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Clinical Medicine Insights: Pediatrics

Normal Growth of Healthy Infants Born from HIV+ Mothers Fed a Reduced Protein Infant Formula Containing the Prebiotics Galacto-Oligosaccharides and Fructo-Oligosaccharides: A Randomized Controlled Trial

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ABSTRACT

OBJECTIVE: The aim of the current study was to evaluate the safety of a new reduced protein (2.1 g/100 kcal) infant formula containing 4 g/L of 90% galacto-oligosaccharides (GOS) and 10% fructo-oligosaccharides (FOS).

METHODS: Healthy term infants from Brazil were enrolled. Those born to human immunodeficiency virus (HIV)-positive mothers were randomized to a test ($n = 65$) or control ($n = 63$) formula group. Infants born to HIV-negative mothers were either exclusively breast-fed ($n = 79$) or received a mixed diet (breast milk and test formula, $n = 65$). Between 2 weeks and 4 months of age, infants were exclusively fed according to their assigned group. Anthropometric measurements were taken at baseline, 1, 2, 3, 4, 6, 8, 10, and 12 months. Digestive tolerance was evaluated during the first 4 months. The primary outcome was mean daily weight gain between 2 weeks and 4 months in the test formula and breast-fed groups.

RESULTS: Data from all infants ($N = 272$) were used in the intention-to-treat (ITT) analysis and data from 230 infants were used in the per-protocol (PP) analysis. The difference in mean daily weight gain between 2 weeks and 4 months in the test formula and breast-fed groups was 1.257 g/day (one-sided 95% confidence interval [CI]: −0.705 to inf, $P < 0.001$) in the PP analysis, showing that the lower bound of the 95% CI was above the −3.0 g/day non-inferiority margin. Results were similar in the ITT analysis. Symptoms of digestive tolerance and frequency of adverse events were similar in the two groups.

CONCLUSIONS: The formula containing 2.1 g/100 kcal protein and GOS and FOS was safe and tolerated well.

KEYWORDS: infant formula, galacto-oligosaccharides, fructo-oligosaccharides, safety, weight gain

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Introduction

Breast-feeding is the gold standard for infant nutrition, and consequently development of infant formulas aims at emulating the properties of human milk as closely as possible. Better understanding of human milk properties has provided opportunities to improve infant formulas accordingly for infants who cannot be breast-fed.

Most infant formulas are based on cow milk. However, human milk and cow milk have important differences, and optimization of infant formulas requires adapting some of the properties of cow milk to better resemble the nutritional and functional properties of human milk. Human milk contains higher concentrations of whey proteins than casein proteins: during the early phases of lactation, the whey to casein ratio is about 80:20, and this ratio decreases to about 50:50 later in lactation.[1] On average, the whey to casein ratio in human milk is about 60:40, whereas in cow milk it is 20:80.[2] Moreover, because human and cow milk whey and casein proteins have different amino acid profiles, merely adjusting the whey and casein protein concentrations in cow milk-based formulas does not yield protein quality similar to human milk.[2] As a result, until fairly recently, higher protein concentrations had to be used in infant formulas (even in those that were whey-predominant) in order to obtain protein quality similar to human milk.

Technological advances have now allowed the protein concentration in infant formulas to be decreased to as low as 1.8 g/100 kcal without compromising protein quality. These cow milk-based low protein, whey-predominant infant formulas have been shown to be safe in healthy term infants.[3–6]

Another feature of breast milk that can, to a certain extent, be mimicked in infant formulas is its ability to stimulate bifidobacterial growth. A bifidobacteria-enriched gut microbiota has been associated with beneficial effects, such as reduced incidence of infections and allergies.[7–11] Among human milk components, some of the numerous undigestible oligosaccharides, which constitute a major part of the solid content of human milk,[12] have been shown to contribute to increased bifidobacteria and *Lactobacillus* growth.[13] These oligosaccharides can also act directly to protect against infections by binding to pathogens and preventing them from attaching to their host receptor sites. Moreover, when fermented in the gut, these oligosaccharides create an acidic environment that is hostile to pathogenic bacteria.[14,15]

The prebiotics galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS) have been used in infant formulas to provide some of the benefits of human milk oligosaccharides for infants who are not breast-fed. These oligosaccharides have been shown to selectively stimulate bifidobacteria and *Lactobacillus* growth.[16,17] Furthermore, some studies suggest that these prebiotics may have beneficial effects in reducing infections and atopic dermatitis in infants.[18–20]

We have developed a new whey-predominant infant formula with lower protein content than in standard infant formula and containing a prebiotic mixture of GOS (90%) and FOS (10%) to mimic some of the properties of human milk. The GOS and FOS concentrations in this test formula (4 g/L) were below the concentrations reported in other studies because we wanted to reduce any possible discomfort for the infants. The current study was aimed at evaluating the safety of this new infant formula in healthy term infants born to mothers who had tested positive for the human immunodeficiency virus (HIV) and who, therefore, were recommended to avoid breast-feeding, as per the Brazilian Public Health policy applicable at the time.

Participants and Methods

Study design. This was a prospective, parallel group, controlled, non-inferiority trial performed between February 2008 and June 2010. It was conducted in Brazil in the following centers: Centro de Pesquisas Fima Lifshitz da Universidade Federal da Bahia, Faculdade de Medicina do ABC (Santo André); Universidade de Ribeirão Preto; Universidade de São Paulo; Universidade Federal de Minas Gerais; Hospital dos Servidores do Estado (Rio de Janeiro); University of Ribeirão Preto (Ribeirão Preto) and Escola de Medicina de Ribeirão Preto - Universidade de São Paulo (FMRP-USP).

The trial was performed in accordance with the Declaration of Helsinki and complied with good clinical practices as laid out in the International Conference on Harmonization guidelines. It was approved by the institutions' ethics committees (Comitê de Ética em Pesquisa do HUPES/UFBA, Comitê de Ética em Pesquisa da UNAERP, Comissão de Ética para Análise de Projetos de Pesquisa, Comitê de Ética em Pesquisa da UFMG-COEP, and Comitê de Ética em Pesquisa da FMRP-USP). Parents/legal guardians and investigators signed the informed consent.

The primary objective of the trial was to demonstrate the safety of a new test formula containing 2.1 g/100 kcal of protein and 90% GOS and 10% FOS by showing the non-inferiority in mean daily weight gain of infants fed on the test formula compared with those breast-fed between 14 days and 16 weeks of age.

The secondary objective was to compare other anthropometric measurements, digestive tolerance, and morbidity between infants in these two feeding groups. Additionally, these outcomes were also compared between the test formula group and two additional groups: one fed on a control formula and another fed on a mixed diet (breast milk and test formula).

Study population. Healthy, term (≥37 weeks and ≤42 weeks), singleton infants with birth weight between 2500 g and 4500 g were recruited to the study. Infants in the exclusively formula-fed groups were recruited from HIV-positive mothers who had received pre-natal and intrapartum prophylaxis and whose infants were receiving post-partum HIV prophylactic therapy at the time of the study. Absence of HIV infection was defined by two HIV-RNA

negative tests performed at 3 months of age. Infants in the exclusively breast-fed and mixed diet groups were recruited from HIV-negative mothers. Those recruited to the exclusive formula- or breast-fed groups had to be ≤14 days old at enrollment whereas those recruited to the mixed diet group could be up to 1 month old. Additional inclusion criterion was having parents/guardians who were able to adhere to the infants' dietary regimen according to their assigned group, ie, exclusive formula, breast milk, or mixed diet from enrollment until 16 weeks of age.

Infants were excluded from the study for the following reasons: having congenital illness or malformation that could affect normal growth, having significant pre-natal or post-natal disease (including HIV infection diagnosed by virus-based confirmatory tests), being re-hospitalized for >2 days during the first 14 days of life for reasons other than jaundice, or participating in another clinical trial.

Study formulas and blinding. The two study formulas contained proteins, carbohydrates, fats, vitamins, and minerals in amounts intended for full nutritional support of infants from birth to 6 months of age. They were isocaloric, containing approximately 67 kcal/100 mL. The test formula contained 2.1 g protein/100 kcal, which was predominantly whey protein (casein:whey ratio of 40:60), and 4 g/L of GOS (90%) and FOS (10%). The control formula contained 2.6 g protein/100 kcal, which was predominantly casein protein (casein:whey ratio of 80:20), and contained no prebiotics. Concentrations of all nutrients except protein were the same in the two formulas.

The formulas were produced by Nestlé (Konolfingen, Switzerland) and were packaged in identical cans, which were coded with single-letter codes by the manufacturer. The investigators, staff, and study participants were all blinded to the identity of the products.

Randomization. At enrollment, infants in the exclusively formula-fed groups (test or control formula groups) were randomized with stratification by gender and delivery mode (cesarean or vaginal) using an in-house computer program, TriBalance.

Trial procedure. Infants' demographic data, mode of delivery, gestational age, birth date, anthropometric data (weight, length, and head circumference measurements) at birth, and medical history including any disease or intake of medication were recorded at enrollment. Additionally, the parents' anthropometric data as well as the mothers' smoking and drinking habits and educational level were also recorded.

Infants in the formula groups received their assigned formulas starting at enrollment (ie, at ≤14 days of age) and were fed ad libitum until 4 months of age. Infants in the breast-fed and mixed feeding groups continued with their respective feeding regimens. Infants in all groups visited the study sites at the age of 14 ± 3 days (0.5 months), 28 ± 5 days (1 month), 56 ± 7 days (2 months), 84 ± 7 (3 months), 112 ± 7 (4 months), 182 ± 7 (6 months), 224 ± 7 (8 months), 280 ± 7 days (10 months), and 364 ± 7 days (12 months). For the first 4 months, parents recorded volume of formula intake and infants' digestive tolerance during the 3 days preceding each visit.

At each visit, investigators took anthropometric measurements, performed clinical examinations, and reviewed medical histories since the previous visit and any adverse event (AE) or intake of medication.

Outcome measures. The primary outcome was daily weight gain between 0.5 months (14 days) and 4 months (112 days) of age. Secondary outcomes were length and head circumference measurements, digestive tolerance, and morbidity at each visit.

Infants were weighed nude on electronic scales that were calibrated each month according to the manufacturer's specifications. Infants in each center were weighed on the same scale, and measurements to the nearest 10 g were recorded. Recumbent length was measured to the nearest millimeter on standardized length boards with the infants' feet flexed and with at least two study staff ensuring proper body alignment. Head circumference was measured to the nearest millimeter at approximately 2.5 cm above the eyebrows, at the largest measurement of the head circumference, using standard plastic-coated measuring tape.

Digestive tolerance was assessed based on the 3-day diaries kept by parents where they recorded daily stool frequency, predominant stool consistency (hard/lumps, formed/normal, soft/creamy, liquid, or watery), and the occurrence of flatulence (never, sometimes, or often). Additionally, parents also recorded infants' behavior that could suggest issues with tolerance. These were the frequency of spitting up, which was defined as non-projectile emission of small volumes of milk shortly after feeding; the frequency of vomiting, defined as projectile emission of relatively large volumes of stomach content; the length of crying time (<1 hour, 1–3 hours, or >3 hours); occurrence of restlessness/irritability (never, sometimes, or often); and the presence or absence of colic. The characteristics of colic in infants 0–3 months of age included all of the following symptoms but without failure to thrive: fits of irritability, fussing, or inconsolable crying that starts and stops without obvious cause; episodes lasting ≥3 hours per day; and episodes occurring ≥3 days per week for at least 1 week.

Compliance was assessed based on the volume of formula intake recorded by parents. The number of cans of formula distributed at each visit was also recorded and allowed the study staff to evaluate if appropriate volumes of formula were being consumed.

Adverse events. Investigators assessed AEs at each visit by interviewing caregivers about any hospitalization, prescription of medication, the occurrence of respiratory tract infections (including bronchiolitis and otitis media) and other respiratory symptoms, diarrhea, fever, and atopic eczema. Respiratory symptoms were runny nose and chronic coughing and were rated as 0, absent; 1, mild; 2, moderate; and 3,

severe. Diarrhea was defined as the presence of three or more loose or watery stools per day, and an episode of diarrhea was considered to have ended once there were two consecutive non-watery stools, or no stools for 24 hours. Fever was defined as the increase in body temperature to 38.5 °C at least once during a 24-hour period. Atopic eczema was rated as 0, absent; 1, mild; 2, moderate; and 3, severe.

Investigators evaluated AEs for seriousness and causality with study formulas. AEs were coded using the World Health Organization Adverse Reactions Terminology (WHOART) and reported by system organ class and preferred term.

Statistical analyses. Sample size calculation was based on showing non-inferiority in daily weight gain of the infants in the test formula group compared with those in the breast-fed group. The margin of –3 g/day for the difference in weight gain was used based on the criterion of the U.S. Food and Drug Administration/American Association of Pediatrics.[21] The standard deviation (SD) was set at 6.1 g/day based on a previous Nestlé study. With a type I error rate of 5% and power of 80%, 52 infants were required in each group. Assuming a 20% dropout rate, 65 infants had to be enrolled per group.

The intention-to-treat (ITT) analysis consisted of data from all randomized infants. The per-protocol (PP) analysis included data from infants that had continuous intake of the study formulas (for those in the exclusive formula group) for a whole year with a break of no more than 3 days. Additionally, the following were also considered major protocol deviations and infants with these deviations were excluded from the PP analysis: occurrence of a life-threatening event during the study period; hospitalization for >3 days; and non-exclusive feeding of assigned formula during the first 4 months of the study. Non-exclusive formula feeding was defined as intake of more than one bottle per week of a different formula, being off the study formula for >3 consecutive days, or introduction of ≥4 teaspoons (20 g) per day of complementary foods (ie, cereals, fruits, meat, fish, eggs, and other protein-rich foods, vegetables, milk-containing cereals, cereal drinks, or any other foods intended for babies) before 4 months of age. Both the primary and secondary analyses were performed in the PP and ITT populations, and results from both analyses are presented for the primary outcome. Only the ITT data are presented for the secondary outcomes. There was no imputation for missing data.

Baseline characteristics were summarized as mean ± SD. Formula intake was adjusted for birth weight and analyzed using analysis of covariance (ANCOVA). The primary endpoint was the difference in mean daily weight gain from 0.5 to 4 months of age between the test formula and breast-fed groups. The one-sided 95% confidence interval (CI) was estimated using a mixed model (PROC MIXED) for the difference in weight gain between groups. Weight was modeled as a linear function of age, treatment, sex, age*treatment, age*sex, age^2*sex, and age^2*treatment. Each subject had their own intercept and slope described by random effects. In addition, the model assumed a variance covariance matrix with type I autocorrelation structure for outcome in adjacent visits. The origin of the age scale was (14 + 112)/2 = 63 days after birth. In order to improve convergence of the iterative computations, age was introduced in months and weight in kg. If the above analysis was significant, confirmatory analysis of the primary endpoint was performed using a one-sided 97.5% CI.

Differences in mean daily weight gain between the test formula group and the control formula and mixed feeding groups were analyzed as with the primary endpoint except that two-sided superiority testing with 95% CI was used. Similarly, weight, length, head circumference, and body mass index (BMI) were also compared between the test formula and the breast-fed, control formula, and mixed feeding groups as with the primary endpoint but using a two-sided superiority testing. Anthropometric measurements were also compared with the World Health Organization (WHO) Child Growth Standards using the WHO software (WHO Anthro [Version 3.2.3, January 2011] available at the WHO web site: http://www.who.int/childgrowth/software/en/). For each variable, the analysis of treatment difference at each visit was performed by comparing the model-adjusted means of the corresponding visits based on a model for repeated-measures that included terms for treatment, visit, and treatment-by-visit with autocorrelation type I structure to model the within-subject variability. If results were significant, then comparison with the test formula was adjusted for three multiple tests according to Hommel.

Stool frequency was determined by summing the total number of stool counts and dividing it by the total number of days for which it was recorded. The percentage of days for which specific stool consistencies, flatulence, spitting up, vomiting, crying, and colic were observed was determined by summing up the number of days for which a characteristic was observed and dividing it by the total number of days for which it was recorded. Stool frequency, stool consistency, and gastrointestinal symptoms were analyzed by Poisson regression. Two-sided superiority testing for treatment differences between groups was performed using Dunnett type of contrasts and adjusted for three multiple tests according to Hommel.

The percentage of infants with at least one serious AE (SAE) or non-serious AE during the exclusive feeding period (up to 4 months) and during the entire study (12 months) was compared between the test group and the three other feeding groups using Chi-square test and adjusted for the three comparisons using the Hommel method.

Statistical analyses were performed using SAS version 9.2 (Cary, NC, USA).

Results

Study population. Two hundred and seventy-two infants were enrolled in the study between February 2008 and July 2009. Of these, 128 were from HIV-positive mothers and were randomized into the two study formula groups. The rest were from HIV-negative mothers and were in either the mixed

feeding or breast-fed groups (Fig. 1). A total of 42 infants were excluded from the PP analysis because of major protocol deviations (Fig. 1). Growth data for primary outcome analysis (from breast-fed and test formula-fed infants) were not available for 22 infants, who were lost to follow-up before 4 months (Fig. 1).

Demographics and baseline characteristics. Infants randomized to the formula- or breast-fed groups were enrolled earlier than those in the mixed feeding group and tended to be younger at baseline. Overall, demographics and baseline characteristics were balanced between groups. Nevertheless, there were some differences between groups: infants in the mixed feeding group tended to weigh slightly more at birth than those in the other groups; the proportion of Caucasian infants in the breast-fed group was smaller than in the other groups; a larger proportion of infants in the breast-fed group were delivered vaginally compared with the other groups; and a larger proportion of mothers in the control formula group smoked compared with those in the other groups (Table 1).

Formula intake among infants fed the study formulas. Among the exclusively formula-fed infants, mean daily volume of formula intake was not significantly different between the test and control formula groups (Fig. 2).

Weight measurements. Mean daily weight gain between 14 days and 4 months in infants fed on the test formula and those in breast-fed groups was 30.02 g/day and 28.71 g/day, respectively. The lower bound of the one-sided 95% CI of the difference in mean daily weight gain between the two groups was above −3.0 g/day for both the PP and ITT populations (Table 2), demonstrating the non-inferiority in weight gain of the test formula group to breast-feeding group. Additionally, mean daily weight gain in the test formula group was not significantly different from that of the control formula and the mixed feeding groups during the exclusive feeding period (Table 3).

Mean weight measurements were not significantly different between the test formula group and the other feeding groups throughout the study (data not shown) except at 12 months, when the mean weight was higher in the test formula group than in the breast-fed group: difference in weight of 0.398 kg (95% CI: [0.116 to 0.679 kg], $P = 0.017$ with adjustment for multiplicity).

Compared with WHO standards, weight-for-age *z*-scores beginning at 2 weeks were within 0.5 SD for all groups except the control group, which had *z*-scores between −0.33 and −0.82 from 2 weeks to 4 months (Fig. 3A). At the 12-month visit, infants in the test formula group had significantly higher mean weight-for-age *z*-scores than infants in the breast-fed group (treatment effect of 0.359, 95% CI [0.064 to 0.655], $P = 0.017$, Fig. 3A), but this was not significant after adjusting for multiplicity ($P = 0.052$). There were no other significant differences in mean weight-for-age *z*-scores between the test formula group and any of the other feeding groups at any other time during the study (Fig. 3A).

Length-, BMI-, and head circumference-for-age z-scores. Early in the study (0.5 and 1 month) mean length-for-age *z*-scores were lower in the test formula group than in the breast-fed and mixed feeding groups ($P \leq 0.001$ following adjustment for multiplicity) for both but not at any time thereafter (Fig. 3B). Similarly, length measurements in cm

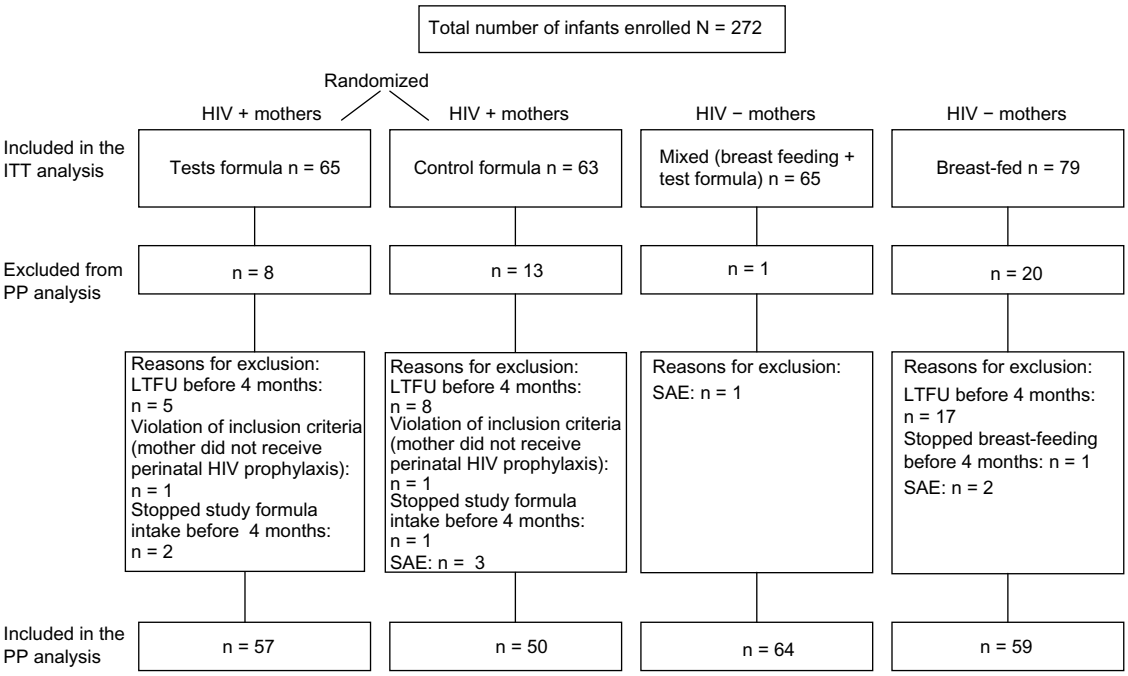

Figure 1. Flow of infants participating in the study.
Abbreviations: ITT, intention-to-treat; PP, per protocol; LTFU, loss to follow-up; SAE, serious adverse event.

Table 1. Demographics and baseline characteristics* of infants enrolled in the study and their mothers, ITT.

CHARACTERISTICS N (%)† OR MEAN (SD)	TEST FORMULA N = 65	CONTROL FORMULA N = 63	MIXED FEEDING N = 65	BREAST-FED N = 79
Infants				
Age at enrollment, days	6.9 (4.6)	6.6 (4.9)	18.7 (8.2)	10.8 (4.3)
Gestational age, weeks	38.6 (1.0)	38.7 (1.1)	39.1 (1.1)	39.2 (1.2)
Gender, boys	35 (53.8%)	33 (52.4%)	31 (47.7%)	40 (50.6%)
Mode of delivery: vaginal	27 (41.5%)	25 (39.7%)	29 (44.6%)	54 (68.4%)
Ethnic origin				
Caucasian	32 (49.2%)	35 (55.6%)	44 (67.7%)	21 (26.6%)
African	18 (27.7%)	17 (27.0%)	16 (24.6%)	18 (22.8%)
Asian	2 (3.1)	0	0	0
Other	13 (20.0%)	11 (17.5%)	5 (7.7%)	40 (50.6%)
Having siblings	45 (69.2%)	55 (87.3%)	31 (47.7%)	40 (50.6%)
APGAR score 1 minute	8.5 (0.8)	8.3 (1.7)	8.4 (1.6)	8.4 (1.0)
5 minutes	9.4 (0.6)	9.5 (0.8)	9.7 (0.6)	9.3 (0.7)
10 minutes	10.0 (0.0)	10.0 (0.0)	9.6 (0.7)	10.0 (0.0)
Weight at birth, kg	3.14 (0.37)	3.09 (0.36)	3.35 (0.37)	3.27 (0.44)
Length at birth, cm	48.4 (1.9)	48.1 (2.0)	48.7 (1.7)	48.5 (2.0)
BMI at birth, kg/m^2	13.4 (1.2)	13.3 (1.2)	14.1 (1.3)	13.8 (1.3)
Head circumference at birth, cm	34.0 (1.3)	34.1 (1.4)	34.6 (1.2)	34.0 (1.5)
Mothers				
Age	27.9 (6.0)	28.5 (7.1)	27.1 (6.4)	26.8 (6.2)
Weight, kg	68.4 (12.3)	67.5 (14.8)	70.2 (13.7)	66.7 (13.1)
Height, cm	160 (6)	160 (7)	160 (6)	159 (6)
BMI, kg/m^2	26.6 (4.4)	26.3 (5.0)	27.5 (5.4)	26.3 (5.1)
Smoking during pregnancy	9 (14.1%)	19 (30.2%)	6 (9.2%)	4 (5.1%)
Daily number of cigarettes	9.0 (11.9)	8.1 (7.2)	8.8 (6.1)	6.8 (8.8)
Alcohol intake during pregnancy: None	53 (82.8%)	54 (85.7%)	60 (92.3%)	63 (79.7%)
Occasional	9 (14.1%)	4 (6.3%)	5 (7.7%)	12 (15.2%)
Regular	2 (3.1%)	5 (7.9%)	0	4 (5.1%)
Years of education				
<4	4 (6.3%)	3 (4.8%)	5 (7.7%)	0
4–7	21 (32.8%)	22 (35.5%)	19 (29.2%)	11 (13.9%)
8–9	16 (25.0%)	13 (21.0%)	22 (33.8%)	18 (22.8%)
>10	23 (35.9%)	24 (38.7%)	19 (29.2%)	50 (63.3%)

Note: *At recruitment; †data were not always available, and % indicates the proportion relative to the available data.
Abbreviations: ITT, intention-to-treat; SD, standard deviation; BMI, body mass index.

were significantly smaller in the test formula than in the breast-fed and mixed feeding groups at these time points (data not shown).

BMI-for-age *z*-scores were consistently higher in the test formula group than in any of the other groups starting at 4 months (Fig. 3C). This difference was significant only for the comparison with mixed feeding and the control groups at 4 months ($P \leq 0.05$ for both following adjustment). Although head circumference *z*-scores were lower in the test formula group than in the breast-fed group at 0.5 months, there were no significant differences between the test formula and any of the feeding groups at any time thereafter (Fig. 3D).

Gastrointestinal tolerance. Mean ± SD daily stool counts were not significantly different between the test formula group (2.66 ± 1.67) and the breast-fed (3.43 ± 2.29), control formula (2.98 ± 1.65), or mixed feeding (2.33 ± 1.58), $P > 0.1$ for all comparisons groups. The frequency of liquid and watery stools tended to be lower in the test formula group than in the breast-fed and mixed feeding groups (Fig. 4). By contrast, infants in the test formula group tended to have

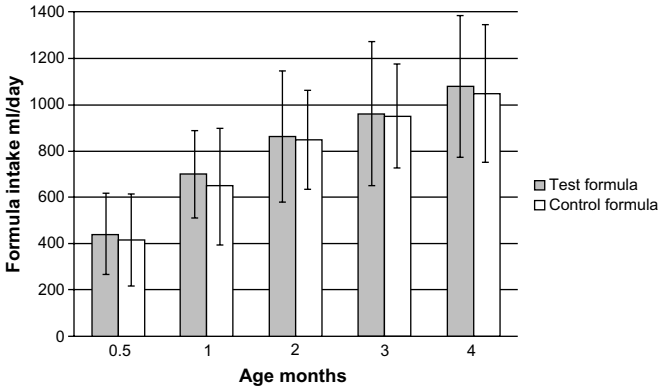

Figure 2. Mean volume of formula intake during the exclusive feeding period, intention-to-treat (test formula, $n = 65$ and control formula, $n = 63$). Error bars indicate standard deviation. There were no significant differences between groups (analysis of covariance adjusted for birth weight $P > 0.1$ at all time points).

fewer hard and formed stools and more soft and liquid stools compared with the control group (Fig. 4).

The frequency of spitting up and vomiting and the proportion of days with crying were not significantly different between the test formula group and the other feeding groups (data not shown). Flatulence occurred slightly more frequently in the test formula group than in the control and breast-fed groups and colic occurred less frequently in the test formula group compared with the mixed feeding group (data not shown).

Adverse events. During the 4-month exclusive feeding period, SAEs and non-serious AEs were reported in 80.0% of infants in the test formula group, 77.8% of infants in the control formula group, 66.2% of infants in the mixed feeding group, and 87.3% of infants in the breast-fed group. The proportion of infants with SAEs was 3.1% in the test formula, 6.3% in the control formula, 1.5% in the mixed feeding, and 8.9% in the breast-fed groups. The frequency of infants with all (serious and non-serious) AEs and the frequency with SAEs alone during the 4-month exclusive feeding period and at the end of the study (12 months) were not significantly different between the test and control formula groups (data not shown). The frequency of SAEs in all feeding groups during the 12-month period is shown in Table 4. The investigators considered two of the SAEs (wheezing and pneumonia in the mixed feeding group) to have a probable relationship with the feeding. None of the remaining SAEs in any of the groups were considered to have any relationship with the feeding.

Table 2. Comparison of mean daily weight gain (g/day) between the test formula and breast-fed groups during the exclusive feeding period (0.5–4 months).

	TREATMENT EFFECT	1-SIDED 95% CI	1-SIDED 97.5% CI	NON-INFERIORITY *P*-VALUE*
ITT	1.309	−0.574 to inf	−0.936 to inf	<0.001
PP	1.257	−0.705 to inf	−1.082 to inf	<0.001

Note: **P*-values calculated as 1-Probt ((diff-margin)/SE,df).
Abbreviations: CI, confidence interval; ITT, intention-to-treat; PP, per protocol.

Table 3. Comparison of mean daily weight gain (g/day) during the exclusive feeding period (0.5–4 months).

	TEST FORMULA VS. CONTROL FORMULA		TEST FORMULA VS. MIXED FEEDING	
	ITT	PP	ITT	PP
Treatment effect	1.486	1.352	0.395	0.428
2-sided 95% CI, *P*-value*	−0.838 to 3.809, $P = 0.419$	−1.084 to 3.788, $P = 0.552$	−1.950 to 2.739, $P = 0.741$	−1.953 to 2.809, $P = 0.724$

Note: **P*-values were adjusted by Hommel's method.
Abbreviations: CI, confidence interval; ITT, intention-to-treat; PP, per protocol.

Discussion

According to current knowledge of infant protein needs, the total protein concentration in standard infant formulas is typically much higher than the amounts required for normal growth and development of infants.[2] Although in the past, high protein concentrations were necessary primarily to make up for the deficiencies in the quality of protein in cow milk, current technology allows for the reduction in protein concentration without compromising quality, thus making them closer to human milk with respect to protein concentration. Infant formulas can further be improved by supplementing them with other ingredients such as undigestible oligosaccharides, which are thought to have beneficial health effect either because they stimulate bifidobacteria and *Lactobacillus* growth or because they have immunomodulatory activity.[16,17,22]

The current study was performed to evaluate the safety of a test formula that had both reduced protein (2.1 g/100 kcal) content and was supplemented with 90% GOS and 10% FOS mixture (4 g/L). Our results showed that infants exclusively fed on the test formula between 14 days and 4 months grew normally, showing mean daily weight gain that was not inferior to that of infants exclusively breast-fed during the same period. These data were consistent in both the PP and ITT populations, demonstrating safety of the test formula. The study included two additional feeding groups, a group of infants exclusively fed (between 14 days and 4 months) on a control formula containing a higher protein concentration (2.6 g/100 kcal) than the test formula but lacking prebiotics and a group of infants that had mixed feeding (breast and test formula feeding). The test formula group had non-inferior mean daily weight gain to both these feeding groups during the exclusive feeding period. The volume of formula intake in the two exclusively formula-fed groups was not significantly different at any time until 4 months, indicating that growth of infants in the test formula group was not because of greater than normal feeding.

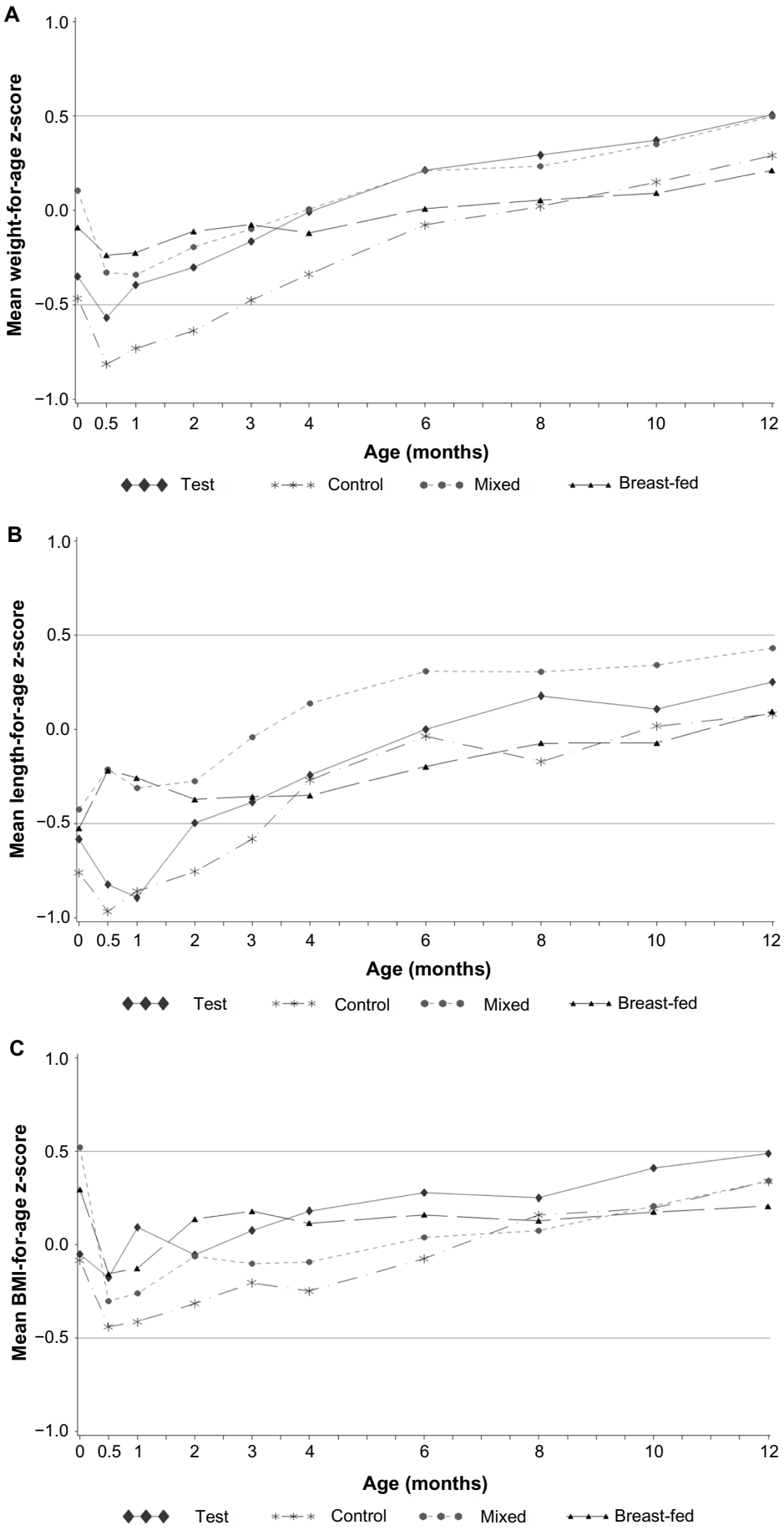

Figure 3. (*Continued*)

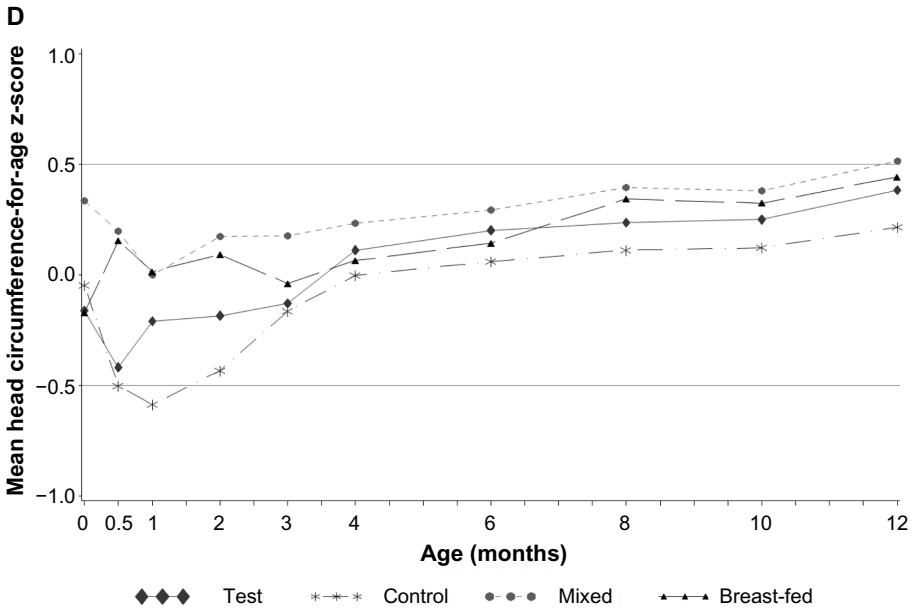

Figure 3. Mean anthropometric measurements relative to World Health Organization child growth standards, intention-to-treat. (**A**) Weight-for-age *z*-scores, (**B**) length-for-age *z*-scores, (**C**) body mass index (BMI)-for-age *z*-scores, (**D**) head circumference-for-age *z*-scores.

Other anthropometric measurements (weight, length, BMI, and head circumference) during the 12-month study period were within ±0.5 SD of the WHO child growth standards in all feeding groups except the control formula groups, which had length-for-age *z*-scores below −0.5 at a few time points.

Data on gastrointestinal symptoms showed no important differences that would indicate a problem with tolerability of the test formula. Infants fed on the test formula had hard and formed stools less frequently and soft stools more frequently compared with the control formula group. Compared with the breast-fed infants, those fed on the test formula had more soft and formed stools and fewer liquid and watery stools. This indicates that compared to control formula intake, intake of the test formula resulted in overall stool consistency that was more like that of breast-fed infants. The higher frequency of softer stools in the test formula group was probably because of

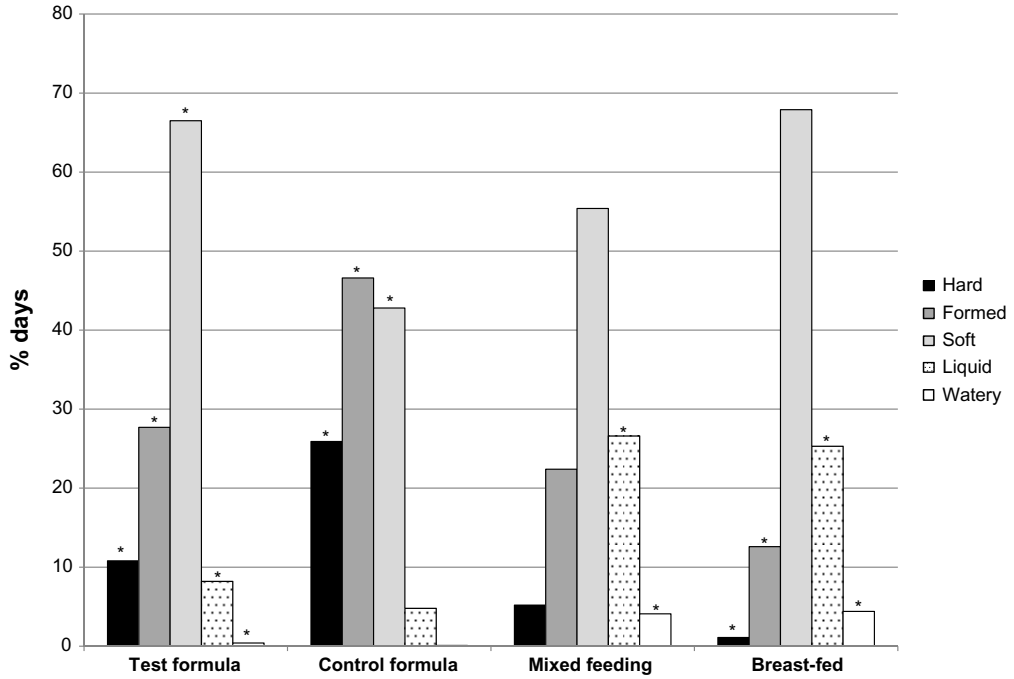

Figure 4. Stool consistency, intention-to-treat. The proportion of days with predominant stool consistencies is shown. Significant differences (Dunnett test, $P < 0.05$, adjusted by the Hommel method) between the test formula and the other groups are shown by an asterisk (*).

Table 4. Serious adverse events (SAE), Intention-to-treat population.

SAE	BREAST-FED (N = 79)	TEST (N = 65)	CONTROL (N = 63)	MIXED (N = 65)
Total	16	3	16	6
Bronchiolitis	4	1	1	0
Fever	2	0	2	1
Diarrhea	1	0	2	0
Pneumonia	2	0	1	1
Wheezing	0	0	0	1
Bronchospasm	1	0	0	0
Respiratory discomfort	0	0	0	1
Poor weight gain	1	0	0	0
Vomiting	0	0	1	0
Impetigo	0	0	1	0
Dehydration	0	0	3	0
Ophthalmologic evaluation	0	0	1	0
Social condition	0	0	1	0
Acute febrile illness	0	1	0	0
Cough	1	1	1	0
Inguinal hernia	0	0	1	0
Gastroesophageal reflux	2	0	0	0
Bronchopneumonia,	0	0	1	0
Dyspnea	1	0	0	0
Surgery of appendectomy and intestinal intussusception	0	0	0	1
Kasai surgery	0	0	0	0
Death	1	0	0	0
Jaundice	0	0	0	1

Note: *Some infants had >1 SAE.

the presence of GOS and FOS, which are known to increase stool water content and have previously been shown to result in softer stools in infants.[17,23,24]

The frequency of SAEs and non-serious AEs in the test formula and control formula groups was consistent with the growth and tolerability data showing no adverse effect of the test formula. The occurrence of gastrointestinal, respiratory, and skin disorders (which include symptoms of infections and allergy) was not significantly different between the test and control formula groups. However, this cannot be interpreted as the absence of an effect of GOS/FOS mixture on these symptoms since the study was not designed to test effects on these outcomes and may not be powered to detect any differences.

In the current study, infants from HIV-positive mothers were included in the exclusive formula-fed groups. This is in accordance with the public health policies of Brazil, which recommend avoiding breast-feeding by HIV-positive mothers.[25] Consistent with this policy, the Brazilian government provides infant formulas free of charge to HIV-positive mothers until their infants are 7 months old.[26] In the present study, the socio-economic level of the families was not evaluated. We considered that in a multi-center study involving families from different parts of the country, and in all cases from the public health system, it is expected that the socio-economic levels would be low in all groups.

In conclusion, our study showed that a formula containing lower protein concentration and the prebiotics GOS and FOS is safe for HIV-exposed but uninfected infants based on its ability to promote daily weight gain that is not inferior to that of breast-fed infants.

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Author Contributions

Conceived and designed the experiments: HR, PS. Analyzed the data: HR, PS, CN. Wrote the first draft: PS. Contributed to the writing of the manuscript: HR, MM, RS, RN, PS, MP. Agree with manuscripts results and conclusions: All. Jointly developed the structure and the

arguments for the paper: HR, MM, PS. Made critical revisions and approved the final version: HR, CN, PS, AM, MC, MP, TR. All authors reviewed and approved the final manuscript.

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