

Title page

Four-week short chain fructo-oligosaccharides ingestion leads to an increase in fecal bifidobacteria and cholesterol excretion in healthy elderly volunteers

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Abstract

Background: Short-chain fructo-oligosaccharides (scFOS) are increasingly used in human diet for their prebiotic properties. We aimed at investigating the effects of scFOS ingestion on the colonic microflora and oro-fecal transit time in elderly healthy human.

Methods: Stools composition, oro-fecal transit time and clinical tolerance were evaluated in 12 healthy volunteers, aged 69 ± 2 yrs, in three consecutive periods: basal period (2 weeks), scFOS (Actilight®) ingestion period (8 g/d for 4 weeks) and follow-up period (4 weeks). Two-way ANOVA, with time and treatment as factors, was used to compare the main outcome measures between the three periods.

Results: Fecal bifidobacteria counts were significantly increased during the scFOS period (9.17 ± 0.17 log cfu/g vs 8.52 ± 0.26 log cfu/g during the basal period) and returned to their initial values at the end of follow-up (8.37 ± 0.21 log cfu/g; $P < 0.05$). Fecal cholesterol concentration increased during the scFOS period (8.18 ± 2.37 mg/g dry matter vs 2.81 ± 0.94 mg/g dry matter during the basal period) and returned to the baseline value at the end of follow-up (2.87 ± 0.44 mg/g dry matter; $P < 0.05$). Fecal pH tended to decrease during scFOS ingestion and follow-up periods compared to the basal period ($P = 0.06$). Fecal biliary acids, stool weight, percentage of water, and oro-fecal transit time did not change throughout the study. Excess flatulence and bloating were significantly more frequent during scFOS ingestion when compared to basal period ($P < 0.05$), but intensity of these symptoms was very mild.

Conclusions: Four-week 8 g/d scFOS ingestion is well tolerated and leads to a significant increase in fecal bifidobacteria in healthy elderly subjects. Whether the

change in cholesterol metabolism found in our study could exert a beneficial action warrants further studies.

Background

Short-chain fructo-oligosaccharides (scFOS) are a mixture of oligosaccharides consisting of glucose linked to fructose units [1]. They are poorly absorbed in the human small intestine [2], but are fermented in the colon by the resident microflora [3]. It is now well established that scFOS fulfill criteria to be considered as prebiotic, defined as a non digestible food ingredient that beneficially affects the host by stimulating selectively the growth and/or activity of one or a limited number of colonic bacteria, and thus improves host health [4]. We have shown in humans that dietary addition of scFOS at dose of 10 g/d led to an increase in fecal counts of bifidobacteria [5]; moreover, the scFOS administration dose-dependently increases fecal bifidobacteria in healthy volunteers, with an optimal and well-tolerated dose ranging from 2.5 to 10 g/d [6, 7].

Bifidobacteria are considered as beneficial to health [8], even if solid proof of such effect is not yet available [4]. *In vivo* in mice, bifidobacteria administered in association with fructo-oligosaccharides reduced the 1,2-dimethylhydrazine induced carcinogenesis [9]. In rats, *Bifidobacterium longum*, administered alone or in association with non-digestible oligosaccharides, exerts strong anti-tumor activity [10, 11]. This effect could be due to colonic acidification which inhibits bacterial degradation of primary to carcinogenic secondary bile acids [12] and/or to an increase in bifidobacteria population. Indeed, bifidobacteria *per se* could have an anti-tumorigenic activity. Bifidobacteria reduce the mutagenicity of nitrosamine, and *Bifidobacterium bifidum* administered with *Lactobacillus acidophilus* to healthy humans decreases nitroreductase activity in stools [13]. Lastly, oligosaccharide ingestion could result in an increase in colonic contents and a decrease in transit

time [14] , both factors that may affect the concentration and mucosal contact time of colonic carcinogens [15]. Thus, taking into account the intrinsic anti-tumoral properties of bifidobacteria and the effects of prebiotics on colonic pH, fecal mass and transit time, a potential benefit of scFOS ingestion could be the prevention of colonic cancer, in particular in the elderly in whom colonic cancer is at particularly risk of development [16].

Although the colonic microbiota is relatively stable throughout adult life, age-related changes in the gastrointestinal tract inevitably affect its composition [17].

Bifidobacteria are numerically important colonic species that can occur in adults [18], and the decline in bifidobacteria numbers is one of the most marked changes in the elderly gut [19]. These changes, along with a general reduction in species diversity in most bacterial groups, and changes to diet and digestive physiology such as intestinal transit time, may result in increased putrefaction in the colon and a greater susceptibility to disease. Dietary supplements containing prebiotics have been suggested to counteract these changes in ageing people.

In that context, the aim of our study was to assess in elderly healthy human the effects of four-week scFOS ingestion on the colonic microflora and the oro-fecal transit time (OFTT).

Methods

Subjects

Twelve elderly healthy volunteers, six men and six women aged 69 ± 2 years, participated in the study. None of them had any history of gastrointestinal disease. No antibiotics or laxatives had been taken during the 3 months prior to the study. No other medication was allowed during the investigation period. Subjects gave written informed consent to the protocol, which was approved by the Lariboisière - Saint-Louis Hospital Ethics Committee.

Experimental Design

The study was conducted at the Saint-Lazare Hospital, Paris, France. It was divided into three periods: basal (weeks 1-2), scFOS (weeks 3-6) and follow-up (weeks 7-10) periods. Throughout the study, volunteers consumed their usual diet. Fermented dairy products containing viable bifidobacteria and FOS (onions, asparagus, rye and Jerusalem artichoke) were not allowed, neither foods known to induce abdominal symptoms (beans, cabbage, raisin, banana and wheat bran). During scFOS period, subjects received 8 g/d scFOS in two oral doses at the end of the breakfast and dinner. This dose has been defined as a good compromise between efficacy and tolerance. We used scFOS from Actilight® (Beghin Meiji, Marckolsheim, France), which consist of 44 % 1-ketose (GF₂), 46 % nystose (GF₃) and 10 % 1^F-β-fructofuranosyl nystose (GF₄).

To measure the mean oro-fecal transit time (OFTT), subjects ingested with the morning meal 20 radio-opaque pellets of different shapes for three consecutive days. The first stool passed after rising on the fourth day was collected and its marker

content analyzed. Stools were recovered for three consecutive days before the end of weeks 2 (basal period), 6 (ingestion period) and 10 (follow-up period) [20].

Tolerance to administered scFOS was evaluated using a daily chart where the symptoms (excess flatus, borborygmi, bloating, abdominal pain) were graded from zero (no symptom) to three (severe symptom). Frequency and consistency of stools were also noted by the volunteers, and diarrhea was defined as one or more watery stool, or more than three stools per day.

Stool collection

Stools were recovered thrice, for 48 h at the end of each period (weeks 2, 6 and 10). Samples were collected in plastic containers rendered anaerobic (Anaerocult A; Merck, Darmstadt, Germany) and immediately transferred to the laboratory and analyzed for bacterial counts and pH within 2 hours. Stools were then frozen at -20 °C for further analysis.

Bacterial counts and pH

Fecal samples (1 g) were introduced in the first pre-weighed tube of the dilution series and thoroughly mixed, then further tenfold dilutions were made up to 10^{-9} in a reduced diluant (cysteinated $\frac{1}{4}$ strength Ringer diluant). 0.1 ml of each dilution was spread on plates with different selective media to outnumber several bacterial genera: total anaerobic counts (Wilkins-Chalgren agar), Bifidobacterium (Beerens' medium), Clostridium (TNS medium) and enterobacteria (McConkey agar). The tests were duplicated for the first two media. Plates of the first three media were incubated anaerobically for 5 to 7 d, and McConkey agar aerobically for 48 hours. Colony

counts were obtained and expressed as a log of the colony-forming units (CFU) per gram of fresh feces. Extemporarily, the fresh stool pH was measured by pH meter (Bioblock, Illkirch, France).

Bile acids

For the analysis of bile acids and neutral sterols, frozen stools were lyophilized and lipids were extracted with ethanol for 24 h in a Soxhlet apparatus. Lipid fractions were saponified in boiling ethanolic 2 m potassium hydroxide for 1 h. The sterols were extracted with hexane, and bile acids were deconjugated. [21] Total bile acids were measured by 3-hydroxy-steroid-dehydrogenase, according to a slight modification of the technique of Stempfel and Sidbury [22]. Prior to enzyme determination, bile acids were dissolved in 2-propanol. Free bile acids were methylated with diazomethane, silylated with Deriva-sil (Chrompack, Middelburg, The Netherlands) and assayed on a Carlo Erba (Milan, Italy) HGRC 5160 gas chromatograph equipped with a standard fused silica WCOT capillary column cross-linked with OV1701 (Spiral, Dijon, France) (length, 25 m; film thickness, 0.2 μ m; oven temperature, 240°C; flow rate of hydrogen carrier gas, 2 mL/min). Fecal sterols were silylated with bis(trimethylsilyl)tri-fluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) (Pierce, Rockford, IL, USA) and quantified using gas chromatography described above, with the following modifications: fused silica WCOT OV 101 capillary column (Spiral, Dijon, France) (length, 25 m; film thickness, 0.2 μ m; oven temperature, 220°C).

Data analysis

Fecal concentrations of bacteria were expressed as log colony forming unit (cfu)/g wet weight. The results were expressed as means \pm SEM for each period. Two-way ANOVA, with time and treatment as factors, was used to compare the bacterial concentrations and pH between the three periods. Following a significant F test ($P < 0.05$), the Newman-Keuls test was used to identify differences between individual means. Symptoms experienced with scFOS were compared to those with placebo using the Wilcoxon signed rank test.

Results

Bacterial counts and pH

Table 1 summarizes the bacterial counts and pH during basal, scFOS, and control periods. Fecal bifidobacteria counts were significantly increased during the scFOS period (9.17 ± 0.17 log cfu/g vs 8.52 ± 0.26 log cfu/g during the basal period; $P < 0.05$) and returned to their initial values during the follow-up period (8.37 ± 0.21 log cfu/g). Total anaerobe counts did not change during scFOS period compared to the basal period, but decreased in the follow-up period compared to the ingestion period ($P < 0.05$). Fecal *Clostridium* counts were significantly increased during the follow-up period compared to the basal and scFOS periods ($P < 0.05$). Fecal enterobacteria counts did not change during the three periods. Fecal pH tended to decrease during scFOS and follow-up periods compared to the basal period ($P = 0.06$).

Fecal neutral sterols and bile acids

Fecal cholesterol concentration increased during the scFOS period (8.18 ± 2.37 mg/g dry matter vs 2.81 ± 0.94 mg/g dry matter during the basal period; $P < 0.05$) and returned to the baseline value during the follow-up period (2.87 ± 0.44 mg/g dry matter (figure 1). However, no statistical differences were noted for coprostanol, cholestanol and ketones for the three periods (Table 2). Total neutral sterol concentrations and outputs did not change but tended to increase ($p = 0.08$) during the scFOS period.

Total bile acid concentrations and outputs were similar in the three periods.

Concentrations of secondary (lithocholic and deoxycholic acids) and primary bile

acids (cholic and chenodeoxycholic acids) did not change for the three periods (Table 2).

Stool weight and oro-fecal transit time

Stool wet weight, dry matter and fecal water did not change throughout the study.

Oro-fecal transit time was not significantly modified by the ingestion of scFOS compared to the basal and follow-up periods (Table 3).

Digestive tolerance

During scFOS ingestion, excess of flatus and bloating were significantly more frequent when compared to the basal period ($P < 0.05$), but intensity of these symptoms was very mild (Table 4). Borborygmi and abdominal pain were not significantly different in all periods.

Discussion

The present experiment showed that four-week scFOS ingestion at the dose of 8 g/d is well tolerated and leads to a significant increase in fecal bifidobacteria and cholesterol excretion in healthy elderly subjects. To our knowledge, this is one of the first times that the bifidogenic properties of a prebiotic compound are demonstrated in elderly subjects, confirming in this population what has been found many times in adults [6, 23-25]. Of the very few available studies dealing with functional foods in elderly subjects, one recent double-blind trial testing a synbiotic (*B. lactis* BL-01, *B. bifidum* BB-02 and an inulin-based prebiotic) also found promising results [26]. Significant increase in total bifidobacteria numbers was indeed observed in the synbiotic group compared with the placebo group.

In our study, several parameters were assessed with the aim to better understand the physiological effects of scFOS in healthy elderly, such as the transit time, stool characteristics and colonic environment. We did not find that scFOS ingestion changed the fecal weight and oro-fecal transit time in elderly. Gibson et al. have shown that prebiotics can increase stool output: they studied 8 volunteers under a controlled diet and showed that with 15 g/d fructo-oligosaccharides, stool output significantly increased from 136 to 154 g/d [24]. Two other human studies have not shown an increase in stool output [2, 27] but in none of these, the diet was controlled, which may have masked a small effect. In the study of Alles et al., subjects started with unusually high fecal weights under control diet, 272 ± 26 g/d gave 4.8-19.2g/d oligomate (52% galacto-oligosaccharides) to 12 healthy subjects and did not show any change in bowel habit. On the other hand, studies using

probiotics have demonstrated that bifidobacteria could shorten the colonic transit time in human, but not all bifidobacteria strains have the same effects [28]. This specific strain-dependant effect could explain why our prebiotic, which stimulate global endogenous bifidobacteria, had no effect.

The microbial transformation of cholesterol into coprostanol was not influenced by the scFOS ingestion in our study. Another study has observed that the biohydrogenation of sterols and fatty acids by the intestinal microflora is altered by the fermentation of oligosaccharides [29]. The production of coprostanol results from the action of intestinal anaerobic bacteria [30]. Concerning bile acid metabolism, no differences were observed during the three periods. Furthermore, the use of poorly digestible carbohydrate in rats, hamsters and pigs demonstrated that the prevention of microbial conversion of bile acids were dependant of the dose of those carbohydrates in the diet [20, 31]. This suggests that the low dose of carbohydrate, 8 g/d scFOS, used in this experiment are unable to modify microbial conversion of bile acids.

Endogenous or exogenous bile acids and dietary cholesterol are carcinogenic factors involved in colon cancer in laboratory animals [32, 33]. Various epidemiological studies suggest that those steroids could be also involved in colon cancer in man [12, 34]. Therefore, ingestion of low dose of scFOS by human, which prevented microbial conversion of cholesterol to cytotoxic molecule, coprostanol, potentially carcinogenetic could be interesting for human. In our study, consumption of scFOS at 8 g/d led to an increase in fecal cholesterol with no effects on cholesterol bacterial

metabolisms. The low dose of scFOS used in our study was probably not sufficient to reduce microbial conversion of bile acids. However, in our previous study evaluating a higher dose of scFOS (12.5 g/d), we also failed to show any significant effect in bile acids and neutral sterol [25]. These negative results could be explained by a questionable capacity of various bifidobacteria to take up cholesterol into their cellular membrane [35].

Conclusion

Overall, we showed that scFOS ingested at 8 g/d are well tolerated and led to a significant increase in fecal bifidobacteria in healthy elderly subjects. Whether the change in cholesterol metabolism found in our study could exert a protective action against colonic cancer warrants further studies.

List of abbreviations used

Abbreviations: OFTT: Oro-fecal transit time; scFOS: Short-chain fructo-oligosaccharides

Competing interests

The authors have no competing interests.

Author's contribution

Yoram Bouhnik participated in the study design, the data collection, the data analysis and the writing of the manuscript. Lotfi Achour participated in the data collection.

Michel Riottot carried out bile acids and neutral sterols analysis. Damien Paineau participated in the data analysis and the writing of the manuscript. Francis Bornet conceived of the study and participated in the writing of the manuscript. All authors read and approved the final manuscript.

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Figure legends

Figure 1. Effect of 4-wk scFOS ingestion (8 g/d) on fecal cholesterol in healthy volunteers (means \pm SEM, n= 12)

Tables

Table 1: Fecal bacterial counts (log cfu/g wet weight) and pH in elderly healthy volunteers during basal (2 wks), scFOS (4 wks) and follow-up (4 wks) periods (n=12, mean \pm SEM)

	Basal period	scFOS period	Follow-up period
Bifidobacteria	8.52 \pm 0.26	9.17 \pm 0.17 ^a	8.37 \pm 0.21
Total anaerobes	10.09 \pm 0.07	10.22 \pm 0.06 ^b	9.94 \pm 0.09
<i>Clostridium</i>	3.25 \pm 0.25	3.45 \pm 0.26 ^b	4.29 \pm 0.30 ^c
<i>Enterobacteria</i>	7.69 \pm 0.21	7.45 \pm 0.28	7.48 \pm 0.24
pH	6.57 \pm 0.10	6.32 \pm 0.10	6.26 \pm 0.07

^a different from basal and follow-up periods (P<0.05)

^b different from follow-up period (P<0.05)

^c different from basal period (P<0.05)

Table 2: Fecal neutral sterols and bile acids (mg/g dry matter) in elderly healthy volunteers during the basal (2 wks), scFOS (4 wks) and follow-up (4 wks) periods (n=12, mean \pm SEM)

Neutral sterols	Basal period	scFOS period	Follow-up period
Coprostanol	9.00 \pm 1.40	8.29 \pm 2.09	7.99 \pm 1.62
Cholestanol	0.29 \pm 0.11	0.13 \pm 0.05	0.23 \pm 0.17
Cholesterol	2.81 \pm 0.94 ^a	8.18 \pm 2.37 ^b	2.87 \pm 0.44 ^a
Ketones	0.27 \pm 0.05	0.16 \pm 0.08	0.21 \pm 0.06
Total	12.38 \pm 1.15	16.75 \pm 1.94	11.30 \pm 1.57
Bile acids	Basal period	scFOS period	Follow-up period
Lithocholic	2.00 \pm 0.43	1.29 \pm 0.29	1.26 \pm 0.17
Deoxycholic	1.80 \pm 0.35	2.58 \pm 0.50	2.61 \pm 0.63
Cholic	0.46 \pm 0.21	0.58 \pm 0.19	0.87 \pm 0.24
Chenodeoxycholic	0.24 \pm 0.05	0.20 \pm 0.04	0.30 \pm 0.07
Ketones	1.26 \pm 0.30	1.52 \pm 0.68	1.33 \pm 0.19

Total

5.77 ± 0.66

6.17 ± 1.25

6.37 ± 1.02

a ≠ b : P<0.05

Table 3. Mean oro-fecal transit time (OFTT) and mean 24-h fecal wet weight, dry weight and percentage of fecal water in elderly healthy volunteers during the basal (2 wks), scFOS (4 wks) and follow-up (4 wks) periods (n=12, mean \pm SEM)

	Basal period	scFOS period	Follow-up period
OFTT (h)	37.2 \pm 3.4	39.9 \pm 3.3	37.8 \pm 3.7
Wet weight (g/d)	155.4 \pm 20.9	137.7 \pm 17.3	174.8 \pm 22.0
Dry weight (g/d)	32.8 \pm 3.3	28.8 \pm 2.9	35.2 \pm 3.5
Fecal water (%)	77 \pm 2	77 \pm 1	76 \pm 2

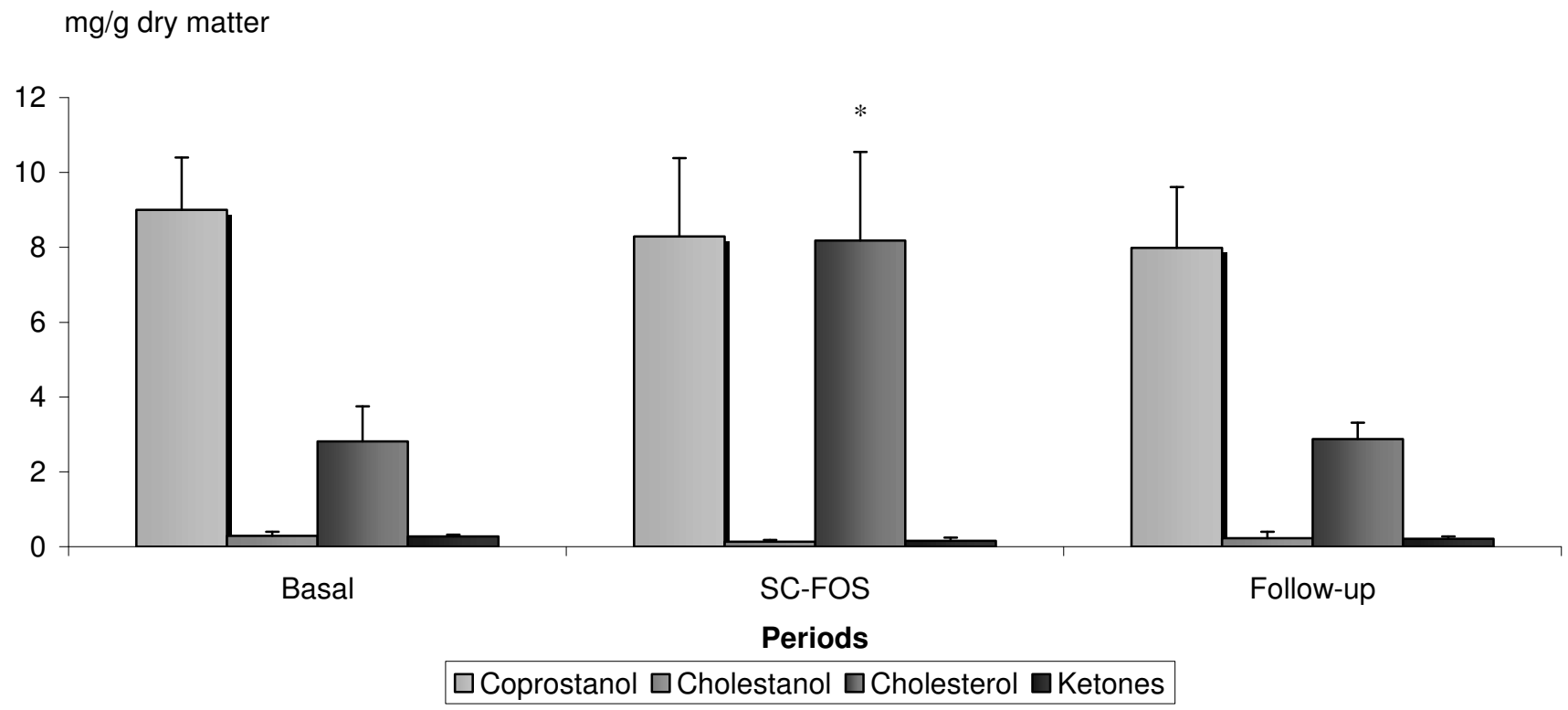
Table 4. Digestive symptom intensity (ranged from 0 to 3) in elderly healthy volunteers during the basal (2 wks), scFOS (4 wks) and follow-up (4 wks) periods (n=12, mean \pm SEM)

	Basal period	scFOS period	Follow-up period
Excess flatus	0	0,83 \pm 0,3 ^a	0,25 \pm 0,13
Bloating	0	0,67 \pm 0,26 ^a	0,33 \pm 0,14
Borborygmi	0	0	0
Abdominal pain	0	0,42 \pm 0,23	0,25 \pm 0,13

Symptoms intensity was noted as follow: 0: no symptom ; 1 : mild symptoms; 2 : moderate symptoms; 3: severe symptoms

^a different from basal period (P<0.05)

Figure 1



*P < 0.05 between scFOS period and both basal and follow-up period