The Turkish Journal of Pediatrics 1997; 39: 195-202

COMPARISON OF GROWTH, SERUM PREALBUMIN, TRANSFERRIN, IgG AND AMINO ACIDS OF TERM INFANTS FED BREAST MILK OR FORMULA*

*Zafer Kurugöl MD**, Mahmut Çoker MD**, Canan Çoker MD****

*Ayten Egemen MD****, Biltan Ersöz MD******

SUMMARY: Kurugöl Z, Çoker M, Çoker C, Egemen A, Ersöz B. (Department of Pediatrics and the Research Laboratory, Ege University Faculty of Medicine, İzmir, Turkey). Comparison of growth, serum prealbumin, transferrin, IgG and amino acids of term infants fed breast milk or formula. Turk J Pediatr 1997; 39: 195-202.

This study was designed to compare growth parameters, biochemical indices of protein metabolism and serum amino acid patterns in newborn infants fed either human milk (n: 22) or formula with protein and amino acid concentrations similar to human milk (n: 19) during the first two months of life. Growth parameters were normal and similar in all infants. Serum levels of transferrin and IgG were not significantly different, whereas prealbumin concentrations were found to be significantly higher in infants fed formula compared to those fed human milk. In the formula group, glutamic acid and cystine levels were significantly lower while phenylalanine and proline were significantly higher compared to the breastfed group.

It was observed that feeding a formula with an amino acid pattern similar to that of human milk does not necessarily give rise to identical patterns of amino acids or indices of protein metabolism. *Key words: breastfed, formula, amino acids, growth.*

Human milk is accepted to be the most appropriate food for infants and is usually recommended during the first four to six months of life without any additional food. However, in some situations it may be necessary to use formulas for infant feeding. Formulas are being modified to be similar to human milk with respect to the composition and concentration of macro and micronutrients. Since optimal growth and development is achieved with mother's milk, the adequacy of formula is usually ascertained by comparing the growth of infants fed such products with that of breastfed infants. Along with other specifications, the protein content of formula is also modified to obtain a whey: casein ratio of 60:40, which is similar to that of human milk (63:37). It is suggested that the use of such formula

* From the Department of Pediatrics and the Research Laboratory, Ege University Faculty of Medicine, İzmir.

** Pediatrician, Ege University Faculty of Medicine.

*** Biochemist, Ege University Faculty of Medicine.

**** Professor of Pediatrics, Ege University Faculty of Medicine.

***** Professor of Biochemistry, Ege University Faculty of Medicine.

This work was awarded the Makbule Haktan mansion prize, and was presented at the following congress: XXIst International Congress of Pediatrics, September 10-15 1995, Cairo, Egypt.

with a protein concentration and amino acid pattern similar to human milk for infant feeding would result in indices of protein metabolism and amino acid patterns like those in breastfed infants[1-6].

Material and Methods

This study included 41 (21 male, 20 female) term, healthy infants who were being followed by the Social Pediatrics Section of the Department of Pediatrics at Ege University Medical School. These infants were registered for the study before birth, and they had no problems during the prenatal, natal or postnatal periods. Twenty-two were bearstfed and 19 received a formula in current use in Turkey, containing 15 g/L protein with a whey: casein ratio of 60/40 and 670 kcal/L. The claimed amino acid composition of the formula as well as that of human milk is shown in Table I. Mothers of the infants fed with formula were trained to prepare it, and the first serving was fed together with a nurse in the hospital. The daily food intake of the infants was regulated and followed on a feeding form completed by the mothers. Neither the formula-fed nor the breastfed infants received any additional nutrients throughout the study.

Table I: Amino Acid Composition of Human Milk[8] and Formula Used in This Study (mg/g of Protein)

Amino Acid	Human Milk	Formula
Threonine	54	40
Valine	59	66
Methionine	14	26
Isoleucine	59	46
Leucine	112	93
Phenylalanine	54	46
Lysine	82	73
Tryptophan	27	13
Cystine	17	6
Tyrosine	58	40
Arginine	44	33
Aspartate	109	73
Serine	58	53
Glutamate	194	193
Prolin	102	113
Glycine	31	20

Infants in both groups were followed up at regular intervals until the end of the second month of life. Measurements of weight, length and head circumference were made at birth and at the end of the first and second months. A digital scale accurate to ± 5 g was used to measure weight, and an infant-measuring board with a millimeter ruler was used to record length. All measurements were made by the same examiner, using the same tools.

Samples of preprandial (2.5-3 hours after the previous meal), peripheral venous blood were obtained at the end of the second month between 9.00 and 10.30 a.m. Serum proteins, prealbumin, transferrin and IgG were measured by immunonephelometry (Beckmann Protein Array). Amino acids in serum were measured by gas chromatography with a flame ionization detector as their n-acetyl-n-propyl ester derivatives, the chromatographic separation being accomplished on a capillary column[7].

The parents' consent to the study and the Ethics Committee Agreement of the Medical Faculty of Ege University were obtained.

Statistical analysis was performed using Student's t and Mann-Whitney U test.

Results

Growth parameters at birth and at the end of the first and second months are summarized in Table II. No significant differences were observed with regard to gains in weight height or head circumference. The recorded data was evaluated at the end of the second month, revealing that the daily intake of formula, protein and energy in the formula group was 130-150 ml/kg/day, 1.95-2.25 g/kg/day and 87-100 kcal/day, respectively.

Table II: Anthropometric Measurements

	Birth	End of First Month	End of Second Month
Weight (g)			
Human milk (n = 22)	3334 ± 499	4457 ± 614	5450 ± 730
Formula (n = 19)	3161 ± 571	4380 ± 348	5341 ± 619
Length (cm)			
Human milk (n = 22)	50.1 ± 1.7	54.9 ± 2.1	58.2 ± 2.4
Formula (n = 19)	49.9 ± 1.7	54.6 ± 2.8	57.8 ± 2.4
Head circumference (cm)			
Human milk (n = 22)	35.1 ± 0.9	37.6 ± 1.0	39.3 ± 1.0
Formula (n = 19)	35.0 ± 0.7	36.9 ± 1.3	39.0 ± 1.5

Values are means ± SD. There were significant differences among groups with regard to gains in weight, height, and head circumference.

Table III shows serum prealbumin, transferrin and IgG values at the end of the second month. Serum levels of transferrin and IgG were similar in the two groups, while serum levels of prealbumin were significantly higher in the group fed formula ($p < 0.01$).

Table III: Serum Biochemical Assessment of Protein Nutriture

Biochemical indices (g/L)	Human Milk (n = 22)	Formula (n = 19)
Transferrin	2.8 ± 0.5	2.8 ± 0.4
Prealbumin	0.13 ± 0.04*	0.17 ± 0.04*
IgG	4.6 ± 1.6	4.6 ± 1.0

Values are mean ± SD. Prealbumin concentrations were significantly higher in infants fed formula than in infants fed human milk ($p < 0.01$).

The amino acid concentrations at the end of the second month are presented in Table IV. No significant difference in serum total and essential amino acid concentrations or in the ratio of essential to total amino acids (0.24 in the breastfed and 0.28 in the formula group) was present between the two groups. However, glutamic acid and cystine were significantly lower while phenylalanine and proline were significantly higher in the formula group compared to the breastfed group. The mean values for the valine to glycine ratio were estimated to be 1.8 in the formula and 1.6 in the breastfed group, with no significant difference between the two groups.

Table IV: Serum Amino Acid Concentrations of Infants fed Human Milk or Formula at the End of the Second Month (μmol/L)

Amino Acid	Human Milk (n = 22)	Formula (n = 19)
Phenylalanine	56.6 ± 8.6*	116.5 ± 22.0*
Methionine	104.1 ± 12.8	104.7 ± 17.1
Aspargine + aspartic acid	33.0 ± 5.7	45.1 ± 8.9
Glycine	96.0 ± 10.9	96.3 ± 13.1
Threonine	79.6 ± 15.0	99.9 ± 22.1
Proline	35.4 ± 5.9*	78.4 ± 13.5*
Arginine	63.0 ± 13.2	64.5 ± 13.1
Isoleucine	19.6 ± 2.4	24.3 ± 2.8
Leucine	50.6 ± 6.1	45.2 ± 6.1
Alanine	117.4 ± 15.1	130.8 ± 29.4
Tyrosine	92.4 ± 19.6	72.9 ± 9.9
Lysine	155.7 ± 20.1	184.2 ± 26.7
Hydroxyproline	133.1 ± 17.8	115.2 ± 22.3
Serine	87.6 ± 11.5	60.7 ± 8.2
Valine	167.1 ± 30.2	141.7 ± 27.8
Glutamine + glutamic acid	863.0 ± 110.0*	505.4 ± 58.2*
Cystine	211.7 ± 64.3*	46.3 ± 9.3*

Values represent means ± SEM.

* In the formula group, glutamic acid and cystine levels were significantly lower while phenylalanine and proline were significantly higher than in the breastfed group ($p < 0.05$).

Discussion

Human milk, the only physiological nutrient for the newborn, is also ideal for growth and development. However, today many commercial formulas are being used either together with or as an alternative to breast feeding. Formulas are modified to be similar to human milk by adding some amino acids and/or by lowering the total protein and amino acid concentrations. It is suggested that the growth and development as well as the indices of protein metabolism and plasma amino acid profiles of the infants fed such formulas are similar to those of breastfed infants[1,4,5]. In formula feeding, over-feeding with a bottle may lead to excessive weight gain. On the other hand, improper preparation of the formula may result in inadequate weight gain. Nonetheless, it has been shown in multiple studies that the growth parameters of breast- and formula-fed, term babies are similar[6,8,9]. In this study, no significant differences were detected between weight gain, increments of length and head circumference of infants fed human milk or formula, all values being in the normal ranges.

The serum albumin level has long been used as an index for evaluating protein energy metabclism[9]. However, the long half-life of albumin (20 days in adults), although somewhat shorter in infants, limits its usefulness in assessing nutritional status. Recently, biochemical parameters with shorter half-lives, such as prealbumin and transferrin, have been gaining interest[4,6,8,10]. Studies on children with kwashiorkor have put forward that prealbumin is a sensitive indicator of protein energy malnutrition[11,12]. It is also considered to be useful for the early diagnosis of malnutrition, reflecting short-term changes in nutritional status[13]. Furthermore, data have been presented that changes in prealbumin levels occur at an earlier stage than changes in anthropometric measurements at different protein intake levels in premature infants[14].

Polberger et al.[2] have shown that prealbumin, RBP and transferrin are the most valuable plasma proteins for assessing protein metabolism in low birth weight infants, particularly stressing prealbumin, which is strongly correlated with protein intake. The formula in this study contained 15 g/L protein, and no difference was observed in the infants' growth. In fact, it is possible to obtain similar growth in formula-fed babies merely by increasing protein intake, thus leading to higher levels of prealbumin in serum.

Transferrin is accepted as an index of nutrition by some authors, while others claim that it is not valuable[2,15]. In accordance with Rigo et al.[6] and Picone et al.[8], no significant difference was observed in serum transferrin concentrations in infants fed either human milk or formula.

There is a controversy between various studies regarding the total amino acid concentration in the serum of infants fed formula with 15 g/L protein and a whey:

casein ratio of 60:40[5, 8, 16, 17]. No difference was observed in the total amino acid concentrations of the two groups of infants in this study.

However, compared to the breastfed group, glutamic acid and cystine levels proved to be significantly lower while phenylalanine and proline significantly higher in the formula group. In the neonatal period, due to a deficiency in cystathionase activity, methionine is not converted to cystine. Thus, cystine may be regarded as an essential amino acid for the neonate. Human milk supplies sufficient cystine. Picone et al.[8] have detected significantly higher levels of plasma total cystine in breastfed compared to formula-fed infants. Also, in this study serum cystine levels were found to be higher in infants fed human milk, which can be explained by the lower cystine content of the formula used.

Glutamine plays a regulatory role in protein synthesis. It has been shown that supplementation of the diet with glutamine speeds up the reparation of intestinal mucosa in infants[18].

The higher levels of glutamic acid in breastfed infants is considered to be a result of the rich glutamic acid content of human milk[19]. An enteresting finding of this study was that, although the glutamic acid content of the formula was equivalent to that of human milk, the glutamic acid levels were higher in breastfed infants.

In accordance with other studies, the phenylalanine concentration was found to be higher in formula-fed infants, even though the phenylalanine content of the formula was decreased in consideration of the fact that phenylalanine metabolism is inadequate in the newborn[3, 8]. However, it was also observed that phenylalanine values did not reach toxic levels in any of the infants.

Similar to phenylalanine, proline levels were determined to be significantly higher in the formula group. Parallel to this result, some previous investigators have found increased proline values in formula-fed infants[3, 8], while others had contrary findings[6]. The reason for the discrepancy in proline levels remains unclear.

Valine is a branched-chain amino acid which has an important role in insulin secretion[20]. The high valine/glycine ratio in formula-fed babies accounts for the hyperinsulinemia that leads eventually to insulin resistance[21]. Recent epidemiological studies have shown that the incidence of insulin-dependent diabetes is higher in children who have not received human milk[22]. Contrary to this finding, in this study the valine levels in serum and the valine/glycine ratio were higher in breast-fed infants, however the difference was not statistically significant.

In spite of continuous efforts to modify formula to be biologically equivalent to human milk, there are differences in the serum levels of micronutrients even though the macronutrients are similar in breast- and formula-fed infants. Also, in this study, the group fed formula had higher levels of prealbumin, phenylalanine and proline and lower levels of glutamic acid and cystine compared to the group fed human milk.

In conclusion, this study provides evidence that formulas with an amino acid profile similar to that of human milk do not necessarily produce plasma patterns similar to those of breast fed infants. Besides the protein and amino acid content of the nutrient, other factors such as the difference in the bioavailability of amino acids in human milk or formula, variable concentration and type of amino acids over the course of lactation, and hormonal response to feeding may also influence amino acid concentrations.

The clinical impacts and the intrinsic results of these discrepancies in the infant remain to be elucidated. It is not known whether the higher concentrations of some amino acids cause metabolic intolerance or whether the lower concentrations of others cause deficiency symptoms in formula-fed infants.

Further modifications in the protein content of infant formulas will depend on more expanded studies which evaluate macro-and micronutrients together to identify and determine the significance of the variables which affect the protein nutritional status.

REFERENCES

1. Moro GE, Minoli I, Fulconis F, Clementi M, Raiha NC. Growth and metabolic responses in low-birth weight infants fed human milk fortified with human milk protein or with a bovine milk protein preparation. J Pediatr Gastroenterol Nutr 1991; 13: 150-154.
2. Polberger SK, Fex GA, Axelsson IE, Raiha NC. Eleven plasma proteins as indicators of protein nutritional status in very low birth weight infants. Pediatrics 1990; 86: 916-921.
3. Priolisi A, Didato M, Gioeli R, Fazzolari-Nesci A, Raiha NC. Milk protein quality in low birth weight infants: effects of protein-fortified human milk and formulas with three different whey-to-casein rations on growth and plasma amino acid profiles. J Pediatr Gastroenterol Nutr 1992; 14: 450-455.
4. Janas LM, Picciano MF, Hatch TF. Indices of protein metabolism in term infants fed either human milk or formulas with reduced protein concentration and various whey/casein ratios. J Pediatr 1987; 110: 838-848.
5. Raiha N, Minoli I, Moro G, Bremerr HJ. Milk protein intake in the term infant. II. Effect on plasma amino acid concentrations. Acta Paediatr Scand 1986; 75: 887-892.
6. Rigo J, Salle BL, Cavero E, Richard P, Putet G, Senterre J. Plasma amino acid and protein concentrations in infants fed human milk or a whey protein hydrolysate formula during the first month of life. Acta Paediatr 1994; 83: 127-131.
7. Adams RF. Determination of amino acid profiles in biological samples by gas chromatography. J Chromatogr 1974; 95: 189-212.
8. Picone TA, Benson JD, Moro G, et al. Growth, serum biochemistries, and amino acids of term infants fed formulas with amino acid and protein concentrations similar to human milk. J Pediatr Gastroenterol Nutr 1989; 9: 351-360.
9. Raiha NC, Heinonen K, Rassin DK, Gaull GE. Milk protein quantity and quality in low-birth-weight infants: I. metabolic responses and effects on growth. Pediatrics 1976; 57: 659-684.
10. Moskowitz SR, Pereira G, Spitzer A, Heaf L, Amsel J, Watkins JB. Prealbumin as a biochemical marker of nutritional adequacy in premature infants. J Pediatr 1983; 102: 749-753.
11. Ingenbleek Y, De Visscher M, De Nayer P. Measurement of prealbumin as index of protein-calorie malnutrition. Lancet 1972; 2: 106-109.

12. Ogunshina SO, Hussain MA. Plasma thyroxine binding prealbumin as an index of mild protein-energy malnutrition in Nigerian children. Am J Clin Nutr 1980; 33: 794-800.

13. Yoder MC, Anderson DC, Gopalakrishna GS, Douglas SD, Polin RA. Comparison of serum fibronectin, prealbumin and albumin concentrations during nutritional repletion in protein calorie malnourished infants. J Pediatr Gastroenterol Nutr 1987; 6: 84-88.

14. Georgieff MK, Sasanow SR, Pereira GR. Serum transthyretin levels and protein intake as predictors of weight gain velocity in premature infants. J Pediatr Gastroenterol Nutr 1987; 6: 775-779.

15. Georgieff MK, Amarnath UM, Murphy EL, Ophoven JJ. Serum transferrin levels in the longitudinal assessment of protein-energy status in preterm infants. J Pediatr Gastroenterol Nutr 1989; 8: 234-239.

16. Janas LM, Picciano MF, Hatch TF. Indices of protein metabolism in term infants fed human milk, whey-predominant formula, or cow's milk formula. Pediatrics 1985; 75: 775-784.

17. Jarvenpaa AL, Rassin DK, Raiha NC, Gaull GE. Milk protein quantity and quality in the term infant. II. Effects on acidic and neutral amino acids. Pediatrics 1982; 70: 221-230.

18. Cooke RJ, Watson D, Werkman S, Corner C. Effects of type of dietary protein on acid-base status, protein nutritional status, plasma levels of amino acids and nutrient balance in the very low birth weight infant. J Pediatr 1992; 121: 44-451.

19. Wu PY, Edwards N, Storm MC. Plasma amino acid pattern in normal term breast-fed infants. J Pediatr 1986; 109: 347-349.

20. Ginsburg BE, Lindblad BS, Lundsjo A, Persson B, Zetterstrom R. Plasma valine and urinary C-peptide in breast fed and artifically fed infants up to 6 months of age. Acta Paediatr Scand 1984; 73: 213-217.

21. Wallensteen M, Lindblad BS, Zetterstrom MR, Persson B. Acute C-peptide, insulin and branched chain amino acid response to feeding in formula and breast fed infants. Acta Paediatr Scand 1991, 80: 143-148.

22. Borch-Johnsen K, Joner G, Mandrup-Poulsen T, et al. Relation between breast-feeding and incidence rates of insulin-dependent diabetes mellitus. A hypothesis. Lancet 1984; 2: 1083-1086.