Growth of infants born to HIV-infected women when fed a biologically acidified starter formula with and without probiotics

Urban MF, Bolton KD, Mokhachane M, Mphahlele RM, Bomela HN, Monaheng L,Beckh-Arnold E and Cooper PA

Department of Paediatrics, University of the Witwatersrand, Johannesburg, South Africa

Correspondence to: Prof P Cooper, e-mail: peter.cooper@wits.ac.za

Abstract

Objectives: To compare the growth of HIV-exposed uninfected infants fed a biologically acidified milk formula with or without probiotics (*Bifidobacterium lactis*) during the first six months of life, with control infants fed a standard starter formula.

Design: Multi-centre, double-blinded randomised controlled trial.

Setting: Infants born to HIV-infected women delivering at one of three academic hospitals in Johannesburg, South Africa.

Subjects: Consenting HIV-positive women, who had previously decided not to breast-feed, were randomised to receive one of three milk formulas for their newborn infants.

Outcome measures: Comparisons of growth parameters through the first four months of life were made between infants fed the acidified formula without probiotics and those fed the control formula ("acidification effect"), and between infants fed the acidified formulas with and without added probiotics ("probiotic effect").

Results: Of 131 randomised infants, 33 (25%) did not complete the study and 13 (10%) were HIV infected, leaving 85 infants available for analysis. Infants receiving the acidified formula with probiotics had more rapid head growth (p=0.04) and showed a trend towards more rapid weight gain (p=0.06) over the first four months of life than the infants receiving the acidified formula without probiotics. No other significant differences between the feeding groups were demonstrated.

Conclusions: Infants in all study groups grew well, with increased head growth and a trend towards increased weight gain for those receiving probiotics. There were no differences in morbidity between the three study groups and no evidence of adverse effects of the study formulas.

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Introduction

Women infected with the human immunodeficiency virus (HIV) type 1 confront a dilemma when deciding whether or not to breast-feed. In the absence of antiretroviral therapy it is estimated that there is a 14% risk of mother-to-child HIV transmission through prolonged breast-feeding.[1] This must be balanced against the many benefits of breast-feeding, most importantly protection against infections and especially diarrhoeal diseases.[2]

For women electing not to breast-feed, it is important to minimise the risks of formula feeding. Non-breast-fed infants are usually given a standard whey-adapted starter formula that is in many respects close to human milk. A biologically acidified starter formula has been widely used in South Africa, as there is some evidence that this inhibits the growth of pathogenic bacteria in vitro [3,4,5] and reduces the incidence of diarrhoea in infants.[6,7]

The protection breast-feeding affords against diarrhoeal disease may, in part, relate to the characteristics of the intestinal micro-flora, which acts as a protective barrier against colonisation with pathogens, and also promotes certain gut immune functions.[8] In breast-fed infants, bifidobacteria are the predominant anaerobic bacteria in the faecal micro-flora,[9,10] whereas in infants receiving standard milk formula the dominant species also include enterobacteria, enterococci and bacteroides.[11] Modulation of the microbial flora towards increased bifidobacteria counts has been successfully achieved by adding either living bifidobacteria (probiotics) or substances enhancing the growth of specific bacteria (prebiotics), or both (synbiotics) to infant feeds.

Some evidence exists that the use of infant milk formula that is biologically acidified and contains bifidobacteria is associated with normal growth in early infancy,[12] and it is possible that such infant formulas could increase infant growth, either by reducing infections or through other mechanisms. Previous controlled trials of the use of probiotics or prebiotics in infants have demonstrated increased infant growth in some studies but not others.[14, 15]

Objectives

The primary objective of this study was to compare growth rates of HIV-exposed, uninfected infants fed a biologically acidified starter

formula with or without probiotics, with growth rates of infants fed a standard non-acidified starter formula. The primary outcome was weight gain (g/d) defined as the increment of weight divided by the increment of age between enrolment and the visit at day 119. Day 119 corresponded to four months of age and this was chosen as the end point for the primary objective as foods other than milk were introduced after that age. Secondary objectives were to assess tolerance of acidified formulas with and without probiotics and to evaluate the frequency of episodes of intercurrent infections.

Methods

This study was a multi-centre, double-blinded randomised controlled trial conducted at three teaching hospitals of the University of the Witwatersrand, Johannesburg, namely the Chris Hani-Baragwanath, Coronation and Johannesburg hospitals.

The study was approved by the Committee for Research on Human Subjects of the University of the Witwatersrand and all mothers of infants enrolled gave written informed consent.

As part of antenatal care, pregnant women attending the antenatal clinics of all three hospitals were routinely offered HIV testing. Those who tested positive received full post-test counselling, which included discussion of infant feeding options. Women who decided against breast-feeding were informed about the study, but enrolment only took place after delivery.

Inclusion criteria were normally grown (birth weight 2500–4200 g), term (gestation 37–42 weeks) male or female infants born to HIV-infected women who had elected not to breast-feed. Infants were excluded if they had major congenital abnormalities or major illness requiring either admission to an intensive care unit or hospital admission for more than three days. Subjects were also excluded if the mother planned to introduce other feeds in the first four months of life, or planned to move out of the area during the six-month study period. Recruitment was within one week of birth.

After enrolment, infants were assigned to one of three groups:

1. A whey-adapted starter formula (control group)
2. A biologically acidified whey-adapted starter formula
3. A biologically acidified whey-adapted starter formula with probiotics

Assignment was by a computer-generated randomisation table, and randomisation occurred separately at each hospital. Both investigators and participants were blinded to the formula assignments. Products were colour-coded and the formula composition did not appear on the tins.

The probiotic strain selected was *Bifidobacterium lactis*, because of its resistance to gastric acidity and because safety and efficacy of this strain have been previously demonstrated when added to follow-up milk.[16] However, this was the first study to evaluate the addition of probiotics to an acidified starter formula.

At the time of enrolment, basic information regarding the pregnancy and delivery were recorded and study personnel measured the infants' weight (to the nearest 10 g), length and head circumference (to the nearest millimetre) using standard equipment and techniques. Follow-up visits were scheduled for 14, 28, 42, 56, 91, 119 and 182 days after enrolment for repeat measurements.

After enrolment and randomisation to study formula, mothers received detailed instructions regarding the safe preparation of formula and were advised to feed on demand. It was stressed that only study formula should be given for the first four months.

Infants were withdrawn from the study if the mother regularly gave more than one bottle per day of another milk formula, if the infant did not receive the study formula (due to the mother not being able to get supplies or illness in the infant) for more than seven consecutive days, or if significant amounts of foods other than formula were introduced before four months of age. Although withdrawn from the study, they were still offered the study formula until six months of age.

At study visits infants were examined by study personnel. Caregivers were interviewed regarding interim health status. A retrospective, two-day questionnaire obtained information on the amount of formula prepared, the amount remaining in the bottle after each feed, whether feeds were tolerated, and use of any other feeds. All unused formula was returned and correlated with reported consumption.

Blood specimens were collected on all infants on days 42, 119 and 182 by study physicians from a peripheral vein, if possible just before the next feed. On each occasion, the following were assayed: haemoglobin, albumin, total protein, blood urea, creatinine, quantitative amino acids (including tryptophan), ferritin, C-reactive protein, calcium, sodium, phosphate, potassium, chloride and glucose. Haemoglobin measurements were done by the Contract Research Laboratory of the National Health Laboratory Service and all biochemical assays were performed by the Centre Hospitalier Universitaire Vaudois, Laboratoire Central de Chimie Clinique in Lausanne, Switzerland. All biochemical test samples were collected in heparinised tubes and put on ice until centrifuged within one hour of collection. The supernatant plasma was stored in a -70°C refrigerator until shipment by a recognised international carrier. Blood was also collected on days 42 and 182 for HIV DNA Polymerase Chain Reaction (PCR) tests, performed by the National Institute for Virology in Johannesburg. All laboratories used were subject to internal and external quality control systems.

The "acidification effect" was estimated by comparing the group receiving acidified formula without probiotics with the standard formula group, while the "probiotic effect" was estimated by comparing the acidified groups with and without probiotics.

The sample size calculation was for non-inferiority of weight gain between enrolment and four months of age. The data collected by Nelson et al[17] was used as a reference for the sample size calculation, with a mean growth of 28 g/day (sd = 5.7). A tolerance of 3.9 g/day was accepted as non-inferior, and non-inferiority would be demonstrated if the 90% confidence interval for weight gain obtained with ANOVA controlling for gender and study site was above the level of -3.9 g/day. Using an alpha value of 0.05 and a power of 80%, it was calculated that 28 infants per formula group were required. Infants who were followed up for at least 119 days (four months) and were not HIV infected qualified for analysis, and it was estimated that 30% of the infants would be lost to follow-up (20% due to dropout and 10% due to a late HIV-positive diagnosis). It was thus estimated that a total enrolment of 120 infants would be required.

The growth parameters were calculated as mean growth velocities (i.e. increment per time interval). Statistical comparisons were made

by analysis of covariance (ANCOVA), controlling for gender and study site effects. Mean anthropometric Z-scores were calculated using Eurogrowth software,[18] but this reference is only reliable after the first month of age.

The data from the two-day diaries were aggregated to visit level and tested per visit by ANCOVA. The statistical calculations were done using NCSS-2000 and SPSS-10.

Results

The study began in February 2000 and was completed in May 2002, with each hospital recruiting a similar number of subjects. During the course of the study, nevirapine prophylaxis against mother-to-child transmission of HIV was gradually introduced to the hospitals in which the study was conducted, potentially increasing the number of infants not infected with HIV. However, loss to follow-up was higher than expected and thus an additional 11 infants were enrolled to ensure that 28 HIV-uninfected infants completed the study in each group, resulting in a total of 131 infants being enrolled.

Figure 1 illustrates the number of infants enrolled in each feeding group, the number that did not complete the study and those found subsequently to be HIV positive. Of the 33 infants (25%) who did not complete the study, 15 did not return at all after the initial enrolment visit. The most common reason for later loss to follow-up was non-attendance and inability to trace the families (14 subjects). Other reasons included parental decision to withdraw (five subjects), non-compliance with the study formula (four subjects), non-exclusive feeding with formula milk (six subjects), illness (five subjects), and

other reasons (six subjects). More than one reason was applicable in some cases. There were no differences between the study groups in numbers of infants not completing the study, or completing but determined to be HIV positive. Of those who did not complete follow-ups to four months (and considered lost to follow-up), four were found to be HIV positive. A total of 21 infants were lost to follow-up prior to the first HIV PCR test at six weeks of age. Although some appointments were not attended at the scheduled dates, the mean age in days for all visits corresponded with the scheduled days except for the fourth visit, which took place at a mean age of 57 rather than 56 days.

Study subjects were generally of low socio-economic status (see Table I), with 62 (73%) having a total household income of less than R1 500 per month. There were no relevant demographic differences between the three feeding groups.

Table I: Characteristics of study subjects by feeding group

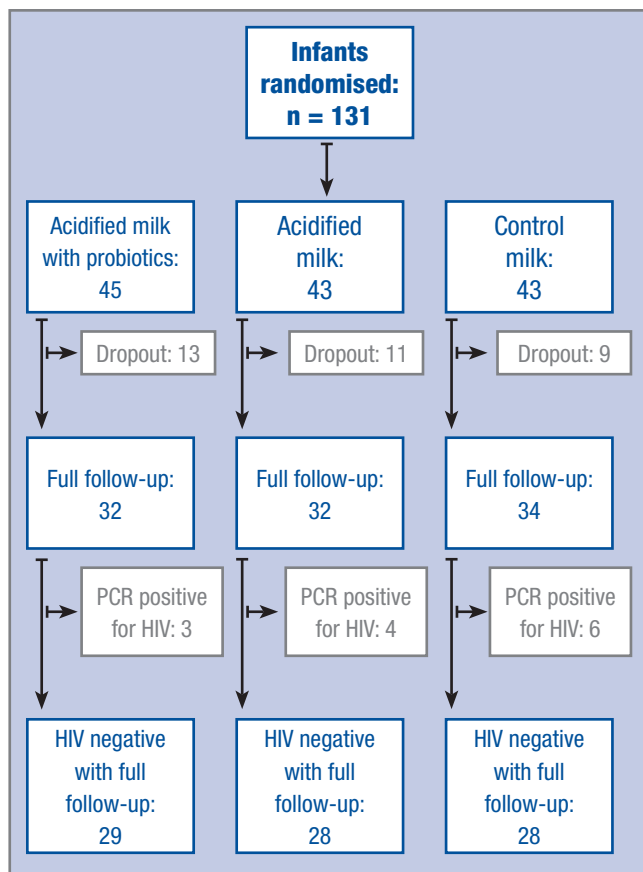|	ACIDIFIED & PROBIOTIC	ACIDIFIED	CONTROL
	n = 29	n = 28	n = 28
Maternal age (years) mean (SD)	28.2 (5.1)	27.4 (5.6)	26.4 (3.9)
Maternal education mean (SD)	10.3 (2.0)	10.2 (2.3)	10.9 (2.1)
Household income < R1 500 pm	23 (79%)	20 (71%)	19 (68%)
Number (%) in formal housing	20 (69%)	16 (57%)	21 (75%)
Number (%) with piped water at their dwelling	27 (93%)	25 (89%)	27 (96%)
Number (%) with flush toilet	29 (100%)	24 (86%)	25 (89%)
Number (%) with electric stove	24 (83%)	22 (79%)	22 (79%)
Number (%) with fridge	18 (62%)	16 (57%)	15 (54%)
Number (%) where parent was caregiver	26 (90%)	27 (96%)	27 (96%)
Number (%) of male infants	17 (59%)	15 (54%)	12 (43%)
Birth weight (grams) mean (SD)	3 206 (350)	3 144 (361)	3 180 (396)
Gestation (weeks) mean (SD)	39.0 (1.1)	39.5 (0.9)	39.2 (1.0)

The effects of acidification and probiotics on anthropometric growth are presented in Table II. The group receiving the acidified formula did not differ in weight gain in comparison with the control group. The lower limit of the 90% confidence interval was equal to -1.4, which is above the level of -3.9 g/day, indicating that the acidification did not have a negative effect on weight gain. The probiotic effect, derived by comparing infants receiving the probiotic formula with those receiving the acidified formula without probiotics, showed that the infants receiving probiotics had an additional weight gain of 3.3 g/day, but this difference was not significant (p=0.06). Comparison of other anthropometric parameters showed a significant increase in head circumference for the probiotic group (p=0.04). Analysis of covariance showed a significant effect of gender on weight gain (p=0.002) but no significant effect of study site.

The weight for age, length for age and head circumference for age Z-scores at each visit between 42 and 182 days are shown in Tables III, IV and V. No significant differences were seen between the groups at any of the time points.

Figure 1: Flow diagram of participants

Infants randomised: n = 131

Acidified milk with probiotics: 45	Acidified milk: 43	Control milk: 43
Dropout: 13	Dropout: 11	Dropout: 9
Full follow-up: 32	Full follow-up: 32	Full follow-up: 34
PCR positive for HIV: 3	PCR positive for HIV: 4	PCR positive for HIV: 6
HIV negative with full follow-up: 29	HIV negative with full follow-up: 28	HIV negative with full follow-up: 28

Table II. Effects of acidification and probiotics on growth parameters

	Male		Female		Formula effect SE)	[c]P-value
	n	Mean (SD)	n	Mean (SD)		
Weight gain (g/day)						
Acidified + probiotic	17	37.6 (7.2)	12	34.3 (6.6)	[a]3.3 (1.7)	0.06
Acidified	15	35.6 (5.5)	13	29.2 (7.2)	[b]1.4 (1.7)	0.40
Control	12	32.8 (4.1)	16	29.0 (6.3)		
Length gain (mm/day)						
Acidified + probiotic	17	1.12 (0.16)	12	1.04 (0.12)	[a]0.04 (0.04)	0.24
Acidified	15	1.17 (0.12)	13	1.0 (0.15)	[b]0.03 (0.04)	0.36
Control	12	1.12 (0.16)	16	1.0 (0.13)		
Head circumference gain (mm/day)						
Acidified + probiotic	17	1.56 (0.52)	12	1.53 (0.52)	[a]0.05 (0.03)	0.04
Acidified	15	1.44 (0.49)	13	1.30 (0.51)	[b]0.001 (0.02)	0.96
Control	12	1.26 (0.25)	16	1.31 (0.55)		

[a] Probiotic effect (acidified + probiotic versus acidified)
[b] Acidification effect (acidified versus control)
[c] p-value of analysis of variance controlling for gender and study site

Table III: Weight for age Z-scores of study subjects by feeding group

AGE*	ACIDIFIED AND PROBIOTIC	ACIDIFIED	CONTROL
6 weeks	0.22 ± 0.94	-0.08 ± 1.05	-0.03 ± 1.13
2 months	0.40 ± 0.94	0.23 ± 0.98	0.12 ± 0.98
3 months	0.80 ± 0.94	0.36 ± 1.16	0.21 ± 0.99
4 months	1.02 ± 1.07	0.41 ± 1.02	0.20 ± 1.01
6 months	0.70 ± 1.25	0.10 ± 1.02	0.03 ± 1.04

No significant intergroup differences were found.
*The ages correspond to the visits at 42, 56, 91,119 and 182 days (see text).

Table IV: Length for age Z-scores of study subjects by feeding group

AGE*	ACIDIFIED AND PROBIOTIC	ACIDIFIED	CONTROL
6 weeks	-0.21 ± 0.80	-0.40 ± 0.96	-0.56 ± 1.14
2 months	-0.38 ± 0.89	-0.42 ± 0.95	-0.32 ± 0.91
3 months	0.04 ± 0.78	-0.18 ± 1.01	-0.14 ± 0.96
4 months	0.05 ± 0.89	-0.33 ± 1.08	-0.60 ± 0.92
6 months	-0.03 ± 1.07	0.28 ± 1.21	-0.57± 0.96

No significant intergroup differences were found.
*The ages correspond to the visits at 42, 56, 91,119 and 182 days (see text).

Table V: Head circumference for age Z-scores of study subjects by feeding group

AGE*	ACIDIFIED AND PROBIOTIC	ACIDIFIED	CONTROL
6 weeks	0.54 ± 0.82	0.67 ± 0.95	0.52 ± 0.95
2 months	0.73 ± 0.75	0.78 ± 1.00	0.67 ±1.14
3 months	0.80 ± 0.96	0.68 ± 0.86	0.81 ± 0.88
4 months	1.02 ± 0.77	0.74 ± 1.09	0.85 ± 1.05
6 months	0.60 ± 0.78	0.63 ± 1.34	0.62 ± 1.14

No significant intergroup differences were found.
*The ages correspond to the visits at 42, 56, 91,119 and 182 days (see text).

The amount of milk formula given in the two days prior to each visit was not significantly different between the groups, except for visit 5 (91 days), when more control formula was consumed than either of the acidified formulas. Up to visit 6 (119 days) there were few cases of giving additional foods: 9 of 174 (5%) assessment days in the acidified plus probiotic group, 5 of 168 (3%) in the acidified formula group, and 9 of 168 (5%) in the control formula group.

Tolerance of feeds was assessed by subjective maternal assessment of the number of stools, number of episodes of spitting up, the number of vomits per day, the frequency of hard or loose stools, the frequency of flatulence, and the proportion of the time when the infant was restless on the two days preceding each of the first six visits. There were no significant differences in these parameters.

A total of 17 infants required hospital admission during the course of the study, five of whom failed to complete the study. There were five cases of proven or suspected septicaemia, five cases of gastroenteritis and dehydration (one of which was also considered to be septicaemic) and three cases of bronchopneumonia or bronchiolitis. Of these seventeen cases, five were in HIV-infected infants, three in infants of unknown HIV status, and a further nine among the 85 infants who completed the study. There was no significant difference in rates of serious illness between the study groups: Seven cases were in infants assigned to the formula with probiotics, four in infants assigned to the acidified formula without probiotics, and six in those assigned to the control formula. The data was also analysed for gastrointestinal symptoms that did not require hospital admission. Episodes of vomiting, diarrhoea or gastroenteritis were found in eight of the infants assigned to each of the acidified formula groups and in eleven of those assigned to the control formula. However, these differences were not significant.

The laboratory results were compared to assess the acidification effect and the probiotic effect using a regression analysis (ANCOVA). Compared to infants taking the control formula, those taking the acidified formulas had significantly higher serum levels of arginine, citrulline and glycine, and lower levels of lysine. Those taking the probiotic formula had significantly lower levels of proline and tyrosine. The magnitude of all these differences was small and not considered clinically significant. With respect to the other laboratory measurements, the infants receiving the control formula had significantly higher levels of chloride and glucose, but the mean differences were small and not considered clinically significant. Infants receiving the control formula also had significantly higher levels of serum ferritin without higher C-reactive protein levels.

Discussion

The primary objective of the study was to assess whether weight gain of infants receiving acidified formula, with or without probiotics, was inferior to that of infants being fed control formula. The data showed no significant effects on growth parameters when using an acidified milk formula. However, the effect of probiotics approached significance for greater weight gain (p=0.06) and was significant for greater gain in head circumference. Since the study was powered as a non-inferiority study, it may not have succeeded in demonstrating increased weight gain using probiotics. Although no statistical differences were seen in Tables III, IV and V, the trends seen in mean Z-scores up to 119 days for weight and head circumference also

suggested an advantage for the group who received the acidified formula with probiotics. The decrease in mean Z-scores between 119 and 182 days corresponded with the introduction of foods other than milk.

The greater gain in head circumference and the trend towards greater weight gain noted in infants receiving formula with probiotics were not related to greater energy or protein intake. The formulas had the same caloric density, and there was no greater intake of study formula or other nutrients. In fact, at visit 5 (mean age 91 days) the history of milk intake was significantly less in the infants randomised to either of the acidified formulas, although this may have been a chance finding since multiple comparisons were performed. Other factors that may affect growth are socio-economic status and gender. There were no differences in socio-economic status detected between the study groups. There were somewhat fewer male infants in the control group and, although it is known that male infants grow faster than females, in this study the trend toward improved growth in infants receiving the formula with probiotics was present in both male and female infants. In addition, the overall effect of formula persisted after controlling for gender in a multivariate analysis.

Probiotics may have functional effects on the gastrointestinal tract that improve digestion or absorption of the energy content of the formula. Previously demonstrated functional effects of specific probiotics include reduced gut permeability and enhanced local intestinal and systemic immunity.[19]

There was a higher than expected rate of loss to follow-up, with 75% of subjects followed to completion. Losses to follow-up were evenly spread across the three study groups and therefore are unlikely to represent a lack of tolerance of any specific milk. Almost half of the losses to follow-up occurred early, with failure to return for the first follow-up visit. Several study subjects mentioned a concern that the use of colour-coded tins may draw attention to their HIV-positive status, as the community increasingly identifies non-breast-feeding with HIV. Some of the loss to follow-up may be due to HIV-related illnesses in women or infants. In addition, many women come from outside of Johannesburg to deliver their infants and then return to their homes soon thereafter. It is probable that some of those lost to follow up were in this category.

Although some infants who were admitted with gastroenteritis and dehydration were noted to be acidotic and concern was expressed during the blinded phase of the study regarding the tolerance of young infants of acidified formulas with respect to acid-base balance, when the code was broken there was no evidence of any differences in this regard between the formula groups. The differences shown between the study groups with respect to some of the biochemical parameters were considered small and not clinically significant.

Conclusions

In this controlled trial of infants fed acidified formulas with and without added probiotics compared to a standard infant formula, there was good growth of infants in all study groups. Growth in head circumference was greater in infants receiving formula containing probiotics compared to formula that was only acidified and there was a trend towards increased weight gain. There were no differences in morbidity between infants in the three study groups, and all types of study milk were well tolerated. Further studies are required to define the separate effects of acidification and probiotics.

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