

ORIGINAL ARTICLE

A double-blind placebo-controlled study to establish the bifidogenic dose of inulin in healthy humans

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Objective: To evaluate the bifidogenic efficacy of two inulin doses in healthy human adults.

Design: A double-blind, placebo-controlled, crossover human study.

Setting: Food Microbial Sciences Unit, The University of Reading, Reading, UK.

Subjects: Thirty healthy volunteers, 15 men, 15 women (age range 19–35).

Interventions: Subjects consumed a chocolate drink containing placebo (maltodextrin, 8 g/day), 5 g/day inulin and 8 g/day inulin for a 2-week treatment period. Each treatment was followed by a 1-week washout at the end of which volunteers progressed to the next treatment. Faecal samples were obtained at the start of the study (baseline) and at the end of each treatment and washout period. Fluorescent *in situ* hybridization was used to monitor populations of *Bifidobacterium* genus, *Bacteroides* – *Prevotella*, *Lactobacillus* – *Enterococcus* and *Clostridium perfringens* – *histolyticum* subgroup.

Results: Bifidobacterial levels increased significantly upon ingestion of both the low ($9.78 \pm 0.29 \log_{10}$ cells/g faeces, $P < 0.05$) and the high inulin dose ($9.79 \pm 0.38 \log_{10}$ cells/g faeces, $P = 0.05$) compared to placebo ($9.64 \pm 0.23 \log_{10}$ cells/g faeces).

Conclusions: Both inulin doses exhibited a bifidogenic effect but a higher volunteer percentage responded to the high dose. A dose response effect was not observed but the magnitude of increase in bifidobacteria levels depended on their initial numbers. The higher the initial concentrations the smaller was the increase upon ingestion of the active treatments.

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Introduction

Fructooligosaccharides (FOS) and inulin are polymers of D-fructose joined by $\beta(2-1)$ bonds with an $\alpha(1-2)$ linked D-glucose at the terminal end of the molecule. Molecules with a degree of polymerization (DP) between 3 and 10 are referred to as oligofructose or FOS and those with a DP between 3 and 65 are known as inulin. Inulin occurs naturally in a range of plants such as chicory, onion, garlic, Jerusalem artichoke, tomato and banana and, as such, it is a part of everyday human diet. According to the US Department of Agriculture 1994–1995 continuing survey of food intakes by individuals, the average daily intake of naturally

occurring inulin and oligofructose was 2.6 and 2.5 g, respectively (Moshfegh *et al.*, 1999). European populations are estimated to consume between 2 and 10 g of inulin per day (Van Loo *et al.*, 1995).

Inulin is not hydrolyzed by digestive enzymes in the upper gastrointestinal tract and reaches the colon intact, where it is then selectively fermented by bifidobacteria. Bifidobacteria have long been regarded among the beneficial members of the human gut microflora. The bifidobacterial dominated gut microbiota of breast fed infants has been associated with improved health benefits (Campbell and Jones, 1996; Vanderhoof and Young, 1998). High numbers of bifidobacteria are also perceived as beneficial for adult health. Bifidobacteria have been shown to inhibit growth of pathogenic bacteria, modulate the immune system, produce digestive enzymes, repress the activities of rotaviruses and restore microbial integrity of the gut microbiota following antibiotic therapy (Bernet *et al.* 1993; Gibson and Wang, 1994; Saavedra *et al.* 1994; Collins and Gibson, 1999; McCracken and Gaskins, 1999).

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The health benefits of bifidobacteria have resulted in research focusing upon the promotion of their growth and activity in the colon. Previous human feeding studies have demonstrated the bifidogenic nature of both inulin and oligofructose. A variety of doses have been thus far reported effective in increasing faecal bifidobacteria *in vivo*, ranging from 4 to 40 g/day (Williams *et al.*, 1994; Gibson *et al.*, 1995; Buddington *et al.*, 1996; Kleessen *et al.*, 1997; Bouhnik *et al.*, 1999; Kruse *et al.*, 1999; Den Hond *et al.*, 2000; Tuohy *et al.*, 2001a; Bouhnik *et al.*, 2004). Although microbial culture techniques were used in the majority of studies, results reported thus far on the prebiotic efficacy of inulin and oligofructose are in agreement with studies employing molecular methodologies of monitoring the prebiotic effect on the bacterial microflora (Tuohy *et al.*, 2001a, b).

It is evident from the above that inulin and oligofructose exhibit bifidogenic efficacy at various daily intakes in healthy humans. Although the safety of inulin ingestion cannot be disputed, it is essential that its efficacy at low daily doses be defined. Results obtained thus far from a multitude of different studies cannot be directly compared as different methodologies, population groups as well as different types of inulin have been used. High daily doses of inulin could result in manifestation of adverse effects such as increased flatulence, through non-selective fermentation by the gut microflora. It is important to establish whether an increase in the daily intake of inulin correlates with an increased bifidogenic effect.

The aim of this study was to establish the minimum daily dose of inulin required to stimulate the numbers of faecal bifidobacteria in healthy humans, without the manifestation of gastrointestinal side effects.

Methods

Subjects

Thirty healthy human volunteers (15 male, 15 female, average age 26.5 ± 3.1 years) participated. Written consent was obtained from all participants and the study protocol was approved by the Ethics and Research Committee of the University of Reading.

Pre-trial assessment

Volunteers were assessed for good health and selected on basis of adherence to exclusion and inclusion criteria.

Eligibility criteria

Inclusion criteria for the participation in the study were: signed consent form, age 18–50 years inclusive, body mass index 20–30 inclusive, good general health and absence of gastrointestinal disorders (chronic constipation, diarrhoea, inflammatory bowel disease, irritable bowel syndrome or

other chronic gastrointestinal complaints) as determined through a medical questionnaire.

Volunteers with a history of physical/mental disease, major surgery, severe allergy, abnormal drug reaction and drug/alcohol abuse were excluded. Volunteers were also excluded if pregnant, lactating or planning pregnancy. Volunteers taking pro/pre/synbiotics, drugs active on intestinal motility, or laxatives of any class, within 4 weeks before the study or antibiotics within 6 months before the study were also excluded.

Requirements for diet and medication during the study

Subjects were instructed not to consume any additional prebiotics, probiotics or synbiotics. They were also asked not to take any antibiotics or drugs that could affect gastric motility. Any medication taken was recorded in diaries. Volunteers were instructed not to alter their usual diet or fluid intake during the trial period.

Treatments

The trial was conducted in a crossover design between two active (high and low inulin dose) and a placebo treatments.

Volunteers consumed each treatment for a 14-day period, which was followed by a 7-day washout. All volunteers consumed the same treatment during each experimental period.

Treatments were supplied by Sensus (Roosendaal, Netherlands) and were colour coded so as to be blind to the investigators as well as the volunteers (Table 1). Each treatment was delivered as one 29 g chocolate powder sachet per day to be dissolved in warm water. Test sachets containing chocolate powder along with inulin, average DP 9–10 (Prutafit IQ, Sensus, Roosendaal, Netherlands) or placebo (maltodextrin) were administered to the volunteers at the start of each 14-day treatment period. Volunteers were asked to drink one chocolate powder sachet per day and were free to consume it at any time during the day.

Faecal sample preparation

Freshly voided faecal samples were collected in sterile plastic pots and were processed within 10 min of collection. Samples were diluted 1 in 10 (w/w) with phosphate-buffered saline (PBS, 0.1 M; pH 7.0) and mixed in a Stomacher[®] 400 (Seward, Norfolk, UK) for 2 min at normal speed.

Bacterial enumeration

Oligonucleotide probes. Synthetic oligonucleotide probes targeting specific regions of the 16S rRNA molecule, labelled with the fluorescent dye Cy3, were utilized for the enumeration of *Bifidobacterium* genus (Bif164, Langendijk *et al.*, 1995), *C. perfringens* – *histolyticum* subgroup (His150, Franks *et al.*, 1998), *Bacteroides* – *Prevotella* (Bac303, Manz *et al.*,

Table 1 Study outline and nutritional information of the 3 treatments ingested throughout study duration

Treatment 1 (placebo)	Washout 1	Treatment 2 (5 g/day inulin)	Washout 2	Treatment 3 (8 g/day inulin)
Days 1–14 1 sachet/day Stool sample on day 1 (baseline) diary for GI symptoms	Days 15–22 No treatment Stool sample at day 22 GI symptom diary	Days 23–37 1 sachet/day Stool sample on day 37 (treatment 2) GI symptom diary	Days 38–45 No treatment Stool sample on day 45 GI symptom diary	Days 46–60 1 sachet/day Stool sample on day 60 (treatment) GI symptom diary
<i>Treatment composition and nutritional information</i> 8 g maltodextrin substituting for the inulin content of the active treatments.		5 g inulin, 3 g maltodextrin. All other ingredients identical to high active treatment		8 g inulin, sucrose, skimmed milk powder, lactose, skimmed cocoa powder (9%), vegetable fat, stabilisers (E339, xanthan gum), salt and aroma.

Each treatment was delivered as 29 g of chocolate powder/day. The sequence the treatments were administered was: placebo, 5 g/day inulin, 8 g/day inulin. Formulae nutritional value per 100 g: energy 410 kcal, protein 12 g, carbohydrates 70 g of which sugars 69 g, sodium 0.4 g, fat 8.5 g of which saturated 8.0 g and monounsaturated 0.5 g, dietary fibre 4.0 g.

1996) and *Lactobacillus – Enterococcus* (Lab158, Harmsen et al., 1999). All probes were provided by MWG-Biotech (London, UK). Total cell counts were achieved by adding the nucleic acid stain 4,6-diamidino-2-phenylindole to the hybridization mixture. Labelled cells were visualized using fluorescent microscopy. The sequences and specific hybridization conditions for each molecular probe are presented in Table 2.

Fluorescent in situ hybridization. The method was carried out as previously described by Rycroft et al. (2001) and Tuohy et al. (2001b).

Samples were fixed overnight (4°C) in 4% (w/v) paraformaldehyde. Fixed cells were centrifuged at 15000g for 5 min and washed twice in 1 ml filtered PBS. The washed cells were re-suspended in 150 µl PBS and stored in ethanol (1/1 v/v) at –20°C.

Following overnight hybridization with each probe, the fixed cells were washed and vacuum filtered (2 µm polycarbonate isopore membrane filter, Millipore UK Ltd, Watford, UK). They were then mounted onto a glass slide with 20 µl of slowfade to prevent fading of fluorescence (Molecular Probes, Leiden, The Netherlands). The fluorescent cells were enumerated using the Fluor 100 lens (Eclipse 400 epifluorescent microscope, Nikon, Kingston upon Thames, UK). Fifteen different random fields of view were counted on each slide.

Gastrointestinal symptoms and stool characteristics

Volunteers were asked to keep diaries throughout the study to record stool frequency (bowel movements per day) and consistency (constipation, hard, formed or diarrhoea) on a daily basis. Abdominal pain, stomach or intestinal bloating and flatulence were also recorded as none, mild, moderate or severe and were given a numerical score ranging from 0 for none, up to 3 for severe symptoms. Data obtained from volunteer diaries were used to determine gastrointestinal tolerance and symptoms throughout the study.

Table 2 Probe sequences and hybridization/washing temperatures

Probe name	Sequence	Hybridization/ washing temperature (°C)
Bac303	5'-CCAATGTGGGGACCTT-3'	45
Bif164	5'-CATCCGGCATTACCACCC-3'	50
Chis150	3'-TTTCCYTCTAATTATGGCGTATT-5'	50
Lab158	5'-GGTATTAGCA(T/C)CTGTTTCCA-3'	50

Bac303 is specific for *Bacteroides – Prevotella*, Bif 164 for *Bifidobacterium* genus, Chis 150 for *Clostridium perfringens histolyticum* subgroup and Lab158 for *Lactobacillus – Enterococcus*.

Statistical analysis

The primary efficacy variable was considered to be the change in bacterial numbers from baseline to the end of each treatment for each bacterial species and total bacterial count. All bacterial concentrations were expressed in log₁₀cells/g faeces.

Paired *t*-tests were used after logarithmic transformation to determine if there was a statistically significant difference between placebo and each of the two treatments. The differences between the treatments were estimated with 95% confidence intervals.

A correlation analysis was performed to determine whether there the magnitude of change in bacterial levels depended on initial bacterial concentrations. The null hypothesis was that the change in bacterial levels after treatment did not depend on initial bacterial levels. The critical value of the correlation coefficient (*r*_{critical}) for 28 degrees of freedom (df = n-2 for a two-tailed test, where *n* is the number of observations) was *r*_{critical} = 0.361 at 95% confidence intervals. Where the absolute calculated value of *r* was greater than the value of *r*_{critical} the null hypothesis was rejected the alternative hypothesis was accepted.

Results

Faecal bacterial populations present in 30 volunteers throughout the study period are presented in Table 3. Changes in total faecal bacteria numbers and the levels of four of the numerically significant and functionally important bacterial populations in human faeces, namely *Bifidobacterium*, *Bacteroides - Prevotella*, *C. perfringens - histolyticum* subgroup and *Lactobacillus - Enterococcus*, were monitored using fluorescent *in situ* hybridization (FISH) and are expressed in log₁₀ cells/g faeces. Bacterial numbers were obtained at baseline (start of trial), at the end of treatment 1 (8 g/day maltodextrin-placebo), end of washout 1, end of treatment 2 (5 g/day inulin, 3 g/day maltodextrin), end of washout 2 and at the end of treatment 3 (8 g/day inulin). Bacterial levels at the end of each treatment period were compared to their respective baseline and statistical significance of the results evaluated using the paired *t*-test at 95% confidence intervals.

At baseline, bacteroides and bifidobacteria were the numerically predominant bacterial populations in faeces (10.06 ± 0.24 log₁₀ cells/g faeces and 9.61 ± 0.31 log₁₀ cells/g faeces, respectively), whereas *C. perfringens - histolyticum* subgroup and *Lactobacilli - Enterococci* existed at lower levels (8.66 ± 0.38 log₁₀ cells/g faeces and 9.22 ± 0.22 log₁₀ cells/g faeces, respectively).

Bifidobacteria did not exhibit any statistically significant change upon ingestion of the placebo sachets (8 g/day maltodextrin) with respect to baseline levels.

A significant increase was observed in bifidobacteria at the end of the second treatment (5 g/day inulin, 3 g/day maltodextrin) with respect to placebo ($P < 0.05$), washout 1 ($P = 0.01$) and baseline ($P < 0.01$) levels. Overall, 20 of the 30 volunteers responded to inulin supplementation at 5 g/day. The average initial *Bifidobacterium* levels were generally lower (9.53 ± 0.25 log₁₀ cells/g faeces) in volunteers that responded to the treatment as compared to mean

Bifidobacterium levels in volunteers that did not respond to treatment (9.76 ± 0.30 log₁₀ cells/g faeces, $P = 0.05$). There was a negative correlation between initial levels and the magnitude of increase in bifidobacterial numbers at the end of the low inulin dose treatment ($r = -0.523$, $P = 0.010$).

Similarly, the higher inulin dose (8 g/day), also resulted in a statistically significant increase in bifidobacterial levels with respect to washout 2 ($P < 0.05$), placebo ($P = 0.05$) and baseline ($P = 0.01$) numbers. Twenty-five volunteers responded to the high inulin treatment. The average initial bifidobacterial levels in the volunteers that responded to the higher dose were lower (9.62 ± 0.32 log₁₀ cells/g faeces) than those of non-responders (9.81 ± 0.30 log₁₀ cells/g faeces), ($P = 0.05$). Seven of the volunteers that did not respond to the low-dose treatment responded to the high dose, whereas three volunteers did not respond to any of the treatments. Two of the volunteers that exhibited an increase in bifidobacteria with the low-dose treatment did not respond to the high inulin dose and only exhibited small decreases in bifidobacterial levels. Again, there was a negative correlation between initial levels and the magnitude of increase in faecal bifidobacteria ($r = -0.438$, $P < 0.05$).

C. perfringens - histolyticum subgroup exhibited no significant increase upon ingestion of the placebo treatment with respect to baseline levels. Numbers showed a significant increase at the end of washout 1 as compared to levels at the end of the placebo treatment ($P < 0.01$). Twenty-five volunteers exhibited increased *C. perfringens - histolyticum* subgroup levels. Their numbers stabilized hereafter and there was no significant change at the end of the low-dose treatment compared to washout 1 levels. There was a significant decrease in *C. perfringens - histolyticum* subgroup at the end of the high-dose treatment with respect to levels at the end of washout 2 ($P < 0.01$). Overall, they decreased in 25 out of 30 volunteers but were still significantly higher than baseline and placebo levels ($P < 0.001$).

Table 3 Mean bacterial counts obtained through FISH enumeration, expressed in log₁₀ cells/g faeces, ± s.d.

	Total cells		Bifidobacterium		Bacteroides - Prevotella		Clostridium perfringens - histolyticum subgroup		Lactobacillus - Enterococcus	
	Mean	± s.d.	Mean	± s.d.	Mean	± s.d.	Mean	± s.d.	Mean	± s.d.
Baseline	10.61	0.30	9.61	0.31	10.06	0.24	8.66	0.38	9.22	0.22
Treatment 1	10.69	0.20	9.64	0.23	10.15	0.14	8.68	0.49	9.66	0.31
Washout 1	10.70	0.21	9.61	0.29	10.24	0.14	9.36*	0.56	9.71	0.18
Treatment 2	10.62	0.19	9.78 [†]	0.29	10.15	0.17	9.51*	0.22	9.49 [†]	0.32
Washout 2	10.61	0.22	9.65	0.32	10.22	0.18	9.60*	0.19	9.09	0.21
Treatment 3	10.61	0.26	9.79 [‡]	0.38	10.08	0.24	9.34 ^{†*}	0.28	9.10	0.24

Abbreviations: FISH, fluorescent *in situ* hybridization; s.d., standard deviation.

Samples were obtained at the start of the study (baseline), end of treatment 1 (placebo), end of washout 1, end of treatment 2 (5 g/day inulin), end of washout 2 and at the end of treatment 3 (8 g/day inulin).

[†]Significant increase with respect to baseline ($P < 0.01$), placebo ($P < 0.05$) and washout levels ($P = 0.01$).

[‡]Significant increase with respect to baseline ($P = 0.01$), placebo ($P = 0.05$) and washout levels ($P < 0.05$).

*Significant increase with respect to baseline levels ($P < 0.001$).

[†]Significant decrease with respect to washout levels ($P < 0.05$).

Lactobacillus – Enterococcus increased significantly during the placebo treatment with respect to baseline levels ($P < 0.01$) in 29 volunteers, remained stable during washout 1 and did not return to baseline. Numbers at the end of washout 1 were still significantly higher than baseline ($P < 0.01$). At the end of the low inulin dose treatment, *Lactobacillus – Enterococcus* significantly decreased with respect to washout 1 ($P < 0.05$). At the end of washout 2, they returned to pretreatment levels. No significant change was observed between washout 2 and treatment 3 numbers.

Total bacteria and bacteroides numbers did not exhibit any significant variation throughout the duration of the trial.

Gastrointestinal symptoms

Results from the volunteer diaries are summarized in Table 4. The higher the score the higher was the severity of the gastrointestinal symptom.

Although there was a wide range of responses noted by the volunteers with respect to the frequency and severity of gastrointestinal symptoms the only statistically significant change observed was an increase in stool number ($P = 0.029$), intestinal bloating ($P = 0.011$) and flatulence ($P < 0.001$) upon ingestion of the low-dose inulin treatment with respect to washout levels. The only significant effect of the placebo treatment was an increase in abdominal pain ($P = 0.014$). Volunteers were also asked to record stool consistency as hard, formed or soft throughout the trial duration. Despite the wide range of responses given by different volunteers, there were no significant differences between the different treatments with the majority reporting formed stools.

Discussion

The aim of this study was to determine the bifidogenic efficacy of two doses of inulin formulated as chocolate powder drinks, in a double-blind, placebo-controlled, cross-over study of 30 healthy volunteers. The trial was structured

as three, 2-week test periods during which volunteers consumed a placebo chocolate drink, a low dose (5 g/day) and a high-dose (8 g/day) inulin chocolate drink. The first two test periods were followed by a 1-week washout to avoid the effect of one treatment being carried over to the next. FISH was used to determine populations of faecal *Bifidobacterium* genus, *Lactobacillus – Enterococcus*, *Bacteroides – Prevotella* and *C. perfringens – hystolyticum* subgroup.

A wide variation in bacterial levels as well as magnitude of response on microflora was observed between the 30 volunteers participating in the trial, upon ingestion of the three different treatments. This was expected as composition of the bacterial microflora varies greatly among different individuals both quantitatively and qualitatively (Tuohy et al., 2001b) resulting in a different response to the study treatments.

The placebo treatment had no significant effect upon any of the bacterial groups enumerated for, apart from *Lactobacillus – Enterococcus*, which increased significantly. This effect was retained during washout 1 and levels gradually decreased at the end of the low inulin treatment to reach approximately baseline numbers at the end of washout 2 and the 8 g/day treatment. As all treatments had identical formulations apart from the active ingredients, it can be assumed that the stimulatory effect on *Lactobacillus – Enterococcus* was due to the maltodextrin content of the chocolate powder (Engfer et al., 2000). The maltodextrin used in this study (DP = 50) may have not been accessible to amylase digestion due to the poor solubility of the placebo chocolate drink. The low-dose inulin treatment delivered 3 g/day maltodextrin along with 5 g/day inulin, that sustained *Lactobacillus – Enterococcus* at the end of this treatment at levels significantly higher than baseline concentrations ($P = 0.001$) despite a significant decrease with respect to washout 1.

Although the placebo treatment had no significant effect on *C. perfringens – hystolyticum* subgroup, numbers significantly increased during washout 1 and remained significantly higher than baseline levels throughout the study. Ingestion of active treatments did not suppress *C. perfringens*

Table 4 Summary of data obtained from diaries completed by 30 volunteers during the three treatment and two washout periods of the trial

	Gastrointestinal symptoms							
	Stool number		Abdominal pain		Intestinal bloating		Flatulence	
	Mean	± s.d.	Mean	± s.d.	Mean	± s.d.	Mean	± s.d.
Placebo	1.478	0.674	0.143 [†]	0.165	0.337	0.452	0.672	0.567
Washout 1	1.428	0.888	0.079	0.181	0.251	0.412	0.685	0.603
5 g/day inulin	1.508 [*]	0.633	0.175	0.295	0.418 ^{**}	0.532	0.942 [§]	0.672
Washout 2	1.379	0.567	0.121	0.288	0.159	0.344	0.544	0.558
8 g/day inulin	1.461	0.7345	0.154	0.224	0.363	0.566	0.733	0.655

^{*}Statistically significant increase in stool number ($P = 0.029$) compared to washout.

^{**}Statistically significant increase in intestinal bloating ($P = 0.011$) compared to washout.

[§]Statistically significant increase in flatulence ($P < 0.001$) during the low dose treatment.

[†]Statistically significant increase in abdominal pain ($P = 0.001$) compared to washout.

– *histolyticum* subgroup to baseline/placebo numbers. Apart from certain restrictions concerning the intake of probiotic and prebiotic containing foods, this was otherwise a free-living human study and participants were allowed to ingest their normal diets. As such, changes in the faecal microflora due to seasonal or other factors not related with the intake of the test drinks could be avoided. This may also, in part, account for the increase in *Lactobacillus* – *Enterococcus* levels observed during the placebo period.

The effect on bifidobacteria populations was clearer. Both the low and the high doses had a statistically significant stimulatory effect on the faecal bifidobacterial microbiota. Although more volunteers responded to the higher dose, the bifidogenic effect was approximately the same with both treatments and no dose–response relationship was observed. Nevertheless, a negative correlation existed between initial faecal concentrations of bifidobacteria and the magnitude of increase during both active treatments. The higher the initial numbers of bifidobacteria the smaller was the bifidogenic effect. The same effect has been observed in previous studies (Roberfroid *et al.*, 1998; Tuohy *et al.*, 2001b) and suggests that in cases where initial numbers of bifidobacteria are sufficiently high, a marked bifidogenic effect may not be observed. The majority of volunteers participating in this study had high initial levels of bifidobacteria. There may have been a more apparent dose effect if a study population with lower initial bifidobacteria levels had been investigated but the target group chosen here was the general healthy population. The fact that both inulin doses promoted bifidobacterial growth is of importance as it is essential to establish the efficacy of inulin to exert a bifidogenic effect on the gut microflora at lower doses. When excess fermentable oligosaccharides are delivered to the colon side effects such as bloating and flatulence may occur. If the fermentation capacity of saccharolytic organisms in the colon is saturated, the excess substrate will be available to organisms that may generate gas (unlike the bifidobacteria). As previously mentioned, tolerance to inulin varies among individuals (Tuohy *et al.*, 2001a). In general, both active treatments were well accepted by the human volunteers and all subjects completed the study. There was a significant increase in stool frequency, intestinal bloating and abdominal pain during the low-dose treatment that was not observed during the higher dose. This suggests that recruits may adapt to inulin or that volunteers may become accustomed to experiencing the symptoms, which become less noticeable. On the other hand, during the higher inulin dose supplementation, *C. perfringens* – *histolyticum* subgroup levels exhibited a statistically significant decrease with respect to the low dose ($P < 0.05$). However, as previously mentioned clostridia levels did not return to baseline/placebo and gastrointestinal symptoms were slightly but not significantly elevated with respect to placebo. Bifidobacteria do not produce gas during carbohydrate fermentation, whereas clostridia are prolific gas producers. This may explain why the high dose was better tolerated.

The most important attribute of a prebiotic, along with resistance to digestion in the upper gastrointestinal tract, is a stimulation of beneficial bacterial populations in the colon, with the main target being bifidobacteria. The anatomy of the human colon makes it largely inaccessible to routine sampling and, although a compromise, the use of faeces as an indication of the composition of colonic luminal content provides invaluable insight on the bacterial population changes. The fact that increased faecal bifidobacteria levels were observed shows that inulin ingestion promoted their growth in the gut.

The bifidogenic efficacy of inulin at 5 and 8 g/day formulated in a processed food product was investigated and demonstrated *in vivo* in 30 healthy human volunteers in this study. Previous studies by Tuohy *et al.* (2001b) established the bifidogenicity of prebiotic biscuits, but no attempt was made to investigate the efficacy of different prebiotic doses. Moreover, here, monitoring of bacterial populations throughout the human trial was performed using FISH, a molecular based method that accurately identifies and quantifies bacterial populations *in situ*. Although the technique was developed several years ago and has proven efficiency, the majority of studies investigating the prebiotic effect of inulin have employed bacterial culture methods to monitor bacterial changes. The lack of selectivity of such methods impedes the accurate evaluation of prebiotic efficacy. Indeed, two recent studies on the bifidogenic efficacy of long chain inulin report conflicting results. At 8 g/day long chain inulin was found to effect a significant bifidogenic effect when bacterial changes were monitored through FISH (Tuohy *et al.*, 2001a) but not at 10 g/day when bacterial culture was used (Bouhnik *et al.*, 2004). The latter result may have occurred because of the use of culture-based technologies and their lack of selectivity.

In conclusion, inulin exerted a bifidogenic effect in healthy human adults at doses of 5 and 8 g/day. In light of both doses proving effective, the higher dose may be preferable as no significant side effects were noted and a higher percentage of the study population responded to the treatment.

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