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ORIGINAL CONTRIBUTION

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# Effects of low doses of lactitol on faecal microflora, pH, short chain fatty acids and gastrointestinal symptomology

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**Abstract** *Background* Lactitol (4-$\beta$-D-galactopyranosyl-D-glucitol) is a sugar alcohol used as a sweetener. Previous studies have shown that it has a beneficial effect on intestinal microflora. *Aims of the study* To determine whether low doses of lactitol had beneficial effects without eliciting adverse gastrointestinal symptoms. *Methods* Faecal bacterial populations (total anaerobes, total aerobes, enterobacteria, bifidobacteria and lactobacilli), faecal pH and faecal short chain fatty acids (SCFA) were studied in a randomized longitudinal study of 75 non-adapted healthy adults before and after consumption of low doses of lactitol. Subjects consumed 25 g tablets of milk chocolate containing 10 g sweetener as sucrose:lactitol in ratios of 10:0, 5:5 or 0:10 daily for 7 d. *Results* No significant changes in faecal bacterial counts occurred in the 10:0 or 5:5 sucrose:lactitol groups. There were no significant changes in faecal anaerobes, aerobes, Enterobacteriaceae or lactobacilli during the study period in subjects consuming 0:10 sucrose:lactitol but there was a significant increase ($P = 0.017$) in bifidobacteria. There were no significant changes in faecal pH and SCFA for the 10:0 or 5:5 sucrose:lactitol groups but a significant decrease ($P = 0.02$) in faecal pH and significant increases ($P = 0.001$) in concentrations of propionic and butyric acids were observed in the 0:10 sucrose:lactitol group. There were few adverse symptoms of gastrointestinal intolerance to the daily consumption of 10 g lactitol. *Conclusions* The results show that low doses of lactitol can beneficially affect the faecal flora without eliciting gross symptoms of intolerance and that lactitol can be classified as a prebiotic.

**Key words** *bifidobacterium* – faecal microflora – lactitol – short chain fatty acids – gastrointestinal symptomology

## Introduction

Lactitol (4-$\beta$-D-galactopyranosyl-D-glucitol) is a sweet-tasting sugar alcohol derived from lactose. Because the upper part of the gastrointestinal tract of humans lacks a suitable $\beta$-galactosidase, the major part of ingested lactitol reaches the human large intestine as an intact disaccharide [13]. The clinical benefits of administrating lactitol have been investigated in adults suffering from chronic functional constipation [32, 40] and for the management of episodic and acute hepatic encephalopathy in cirrhotic patients [18, 35, 39].

The microflora of the large intestine comprises more than 500 different bacterial species [38, 42] with approximately $10^{11}$–$10^{12}$ bacteria per gram of contents [8]. Intestinal bacteria may be broadly divided into species that are either harmful (causing diarrhoea, infection, production of carcinogens, intestinal putrefaction) or beneficial (competition with pathogens, stimulation of gut associated lymphoid tissue) to the health of the host [11]. Bifidobacteria are quantitatively one of the most important genera of intestinal bacteria in man and stimulation of bifidobacterial growth in the colon is positive for human health because of their potential health promoting activities [3, 22] e.g. increased production of fermentation products in the form of short chain fatty acids (SCFA) which act as a vital energy sources for colonocytes [23] and exert a trophic impact on the colonic mucosa [36]. Non- or slowly-digestible food ingredients, especially carbohydrates, are the principle substrates for bacterial growth in the large intestine [7]. Specific carbon sources are used as bifidogenic factors to selectively stimulate the growth of bifidobacteria in vivo [11, 28]. The selective stimulation of growth and/or activity of one or a limited number of bacteria in the colon to the benefit of the host by ingestion of non-digestible food ingredients are now widely accepted [11]. In order to be effective, the food ingredient must resist digestion in the upper digestive tract and then be selectively fermented in the colon such that the microflora is altered towards a potentially healthier composition; such food ingredients are known as prebiotics [10, 33].

Fermentation of non-digestible carbohydrates to SCFA is central to many of the proposed health benefits provided by prebiotics [6, 21]. Colonic fermentation of prebiotics produces mainly acetate, propionate and butyrate [9]. SCFA stimulate intestinal mucosal cell proliferation, the effect is dose-dependent and butyric acid is more effective than acetic and propionic acids [34]. SCFA have been shown to have positive effects in maintaining a healthy colonic mucosa and offer a protective role in colonic tumorigenesis [1]. Butyric acid in particular has received attention as a stimulator of increased apoptosis in transformed cells but not in healthy colonic cells [24, 43].

Incorporation of low digestible carbohydrates into foods intended for human consumption offers many potential health benefits [37]. However, also of importance to the consumer is the possible risk of intolerance and its potential to cause unwanted symptoms. Symptoms of intolerance occur depending on the degree of malabsorption, the osmotic activity of the sugar alcohol and whether the fermentative capacity of the colon is exceeded [31]. Therefore, because high doses of lactitol are not well tolerated in healthy adults, the present study investigated the influence of lower doses of lactitol on gastrointestinal function. The lowest dose used previously was 20 g $d^{-1}$ [4]. However as little as 10 g $d^{-1}$ may still significantly increase the amount of unabsorbed carbohydrates reaching the colon. The study describes the effects of low doses of lactitol on selected bacteria, pH and SCFA content in faecal specimens from healthy adults.

## Materials and methods

### Study design

The study was of a randomized, longitudinal double blind design and was conducted in accordance with the ABPI Guidelines for Medical Experiments in Non-Patient Human Volunteers [2]. Subjects were recruited from the student population of The University of Salford by publicity. Subjects were subjected to prescreening to ensure that they had no history of either gastrointestinal or metabolic disorders and were not subject to any dietary restrictions, prescribed diets or supplementary fibre intake and had not received antibiotics, steroids or any drugs for 6 months prior to the study. Medication prescribed during the study was to be reported to enable subject's suitability for continued participation to be reassessed. A total of 75 subjects were successfully recruited onto the study.

Subjects were healthy, non-vegetarian, non-adaptive, naïve consumers of pre- and pro-biotic products aged between 18 and 24 years, 39 were male and 26 were female. Body mass indices (mean ± SD) were: male 22.80 ± 3.01 and female 22.53 ± 2.88.

### Study restrictions

Subjects were required to adhere to certain restrictions 24 h before commencement of the study and throughout the period of the study and were to limit their intake of milk and fruit juice to 300 $cm^3$ each day and their daily alcohol intake to no more than 2 pints of beer or half a bottle of wine or four single measures of spirits. Subjects were also requested not to consume any sugar free/low calorie products, any pre- or pro-biotic products or fermented dairy products during the study. Subjects were individually debriefed during their visits to the laboratory to ensure they had adhered to study restrictions. All subjects who completed the study adhered to the study restrictions.

## Test product and experimental design

The test product, lactitol, was supplied by PURAC Biochem bv, The Netherlands and was in the form of 25 g chocolate bars. The bars were divided into three groups containing 10 g sweetener in the ratios 10:0, 5:5 or 0:10 sucrose:lactitol. To ensure a double blind design, the bars were coded by the supplier and were randomly assigned to each of the 75 subjects (25 subjects in each group). Chocolate bars were supplied in two pieces and subjects were instructed to consume one chocolate bar per day for 7 d, the first piece mid-morning, the second mid-afternoon. The lactitol content of the bars was not revealed to the investigators until after completion of the study. Faecal bacterial analysis.

## Collection and processing of faecal samples

Faecal samples from each subject were supplied on two occasions during the participation of the study. The first sample (d 0:baseline counts) in the morning before commencement of test product consumption, and the second sample on d 7 following completion of test product consumption. Faecal samples were collected into sterile bags (GENbag anaer™, Bio Mérieux, France); an anaerobic sachet was added to the bag before clip sealing. Samples were then brought immediately into the laboratory. Faecal samples were transferred into an anaerobic cabinet (Compact M, Don Whitley Scientific, UK) supplied with 80% nitrogen, 10% carbon dioxide, 10% hydrogen, (BOC UK) and all subsequent assays were performed under anaerobic conditions. Faeces (1.0 g) was transferred into a sterile universal bottle containing 9.0 $cm^3$ pre-reduced buffered peptone water (Oxoid, Unipath Ltd. UK) containing 0.5 g $dm^{-3}$ cysteine-HCl and homogenized using a sterile swab. Culture media.MacConkey agar No. 3 (MacConkey) (Oxoid, Unipath Ltd. UK) was used for *Enterobacteriaceae*. Brain heart infusion blood agar (BHIB) was used for total counts of anaerobes and aerobes: 37 g $dm^{-3}$ brain heart infusion broth (Lab M, Bury, UK) was added to 16 g $dm^{-3}$ agar base No. 1 (Oxoid, Unipath Ltd, UK). After sterilization, the medium was cooled to 45°C and then 50 $cm^3$ $dm^{-3}$ defibrinated sheep blood was added (P & R Lab Supplies, Liverpool, UK). LAMVAB agar [14] was used for *Lactobacillus* and Raffinose-Bifidobacterium (RB) agar [15] for *Bifidobacterium*. Chemicals were obtained from Sigma Aldrich, (Poole, UK). Quantification of faecal microflora. Ten-fold serial dilutions of the faecal suspension were prepared in pre-reduced buffered peptone water and non - selective and selective media inoculated in triplicate with 0.1 $cm^3$ of appropriate dilutions all media were incubated at 37°C. After the appropriate incubation (BHIB, 1–3 d aerobically for total aerobes, 5–7 d for total anaerobes; 1–3 d aerobically for McConkey agar; 3–5 d anaerobically for LAMVAB and 3–5 d anaerobically for RB), total colonies were counted on the non-selective media. Typical colonies were counted on selective media based on colony and gram-staining characteristics. Subsequent biochemical identification confirmed colony identification to genus level. Pink or colourlesss, Gram-negative, oxidase negative, aerobic rods isolated from MacConkey agar were confirmed as *Enterobacteriaceae* using the Vitek Gram-negative identification system (Bio Mérieux, France). Identification of *Lactobacillus* was based on colony morphology using the criteria of Hartemink et al. (green colonies >1.0 mm diam) [14]. Identification of *Bifidobacterium* was identified using the colony morphology criteria of Hartemink et al. (yellow colonies >1.0 mm diam with a yellow opaque halo) [15]. Selected colonies were Gram stained and identification of *Lactobacillus* and *Bifidobacterium* confirmed using the rapid ID 32A identification system (BioMérieux, France).

## Faecal pH

Faeces (1.0 g) were homogenised in 10 $cm^3$ sterile distilled water using a sterile swab. The pH's of the suspensions were measured using a glass pH electrode (Corning 220 pH meter).

## Faecal SCFA

Short chain fatty acids analyses were carried out by gas chromatography using a BP21 capillary column (internal diameter 0.53 mm, length 12 m, SGE, UK) fitted with 1.0 m, deactivated silica pre-column (SGE, UK). These were fitted into a Hewlett Packard 5790A series Gas Chromatograph with a Flame Ionisation Detector (FID) using nitrogen (BOC, UK) as carrier gas. Air (BOC, UK) and hydrogen (Hewlett Packard 9100 series hydrogen generator), provided the gases for the FID flame. Faecal samples were filtered by membrane filtration (cellulose nitrate 0.22 μm pore size, Whatman International UK) before injection onto the column. Faecal SCFA concentrations were expressed as mean μmol g wet weight $faeces^{-1}$.

## Gastrointestinal symptomatology and laxation

Subjects were provided with pre-printed forms to record daily symptomatology and laxation data. Gastrointestinal intolerance symptoms of nausea,

colic, bloating, borborygmi and flatus were recorded and ranked on a hedonic scale according to the following: 0 (Normal) no symptom or no more symptom than usual; 1 (Mild) slightly more symptom than usual; 2 (Moderate) noticeably more symptom than usual and 3 (Severe) considerably more symptom than usual. From this data total symptom scores for each subject were then calculated along with the number of toilet visits and the consistency of faeces passed on each day of the study. The toilet visits data was recorded as follows: 0 = 0 toilet visits to pass watery faeces; 1 = 1 toilet visit to pass watery faeces; 2 = 2 toilet visits to pass watery faeces etc.

### Statistical analysis

Quantitative results were expressed as mean ± SEM g wet weight faeces$^{-1}$. The differences between means on day 0 and day 7 were compared for each test group using the Student's *t* test. Wilcoxon Rank-Sum test was performed to compare the incidence of mean total symptoms in the three study groups.

## Results

### Quantification of faecal microflora

The mean ± SEM numbers of total anaerobes, total aerobes, *Enterobacteriaceae*, *Lactobacillus* spp. and *Bifidobacterium* spp. ($Log_{10}$ CFU g wet weight faeces$^{-1}$) and faecal pH for subjects consuming 10:0, 5:5 or 0:10 sucrose:lactitol for 7 d are shown in Fig. 1. The results show that the mean counts for the groups consuming 0 and 5.0 g lactitol remained relatively stable and no significant differences were observed ($P > 0.05$). The mean bacterial counts of total anaerobes, aerobes, *Enterobacteriaceae* and lactobacilli also remained quite stable following consumption of 10 g lactitol, however

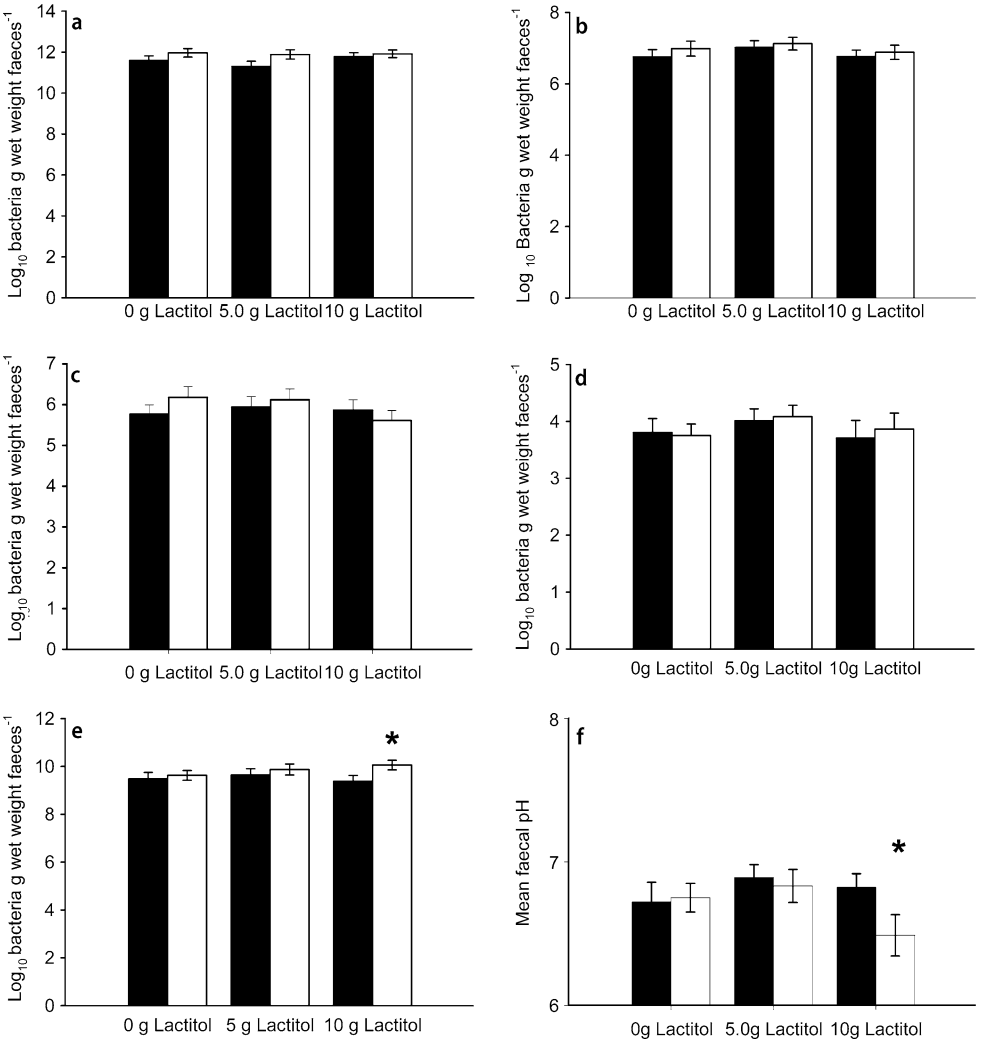


**Fig. 1** Effects of lactitol consumption on faecal flora and pH. Mean ± SEM $log_{10}$ CFU g wet weight (w/w) faeces$^{-1}$ and pH of faecal bacteria were determined on day 0 and day 7 for subjects consuming 0, 5.0 and 10 g lactitol daily for 7 days ($n = 25$ for each group). (**a**) total anaerobes, (**b**) total aerobes, (**c**) *Enterobacteriaceae*, (**d**) *Lactobacillus* spp., (**e**) *Bifidobacterium* spp. (**f**) pH. (■ Day 0, □ Day 7). Levels of significance (Student's *t* test) *$P \leq 0.05$

*Bifidobacterium* counts showed a significant increase from 9.37 to 10.06 (Fig. 1e, $P = 0.017$). There was no significant difference in mean pH for the groups consuming 0 and 5.0 g lactitol (Fig. 1f; $P > 0.05$). Faecal pH decreased significantly ($P = 0.023$) for subjects consuming 10 g lactitol (day 0, 6.82; day 7, 6.48).

The percentage molar proportions of faecal SCFA (acetic, propionic and butyric) from subjects consuming 0, 5.0 and 10 g lactitol are shown in Fig. 2. There were no significant differences in SCFA for the groups consuming 0 g (Fig. 3a) and 5.0 g lactitol or in acetic acid for the group consuming 10 g lactitol (Fig. 3b, $P > 0.05$). There were however, significant differences in propionic ($P = 0.001$) and butyric ($P = 0.001$) acids for the group consuming 10 g lactitol (Fig. 3c). Between day 0 and day 7 the mean (SEM) concentration of propionic acid increased from 11.7 (19.25) to 19.3 (32.69) µmol g wet weight faeces$^{-1}$ and butyric acid concentrations increased from 9.1 (15.57) to 15.4 (27.53) µmol g wet weight faeces$^{-1}$.

### Gastrointestinal symptomatology and laxation

Total scores for symptoms of nausea, colic, bloating, borborygmi and flatus during consumption of the test product were calculated (Fig. 3). Frequency of toilet visits and faecal consistency were also noted. Mean total symptom scores were lowest for the 0 g lactitol group with mean (SD) scores of individual subjects ranging from 0.44 (0.26) to 1.0 (0.51) during the study. Mean (SD) total symptom scores for the 5.0 g lactitol group were between 0.36 (0.35) and1.12 (0.67), and between 0.76 (0.44) and 1.96 (0.71) for the 10 g lactitol group. Analysis of the individual results showed that three subjects reported moderate to severe symptoms of flatus for the first few days of consumption of 10 g lactitol. One of the subjects returned to normal within two d and the other reported mild symptoms for the remainder of the study. Two further subjects reported mild symptoms of flatus throughout the study but there was no increase in the number of toilet visits or a change in faecal consistency. Only one subject reported moderate/severe symptoms of flatus and a change in faecal consistency for the duration of the study. This was associated with an increase in toilet visits but not on every day of the study. There was a significant difference in total symptom scores between the 0 and 10 g lactitol groups ($P = 0.004$).

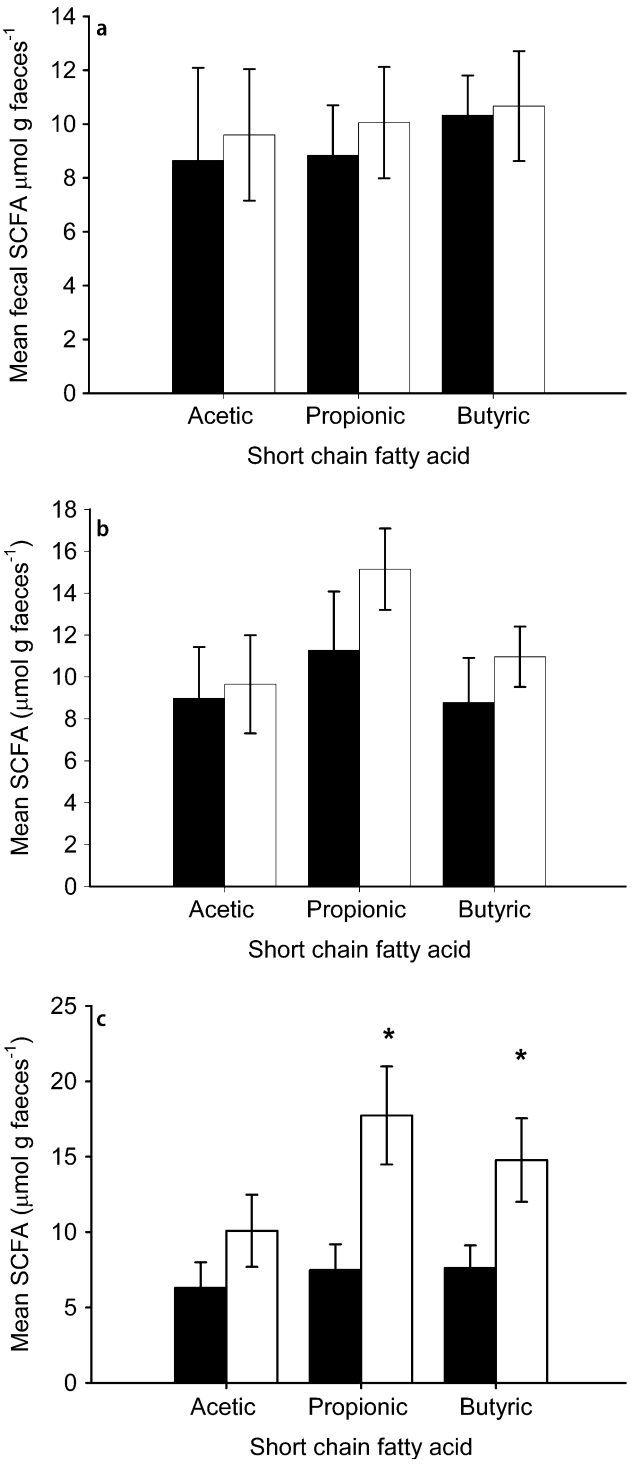


**Fig. 2** Effects of lactitol consumption on faecal short chain fatty acids. The percentage molar proportions of faecal SCFA (acetic, propionic and butyric) were determined on day 0 (■) and day 7 (□) for subjects consuming (**a**) 0 g, (**b**) 5.0 g and (**c**) 10 g lactitol daily for 7 d. Levels of significance (Student's *t* test) *$P \leq 0.05$

## Discussion

The results showed that consumption of 10 g d$^{-1}$ lactitol induced changes in intestinal bacteria in healthy adults similar to those demonstrated for higher doses without inducing undue adverse symptoms. No significant changes were observed in the

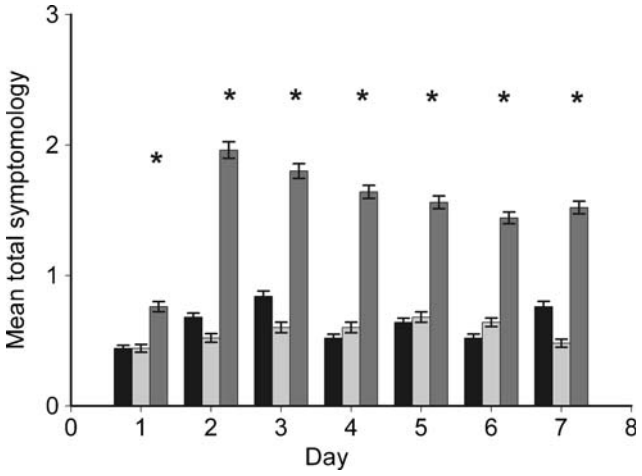


**Fig. 3** Effects of lactitol consumption on severity of self-reported gastrointestinal symptoms Mean ± SEM total symptom score of subjects consuming 0 g (■) and day 7 (■) and 10 g (■) lactitol daily for 7 d ($n = 25$ per group). Mean total symptom score for the 0 and 10 g lactitol groups were significantly different. Levels of significance (Wilcoxon rank sum test) $^*P \leq 0.05$

counts of total anaerobes, total aerobes, *Enterobacteriaceae* or *Lactobacillus*. There was, however, a significant increase in counts of *Bifidobacterium*. This confirms that lactitol has the properties of a prebiotic. Ballongue et al. [4] found increased faecal bifidobacteria and lactobacilli when daily doses of lactitol (20 g) were administered to healthy volunteers. Similarly, increased numbers of saccharolytic bacteria and decreased numbers of proteolytic bacteria were found with 40 g $d^{-1}$ lactitol in patients with liver disease [35].

An Important criterion for classification of a food component as a prebiotic is the selective fermentation of the substrate such that the composition of the large intestine is altered toward a potentially healthier community [10, 33]. The results from this study indicated that both propionic and butyric acids increased significantly following the consumption of 10 g lactitol. Despite being randomly allocated to the three groups, the mean SCFA in the 10 g $d^{-1}$ lactitol group were lower than the other groups. This is possibly due to the great variability shown in faecal flora in different individuals [30]. The increase in SCFA is in agreement with previous authors who examined SCFA concentrations in faecal samples of adult subjects following daily consumption of lactitol [4, 32]. Ravelli et al. [32] showed that acetate, propionate and butyrate all increased significantly and Ballongue et al. [4] reported increases in acetate and lactate. Subjects in both these studies consumed 20 g $d^{-1}$ lactitol compared to the maximum 10 g $d^{-1}$ lactitol in this study and the differences in SCFA between the two studies probably reflect differences in the amounts of lactitol consumed. Minekus et al. [27] reported that lactitol fermentation in vitro resulted in relatively low production of acetic acid by mixed intestinal flora and a higher molar ratio of butyric acid. They reported that the fermentation of lactitol was slow compared to the other substrates investigated but did not account for the slower fermentation or could not specifically account for the different molar ratios obtained. Ballongue et al. [4] found that lactitol fermentation in healthy volunteers was slower than that of lactulose. Lactitol utilization was also slower than lactitol oligosaccharide and this was attributed to the intermolecular hydrogen bonding between the oxygen molecule of galactose and the hydroxyl of sorbitol giving lactitol molecule a more rigid structure than lactose-oligosaccharide [16, 44]. Measurement of SCFA production from the in vivo fermentation of dietary substrates by determining the concentration in faeces has its limitations because it does not give information on the events occurring along the length of the bowel. Around 95% of the SCFA generated in the colon is absorbed [8], however, faecal SCFA are frequently used as an indicator of intestinal concentrations due to the difficulty in accessing intestinal contents.

The findings from this study also indicated that there was a significant decline in faecal pH in the 10 g lactitol study group which may have been due to production of increased SCFA. The lower pH may have created a more favourable environment for the growth of bifidobacteria and lactobacilli. Many intestinal pathogens and putrefactive bacteria prefer a neutral pH [41] and SCFA production from prebiotic fermentation and the concomitant decrease in pH may contribute to the reduction of these bacteria [26]. The increase in bifidobacteria shown here cannot be directly responsible for the increased levels of butyrate since the main fermentation products produced by bifidobacteria are acetate and proprionate. The production of butyrate in the intestine is however complex and production of acetate may stimulate butyrogenic species [30]. Future studies should look for a wider range of bacteria including butyrogenic species.

Although there was a significant increase in total gastrointestinal symptom scores, the symptoms reported by most subjects were generally "no more than normal". Undesirable symptoms following consumption of non-digestible carbohydrates result from osmotic effects resulting in liquid secretion into the bowel leading to laxation or production of gas leading to flatulence and colic [20]. Flatus appeared to be the main intolerance symptom reported by subjects consuming 10 g lactitol. The subjects who experienced moderate to severe symptoms of flatus at the start of the study adapted to the consumption of lactitol whereas the two subjects who experienced

mild symptoms of flatus throughout the study and the single subject with moderate to severe flatus throughout the study did not adapt to the consumption of lactitol. Various studies have been carried out to establish the gastrointestinal response and the laxative threshold following the ingestion of different quantities of lactitol. A dose of 20 g $d^{-1}$ was tolerated in one study [5] but gastrointestinal symptoms occurred in a second study [12]. Koutsou et al. [17] reported a significant increase in gastrointestinal intolerance following the ingestion of 30 or 40 g lactitol and found that the incidence and severity of symptoms was dose dependent. It has also been demonstrated that there is an important inter-subject variability in the tolerance of low digestible carbohydrates and the risk of intolerance may also vary in the same individual [29]. The variability between subjects is probably due to differences in absorption capacities, motility patterns, colonic responses and intestinal sensitivity [25]. The results of the present study show that consumption of 10 g $d^{-1}$ lactitol did not elicit gross symptoms of intolerance yet produced beneficial changes in the human intestinal bacteria, faecal pH and the quantities of the SCFA produced. The modulation in gut flora may be of particular benefit in liver disease [19].

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