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The effect of a fructo-oligosaccharide supplemented formula on gut flora of preterm infants

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Abstract

Aim: The intestinal flora of breast-fed infants is generally dominated by bifidobacteria which have beneficial properties. Their presence is due to various components of breast milk, including prebiotic substances. This prospective double-blind study compared the numbers of bifidobacteria in the stool flora of bottle-fed preterm infants randomized to receive for 14 days either a formula with prebiotic fructo-oligosaccharides at a concentration of 0.4 g/dL or the same formula with maltodextrin as a placebo.
Methods: Within 0–14 days after birth, 56 healthy bottle-fed infants were enrolled to receive either the prebiotic or placebo. Faecal samples were taken at inclusion day and at study day 7. The number of bifidobacteria in the stools, stool characteristics and somatic growth were recorded during the study.
Results: In the group fed fructo-oligosaccharides, both the numbers of bifidobacteria in the stools and the proportion of infants colonized with them were significantly higher as compared to the placebo group ($p=0.032$ and $p=0.030$ respectively). There was also a higher number of bacteroids in the fructo-oligosaccharide group as compared to the placebo ($p=0.029$). At the same time, reduction was noted in the numbers of *Escherichia coli* and enterococci. ($p=0.029$, and $p=0.025$, respectively). Supplementation had also significant influence on stool frequency per day ($p=0.0080$).
Conclusion: An infant formula containing a small quantity of prebiotic oligosaccharides is well accepted and leads to rapid growth of bifidobacteria in the gut of bottle-fed preterm infants while decreasing the numbers of pathogenic microorganisms.

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1. Introduction

Breast-fed infants suffer from fewer infections than formula-fed infants. This beneficial effect is attributed among others to the characteristic gastrointestinal microflora of breast-fed infants in which lactic acid-producing bacteria predominate [1—4]. Galacto-oligosaccharides (GOS) which are a major component of human milk have been identified as a "bifidogenic" factor of human milk [5—12]. Not only GOS but also fructo-oligosaccharides (FOS) derived from plants have been shown in adult humans and infants to increase the gut populations of beneficial bacteria such as bifidobacteria (BB) [13—15]. Interest in bifidogenic diets has increased recently since the publication of studies showing that the addition of BB to infant formula can reduce the risk and the severity of necrotizing enterocolitis [16,17] and the incidence of allergic disease in preterm infants [18,19].

The aim of the present prospective double-blind placebo study was to determine whether a relatively small dose of FOS has a probiotic effect on the gastrointestinal flora of preterm infants.

2. Patients and methods

Preterm infants with a maximum gestational age of 36 weeks admitted to the Neonatal Unit of the Alexandra Regional General Hospital in Athens were eligible if they were healthy and exclusively formula fed. Infants with major congenital abnormalities, chromosomal disorders or with disease requiring systemic antibiotic treatment were excluded. The primary study parameters was bifidogenic effect and stool characteristics. Secondary parameters were somatic growth and well-being. Analysis was carried on an intention-to-treat basis. The sample size was based on the described concentration of bifidobacteria in faecal samples. With a sample size of at least 15 infants per group, it is possible to detect a mean difference of 30% in bifidobacteria, with a probability of 80% and a significance level of 0.05 [20]. After allowing for dropouts (20%), the number of infants required to be recruited per group was 20 infants.

2.1. Analysis of data

Results of the two formula groups were evaluated using *t*-tests for parametric data and Mann—Whitney test for non-parametric data. Binary and categorical data were assessed by chi-squared analysis.

The study protocol was approved by the Ethical Committee of the hospital and informed parental consent was obtained for each infant prior to enrollment in the study. Infants were randomly assigned by closed envelopes to one of two formula groups. The composition of the two formulas was, apart from the supplemented oligosaccharides, identical. The study formula, which was a standard preterm formula, was supplemented with FOS (0.4 g/100 ml), while the control formula was the same but supplemented with 0.4 g of maltodextrins as placebo. The FOS used in the study was inulin, a non-nutrient carbohydrate produced by partial enzymatic hydrolysis of chicory inulin. The feeding regimen was performed according to the practice of the hospital and was not influenced by the study protocol. The total duration of supplementation was 14 days. Table 1 gives the most relevant clinical data of he infants. The first day of full formula feeding was defined as measurement day 1. The following parameters were evaluated on day 1 of the study: body weight, length, head circumference, mid-arm circumference, concentration of bifidobacteria and other organisms in stools. A diary recording daily formula intake, stool frequency, size, consistency and color was completed. Stool consistency was recorded using a descriptor corresponding to an assigned numerical value as follows: watery = 5, loose = 4, soft = 3, firm = 2 and hard = 1. Stool frequency was reported as the number of stools per 24 h. Somatic measurements were repeated after 7 and 14 days. Faecal flora was analysed again on day 7 of the study. This was done by collecting 0.5 g of freshly voided stools in sterile plastic vials which were immediately transferred to the laboratory. Identification of isolates was made with standard laboratory methods using MacConkey agar, manitol salt agar, blood agar, Saburaud agar for enterobacteria, staphylococci, and enterococci. Prereduced Columbia blood agar with hemin, vitamin K, colimycin, vancomycin, Beerens agar as well as Rogosa agar (Oxoid) were used for anaerobic bacteria.

Table 1 Clinical data of the infants enrolled in the study; expressed as mean (S.D.)

Parameter	FOS* (*N* = 36)	Placebo (*N* = 20)	*p*
Gestational age (weeks)	33.9 (1.3)	33.4 (1.8)	NS
Sex *N* (M/F)	16/17	8/12	NS
Type of delivery (caesarian/vaginal)	16/20	8/12	NS
Birth weight (g)	1592 (333)	1639 (170)	NS
Birth length (cm)	43.(2.9)	42.8 (2.4)	NS
Head circumference (cm)	29.5 (2.2)	29.3 (1.4)	NS
Arm circumference (cm)	7.33 (0.6)	7.3 (0.5)	NS
Age at study entry (days)	6.4 (4.9)	8.2 (3.8)	NS
Weight gain during study (g/day)	22.8 (6)	27.4 (7)	<00.5
Length gain during study (cm/week)	1.5 (0.06)	1.2 (0.06)	NS
Head growth during study (cm/week)	0.95 (0.03)	0.96 (0.02)	NS
Arm circumference growth (cm/week)	0.35 (0.01)	0.6 (0.01)	<0.001
Stool frequency (number/day)	3.05 (0.95)	2.3 (0.6)	<0.001
Stool consistency score	2.66 (0.58)	3.0 (0.6)	NS

*FOS = fructo-oligosaccharides.

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Table 2 Faecal flora before and after 7 days supplementation with FOS, expressed as mean (S.D.) log 10 CFU/g wet faeces

Type of diet						
Microorganism	Day 1		*P*	Day 7		*P*
	FOS	Placebo		FOS	Placebo	
Bifidobacteria	9.0 (0.2)	9.0 (0.3)	NS	9.5 (0.32)	9.02 (0.4)	<0.05
Percentage (%)	59	58	NS	90	70	<0.05
Staphylococci	8.0 (0.32)	8.0 (0.31)	NS	8.0 (0.31)	8.0 (0.32)	NS
E. coli	9.8 (0.4)	9.8 (0.3)	NS	8.0 (0.3)	9.69 (0.3)	<0.05
Bacteroides	9.1 (0.33)	9.1 (0.4)	NS	9.6 (0.1)	9.1 (0.3)	<0.05
Enterococci	9.1 (0.6)	9.2 (0.2)	NS	8.3 (0.4)	9.1 (0.3)	<0.05

FOS = fructo-oligosaccharides.

Differing colonies appearing on those media were identified by colony appearance, Gram stain and using Biochemical Api Systems. Colony counts were expressed as number of microorganisms per gram of faeces or as the log of colony forming units/g wet weight of faeces.

3. Results

Of the initial number of 65 infants recruited for the study, 9 dropped out (5 from the FOS group and 4 from the control group). The reasons for excluding participants from the study were development of sepsis, necrotizing enterocolitis, ingestion of non-study feeds, inability to collect stools. So 56 infants completed the study (36 in the FOS group and 20 in the placebo group). There were no significant differences in the infant characteristics between the two groups at the beginning of the study. Mean weight gain and arm circumference at day 14 of the study were higher in the placebo group. There was no significant difference in other somatic parameters between the two study groups (Table 1). Mean daily stool frequency was significantly higher in the FOS group. Stool consistency also differed in the two groups with infants fed the placebo diet having harder stools (Table 1). All infants tolerated well the two formulae. At the first day of the study, the numbers of faecal BB and the percentage of infants colonized with BB did not differ between the groups. During the study period, the number of faecal BB increased significantly in the FOS group, while the increase was only marginal in the placebo group. Therefore, at the end of the 7-day feeding period, the number of BB in the stools was significantly higher in the FOS group. The percentage also of infants colonized with BB was significantly higher in the FOS group. The number also of bacteroids was significantly higher in the FOS group. On the other hand, the numbers of *Escherichia coli* and enterococci were significantly lower in the FOS group as compared to placebo (Table 2).

4. Discussion

The most consistently observed difference between breast-fed and bottle-fed infants is that breast-fed infants have lower counts of clostridia, enterococci and enterobacteria and higher counts of staphylococci and bifidobacteria. Bacteroids do not seem to be much influenced by breast-feeding and even less so do the lactobacilli [21]. Much of the action of human milk on stool flora is due to the presence of prebiotics [10].

Prebiotics are non-digestible food components that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thereby improving host health [10—11].

In the majority of studies conducted in term neonates, a prebiotic mixture with 90% GOS and 10% FOS was used in order to mimic the prebiotic effect of neutral human milk oligosaccharides [6,22—29]. Only one study used FOS as the sole prebiotic [30]. Bifidogenic effect was noted in all studies except one [29]. This stimulating effect on BB was clearly demonstrated after at least 2 weeks of prebiotic supplementation. One of the studies reported a dosage-related bifidogenic effect (higher dosage of prebiotics leading to increased numbers of BB in the faeces) [24], while another study reported exactly the opposite effect (higher doses leading to smaller concentration of BB as compared to lower dosage) [30]. In two of these studies, the increasing number of BB was constantly accompanied by a reduction of the most clinically relevant pathogens [26,27]. In studies where stool characteristics were recorded, stool consistency was significantly lower than in the control groups, while stool frequency was increased [20,24,34].

Experience on the use of prebiotics in preterm infants is extremely limited. In the only published study, preterm infants were given a mixture of GOS and FOS at a concentration of 1 g/dL. Bifidogenic effect was observed after 14 days of supplementation [31]. This effect was more pronounced at 28 days. Further analysis of data on the same infants showed, in addition, a simultaneous reduction in the numbers of pathogenic microorganisms [32]. In the present study on preterm infants, the effect of the supplementation on the number of faecal BB was already detectable after only 7 days of supplementation. This was followed by a reduction in the numbers of *E. coli* and enterococci. The level of supplementation in our study was much lower (0.4 g/dL) in comparison to the previous study, and FOS was the only prebiotic used. Another characteristic of our study is that infants enrolled were exclusively bottle-fed, and they never received breast milk, thus excluding any possible beneficial effect on stools of previous breast milk. It is of interest to note that the addition of FOS in the present study led to higher colonization with *Bacteroides*. Similar increase in *Bacteroides* counts following FOS supplementation was also reported by Euler et al. [30]. *Bacteroides*

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commonly colonise the large bowel of preterm infants. In fact, Rotimi et al. report an 80% rate of colonization within the first 6 days of life [33]. Their role has not been clearly defined, although they are usually considered as non-pathogenic. It was recently shown that intestinal colonization of infants with the *Bacteroides fragilis* group was more closely associated with maturation of IgA and IgM secreting cells than colonization with the other bacterial genera studied [34].

In accordance with previous studies stool frequency was significantly higher in the FOS group. This group had also softer stools in comparison to the placebo group. Infants fed the FOS diet in the present study had normal somatic growth, although they gained less weight at day 14 of the study and their arm circumference was also smaller as compared to placebo. However, length growth and head growth were similar. Higher weight gain in the control group was expected since FOS by definition are not absorbed in the small intestine, while maltodextrins are fully absorbed and provide extra calories [35]. Normal somatic growth following FOS supplementation was also reported in previous studies [24,27].

4. Conclusion

We have documented that the addition of a small quantity of FOS in the normal diet of preterm infants was well tolerated and resulted in a rapid increase in the numbers of BB and the proportion of infants colonized by BB. It remains, however, to be seen whether such a diet has a major clinical or long-term benefit to preterm infants.

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