

# Effects of $\alpha$ -lactalbumin-enriched formula containing different concentrations of glycomacropeptide on infant nutrition<sup>1–3</sup>

Olof Sandström, Bo Lönnerdal, Gitte Graverholt, and Olle Hernell

## ABSTRACT

**Background:** Formula-fed infants have growth and plasma amino acid patterns different from those of breastfed infants.

**Objective:**  $\alpha$ -Lactalbumin is a major protein in human milk, and the addition of bovine  $\alpha$ -lactalbumin to infant formula has been proposed to modify the plasma amino acid pattern of the recipient infant, possibly allowing a reduction in the protein content of the formula, which may affect growth.

**Design:** We compared breastfed infants and infants fed standard formula or  $\alpha$ -lactalbumin-enriched formulas (25% of protein) with glycomacropeptide accounting for 15% or 10% of the protein. The protein content of each formula was 13.1 g/L. Ninety-six infants aged  $6 \pm 2$  wk were recruited. Anthropometric measures were recorded, and interviews were conducted at enrollment and monthly until 6 mo of age. Blood samples were collected at enrollment and at 4 and 6 mo.

**Results:** Formula intake did not differ between groups, and weight gain in the  $\alpha$ -lactalbumin-enriched formula groups were similar to that of the breastfed infants. The standard formula group gained significantly more weight than did the breastfed infants. All formula-fed infants had significantly higher plasma concentrations of most essential amino acids at 4 and 6 mo than did the breastfed infants, and serum urea nitrogen was also higher in the formula-fed infants. Insulin and leptin concentrations did not differ between groups.

**Conclusions:** Compared with standard formula-fed infants, infants fed formula with a modified protein composition had growth patterns more similar to those of breastfed infants. All formula-fed groups had plasma amino acid concentrations similar to or higher than those of breastfed infants. This indicates that the protein content of  $\alpha$ -lactalbumin-enriched formula can be further reduced, which should be evaluated. *Am J Clin Nutr* 2008;87:921–8.

## INTRODUCTION

The use of cow milk as the protein source in infant formula results in a casein-dominant formula, which differs from the whey protein predominance in human milk. Consequently, the whey:casein ratio of infant formula has been adjusted to resemble that of human milk ( $\approx 60:40$ ) by the addition of whey protein concentrate. Despite the adjustment, there are considerable differences in the protein composition of infant formula and human milk. A large part of that difference is due to a higher concentration of  $\alpha$ -lactalbumin in human than in bovine milk. Furthermore, bovine whey contains a high concentration of  $\beta$ -lactoglobulin, which is absent in human milk (1). Current dairy technology makes it possible to produce bovine

whey fractions that are reduced in  $\beta$ -lactoglobulin and enriched in  $\alpha$ -lactalbumin (2, 3). Adding bovine  $\alpha$ -lactalbumin to infant formula has been proposed, because it modifies the plasma amino acid pattern in infants, making it more similar to that of breastfed infants (4–6).

The optimal protein content of infant formula is a matter of controversy. Although there is general agreement that the protein content in currently used formula exceeds infant requirements, the extent to which protein can be safely reduced is not clear. This is because some amino acids, notably tryptophan and arginine, become limiting by the current protein composition of formula. The alternatives are to maintain a protein concentration higher than necessary or reduce the protein concentration and add free amino acids. It has been argued that excess protein places unnecessary strain on immature metabolic organs (7, 8), that adding free amino acids appears unphysiological, and that the metabolic consequences of doing so are largely unknown. A more physiologically relevant approach would be to modify the protein composition of formula to make it more similar to human milk, which would presumably lead to plasma amino acid patterns more similar to those of breastfed infants. The amino acid composition of  $\alpha$ -lactalbumin is well balanced, with a high proportion of certain essential amino acids. Increasing the  $\alpha$ -lactalbumin concentration in formula, and thus concentrations of these essential amino acids, would allow a reduction of the total protein concentration and result in a more balanced plasma amino acid profile in formula-fed infants (9). In addition to being a source of amino acids,  $\alpha$ -lactalbumin is believed to have several physiologic effects, such as antimicrobial activity, enhanced immune function, prebiotic function, and increased trace element absorption (10, 11). Thus, there are several potential benefits of adding  $\alpha$ -lactalbumin to formula that need to be evaluated in clinical studies.

Glycomacropeptide (GMP) is a large acidic glycoprotein that is cleaved from  $\kappa$ -casein by pepsin hydrolysis in the stomach.

<sup>1</sup> From the Department of Clinical Sciences, Pediatrics, Umeå University, Umeå, Sweden (OS and OH); the Department of Nutrition, University of California, Davis, CA (BL); and Arla Foods Ingredients, Aarhus, Denmark (GG).

<sup>2</sup> Supported by Arla Foods Ingredients, Aarhus, Denmark.

<sup>3</sup> Address reprint requests and correspondence to O Hernell, Department of Clinical Sciences, Pediatrics, Umeå University, S-901 85 Umeå, Sweden. E-mail: olle.hernell@pediatri.umu.se.

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GMP is not present in breast milk, but it is formed during digestion and is believed to have antimicrobial, mineral-absorption-enhancing (12), and prebiotic (11–14) effects. GMP is also formed from bovine  $\kappa$ -casein by the action of rennet during the manufacturing of cheese and has recently become commercially available (2). Consequently, it is of interest to study the clinical effects of adding GMP to infant formula. In our initial study, we found only minor effects of the experimental formulas studied herein on intestinal microbiota (15).

## SUBJECTS AND METHODS

### Experimental formula

Bovine whey fractions rich in  $\alpha$ -lactalbumin with various concentrations of GMP were provided by Arla Foods Ingredients (Aarhus, Denmark) and were produced as previously described (2, 3). The formulas were as follows: 1) whey-predominant standard infant formula (11%  $\alpha$ -lactalbumin, 14% GMP); 2)  $\alpha$ -lactalbumin-enriched formula (25%  $\alpha$ -lactalbumin), with GMP accounting for 15% of the protein content ( $\alpha$ -LAC); and 3)  $\alpha$ -lactalbumin-enriched formula (25%  $\alpha$ -lactalbumin), with GMP accounting for 10% of the protein content (RGMP; **Table 1**).

### Study population

The study was controlled, blind, and randomized. Ninety-six infants were recruited from well-baby clinics in Umeå, Sweden. Formula-fed infants were randomly assigned to the standard ( $n = 21$ ),  $\alpha$ -LAC ( $n = 20$ ), or RGMP ( $n = 21$ ) formula groups. The first infant was assigned to group A, the second to group B, and the third to group C and so on. The type of formula was blinded to the parents and the research nurse. Breastfed infants served as a reference group ( $n = 34$ ). Each participating infant fulfilled the inclusion criteria of being healthy, having a gestation period of 36 to 42 wk, and having a birth weight between 2500 and 5000 g. Infants born by cesarean delivery were excluded. The study was approved by the Ethics Committee on Research Involving Human Subjects of the Faculty of Medicine, Umeå University, and informed consent was obtained from each infant's parents.

### Study design

Infants aged  $6 \pm 2$  wk were recruited for the study. The infants were exclusively breastfed or exclusively formula fed 1 of the 3 experimental formulas until they were 4 mo of age. At 4 mo of age, the infants were permitted 1–2 Tbsp fruit and/or vegetable puree each day. Subsequent to the initial enrollment visit, an experienced research nurse visited each participating infant when the infant was 2, 3, 4, 5, and 6 mo of age. Anthropometric measurements, structured interviews concerning sleeping time and general well-being, and questionnaires regarding formula intake, bowel habits, stool consistency on the last 3 days before the visit, and morbidity during the whole month were obtained at all visits (15). Formulas were fed with bottles graded to the nearest 5 mL, and parents recorded the volume at the beginning and the end of each meal. Weight was measured with a Seca 835 digital baby scale (Seca, Hamburg, Germany), and recumbent length was measured with a Harpenden infantometer (CMS Weighing Equipment, London, United Kingdom). Knee-heel length was measured with an Infant Knemometer (BK5; FORCE Institutet, Brøndby, Denmark). Head circumference was measured with a nonstretchable measuring tape. Blood samples were

**TABLE 1**

Nutrient content of the formulas<sup>1</sup>

Nutrient	Standard	$\alpha$ -LAC	RGMP	Recommendations <sup>2</sup>
Energy (kcal/100 mL)	66.9	66.9	66.9	60–70
Protein (g)	1.96	1.96	1.96	1.8–3
Carbohydrate (g)	11.1	11.2	11.2	9–14
Fat (g)	5.3	5.3	5.3	4.4–6
Calcium (mg)	67.6	68.9	65.6	50–140
Phosphorus (mg)	44.8	45.4	44.1	25–90
Sodium (mg)	30.2	30.9	35.4	20–60
Potassium (mg)	113.3	114.8	109.6	60–160
Chloride (mg)	74.7	75.2	74.9	50–160
Magnesium (mg)	7.5	7.3	6.9	5–15
Iron (mg)	0.6	0.6	0.6	0.3–1.3
Zinc (mg)	0.6	0.6	0.6	0.5–1.5
Copper ( $\mu$ g)	60.4	60.5	60.5	35–80
Iodine ( $\mu$ g)	14.6	14.6	14.6	10–50
Selenium ( $\mu$ g)	2.4	2.4	2.4	1–9
Vitamin A ( $\mu$ g RE)	101.5	101.6	101.6	60–180
Vitamin D ( $\mu$ g)	2.1	2.1	2.1	1–2.5
Vitamin E (mg)	4.0	4.0	4.0	0.5–5
Vitamin K ( $\mu$ g)	6.0	6.0	6.0	4–25
Thiamine ( $\mu$ g)	97.6	97.9	97.9	60–300
Riboflavin ( $\mu$ g)	138.4	138.7	138.7	80–400
Niacin ( $\mu$ g)	1186	1190	1189	300–1500
Pantothenic acid ( $\mu$ g)	588.2	589.8	589.5	60–300
Pyridoxin, vitamin B-6 ( $\mu$ g)	99.1	99.4	92.3	35–175
Cobalamin, vitamin B-12 ( $\mu$ g)	0.58	0.58	0.58	0.1–0.5
Folic acid ( $\mu$ g)	13.9	13.9	13.9	10–50
Vitamin C (mg)	14.2	14.3	14.3	10–30
Choline (mg)	23.0	18.5	19.9	7–30
Biotin ( $\mu$ g)	5.8	5.8	5.8	1.5–7.5
Inositol (mg)	6.3	6.3	6.3	4–40
Histidine (mg)	41.0	44.2	45.0	—
Isoleucine (mg)	116.2	120.6	114.9	—
Leucine (mg)	187.6	180.9	185.4	—
Lysine (mg)	154.0	156.5	159.3	—
Methionine (mg)	41.9	37.4	35.7	—
Phenylalanine (mg)	71.0	74.3	75.0	—
Threonine (mg)	104.6	103.6	102.7	—
Tryptophan (mg)	28.5	36.9	36.5	—
Valine (mg)	119.1	114.7	114.6	—
Tyrosine (mg)	69.8	75.0	77.0	—

<sup>1</sup> All nutrients are expressed per 100 kcal, except for the energy content, which is per 100 mL.  $\alpha$ -LAC,  $\alpha$ -lactalbumin enriched (15% glycomacropeptide); RGMP,  $\alpha$ -lactalbumin enriched (10% glycomacropeptide).

<sup>2</sup> Global standard for the composition of infant formula. Recommendations of a coordinated international expert group of the European Society for Paediatric Gastroenterology (16).

drawn at enrollment and at 4 and 6 mo of age. Blood samples were drawn before a meal, generally 2–4 h after the latest meal.

Hematology and iron status were assessed at the Department of Clinical Chemistry, Umeå University Hospital. Iron status was analyzed by a Sysmex SE 9000 Autoanalyzer (Tillqvist, Kista, Sweden). Hemoglobin was analyzed with a Sysmex Sulfolyser automated hemoglobin reagent (Toa Medical Electronics Co, Ltd, Los Alamitos, CA), and mean corpuscular volume was automatically calculated from the erythrocyte particle concentration and hematocrit. Serum iron (S-Fe) was analyzed by the ferrozine method (Iron kit 1553712 and UIBC kit 1030600;



Boehringer Mannheim, Scandinavia AB, Bromma, Sweden). Serum ferritin (S-Ft) was analyzed with an immunoturbidimetric technique (BM/Hitachi 704/717/911; Boehringer Mannheim) calibrated against World Health Organization standard 80–602. Serum urea nitrogen (BUN) was measured with a commercial kit using urease (Sigma, St Louis, MO). Proteins were separated from plasma with 6% sulfosalicylic acid precipitation, and free amino acids were analyzed in the supernatant fluid on a Beckman 6300 amino acid analyzer (Beckman, Mountain View, CA). Plasma insulin and leptin were analyzed by radioimmunoassay (Linco Research, St Louis, MO).

### Statistical analysis

SPSS version 11 (SPSS Inc, Chicago, IL) was used for all statistical analyses. Analysis of variance was performed, and Bonferroni post hoc tests were used to adjust for multiple comparisons. Values are expressed as means  $\pm$  SDs. For those variables measured at study enrollment (anthropometric data, hematology, and iron status), analysis of covariance was used to adjust for differences at baseline. Initial values ( $6 \pm 2$  wk) were used as covariates. For variables for which there were differences between males and females, sex was a covariate.

Anthropometric data are reported as mean increments per month, and  $z$  scores were compared with a World Health Organization/National Center for Health Statistics reference population (17). Data were converted to  $z$  scores by using EPI-INFO version 1.1.1 (Centers for Disease Control and Prevention, Atlanta, GA) and the 2000 Centers for Disease Control and Prevention reference growth data.

## RESULTS

Eighty (83%) of the 96 recruited infants completed the study. In the standard formula group, 2 infants developed allergies to cow milk protein, and 2 families chose to discontinue participation in the study. In the  $\alpha$ -LAC group, 2 infants developed allergy to cow milk protein, and one family chose to discontinue participation in the study. In the RGMP group, 1 child developed an allergy to cow milk protein, and 3 families chose to discontinue participation in the study. Five of the breastfed infants dropped out of the study because of inadequate milk production. There were no statistically significant between-group differences in dropout rate or the reasons for doing so. No serious adverse events were recorded for any of the groups. Mean gestational age, birth weight, and recumbent length were similar for the formula-fed and breastfed groups. However, there were significant differences in sex distribution between the groups (Table 2). Post

hoc analyses were performed, and we found that amino acid concentrations differed between males and females. Therefore, sex was included as a covariate when plasma amino acid patterns were analyzed. Other variables did not differ significantly between groups.

### Formula intake and infant growth

Infants in the standard formula group consumed the most formula, although the amount did not differ significantly between groups (Table 3), and they gained significantly more weight than did the breastfed infants ( $P = 0.03$ ). There were no significant differences between infants in the  $\alpha$ -LAC, RGMP, or breastfed groups, nor were there significant group differences in the increase in recumbent length, knee-heel length, or head circumference (Table 3).

Weight-for-age  $z$  (WAZ) and length-for-age  $z$  (LAZ) scores were higher than the international reference population in all groups (Figure 1). Breastfed infants had a WAZ score of  $\approx 1.0$  at enrollment, and their score gradually decreased during the study. Infants in the standard formula group had lower scores than did the breastfed infants at enrollment, but their scores increased during the study. Infants in the  $\alpha$ -LAC and RGMP groups had the lowest scores at enrollment, and their scores increased only marginally during the study. The WAZ scores of the breastfed group were significantly different from each of the formula groups at 6 mo of age ( $P < 0.05$ ), but there were no significant differences between the formula groups. The LAZ score of the standard formula group was significantly higher than that of the RGMP group at 6 mo of age ( $P < 0.05$ ). There were no other statistically significant differences in the WAZ and LAZ scores between groups.

Head circumference-for-age  $z$  (HCAZ) scores increased rapidly from 3 mo of age in the standard formula group and were significantly higher than those of the RGMP and breastfed groups at 6 mo of age ( $P < 0.05$ ).

### Health and sleep patterns

There were no group differences in the number of infections, days with fever, or days with symptoms of upper respiratory infection (Table 3). We did not observe any differences in bowel habits or stool consistency between groups, nor were there any differences in sleep patterns between the formula-fed groups. Total daily sleep time did not differ between groups (Table 4).

### Hematology

There were no statistical differences between groups with respect to hemoglobin. Infants fed  $\alpha$ -LAC and standard formula

**TABLE 2**  
Baseline characteristics of participating infants<sup>1</sup>

	Study group				<i>P</i>
	Standard ( <i>n</i> = 21)	$\alpha$ -LAC ( <i>n</i> = 20)	RGMP ( <i>n</i> = 21)	Breastfed ( <i>n</i> = 34)	
Birth weight (g)	3487 $\pm$ 475 <sup>2</sup>	3407 $\pm$ 496	3638 $\pm$ 662	3713 $\pm$ 433	0.205
Birth length (cm)	50.4 $\pm$ 2.6	49.9 $\pm$ 2.8	50.8 $\pm$ 2.4	51.1 $\pm$ 2.4	0.473
Gestational age at birth (wk)	39.8 $\pm$ 1.3	39.7 $\pm$ 1.5	39.2 $\pm$ 1.8	39.9 $\pm$ 1.3	0.532
Girls (%)	35 <sup>a</sup>	76 <sup>b</sup>	41 <sup>a</sup>	34 <sup>a</sup>	0.038

<sup>1</sup> Groups were compared by using ANOVA and a Bonferroni post hoc test. Values with different superscript letters are significantly different ( $P < 0.05$ ).

<sup>2</sup>  $\bar{x} \pm$  SD (all such values).  $\alpha$ -LAC,  $\alpha$ -lactalbumin enriched (15% glycomacropeptide); RGMP,  $\alpha$ -lactalbumin enriched (10% glycomacropeptide).

**TABLE 3**Volume of formula consumed, growth, and infection variables from enrollment to 6 mo of age<sup>1</sup>

	Study group				<i>P</i>
	Standard	$\alpha$ -LAC	RGMP	Breastfed	
Formula intake (mL)					
Entry	880 $\pm$ 199	820 $\pm$ 172	792 $\pm$ 143	—	0.385
2 mo	938 $\pm$ 130	870 $\pm$ 304	838 $\pm$ 90	—	0.372
3 mo	960 $\pm$ 151	906 $\pm$ 156	893 $\pm$ 110	—	0.361
4 mo	1062 $\pm$ 193	984 $\pm$ 262	928 $\pm$ 171	—	0.206
5 mo	1009 $\pm$ 176	991 $\pm$ 208	943 $\pm$ 141	—	0.590
6 mo	930 $\pm$ 136	866 $\pm$ 304	852 $\pm$ 230	—	0.640
Growth (gain/mo)					
Length (cm)	2.57 $\pm$ 0.32	2.50 $\pm$ 0.29	2.46 $\pm$ 0.26	2.48 $\pm$ 0.89	0.961
Weight (g)	730 $\pm$ 134 <sup>a</sup>	707 $\pm$ 145 <sup>a,b</sup>	697 $\pm$ 123 <sup>a,b</sup>	595 $\pm$ 144 <sup>b</sup>	0.014
Head circumference (cm)	1.25 $\pm$ 0.29	1.12 $\pm$ 0.24	1.16 $\pm$ 0.15	1.09 $\pm$ 0.13	0.118
Knee-heel length (cm)	0.35 $\pm$ 0.08	0.33 $\pm$ 0.07	0.35 $\pm$ 0.08	0.32 $\pm$ 0.07	0.753
Infections (n/mo)					
Fever episodes	0.14 $\pm$ 0.23	0.12 $\pm$ 0.16	0.16 $\pm$ 0.24	0.13 $\pm$ 0.17	0.908
Days of fever	0.36 $\pm$ 0.53	0.27 $\pm$ 0.40	0.33 $\pm$ 0.58	0.36 $\pm$ 0.49	0.908
Episodes of airway infections	0.36 $\pm$ 0.28	0.36 $\pm$ 0.25	0.38 $\pm$ 0.34	0.39 $\pm$ 0.33	0.956

<sup>1</sup> All values are  $\bar{x} \pm$  SD.  $\alpha$ -LAC,  $\alpha$ -lactalbumin enriched (15% glycomacropeptide); RGMP,  $\alpha$ -lactalbumin enriched (10% glycomacropeptide). Groups were compared by using ANOVA and a Bonferroni post hoc test. Values with different superscript letters are significantly different ( $P < 0.05$ ).

had a higher S-Fe concentration than did the breastfed infants at 6 mo of age ( $P < 0.05$ ), and  $\alpha$ -LAC-fed infants had a lower total iron-binding capacity at 6 mo than did the infants fed standard or RGMP formula ( $P < 0.05$ ) (Table 5). There was no significant difference in S-Ft concentrations between groups at any time point.

#### Plasma leptin and insulin and serum urea nitrogen

Serum concentrations of leptin and insulin were measured at 4 and 6 mo of age. At 6 mo of age, the serum insulin concentration was highest in the standard formula group, but there were no significant group differences. Serum BUN was significantly greater in all formula groups than in the breastfed group at 4 and 6 mo of age ( $P < 0.001$ ; Table 6).

#### Plasma amino acids

Formula-fed infants had significantly higher plasma concentrations of the essential amino acids threonine, isoleucine, valine, methionine, isoleucine, lysine, and phenylalanine than did the breastfed infants at 6 mo of age. At 4 mo of age the plasma concentration of tryptophan was significantly lower in infants fed standard formula than in breastfed infants (Figure 2A). Of the nonessential amino acids, tyrosine was significantly higher in all formula groups than in the breastfed group at 6 mo, whereas proline was lower in the  $\alpha$ -LAC group than in the breastfed group (Figure 2B).

The concentration of insulinogenic amino acids (leucine, isoleucine, and valine) was significantly greater in the standard formula group than in the breastfed group at 4 mo of age ( $P < 0.05$ ) and was significantly greater in the  $\alpha$ -LAC group than in the breastfed group at 6 mo of age ( $P < 0.05$ ; Figure 3).

#### DISCUSSION

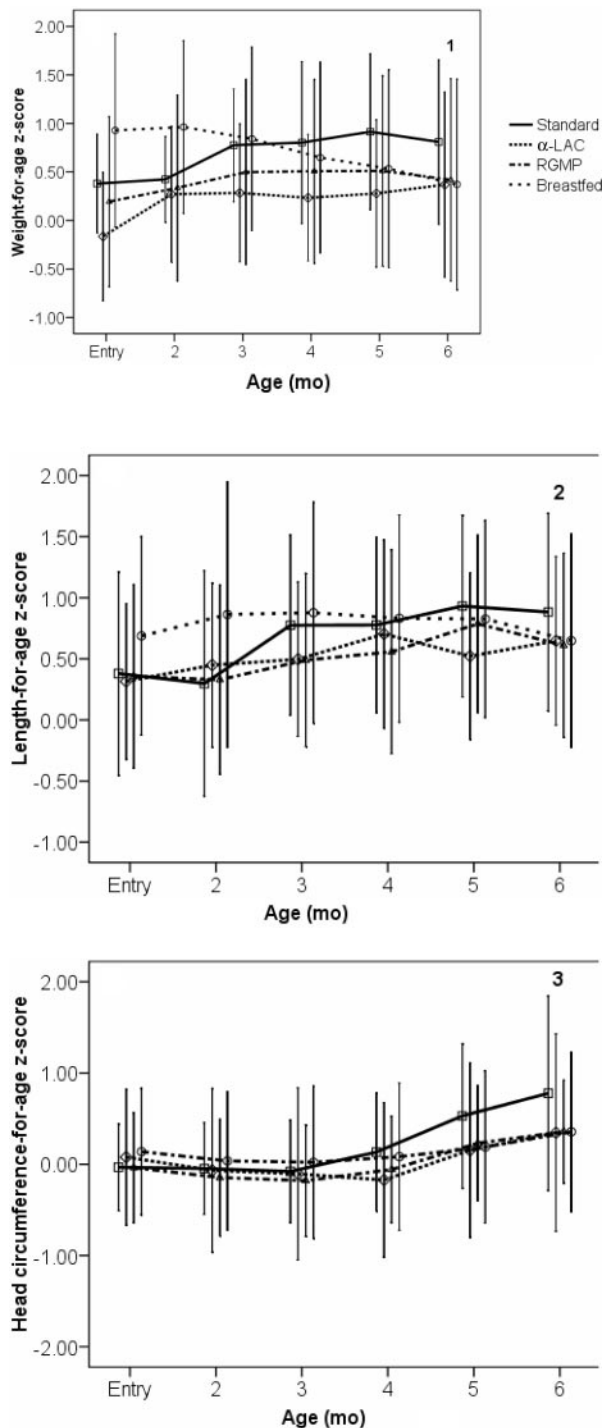
The standard,  $\alpha$ -LAC, and RGMP formulas were well tolerated and caused no adverse effects. It is notable that infants in the  $\alpha$ -LAC and RGMP groups consumed less formula each day than

did infants in the standard formula group, although the difference did not reach statistical significance, possibly because of large individual variations in intake. However, the difference may be biologically significant because there were no significant differences in growth patterns between the  $\alpha$ -LAC and RGMP groups and breastfed infants, whereas infants in the standard formula group gained significantly more weight than did the breastfed infants. Although the reason for greater weight gain in infants fed standard formula is not known, greater protein intake, due to consuming larger volumes, was reflected by elevated plasma concentrations of the branched-chain amino acids (leucine, valine, and isoleucine) that are known to be insulinogenic (18) at 4 mo of age. Furthermore, the plasma insulin concentration tended to be highest in the standard formula group, although the group differences were not statistically significant. Factors affecting food intake regulation are not well understood, but leptin, also acquired from breast milk, has been suggested to be a contributing factor (19, 20) in infants. However, we did not observe group differences in plasma leptin concentration.

There has been considerable discussion regarding the optimal concentration of protein in infant formula and the risks of excessive protein intake. In the present study, all 3 formulas had the same protein concentration (ie, 13 g/L, 1.95 g/100 kcal). In a randomized intervention trial, R  ih   et al (21) compared healthy infants who were breastfed or fed 1 of 2 formulas: one with a protein concentration of 12.1 g/L, 1.8 g/100 kcal, and a whey: casein ratio of 70:30 and an increased proportion of  $\alpha$ -lactalbumin and no GMP and a control formula with 14.7 g protein/L (2.2 g/100 kcal). After 30 d, infant growth patterns did not differ between the 2 formula groups, but concentrations of BUN were lower in infants that were breastfed or fed the formula with 12.1 g protein/L than in infants that were fed the formula with 14.7 g protein/L. At 2 and 4 mo of age, BUN was greater in formula-fed infants than in breastfed infants.

Lien et al (22) studied healthy term infants from birth until 12 wk of age who were fed standard formula with 15.1 g protein/L (2.25 g/100 kcal), of which  $\alpha$ -lactalbumin accounted for 1.2 g/L





**FIGURE 1.** Mean ( $\pm$ SD) weight-for-age, length-for-age, and head circumference-for-age z scores from enrollment to 6 mo of age. Groups were compared by using ANCOVA (adjusted for baseline values) and a Bonferroni post hoc test. At enrollment the weight-for-age z score was significantly higher for the breastfed group than for the  $\alpha$ -LAC formula group ( $P < 0.05$ ).  $\alpha$ -LAC,  $\alpha$ -lactalbumin enriched (15% glycomacropeptide); RGMP,  $\alpha$ -lactalbumin enriched (10% glycomacropeptide). <sup>1</sup>When adjusted for entry values, the weight-for-age z score at 6 mo of age was significantly lower ( $P < 0.05$ ) in the breastfed group than in all of the formula-fed groups. <sup>2</sup>When adjusted for entry values, the length-for-age z score at 6 mo of age was significantly higher ( $P < 0.05$ ) in the standard formula group than in the RGMP group. <sup>3</sup>When adjusted for entry values, the head circumference z score at 6 mo of age was significantly higher ( $P < 0.05$ ) in the standard formula group than in the RGMP and breastfed groups.

(8%) of the total protein or experimental formula with 14.4 g protein/L (2.15 g/100 kcal), of which 2.2 g/L (15%) of the total protein accounted for  $\alpha$ -lactalbumin. Infants fed the experimental and standard formulas had similar growth patterns and similar serum concentrations of albumin, and the authors concluded that all of the infants were well nourished. However, infants that were fed experimental formula had a lower BUN concentration. The difference in the protein content of the experimental and standard formulas was a modest 0.7 g/L, and there were no breastfed infants in the study to serve as reference.

Räihä et al (21) argued that elevated BUN is indicative of increased waste nitrogen and increased renal solute load and that a reduction in protein concentration would be beneficial. In the studies cited above, BUN was higher in formula-fed infants than in breastfed infants, although breastfed infants were not included in the Lien et al (22) study. In the present study, BUN values were  $\approx$ 50% higher in formula-fed than in breastfed infants, whereas they were  $\approx$ 35% higher in the study by Räihä et al (21), which is consistent with the slightly higher protein concentration in the experimental formulas we studied. Considered with the fact that infants fed the experimental formulas grew at the same rate as did the breastfed infants, the implication is that it is possible to reduce the total protein content in infant formula, provided that the remaining protein is of high quality.

Given the fact that the mere composition of a formula is not the proper reference but rather the performance of formula-fed infants compared with that of exclusively breastfed infants is, we assessed plasma amino acid patterns in formula-fed and breastfed infants. Formula-fed groups had higher plasma concentrations of all essential amino acids, except tryptophan. The results suggest that it is possible to reduce the protein concentration in the formula to  $<13$  g/L. However, a reduction in the protein concentration will also result in a reduction in plasma tryptophan, the first limiting amino acid. Indeed, at 4 mo of age, infants fed standard formula had significantly lower plasma tryptophan concentrations than did breastfed infants. It should be noted that Räihä et al (21) did not provide the proportion of  $\alpha$ -lactalbumin in the formula that they used. Furthermore, the investigators used sweet whey, which contains relatively more tryptophan than does conventional acid whey and led to tryptophan concentrations similar to those of breastfed infants.

In the present study, we used an  $\alpha$ -lactalbumin-enriched protein fraction to increase the tryptophan content of the formula, and the  $\alpha$ -LAC and RGMP formulas contained  $\approx$ 20% more tryptophan than did breast milk and standard formula. Thus, feeding infants with those formulas resulted in plasma tryptophan concentrations that were not significantly different from those of breastfed infants, even though free tryptophan had not been added to the formulas. In contrast, in the study by Räihä et al (21), free tryptophan was added to one of the reduced protein formulas to overcome the potential problem of too-low tryptophan concentrations. However, plasma amino acid concentrations were not reported in that study. Thus, it is not known whether a reduction in protein concentration to  $<13$  g/L results in plasma tryptophan concentrations that are similar to those of breastfed infants. GMP is rich in threonine, and infants fed whey-predominant infant formulas have higher plasma threonine concentrations than do breastfed infants. Therefore, formula with a reduced GMP concentration is likely to decrease the difference between formula-fed and breastfed infants. In fact, this was reported by Rigo et al (23) when they studied the effects of GMP-free formula on



**TABLE 4**Duration of sleep<sup>1</sup>

	3 mo			6 mo		
	Daytime sleep	Nighttime sleep	Total sleep	Daytime sleep	Nighttime sleep	Total sleep
		<i>h</i>			<i>h</i>	
Standard	4.2 ± 1.3	8.9 ± 1.4	13.1 ± 1.6	5.0 ± 1.1	8.9 ± 1.5	13.7 ± 1.6
α-LAC	4.0 ± 1.2	9.2 ± 1.0	13.2 ± 1.3	5.0 ± 1.7	9.0 ± 1.4	14.2 ± 1.8
RGMP	4.2 ± 1.1	9.2 ± 1.5	13.4 ± 1.3	5.4 ± 1.7	8.7 ± 1.1	14.1 ± 1.8
Breastfed	4.4 ± 1.2	8.8 ± 1.1	13.2 ± 1.2	5.7 ± 1.4	8.9 ± 1.2	14.8 ± 1.5

<sup>1</sup> All values are  $\bar{x} \pm$  SD. Groups were compared by using ANOVA. No significant differences were found. α-LAC, α-lactalbumin enriched (15% glycomacropeptide); RGMP, α-lactalbumin enriched (10% glycomacropeptide).

preterm infants. However, although one of the present experimental formulas had a reduced GMP content, it did not result in a lower plasma threonine concentration, which may be explained by the too-small difference in threonine content between the formulas. However, the lack of difference suggests that a reduction in the GMP content in formula is not necessary in term infants. Interestingly, it has been suggested that the

difference in plasma threonine concentrations between formula-fed and breastfed infants is due to a difference in threonine metabolism (24). This suggestion should be studied further.

There were no significant group differences in hemoglobin and S-Ft. However, S-Fe was significantly higher in the α-LAC group than in the breastfed group. Consistent with this finding,

**TABLE 5**Red blood cell counts and iron status<sup>1</sup>

	Study group				<i>P</i> <sup>2</sup>
	Standard	α-LAC	RGMP	Breastfed	
Red blood cells ( $\times 10^9/L$ )					
Entry	3.62 ± 0.50	3.98 ± 0.76	3.78 ± 0.69	3.78 ± 0.50	
4 mo	4.12 ± 0.30	4.02 ± 0.35	4.12 ± 0.25	4.22 ± 0.63	
6 mo	4.33 ± 0.32 <sup>a,b</sup>	4.29 ± 0.33 <sup>a</sup>	4.39 ± 0.29 <sup>a,b</sup>	4.41 ± 0.39 <sup>b</sup>	0.048
Hemoglobin (g/L)					
Entry	117 ± 17	121 ± 15	123 ± 21	123 ± 17	
4 mo	115 ± 9	111 ± 9	113 ± 7	114 ± 13	
6 mo	115 ± 9	114 ± 7	115 ± 7	114 ± 7	
Hematocrit (g/L)					
Entry	34.1 ± 4.7	35.4 ± 4.5	35.5 ± 5.8	35.3 ± 4.5	
4 mo	33.4 ± 2.4	28.6 ± 11.1	32.9 ± 1.7	33.7 ± 4.4	
6 mo	31.7 ± 8.9	29.9 ± 12.0	31.6 ± 8.8	32.5 ± 6.9	
MCV (fL)					
Entry	93.7 ± 4.2	92.7 ± 3.6	92.4 ± 4.1	93.4 ± 3.9	
4 mo	81.3 ± 3.0	80.8 ± 2.7	79.6 ± 4.4	80.1 ± 4.0	
6 mo	77.9 ± 2.2	78.4 ± 4.3	77.2 ± 4.6	77.3 ± 4.1	
Platelets ( $\times 10^9/L$ )					
Entry	340 ± 98	394 ± 99	411 ± 126	404 ± 130	
4 mo	416 ± 99	440 ± 70	458 ± 61	459 ± 129	
6 mo	385 ± 129	414 ± 87	425 ± 78	413 ± 104	
Serum iron (μmol/L)					
Entry	19.6 ± 5.1	19.6 ± 5.2	20.2 ± 8.4	18.1 ± 6.3	
4 mo	12.7 ± 3.2	11.4 ± 3.5	10.4 ± 3.9	10.2 ± 3.1	
6 mo	12.1 ± 5.2 <sup>a</sup>	14.5 ± 12.1 <sup>a</sup>	10.0 ± 3.9 <sup>a,b</sup>	7.6 ± 3.4 <sup>b</sup>	0.01
TIBC (μmol/L)					
Entry	38.1 ± 6.9	37.9 ± 8.0	41.6 ± 10.7	38.2 ± 11.5	
4 mo	56.5 ± 8.4	55.9 ± 8.6	55.1 ± 12.5	52.6 ± 11.5	
6 mo	60.0 ± 5.9 <sup>a</sup>	54.0 ± 12.9 <sup>b</sup>	59.1 ± 8.5 <sup>a</sup>	56.3 ± 10.1 <sup>a,b</sup>	0.048
Serum ferritin (μg/L)					
Entry	285 ± 140	272 ± 99	273 ± 111	242 ± 125	
4 mo	80.9 ± 48.4	72.2 ± 36.3	100.6 ± 81.8	88.9 ± 69	
6 mo	57.8 ± 64.4	44.7 ± 18.7	69.8 ± 57.8	51.6 ± 48.9	

<sup>1</sup> All values are  $\bar{x} \pm$  SD. α-LAC, α-lactalbumin enriched (15% glycomacropeptide); RGMP, α-lactalbumin enriched (10% glycomacropeptide); MCV, mean corpuscular volume; TIBC, total-iron-binding capacity.

<sup>2</sup> Groups were compared by using ANCOVA (adjusted for baseline values) and a Bonferroni post hoc test. Values with different superscript letters are significantly different ( $P < 0.05$ ).



**TABLE 6**

Plasma insulin and leptin and serum blood urea nitrogen (BUN) concentrations<sup>1</sup>

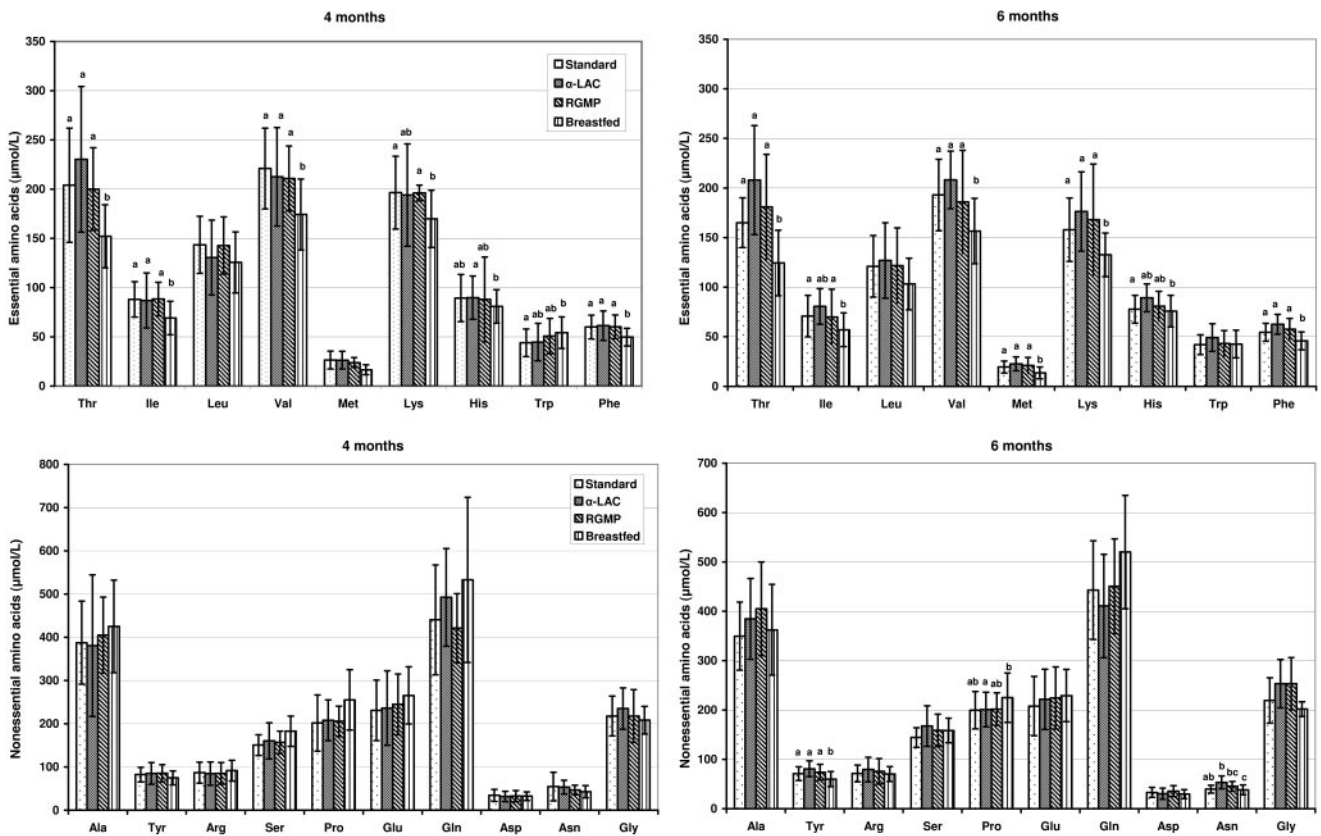
	Study group				<i>P</i> <sup>2</sup>
	Standard	α-LAC	RGMP	Breastfed	
Insulin (μU/mL)					
4 mo	24.6 ± 10.9	24.0 ± 10.5	21.1 ± 5.5	21.7 ± 10.5	0.694
6 mo	23.4 ± 14.8	14.8 ± 6.0 <sup>2</sup>	20.2 ± 8.4	17.5 ± 10.0	0.109
Leptin (ng/mL)					
4 mo	5.0 ± 4.8	3.6 ± 1.4	5.6 ± 6.1	4.7 ± 1.9	0.480
6 mo	5.2 ± 1.8	5.2 ± 1.9	6.1 ± 4.9	5.5 ± 2.3	0.797
BUN (mg/dL)					
4 mo	9.0 ± 1.2 <sup>a</sup>	8.4 ± 1.7 <sup>a</sup>	8.1 ± 2.0 <sup>a</sup>	5.9 ± 1.5 <sup>b</sup>	< 0.001
6 mo	8.1 ± 2.0 <sup>a</sup>	7.9 ± 1.6 <sup>a</sup>	7.2 ± 1.8 <sup>a</sup>	5.3 ± 1.6 <sup>b</sup>	< 0.001

<sup>1</sup> All values are  $\bar{x} \pm$  SD. α-LAC, α-lactalbumin enriched (15% glycomacropeptide); RGMP, α-lactalbumin enriched (10% glycomacropeptide).

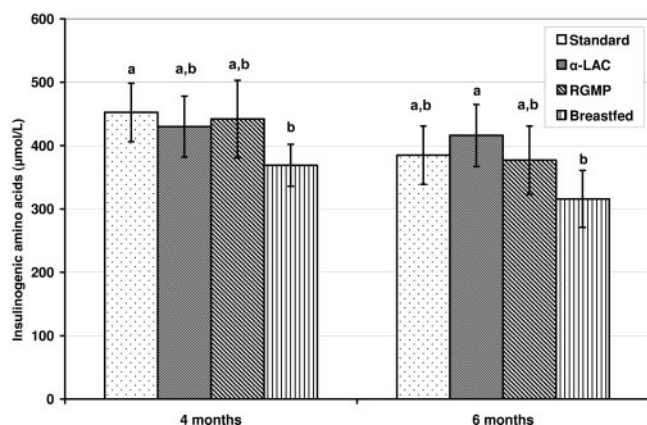
<sup>2</sup> Groups were compared by using ANOVA and a Bonferroni post hoc test. Values with different superscript letters are significantly different (*P* < 0.05).

iron-binding capacity in the α-LAC group was significantly lower than that in the standard formula and RGMP groups. This suggests that iron absorption was greater in the α-LAC group than in the other groups. We previously reported that infant rhesus monkeys fed α-lactalbumin-enriched formula had greater iron absorption and hematocrit than did monkeys that were fed control formula (25). Although the reason for the greater iron utilization in infants fed α-lactalbumin formula is not clear, α-lactalbumin is known to bind divalent cations (6), and it is possible that smaller peptides formed during digestion facilitate iron absorption.

In conclusion, modification of the protein composition of infant formula by increasing its proportion of α-lactalbumin resulted in growth patterns in infants that were similar to those of breastfed infants, possibly because of a consequent reduction in plasma concentrations of insulinogenic amino acids. Plasma tryptophan concentrations in infants fed α-lactalbumin-enhanced formulas were more similar to those of breastfed infants than to those of infants fed standard formula, which suggests that the protein quality of the formula was enhanced. The high plasma concentrations of all other essential amino acids and BUN suggest that it is possible to reduce the protein concentration in



**FIGURE 2.** Mean (±SD) plasma concentrations of essential amino acids and nonessential amino acids at 4 and 6 mo of age. Groups were compared by using ANOVA and a Bonferroni post hoc test. Values with different superscript letters are significantly different (*P* < 0.05). α-LAC, α-lactalbumin enriched (15% glycomacropeptide); RGMP, α-lactalbumin enriched (10% glycomacropeptide).



**FIGURE 3.** Mean ( $\pm$ SD) total plasma concentrations of isoleucine, leucine, and valine combined at 4 and 6 mo of age. Groups were compared by using ANOVA and a Bonferroni post hoc test. Values with different superscript letters are significantly different ( $P < 0.05$ ).  $\alpha$ -LAC,  $\alpha$ -lactalbumin enriched (15% glycomacropeptide); RGMP,  $\alpha$ -lactalbumin enriched (10% glycomacropeptide).

formula to  $<13$  g/L, provided that high-quality protein sources are used. Further studies should be conducted to verify the safety of such formulas.

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