

Fortified complementary foods with or without α -amylase treatment increase hemoglobin but do not reduce breast milk intake of 9-mo-old Zambian infants^{1–3}

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ABSTRACT

Background: Malnutrition in late infancy in developing countries may result from poor-quality complementary foods that displace breast milk.

Objective: The objective of the study was to assess the effects of fortified complementary blends of different energy densities on growth, hemoglobin concentrations, and breast milk intake of 9-mo-old Zambian infants.

Design: Infants were randomly assigned at 6 mo of age to receive for 3 mo a fortified blend of maize, beans, bambaranuts, and groundnuts [Chilenje Baby Mix (CBM); energy density: 68 kcal/100 g; $n = 37$] or a similar blend with α -amylase (CBMA; energy density: 106 kcal/100 g; $n = 44$). Cross-sectional data were obtained at 9 mo for a control group of infants ($n = 69$) not given the diets. Breast milk intake was measured by using the dose-to-the-mother deuterium dilution technique.

Results: No differences in weight or length z scores, all of which were within normal ranges, were seen between groups at 9 mo. Percentage fat mass was significantly ($P = 0.01$) greater in the infants in both the CBM ($23.2 \pm 2.7\%$) and CBMA ($23.4 \pm 2.5\%$) groups than in the control group ($21.6 \pm 2.6\%$). Hemoglobin concentrations were significantly ($P = 0.03$) greater in both intervention groups (CBM group: 104 ± 12 g/L; CBMA group: 103 ± 12 g/L) than in the control group (98 ± 14 g/L). Breast milk intake was not significantly ($P = 0.87$) different between groups (CBM group: 614 ± 271 g/d; CBMA group: 635 ± 193 g/d; control group: 653 ± 221 g/d).

Conclusions: The study foods improved hemoglobin concentrations without reducing breast milk intake and may be used to improve the nutritional status of infants in developing countries. *Am J Clin Nutr* 2007;86:1094–103.

KEY WORDS Complementary food, micronutrient-fortified foods, α -amylase, infant growth, hemoglobin, breast milk intake, deuterium dilution, Zambia

INTRODUCTION

Growth faltering from early infancy is a major public health problem in developing countries (1). It may be exacerbated by the introduction of traditional complementary foods (2), which often are inadequate to meet the nutritional requirements of infants because of low energy and nutrient density and deficiency and the poor bioavailability of essential micronutrients such as iron,

zinc, and calcium (3). Complementary foods may partially displace breast milk (4–6) and also may interfere with the absorption of the nutrients in breast milk (7), thereby leading to a greater likelihood of nutrient deficiencies.

The application of enzymes such as α -amylase to increase energy density and the fortification with micronutrients may be used to improve complementary food quality (8, 9). The effects of α -amylase (10–13) and of food fortification (10, 14–16) on the energy intakes, growth, and micronutrient status of infants and young children were assessed previously. However, most of those studies were carried out in acutely ill or severely malnourished infants and young children in rural areas, and the results were inconsistent (17).

Because deficiencies of micronutrients such as iron may affect a much larger proportion of the population than those who have evident clinical symptoms and may have adverse effects on infant survival and later work capacity (18), it is essential to assess the effect of improved complementary foods on the growth and micronutrient status of infants from better-off communities (19).

Few data are available on the breast milk intake of older infants. The dose-to-the-mother deuterium dilution method is a noninvasive, simple, safe, and accurate method for measuring breast milk intake, especially in nonexclusively breastfed infants (20), and it has been applied successfully in field conditions in developing countries (4, 21). Unlike test weighing, it does not disrupt infant feeding patterns, and it measures both daytime and nighttime breast milk intake.

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The aims of the present study were 1) to assess the effects on growth and hemoglobin concentrations of 2 industrially processed, multimicronutrient-fortified complementary foods made with maize (*Zea mays*), beans (*Phaseolus vulgaris*), groundnuts (*Arachis hypogea*), and bambaranuts (*Voandzeia subterranea*) without α -amylase [Chilenje Baby Mix (CBM); Quality Commodities Limited, Lusaka, Zambia] and with α -amylase (CBMA; Quality Commodities Limited); and 2) to assess the effect of these diets on breast milk intake of 9-mo-old Zambian infants.

SUBJECTS AND METHODS

Study site

The present study was based at the Chilenje Clinic in Lusaka, Zambia. Lusaka is a middle-income urban area in which most households have running water and flush toilets.

Subjects

Women were invited to participate in the study when they brought their infants to the clinic for diphtheria and oral polio vaccinations at 5 mo of age. The selection criteria for infants were that the mothers gave informed consent to prepare and feed their children with the study blends, to attend the clinic for growth monitoring, and to allow blood sampling from the infant for hemoglobin measurements at the clinic. Antenatal HIV prevalence in the study population was estimated to be 30%. Maternal and infant HIV status were not determined in the present study. The infants were excluded from the study if they had evidence of chronic disease (eg, active tuberculosis or symptomatic HIV) and if they had a birth weight <2500 g.

We considered it unethical and likely to result in resentment and consequent lack of compliance to recruit control infants at 6 mo without supplying them complementary foods. Therefore, control infants were recruited at 8 mo of age (ie, 1 mo before the end of the feeding program) and were measured at 9 mo together with the study group to avoid seasonal variation in data. The selection criteria for control infants were that the mothers gave informed consent to attend the clinic for growth indicator measurements and to allow blood sampling from the infant for hemoglobin measurements. Potential control infants were excluded if they had evidence of chronic disease (eg, active tuberculosis or symptomatic HIV), if they had a birth weight <2500 g, and if weight data for ages 6–8 mo were incomplete, which would indicate irregular clinic attendance. Thus, although the inclusion criteria of the control and intervention groups appeared identical, in fact, control infants may have been healthier because the intervention groups had had no severe illness at 6 mo but could have become ill by 9 mo, whereas control infants were well at 9 mo.

Ethical approval was obtained from the ethics committees of the University of Zambia, the Great Ormond Street Hospital, and the Institute of Child Health. Subjects gave written informed consent.

Sample size calculation

Sample size was calculated on the basis of the primary hypothesis that infants consuming CBMA would have higher total energy and micronutrient intakes than would infants consuming traditional nonbreast milk foods and that the former group would therefore have better growth and micronutrient status than the

TABLE 1

Micronutrient amounts in the fortified maize-beans-groundnuts-bambaranuts blend with or without amylase¹

Nutrient	Amount obtained from 100-g blend
Vitamin A (μg)	700
Vitamin C (mg)	200
Vitamin D (μg)	10
Thiamine (mg)	0.9
Riboflavin (mg)	1.0
Niacin (mg)	12
Pyridoxine (μg)	860
Folate (μg)	220
Pantothenic acid (mg)	4
Calcium (mg) ²	700
Phosphorus (mg) ²	550
Magnesium (mg)	100
Electrolytic iron (mg) ³	41.8
Zinc oxide (mg)	10
Copper (μg)	400
Manganese (μg)	1200
Selenium (μg)	20

¹ Blend composition (wet wt) was 65% maize, 15% beans, 15% groundnuts, and 5% bambaranuts.

² As tricalcium phosphate.

³ Low iron bioavailability (23) is assumed.

latter group. Because micronutrient deficiencies, especially of iron, were expected to be of more concern than was energy deficiency in this population, the sample size was calculated to allow for the detection of differences of ≥ 5 g/L in hemoglobin concentrations at 80% power and 5% significance. One-tailed distribution was assumed because of the fact that giving free nutrient-dense food was unlikely to decrease nutrient intake. At least 58 infants per group were needed. The sample size for the measurement of breast milk (25 infants/group) was based on the detection of mean \pm SD differences of 100 ± 130 g/d in breast milk intake between groups as in a Brazilian study (4).

Preparation and allocation of the study foods

The details of the development and the processing of the study blend were discussed elsewhere (22). Briefly, a complementary blend that contained 65% maize, 15% kidney beans, 5% bambaranuts, and 15% groundnuts was industrially processed by using an extrusion cooking technique at Quality Commodities Limited (Lusaka, Zambia). The processed batch was divided into 2 equal portions. Both portions were fortified with multimicronutrients (Table 1) as currently recommended for infants aged 6–11 mo (23) and included overages for water-soluble vitamins to account for losses during preparation. We used 41.8 mg electrolytic iron/100 g study blend because of the fact that infant foods in Lusaka are made mainly from maize, beans, and groundnuts that are likely to have high phytate and hence low iron bioavailability. Electrolytic iron was chosen because it was deemed to be more stable in cereal foods and generally more consumer acceptable than other forms of iron. In addition, electrolytic iron is relatively inexpensive (24).

One-half of the batch was treated with α -amylase at 0.04% wet weight and was labeled Chilenje Baby Mix with amylase (CBMA). The nonamylase-treated half was labeled Chilenje Baby Mix (CBM). The blends were packed in 1-kg packets and

were labeled with computer-generated random numbers that corresponded to CBM or to CBMA. The list of random numbers assigned to the 2 blend treatments was kept separate from the Chilenje clinic to blind the researcher and the assistants who distributed the blends to the mothers.

Mother-infant pairs were randomly assigned in a block design to receive either CBM or CBMA when the infants were 6 mo old. The assignment to CBM or to CBMA was made by asking the mothers to pick 1 number from an envelope with 10 random numbers (5 numbers corresponded to each blend treatment). The next envelope was opened only after all the numbers in the previous envelope had been allocated. Each mother received 2 kg/mo of the same blend for the study infant and another 2 kg/mo of the same blend for every additional child aged <3 y. The infants in the control group were supplied 4 kg of the blend (2-mo supply of either CBM or CBMA) after 9 mo. A suggested basic recipe for preparing the porridge was written on the bags. Practical demonstration to the mothers on how to prepare the porridge was not done because the presence of α -amylase in one of the study blends would have resulted in a different porridge consistency and hence would have adversely affected the blinding of both mothers and study nurses.

Anthropometric measurements

All anthropometric measurements of the infants in the intervention groups and the 9-mo measurements of those in the control group were carried out by the same 2 trained assistants, who had had experience with growth monitoring in the Chilenje Clinic's Maternal and Child Health department. To determine inter-observer variation in measurements, each nurse took 3 measurements daily for 3 d of weight, height, circumferences (chest, abdomen, thigh, and midupper arm) and skinfold thicknesses (triceps, biceps, subscapular, and suprailiac) in the same 15-y-old girl who had volunteered to be measured with permission from the guardian. No significant differences in anthropometric values were obtained by the 2 nurses. Although we were not able to perform repeated measurements of the same infant to compare the anthropometric values obtained by the 2 nurses, each nurse had taken rehearsal measurements of the infants until 3 measurements were obtained that agreed within 0.5 units. Measurements of growth and body-composition indexes were made monthly in triplicate by using standardized anthropometric techniques and calibrated equipment (25). The infant nude weights were measured to the nearest 100 g by using a Salter scale (Fairfield, NJ). The infant recumbent lengths were measured to the nearest 0.1 cm with the use of a portable measuring board. Triceps, biceps, subscapular, and suprailiac skinfold thicknesses were measured to the nearest 0.1 mm by using Holtain skinfold calipers (Crymych, United Kingdom). The abdomen, thigh, chest, head, and midupper arm circumferences were measured to the nearest 0.1 cm by using nonstretchable measuring tape. Weight records (based on routine weight measurements at the clinic) for the control infants from 6 to 8 mo of age were obtained from the growth monitoring cards with the permission of the Lusaka District Health Management Team. Although the anthropometric measurements in the present study were not as rigorously controlled as those of other studies, in our experience, the routine weight measures provided by the clinic were reliable.

Hemoglobin measurements

Finger-prick blood samples were used to measure hemoglobin concentrations by use of an HemoCue hemoglobinometer (HemoCue AB, Sheffield, United Kingdom) in the clinic at 6 and 9 mo. Anemia was defined as hemoglobin concentrations <110 g/L.

Breast milk measurements

Measurement of breast milk intake required an additional consent form and was conducted only in a subset from each group (Figure 1). Furthermore, 3 infants in the CBM group and 5 infants in the control group were no longer breastfed at 9 mo and thus were excluded from breast milk intake assessment. Breast milk intake was determined by a dose-to-the-mother deuterium dilution method as described previously (4). Briefly, each mother received an accurately weighed oral dose of $\approx 10 \text{ g } ^2\text{H}_2\text{O}$ diluted in 50 mL ordinary drinking water in a clean plastic bottle. The amount of dose taken was determined by weighing the bottle before and after the administration of the dose. Baseline urine samples were obtained from mother and infant before the deuterium dose was administered. Additional urine samples were collected from the mother on days 1, 4, and 14 and from the infant on days 1, 3, 4, 13, and 14. To obtain infant urine samples, a cotton wool ball was placed in the diaper and was checked every 30 min for wetness. The times of the infant urine samples were recorded as the average of the time at which the cotton ball was noted to be wet and the time, within the previous 30 min, at which it was last checked and found dry. The wet cotton wool ball was retrieved and put into a syringe barrel. The plunger was inserted, and the urine was pushed into a 2-mL cryotube. Urine sample tubes were stored at $-20 \text{ }^\circ\text{C}$. The urine samples and the predose aliquots were transported frozen to the United Kingdom to be analyzed by isotope ratio-mass spectrometry. The urine samples were analyzed for ^2H enrichment by using isotope ratio-mass spectrometry after equilibration of $^2\text{H}_2\text{O}$ with 2% ^2H in helium for measurement of $^1\text{H}/^2\text{H}$. Briefly, 500- μL urine samples were flush-filled for 7 min at 75 mL/min with a 2% $^2\text{H}/\text{He}$ mixture and were equilibrated for a minimum of 5 h. Equilibration was done by using platinum rods (Thermo, Howell, MI) and nonevacuated 12-mL Exetainer tubes (Labco, Howell, MI). Laboratory standards calibrated back to Vienna Standard Mean Ocean Water, Standard Light Antarctic Precipitation, and Greenland Ice Sheet Program international standards were used at the start and end of every run. Samples were analyzed on a Delta XP infrared mass spectrometer (Thermo). Sample duplicates were accepted if within 5 δ of one another; otherwise, the analysis of the sample was repeated. Within these specifications, precision within the same run was within 0.5 δ .

Dietary intake measurements

A single interactive 24-h recall (26) was performed monthly for 3 consecutive months in the CBM and CBMA groups and once in the control group to determine the infant dietary intakes. This method was previously validated by 12-h observed records on the day of the 24-h recall (27). A total of 65 types of foods were identified, and their as-is-eaten nutrient contents were calculated on the basis of the Zambian National Nutrition Commission food composition table (28). Ten food groups were generated from the identified recipes, namely, 1) CBM or CBMA, 2) cereals and cereal products, 3) porridge with groundnuts, 4) milk and milk



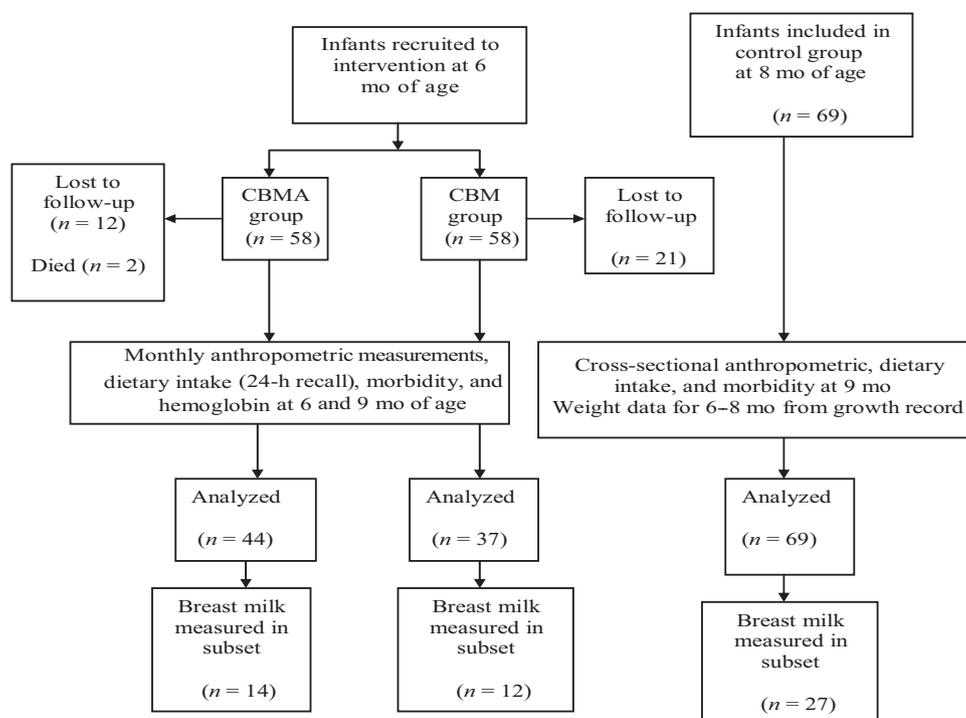


FIGURE 1. Flow chart of the recruitment and follow-up of subjects. Infants were randomly assigned at 6 mo of age to receive for 3 mo either Chilenje Baby Mix without α -amylase (CBM) or CBM with α -amylase (CBMA). Cross-sectional data were obtained for the control group at 9 mo. The nonrandom subset of mother-infant pairs was sampled at 9 mo for measurements of breast milk intake.

products, 5) commercial complementary foods (milk and soy based), 6) eggs, 7) soups, 8) fruits, 9) beverages (fruit juices and soft drinks), and 10) others.

Data analysis

Data were double entered by using EPI INFO software (version 3.2; Centers for Disease Control and Prevention, Atlanta, GA) and were cross-checked. Standard statistical analyses were conducted by using SPSS software (version 11.0; SPSS Inc, Chicago, IL). Significant differences in noncontinuous variables between interventions and between intervention and control were determined by using the Kruskal-Wallis test for K independent samples. Significant differences in continuous variables between the intervention and control groups were tested by using 1-factor analysis of variance, and significant differences in continuous variables within groups were tested by using the least-squares method. Differences in the continuous variables between CBM and CBMA from 6 to 8 mo were determined by an independent sample t test.

Weight and length measurements were converted to z scores by using ANTHRO software (version 2005 PC; World Health Organization, Geneva, Switzerland). Body-composition data were used to calculate body fat, fat-free mass, midarm muscle area, and midarm fat area.

Percentage fat mass, based on anthropometric measurements, was calculated from the sum of 4 skinfold thicknesses according to the following equation (29):

$$\% \text{ Fat mass} = \left\{ \left[\frac{4.95}{1.1451 - 0.0621 \times \log S_1} \right] - 4.5 \right\} \times 100 \quad (1)$$

where S_1 is the sum of biceps, triceps, subscapular, and suprailiac

skinfold thicknesses (mm). Equation 1 was designed for adolescents and may not be applicable to infants; however, intergroup differences will not be affected.

Midarm muscle area and midarm fat area were calculated on the basis of published equations (30):

$$A \text{ (mm}^2\text{)} = \pi \times d^2/4 \quad (2)$$

$$M \text{ (mm}^2\text{)} = (MUAC - \pi T)^2/4\pi \quad (3)$$

and

$$F \text{ (mm}^2\text{)} = A - M \quad (4)$$

where A is arm area, MUAC is midupper arm circumference, d is MUAC/ π , M is arm muscle area, F is arm fat area, and T is triceps skinfold thickness.

Dietary data were used to calculate the age-specific amount of CBM and other food groups consumed in grams and kilocalories and to calculate the proportion of the total daily energy and dietary intakes contributed by the CBM and the other food groups. Dietary data were analyzed in 5 steps, namely, 1) the creation of the as-is-eaten food-composition table on the basis of the original 24-h recall sheets with the use of EXCEL WINDOWS software (version 7.0; Microsoft Corp, Redmond, WA), 2) the creation of the SPSS syntax command from the as-is-eaten food-composition table, 3) the creation of the SPSS database of all foods and amounts eaten by each infant, 4) the running of the SPSS syntax in step 2 over the database in step 3 to calculate nutrient amounts, and 5) the aggregation of nutrient data for each infant.

The calculated energy densities for CBM and CBMA to maintain the same porridge viscosity were 76 kcal/100 g and 106 kcal/100 g, respectively. However, 24-h recall data showed that

TABLE 2Infants' birth weight and household baseline characteristics¹

	CBM group (n = 37)	CBMA group (n = 44)	Control group (n = 69)	Dropouts ² (n = 35)	P ³
Birth weight (kg)	3.1 ± 0.5 ⁴	3.2 ± 0.5	3.1 ± 0.5	3.0 ± 0.3	0.70
Maternal age (y)	27.2 ± 5.9	27.2 ± 4.7	25.6 ± 5.3	25.2 ± 5.3	0.34
Maternal marital status [n (%)]					0.60
Married	29 (83)	36 (82)	49 (71)	27 (85)	
Widowed	1 (3)	2 (4.5)	0	0	
Single	5 (14.3)	6 (24)	18 (16)	5 (15)	
Divorced	0	0	1 (1.4)	0	
Maternal education [n (%)]					0.90
Primary	6 (17)	10 (23)	15 (22)	11 (31)	
Secondary	21 (60)	20 (46)	36 (52)	19 (54)	
Tertiary college	6 (17)	14 (32)	16 (24)	5 (15)	
University	2 (6)	0	1 (1.4)	0	
Maternal occupation [n (%)]					0.25
Salaried	5 (14)	7 (16)	11 (16)	5 (15)	
Self-employed	5 (14)	7 (16)	6 (9)	0	
Housewife	22 (63)	25 (57)	35 (51)	24 (69)	
Student or dependent	3 (9)	4 (9)	16 (23)	5 (15)	
Paternal age (y)	34.2 ± 5.7	33.6 ± 5.5	32.8 ± 7.4	33.6 ± 5.7	0.80
Paternal education [n (%)]					0.87
Primary	1 (3)	3 (7)	2 (3)	3 (8)	
Secondary	14 (40)	17 (39)	26 (38)	18 (51)	
Tertiary college	11 (31)	13 (30)	15 (22)	7 (20)	
University	4 (11.4)	5 (11.4)	5 (7.2)	5 (15)	
Paternal occupation [n (%)]					0.67
Salaried	23 (77)	30 (79)	35 (71)	—	
Self-employed	4 (13)	7 (18)	10 (20)	—	
Other	0	0	1 (2)	—	
Persons in household (n)	6.1 ± 2.2	6.9 ± 2.7	6.6 ± 3.2	6.0 ± 2.0	0.55
Children in household (n)	3.0 ± 1.9	2.9 ± 1.5	3.0 ± 1.7	3.0 ± 1.6	0.97

¹ CBM, Chilenje Baby Mix without α -amylase; CBMA, CBM with α -amylase.² From combined intervention groups (n = 21 from CBM and n = 14 from CBMA).³ Mann-Whitney U test for independent samples, P < 0.05.⁴ \bar{x} ± SD (all such values).

mothers used the same amounts of flour and water in both groups. The combined mean energy density of the study blends was 94 kcal/100 g (95% CI: 68, 121) as reported during the 24-h recall sessions. The combined energy densities of the 3 recipes for CBM and CBMA—ie, blend only, blend with cooking oil, and blend with milk—were 76, 86, and 121 kcal/100 g, respectively. The mean energy densities (kcal/100 g) of the other main food groups were as follows: 76 (95% CI: 65, 87) for cereals and cereal products; 126 (95% CI: 88, 163) for porridge with groundnuts; 67 (95% CI: 49, 84) for milk and milk products; 129 (95% CI: 85, 174) for commercial complementary foods; 21 (95% CI: 12, 31) for soups; and 51 (95% CI: 46, 56) for beverages.

Breast milk transfer was calculated by fitting the isotopic (tracer) data to a model for water (tracee) turnover in the mothers and infants and for the transfer of milk from mother to the infant on the basis of equations and assumptions previously described in detail (4). Breast milk was assumed to be 87.1% water (4) and to have an energy density of 0.67 kcal/g (8).

RESULTS

The flow diagram of the recruitment and follow-up of intervention infants (CBM and CBMA groups) from 6 to 9 mo of age and of control infants at 9 mo of age is shown in Figure 1. A total

of 116 mother-infant pairs were recruited into the 2 intervention groups. Two infants in the CBMA group died of upper respiratory illnesses during the 3-mo follow-up. Twenty-one (36%) of the CBM group subjects and 12 (21%) of the CBMA group subjects were lost to follow-up. The main reasons for these losses were the relocation of residences and the opposition of some fathers to their children's receiving food from the clinic. Two mothers, one in each group, cited dark porridge color as the cause of their withdrawal. Measurements were obtained from all 69 mother-infant pairs included in the control group.

Birth weight and household demographic characteristics

There were no significant differences in the infant birth weights or in household demographic and socioeconomic characteristics between groups (Table 2). Infants lost to follow-up did not differ significantly from those who remained in the study.

Growth

The mean (± SD) length at 6 mo was not significantly different between the 2 supplemented groups of infants (67.2 ± 2.7 cm for the CBM group; 67.4 ± 2.5 for the CBMA group). There were no significant differences between the 2 intervention groups in length gain (4.2 ± 1.4 cm for the CBM group; 4.0 ± 1.4 cm for

TABLE 3

Weight-for-age *z* scores of infants in the Chilenje Baby Mix without α -amylase (CBM), CBM with α -amylase (CBMA), and control groups at 6, 7, 8, and 9 mo of age

Age	CBM group	CBMA group	Control group	<i>P</i> ¹
6 Mo	0.22 ± 0.9 ²	0.19 ± 1.3	0.12 ± 1.1	0.90
7 Mo	0.24 ± 0.9	0.22 ± 1.3	0.08 ± 1.1	0.74
8 Mo	0.47 ± 1.0	0.36 ± 1.4	0.04 ± 1.1	0.26
9 Mo	0.24 ± 1.2	0.13 ± 1.4	0.09 ± 1.1	0.86

¹ One-factor ANOVA and least-squares mean difference, *P* < 0.05. There were no significant differences in weight-for-age *z* scores among different time points or among the 3 groups.

² $\bar{x} \pm$ SD (all such values).

the CBMA group; *P* = 0.56 for both) and in length-for-age *z* scores (-0.1 ± 0.5 cm for the CBM group; -0.12 ± 0 for the CBMA group; *P* = 0.87 for both) during the study. The mean lengths at 9 mo were 71.8 ± 2.5 , 71.3 ± 1.5 , and 70.9 ± 2.4 cm (*P* = 0.06, analysis of variance) for infants in the CBM, CBMA, and control groups, respectively. Infants in the CBM group had a significantly greater length (*P* = 0.04, *t* test) at 9 mo than did the infants in the control group. There were no significant differences in length-for-age *z* scores between the 3 groups (0.10 ± 0.9 cm for the CBM group; 0.07 ± 0.6 cm for the CBMA group; -0.20 ± 0.9 cm for the control group) (*P* = 0.14, analysis of variance).

The mean weight of the infants at 6 and 9 mo, respectively, was 7.8 ± 0.8 and 9.0 ± 1.5 kg for the CBM group, 7.9 ± 0.9 and 8.9 ± 1.4 kg for the CBMA group, and 7.9 ± 1.0 and 8.6 ± 1.1 kg for the control group. There were no significant differences in weight between the groups at 6 and 9 mo. Weight-for-age *z* scores for infants in the CBM, CBMA, and control groups at 6, 7, 8, and 9 mo of age are shown in **Table 3**. There were no significant differences in the weight-for-age *z* scores between the 3 groups

at any time point. There were no significant differences in weight-for-age *z* scores within groups at any time point.

Body composition

Body circumferences (head, chest, abdomen, and thigh), skinfold thicknesses (biceps, triceps, subscapular, and suprailiac), and body-composition indexes for the infants in the CBM, CBMA, and control groups at 9 mo are shown in **Table 4**. Infants in both the CBM and CBMA groups had significantly greater skinfold thicknesses [biceps (*P* = 0.02), subscapular skinfold (*P* < 0.001), and suprailiac (*P* < 0.001)] and percentage fat mass (*P* = 0.01) than did the infants in the control group. There were no significant differences between the 3 groups in body circumferences, fat-free mass, midarm muscle area, and midarm fat area.

Maternal anthropometric data

There were no significant differences in maternal weight (60.3 ± 14.5 kg for the CBM group; 60.1 ± 14.4 kg for the CBMA group; 58.5 ± 9.6 kg for the control group, *P* = 0.71), height (159 ± 5.2 cm for the CBM group; 161.5 ± 5.9 cm for the CBMA group; 158.9 ± 6.1 cm for the control group, *P* = 0.06), or MUAC (27.9 ± 4.9 cm for the CBM group; 27.0 ± 4.4 cm for the CBMA group; 26.9 ± 3.3 cm for the control group, *P* = 0.63). There were no significant differences in maternal percentage fat mass ($28.3 \pm 6.1\%$ for the CBM group; $27.7 \pm 5.2\%$ for the CBMA group; $26.7 \pm 5.1\%$ for the control group, *P* = 0.35) or fat-free mass ($42.3 \pm 6.7\%$ for the CBM group; $42.9 \pm 7.6\%$ for the CBMA group; $42.5 \pm 5.6\%$ for the control group; *P* = 0.95).

Hemoglobin concentrations

Hemoglobin concentrations and the numbers and percentages of infants with hemoglobin concentrations that were less than the specified cutoff at 6 and 9 mo of age are presented in **Table 5**.

TABLE 4

Body circumferences, skinfold thicknesses, and body-composition indexes for infants at 9 mo of age¹

	CBM group (<i>n</i> = 37)	CBMA group (<i>n</i> = 44)	Control group (<i>n</i> = 69)	<i>P</i>
Body circumference (cm)				
MUAC	15.2 ± 1.5 ²	14.7 ± 1.3	14.7 ± 1.2	0.18
Head	45.2 ± 1.6	44.9 ± 1.5	44.8 ± 1.4	0.38
Chest	45.5 ± 3.0	45.8 ± 3.2	45.2 ± 2.5	0.48
Abdomen	48.2 ± 3.9	48.1 ± 3.7	46.6 ± 3.9	0.05
Thigh	24.7 ± 3.1	24.8 ± 3.6	24.0 ± 3.5	0.77
Skinfold thickness (mm)				
Biceps	6.5 ± 1.4 ^a	6.6 ± 1.3 ^a	5.9 ± 1.4 ^b	0.02
Triceps	8.9 ± 1.8	9.1 ± 1.7	8.7 ± 1.9	0.51
Subscapular	9.4 ± 2.4 ^a	9.5 ± 2.1 ^a	8.2 ± 1.7 ^b	< 0.001
Suprailiac	5.1 ± 1.3 ^a	5.0 ± 1.0 ^a	3.8 ± 0.9 ^b	< 0.001
Body composition				
Fat-free mass (kg)	6.9 ± 0.9	6.8 ± 0.9	6.9 ± 0.8	0.73
Fat mass (%)	23.2 ± 2.7 ^a	23.4 ± 2.5 ^a	21.6 ± 2.6 ^b	0.01
Body fat (kg)	2.2 ± 0.9 ^a	2.0 ± 0.4 ^{a,b}	1.9 ± 0.3 ^b	0.02
Midarm muscle area (mm ²)	1236 ± 234	1174 ± 235	1184 ± 232	0.27
Midarm fat area (mm ²)	622 ± 163	621 ± 161	584 ± 147	0.35

¹ CBM, Chilenje Baby Mix without α -amylase; CBMA, CBM with α -amylase. Values in the same row with different superscript letters are significantly different, *P* < 0.05 (1-factor ANOVA and least-squares mean difference).

² $\bar{x} \pm$ SD (all such values).

TABLE 5Hemoglobin concentrations and the number (%) of infants with hemoglobin concentrations lower than the cutoff at 6 and 9 mo of age¹

	CBM group (n = 37)	CBMA group (n = 44)	Control group (n = 69)	P
Hemoglobin concentrations (g/dL)				
At 6 mo	10.8 ± 1.2 ²	10.2 ± 1.7	— ³	0.07
At 9 mo ⁴	10.5 ± 1.2 ^a	10.4 ± 0.9 ^a	9.8 ± 1.5 ^b	0.02
Prevalence of anemia [n (%)] ⁵				
<11 g/dL at 6 mo	19 (51)	30 (68)	—	0.42
<11 g/dL at 9 mo	23 (62) ^a	30 (68) ^a	53 (77) ^b	0.01
<10.5 g/dL at 6 mo	14 (38)	25 (57)	—	0.32
<10.5 g/dL at 9 mo	18 (49) ^a	23 (52) ^a	44 (64) ^b	0.003
<10 g/dL at 6 mo	10 (27)	16 (36)	—	0.31
<10 g/dL at 9 mo	12 (32)	10 (23)	35 (51)	0.02
<9.5 g/dL at 6 mo	3 (8)	13 (29.5)	—	0.43
<9.5 g/dL at 9 mo	9 (24) ^a	5 (11) ^a	27 (39) ^b	0.01

¹ CBM, Chilenje Baby Mix without α -amylase; CBMA, CBM with α -amylase.² $\bar{x} \pm$ SD (all such values).³ Not measured (all such).⁴ Values in the same row with different superscript letters are significantly different, $P < 0.05$ (1-factor ANOVA and least-squares mean difference).⁵ Values in the same row with different superscript letters are significantly different, $P < 0.05$ (Mann-Whitney U test for independent samples).

There were no significant differences in hemoglobin concentrations and anemia prevalence at 6 mo between the CBM and CBMA groups.

At 9 mo of age, the infants in both the CBM and CBMA groups had significantly higher hemoglobin concentrations ($P = 0.02$) than did the infants in the control group, but the 2 intervention groups did not differ significantly. There were no differences in anemia prevalence (hemoglobin concentrations < 110 g/L) between the CBM and CBMA groups at 9 mo, but the control group had a significantly ($P = 0.01$) greater proportion of infants with anemia. Nonbreastfed infants in the control group ($n = 5$) had significantly ($P = 0.04$) lower hemoglobin concentrations than did the breastfed infants in the control group (ie, 85 ± 19 and 99 ± 14 g/L, respectively); the hemoglobin concentrations of the 3 nonbreastfed infants in the CBM group did not differ significantly from those of the other infants in the group (data not shown).

Breast milk intake

Breast milk was measured in fewer mother-infant pairs in the study groups than in the control group because the former groups were less likely to allow for more measurements than were the subjects in the control group, who had just been recruited. There were no significant differences in daily breast milk intake between the 3 groups [CBM group: 614 g/d (95% CI: 418, 771 g/d); CBMA group: 635 g/d (512, 758); control group: 653 g/d (566, 741)] (Figure 2). There were no significant differences in non-breast milk oral water intake between the 3 groups [CBM group: 451 g/d (196, 705); CBMA group: 484 g/d (204, 764); control group: 434 g/d (317, 551)].

Nutrient intake

There were no significant differences in anthropometric and demographic indicators between the subset of infants whose breast milk intake was measured and the rest of the infants in all groups except the control group, in which infants in the breast milk subset had significantly greater head circumference. Hence, the average breast milk values obtained for each group subset

were used to calculate total nutrient intakes for the entire CBM, CBMA, and control groups.

The total daily macronutrient intake from breast milk and complementary foods and the energy composition for infants at 9 mo of age are shown in Table 6. There were no significant differences between the groups in macronutrient intake. The proportions of energy intake contributed by protein and fat were slightly greater than the currently recommended values of 6–10% protein and 24% fat for infants aged 6–11 mo (24). The proportion of energy from the study blend and commercial complementary foods decreased in both the CBM and CBMA groups between 7 and 9 mo, whereas the proportion of energy from traditional cereals and cereal products increased within the same period in both groups (Figure 3). There were no significant differences between the CBMA and CBM groups in the proportion of energy obtained from any complementary food group; hence, the results were merged for the 2 groups.

Compared with the infants in both intervention groups, the infants in the control group received a greater proportion of energy from cereals and cereal products (CBM group: 30.5%; CBMA group: 33.5%; control group: 37%), porridge with groundnuts (CBM group: 12%; CBMA group: 17%; control group: 24%), and commercial complementary foods (CBM group: 17.5%; CBMA group: 15%; control group: 27%), which suggested that the study blends had displaced these food groups.

DISCUSSION

The results show that consumption of the study blends, regardless of amylase treatment, resulted in improved hemoglobin concentrations and increased body fat. Only modest effects on length and no effects on weight were seen, possibly because weight and length z scores were in the normal range for all groups. Breast milk intake did not differ between any of the groups, and it is apparent that traditional complementary foods were displaced by the provision of higher-quality complementary foods.



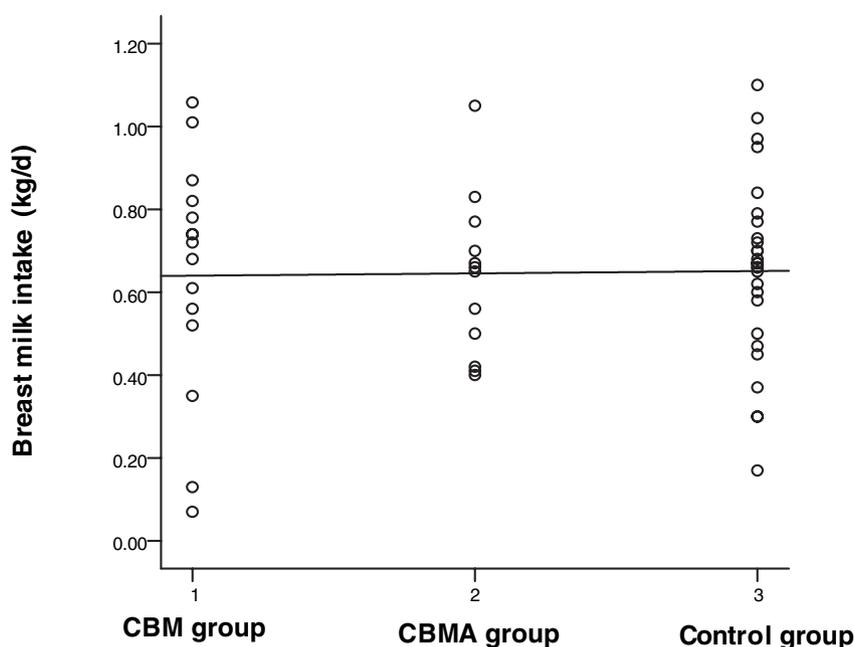


FIGURE 2. Breast milk intakes of individual infants in the Chilenje Baby Mix without α -amylase (CBM) group, CBM with α -amylase (CBMA) group, and the control group. There were no significant differences in breast milk intake between groups. The horizontal line is the mean breast milk intake (0.64 kg/d) for the 3 groups.

Study limitations

Although we were not able to achieve the initially planned sample size, we recruited sufficient numbers to achieve statistical significance. Any benefit of the blends on growth was possibly confounded by the fact that some infants may have been exposed to HIV. HIV-infected infants are usually shorter and lighter than uninfected infants (31–33). Although we were not able to assess infant or maternal HIV status in the present study, we excluded seriously ill infants and assumed random distribution of HIV between the groups. It is possible that the infants in

the intervention group, although healthy at 6 mo, developed disease symptoms between 6 and 9 mo of age; indeed, 2 infants died. However, morbidity data (data not shown) showed that there were no significant differences between intervention groups and the control group.

Infant growth and hemoglobin concentrations

The observed trend toward greater weight-for-age and length-for-age z scores in the 2 intervention groups than in the control

TABLE 6

Daily mean (and 95% CIs) intakes of macronutrients from breast milk and complementary foods and energy composition at 9 mo¹

Nutrient	CBM group ² (n = 37)			CBMA group ³ (n = 44)			Control group ⁴ (n = 59)			P ⁵
	Intake from breast milk	Intake from solid foods	% RDA	Intake from breast milk	Intake from solid foods	% RDA	Intake from breast milk	Intake from solid foods	% RDA	
			%			%			%	
Energy (kcal)	412 (307, 516) ⁶	390 (315, 467)	116	425 (343, 508)	344 (267, 422)	112	438 (379, 496)	353 (299, 407)	115	0.63
Protein (g)	8.6 (6.4, 10.8)	13.7 (10.4, 17.1)	232	9.0 (6.8, 11.1)	10.5 (8.4, 12.6)	203	9.1 (7.8, 10.4)	11.5 (9.6, 13.5)	214	0.21
Fat (g)	11.1 (8.2, 13.9)	14.2 (9.0, 19.5)	—	11.5 (8.8, 14.3)	11.2 (7.8, 14.5)	—	11.7 (10, 13.4)	11.6 (9.5, 13.5)	—	0.43
Carbohydrate (g)	44.8 (33.4, 56.2)	63.4 (50.4, 76.5)	—	46.8 (35.7, 57.9)	53.0 (42.3, 63.3)	—	47.5 (40.5, 54.4)	53.9 (46.1, 61.8)	—	0.31
Fiber (g)	0	10.1 (3.2, 16.9)	—	0	5.4 (3.9, 6.9)	—	0	12.4 (6.2, 18.6)	—	0.24

¹ CBM, Chilenje Baby Mix without α -amylase; CBMA, CBM with α -amylase. Macronutrient intakes from breast milk are based on average breast milk intakes (614 g/d in the CBM group, 635 g/d in the CBMA group, and 653 g/d in the control group).

² Energy composition (% of energy) in CBM group (\bar{x} and 95% CIs): protein, 11.9 (11.4, 12.5); fat, 28.9 (27, 30.7); carbohydrate, 59.2 (57.3, 61). $P = 0.76$.

³ Energy composition (% of energy) in CBMA group (\bar{x} and 95% CIs): protein: 11.2 (10.8, 11.6); fat: 29.4 (26, 32.8); carbohydrate: 59.4 (56.1, 62.7). $P = 0.65$.

⁴ Energy composition (% of energy) in controls (\bar{x} and 95% CIs): protein: 11.8 (11.0, 12.5); fat: 28 (26, 30); carbohydrate: 60.3 (57.9, 62.6). $P = 0.45$.

⁵ Because there were no significant differences between groups in breast milk intake, total differences are based on nutrient intake from complementary foods. Values in the same row with different superscript letters are significantly different, $P = 0.05$ (1-factor ANOVA).

⁶ \bar{x} ; 95% CIs in parentheses (all such values).



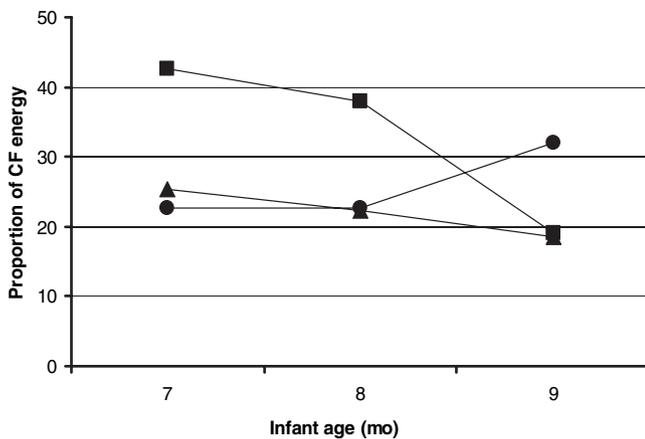


FIGURE 3. Proportion of energy from the different complementary food (CF) groups: study blends (■), commercial complementary foods (▲), and traditional cereals and cereal products (●) between 7 and 9 mo. Proportion of energy from the study blends and the commercial complementary foods decreased in both the CBM and CBMA groups between 7 and 9 mo, whereas the proportion of energy from traditional cereals and cereal products increased the same period in both groups. There were no significant differences between the CBMA and CBM group in the proportion of energy obtained from any complementary food group; hence, the results were merged for the 2 groups.

group may be attributed to the provision of high-quality complementary foods. The observed decline in weight-for-age *z* scores after 8 mo in the 2 intervention groups may be attributed to the decrease in the amount of study blends and commercial complementary foods consumed as the infants grew older. The lack of significant differences in weight, length, and hemoglobin concentrations between the 2 intervention groups may be explained by the fact that both groups had comparable nutrient intakes.

No significant effect of α -amylase addition on length was observed in the current study in contrast with a study from Congo (10), which found an improvement in length gain velocity between 24 and 31 wk in infants given multimicronutrient-fortified maize-soy blend. The main differences between the current study and the Congolese study (10) were that, in the latter case, complementary foods were introduced at a younger age (13 wk), and the initial stunting rate (15.5%) was higher than that observed at 6 mo in the current study. A Ghanaian study (14) found an intervention effect on weight-for-age and length-for-age between 9 and 12 mo, but not between 6 and 9 mo.

The improvement in hemoglobin concentrations observed in the current study is consistent with findings from South Africa (17) but is contrary to the results from a Ghanaian study (14). The lack of improvement in hemoglobin concentrations with intervention in Ghana (14) may have been due to the high prevalence of malaria in the study area (34).

The differences between the current study and similar studies may be further explained by the different amounts of micronutrients added to complementary foods in the various studies. In Ghana (14), the highly fortified blend had higher calcium concentrations (1736 mg/100 g) and higher zinc concentrations (17.1 mg/100 g) but lower iron concentrations (36.6 mg/100 g) and lower vitamin C concentrations (78 mg/100 g) than those in the current study. A South African study (17) found no effect on growth of giving infants fortified maize meal porridge for 6 mo, and this lack of effect, according to those authors, was likely due to the insufficient amounts of zinc fortification. Although the

effect on infant growth of food fortification with zinc alone has not been assessed, supplementation with zinc alone improved growth in stunted infants (35, 36).

The significantly greater percentage fat mass without significant differences in weight gain in the treatment groups was unexpected. It is possible that the presence of zinc in the fortification premix resulted in fat increments. A recent study (37) that analyzed data from 4 longitudinal studies on infant growth in the first 12 mo of life showed that initial skinfold thickness at 3 and 4 mo of age was positively associated with later length gain.

Breast milk intake

The interindividual variations in breast milk intake observed in the present study are consistent with a similar pattern observed in Brazil (4), in which the intake range was 357–987 g/d in partially breastfed infants. A recent Bangladeshi study (6) also found large variations in milk intake between individuals. The overall SD in the breast milk intake in the present study (228 g/d) is comparable with that observed (203 g/d) in Congo (10). The lack of displacement of breast milk by the study blends shows that high-quality complementary foods can be incorporated into infant diets without a significant reduction in breast milk intake. This is the first study to report breast milk intake of older infants given improved complementary foods by using a stable isotope technique. Previous studies have been done in young infants (4) and were based on test weighing (5, 6, 10). Two studies found significant reductions (5, 6) in breast milk intake when infants were given complementary foods of high energy density, whereas the third study (10) observed no differences. Reported breast milk intakes measured by test weighing are lower than those observed in the current study, which was likely due to insensible water loss during breastfeeding or to a lack of nighttime intake data. Although workers in India (5) corrected for any possible insensible water loss, breast milk intake was still low, which suggested that the technique may not have been easy to perform at nighttime.

Policy implications

The current World Health Organization recommendation for infant and young child feeding (2) is that infants be exclusively breastfed for the first 6 mo of life and thereafter should receive appropriate complementary foods with continued breastfeeding to at least 2 y of age. The present study proposes that, for infants aged 6–11 mo, CBM or similar blends be fed in combination with traditional complementary foods and family dishes. A strategy to integrate food aid with programs for the prevention of mother-to-child transmission of HIV is currently being developed by the World Food Programme to provide infants and young children of HIV-positive mothers in resource-poor settings with replacement foods such as corn-soy blend or an equivalent after the cessation of breastfeeding (38). The evaluation of the benefits of blends such as CBM on growth and nutritional status of non-breastfed infants in developing countries, the optimum age at which such replacement foods may be introduced, and the optimum frequency of feeding is necessary.

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