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Effects of Short-Chain Fructooligosaccharides on Faecal Bifidobacteria and Specific Immune Response in Formula-Fed Term Infants: A Randomized, Double-Blind, Placebo-Controlled Trial

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Summary The aim of this study was to evaluate the effect of an infant formula supplemented with short-chain fructooligosaccharides (scFOS) on faecal concentration of bifidobacteria. Sixty-one healthy formula-fed infants participated in this double-blind controlled trial and were randomized to receive either the scFOS-supplemented formula (4 g/L scFOS) or the placebo-supplemented formula (4 g/L maltodextrins) until the age of 4 mo. Stool samples were analyzed for bifidobacteria at enrolment and at the age of 2 and 3 mo and for antipoliovirus IgA at the age of 4 mo. Parents completed a questionnaire to assess digestive tolerance. Change in faecal bifidobacteria after 2 mo were higher with scFOS compared to the placebo. At 4 mo, specific IgA tended to be higher with the scFOS group than with the placebo. Somatic growth and digestive tolerance were similar between groups. This study confirms that scFOS-supplemented formula can increase the concentration of faecal bifidobacteria while being well tolerated.

Key Words microbiota, infant formula, prebiotic

Breast feeding exerts a number of beneficial effects in infants, including protection against infectious and allergic diseases (*1*). Those effects are probably partly mediated through intestinal microflora modulation: compared to formula-fed infants, breast-fed infants indeed exhibit a higher proportion of bifidobacteria and lactobacilli (*2*, *3*). Oligosaccharides, the third largest component in human milk (10 to 12 g/L), are likely to be involved in this beneficial modulation of microflora in breast-fed infants (*4*). They are implicated in the gastrointestinal tract development and in the reduction of respiratory and gastrointestinal illness in infants (*5*, *6*). For these reasons, new infant formulas containing prebiotic oligosaccharides have been developed in the past 10 y to mimic human milk's beneficial effects on infant health. A mixture of short chain galactooligosaccharides and long chain fructo-oligosaccharides (scGOS/lcFOS) at 2 to 10 g/L increase bifidobacteria and boost local immunity, which has been associated with a reduced incidence of gastrointestinal and respiratory infections and atopic dermatitis (*7*, *8*). This formula is well tolerated and improves stool consistency in infants (*9*, *10*).

So far, no studies have been carried out to investigate the effects of infant formula supplemented with short-chain fructooligosaccharides (scFOS) alone. ScFOS are non digestible fructans, which mainly differ from lcFOS by their low degree of polymerization (3–5 monomers versus 2–60 monomers). Along with GOS and lactulose, they are considered as prebiotic compounds (*11*, *12*). ScFOS have been extensively studied in healthy adults, showing dose-response bifidogenic effects for doses ranging from 2.5 to 10 g/d and positive effects on colonic environment and digestive comfort (*13*, *14*). In children they have been shown to reduce duration of diarrhoea episodes when consumed at around 2.5 to 5 g per day (*15*, *16*).

The objective of this study was to evaluate the effects of scFOS-enriched infant formula on faecal concentration of bifidobacteria and on specific immune response towards poliovirus vaccination in comparison to the same not supplemented infant formula.

MATERIALS AND METHODS

The study protocol was approved by the ethical committee of Lille (France) under the number CP04/43 and was performed in accordance with the guidelines of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use and the principles laid down in the current version of the Declaration of Helsinki.

Subjects. Sixty-one healthy term infants were recruited via 6 paediatricians practising in two different French maternity wards from October 2004 to December 2005. Vaginally delivered infants between 0 and 7 d

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Table 1. Nutritional composition of the formulae.

Composition	Control formula		scFOS formula	
	Per 100 g	Per 100 mL at 13.5%[2]	Per 100 g	Per 100 mL at 13.5%
Energy (kcal)	510	68.85	504	68.07
Proteins (g)	11.2	1.51	11.2	1.51
Carbohydrates (g)	55.2	7.52	55.2	7.52
As pure scFOS[1]	—	—	2.9	0.40
Fat	27.2	3.67	27.2	3.67

[1] Caloric value of pure scFOS is 2 kcal/g.

[2] 13.5% of powder in 100 mL of reconstituted formula.

List of ingredients: demineralised lactoserum, vegetable oil (palm and soya), skimmed milk, maltodextrins, minerals (calcium carbonate, sodium citrate, sodium chloride, magnesium chloride, iron sulphate, manganese sulphate, zinc sulphate, copper sulphate, potassium iodine, sodium selenite, potassium phosphate), emulsifying agent: soya lecithin, vitamins (A, D3, E, K1, B1, B6, B12, C, niacin, biotine, folic acid, beta-carotene), choline chloride, taurine, inositol, and carnitine.

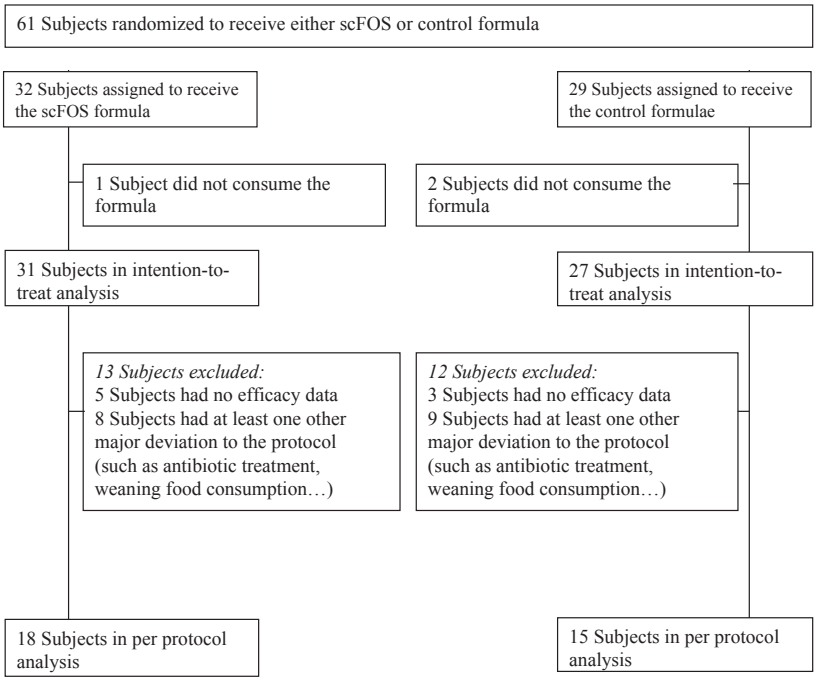

Fig. 1. Flow chart of progression of infants during the study.

of age were included in the study if gestational age was between 37 and 42 wk, if their mother had previously decided to not breast-feed, if they had received only standard formula feeding without pre- or probiotics from birth to enrolment in the study and if their parents gave written informed consent. Infants with any of these characteristics were excluded from the study: in utero growth retardation, cow's milk allergy, and lactose intolerance.

Study design. Infants were allocated to receive either a scFOS-enriched formula or a placebo-enriched formula during an intervention period of 4 mo. Randomisation was done in each centre and included a block size of 4 done by a person not involved in the study; both parents and investigators were blinded for the type of formula that infants were receiving. Both formulas were prepared from the same standard formula (Table 1). scFOS formula contained short-chain fructooligosaccharides (ACTILIGHT 950P®, 95% pure scFOS with a degree of polymerisation between 3 and 5, Beghin Meiji, Marckolsheim, France) at a concentration of 3.1% in dry matter, corresponding to 4 g/L scFOS in reconstituted formula. Control formula contained added maltodextrins (CERESTAR DRY MD 01915®, Cerestar, Vilvoorde, Belgium) at the same concentration throughout the 4 mo of the study, parents were instructed to fed their infant ad libitum, exclusively with the provided formula; recommended daily doses of formula were determined by paediatricians according to the infant's weight, and therefore ranged from 0.5 to 1 L, corresponding to about 2 to 4 g scFOS/d for scFOS formula. This dosage was based on the fact that scFOS start to increase fae-

cal bifidobacteria from 2.5 g/d in adults (*13*). Adequate dietary compliance was defined as exclusive formula consumption during at least 75% of the study duration and no consumption of weaning products (especially infant cereals).

Four visits were programmed during the study, at inclusion (M0), and at 2/3/4 mo of age (M2/M3/M4) (Fig. 1). At M0, inclusion criteria were checked and informed consent was collected; clinical examination was performed by a paediatrician to ensure enrolment of healthy infants. Products were given to the families, along with the material required for proper follow-up, including a personal digital assistant (PDA).

Vaccine-specific secretory IgA is considered a suitable marker to assess diet-induced changes in immune functions (*17*). In the current study, anti-poliomyelitis vaccine (Pentavac®, Aventis Pasteur MSD, Lyon, France) was chosen to assess the effects of formula on antipoliovirus-specific immune response. This pentavalent vaccine is used in France for prevention of poliomyelitis, diphtheria, tetanus, pertussis, and invasive infections of *Haemophilus influenzae* type b. Pentavac® administration occurred at M2, M3 and M4, according to health authorities' recommendations in France.

The primary efficacy objective was to determine, by molecular technique, whether scFOS-enriched infant formula was associated with increased concentration of faecal bifidobacteria 2 and 3 mo after birth in comparison to a control infant formula.

The secondary efficacy objective was to evaluate whether of scFOS-enriched infant formula was associated with higher specific immune response towards antipoliovirus after vaccination. The safety objective was to compare digestive tolerance between the 2 infant formulas.

Faecal samples. Stool samples were collected by parents at inclusion (M0), and by the age of 2 mo (M2), 3 mo (M3) and 4 mo (M4) and immediately stored at −20°C before frozen transport to the laboratory (Advance Analytical Technologies, Milan, Italy). Prior to the study initiation, parents were instructed on the detailed method for stool sampling. When stool collection was programmed, parents used special cotton-made diapers in order to avoid interactions between synthetic diapers and biological parameters. All analyses were performed at the same time at the end of the study.

Analysis of faecal bifidobacteria. Faecal total bifidobacteria counts at M0, M2 and M3 were measured using quantitative Real-Time Polymerase Chain Reaction (RT PCR) (*18–21*). Briefly, we used 1 mL aliquots of faecal samples to extract total DNA of bacterial populations by means of a commercial kit (QIAmp DNA Stool Mini Kit; Qiagene, Hilden, Germany). Quantification of total bifidobacteria was performed with the LightCycler system® (Roche Applied Science, Mannheim, Germany), using genus-specific primers (*22*). All Polymerase Chain Reactions (PCRs) were performed in triplicate in a final volume of 20 μL in LightCycler® Capillaries. An analytical kit was used for total bifidobacteria determination (QuantiTect SYBR Green PCR Kit; Qiagene). A reaction mixture for the optimized SYBR Green I based assay was prepared following the kit manufacturer's instructions, with the correction applied to $MgCl_2$ concentration, adjusted to 3.3 mM $MgCl_2$. The thermal cycling conditions used were an initial DNA denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 15 s, primer annealing at an optimal temperature of 58°C for 20 s, extension at 72°C for 30 s, and an additional incubation step at 85°C for 30 s to measure the SYBR Green I fluorescence. Finally, melt curve analysis was performed by slowly cooling the PCRs from 95 to 60°C with simultaneous measurement of the SYBR Green I signal intensity. *Bifidobacterium infantis* 15697D DNA purchased from the American Type Culture Collection (ATCC) was used for optimizing real-time PCR and generating quantification standards.

Analysis of antipoliovirus-specific IgA. Antipoliovirus-specific IgA by the age of 4 mo (M4, 1 mo after second injection of the vaccine) was measured through Enzyme-Linked Immunosorbent Assay (ELISA) (*23*). Faecal samples were initially diluted 1 : 1 with PBS and centrifuged at 20,000 $\times g$ 4°C for 30 min. Clear supernatant was supplemented with protease inhibitors to avoid further IgA destruction. A mixture of 3 inactivated poliovirus types 1, 2, and 3 (Pasteur-Merieux, Swiftwater, PA) was used as a capturing antigen (30 μg/mL per serotype in 0.05 mol/L carbonate buffer, pH 9.6, in a total well volume of 50 μL). After 16 h wells were extensively washed and we added each sample diluted in PBS-albumin 0.1%. Each sample was run in triplicate with dilutions ranging from 1 : 1 to 1 : 100 to obtain more accurate readings. After 2 h incubation plates were extensively washed and then we added 50 μL of a solution of goat-anti human IgA-HRP labelled (Biodesign, Saco, ME) in PBS-albumin 0.1% Tween 80 0.05%. Following 2 h of incubation plates were extensively washed again and incubated with the HRP substrate. After 15 min the reaction was stopped by the addition of an isovolume of 0.1 M HCl. To quantify the amount of IgA present in each sample, reference curves were obtained by adsorbing in the microplate wells known amounts of purified human IgA.

Evaluation of digestive tolerance and somatic growth. The PDA was used daily by the parents throughout the study to collect data on product consumption, digestive tolerance and adverse events. Digestive tolerance was assessed using incidence of the following digestive symptoms between M0 and M4: abdominal pain evaluated by infant crying without any apparent reason, diarrhoea (number of liquid stools defined in a scale: hard, formed, soft, liquid) and vomiting. Nutrition adequacy of the tested formula was assessed using changes in body weight and length at M3 and M4. Anthropometric parameters were measured at inclusion and at each follow-up visit. Weight of the naked infants was measured to the nearest 10 g using a standard beam balance. Recumbent length was measured to the nearest 0.5 cm using a measuring board fitted with a head and footboard. Prior to the study, investigators were instructed on how to perform standardized measurements.

Statistical analyses. The sample size calculation for this study was based on the previously reported effects of prebiotic-enriched infant formula on bifidobacteria (*10*). With a 2-sided 0.05 significance level (α=0.05), and bifidobacteria after product consumption as the primary outcome measure, studying 11 infants in each group makes it possible to detect a mean difference of 50% in total bifidobacteria at 80% power. However, it was decided to target a total per protocol population of 30 infants in order to avoid a hypothesis on data normality. Since previous studies in this area exhibited high levels of drop-outs (30 to 40%) (*24*, *25*), we aimed at including at least 21 infants in each group.

Statistical analyses were all conducted using the SAS statistical program, version 8.2 (SAS Institute). Efficacy data (effects on faecal bifidobacteria and specific immune response) were analyzed on an Intention to Treat (ITT) and Per Protocol (PP) basis, and safety data (digestive tolerance and somatic growth) were analyzed on an intention-to-treat basis. Categorical data were compared by χ^2 or Fisher exact tests. For continuous variables, normality was assessed using the Shapiro-Wilk test; variables were compared using *t* tests for parametric data and the Wilcoxon test for nonparametric data. Student's *t* tests were performed using the Satterthwaite procedure if variance was not homogenous. All analyses were 2-sided, with a level of $p \leq 0.05$ being considered significant.

Table 2. Baseline characteristics in the intention-to-treat group[1].

	Control group (n=31)	scFOS group (n=27)
Gender (n male/n female)	14/13	15/16
Birth weight (kg)	3.31±0.46	3.62±0.47[a]
Birth length (cm)	49.3±2.2	49.6±1.9
Age at enrolment (d)	4.2±0.7	4.0±0.8
Weight at enrolment (kg)	3.19±0.45	3.48±0.43
Length at enrolment (cm)	49.4±2.1	49.7±2.0

[1] Mean±standard deviations.
[a] $p<0.05$ versus control group (Student's *t* test).

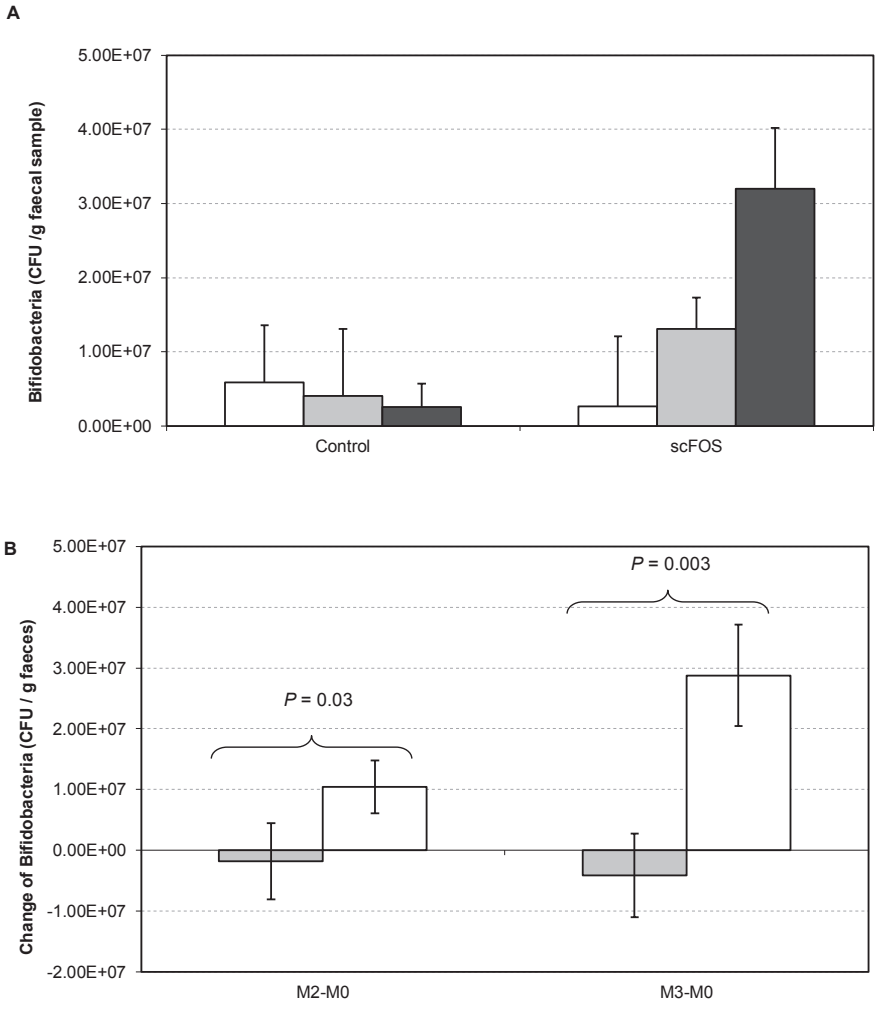

Fig. 2. Absolute numbers of bifidobacteria (A) and change of bifidobacteria (B) from inclusion (M0) to month 2 (M2) and from inclusion to month 3 (M3) in the per protocol sc-scFOS group (white bars) and the per protocol control group (grey bars). Data are presented as means±SD. The *p* values represent the significance level of the difference in the change from M0 to M2 and from M0 to M3 between the 2 groups.

RESULTS

Study population

Sixty-one infants were included in the study (Fig. 1). Mean age at enrolment was 4.1±0.8 d. Baseline characteristics of all infants in the ITT analysis are shown in Table 2. These characteristics did not differ between groups except for birth weight, which was slightly higher in the scFOS group (p=0.04). Infants who presented at least one of the following deviations to the protocol were not included in the PP analysis: no visit at M2 or no complete data for efficacy analysis, antibiotic treatment or other medical treatment likely to modify colonic microflora, consumption of weaning food, consumption of another infant formula at least once during the 15 d before a visit, and consumption of the tested formula starting more than 24 h before the first stool collection. Overall, 49 infants were included in the ITT analysis (25 scFOS group, 24 Control group) and 33 (18 scFOS group, 15 Control group) were included in the PP analysis. Mean duration of formula consumption throughout the study did not differ between groups (scFOS: 86.71±42.18 d; Control: 100.62±36.59; p=0.37).

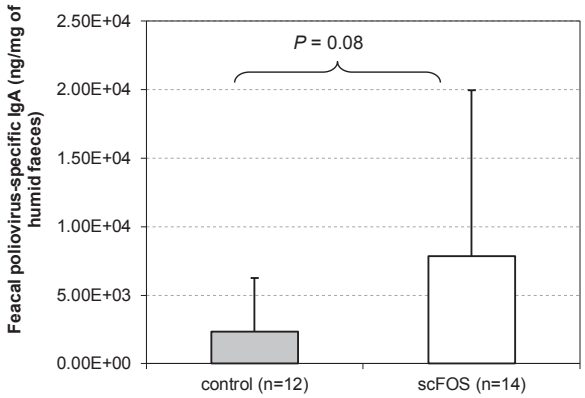

Fig. 3. Faecal levels of antipoliovirus IgA in the per protocol scFOS group (white bar) and the per protocol control group (grey bar) at M4. Data are presented as means±SD.

Table 3. Frequency of digestive symptoms in the intention-to-treat group during the 4-mo supplementation period.[1]

	Control group (n=26)	scFOS group (n=25)
Abdominal pain (with crying)	0.22±0.28	0.23±0.23
Diarrhoea (with liquid stools)	0.10±0.16	0.18±0.24
Nausea	0.05±0.12	0.03±0.07

[1] Mean±standard deviations (number of days with each symptom/number of days with formula consumption). No significant difference throughout the study between the two groups (Wilcoxon tests).

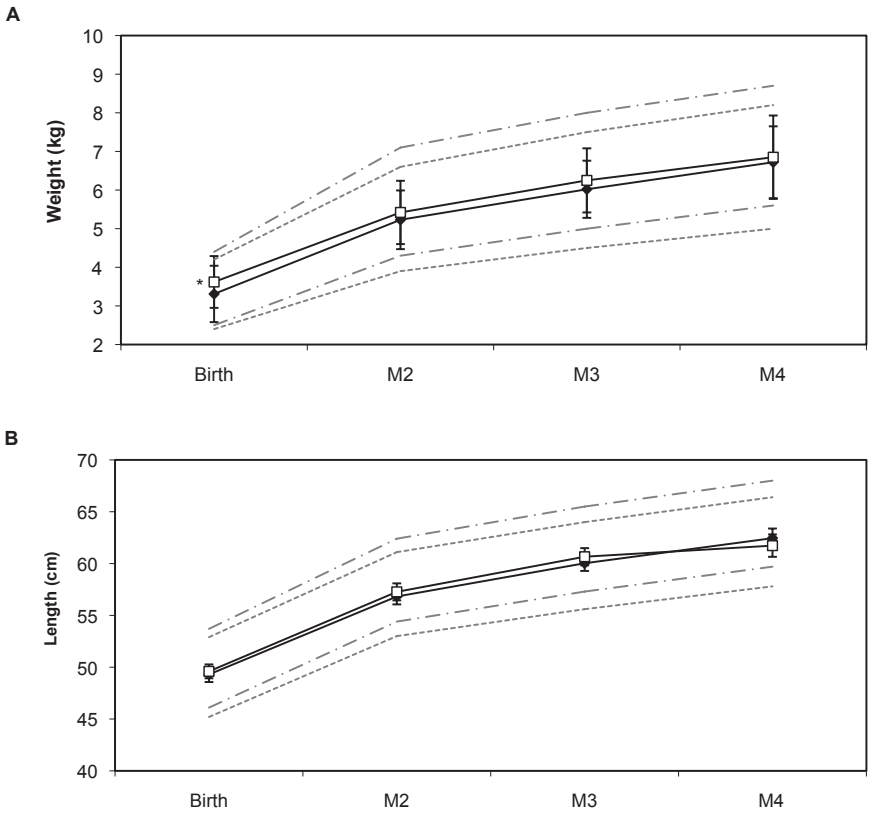

Fig. 4. Body weight and body length of infants in the ITT scFOS group (white squares) and in the ITT control group (black squares) from birth to 4 mo of age versus the WHO standards. Data are presented as means±SD. The WHO standards for weight and length are presented as ±2SD for girls (---------) and boys (— · — · —)

Moreover no differences were found regarding mean daily intake of formula between M0 and M2 (scFOS: 575±156 mL; PF: 618±104 mL; $p=0.28$), between M2 and M3 (scFOS: 715±164 mL; PF: 694±151 mL; $p=0.93$) or between M3 and M4 (scFOS: 728±180 mL; PF: 746±166 mL; $p=0.75$).

Faecal bifidobacteria and specific immune response

The mean absolute number of faecal bifidobacteria (expressed as colony forming unit (CFU)/g dry matter after log transformation) increased in the scFOS group and decreased in the PF group throughout the study. As a result, a significant difference was observed regarding changes in bifidobacteria counts between M0 and M2 and between M0 and M3 for the PP population, the scFOS group being higher than the control group ($p=0.03$) (Fig. 2). In the intention-to-treat analysis, changes in bifidobacteria counts between M0 and M2 were not different between groups but a trend was observed (scFOS: $8.08\times10^6\pm4.07\times10^7$ CFU/g faeces; control: $-149\times10^5\pm7.07\times10^7$ CFU/g faeces; $p=0.08$). Changes between M0 and M3 were highly significant (scFOS: $2.30\times10^7\pm7.73\times10^7$ CFU/g faeces; control: $-2.51\times10^5\pm9.36\times10^7$ CFU/g faeces; $p=0.008$).

Faecal levels of poliovirus-specific IgA were not statistically different between the two groups at M4 (Fig. 3). However, a trend was observed towards higher levels in the prebiotic group both in PP analysis (scFOS: 7,852±12,107 vs control: 2,334±3,896 ng of antipoliovirus IgA per mg of humid faeces; $p=0.08$) and in ITT analysis (scFOS: 7,685±11,147 vs control: 3,650±7,022 ng of antipoliovirus IgA per mg of humid faeces; $p=0.06$).

Digestive tolerance and somatic growth

Digestive symptoms showed similar frequencies in both groups, whatever the symptom considered (Table 3). Only one infant, belonging to the scFOS group, reported a serious adverse event during the study; however, this event was not related to the tested formula (hospital stay due to bronchitis). For the 4 mo of product consumption, body weight and body length (Fig. 4) remained similar between groups and close to the median range of the WHO standards for children's growth (*26*).

DISCUSSION

Bifidogenic effect

In this study it was decided to evaluate the effect of scFOS on the faecal concentration of bifidobacteria in infants from birth to 4 mo of age because breast-fed infants generally have higher counts of bifidobacteria than formula-fed infants (*3*) and because in previous studies in adults, scFOS have shown a specific effect on bifidobacteria while not influencing total anaerobes or lactobacilli populations (*13*). Absolute bifidobacteria counts were lower in our study than in previous trials in infants (about 10^7 versus 10^9), which may be related to the storage duration of faecal samples. Since we decided to carry out molecular analyses at the end of the study, stool samples had indeed been kept frozen for several months, which may have resulted in partial DNA degradation (*27*, *28*). However, we can suppose that storage conditions had a similar impact in both groups as all the samples were treated identically, and thus did not introduce a bias in comparative analysis of the data. Other parameters like the geographical area where the study was conducted might have influenced the level of bifidobacteria without confounding effect of feeding or mode of delivery as revealed by a European study (*3*). Moreover, the effects of scFOS to stimulate the growth of Bifidobacteria and increased faecal concentrations have already been observed with initial levels varying from 10^5 to around 10^{10} CFU/g (*29*).

Quantitative RT-PCR has been previously used in infants receiving a prebiotic infant formula to detect faecal Lactobacillus and bifidobacterium species (*18*, *19*). We found that mean bifidobacteria counts increased in the scFOS group between M0 and M3 compared to controls, with daily scFOS doses ranging from 2 g at the start of the study to 4 g at the end of the study. Previous studies using other type of prebiotic compounds like GOS or high-molecular weight FOS found similar results but at a higher daily intake level than the one tested here. In 35 infants aged 4 to 6 mo, Scholtens et al. found that a mixture of low-molecular-weight GOS and high molecular-weight FOS (scGOS/lcFOS) with a ratio of 9 : 1 increased faecal bifidobacteria after 6 wk of intervention at 4.5 g/d (*25*). The same formula was shown bifidogenic in preterm infants at 10 g/L (*30*) and in healthy term infants at 8 g/L (*24*), with a dose-dependent bifidogenic effect from 4 to 8 g/L (*10*). Contradictorily, Costalos et al. found that this GOS/FOS formula had no bifidogenic effect in 140 infants, but the authors noticed a relative decrease in the percentages of faecal clostridia and *Escherichia coli* in the prebiotic-supplemented group (*31*). Using FOS alone in 72 healthy term infants 2 to 6 wk of age, Euler et al. found that limited FOS intake (1.5 g/L) increased mean counts of bifidobacteria after 1 wk of consumption compared to controls and also to the same formula at 3 g/L FOS (*32*).

Regarding colonic environment, previous studies demonstrated that prebiotic-enriched formula can decrease stool mean pH, increase proportion of acetate and decrease proportion of propionate (*33*). Those modulations tended to modify the fermentation profile towards that observed in breast-fed infants. Further studies are required to investigate the effects of scFOS-supplemented formula on the metabolic activity of the total intestinal flora. In this study, we observed a high percentage of dropouts (25 subjects, 40%), the main reasons being that many parents found the stool collections and the electronic questionnaire completion to be too time consuming. Other minor reasons were antibiotic treatment (scFOS: 1 infant; PF: 0 infant), insufficient satiety with the tested formula (scFOS: 1 infant; control: 0 infant), and digestive intolerance (scFOS: 0 infant; control: 4 infants). Previous studies in infants also found a high percentage of dropouts, even with shorter study duration and/or limited monitoring constraints: for instance in two randomized controlled trials

lasting between 1.5 and 3 mo, one third of the infants dropped out (*24*, *25*). In this study, the dropout rate and reasons were similar in the two groups (scFOS: 38% vs control: 43%). The number of subjects included in the final per protocol analysis remained high enough to detect a 50% difference in bifidobacteria change at M2 between groups.

Immune effects

The increase of bifidobacteria in the FOS group was associated with a trend toward a relative increase in specific immune response. Those results are consistent with previous findings. In healthy term infants, Bakker-Zierikee et al. showed that a scGOS/lcFOS-supplemented formula at 6 g/L increased the quantity of faecal secretory IgA by age 16 wk (*34*). In another trial testing a fermented infant formula in infants from birth to 4 mo, antipoliovirus IgA titres increased after vaccination in the intervention group compared to the control group (*35*). It is now admitted that changes in colonic microflora during the first months of life influence the development and expression of the gut mucosal immune system, both at short and long terms (*36*). We cannot surely conclude that the immunomodulatory effects of scFOS-supplemented formula was uniquely mediated via the direct contact of bifidobacteria (*37*) or their bacterial products (*38*) with intestinal immune cells as other bacterial strains may also have interfered. Furthermore it would have been interesting to also analyse the concentrations of faecal bifidobacteria also after 4 mo. At least, the increase of faecal bifidobacteria observed until 3 mo of age confirms that the effect of scFOS on immune response was likely linked to modification of the intestinal microbiota. Experimental studies in animal models demonstrated that dietary scFOS increases the intestinal IgA response in the small intestine as well as in the colon (*39*, *40*). More particularly it was shown that scFOS increased the relative expression of polymeric Ig receptor at the ileum and colonic level in infant mice. This latter plays a critical role in transporting intestinal IgA onto the mucosal surface and thus can contribute to the higher level of sIgA observed in digestive contents during scFOS dietary supplementation (*40*). Increased vaccine-specific secretory IgA is considered a surrogate marker of well functioning of the mucosal immune system that reduces the need for systemic immune response that is primarily proinflammatory (*17*, *41*). Clinical implications of immunomodulatory effects of prebiotic-supplemented infant formula are still under investigation, but recent results suggest a protective effect against infections and atopy (*7*, *8*). Two studies demonstrated that dietary supplementation with scFOS can reduce the duration of diarrhoea episodes in children (*15*, *16*). Using a scGOS/lcFOS formula at 8 g/L in 259 infants at risk for atopy, Moro et al. indeed found that the incidence of atopic dermatitis during the first 6 mo of life was lower in the intervention group (*42*). The same formula was proved efficient in reducing infections and recurring infections during the first 6 mo of life (*43*). Another study found that a synbiotic (4 probiotic strains and galacto-oligosaccharides) significantly prevented eczema and especially atopic eczema in children by age 2 y, with a concomitant increase in lactobacilli and bifidobacteria (*44*). An increase in bifidobacteria was also associated with a reduced atopic sensitization at 12 mo of age in infants with a high risk for atopic disease (*45*).

Digestive tolerance

In our study, digestive tolerance was similar between groups. Among the 45 infants with data on digestive tolerance, 9 infants reported digestive symptoms, and 36 infants had no symptom. For exploratory purposes, we aimed at analysing possible differences in bifidobacteria counts between infants with or without digestive symptoms. Those two groups were not different regarding absolute bifidobacteria counts at M0 ($p=0.67$) or change in bifidobacteria counts between M0 and M2 ($p=0.17$). In a previous study, a mixture of scGOS/lcFOS at 8 g/L was also well tolerated and improved stool consistency, with a dose-dependant effect (*10*). The same formula was well tolerated at 4 g/L in healthy term infants (*31*) and at 10 g/L in preterm infants (*30*). In the study by Costalos et al., it increased stool frequency and modified stool consistency towards softer stools (*31*).

CONCLUSION

This study demonstrates that a milk-based infant formula supplemented with scFOS at 4 g/L will increase the faecal content of bifidobacteria in healthy term infants in comparison to a placebo formula without inducing any problem of digestive tolerance. A further study with comparison to breast-fed infants could be interesting to check that effects of scFOS are close to the ones of mother's milk on the modulation of intestinal microbiota. And as already done in children from 1 to 4 y of age (*15*, *16*), other studies in infants should investigate other types of bacterial groups and associated clinical implications of the modifications induced by scFOS at the intestinal level.

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Conflict of interest

The co-authors Damien Paineau, Vincent Menet, Roland Sauvage and Francis Bornet declare that they have no conflict of interests. Co-authors Frédérique Respondek and Anne Wagner are employed by Tereos-Syral, company belonging to the Tereos Group, which includes Beghin-Meiji. There are no further patents, products in development or marketed products to declare.

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