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ORIGINAL ARTICLE

Effect of an α-lactalbumin-enriched infant formula with lower protein on growth

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Background/Objectives: Protein concentration is lower in human milk (HM) than in infant formula. The objective of this study was to evaluate the effect of an α-lactalbumin-enriched formula with a lower protein concentration on infant growth, protein markers and biochemistries.
Subjects/Methods: Healthy term formula-fed (FF) infants 5–14 days old were randomized in this controlled, double-blind trial to standard formula (SF: 14.1 g/l protein, 662 kcal/l) group ($n = 112$) or experimental formula (EF: 12.8 g/l protein, 662 kcal/l) group ($n = 112$) for 120 days; a HM reference group ($n = 112$) was included. Primary outcome was weight gain (g/day) from D0 to D120. Secondary outcomes included serum albumin, plasma amino acids insulin and incidence of study events. Anthropometric measures were expressed as Z-scores using 2006 World Health Organization growth standards.
Results: A total of 321 of the 336 infants (96%) who enrolled, completed the study. Mean age was 9.6 (± 2.9) days; 50% were girls. Mean weight gain (g/day) did not significantly differ between SF vs EF ($P = 0.67$) nor between EF vs HM ($P = 0.11$); however weight gain (g/day) was significantly greater in the SF vs HM group ($P = 0.04$). At day 120, mean weight-for-age Z-score (WAZ) and weight-for-length Z-score (WLZ) did not significantly differ between SF vs EF nor EF vs HM; however the WAZ was significantly greater in SF vs HM ($P = 0.025$). Secondary outcomes were within normal ranges for all groups. Incidence of study events did not differ among groups.
Conclusions: α-Lactalbumin-enriched formula containing 12.8 g/l protein was safe and supported age-appropriate growth; weight gain with EF was intermediate between SF and HM groups and resulted in growth similar to HM-fed infants in terms of weight gain, WAZ and WLZ.

Introduction

Human milk (HM) is the gold standard of early infant nutrition. The World Health Organization (World Health Organization, 2003) and the American Academy of Pediatrics (Koosha *et al.*, 2008; CON AAP, 2009) recommend that infants be exclusively breastfed for the first 6 months of life. However, this recommendation is not consistently achieved; reported rates of exclusive breast feeding at 6 months of age range from 10 to 46% (World Health Organization, 2003; Koosha *et al.*, 2008). The World Health Organization

and AAP recognize infant formula as a suitable feeding alternative when HM is not available (World Health Organization, 2003; CON AAP, 2009). Mixed feeding (breastfed and infant formula) does occur, and it is estimated that between 35 and 50% of the infants receive some feedings of infant formula during the first 6 months of life (Bolling *et al.*, 2007; Shealy *et al.*, 2008). Given the number of infants receiving mixed feedings (HM and formula), and the importance of early life nutrition for optimal growth and development, continued research related to the composition of infant formula and health outcomes of formula fed (FF) infants is warranted.

One of the areas in which HM and infant formula differ is in protein composition and concentration. Mature HM provides 10–12 gm/l total protein (Lonnerdal *et al.*, 1976; Raiha *et al.*, 1986; Darragh and Moughan, 1998; Feng *et al.*, 2009) and is rich in essential amino acids. Standard infant

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formulas are typically made from cow's milk, which has a higher total protein concentration and contains a different amino acid profile than HM. A higher total protein concentration in infant formula (14–15 gm/l) has been necessary to provide sufficient quantities of all the essential amino acids.

Higher protein concentration in infant formula is hypothesized to be a factor that contributes to growth differences observed between FF- and HM-fed infants (Koletzko et al., 2009). FF infants tend to have higher post prandial (Tikanoja and Simell, 1983) and fasting (Picone et al., 1989; Hanning et al., 1992; Akeson et al., 1998) concentrations of branched chain amino acids compared with HM-fed infants. Plasma concentrations of these amino acids have been positively correlated with insulin release (Ginsburg et al., 1984), and insulin is known to increase cellular glucose uptake and inhibit lypolysis. These metabolic alterations are proposed mechanisms for greater weight gain and greater weight-for-length z-scores (WLZ) or body mass index in FF infants compared with HM-fed infants (Lucas et al., 1980). Rapid weight gain, upward crossing of growth percentiles and a greater weight-for-length at 6 months have been identified as risk factors for overweight and obesity, later in life (Stettler et al., 2002, 2003; Karaolis-Danckert et al., 2007; Taveras et al., 2009).

The primary limiting factor in reducing the total protein concentration in infant formula is the ability to provide sufficient quantities of essential amino acids. This can, in part, be addressed by enriching whey protein fractions in formula with substantially higher concentrations of α-lactalbumin; α-lactalbumin is the predominant whey protein found in HM and is a rich source of essential amino acids (Lien et al., 2004). α-Lactalbumin accounts for 28% of the total protein in HM and only 3% of the total protein in bovine milk (Heine et al., 1991). Human and bovine α-lactalbumin share 72% amino acid sequence homology and both consist of 123 amino acids (Findlay and Brew, 1972). Infants fed a formula enriched with bovine α-lactalbumin (14 g/l protein, 2.2 g/l α-lactalbumin) demonstrated appropriate growth and biomarkers of protein status (Lien et al., 2004), as well as good gastrointestinal tolerance to the formula (Davis et al., 2008). It was notable that the plasma amino acid concentrations in the infants consuming the 14 g/l protein formula were similar to but slightly higher than those fed HM (Davis et al., 2008). These data suggested that a further reduction in protein concentration of an α-lactalbumin-enriched formula might be achievable, while still supporting appropriate growth.

This study sought to evaluate the safety and efficacy of a new, lower protein, α-lactalbumin-enriched formula. The primary hypothesis was that weight gain of infants fed this lower protein experimental formula (EF) would be less than but within 3 g/day of those fed the higher protein standard formula (SF), which in addition to other parameters, would indicate that the lower protein concentration was indeed suitable for term infants. We also sought to evaluate whether the modest reduction in the formula's total protein concentration would result in growth outcomes in EF infants that were similar to HM-fed infants.

Subjects and methods

Study design
This study was a randomized, controlled, double-blind study of healthy term FF infants; FF infants were randomized to receive either SF or EF, and a HM group was included as a reference. The protocol and informed consent form were reviewed and approved by the National Ethics Committee and the Bureau of Food and Drugs in the Philippines. Written informed consent was obtained from the parent or legal guardian of each infant before enrollment.

Subjects
Inclusion criteria required that infants were at birth, a singleton and term infant; at enrollment were 5–14 days of age, and had a weight, length and head circumference ⩾fifth and ⩽ninety-fifth percentile for age according to Filipino growth reference standards (Florentino et al., 1992), and exclusively consuming and tolerating a cow's milk infant formula (to be eligible for FF group), or exclusively consuming and tolerating HM (to be eligible for HM group). Main exclusion criteria included: a family history of allergy to a cow's milk protein formula, major congenital malformations, infection or other systemic disorders.

Methods
Randomization. Allocation of FF infants to one of the two study formulas proceeded through the use of a gender-stratified randomization schedule. To ensure double blinding, the packaging of the study formula was identical aside from the package number. Formula feeding began after randomization (baseline) and continued for 120 days.

Nutrient composition of study formulas. Study formulas, standard S-26 GOLD (SF) or experimental S-26 GOLD (EF) (Wyeth Nutrition, Askeaton, Ireland) were packaged in 250 ml ready-to-feed Tetra Brik cartons (Tetra Pak, Lund, Sweden). The main difference between the formulas (Table 1) was the total protein concentration. The ability to provide the required amount of essential amino acids in a formula with a lower protein concentration was achieved primarily through enrichment of the formula with α-lactalbumin and supplementation with small amounts of L-tyrosine and L-tryptophan. The vitamin and mineral concentrations of the study formulas were similar. Both formulas satisfied the essential composition requirements of Codex and EU Commissions (Codex Alimentarius Commission, 1981; European Commission, 2006) and the US Infant Formula Act (United States Food and Drug Administration, 2004).

Table 1 Macronutrient composition of study formulas[a]

| | SF | EF | Recommendations[b] | | | |
| | | | Codex alimentarus | | EU commission | |
			Min	Max	Min	Max
Energy, kcal/l	672	666	600	700	600	700
Protein: energy ratio, g protein per 100 kcal	2.1	1.9	1.8	3.0	1.8	3.0
Protein, g/l	14.1	12.8	See above		See above	
Histidine, mg/100 kcal	51	45	40		41	
Isoleucine	113	103	90		92	
Leucine	205	186	166		169	
Lysine	182	170	113		114	
Threonine	122	110	77		77	
Tryptophan	36	37	32		33	
Methionine + cysteine[c]	84	76	61		62	
Tyrosine + phenylalanine[d]	176	170	159		156	
α-Lactalbumin, g/l	2.2	2.3	No recommendations		No recommendations	
Whey:casein	60:40	66:34	No recommendations		No recommendations	
Carbohydrate, g/100 kcal	10.8	10.8	9.0	14.0	9.0	14.0
Fat, g/100 kcal	5.4	5.4	4.4	6.0	4.4	6.0

Abbreviations: EF, experimental formula; EU, European union; Min, minimum; Max, maximum; SF, standard formula.
[a]Nutrient composition analyzed by Covance Laboratories, Madison, WI, USA; total protein calculated as total nitrogen × 6.25.
[b]Codex and EU recommendations for infant formula composition are based on a compilation of published literature values on human milk composition.
[c]The concentration of methionine and cystine/cysteine may be added together if the ratio between methionine and cystine is not greater than 2.
[d]The concentration of tyrosine and phenylalanine may be added together if the ratio between tyrosine:phenylalanine is not greater than 2.

Anthropometric measures. Weight, recumbent length and head circumference were measured at baseline, days 30, 60, 90 and 120. Weight of naked infants was measured on an infant scale (Seca 374, Hamburg, Germany), recumbent length was measured on a pediatric length board (Ellard Instrumentation, Washington, DC, USA) and head circumference was measured utilizing a pediatric tape measure (Seca 212, Hamburg, Germany). All anthropometric measures were taken twice at each visit and the mean was calculated.

Serum/plasma biochemistries. Blood samples were collected at baseline, study days 60 and 120. At day 60, a timed blood sample (2 h post-prandial) was collected to assess plasma essential and conditionally essential amino acids, insulin and glucose concentrations. Serum chemistries were analyzed by spectrophotometry, serum insulin was measured by radio-immunoassay and serum glucose was measured by spectro-photometry (Quest Diagnostics Clinical Trials, Valencia, CA, USA). Plasma amino acids were analyzed by ion-exchange chromatography with the exception of tryptophan, which was analyzed by tandem liquid chromatography mass spectro-scopy (Mayo Clinical Trial Services, Rochester, MN, USA).

Study events. Study events were recorded throughout the study. A study event was defined as any untoward, undesired or unplanned event in the form of signs, symptoms, disease or laboratory or physiological observations occurring in a person given a test article (study formula) or enrolled in the study. The investigator assigned each event as 'related' if there was a reasonable causal relationship to the test article,

or 'not related' if there was not a reasonable causal relation-ship to the test article. A subset of symptoms related to the digestive system and gastrointestinal tolerance were identi-fied of particular interest *a priori*: hard stool, constipation, difficulty having a bowel movement, acute diarrhea, chronic diarrhea, spitting up, regurgitation, vomiting, gastroesopha-geal reflux disease, colic and crying/neonatal abnormal crying. To ensure consistency in diagnosis, investigators were provided with standard definitions for these symptoms.

Data management. Data management was performed by Accenture (Philadelphia, PA, USA). Remote data capture was used to electronically record data; study monitors verified data in RDC against the source data and identified and resolved discrepancies. Data collection, data entry, query process, data review and database lock were performed according to standard operating procedures.

Statistical analysis. Data analysis was conducted as outlined in the statistical analysis plan utilizing SAS software version 9.1.3 (Cary, NC, USA). Analysis populations were defined as follows, intent to treat: subjects with a randomization number and efficacy analyzable: all randomized infants who took at least one feed of the study formula and have a measurable primary endpoint. Anthropometric measures were evaluated as raw data and also expressed as z-scores relative to the growth standards of the World Health Organization (WHO Multicentre Growth Reference 2006) using a World Health Organization software program (http://www.who.int/childgrowth/software/en/). Baseline z-score

values are raw means; study day *z*-score values are adjusted means based on an ANCOVA with baseline and feeding group in the model.

Comparison between the EF and SF groups was of interest, as was the comparisons between each formula group and the HM group. Analyses were conducted as independent sets of pairwise comparisons because the HM group was included as a reference group and was not a randomized group. A sample size of 90 subjects per arm (45 subjects per gender per arm) was required to have 80% power to detect a difference in weight gain of 3 g/day (baseline to day 120) between groups, when testing at an α level of 0.025 (one-tailed). The error rate was held constant at the α = 0.05 level for all outcomes. Given the multiple *t*-tests performed for the plasma essential amino acid analysis, results are also present using the Bonferroni adjustment for multiplicity $\alpha_{adj} = 0.0015$.

Results

Subject disposition, study events and subject demography
Of the 336 infants enrolled (112 infants per group), 321 infants completed the study (96%). The number of discontinuations for study events was low and not significantly different between groups (2.7, 2.7 and 0% in the SF, EF and HM groups, respectively). The primary safety endpoint was the frequency of study events; study events were distributed across all treatment groups. A total of 25 treatment-related gastrointestinal (GI) study events occurred (see Methods): 6.3% in the EF group, 11.6% in the SF group and 4.5% in the HM group. Baseline infant characteristics were comparable between the groups (Table 2), with the exception of birth weight, which was significantly higher in the formula groups compared with HM (*P* = 0.005, HM vs EF or SF). Mothers of FF infants gained significantly (*P* = 0.014) more weight during pregnancy compared with mothers of HM-fed infants (EF: 11.8 kg, SF: 11.2 kg and HM: 10.2 kg).

Table 2 Subject demography by feeding group[a]

	SF (n = 112)	EF (n = 112)	HM (n = 112)
Gestational age (weeks)	38.5 (0.9)	38.6 (0.8)	38.6 (0.9)
Birth weight (kg)	3.14 (0.42)[b]	3.17 (0.43)[c]	3.00 (0.40)
Birth length (cm)	49.4 (2.0)	49.5 (2.0)	49.4 (1.7)
Age at enrollment (days)	9.5 (3.0)	9.5 (2.7)	9.8 (3.0)
Gender (% male)	50	50	50
Race (% Asian)	100	100	100

Abbreviations: HM, human milk; EF, experimental formula; SF, standard formula; values presented are means (s.d.).
[a]Intent-to-treat (ITT)population.
[b]Significant difference between SF vs HM, *P* < 0.05 (*P*-value based on two-sample *t*-test).
[c]Significant difference between EF vs HM, *P* < 0.05 (*P*-value based on two-sample *t*-test).
Differences observed for both ITT population (data shown in table) and efficacy analyzable population (data not shown).

Growth velocity
Mean weight gain (baseline to day 120 g/day) did not significantly differ between neither EF vs SF (*P* = 0.67) nor EF vs HM (*P* = 0.11); however, weight gain was significantly greater in the SF vs HM group (*P* = 0.04) (Table 3). Mean length gain (baseline to day 120) was comparable and did not significantly differ between EF vs SF. Mean length gain of the EF group was slightly greater than that of the HM infants (*P* = 0.03).

Z-scores
At baseline, there were no significant differences between the EF and SF groups in weight-for-age *z*-score (WAZ), length-for-age *z*-score, head circumference-for-age *z*-score or WLZ (Figure 1). Both formula groups were significantly higher than HM group at baseline for WAZ (*P* = 0.008 and *P* = 0.012 for EF and SF, respectively) and head circumference-for-age *z*-score (*P* = 0.03 and *P* = 0.003 for EF and SF, respectively). The mean WLZ of SF group was significantly higher than the HM group (*P* = 0.03) at baseline.

At day 120, there were no significant differences between the EF and SF groups for any mean *z*-score parameters (adjusted for baseline). The adjusted mean WAZ of the SF group was significantly higher than the HM group (mean difference 0.245, *P* = 0.03). The adjusted mean length-for-age *z*-score of the EF and SF groups were significantly higher than the HM group (mean difference 0.269 and 0.243, *P* = 0.001 and *P* = 0.002, for EF and SF groups, respectively). The adjusted mean head circumference-for-age *z*-score of the EF and SF groups were significantly higher than HM (mean difference 0.227 and 0.219, *P* = 0.007 and *P* = 0.009 for EF and SF groups, respectively). There was no significant difference in the adjusted mean WLZ for either formula group against HM at study day 120.

Serum biochemistries and amino acids
Mean concentrations of serum albumin, total protein, blood urea nitrogen and creatinine were within the normal range

Table 3 Growth velocity by feeding group[a]

	SF (n = 108)	EF (n = 103)	HM (n = 110)
Weight gain, g/day	28.1 (5.4)[b]	27.8 (5.3)	26.6 (5.4)
Length gain, cm/month	3.21 (0.33)	3.22 (0.35)[c]	3.12 (0.32)
Head circumference, cm/month	1.60 (0.20)	1.61 (0.22)[c]	1.55 (0.18)

Abbreviations: HM, human milk; EF, experimental formula; SF, standard formula; values presented are means (s.d.).
[a]Efficacy analyzable population; growth velocity = rate of change from baseline to day 120.
[b]Significant difference between SF vs HM group, *P* < 0.05 (*P*-value based on two-sample *t*-test).
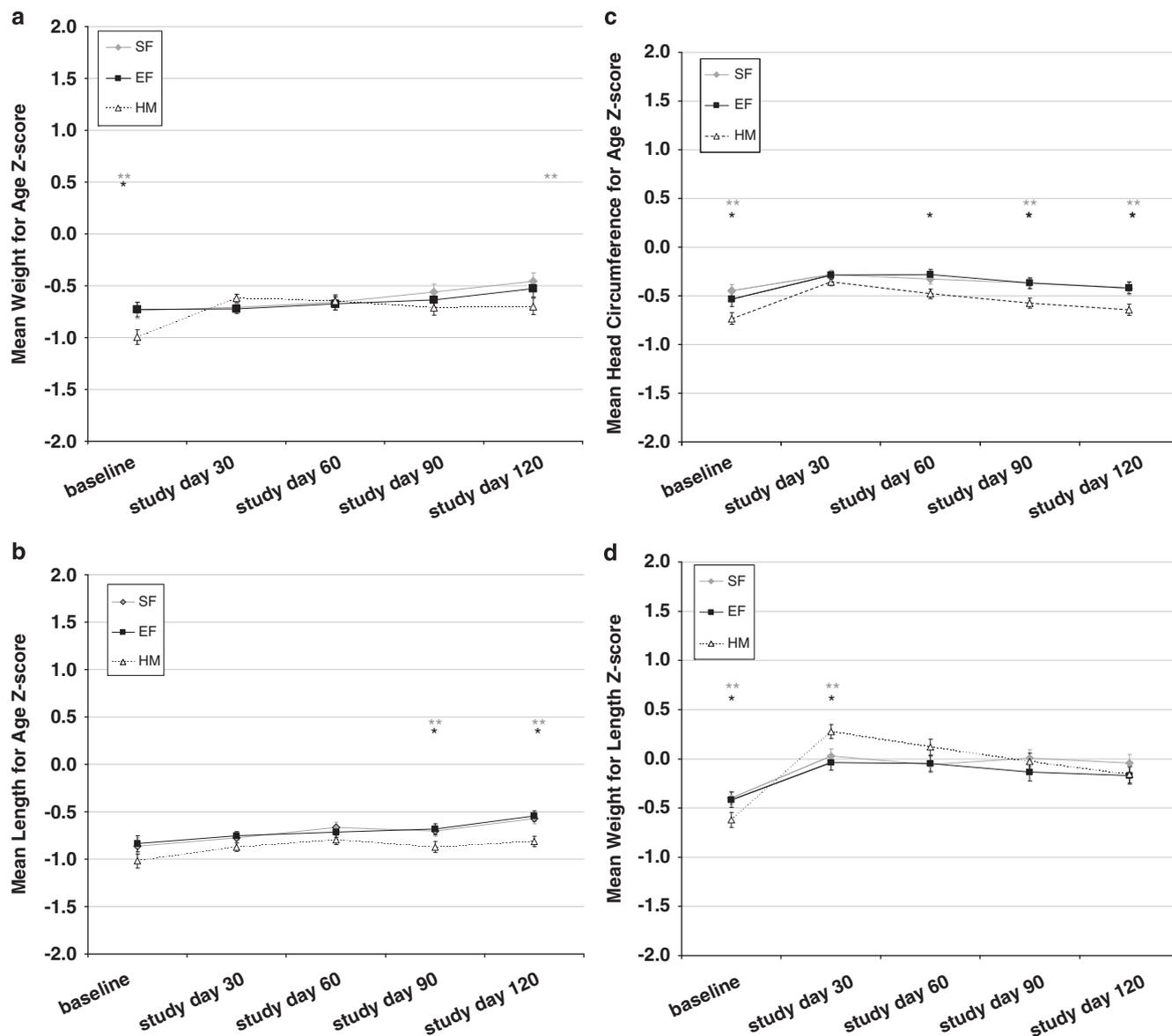[c]Significant difference between EF vs HM group, *P* < 0.05 (*P*-value based on two-sample *t*-test).

Figure 1 Z-scores are based on the World Health Organization reference data (World Health Organization, 2003). (**a**) weight-for-age Z-score, (**b**) length-for-age Z-score, (**c**) head circumference-for age-Z-score, (**d**) weight-for-length Z-score. Baseline values are means; P-values are based on a two-sample t-test. Study day values are least square (LS) means (study day mean adjusted for baseline); P-value is based on ANCOVA with baseline value and feeding group in the model. Significance declared at $P < 0.05$; **Significant difference between SF and HM groups; *Significant difference between EF and HM groups.

for all groups at all time points (Table 4). Mean concentrations of glucose and insulin did not differ between groups. There were no significant differences in the mean amino acid concentrations between the EF and SF groups (Table 5). EF and SF infants had a significantly greater concentrations of some amino acids (lysine, methionine, phenylalanine and threonine) compared with the HM group, however, the mean concentrations of these amino acids were within the normal range for all groups. In general, the plasma essential amino acid concentrations of both the EF and SF formula groups were similar to and within one s.d. of the HM group.

Discussion

This study demonstrated that healthy term infants fed a new lower protein formula (EF) had an age-appropriate growth, biomarkers of protein status and biochemistries were normal, and no difference in the incidence of study events compared with the SF and HM groups. Weight gain with the new lower protein formula (EF) was intermediate between SF and HM groups; infants who consumed the new formula had weight gain, WAZ and WLZ similar to the HM-fed infant. This study compared growth of infants fed formulas that had

Table 4 Serum biochemistries by feeding group[a]

	Units	SF (n = 108)	EF (n = 103)	HM (n = 110)
Albumin				
Baseline	g/l	40.0 (3.0)	40.9 (2.5)	40.5 (2.8)
Day 60		42.7 (2.2)	42.6 (2.2)	42.3 (2.3)
Day 120		44.5 (2.2)	45.0 (2.3)[b]	44.0 (2.1)
Total protein				
Baseline	g/l	61.4 (4.6)	61.9 (4.4)	61.3 (4.5)
Day 60		61.4 (4.3)	60.8 (6.3)	61.2 (3.6)
Day 120		63.8 (4.0)	64.1 (4.2)	63.9 (4.2)
BUN				
Baseline	mg per 100ml	8.4 (2.9)	8.4 (2.3)	8.8 (2.8)
Day 60		6.7 (1.3)[c]	5.7 (1.4)	5.3 (1.8)[c]
Day 120		6.9 (1.8)[c]	6.4 (1.8)[b]	4.8 (1.4)[b,c]
Creatinine				
Baseline	μmol/l	42.5 (9.5)	41.7 (9.3)	41.1 (8.1)
Day 60		26.8 (3.3)	26.5 (3.5)	27.1 (2.2)
Day 120		27.3 (3.2)	26.9 (3.6)	27.3 (2.3)
Insulin				
Day 60	μU/ml	6.48 (4.18)	7.35 (4.83)	6.58 (4.47)
Glucose				
Day 60	mg per 100ml	67.9 (10.1)	68.1 (10.2)	70.2 (8.84)

Abbreviations: BUN, blood urea nitrogen; HM, human milk; EF, experimental formula; SF, standard formula; values presented are means (s.d.).
[a]Efficacy analyzable population.
[b]Significant difference between EF vs HM, $P<0.05$ (P-value based on two-sample t-test).
[c]Significant difference between SF vs HM, $P<0.05$ (P-value based on two-sample t-test).

Table 5 Mean plasma essential amino acid concentrations by feeding group[a]

	SF (n = 108)	EF (n = 103)	HM (n = 110)
Cystine, μmol/l	13.67 (7.24)	13.80 (6.27)	12.47 (5.73)
Histidine	85.36 (12.02)[b]	84.45 (12.89)	81.86 (9.61)
Isoleucine	60.80 (2.64)[b,c]	58.66 (13.86)[d]	53.45 (12.52)
Leucine	105.24 (19.37)	101.59 (21.77)	104.23 (20.72)
Lysine	193.74 (33.74)[b,c]	192.52 (39.71)[d,e]	169.93 (35.08)
Methionine	33.73 (7.77)[b,c]	33.49 (6.76)[d,e]	29.99 (5.86)
Phenylalanine	54.47 (8.06)[b,c]	52.83 (8.99)[d,e]	48.57 (9.65)
Threonine	182.11 (44.02)[b,c]	183.65 (40.84)[d,e]	132.50 (29.42)
Tryptophan	62.79 (13.13)	64.78 (11.78)[d]	60.89 (11.73)
Tyrosine	79.86 (15.87)	88.31 (21.48)[d,e]	77.95 (15.11)
Valine	166.17 (27.44)[b,c]	154.26 (29.52)[d]	143.75 (31.93)

Abbreviations: HM, human milk; EF, experimental formula; SF, standard formula; values presented are means (s.d.).
[a]Efficacy analyzable population.
[b]Significant difference between SF vs HM, $P<0.05$ (P-value based on two-sample t-test).
[c]Significant difference between SF vs HM adjusted for multiplicity, $P<0.0015$ (P-value based on two-sample t-test).
[d]Significant difference between EF vs HM, $P<0.05$ (P-value based on two-sample t-test).
[e]Significant difference between EF vs HM adjusted for multiplicity, $P<0.0015$ (P-value based on two-sample t-test).

a very modest difference (∼10%) in protein concentration; both formulas were found to be safe and support growth, but notably the weight gain in the lower protein (EF) group was not significantly different from the HM group.

Evidence of a positive relationship between the protein concentration of feeding and weight gain is hypothesized (Koletzko *et al.*, 2009), and supported by the results of randomized and observational studies. In prospective randomized trials, infants receiving a higher protein formula have been shown to have greater weight gain (Raiha *et al.*, 1986; Axelsson *et al.*, 1989) or greater WAZ (Koletzko *et al.*, 2009) compared with infants receiving a lower protein formula. Randomized trials that have not found this difference (Janas *et al.*, 1987; Picone *et al.*, 1989; Turck *et al.*, 2006) tended to have a smaller difference in protein concentration between the formulas as well as a smaller sample size. In observational cohort studies, a higher protein intake (Gunnarsdottir and Thorsdottir, 2003) and a higher percentage of dietary energy as protein (Rolland-Cachera *et al.*, 1995; Scaglioni *et al.*, 2000) have been associated with overweight or increased body mass index at an age of 5–8 years, and an association between weight gain in infancy and later life obesity has also been shown (Stettler *et al.*, 2002; Ong *et al.*, 2009). In summary, the literature suggests that the protein concentration of infant feeding has a role in weight gain during infancy, which in turn may have effect on weight in later life; this underscores the importance of the results of weight gain observed in the lower protein (EF) group in this study.

The difference in weight gain between the EF and SF groups was 0.3 g/day and although this represents only a modest reduction on a daily basis, it is cumulative and may be meaningful when projected over a longer time period. Indeed, at the conclusion of the study the mean adjusted WAZ of infants fed EF was not significantly different from that of HM group, whereas those receiving the SF had significantly greater WAZ compared with that of HM group. Both the formula groups had a significantly greater rate of length gain and length-for-age Z-scores compared with that of the HM group. Although the formula groups were longer than their HM-fed counterparts, their weight was proportional to their length as demonstrated by no significant difference in the WLZ of either of the formula group against the HM group at the conclusion of the study.

The plasma essential and conditional amino acid concentrations of infants fed with EF were for the most part intermediate between the SF and HM groups. Higher concentrations of the branched chain amino acids leucine, isoleucine and valine have been positively correlated with insulin concentrations (Ginsburg *et al.*, 1984), which in turn may affect weight gain. This study found no significant difference in insulin concentrations between any of the groups. Higher insulin concentration in FF infants compared with HM-fed infants found in an earlier study (Lucas *et al.*, 1980) may be because of substantially higher total protein concentration of infant formulas at that time.

The strengths of this study include the sample size, gender stratification and completion rate. The completion rate exceeded the minimum sample size needed to test the primary hypothesis, and gender balance was achieved within each group. A limitation of the study relates to the duration of the clinical trial infants received the study formula until they were 4.5 months of age. We do not know whether the weight gain observed in the EF group will have an impact on weight status in later age.

In summary, a randomized clinical trial demonstrated that the new lower protein α-lactalbumin-enriched formula (EF) is appropriate for term infants as evidenced by age-appropriate growth, markers of protein status, plasma essential amino acid concentrations and gastrointestinal tolerance. Infants randomized to the lower protein formula had growth outcomes similar to HM-fed infants in terms of weight gain, WAZ and WLZ. These data suggest that a modest reduction in the total protein concentration of the formula has an effect on growth in the short term; further studies are needed to determine if the trend in weight gain observed in the EF group has an impact on weight status in later life.

Conflict of interest

Acknowledgements

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