull GE. Milk protein quantity and quality in the term infant. I. Metabolic responses and effects on growth. Pediatrics 1982;70:214–20.
26. Smith JL, Arteaga C, Heymsfield SB. Increased ureagenesis and impaired nitrogen use during infusion of a synthetic amino acid formula. N Engl J Med 1982;306:1013–8.
27. Räihä NCR, Minoli I, Moro G, Bremer HJ. Milk protein intake in term infants II. Effects on plasma amino acid concentrations. Acta Paediatr Scand 1986;75:887–92.
28. Janas LM, Picciano MF, Hatch TF. Indices of protein metabolism in term infants fed either human milk or formulas with reduced protein concentrations and various whey/casein ratios. J Pediatr 1987;110: 838–48.
29. Lönnerdal B, Chen C-L. Effects of formula protein level and ratio on infant growth, plasma amino acids and serum trace elements. II. Follow-up formula. Acta Paediatr Scand 1990;79:266–73.
30. Hauser B, Blecker U, Keymolen K, Suys B, Gerlo E, Vandenplas Y. Plasma amino acid concentrations in term-born infants fed a whey predominant or a whey hydrolysate formula. JPEN J Parenter Enteral Nutr 1997;21:27–30.
31. Hurrell RF, Lynch SR, Trinidad TP, Dassenko SA, Cook JD. Iron absorption as influenced by bovine milk proteins. Am J Clin Nutr 1989;49:546–52.
32. Rudloff S, Lönnerdal B. Calcium and zinc retention from protein hydrolysate formulas in suckling rhesus monkeys. Am J Dis Child 1992;146:588–91.
33. Krebs NF, Reidinger CJ, Miller LV, Borschel MW. Zinc homeostasis in healthy infants fed a casein hydrolysate formula. J Pediatr Gastroenterol Nutr 2000;30:29–33.
34. Lönnerdal B, Cederblad Å, Davidsson L, Sandström B. The effect of individual components of soy formula and cow's milk formula on zinc bioavailability. Am J Clin Nutr 1984;40:1064–70.
35. Wapnir RA, Khani DE, Bayne MA, Lifshitz F. Absorption of zinc by the rat ileum: effects of histidine and other low-molecular-weight ligands. J Nutr 1983;113:1346–54.