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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.