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The prebiotic effects of biscuits containing partially hydrolysed guar gum and fructo-oligosaccharides – a human volunteer study

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Prebiotics are non-digestible food ingredients that target selected groups of the human colonic microflora, thus having the ability to alter the composition towards a more 'beneficial' community, i.e. selectively increasing populations of bifidobacteria and/or lactobacilli. In the present study the prebiotic potential of partially hydrolysed guar gum (PHGG) and fructo-oligosaccharides (FOS) in a biscuit was assessed in human volunteers. Fluorescent *in situ* hybridization using oligonucleotide probes targeting *Bacteroides* spp., *Bifidobacterium* spp., *Clostridium* spp. and *Lactobacillus–Enterococcus* spp. were used for the bacteriology and total bacteria were enumerated using the fluorescent stain 4′,6-diamidino-2-phenylindole. Thirty-one volunteers consumed daily either three experimental biscuits (providing a total (g/d) of 6·6 FOS and 3·4 PHGG) or three placebo biscuits for two 21-d crossover periods. Bifidobacteria significantly increased in number on ingestion of the experimental biscuits compared with pre-treatment and placebo population levels. Bifidobacterial numbers returned to pretreatment levels within 7 d of the cessation of intake of experimental biscuits. A correlation was observed between the initial faecal bifidobacterial numbers and the magnitude of bifidogenesis, with volunteers who possessed low initial population levels of bifidobacteria experiencing the greatest increase in bifidogenesis. No changes were observed in the other bacterial groups monitored during the trial. Thus, the prebiotic nature of FOS and PHGG was maintained in a final food product as evidenced from the selective increase in bifidobacterial numbers.

Gut microflora: Prebiotic: Gene probes

The importance of the human colon in health and disease has become increasingly recognized with a greater understanding of the ecology and biological importance of the human gastrointestinal microflora. More than 500 different bacterial species are thought to make up the gut microbiota, with the total number of bacterial cells present in the colon far exceeding the total number of eukaryotic cells in the body (Conway, 1995; Gibson & Beaumont, 1996; Tannock, 1999). Although it is known that many disease states involve bacterial metabolism, the human gut microflora may also be considered as very relevant to improved host health (Gibson & Beaumont, 1996). For instance, bifidobacteria and lactobacilli are thought to improve resistance to gut infections by inhibiting the growth of harmful bacteria, to reduce cholesterol levels, to improve the immune response and to produce vitamins (Gibson, 1998; Holzapfel *et al.* 1998; Vanderhoof & Young, 1998; Ziemer & Gibson, 1998). In the present study the efficacy of biscuits containing fructo-oligosaccharides (FOS) and partially hydrolysed guar gum (PHGG) in bringing about a beneficial modulation of the gastrointestinal microflora was determined. Particularly, the ability of the prebiotic biscuits to increase numbers of bifidobacteria selectively was assessed.

The complexity of the gut microflora presents difficulties in monitoring this ecological phenomenon (Steer *et al.* 2000). Selective agars and growth conditions exist for only a small percentage of the most numerically dominant species, and it has been estimated that only between 10 and 50 % of bacteria present in the human gut have been cultivated (Langendijk *et al.* 1995; Wilson & Blitchington, 1996). The phylogenetic information encoded by 16S rRNA has enabled the development of molecular biology techniques to allow characterization of the whole human gut microflora (Collins & Gibson, 1999; Suau *et al.* 1999). Similarly, fluorescent *in situ* hybridization (FISH), employing

Abbreviations: FISH, fluorescent *in situ* hybridization; FOS, fructo-oligosaccharides; PHGG, partially hydrolysed guar gum.

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oligonucleotide probes targeting 16S rRNA, allows the enumeration of both culturable and non-culturable moieties of the gut microflora *in situ* in gastrointestinal and faecal samples (Amann *et al.* 1995; Langendijk *et al.* 1995; Zoetendal *et al.* 1998; Ames *et al.* 1999). Using FISH, changes in population levels of specific phylogenetically related groups of bacteria may be monitored in response to altering ecological conditions, such as the presence of fermentable substrate.

Manipulation of the gut microflora for improved health is not a new concept. Probiotics, which are live microbial feed additives thought to confer health advantages, have a long history (Fuller & Gibson, 1997). Whilst the probiotic approach is conceptually sound, it does have problems in practice. Many of the health-promoting attributes of selected probiotic strains may be determined by survival of relatively high numbers ($> 10^7$ colony-forming units/ml lumen contents) of metabolically active cells in the human colon (Ducluzeau, 1989). The disparity in scientific reports investigating the health benefits of probiotics in human studies may at least in part be related to survivability problems of the probiotic strain in the product, but mainly after ingestion (MacFarlane & Gibson, 1997; Roberfroid *et al.* 1998; Collins & Gibson, 1999). Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that can improve host health (Gibson & Roberfroid, 1995). Efficient prebiotics need to induce a specific fermentation in the colon. This process should occur by the stimulation of benign, or potentially health-promoting, indigenous bacterial genera but not harmful organisms (Gibson & Roberfroid, 1995). The premise is therefore similar to that of dietary fibres, but with a much more tailored fermentation, e.g. towards lactic acid-producing bacteria (Gibson *et al*. 1996; Collins & Gibson, 1999).

The identification of potential prebiotics is of considerable scientific and commercial interest. FOS are most commonly thought of as prebiotics and occur naturally in onion (*Allium cepa*), asparagus (*Asparagus officinatis*) and artichoke (*Cynara scolymus*). They are poorly digested in the human small intestine, but are fermented in the colon by the resident microflora (Andersson *et al.* 1999). *In vivo* human studies have shown that dietary addition of FOS leads to an increase in faecal bifidobacteria (Gibson *et al.* 1995; Roberfroid *et al.* 1998; Bouhnik *et al.* 1999). However, such studies were conducted using FOS as a dietary supplement. There is no information on the prebiotic capabilities of FOS present in a processed or final food product consumed as part of the daily diet. Guar gum is a soluble fibre derived from the Indian cluster bean (*Cyamopsis tetragonoloba* (*psoralioides*)); it forms viscous solutions and is used worldwide in the food industry as a thickener and emulsion stabilizer. Partially hydrolysing the guar gum causes its viscous consistency to be lost, so that it can be added to food in high amounts. It has been shown that PHGG has a positive effect on diarrhoea, e.g. reducing its duration (Alam *et al.* 1997) or incidence (Homann *et al.* 1994), as well as having an ameliorating effect in human subjects suffering from constipation (Takahashi *et al.* 1994). Similarly, Okubo *et al.* (1994) have demonstrated that ingestion of PHGG at 21 g/d for 14 d resulted in a small increase in bifidobacteria and a sizable increase in lactobacilli in faecal samples collected from nine healthy male volunteers. This response occurred despite the fact that neither bifidobacteria nor lactobacilli were able to grow *in vitro* using PHGG as the only C source. The addition of fermentable fibre (21 g/d) into the colon represents a considerable contribution to the total carbohydrate load available to bacteria in the colon, estimated to be between 40 and 60 g/d (Hudson & Marsh, 1995). Little is known about the prebiotic capabilities of PHGG when delivered into the colon at lower doses.

To date, no data exist on the prebiotic effects of a final food product containing FOS. Here we determined in a human volunteer study the prebiotic potential of PHGG and FOS incorporated into a biscuit. To overcome the limitations of traditional microbiological culture in the recovery and enumeration of specific groups of bacteria, FISH employing 16S rRNA-targeted probes was used for the bacteriology.

Experimental design

Experimental treatment

Experimental biscuits provided 6·6 g FOS/d and 3·4 g PHGG/d. Active treatment was delivered as three biscuits (37·5 g) daily, containing (w/v): wheat flour 40 %, sugar 5 %, vegetable fat 15 %, FOS 20 %, PHGG 11 %, wheat flakes 6 %, baking powder 1 %, salt 0·2 %. Volunteers were free to eat the biscuits at any time of day.

Placebo treatment

Placebo treatment did not contain FOS or PHGG and was delivered as three biscuits daily, containing (w/v): wheat flour 60 %, sugar 12 %, vegetable fat 15 %, wheat flakes 6 %, baking powder 1 %, salt 0·2 %.

Biscuits were supplied by Novartis Nutrition Research AG, Neuenegg, Switzerland and colour coded (experimental or placebo) so as to be blind to investigator as well as volunteers. Volunteers consumed experimental biscuits for one 21 d period and the placebo biscuits for a second 21 d period, i.e. the trial was conducted with a crossover between experimental and placebo treatments. Volunteers were randomly chosen to consume either the experimental or the placebo biscuits during the first treatment. Sixteen volunteers consumed the experimental biscuits during the second treatment. Volunteers were asked to keep diaries while ingesting biscuits, to record stool frequency and consistency (constipation, hard, formed, soft or diarrhoea), abdominal pain (none, mild, moderate or severe), intestinal bloating (none, mild, moderate or severe) and flatulence (none, mild, moderate or severe) on a daily basis. Any concomitant medication, adverse events or volunteer comments were also recorded.

Outline of study protocol

Subjects. Thirty-one healthy volunteers (seventeen females, fourteen males) participated in the present study. Written

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consent was obtained from each individual and the study was approved by the Ethics Committee of the University of Reading. Test biscuits were administered to volunteers at the start of each 21 d treatment period. Volunteers were asked to eat three biscuits daily, and were free to consume the biscuits at any time during the day. Volunteers were asked to return any uneaten biscuits. There was a high level of compliance (judged to be at least 90 %) with only a few volunteers returning one or two packets of biscuits.

Pretrial assessment. Volunteers were assessed for good health and selected on the basis of adherence to inclusion or exclusion criteria. Two pretreatment stool samples were taken, between day −3 and 0.

Treatment 1 (days 1–21). Volunteers consumed biscuits (experimental or placebo) and kept diaries during treatment 1. Stool samples were obtained on two consecutive days starting on day 19, 20, 21 or 22.

Treatment 2 (days 23–44). Volunteers consumed a second set of biscuits (experimental or placebo) and kept diaries during treatment 2. Stool samples were obtained on two consecutive days starting on day 40, 41, 42 or 43.

Volunteers returned unused biscuits and completed diaries.

Post-treatment sampling (days 49, 56 and 63). Single post-treatment stool samples were obtained on day 47, 48, 49, 50 or 51 and on day 54, 55, 56, 57 or 58. Two stool samples were obtained, on two consecutive days starting on day 61, 62, 63 or 64.

Inclusion and exclusion criteria

Inclusion criteria. The criteria were: signed consent form, age 18–50 years inclusive, BMI 20–30 kg/m^2 inclusive and good general health as determined by a medical questionnaire.

Exclusion criteria. Volunteers were excluded from the trial if there was evidence of physical or mental disease or major surgery, as revealed by medical history, that might limit participation in or completion of the study. Volunteers with a history of drug abuse, including alcohol, were excluded. Volunteers were excluded if they were pregnant, lactating or planning pregnancy, had severe allergy or a history of a severe abnormal drug reaction. Other exclusion criteria included: participation in a clinical trial (e.g. for an experimental drug) within 4 weeks before the study, former participation in a prebiotic or laxative trial within the previous 3 months, use of antibiotics within the previous 6 months, chronic constipation, diarrhoea or other chronic gastrointestinal complaints, and intake of other specific prebiotics or probiotics, drugs active on gastrointestinal motility, or a laxative of any class, for 4 weeks before the study.

Requirements for diet and medication during the study. The following were not permitted: intake of additional prebiotics (such as oligosaccharides, e.g. FOS or inulin), probiotics, live yoghurts, drugs active on gastrointestinal motility, antibiotic treatment or any class of laxative. Any medication taken was recorded in diaries. Volunteers were instructed not to alter their usual diet or fluid intake during the trial period.

Bacterial enumeration

Faecal samples were collected in sterile plastic pots and stored at 4°C, and processed within 2 h of collection. Freshly voided faecal samples were diluted 1:10 (w/w) with phosphate buffer (0·1 M; pH 7·0) and mixed in a stomacher for 2 min. Changes in faecal bacterial populations were assessed through the use of FISH with molecular probes targeting 16S rRNA. Genotypic probes targeting predominant components of the gut microflora (bacteroides, bifidobacteria, clostridia and lactobacilli) were manufactured and tagged with fluorescent markers so that quantifiable changes in faecal bacterial populations were determined. The probes used were Bif164 (Langendijk *et al.* 1995), Bac303 (Manz *et al.* 1996), His150 (Franks *et al.* 1998) and Lab158 5′GGTATTAGCA(T/C)CTGTTTCCA (Harmsen *et al.* 1999), specific for bifidobacteria, bacteroides, clostridia (*Clostridium perfringens/histolyticum* sub.grp.) and *Lactobacillus–Enterococcus* spp. respectively. The nucleic acid stain 4′6-diamidino-2-phenylindole was used for total bacterial counts. Faecal samples were diluted and fixed overnight in paraformaldehyde. These cells were then washed with phosphate-buffered saline (0·1 M-NaCl; pH 7·0), resuspended and stored at −20°C. The cell suspension was then added to the hybridization mixture. Hybridization was carried out at appropriate temperatures for the probes. Subsequently, the hybridization mix was vacuum filtered and the filter was mounted onto a microscope slide and examined under a fluorescent microscope. The significance of changes in bacterial numbers measured on ingestion of experimental biscuits compared with bacterial numbers at pretreatment, after ingestion of placebo biscuits, 7 and 14 d after cessation of the intake of experimental biscuits and return to pretreatment population levels, were assessed using the matched paired *t* test.

Results

Bacteriology

Bacterial populations present in the thirty-one volunteers over the experimental period are shown in Table 1. Bacterial counts were determined using FISH and are expressed as $\log_{10}$ cells/g faeces. No significant difference was observed in the numbers of total bacteria, *Bacteroides* spp., *Clostridium* spp. or *Lactobacillus* spp. on ingestion of either experimental or placebo biscuits. However, consumption of experimental biscuits resulted in an increase in bifidobacterial numbers of 0·487 $\log_{10}$ cells/g faeces from pretreatment levels. A significant increase in *Bifidobacterium* spp. was observed on ingestion of experimental biscuits compared with ingestion of the placebo biscuits ($P = 2{\cdot}15 \times 10^{-5}$). Bifidobacterial numbers increased from pretreatment levels of 9·10 $\log_{10}$ cells/g faeces and placebo levels of 9·18 $\log_{10}$ cells/g faeces, to 9·59 $\log_{10}$ cells/g faeces after ingestion of the experimental biscuits.

In the sixteen volunteers who ingested experimental biscuits during the second treatment, no significant difference was observed between bifidobacterial numbers 7 d after cessation of treatment or from pretreatment population levels (Table 2). A bifidobacterial population

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Table 1. Faecal bacterial numbers ($\log_{10}$ cells/g faeces) determined by fluorescent *in situ* hybridization for thirty-one volunteers over the trial period during which they were given placebo biscuits or experimental biscuits containing partially hydrolysed guar gum and fructo-oligosaccharides†

(Mean values and standard deviations)

Treatment group‡…	Pretreatment		Placebo		Experimental		Post-treatment	
Bacterial group	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total bacteria	10·60	0·22	10·61	0·21	10·68	0·22	10·62§	0·44
Bacteroides spp.	9·76	0·24	9·67	0·30	9·71	0·32	9·71§	0·36
Bifidobacterium spp.	9·10	0·42	9·18	0·51	9·59*	0·26	8·98§	0·41
Clostridium spp.	7·93	0·89	7·81	0·79	7·76	0·73	7·68§	0·51
Lactobacillus spp.	7·71	0·44	7·71	0·38	7·72	0·55	7·62§	0·43

Mean value was significantly different from that for the placebo group (paired *t* test): * $P = 2{\cdot}15 \times 10^{-5}$.
† For details of subjects and procedures, see p. 342.
‡ Stool samples were collected at time points between days −14 and 0 (pretreatment), days 0 and 19–23 (placebo), days 19–23 and 40–44 (experimental) and days 61–65 (post-treatment).
§ Mean values were not significantly different from those for the pretreatment group.

level of 9·55 $\log_{10}$ cells/g faeces after experimental treatment decreased to 9·08 $\log_{10}$ cells/g on day 7 after the end of intake of experimental biscuits. Clearly, bifidobacterial population levels returned to approximately pretreatment levels once volunteers stopped eating the experimental biscuits (or at least within 7 d after experimental treatment ceased).

Not all the volunteers responded in a similar manner to ingestion of the experimental biscuits. Fig. 1 correlates the change in bifidobacterial population levels observed in faecal samples with the initial numbers for the thirty-one volunteers. Those volunteers showing the lowest initial bifidobacterial population levels gave the largest increase on ingestion of the experimental biscuits. Fifteen of the volunteers gave increases in bifidobacterial number of $\geq 0{\cdot}5$ $\log_{10}$ cells/g faeces, twelve gave increases of $<0{\cdot}5$ $\log_{10}$ cells/g faeces and in four volunteers bifidobacteria number decreased after ingestion of the experimental biscuits (Fig. 1). In volunteers showing initial bifidobacterial levels of $\geq 9{\cdot}45$ $\log_{10}$ cells/g faeces, no major change in number was observed after ingestion of the experimental biscuits. Overall, bifidobacteria increased in number by more than 0·2 $\log_{10}$ cells/g faeces in 70 % of the volunteers. Little or no change in numbers of total bacteria, *Bacteroides* spp., *Clostridium* spp. or *Lactobacillus–Enterococcus* spp. was observed when data from this group were analysed separately.

Gastrointestinal symptoms and digestive tolerance

Faecal pH changed little during the trial period. Mean faecal pH for the thirty-one volunteers was 6·96, 7·08 and 7·05 for pretreatment, placebo treatment and experimental treatment faecal samples respectively. Similarly, no significant difference was observed in mean daily stool frequency, which was 1·61 (SD 0·94; *n* 31) during placebo treatment and 1·59 (SD 0·67; *n* 31) during experimental treatment. Some volunteers did report increased occurrence of soft stools during the first few days of intake of experimental biscuits.

Table 3 summarizes data on digestive tolerance (flatulence, abdominal pain, bloating) and stool consistency recorded by volunteers during biscuit intake. Stool consistency, qualitatively graded by volunteers as constipation, hard, formed, soft or diarrhoea, varied greatly between individuals. The percentage coverage of each category over the total number of responses given per volunteer was determined. Ingestion of the placebo biscuits gave a small increase in the percentage of stools described as formed–constipation. Stools during experimental biscuit intake were more often described as formed–soft. This description was especially true of stool samples collected during the first few days of ingestion of experimental biscuits. The occurrence of abdominal pain, intestinal bloating and flatulence were also qualitatively recorded daily by volunteers in diaries under the categories none,

Table 2. *Bifidobacterium* spp. population levels ($\log_{10}$ cells/g faeces) determined by fluorescent *in situ* hybridization in sixteen volunteers given experimental biscuits containing partially hydrolysed guar gum and fructo-oligosaccharides during the second treatment (*T*2) stage*

(Mean values and standard deviations)

Pretreatment		Experimental treatment (T2)		T2 + 7 d		T2 + 14 d		Return to pretreatment	
Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
8·98[a]	0·49	9·55[b]	0·25	9·08[a]	0·45	8·83[a]	0·72	8·91†	0·50

[a,b]Mean values with unlike superscript letters were significantly different (paired *t* test; $P = 4{\cdot}99 \times 10^{-7}$).
* For details of subjects and procedures, see p. 342.
† Mean value was not significantly different from pretreatment value.

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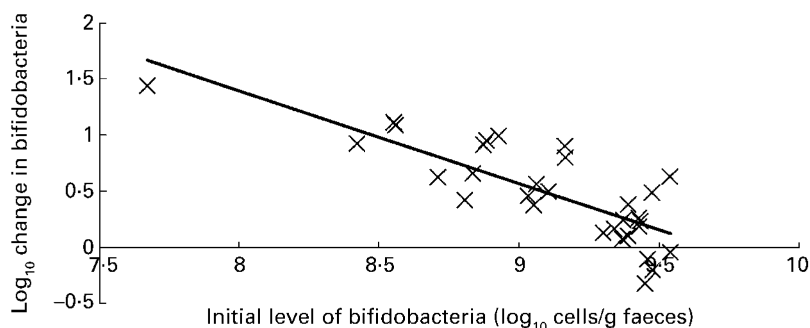

Fig. 1. Increase in bifidobacterial numbers in stool samples collected from thirty-one volunteers as a function of initial pretreatment bifidobacterial population levels. Bacterial counts are expressed as log_{10} cells/g faeces (mean of two samples) and were determined using fluorescent *in situ* hybridization. The line of best fit through the data (R^2 0·6465) shows that volunteers with the lowest initial levels of bifidobacteria gave the largest increase in bifidobacterial numbers on ingestion of the experimental biscuits containing partially hydrolysed guar gum and fructo-oligosaccharides.

mild, moderate and severe. The percentage coverage of each category over the total number of responses given by each volunteer was determined (Table 3). As with stool consistency, the range and frequency of responses given by different volunteers varied greatly. During experimental treatment, a small increase in mild (6·09 %) and moderate (7·28 %) abdominal pain was reported. There was also a general trend towards a reporting of flatulence and intestinal bloating as moderate during consumption of experimental biscuits compared with mild during placebo ingestion. Only two volunteers reported any severe or significant increase in flatulence (and intestinal bloating in one of these volunteers) during ingestion of experimental biscuits.

Moderate and severe intestinal bloating increased by 5·09 and 18·38 % respectively, during experimental treatment compared with placebo treatment. Moderate and severe flatulence increased by 10·84 and 8·96 % respectively during ingestion of the experimental biscuit.

Table 3. Summary of data recorded* in diaries by thirty-one volunteers over the course of the trial of intake of placebo biscuits or experimental biscuits containing partially hydrolysed guar gum and fructo-oligosaccharides†

(Mean values and standard deviations)

Treatment group…	Placebo		Experimental	
	Mean	SD	Mean	SD
Stool consistency				
Constipation	30·95	10·11	14·46	11·88
Hard	17·87	9·94	12·03	8·75
Formed	70·78	28·43	63·15	23·98
Soft	31·40	29·41	31·05	24·71
Diarrhoea	8·33	5·59	7·66	3·79
Digestive tolerance				
Abdominal pain				
None	89·04	15·76	77·16	23·64
Mild	15·34	11·58	21·43	18·87
Moderate	10·57	7·51	17·85	22·62
Severe	5·3		13·6	
Intestinal bloating				
None	80·95	25·44	64·21	30·91
Mild	32·70	28·25	32·30	21·30
Moderate	15·38	12·63	20·47	19·58
Severe	–	–	18·38	24·00
Flatulence				
None	60·11	29·51	42·92	33·48
Mild	51·35	27·91	43·30	22·96
Moderate	18·38	12·01	29·22	15·78
Severe	6·75	2·76	15·71	20·53

* Percentage coverage of each category over the total number of responses given per volunteer was determined.

† For details of subjects and procedures, see p. 342.

Discussion

In the present study the prebiotic potential of PHGG and FOS incorporated into a biscuit was assessed in thirty-one healthy volunteers. The present study was carried out in a double-blind randomized crossover manner, with volunteers consuming experimental biscuits for 21 d and placebo biscuits for 21 d. *Bifidobacterium* spp., *Bacteroides* spp., *Clostridium* spp. and *Lactobacillus–Enterococcus* spp. were enumerated using FISH, and total bacteria were determined using the fluorescent stain 4′,6-diamidino-2-phenylindole.

The prebiotic nature of FOS has been demonstrated previously in various human volunteer studies (Gibson *et al.* 1995; Roberfroid *et al.* 1998), and PHGG has also been shown to have a positive effect on gut motility (Homann *et al.* 1994; Takahashi *et al.* 1994). However, there is little information on the ability of specific prebiotics to retain their functionality in final food products. Similarly, previous studies have relied on traditional microbiological culture techniques to monitor changes in gut microflora in response to prebiotics. In view of recent observations relating to the lack of specificity shown by so-called 'selective agars', and the proportion of bacteria in the gut microflora that remain unculturable, direct enumeration of micro-organisms in environmental samples by the use of novel molecular procedures constitutes a significant technological advance (Ward *et al.* 1992; Amann *et al.* 1995; Nelson & George, 1995).

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In the present study consumption of biscuits containing FOS and PHGG gave a significant increase in faecal bifidobacterial numbers ($P = 2{\cdot}15 \times 10^{-5}$). Bifidobacteria increased from 9·18 $\log_{10}$ cells/g faeces after the placebo treatment to 9·59 $\log_{10}$ cells/g faeces after the experimental treatment. Little or no change in the numbers of total bacteria, *Bacteroides* spp., *Clostridium* spp. or *Lactobacillus–Enterococcus* spp. present in faecal samples collected over the course of the trial was observed. No major changes in bacterial populations were observed on ingestion of the placebo biscuits. In volunteers fed the experimental biscuits during the second treatment period, no significant difference was observed between bifidobacterial populations 7 d after the end of active treatment and those in pretreatment samples. This finding indicates that the bifidogenesis observed on ingestion of the experimental biscuits did not persist once treatment had ceased. Similarly, in previous prebiotic feeding studies (Roberfroid *et al.* 1998), increases in the number of faecal bifidobacteria in volunteers fed prebiotics returned to prefeeding levels once prebiotic ingestion had ceased. Such observations illustrate the selective nature of prebiotic fermentation in the colon, and add weight to the concept of beneficial modulation of the gut microflora through dietary supplementation with functional foods. No changes in stool frequency or faecal pH were observed in the present study. The severity and frequency of reported changes in gastrointestinal symptoms varied greatly between volunteers. In general, ingestion of the experimental biscuits resulted in stools more often described as formed–soft and a reduction in the reported incidence of hard stools and constipation. However, a small increase in abdominal pain, intestinal bloating and flatulence was reported by some volunteers on ingestion of the experimental biscuits. No correlation between the level of increase in faecal bifidobacteria and gastrointestinal symptoms was discerned, however. Bifidobacteria do not produce gas during carbohydrate fermentation and, in the present study, little difference in numbers of clostridia (prolific gas producers) was observed on ingestion of the experimental biscuits. The relationship between numbers of specific bacteria in the gut microflora and gas production is not well understood (Levitt *et al.* 1995). It is possible that consumption of FOS or PHGG may result in increased gas production, and associated increases in abdominal symptoms, without a concomitant increase in the numbers of the bacterial groups that were monitored in the present study. The relationship between gas economy in the colon, the bacterial species involved and their metabolic response to dietary components, including prebiotics, remains to be clarified.

As noted previously (Roberfroid *et al.* 1998), a correlation may exist between initial *Bifidobacterium* spp. population levels and the magnitude of the increase in bifidobacterial numbers observed in the volunteers fed prebiotics. In the present study volunteers showing no response to the experimental biscuits all possessed relatively large populations of bifidobacteria ($>9{\cdot}3$ $\log_{10}$ cells/g faeces) in pretreatment faecal samples (Fig. 1).

The bifidogenic nature of a final food product containing FOS and PHGG has been demonstrated in the present study. Previous studies in human volunteers have determined the bifidogenic nature of FOS (Gibson *et al.* 1995; Roberfroid *et al.* 1998). Gibson *et al.* (1995) showed that its consumption reduced numbers of *Clostridium* spp. and *Bacteroides* spp. as well as increasing numbers of bifidobacteria in human volunteers. No such reductions in numbers of *Clostridium* spp. or *Bacteroides* spp. were observed in the present study. Here, we employed a powerful molecular technique (FISH) to enumerate bacteria directly *in situ* in faecal samples, whereas previous studies have relied on traditional microbiological culture techniques. Selective agars rely on differing growth characteristics of different bacterial groups. However, it is becoming clear that phylogenetically unrelated bacterial species often share phenotypic traits, allowing them to grow on purportedly ‘selective’ agars (Woese, 1987; Nelson & George, 1995). FISH, on the other hand, allows the enumeration of bacteria that are closely related at the phylogenetic level. Thus, in order to elucidate the effects of FOS on clostridia and bacteroides populations in the human gut microflora, it may be necessary to use a number of different molecular and microbiological culture-based techniques.

Bifidobacteria have long been regarded among the beneficial members of the human gut microflora. One aspect is the improved health benefits associated with the bifidobacteria-dominated gut microflora of breast-fed infants (Campbell & Jones, 1996; Vanderhoof & Young, 1998). High numbers of bifidobacteria are also seen as positive for adult health. Bifidobacteria have been shown to inhibit the growth of pathogenic bacteria, to modulate the immune system, to produce digestive enzymes, to repress the activities of rotaviruses and to restore the microbial integrity of the gut microflora following antibiotic therapy or antibiotic-associated diarrhoea (Bernet *et al.* 1993; Gibson & Wang, 1994; Saavedra *et al.* 1994, Collins & Gibson, 1999; McCracken & Gaskins, 1999). Application of prebiotic ingredients to food products conducive to the modern way of living (e.g. biscuits and cereals) has the potential to improve the gastrointestinal health of the population as a whole. However, it is on the health of specific groups of individuals with lowered bifidobacterial numbers in their gut microflora that prebiotic foods may have the greatest impact. These groups may include the elderly, individuals undergoing antibiotic therapy or those with antibiotic-associated diarrhoea and individuals with persistent gastrointestinal conditions, such as inflammatory bowel disease or gastroenteritis. In the present study we have shown that healthy volunteers with lower initial bifidobacterial populations in faecal samples displayed the largest increases on consumption of prebiotic-containing biscuits. Similar observations have been made previously with FOS and inulin (Roberfroid *et al.* 1998). Thus, prebiotic biscuits containing PHGG and FOS may prove efficacious for increasing bifidobacterial numbers in the gut microflora of those individuals with lowered colonic bifidobacterial numbers. However, well-designed placebo-controlled and, at least, double-blind clinical trials are required to determine any health benefits associated with increased gut bifidobacterial numbers in such groups as patients with inflammatory bowel disease and hospitalized elderly patients at risk of nosocomially and antibiotic-acquired diarrhoea.

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